



**The effectiveness of different lifestyle interventions on
body composition, insulin sensitivity, and novel
biomarkers of insulin resistance in obese individuals**

Diane Cooper

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Requirement for the degree of Doctor of Philosophy

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Supervised by:

Dr. Donal O’Gorman

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Authors Declaration

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of Doctor of Philosophy is entirely my own work, and that I have exercised reasonable care to ensure that the work is original, and does not to the best of my knowledge breach any law of copyright, and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

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ID: **52418568**

Date: _____

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List of Abbreviations

1RM	1 Repetition Maximum Strength Test
3RM	3 Repetition Maximum Strength Test
ACSM	American College of Sports Medicine
AUCG	Area under the Glucose Curve
AUCI	Area under the Insulin Curve
AUCG/AUCI	Area under the insulin curve / area under the glucose curve index
BMI	Body Mass Index
CSA	Cross Sectional Area
DEXA Scan	Dual Energy X-Ray Absorptiometry
ECG	Electrocardiogram
FFA's	Free Fatty Acids
HDL	High Density Lipoprotein Cholesterol
HOMA-B	Model that reflects fasting insulin secretion
LDL	Low Density Lipoprotein Cholesterol
MRI	Magnetic Resonance Imaging
OGTT	Oral Glucose Tolerance Test
RMR	Resting Metabolic Rate
TG's	Triglycerides
TC	Total Cholesterol
$\dot{V}O_{2max}$	Maximal Oxygen Uptake

Glossary of Terms

Concentric Muscle Contraction

The phase of muscle contraction where the muscle is shortening under tension. The actin and myosin filaments are being pulled towards the centre of the sarcomere.

Concurrent Training

The performance of both strength and endurance training during the same phase of a training programme in order to achieve multiple training goals.

Eccentric Muscle Contraction

The phase of muscle contraction where the muscle is lengthening under tension. The actin and myosin filaments are being pulled away from the centre of the sarcomere.

Gluconeogenesis

The formation of glucose from non- glucose sources such as amino acids and lactate.

Glycogenolysis

The breakdown of glycogen to form glucose.

Glycolysis

The breakdown of carbohydrate (glycogen or glucose) to form lactate and ATP (anaerobic) or pyruvate and ATP (aerobic).

Insulin Resistance

Resistance to the action of insulin. The circulating concentrations of insulin that are effective in controlling blood glucose in normal glucose tolerant individuals are not as effective in insulin resistance individuals.

Maximal Oxygen Uptake ($\dot{V}O_{2\max}$)

The maximal ability of an individual to take up, transport and utilise oxygen by the working muscles.

Metabolic flexibility

The ability of the muscle to switch effectively between fuel sources for oxidation and storage when challenged with conditions such as fasting, feeding, exercise and the post exercise period.

Resting Metabolic Rate (RMR)

Includes basal metabolic rate (the minimum energy requirements to sustain the body's functions in the waking state) plus the added cost of arousal.

Abstract

Title: The effectiveness of different lifestyle interventions on body composition, insulin sensitivity, and novel biomarkers of insulin resistance in obese individuals.

Author: Diane Cooper.

Obesity is a serious global health problem (WHO, 2009) and an independent risk factor for a number of chronic diseases (Quilliot, Petit et al. 2002). Obesity is caused by a mismatch between energy intake and energy expenditure leading to storage of surplus energy and expansion of fat stores which causes metabolic disturbances (Speakman, 2004). Diet and exercise interventions remain the cornerstone of treatment for obesity but they have been largely ineffective (Franz et al. 2007). The purpose of this thesis was to identify components of a lifestyle intervention that are most likely to improve body composition, insulin sensitivity, and novel biomarkers of insulin resistance in obese individuals. This was achieved by investigating 4 different short term interventions. In study 1, isocaloric diet and exercise interventions were compared, while in study 2 concurrent training (regular resistance training combined with aerobic exercise) and concurrent training incorporating an eccentric component were compared.

Briefly, the key findings of this PhD thesis were that isocaloric diet and exercise interventions lead to similar reductions in body weight, but exercise training may lead to greater improvements in body composition and metabolic health. Importantly, aerobic fitness was the single best predictor of improvements in metabolic health in this population. Resistance training is important for improving lean tissue mass, fat oxidation and resting metabolic rate. The novel biomarkers of insulin resistance are differentially regulated by diet, exercise and different modes of exercise training. Improvements in body composition and fitness drive improvements in insulin sensitivity and the circulating concentration of the biomarkers, and there is a cyclical relationship between the biomarkers and metabolic health. It is important to study the biomarkers for better understanding of metabolic processes but larger scale studies may be required to determine their role. A combination of calorie restriction, aerobic exercise and resistance training will optimise improvements in insulin resistance and body composition in obese individuals.

Published Abstracts and Articles

Articles:

Cooper, D and O’Gorman, D.J. Exercise and type 2 diabetes: the metabolic benefits and challenges. *Diabetes Management* Vol. 1, issue 6 pg 575-587, 2011.

Poster Presentations:

Diane Cooper ¹, Declan O’Hanlon², Paul O’Connor¹, Noelle Collura², Shah Syed², Krzysztof Wanic², Fiona Lithander², Prof. John Nolan², Donal O’Gorman¹. Cellular mechanisms of insulin resistance and exercise resistance in early onset type 2 diabetes. IRCSET conference, Dublin, Ireland, September 2009.

Dublin City University, Dublin¹, St. James Hospital, Dublin²

Agneiszka Pazderska², Diane Cooper ¹, Declan O’Hanlon², Noelle Collura², Shah Syed², Krzysztof Wanic², Fiona Lithander², Prof. John Nolan², Donal O’Gorman¹. 12 weeks aerobic exercise improves intrinsic muscle mitochondrial function in patients with type 2 diabetes. Irish Endocrine Society Meeting, Dublin, Ireland, September 2010.

Dublin City University, Dublin¹, St. James Hospital, Dublin²

Chapter 1. Introduction

1. 1. Introduction

There has been an exponential rise in the prevalence of overweight and obesity in recent years, which has reached epidemic proportions. In fact, the worldwide prevalence of obesity has almost doubled in the last 20 years and it is currently the fifth leading risk for global death (WHO 2010). Obesity is an independent risk factor for a number of chronic diseases including hypertension, cardiovascular disease, and diabetes. Obesity also increases the risk of developing certain cancers such as breast and colon cancer, in addition to respiratory disorders such as sleep apnea (Quilliot, Petit et al. 2002). An obese individual is also more likely to be depressed, suffer from joint problems, and suffer from skin disorders (Speakman 2004). The cost of treating obesity and its related complications puts considerable stress on the global economy. The direct costs of obesity include hospital admissions, medical consultations, and medications. In 2008, this amounted to \$113.9 billion in the USA, which accounted for almost 10% of health care spending (Tsai, 2011). In the UK the direct costs were £990-1225 million in 2004 and represented 2.3-2.6% of the National Health Service expenditure (HSMO 2004). In 2009, the direct cost of overweight and obesity was estimated at €399 million in the Republic of Ireland and €127 million in Northern Ireland (SafeFood, 2012). This represented 2.7% and 2.8% of total health care costs in that year (SafeFood, 2012). The indirect costs are more difficult to quantify but include absenteeism, disability and premature mortality and workers compensation (Trogdon, Finkelstein et al. 2008). These were estimated to be €729 million and €383 million in the Republic of Ireland and Northern Ireland respectively in 2009 (SafeFood, 2012).

The primary cause of obesity is a mismatch between energy intake and energy expenditure leading to storage of surplus energy. The industrialisation of food production has resulted in greater processing and preservation and a consequence of this is a dramatic increase in the availability of cheap foods that are energy dense, high in fat, sugar and salt (Ferder et al, 2010). A growing number of individuals in Western society are increasing their consumption of these foods and this is occurring alongside an epidemic of physical inactivity, which is now the fourth leading risk factor for global mortality (WHO 2009). In addition to these behavioural factors, evidence is emerging to suggest that genetics also plays an important role, and it is the interaction between an individuals environment and their genetic composition that determines the extent of their obesity (Speakman and O'Rahilly 2012).

Over the last number of years a considerable amount of resources have been devoted to the development of therapies for the treatment of obesity. The most common include lifestyle intervention, consisting of dietary restriction and/or physical activity, weight loss promoting drugs such as Orlistat, and surgery in the case of the morbidly obese individual. Although these different treatment options are available, long term lifestyle intervention has been largely ineffective, surgery is impractical to implement at a population level and many of the pharmacological treatments have additional side effects. There is a need to develop more innovative approaches to weight management and, in particular, to develop effective lifestyle intervention strategies.

1. 2. Statement of the problem

It is widely acknowledged that lifestyle interventions that promote healthy eating and daily physical activity effectively reduce fat mass in the obese population. However, translating research interventions into population based programmes has not been effective. One major challenge has been to identify the most effective intervention given the high degree of variability in study outcomes related to differences in study design. Interventions that have focused on dietary restriction differ with regard to the calorie deficit, the nutrient composition, and the quantification of nutrient intake (Franz, VanWormer et al. 2007). Similarly, exercise interventions vary in the number and intensity of sessions in addition to the mode of exercise (Franz, VanWormer et al. 2007). Both interventions vary in total duration and level of supervision and monitoring. The most effective interventions are those that are tightly controlled, heavily supervised, and of short duration (~12 weeks) (Tessier, Menard et al. 2000) with greater variation for studies lasting 6-24 months (Franz, VanWormer et al. 2007).

A second challenge is the perception that dietary restriction is superior to exercise training in treating obesity when weight loss is the primary outcome measure (Franz, VanWormer et al. 2007). However, it is difficult to accept this conclusion since the literature contains very few diet and exercise interventions that are isocaloric (energy expended in exercise intervention is equal to energy restricted in dietary intervention) making a direct comparison impossible. The strategy most commonly prescribed in dietary interventions is a caloric restriction of 500kcal per day, which accumulates to an energy deficit of 3,500kcal per week (Franz, VanWormer et al. 2007). Assuming physical activity is controlled at pre

intervention levels; this calorie deficit would yield a weight loss of 0.39kg per week since there is approximately 9000 kcal in 1kg of body fat. On the other hand, many of the exercise interventions are based on the ACSM's recommendations for health which is a minimum of 150 minutes per week of moderate intensity physical activity (Donnelly, Blair et al. 2009). The energy expenditure derived from this amount of physical activity depends on many factors including the perception of moderate intensity activity, their current level of fitness and body mass, but it generally yields an energy expenditure in the region of 1,000kcal per week. This would result in a weight loss of approximately 0.11kg per week when energy intake is maintained at pre intervention caloric intake. Clearly there is a mismatch between the current design of diet and exercise interventions making it difficult to compare them.

A third challenge is to devise physical activity recommendations for obesity and not just for health-enhancing benefits. The ACSM have recognised this and now state that a minimum of 250-300 minutes of moderate intensity physical activity per week is needed to produce clinically significant long term weight loss (Donnelly, Blair et al. 2009). The energy expenditure derived from this prescription is more in line with the caloric restriction currently used in diet interventions. This is also complicated by the failure to clearly differentiate between exercise and physical activity. One reason for suggesting the recommendations are unattainable is the perception that the targets must be met in structured exercise such as walking, running, cycling, etc. On the contrary, a shift is required to focus on total daily energy expenditure irrespective of how this is achieved but could include some structured exercise.

1. 3. Significance of the study

The literature reports that lifestyle interventions are successful in reducing fat mass in the obese population. However, translating research findings into successful population based programmes has not been effective. A change is required in our current approach and 3 major challenges must be overcome to bring about this change. Firstly, the literature reports wide variation in the effectiveness of lifestyle interventions and this is due to the wide variation that exists in study design. There is a need to determine the study design that maximises fat loss and improvements in health in the obese population. Secondly, few isocaloric diet and exercise interventions exist making direct comparisons between them impossible. Isocaloric interventions must be designed and administered so that the

effectiveness of diet and exercise can be compared. Finally, the exercise prescription used in many training studies is based on the ACSM's recommendations for health which does not lead to any meaningful energy expenditure or weight loss. These recommendations must be revised to promote significant fat mass loss for obese individuals. This PhD thesis addresses these 3 challenges and uses the findings to develop a revised lifestyle intervention that maximises improvements in fat mass and metabolic health in this population.

1. 3. 1. Body composition or body weight change?

A systematic review and meta-analysis of diet, exercise and combined lifestyle interventions shows that the success of most lifestyle interventions is based on body weight loss as the primary outcome variable (Franz et al, 2007). This is a crude descriptor of success and is not necessarily the best indicator of health enhancement (De Souza et al, 2012). Body weight can be compartmentalised into body fat mass and lean tissue but most interventions do not differentiate between the changes in each compartment. Lean tissue has high metabolic activity and accounts for the majority of resting energy expenditure (Poehlman 1989). The metabolic activity of most tissues remains relatively stable to sustain life but skeletal muscle mass, which accounts for 60-75% of resting metabolic rate, can be influenced by physical activity and physical inactivity (Poehlman 1989). Caloric restriction, especially very low calorie diets, report significant weight loss but this often includes significant reductions in lean tissue. The loss of lean tissue consequently reduces metabolic rate and may have longer term implications for further weight loss and metabolic health (Martin, Heilbronn et al. 2007). The loss of lean tissue may even contribute to weight regain that is also often seen with these types of intervention over a 6 to 24 month period (Franz, VanWormer et al. 2007). Lifestyle interventions should ideally aim to reduce fat mass and maintain or increase skeletal muscle mass in obese individuals (Capurso and Capurso 2012). Exercise training is known to simultaneously reduce fat mass and maintain or increase lean tissue mass especially in previously sedentary individuals (Stewart, Bacher et al. 2005). The improvements in body composition with exercise training can be quite significant and carry a range of health benefits, but the net result is often only a minimal change in body weight, thus leading to a poor perception of success when weight loss is the primary outcome (Catenacci, 2007). Unrealistic expectations of the energy expenditure derived from exercise training, combined with poor weight loss outcomes and a poor understanding of the significance of favourable changes in body composition, can negatively affect adherence to such interventions (Foster, Wadden et al. 1997). It is

important that body composition is a primary outcome measure for diet and physical activity interventions and not body weight.

1. 3. 2. Combining biology and physiology for individualised interventions.

Another difficulty encountered is our incomplete understanding of the physiological regulation of energy balance. Even within tightly controlled and supervised programmes there is still a large variation in individual response to an intervention (Bouchard, Rankinen, 2001). This is true for changes in body composition and body weight, improvements in fitness levels, rate of adaptation to training, and metabolic improvements (Bouchard, Rankinen, 2001). It is clear that one size does not fit all when it comes to exercise prescription for obese individuals and efforts must be made to consider a more individualised approach to intervention. The same is true for dietary interventions where nutrient composition may be need to be considered in conjunction with total caloric restriction due to the influence of different food types and fat content on metabolism (Astrup, Buemann et al. 2002).

Our understanding of the biology of adipose tissue itself is also still incomplete. It is now recognised that adipose tissue is an important endocrine organ that produces and secretes a number of cytokines involved in glucose and lipid metabolism (Bays, Gonzalez-Campoy et al. 2008). Adipose tissue dysfunction is a key characteristic of obesity which results in increased production of disease promoting cytokines concomitant with a decreased production of health promoting cytokines (Arner, Pettersson et al. 2008). Leptin and Adiponectin were two of the first cytokines to be identified and have been studied extensively, but little is known about the effects of diet and exercise on novel cytokines that have recently been identified. A greater focus on the effects of diet and exercise interventions on the novel cytokines may assist in determining success of programmes from a metabolic perspective.

In summary, more basic research is warranted to understand the impact of different nutrient intakes, and different physical activity interventions on the mechanisms at play in different types of body tissue, so that we may be better able to advise overweight and obese individuals. It is also important to investigate novel modes of exercise training that increase daily energy expenditure since it is difficult for obese, previously sedentary, individuals to

achieve and sustain the required amount of physical activity. For example, a single bout of eccentric exercise, where the muscle is lengthening under tension, has been shown to increase energy expenditure and fat oxidation in obese individuals (Dolezal, Potteiger et al. 2000) but little is known about the effects of chronic eccentric training. It is also important that comparisons between dietary restriction and physical activity are based on isocaloric interventions, to assess the relative importance and contribution of both approaches. Finally, a re-evaluation of the criteria used to evaluate interventions is required and should focus more on the interface between physiological changes in body composition and biological regulation to maximise the chance of successful long term health outcomes. Therefore, the aims and objectives of this thesis are:

1. 4. Aims

The primary aim of this thesis is to identify components (calorie restriction or increased energy expenditure by exercise) of a lifestyle intervention that is most likely to improve weight loss, body composition, insulin sensitivity and biomarkers of insulin resistance in obese individuals.

The second aim of this thesis is to identify the components of an exercise regime (aerobic versus resistance) that are most likely to improve weight loss, body composition, insulin sensitivity and biomarkers of insulin resistance in obese individuals.

1. 5. Objectives

1. To compare the effects of an isocaloric diet and aerobic exercise intervention on body composition, insulin sensitivity, and the circulating concentrations of novel biomarkers of insulin resistance.
2. To measure the impact of eccentric exercise, as part of an aerobic and resistance training intervention, on body composition and biomarkers of insulin resistance in obese individuals.
3. To compute the most effective combination of dietary restriction and physical activity based on improvements in body composition and changes in biomarker profile in the obese population.

1. 6. Hypothesis

1. Dietary restriction and exercise interventions will result in distinctive physiological and biological profiles following a 12-week intervention in obese individuals.
2. Exercise training will confer more favourable changes on body composition than dietary restriction with concurrent training being most effective.
3. A combination of dietary restriction, aerobic exercise and resistance training will be necessary to maximise the impact on physiological variables and biomarkers of insulin resistance.

1. 7. Thesis Overview

In this thesis, two separate interventions were conducted to test our hypotheses. In both studies obese individuals volunteered to participate in a 12-week diet or exercise intervention. All interventions were isocaloric and comprised 2500 kcal/week dietary restriction or exercise.

1. 7. 1. Intervention 1: Comparison of dietary restriction and aerobic exercise

Subjects were randomised to a dietary restriction or exercise intervention for 12-weeks. Dietary restriction was monitored by food diaries completed every 2-weeks and evaluated by a dietician. The exercise programme consisted of supervised exercise in DCU Sport of 60-75 minutes of aerobic exercise 4 times per week working at an intensity of 70% of $\dot{V}O_{2max}$. Baseline measures of glucose tolerance, aerobic capacity and body composition and biomarker profiles were repeated at the end of the intervention and all programmes were updated at week 4 and week 8 to ensure continued adaptation and progression.

1. 7. 2. Intervention 2: Eccentric exercise as a novel mode of increasing energy expenditure and fat oxidation

All subjects performed aerobic exercise for 10-weeks and were randomised to complete a standard resistance training programme or an eccentric resistance training programme. The response to both interventions was measured by changes in body composition, glucose

tolerance, resting energy expenditure, fat oxidation, and skeletal muscle cross sectional area and biomarker profiles.

1. 7. 3. Study 3: Impact of exercise training on body composition and biomarker profile

Exercise data from the two intervention studies was combined for a comprehensive statistical analysis to determine the effects of training on body composition and biomarker profile.

1. 7. 4. Study 4: Comparison of 4 isocaloric interventions

All data from the two intervention studies was combined for a comprehensive statistical analysis to determine the intervention or combination of interventions that resulted in the most favourable physiological and biological outcomes.

1. 8. Delimitations

- ❖ The subjects in all of the experiments were recruited exclusively from staff and students in Dublin City University due to ease of access to testing and training facilities. This group is only one sample of an obese population.
- ❖ In the first study, the subjects recruited were delimited to being young (<30 years) or older (>50 years) due to original study design.

1. 9. Limitations

- ❖ With regard to intervention 1, the original study was a multi-centre cross-over experiment but the complexity of the design, analysis and the interaction effects led us to exclude the second phase of the intervention. The complex nature of this original design also resulted in a smaller sample size than originally anticipated. The data presented in this PhD thesis excluded a lot of the data that was actually collected as part of the original project but this allowed for more robust comparisons and conclusions.
- ❖ We were not able to measure body composition on all subjects as the DEXA scanner was damaged in a flood during the first year of data collection.
- ❖ For intervention 2, the original study design was a 12 week intervention. However, due to the time required to conduct the extensive baseline testing, the intervention

was reduced to 10 weeks to ensure that the post tests could be completed prior to end of semester.

- ❖ The time commitment required for the training studies, in addition to the extensive pre and post tests that were carried out resulted in a smaller sample size than originally recruited. This limited the statistical power to detect change.

Chapter 2. Literature Review

This chapter will provide a focused review of the obesity related scientific literature. The overall body of knowledge is beyond the scope of a single review but the relevant literature across three key main sections will be critically appraised. The first section will look at the factors that contribute to obesity, the health consequences associated with obesity, and the current modes of intervention used to treat and manage this condition. The second section will focus specifically on the physiological and metabolic adaptations that occur in response to exercise training and the role of these adaptations in treating obesity. The third and final section will begin with an overview of adipose tissue biology, and will subsequently focus on 6 novel biomarkers that are reported to play a role in obesity and insulin resistance. The known effects of lifestyle intervention on these biomarkers will also be presented.

2. 1. Obesity - Cause, consequence and current modes of treatment.

2. 1. 1. Introduction

The prevalence of overweight and obesity has reached epidemic proportions in recent years. Overweight and obese individuals carry around excessive fat which is generally estimated by combining measures of height and weight, a method particularly relevant in the sedentary adults (Speakman, J.R, 2004). This is reflected in the Body Mass Index (BMI) measurement where weight (kg) is divided by height (m^2) (Speakman, J.R, 2004). Overweight is defined as a BMI $>25\text{kg}/m^2$ but $<30\text{kg}/m^2$, obesity is defined as a BMI $>30\text{kg}/m^2$ (Speakman, J.R, 2004). By the year 2000 obesity had grown to such an extent that the World Health Organisation declared it to be the greatest threat facing Western Society. In 2000, 300 million adults worldwide were classified as being obese but this figure has since increased to >520 million with an additional 1 billion adults classified as being overweight. The worldwide prevalence of obesity has almost doubled between the years 1980 and 2008. In 1980 5% of men and 8% of women were classified as being obese compared to 10% of men and 14% of women in 2008. These figures are continuing to rise and it is predicted that by the year 2015, 2.3 billion adults worldwide will be overweight and 700 million will be obese (WHO, 2009).

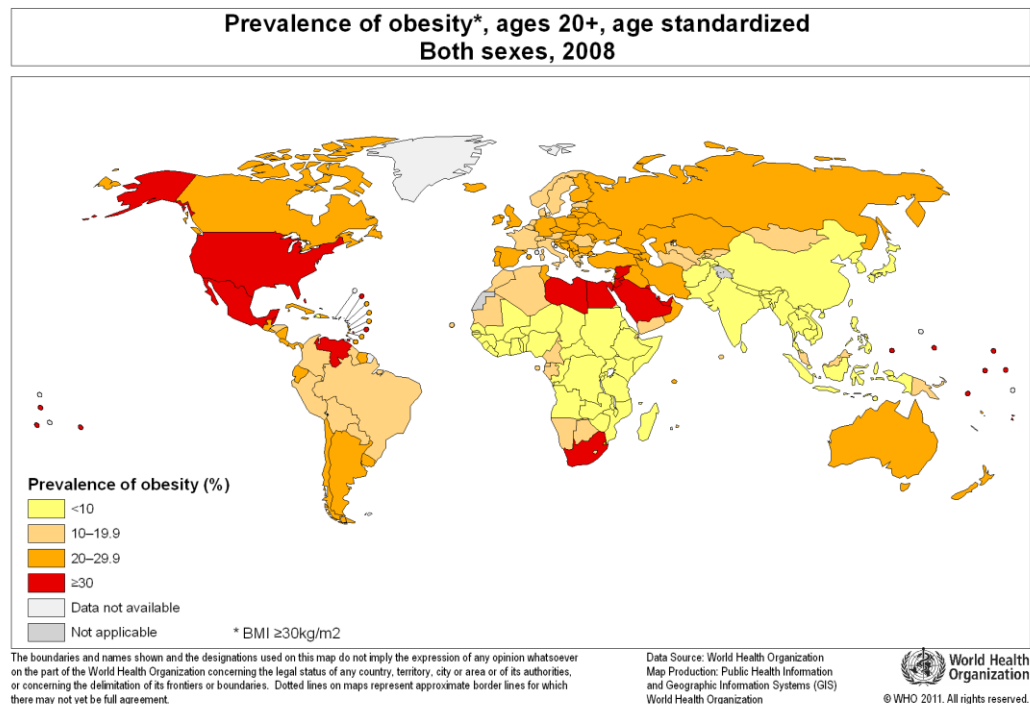


Figure 2.1 World Health Organisation global prevalence of obesity, ages 20+, both sexes, 2008

The rise in the level of obesity over the last 20 years is particularly evident in the USA (Figure 2.2). Data from the National Health and Nutrition Examination Survey 2009-2010 revealed that more than 2 in 3 or 68.8% of adults over the age of 20 in the US are overweight or obese. Furthermore, 1 in 20 adults or 6% are considered to have extreme obesity. This condition is not just confined to adults, alarmingly an estimated 33% of children aged 6 to 19 years are classified as being overweight or obese. It is predicted that by the year 2025 50% of the US population will be obese if these trends are maintained (NHANES, 2009-2010).

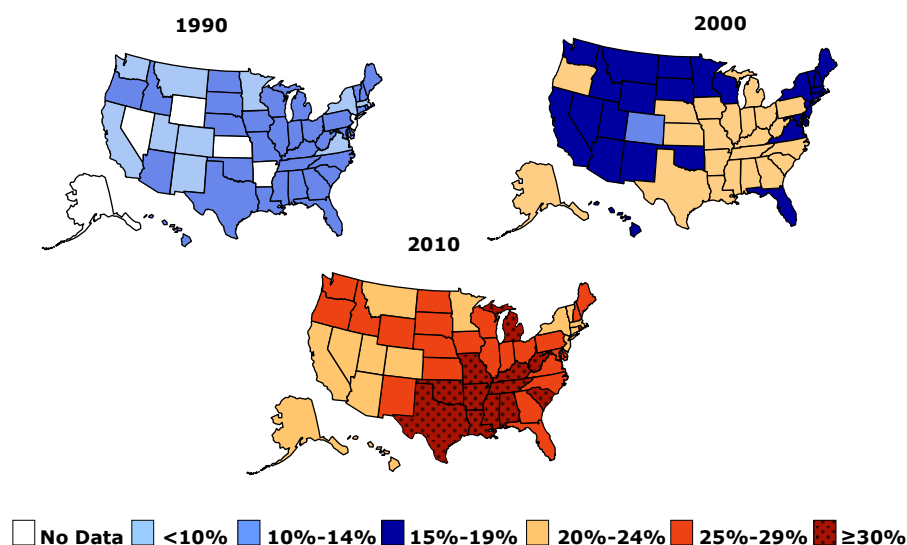


Figure 2.2 Obesity trends in U.S. adults over a 20 year period from 1990 to 2010. BRFSS, 1990, 2000, 2010. The percentages refer to individuals with a BMI ≥ 30 , or about 30 lbs overweight for 5'4" person. This figure has been adapted from the Center for Disease Control (2010).

The latest available data from the Eurostat Statistics Database in 2012 revealed a similar picture in the EU. At least 52% of adults in all 27 member states were classified as overweight or obese, but this figure is even greater in 18 of the 27 member states (Eurostat Statistics Database, 2012).

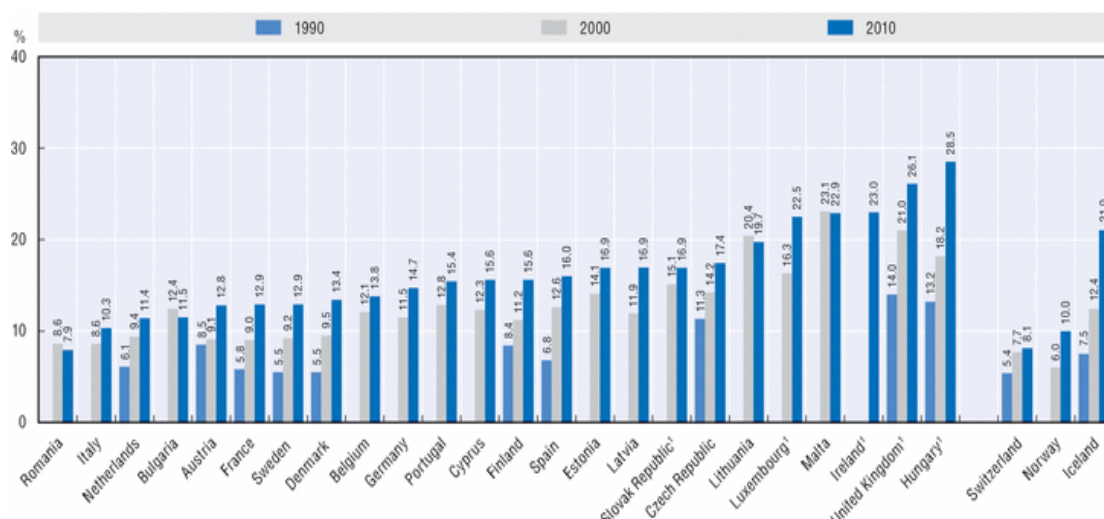


Figure 2.3 Depicts the increasing obesity rates among adults in European countries over a 20 year period from 1990 to 2000 to 2010 (or nearest years). Source: *OECD Health Data 2012; Eurostat Statistics Database; WHO Global Infobase.*

Unfortunately, a similar trend is also evident in Ireland. In 1990 11% of the population were obese, this increased to 18% in 2000 and 25% in 2010. This was in addition to 39% of the population being classified as overweight at both of those time points. The most recent representative data for Ireland shows that 37% of the Irish adult population is overweight and a further 24% is obese (IUNA, 2010). By the year 2030 it is predicted that the prevalence of overweight and obesity in Irish adults will reach 89% and 85% in males and females respectively (Keaver et al. 2013). This issue is not exclusive to Irish adults. Recent research shows that 19% of 9 years olds are overweight and 7% are obese (Growing up in Ireland, 2011). This translates into a total of 30% of girls and 22% of boys being classified as either overweight or obese (Growing up in Ireland, 2011). The last prediction of childhood obesity in 2005, based in UK values, estimated the prevalence of obesity in Irish children is expected to rise by 10,000 per year (National Obesity Task Force, 2005). The rate of overweight and obesity in Ireland is greater than the EU average and creates an even stronger rationale for developing interventions to prevent and reduce excess fat accumulation.

2. 1. 2. Health consequences of obesity

Obesity, defined as a body mass index of $\geq 30\text{kg/m}^2$, is a serious global health issue. It is currently the fifth leading risk for global death, and at least 2.8 million adult deaths each year are a direct result of being overweight or obese (WHO, 2009). Mortality increases as

BMI increases above 25kg/m². In the 1930's, US life insurance companies analysed the factors that influenced the probability that someone would redeem a life insurance policy. Based on the redemption of 5,000,000 policies in that year, they deemed BMI to be the most effective predictor of mortality (Engeland et al., 2003). The reason for this was due to the direct link between body fatness and many chronic diseases. An individual with a BMI of 35kg/m² is 40-90 times more likely to develop type 2 diabetes than an individual with a BMI of 22kg/m² (Chan et al., 1994). Obesity is an independent risk factor for a number of chronic diseases including hypertension and cardiovascular disease. The risk of certain cancers such as breast and colon cancer are also increased with obesity (WHO, 2009), as is the risk of developing of clinical respiratory disorders such as sleep apnea (Quilliot et al., 2002). The obese individual is also more likely to on anti-depressant medication, suffer from joint problems, and suffer from skin disorders (Speakman, 2004). Based on current trends, by the year 2020 non communicable chronic diseases are expected to account for 70% of global deaths and 60% of the global disease burden (WHO, 2009).

2. 1. 3. Economic cost of obesity

The health consequences of obesity combined with the large number of people who are affected by it imposes significant costs to the economy both directly and indirectly. Firstly, the treatment of obesity and related morbidities impose direct medical costs on the health care system in terms of hospital admission, hospital days, patient care, medical consultations, drugs and other allied health care practitioners. In 2008, the direct cost of overweight and obesity per person in the USA was estimated at \$266 and \$1723 respectively. This amounted to a total national cost of \$113.9 billion in that year, which accounted for almost 10% of U.S. health care spending (Tsai, 2011). In 2009, the direct cost of overweight and obesity was estimated at €399 million in the Republic of Ireland and €127 million in Northern Ireland (Safefood, 2012). This represented 2.7% and 2.8% of total health care costs in that year (Safefood, 2012). It is estimated that over the next 20 years non communicable disease will cost the global economy \$30 trillion dollars representing 48% of the global Gross Domestic Product in 2010 (WHO, 2010b). Secondly, the indirect costs associated of obesity are those that are incurred through loss of productivity due to absenteeism, disability, and premature mortality. The evidence that is currently available suggests that obesity incurs high indirect costs but it is difficult to measure this and more research is required to determine the total spectrum of these costs (Trogdon et al., 2008). The indirect costs associated with overweight and obesity in the

Republic of Ireland and Northern Ireland was estimated at €729 million and €383 million respectively in 2009 (SafeFood, 2012). It is likely that these costs will continue to rise in the absence of effective intervention.

2. 1. 4. Risk factors for obesity

Obesity has only become an epidemic in the past 50 years and a lot of research has been conducted to investigate why this is the case. The most significant contributing factors are thought to be changes that have occurred to our eating and physical activity habits, which ultimately favour the storage of excess energy. This excess energy is stored in the adipose tissue as fat, and chronic exposure to excess energy leads to an expansion of fat stores so that the person initially becomes overweight and eventually obese (Speakman, 2004). Evidence has recently emerged to suggest that there may also be a genetic component to the development of obesity. The contribution of energy intake, energy expenditure, and genetics in the development of obesity is outlined below.

2. 1. 4. 1. Trends in dietary intake and its contribution to obesity

There is an ongoing debate as to whether chronic intake of excess energy, chronic physical inactivity, or a combination of both is the main contributor to energy storage and weight gain. Eating patterns have undoubtedly changed over the last 50 years. There has been a shift towards eating away from home (Binkley et al., 2000), and a marked increase in the consumption of energy dense convenience foods that are high in fat and added sugar. Fructose is a sugar that is added in the processing of convenience foods and is enzymatically produced from corn starch (Ferder et al, 2010). Unlike glucose, fructose is almost completely metabolised in the liver, does not stimulate insulin secretion or satiety, is more lipogenic than glucose and is associated with an adverse lipid profile and an accumulation of lipid in tissues of metabolic importance (Ferder et al, 2010). A positive relationship has been found between fructose ingestion, excess energy intake, body weight, T2DM and CVD (Tappy et al., 2010). This is coupled with a greater intake of saturated fat, predominantly from animal products, a reduced intake of complex carbohydrates and fiber, and a reduced intake of fruit and vegetables (Drewnowski and Popkin, 1997). The global availability of cheap vegetable oils and fats means that a high fat intake is particularly evident among low income nations (Drewnowski and Popkin, 1997). While it is widely accepted that patterns of dietary intake have changed over the past 30-50 years there is still debate about total energy intake as some report an increase (Nielsen et al., 2002), no

change (Alexy et al., 2002, Arnett et al., 2000) or a decrease (Popkin et al., 1989). However, this information must be interpreted with caution since the tools used to quantify food intake, such as dietary questionnaires and subject recall, are prone to misreporting (Black et al., 1993). There is more consensus with regard to the change that has occurred in macronutrient intake over the last 20 years with both the USA (Willett and Leibel, 2002) and Europe (Alexy et al., 2002) reporting a decrease in fat intake and an increase in the consumption of carbohydrate, particularly simple sugars (Alexy et al., 2002, Arnett et al., 2000), which when not used for energy are stored as fat.

The composition of food consumed in Ireland over the last number of years has also changed. The 2007 National Survey on Lifestyle, Attitudes and Nutrition reported that 86% of respondents consumed greater than 3 servings per day of energy dense foods that are high in fat, salt and sugar, similar to reports in the years 1998 and 2002. There has been a reduction in the intake of complex carbohydrates over the past number of years with 26% of respondents consuming the recommended 6 or more daily servings of cereals, breads and potatoes compared to 40% in 1998 and 36% in 2002. Fruit and vegetable consumption has also changed as 65% of respondents met the recommended servings of fruit compared to 56% in 1998 and 68% in 2002. Half (48%) of the individuals snacked between meals mostly on carbohydrate based convenience snacks such as biscuits and cakes but there was no information available on total calorie intake or how this has changed since the previous survey in 2002 (SLAN, 2007).

Dietary intake has changed over time in line with the rise in the prevalence of obesity. The literature suggests that total caloric intake may not have changed considerably but this finding must be interpreted with caution since it is based on self reported questionnaires that may be subject to underreporting. There is more consensus in the literature regarding the change in the composition of dietary intake over time which identifies a definite shift towards increased snacking between meals and increased consumption of energy dense, high fat, high processed food, all of which are linked to obesity and related health consequences (Tappy et al., 2010).

2. 1. 4. 2. Trends in physical inactivity and its contribution to obesity

On a global scale the literature suggests that calorie intake hasn't increased significantly over the last 20 years (Willett and Leibel, 2002, Alexy et al., 2002), which implies that the

contribution of physical inactivity to obesity may be quite significant. Our environment has changed dramatically over the last 50 years to make daily tasks more efficient in terms of time required to complete them, but also in terms of the energy required to complete them. Advances in technology have significantly affected physical activity patterns. In the 1950's the dominant mode of transport was walking. Most people in western society had no other option and walked to various small shops to do their weekly shopping and other tasks of daily life. In modern society the dominant mode of transport is by car and the numerous smaller shops have been replaced with larger hypermarket or convenience stores making weekly food shopping and general daily activities more energy conserved (Speakman, 2004).

Advances in household appliances have also had a significant impact on energy expenditure. The introduction of washing machines, dishwashers, vacuum cleaners and other appliances have made traditional domestic chores more time efficient but also more energy efficient since they were traditionally manual tasks. The appliance market has changed so dramatically over time that there are now electrical appliances for even the smallest of tasks such as cutting food with carving knives. All of these advances mean that daily energy expenditure is decreasing and daily sedentary time is increasing, contributing to positive energy balance (Speakman, 2004).

One of the most significant factors linked to positive energy balance is an increase in the number of hours per day spent watching television (Dennison et al., 2002, Armstrong et al., 1998, Salmon et al., 2000). In 1970, almost all households in the USA and in Europe possessed a television but the prevalence of obesity was much lower then. In the late 1990's it was estimated that 20% of children in the USA watched more than 6 hours of television per day and this was positively associated with BMI (Armstrong et al., 1998). Having a television in a child's room significantly increases the risk of the child becoming obese (Dennison et al., 2002). A similar picture is also true for adults (Salmon et al., 2000). The increase in time spent watching television has brought about changes in behaviour. These individuals are less likely to be active (Eisenmann et al., 2002), and more likely to increase their consumption of snack foods.

Finally, the time required for earning a living and domestic work has also declined over the last few decades in Western societies and this trend is associated with a marked decline in

energy spent on these activities. The reduction in work time means that more leisure time is available, but the majority of this time is spent on passive activities. Thus daily energy expenditure has fallen and lifestyles have become increasingly sedentary (Ferro-Luzzi and Martino, 1996).

The epidemiological data that is available regarding the change in physical activity patterns over time shows that in the year 2000 only 26.2% of US adults engaged in enough physical activity to meet the basic ACSM guidelines for health, which is 30 minutes of moderate physical activity at least 5 times per week, or vigorous activity for 20 minutes at least 3 times per week. The likelihood of reaching these recommendations was related to years spent in education where 14.5% of individuals with less than 12 years of education met the guidelines compared to 34.2% of individuals with a college education (CDC, 1996). In the Irish context, the SLAN survey 2007 revealed that only 41% of Irish adults took part in moderate or vigorous activity for at least 20 minutes on 3 or more days per week (SLAN, 2007). The 2008 report by the Health Behaviours in School Aged Children established that 53% of Irish children were active for 60 minutes or more on 5 or more days of the week. The reported frequency of physical activity is 25% lower in children aged 15-17 years compared to children aged 10-11 years, indicating that children become less active in their teenage years, and girls consistently reported less activity than aged matched boys (HSBC, 2008).

Over the last number of decades our environment and our lifestyles have been engineered to favour increased sedentary time. Worldwide more than 60% of adults do not engage in sufficient levels of physical activity. Physical inactivity decreases with age and is more prevalent in women than men across all age groups. The rise in the prevalence of obesity is attributed to physical inactivity in leisure time combined with increased sedentary behaviour throughout the day. The health consequences of physical inactivity are significant (WHO, 2003).

2. 1. 4. 3. The contribution of genetics to obesity

It is clear that the changes in energy intake and energy expenditure over the last 50 have favoured positive energy balance which contributes to obesity. However, within all environments there is a mixture of lean and obese individuals indicating that the environment is not the only factor responsible for obesity. The possible genetic

contribution to obesity has received growing attention in recent years. Some rare cases have been reported where children were found to have defects in their Leptin genes (Farooqi et al., 1999). This meant that they did not produce Leptin, a hormone that would normally act to regulate appetite, and its absence meant that these children had ravenous, insatiable appetites resulting in extreme obesity at a very early age. Treatment with daily Leptin injections reversed their compulsive eating behaviour and dramatically reduced their weight (Farooqi et al., 1999). This is a rare genetic disorder but an example of the role of the interaction between genetics and the environment in the development of obesity. It is important to note that no direct causal link has been established between genes and obesity. Rather, some people develop obesity because their genetic makeup makes them more likely to adopt behaviours, such as over eating, or have physiological characteristics, such as low resting metabolic rates, that put them in positive energy balance. An individual does not develop excessive fat stores without consuming too much energy, expending too little energy, or a mixture of both, but genetic variation may explain the inter individual variation in the extent of obesity (Speakman, 2004). This is particularly evident in twin studies. Bouchard et al 1990 conducted a study to investigate the response of twins to long term overfeeding. The investigators took 12 pairs of young adult male identical twins and housed them in a closed section of a dormitory for 120 days with 24 hour supervision. The subjects were monitored over the first 14 days to establish baseline calorie intake. For the next 100 days, all individuals were fed 1,000kcal above their baseline intake for 6 days per week, and only their baseline intake on the last day of each week. Physical activity levels were tightly controlled. At the end of the study the weight gained within twin pairs was similar, but the weight gain between twin pairs varied greatly from 4.3kgs to 13.3kgs despite all of them being exposed to the same overfeeding conditions and the tight control of the study. Similar results were found for gains in fat mass. The authors concluded that the twins genetics may govern their tendency to store excess energy as fat mass or lean tissue, and may also influence various determinants of resting energy expenditure (Bouchard et al., 1990). It is proposed that genetics may account for up to 65% of the variance in obesity (Speakman, 2004).

The literature that is currently available regarding the genetics of obesity surmises that individuals do not become obese without consuming too many calories, being physically inactive, or a combination of both. Dietary intake patterns have changed over the last number of decades to favour increased snacking between meals and increased

consumption of energy dense foods. This has occurred alongside an epidemic of inactivity in leisure time and increased sedentary time throughout the day. Over a prolonged period of time this lifestyle favours energy storage which can accumulate to obesity. Only in very rare cases is genetics directly responsible for the development of obesity; however genetics may be responsible for individual variation in weight gain. The obesity epidemic is continuing to rise at an alarming rate and efforts must be made to treat and manage this condition.

2. 1. 5. Treatment and management of obesity

A considerable amount of resources have been devoted to the development of therapies for the treatment and management of obesity. The first mode of treatment is usually diet and exercise intervention, which is complimented with weight loss enhancing drugs such as Orlistat for some individuals. Bariatric surgery occurs in the morbidly obese after all other treatments have been administered (Franz et al, 2007). A review of the effectiveness of these therapies is presented below (Franz et al, 2007).



Figure 2.4 Therapies currently used in the treatment and management of obesity.

2. 1. 5. 1. Bariatric Surgery

Bariatric surgery includes a number of different procedures for obese individuals. The procedures include gastric banding, which involves reducing the size of the stomach with a gastric band, gastric bypass surgery which involves resecting and re-routing the small intestines to a small stomach pouch, and surgery to remove a portion of the stomach. These procedures were designed to cause weight loss by food restriction and nutrient malabsorption but there is evidence to suggest that they work by decreasing hunger, increasing satiation during a meal, changing food preferences and energy expenditure (Miras and le Roux, 2013). Bariatric surgery is recommended for individuals with a BMI of at least $40\text{kg}/\text{m}^2$, but if serious co-morbidities such as diabetes exist, the BMI criteria is $\geq 35\text{kg}/\text{m}^2$ (Robinson, 2009). Many studies report significant long term reductions in weight and mortality (Robinson, 2009). This treatment option is only considered when the patient has at least undergone a lifestyle intervention, in addition to pharmacological therapy in some cases (Maggard et al., 2005) but studies show that bariatric surgery is associated with substantial weight loss at 12-months. Schauer et al (2012) reported a weight loss of $29.4\text{kgs} \pm 9\text{kgs}$ 12 months post Roux-en-Y gastric bypass ($n=50$) or sleeve gastrectomy ($n=49$), which was accompanied by significant metabolic and cardiovascular improvements. The drugs that were being used to control glucose, blood pressure and lipids in these subjects decreased significantly after both surgical procedures. Insulin resistance also improved significantly (Schauer et al., 2012) and these findings are supported by other studies (Guo et al., 2013). There were no deaths or life threatening complications in the Schauer (2012) study but other research shows that adverse events occur in approximately 20% of cases (Maggard et al., 2005). Bariatric surgery is undoubtedly very effective in producing weight loss in the obese population and studies show that this weight loss is highly correlated with improved quality of life as assessed by the Health Related Quality of Life Questionnaire (HRQOL) (Billy et al. 2014, Hansen et al. 2014, Efthymiou et al. 2014) and the Obesity and Weight Loss Quality of Life questionnaire (OWLQOL) (Billy et al, 2014). Billy et al, 2014 reported a significant ($p<0.0001$ for both) improvement in OWLQOL scores in 174 patients within 6 months post implantation of the laparoscopic adjustable gastric band which continued to improve over a 3 year period. The mean improvement in quality of life from baseline scores was 52%. Percent weight loss was maintained through the 3 years and the improvement in OWLQOL scores was associated with the percent weight loss at 3 years. The authors conclude there are meaningful improvements in quality of life following this type of surgery

(Billy et al, 2014). Many of the surgical interventions report weight loss at 12 to 36 months and so it is difficult to obtain results after only 12 weeks for comparison to common lifestyle interventions.

2. 1. 5. 2. Weight loss promoting drugs

In the USA in 2011, approximately 2.74 million patients were using some form of antiobesity drug (Hampp et al., 2013). These drugs are commonly used in conjunction with lifestyle intervention and have consistently shown a weight loss of 2-3kgs additional to that obtained by lifestyle intervention alone over a one year period (Rucker et al., 2007). A number of anti-obesity drugs have been developed. Some such as sibutramine and rimonabant work by acting centrally on the brain to suppress appetite, thereby reducing food intake and promoting weight loss (Akbas et al., 2009). However, their potentially severe side effects including psychiatric disorders with rimonabant and elevated blood pressure and insomnia with sibutramine, limit their clinical use (Akbas et al., 2009). Sibutramine has since been removed from the market due to these harmful side effects (FDA, 2010). Exenatide is a GLP-1 agonist prescribed to treat diabetes. It works by augmenting the insulin response to a glucose load, suppressing the glucagon response to a glucose load, delaying gastric emptying and thus the rate at which glucose appears in the blood stream, in addition to reducing appetite and promoting satiety (Bawa et al., 2013). When prescribed in addition to regular diabetic medications (insulin, metformin and sulphonylureas) the literature shows a significant improvement in glucose control in addition to a significant weight loss of 1.7 ± 1.3 kgs at one month, 3.8 ± 2.5 kgs at 3 months, 6.3 ± 3.4 kgs at 6 months and 8.3 ± 4.3 kgs at 12 months (Bawa et al., 2013). Currently, orlistat is the only registered drug for the pharmacotherapy of obesity in Europe (Avenell et al., 2004). The drug works by inhibiting an enzyme in the intestine known as lipase, which would normally act to break down triglycerides into absorbable free fatty acids. Blocking this enzyme reduces the absorption of dietary fat, which is excreted undigested instead (Cahill and Lean, 1999). Orlistat therapy has been shown to reduce serum concentrations of lipid and glucose, as well as reducing blood pressure (Avenell et al., 2004, Cahill and Lean, 1999) but this may be due to weight lost. Franz et al 2007 carried out a systematic review on the effectiveness of orlistat combined with lifestyle intervention to assist weight loss and maintain it in the long term. Following a review of 13 studies, weight loss at 6 months was 8.3kgs or 8%, and this was maintained at 8.2kgs or 8% at 12 months, 7.7kgs or 7% at 24 months, 7.8kgs or 7% at 36 months, and 5.8kgs or 5% at 48 months (Franz et al.,

2007). Weight loss achieved by orlistat combined with lifestyle intervention is reported to be significantly greater than lifestyle intervention alone (Olszanecka-Glinianowicz et al., 2013) and may be beneficial in long term weight loss maintenance (Cahill and Lean, 1999).

2. 1. 5. 3. Dietary interventions

The general consensus in the literature is that caloric restriction is successful in the treatment of obesity and there appears to be a dose response relationship between the amount of calories restricted and the amount of weight lost (Franz et al., 2007). However a closer inspection of this body of work reveals that it is true in the short term but weight regain is common in the longer term, particularly following very low calorie diets (Franz et al., 2007). There is large variation in the reported success of dietary interventions, which is related to the wide variation in study design in terms of the number of calories restricted, the composition of the diets, and also the duration of the interventions and the level of supervision. Franz et al 2007 carried out a systematic review of 51 dietary interventions. The aim of this review was to evaluate the short term and long term success of the interventions, as measured by the amount of weight lost. The interventions reviewed included diet alone, meal replacements, and very low calorie diets (VLCD). All 51 studies were randomised clinical trials of different duration ranging from 6 weeks to 36 weeks with ≥ 1 year follow up and contained ≥ 50 subjects. Weight loss was measured at 6 months and again at 12, 18, 24, 36 and 48 months (Franz et al., 2007).

The majority of the 51 interventions used a caloric deficit of 500kcal per day below that required by the individual as determined by a Resting Metabolic Rate (RMR) test. The mean weight loss at 6 months was 4.9kg (5%), which was maintained at 4.6kg (4.6%) at 12 months, 4.4kg (4.4%) at 24 months, and 3.0kg (3%) at 48 months. The meal replacement studies (n=7) achieved a weight loss of 8.6kg (9.6%) at 6 months which was maintained at 6.7kg (7.5%) at 12 months. Studies adopting a very low calorie diet (calorie intake <800kcal/day) achieved substantial weight loss at 6 months (17.9kg (16%)) that was not sustained at 12months (10.9 kg (10%)) or 36 months (5.6 kg (5%)), even though the results are comparable or better than other dietary approaches. The authors concluded that caloric restriction was successful in initiating and maintaining weight loss (Franz et al., 2007).

To limit the review, the primary outcome measure used to determine success was weight loss. No information was provided on the overall change in body composition of the

subjects and so it is unclear whether this weight loss was derived from body fat mass, lean tissue mass, or a mixture of both. Secondary outcome measures such as blood pressure, lipids, glucose, quality of life, and treatment satisfaction were not described. However there are a number of studies in the literature to support the observation that caloric restriction improves lipid profile, insulin sensitivity and cardiovascular risk in obese individuals (Brinkworth et al., 2004, Clifton et al., 2009, Lee et al., 2013b) Lee et al., 2009, Oberhauser et al., 2012).

A number of short duration, carefully supervised studies have been conducted that report varying degrees of weight loss due to differences in study design. To follow is a discussion of the effectiveness of three typical study designs, a 30% reduction in calorie intake below daily requirements, a comparison of high protein to standard diets, and very low calorie diets.

Klempel et al. (2012) conducted a 10 week intervention where subjects were randomly assigned to one of two groups, intermittent fasting (severe restriction one day per week) with calorie restriction using liquid meal replacements (IFCR-L), or intermittent fasting with calorie restriction based on food (IFCR-F). Each group was restricted by approximately 30% of their baseline caloric needs. They both consumed 240kcal for breakfast, 240kcal for lunch, and 400-600kcal for dinner 6 days per week, and on the last day of each week they fasted, which consisted of water consumption with 120kcal of juice powder only. The difference between groups was that the IFCR-L group consumed the calories in the form of liquid meal replacement supplements, but the IFCL-F consumed their calories in the form of food which was provided by the study. The first 2 weeks consisted of a weight maintenance period and the final 8 weeks involved calorie restriction. The post tests revealed that there was a significant decrease in weight in both groups, which was significantly greater in the IFCR-L group (3.9kgs or 4.1%) ($p=0.04$) compared to the IFCR-F group (2.5kgs or 2.6%). There was a significant decrease in fat mass post intervention in the IFCR-L group (2.8kgs) and in the IFCR-F group (1.9kgs) but there was no difference between groups in the amount of fat mass lost. Fat free mass decreased by an average of approximately 1.2kg in the IFCR-L group and 0.5kg in the IFCR-F group but this did not reach statistical significance and there was no difference between groups. The lipid profile of the subjects and risk factors for cardiovascular disease also improved post intervention (Klempel et al., 2012). The results of this study suggest that when calorie

restriction is similar, weight loss is augmented in those consuming a liquid diet compared to a solid food diet. However, Aslam et al 2009 conducted a 12 week solid food intervention also with a caloric restriction of 30% below actual daily requirements. The weight loss (-7.86kgs) and fat mass loss (-5.22kgs) outcomes were approximately double that of the aforementioned study, but this was concomitant with a reduction of fat free mass in the region of 2.6kgs (Aslam et al., 2009).

The use of high protein diets in weight loss interventions has become prominent in recent years. There is evidence in short term studies to suggest that a higher protein intake during feeding alters total energy intake via the feeling of satiety, and increases total energy expenditure by increasing thermogenesis. Additionally, higher protein intake has proven to lead to a reduction in subsequent energy intake compared lower protein diets. Thus high protein diets have reported greater weight and fat mass loss than lower protein conventional diets, but findings have not been consistent and further long term research is required (Eisenstein et al., 2002).

Clifton et al. (2009) administered a 12 week intervention where subjects were randomised into one of two groups, a high protein (HP) diet, or a standard protein (SP) diet, with the energy allowance being 1300-1550kcal per day. Weight loss after 12 weeks was significant and comparable in both groups (-7.82kg in HP, -7.65kg in SP) as was total fat loss (-6.8kg in HP, -6.4kg in SP), with slight reductions in fat free mass (Clifton et al., 2009). Lee et al. (2009) also carried out a 12 week weight loss intervention comparing the effectiveness of a high protein diet to a conventional diet. Subjects were randomly assigned to one of two different groups, a low calorie diet with partial meal replacement and high protein content (HP), and a low calorie nutritionally balanced conventional plan (C). Weight loss was also comparable in both groups (-5kg in HP, -4.9kg in C) as was the total reduction in fat mass (-2.5kg in HP, -2.3kg in C). Both groups also experienced at least a 2kg reduction in fat free mass. When adherence to dietary intervention was greater than 70%, the HP group experienced greater success in total body fat reductions (Lee et al., 2009). The results of these studies indicate that both types of intervention yield similar weight loss and fat mass loss results. However, the high protein diets report other health outcomes including an improvement in lipid profile in obese subjects at risk for cardiovascular disease (Clifton et al., 2009)

Very low calorie diets have also received attention in the literature in recent years. The strategy often used in these types of interventions is a caloric intake of ≤ 800 kcal per day and so the weight loss results, at least in the short term, have proven to be quite significant. Papadaki et al 2013 investigated the effects of an 8 week VLCD on obesity measures in 773 overweight and obese subjects from eight European cities. The subjects were restricted to 800 kcal per day and all meals were replaced with supplements. The average weight loss was 11.1 ± 3.3 kgs, which consisted of an average fat mass loss of 8.3 ± 4.4 kgs and an average lean tissue loss of 2.8 ± 4.0 kgs (Papadaki et al., 2013). Foster et al. (1992) compared the effects of 3 different VLCD's on body weight and body composition in obese subjects. The total calorie allowance for each of the 3 groups was 420 kcal per day, 660 kcal per day and 800 kcal per day. After 12 weeks of intervention, subjects had an average weight loss of 18.2 kgs, 18.5 kgs and 16.6 kgs respectively. Fat mass accounted for 85.4% of the weight loss in the 420 kcal/day group, 86.1% of the weight loss in the 660 kcal/day group, and 88.2% of the weight loss in the 800 kcal/day group, with the remainder derived from fat free mass. Weight loss was the single best predictor of changes in fat free mass, accounting for 42% of the variance, thus the subjects who lost the most weight also lost the most fat free mass (Foster et al., 1992). It is clear from these studies that VLCD's lead to substantial weight loss and fat mass loss at least in the short term but this also occurs alongside a reduction in lean tissue mass.

Novel types of diet intervention continue to emerge in the literature. The earliest work carried by Dr. Michelle Harvie shows intermittent fasting is as effective as continuous energy restriction with regard to weight loss and improvements in metabolic health, and may be offered as an alternative equivalent to continuous energy restriction for the overweight and obese population (Harvie, 2011). Intermittent fasting involves 2 days of restricted intake per week i.e. low calorie (~ 600 kcal), high protein, low carbohydrate and limited dairy, and 5 days of normal eating per week based on a Mediterranean diet i.e. moderate calorie consumption, moderate protein, unprocessed carbohydrates, low fat, and limited dairy (Harvie, 2013). More recently, when intermittent energy and carbohydrate restriction (IECR) was compared to continuous daily energy restriction (DER) (1,500 kcal/day) for a 3 month weight loss period in overweight women, the results showed that insulin resistance and body fat decreased in both groups but the improvement in insulin resistance was significantly greater in the IECR than the DER group despite both groups having an overall energy deficit of 25% per week. IECR may lead to greater positive

adaptations in metabolic characteristics of overweight and obese individuals but long term studies into the safety and effectiveness of IECR diets are warranted (Harvie et al, 2013).

It is clear from the above research that calorie restriction is effective in reducing body weight in overweight and obese individuals. However, diet induced weight loss simultaneously reduces body fat and lean tissue mass in this population and few studies have investigated whether these changes are influenced by macronutrient composition of the diet intervention (de Souza et al, 2012). The POUNDS LOST trial was a 2 year study involving 811 overweight and obese adults, which investigated the effects of 4 diets with different macronutrient composition on fat mass, lean tissue mass, visceral adipose tissue, and hepatic fat in overweight and obese adults (de Souza et al, 2012). The subjects were randomly assigned to 1 of 4 diets (i) low-fat average-protein (20% fat, 15% protein, and 65% carbohydrate), (ii) low-fat, high-protein (30% fat, 25% protein, and 55% carbohydrate), (iii) high-fat, average-protein (40% fat, 15% protein, and 45% carbohydrate), and (iv) high-fat, high-protein (40% fat, 25% protein, and 35% carbohydrate). For all diets, participants had caloric deficit of 750kcal/day below their energy requirements as determined by a resting metabolic rate test. At 6 months participants showed a significant reduction in fat (4.2 ± 0.3 kg or 12.4%) and lean tissue mass (2.1 ± 0.3 kg or 3.5%), there was no difference between diets. Abdominal fat (2.3 ± 0.2 kg or 13.8%), subcutaneous fat (1.5 ± 0.2 kg or 13.6%) and visceral fat (0.9 ± 0.1 kg or 16.1%) also decreased significantly in all groups but there was no difference between diets. Participants regained 40% of these losses by the end of the 2 year period with no difference between groups. The key findings of this trial was that participants lost more fat mass than lean tissue mass in all groups and there was no difference between diets with regard to improvements in body composition, abdominal fat or hepatic fat. The authors concluded that total calorie restriction as opposed to macronutrient composition of the diet is the most important determinant of fat loss in this population (de Souza et al, 2012).

An overview of the literature suggests that calorie restriction is effective in reducing body weight of obese individuals and this relationship is dose dependent. This is particularly true in the case of short term, structured and supervised interventions. Very low calorie diets (≤ 800 kcal per day) are associated with greater weight loss than diets using moderate calorie restriction (~ 500 kcal per day), but the additional weight loss that occurs when calorie intake is less than 800kcal per day is minimal. Also, weight regain following very low calorie

interventions is common. This may be due to the fact that an individual cannot sustain an 800kcal indefinitely and so calorie intake eventually increases resulting in an increase in body weight. It may also be related to the fact that caloric restriction, particularly severe caloric restriction, results in a loss of lean tissue mass concurrent with reductions in fat mass, which may reduce resting metabolic rate and thus daily energy expenditure, making it increasingly difficult to lose weight, or even maintain weight loss, in the longer term. The literature has reported reductions in fat free mass in the region of 17-25% after a low calorie diet intervention (Foster et al., 1990, Wadden et al., 1990, Barrows and Snook, 1987, Donnelly et al., 1991).

In summary, caloric restriction is effective in reducing body weight in obese individuals and there appears to be a dose response relationship between the amount of calories restricted and the amount of weight lost, particularly in the short term (Franz et al., 2007). Numerous study designs are evident in the literature which explain the large variation in success of these interventions. More basic research is warranted to understand the impact of different nutrient intakes and caloric restriction on weight loss but also on the mechanisms at play in different types of body tissue so that we can determine the optimal intervention for this population.

2. 1. 5. 4. Exercise Interventions

Exercise training interventions are routinely used to treat obesity and improve metabolic health but controversy exists in the literature with regard to the effectiveness of these interventions particularly when weight loss is the primary outcome measure. Catenacci et al. (2007) investigated the effect of exercise on weight loss in obese individuals using 16 randomized controlled trials ranging from 4 to 16 months in duration. The average BMI of the subjects was 25-30kg/m². Eleven of the 16 studies reported significant reductions in body weight ranging from 0.1kg to 5.2kg with the average being 1-3kg. This is a modest weight loss and the results may be perceived to be unsuccessful. However, with closer investigation numerous limitations of the various studies were identified which greatly affect the interpretation of the results. In the majority of studies, exercise induced energy expenditure was not closely controlled or accurately measured. Also, the energy expenditure prescribed in the interventions was modest and not enough to result in substantial weight loss. Most of the interventions were designed based on the ACSM's physical activity recommendations for health, which is 30 minutes of moderate physical

activity on 5 or more days of the week, and averaged 60-180 minutes per week (Catenacci and Wyatt, 2007). Energy expended per week based on this recommendation could be as little as 1000kcal which equates to a modest 0.11kg weight loss per week when energy intake is maintained at pre intervention caloric intake. Given the fact that there are 9000kcal in 1kg of body fat, an energy expenditure of 1,000kcal per week is not sufficient to cause substantial weight loss.

Franz et al. (2007) also carried out a review to establish the effectiveness of exercise training in treating obesity using weight loss as their primary outcome measure. The subjects in these studies were given recommendations for exercise and minimal or no advice on food or meal planning. Only 6 studies were included in this analysis. The mean weight loss at 6 months was 2.4kg (2.7%) maintained at 1.0kg (1%) at 24 months. The authors of the review concluded that exercise interventions are not successful in achieving weight loss in the obese population. However, there are also a number of limitations to this conclusion. Firstly, the review consisted of only 6 exercise interventions, the total treatment duration was 52 and 78 weeks, and the time spent exercising was as little as 90 minutes per week (Donnelly et al., 2000). This volume of exercise is less than the ACSM's physical activity recommendations for health and so it is no surprise that weight loss was minimal (Irwin et al., 2003). Shorter term exercise training interventions based on more structured and supervised training have consistently reported significant changes in weight and physical characteristics in obese subjects (Donnelly et al., 2003, Tessier et al., 2000, Stewart et al., 2005). Secondly, weight loss as a primary measure of success does not reflect the changes that occur to body composition as a result of exercise training. Physical training over a number of weeks reduces body fat mass and concurrently increases lean tissue mass especially in previously sedentary individuals (Stewart et al., 2005). The change that occurs to body composition, especially over the first 10 weeks of training, means that the total net weight loss is often minimal over that time period because the weight gain in lean tissue negates the weight lost in fat mass. Therefore, it is more appropriate to use body composition as a primary measure of success following exercise training interventions. Also it is important to quantify changes in body composition since the literature consistently shows that fat accumulation in obese individuals is significantly inversely correlated with metabolic health (Capurso and Capurso, 2012) and so reductions in fat mass should be a primary outcome measure. Skeletal muscle mass in trained athletes is positively correlated

with metabolic health (Ebeling et al., 1993) and so it is important to quantify gains in lean tissue mass with exercise training in the obese population.

Other reviews have demonstrated that exercise training is successful in the treatment of obesity. They report that the amount of weight loss is directly related to the amount of energy expended per week. Ross et al 2001 conducted a systematic review and meta analysis of the effects of physical activity on reducing obesity, with a particular focus on the influence of exercise induced weight loss on fat mass loss. Short term studies (≤ 16 weeks) were tightly controlled and supervised, and used an energy expenditure target of 2,200kcal per week with an average weight loss and total fat loss of 0.18kg and 0.21kg respectively per week. Long term studies (≤ 16 weeks) used an energy expenditure target of 1,100kcal per week, and yielded an average weight loss and total fat loss of 0.06kg and 0.06kg per week. The review of the short term training studies provide evidence that exercise induced weight loss correlates with fat loss in a dose response manner and that lean tissue mass is at least maintained with exercise training. This review also provides evidence that the amount of weight loss and fat loss achieved with exercise training is directly related to the energy expended throughout the intervention. Greater amounts of energy expenditure per week are associated with greater amounts of body weight and body fat mass loss (Ross and Janssen, 2001).

Two uncontrolled short term randomised studies investigated the effects of higher amounts of exercise induced energy expenditure and reported much larger weight loss post intervention (Lee et al., 1994, Hadjiolova et al., 1982). Lee et al 1994 studied the impact of 5 months of military training on 175 obese male recruits. The recruits exercised 5 days per week for 20 weeks and averaged 29 hours of training per week, 57% of which was deemed to be high intensity activity. No dietary restriction was imposed on the recruits. The average weight loss was 12.5kgs in 5 months or 0.63kg per week. The weight loss was attributed to loss of body fat determined by skin fold measurements (Lee et al., 1994). This study shows that even with substantial weight loss over a short period of time lean tissue mass is at least maintained with exercise training and the reduction in body weight completely accounted for by reductions in body fat mass. Hadjiolova et al 1982 studied the effects of 45 days of training 10 hours per day with an energy expenditure of 3,600-3,700kcal per day. These subjects lost an average of 12.5kgs. Although these interventions

were relatively uncontrolled the findings demonstrate that substantial weight loss can be achieved with substantial energy expenditure (Hadjiolova et al., 1982).

When assessing the effectiveness of exercise training with regard to improvements in body weight and body composition there is an additional difficulty to be considered in the interpretation of the results. In general the standard deviation is quite large indicating a high level of inter-individual variability even when energy expenditure and energy intake are tightly controlled. Thus, the mean result reported for weight loss or change in body composition can often mask the extent of the results achieved by some participants. King et al. (2008) attempted to identify and characterise the individual variability in response to an exercise intervention. The subjects performed supervised, aerobic exercise at 70% heart rate max until 500kcal were expended for 5 days per week. After 12 weeks of training there was a significant reduction in body weight ($3.7 \pm 3.6\text{kg}$), and fat mass ($3.7 \pm 2.6\text{kg}$), indicating again that lean tissue mass was maintained and all weight loss was derived from fat mass loss. However, the investigators reported that there was large individual variability in weight change (-14.7kg to $+1.7\text{kg}$) and the change to fat mass (-9.5kg to $+2.6\text{kg}$) despite the fact that the study was tightly controlled and supervised, and the subjects were deemed to be fully compliant. Due to the tight control and the large individual variation in results, the investigators further subdivided the participants into two groups, compensators and non compensators. Compensators were those whose weight loss was lower than predicted ($-1.5 \pm 2.5\text{kgs}$ and $-2.1 \pm 2.3\text{kgs}$ for body weight and body fat, respectively) and non compensators were those individuals who achieved or exceeded the predicted weight loss ($6.3 \pm 3.2\text{kg}$ and $5.3 \pm 2.2\text{kg}$ for body weight and fat mass, respectively). Both groups had modest reductions in lean tissue mass ($0.47 \pm 1.5\text{ kg}$ vs $0.89 \pm 2.1\text{ kg}$ for compensators and non-compensators, respectively) and so weight loss was mainly attributes to fat mass loss (King et al., 2008). This study highlights a number of important issues.

Firstly, a mean outcome measure should be interpreted with caution since one size does not fit all and individuals respond differently to the same intervention with regard to changes in body weight and body composition. Secondly, it seems that either consciously or subconsciously some individuals compensate for energy expenditure even within tightly controlled interventions. This observation has been reported in other studies and possible explanations include variability in exercise compliance, compensation for energy expenditure with energy intake, and reduced energy expenditure in the non exercising

portion of the day (Bouchard and Rankinen, 2001, Hautala et al., 2006, Levine et al., 2005). Variability in exercise compliance was not an issue in the King et al. (2008) study since all of the sessions were individually designed and supervised and subjects were deemed to be compliant. It is possible that the subjects compensated for energy expenditure with an increase in energy intake or a reduction in total daily energy expenditure. This study highlights the need to educate subjects and make them aware of the effects of compensation either through increasing energy intake or reducing energy expenditure in non exercising time. It also highlights the need to treat obese individuals as individuals within a group in order to prescribe the most effective treatment for them. It is important to note that even the lowest responders exhibited significant improvements in health, characterised by decreased blood pressure, increased fitness and decreased waist circumference. These additional health benefits should be considered important outcome measures and participants should be educated in this regard so that success is not merely based on changes to physical characteristics (King et al., 2008).

A review of the current literature suggests that there is a dose dependent relationship between the amount of energy expended per week and the amount of weight loss achieved. Many exercise training interventions are based on the ACSM's physical activity guidelines for health. These guidelines represent the minimal amount of physical activity required to obtain health benefits and are not sufficient for weight loss. Greater amounts of weekly energy expenditure are required to achieve more substantial weight loss. The dominant mode of exercise reported in the literature is aerobic exercise and / or resistance exercise. It is possible that other modes of training would augment the weight loss results and so more work needs to be carried out to investigate other possible modes or combinations of training. It must be highlighted that significant changes occur to body composition as a result of exercise training, particularly in previously sedentary individuals. Importantly, exercise induced weight loss is derived predominantly from fat mass, and lean tissue mass is at least maintained, if aerobic exercise is the only mode of training, or increased if resistance training is incorporated. Exercise induced changes in body composition have significant favourable implications for the health of this population. For these reasons it is more appropriate to focus on changes in body composition as a primary outcome measure of success in these types of intervention rather than weight loss.

In summary, exercise training is routinely used to treat obesity and improve metabolic health but controversy exists in the literature with regard to the effectiveness of these interventions. The majority of training studies are based on the ACSM's physical activity recommendations for health (Catenacci and Wyatt, 2007) which are completely inadequate for meaningful weight loss. Weight loss is not the most appropriate measure of success of these interventions because exercise training results in reductions in fat mass concomitant with increases in lean tissue mass and the net effect is often minimal weight lost. More basic research is warranted to determine the energy expenditure required for significant improvements in body composition and body weight in this population, but also to investigate the effects of different types of physical activity on the mechanism at play in different types of body tissue so we can determine the optimal intervention for this population.

2. 1. 5. 5. Comparison of diet and exercise interventions

Diet and exercise interventions have been compared in the literature and the general consensus appears to lean towards diet being more effective when weight loss is the primary outcome measure (Franz et al., 2007). However, few isocaloric diet and exercise interventions exist making a direct comparison impossible. Both interventions have already been discussed in detail but a summary of the key differences between them is presented below.

Firstly, the caloric restriction most commonly prescribed in dietary interventions is a minimum of 500kcal per day, which accumulates to an energy deficit of 3,500kcal per week, and a weight loss of ~0.39kgs per week when energy expenditure is controlled for (Franz et al., 2007). In contrast, the energy expenditure prescribed in many exercise interventions is approximately 1,000kcal per week (Donnelly et al., 2009), which yields a weight loss of ~0.11kgs per week controlling for energy intake. Clearly there is a mismatch between the designs underpinning both interventions which is why weight loss is generally greater post diet intervention.

Secondly, using weight loss as a primary measure of success poses an additional problem when comparing the effectiveness of diet and exercise interventions because it does not take into account the different changes that occur to body composition in response to both types of intervention. Exercise training simultaneously reduces fat mass and maintains or

increases lean tissue mass especially in previously sedentary individuals (Stewart et al., 2005). The gains in lean tissue are associated with an increase in RMR which may facilitate weight loss in the longer term (Kirk et al., 2009, Byrne and Wilmore, 2001). The changes that occur to body composition with exercise training can be quite substantial but the net weight loss may be minimal. In contrast, the weight loss achieved by caloric restriction is directly proportional to the amount of calories restricted, but it is derived from reductions in fat mass combined with reductions in lean tissue mass. The loss of lean tissue is reported to be quite significant with very low calorie diets and it has been proposed that this may contribute to the weight regain that is often seen in these interventions over a 6 to 24 month period (Franz et al., 2007).

2. 1. 6. Obesity and poor fitness, an additional problem

Extensive evidence exists to show that inactivity and poor fitness have serious implications for health. There is a strong, independent, inverse relationship between physical fitness and all cause mortality (Kampert et al., 1996, Blair et al., 1989, Bronnum-Hansen et al., 2007, Mokdad et al., 2004) accounting for 3.2 millions deaths each year (WHO, 2010a). The World Health Organisation has declared physical inactivity to be the fourth leading risk factor for global mortality, to an even greater extent than obesity itself, which is currently the fifth leading risk factor (WHO, 2009). Physical inactivity is estimated to account for 21-25% of breast and colon cancers, 27% of diabetes, and 30% of ischemic heart disease globally. When obesity and inactivity are combined the health consequences may be even more drastic than obesity alone.

Individuals with a low fitness level and a diagnosed chronic disease have a higher rate of all cause mortality across all BMI categories after adjustment for age and baseline risk factors (McAuley et al., 2012). Individuals with higher levels of fitness are protected against mortality regardless of their BMI, waist circumference and percent body fat (Blair et al., 1989). Therefore, when fitness is taken into account, the mortality risk associated with obesity is offset (McAuley and Blair, 2011) and, for this reason, obesity should not be used in isolation (McAuley et al., 2012).

In a comparison of risk factors for all-cause mortality, Wei et al. (1999) examined the impact of BMI, aerobic fitness and established factors including baseline CVD, diabetes, cholesterol, hypertension and smoking status in 25,714 men. The key finding of this study

was that low fitness is a strong and independent predictor of all cause mortality but when coupled with obesity was much better than established risk factors and equivalent to having had a cardiovascular event (Wei et al., 1999). On average, physically active people live 5 years longer than physically inactive people, and their expected lifetime without long-standing illness is increased by approximately 8 years compared to inactive individuals (Bronnum-Hansen et al., 2007). The enormity of the health consequences of physical inactivity is such that Pedersen (2010) proposed the concept of the ‘diseasome of physical inactivity’. The theory behind this concept is that physical inactivity, which is associated with chronic systemic inflammation independent of obesity, leads to the accumulation of visceral fat and consequently the activation of inflammatory pathways, which promote the development of insulin resistance and type 2 diabetes, atherosclerosis and a cluster of diseases belonging to the ‘diseasome of physical inactivity’ (Pedersen, 2009).

It is clear that physical activity and physical fitness may play a more important role in the health of the general population and the obese population than previously thought and for this reason the primary focus of this literature review and PhD thesis is the role of exercise training in the comprehensive treatment and management of obesity. To understand this role it is first necessary to understand metabolism in the normal weight healthy individual compared to the obese individual, and subsequently the physiological and metabolic adaptations that occur with acute and chronic exercise training.

2. 1. 7. Metabolism – Normal vs Obese

Blood glucose concentration is regulated by a number of organs and tissues in the body including the liver, skeletal muscle, brain, kidney, adipose tissue, pancreas and intestine (Groop et al., 1989). Of these tissues, skeletal muscle is the most important because it is responsible for almost 80% of insulin-mediated glucose uptake and disposal (Bonen et al., 2004, Coort et al., 2004). The hormones released from the endocrine system play a major role in glucose homeostasis particularly insulin and glucagon, which are both released from the pancreas (Kang, 2008, Jones et al., 2012).

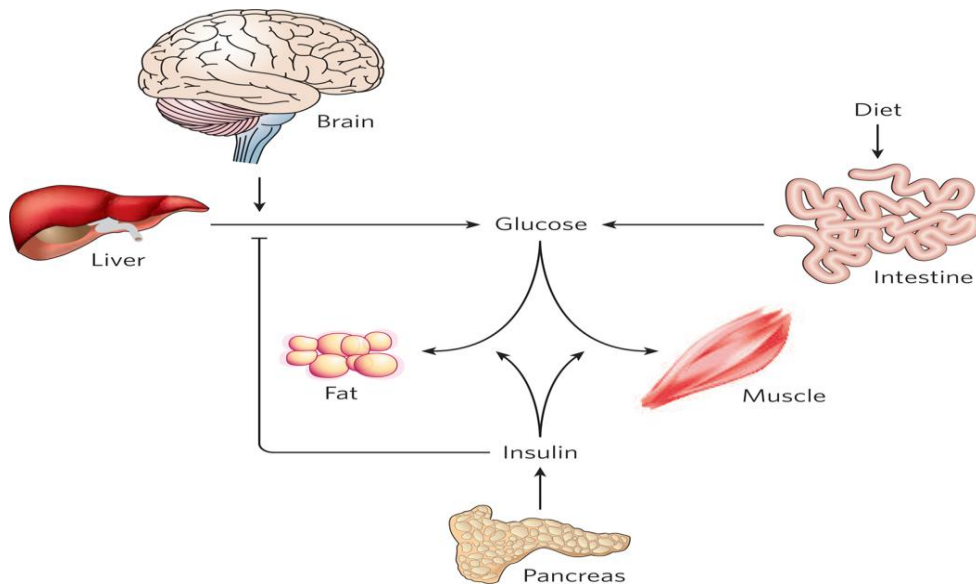


Figure 2.5 Glucose homeostasis requires coordinated interaction between various organs and tissues in the body. Glucose levels rise when absorbed from the intestine and also when released from the liver. The liver plays a key role in elevating glucose levels via glycogenolysis and gluconeogenesis, both of which are inhibited by insulin. Blood glucose levels are reduced by uptake by all cells in the body but predominantly skeletal muscle and adipose tissue. It has recently emerged that the CNS can also sense glucose and may affect glycemia by playing a role in the regulation of gluconeogenesis. Source: Nature 2006 December 14; 444(7121):847-853.

Insulin is produced by the β -cells of the pancreas and is secreted in response to elevated blood glucose levels. It acts on peripheral tissues to regulate both carbohydrate and lipid metabolism by oxidation to produce energy or stored as glycogen or intracellular lipid. In the adipose tissue, insulin suppresses lipolysis and insulin-mediated glucose uptake is used to re-esterify free fatty acids (FFA's) as Triglycerides (TG's). The liver does not require insulin to facilitate glucose uptake but insulin regulates hepatic glucose production by inhibiting gluconeogenesis and glycogenolysis. The kidney also plays an important role in glucose regulation. When blood glucose levels become excessively high, the kidney facilitates the excretion of glucose from the body in the urine. The combined actions of insulin on the muscle, liver and adipose tissue result in the appropriate control of blood glucose concentration (Ferrannini and Mari, 1998, Kang, 2008). Glucagon is produced in the α -cells of the pancreas and functions to oppose the action of insulin. It stimulates glycogenolysis and gluconeogenesis when blood glucose levels fall below the normal range

thus elevating blood glucose concentrations and returning them to normal circulating values (Jones et al., 2012).

Obesity is characterised by an expansion of adipose tissue mass and ectopic fat storage in most tissues, including, the liver, heart and skeletal muscle (Capurso and Capurso, 2012). There is substantial evidence to suggest that the elevated concentrations of FFA's and intracellular lipid in obese individuals induces insulin resistance by disrupting the normal insulin signalling cascade (Belfort and Mandarino 2005, Bevilacqua et al, 1987, DeFronzo, 2004). Insulin stimulated glucose transport into target tissues such as the skeletal muscle, liver, intestine, pancreas, kidney, brain and adipose tissue is impaired (Petersen and Shulman, 2002, Shulman, 2000, Yu et al., 2002), resulting an elevation in blood glucose concentrations over time (Petersen and Shulman, 2006). The impaired suppression of lipolysis and hepatic glucose production further contribute to the metabolic stress of excess substrate availability in circulation (Rosen and Spiegelman, 2006).

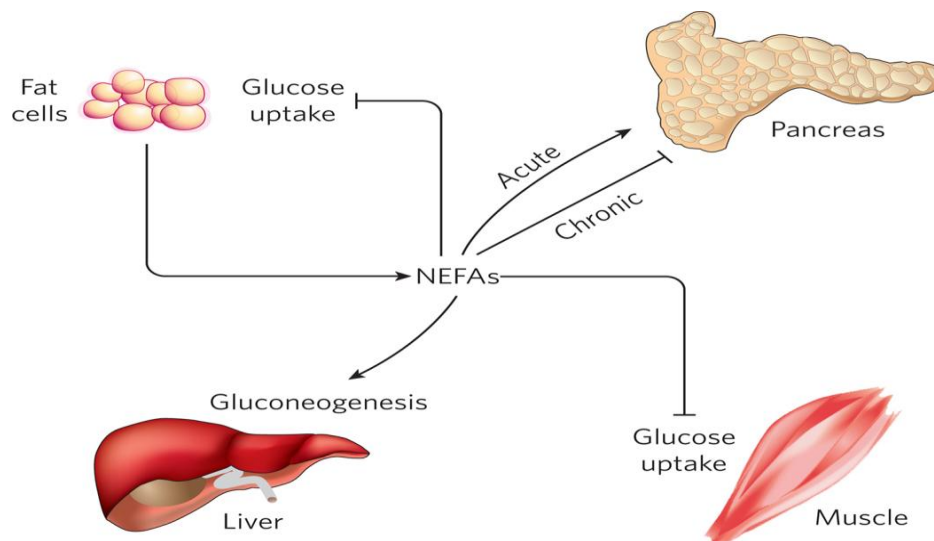


Figure 2.6 Obesity and insulin resistance are associated with increased lipolysis and elevated circulating concentrations of NEFA. NEFA inhibit the ability of insulin to promote glucose uptake in skeletal muscle and adipose tissue, and to suppress hepatic glucose production. Transiently elevated NEFA stimulate insulin release from the pancreas (e.g. after a meal) but chronically elevated NEFA reduce insulin secretion. Source: Nature 2006 December 14; 444(7121):847-853.

Skeletal muscle relies primarily on fat and carbohydrate as fuel for energy production. The contribution that fat or carbohydrate make to energy supply depends on a number of factors including the current metabolic demand, the amount of substrate available, and the ability of the muscle to transport and oxidise the fuel source. Metabolic flexibility in skeletal muscle refers to the ability of the muscle to adapt appropriately to changes in physiological condition by selecting the appropriate type and quantity of fuel for oxidation to meet particular energy demands. During fasting for example there is a greater reliance on fat for fuel. Oxidation of fat during fasting ensures that carbohydrate stores are preserved and available for use by the brain and skeletal muscle when required for energy. In contrast, after a meal there is a switch to glucose oxidation (Storlien et al., 2004). Physical activity also requires metabolic flexibility because skeletal muscle must be able increase glucose and fat oxidation in response to energy demand. The intensity and duration of physical activity determines the mix of substrate metabolised. Short duration higher intensity activities require a greater contribution from carbohydrate than from fat, whereas the energy demands of longer duration lower intensity activities can be met by a greater contribution from fat than from carbohydrate, especially in trained individuals. Metabolic flexibility is evident in lean healthy individuals, particularly those who are trained, but metabolic inflexibility is a characteristic of obesity and insulin resistance (Brooks, 1997).

2. 2. Physiological and metabolic responses to acute exercise, and adaptations to chronic exercise training.

2. 2. 1. Physiological responses to acute exercise

Aerobic fitness is measured by $\dot{V}O_{2\max}$ which is the maximal amount of oxygen that an individual can take in and use to produce energy. $\dot{V}O_{2\max}$ is a function of the ability of the cardiovascular system to deliver blood and oxygen to skeletal muscle, and the ability of skeletal muscle to extract this oxygen and use it to produce energy. At the onset of exercise the physiological systems respond, proportional to the intensity and duration of exercise, to maintain homeostasis. The cardiovascular system meets the increased demand of tissues for oxygen delivery and carbon dioxide removal by increasing heart rate and stroke volume. As stroke volume reaches its maximal capacity at exercise intensities between 40-60% of $\dot{V}O_{2\max}$ in untrained individuals, further increases in cardiac output are met by increasing heart rate. The increase in cardiac output can reach 5 times the resting levels and leads to (i)

an increase in mean arterial pressure, mainly due to increased systolic pressure, despite intrinsic (active hyperemia, chemical messengers) and extrinsic (neural and endocrine) promotion of vasodilation and (ii) a redistribution of blood to the working muscle and to the skin for dissipation of heat (Hughson and Tschakovsky, 1999). In skeletal muscle there is a greater demand for energy to fuel actin-myosin cross-bridging in muscle contraction and the reploarisation of muscle cells. Both glucose and lipids are used as substrates in the mitochondria but carbohydrate is preferentially used with higher intensity exercise, although it has a limited availability relative to fat (Hultman, 1995). The availability and utilisation of substrates for metabolic processes is also regulated by the sympathetic nervous system and the endocrine system with increases in pituitary (growth hormone, adrenocorticotrophin hormone, thyroid stimulating hormone), adrenal (catecholamines, cortisol, aldosterone), thyroid and pancreatic (glucagon) hormones (Richter, 1994).

2. 2. 2. Metabolic responses to acute exercise

A single bout of aerobic exercise has many benefits with regard to glucose and lipid regulation. During exercise there is an increased demand for metabolic substrates in the active muscles, with a 10-20 fold increase in glucose uptake evident during moderate to high intensity exercise (Wahren et al., 1971). The glucose is supplied via increased glycogenolysis in the active muscles as well as increased uptake of glucose from circulation into the working muscles. In this way exercise plays a key role in increasing the usage of circulating blood glucose and well as stored glucose (Hayashi et al., 1997). The hours following an exercise bout is characterised by an increase in insulin sensitivity. The expression of the glucose transporter isoform 4 (GLUT4) remains elevated and so a lower concentration of insulin is required to exert its maximal effect on glucose transport. Glycogen synthase activity, the enzyme responsible for the synthesis of glycogen, is also elevated following exercise, which increases the rate of glucose uptake in to the skeletal muscle in order to replenish depleted glycogen stores (Holloszy, 2005). Evidence in support of this increased insulin sensitivity post exercise is provided by numerous studies that found increased sensitivity post exercise in an exercising leg but not in the resting control limb (Richter et al., 1989).

2. 2. 3. Physiological adaptations to aerobic exercise training

Aerobic exercise leads to significant improvements in aerobic fitness, which in turn is positively associated with metabolic health and reduced risk of all cause mortality (Blair et

al., 1989, Kampert et al., 1996). The improvement in $\dot{V}O_{2\max}$ and thus fitness that occurs with aerobic exercise training is due to a combination of adaptations that occur to the cardiovascular system to facilitate greater oxygen delivery, and the muscles themselves to facilitate greater oxygen use. With regard to the cardiovascular system there are exercise induced adaptations to the heart, blood and blood vessels. The left ventricle hypertrophies and this increase in wall thickness allows for greater force of contraction. With training, heart rate decreases during submaximal exercise which extends ventricular filling time and plasma volume increases due to increased protein content and up regulation of the fluid conservation hormones. As the ventricles stretch in response to the elevated blood volume the heart muscle responds by generating a more forceful contraction, known as the Frank-Starling mechanism, with the result being an increase in stroke volume. Aerobic training is associated with an increase in the number of capillaries infiltrating muscle tissue and an increase in the recruitment of currently available capillaries. Blood flow redistribution is more effective and all of these adaptations combine to facilitate increased blood flow to the metabolically active tissues. Red blood cell volume and thus oxygen carrying capacity also increase with training. Once the blood reaches the active muscles oxygen must be extracted for energy production. Oxygen extraction, represented by the $a-vO_{2\text{diff}}$, is augmented with exercise training, which increases energy production and functional capacity. There is an increase in the size of type 1 muscle fibers, which are characterised by having a high aerobic oxidative capacity. Myoglobin content, the oxygen transporter molecule, increases in the region of 75-80% which augments the oxygen carrying capacity of the muscle. Training results in marked improvements in the functionality of the mitochondria, which increase in size and number. In addition, the activity of the oxidative enzymes in the muscle also increases. All of these adaptations combine to increase the extent to which an individual can use oxygen to produce energy (Blomqvist and Saltin, 1983). An increase in blood flow to exercising tissue combined with an increase in the ability of the tissues to extract oxygen from the blood and remove waste products, results in increased energy production represented by an increase in fitness or $\dot{V}O_{2\max}$.

2. 2. 4. Metabolic adaptations to aerobic exercise training

In addition, aerobic training increases the expression of GLUT4, which facilitates greater uptake of blood glucose into skeletal muscle (Kim et al., 2004). Glycogen synthase expression and activity is augmented, which leads to increased storage of glucose in the form of glycogen (Ebeling et al., 1993). Whether the increased insulin sensitivity results

from exercise training or the previous exercise session has been debated but athletes experience a lower insulin response to a glucose load in comparison to untrained individuals (Horton, 1986) suggesting that these adaptations are due to chronic training and not just a single bout of exercise (Ebeling et al., 1993). The same adaptations are also true for master athletes (King et al., 1987), which has important implications for ameliorating age related insulin resistance in inactive older individuals.

The literature shows that aerobic exercise training augments lipid oxidation and reduces fat stores in the body (Carey et al., 1996). Of particular importance is the reduction in abdominal fat stores associated with aerobic exercise training. This reduces the availability of free fatty acids in circulation (Roden, 2005), which in turn reduces intramuscular triglycerides (O'Leary et al., 2006). Low intensity aerobic exercise training increases the oxidation of intramyocellular lipids. These small lipid droplets lie adjacent to mitochondria and it appears that exercise facilitates the delivery of these droplets to the mitochondria for oxidation (Goodpaster et al., 2001). The expression and activity of enzymes involved in lipid oxidation also increases with training (Kim et al., 2004). This is particularly evident in highly trained endurance athletes, who paradoxically have elevated intra muscular lipid content, but their skeletal muscle is markedly insulin sensitive due to the higher oxidative capacity and a high rates of fat turnover (Goodpaster et al., 2001). Given the adverse relationship between fat accumulation and insulin resistance in obese sedentary individuals, an increased ability to oxidise fat is important in the prevention or treatment of insulin resistance in the obese population. It may also contribute to greater loss of fat mass over time.

2. 2. 5. Physiological adaptations to resistance training

Prior to discussing the physiological adaptations to resistance training, it is important to understand that there are two main types of muscle contraction, concentric and eccentric, and the adaptations that occur are specific to the type of contraction performed. When a muscle is contracted concentrically it is shortening under tension. In physiological terms, the thin filaments of the sarcomere are being pulled towards the centre of the sarcomere, thus shortening the length of the sarcomere. An example of this type of contraction in the context of resistance training is the lift phase of a bicep curl (Figure 2.7). When a muscle is contracted eccentrically it is lengthening under tension. In this case the thin filaments of the sarcomere are being pulled away from the centre of the sarcomere, thus elongating it

but still developing force (Figure 2.7). This type of contraction is the lowering phase of a bicep curl. Most actions whether they are aerobic or resistance type exercise require both types of contraction (McArdle, 2011).

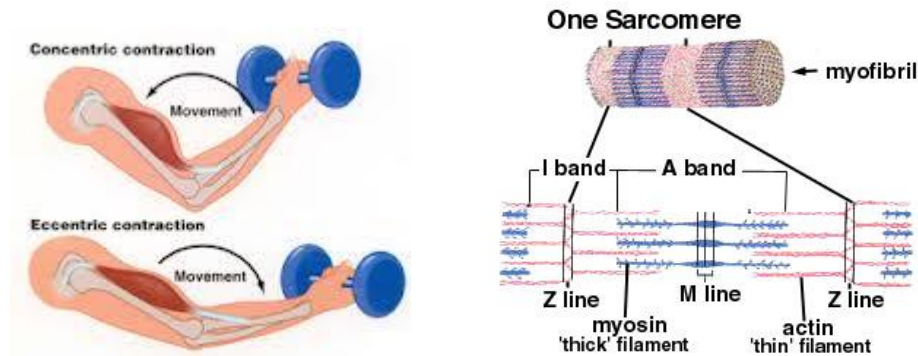


Figure 2.7 Depicts concentric muscle contraction (shortening of the sarcomere under tension) and eccentric muscle contraction (lengthening of the sarcomere under tension).

Resistance training lasting 3-6 months can improve strength in previously untrained individuals by as much as 25-100%. The neuromuscular system is very responsive to training and up to 50% of initial strength gains in untrained individuals can be accounted for by neuromuscular efficiency commonly referred to as the learning effect (Baechele, 2000). In line with this learning effect, the largest gains in strength occur within the first 8-10 weeks of training. Longer term strength gains occur mostly as a result of adaptations to the physical structure of the muscle such as hypertrophy, which means an increase in the size of the muscle (Staron et al., 1994).

For many years the increase in strength that occurs with resistance training was attributed mostly to muscle hypertrophy. While there is a relationship between muscle size and muscle strength, this theory does not explain increased strength in the absence of hypertrophy. The literature reports that neural adaptations account for strength gains in the absence of hypertrophy (Enoka, 1988). The neural adaptations to resistance training include (i) improved synchronization of motor units, which improves the rate of force development and the capability of the muscle to exert steady forces (Duchateau and Enoka, 2002), (ii) increased number of motor units recruited thus leading to greater force production, (iii) increased frequency of stimulation of the muscle fibers with electrical

impulses that summate to produce a greater amount of force (Enoka, 1997), (iv) a reduction in autogenic inhibition allowing muscles to produce greater amounts of force and reach greater levels of strength before they are inhibited to prevent injury (Aagaard et al., 2000), and a reduction in coactivation of antagonist muscles which may allow for greater force generation in the agonist or working muscles (Enoka, 1997).

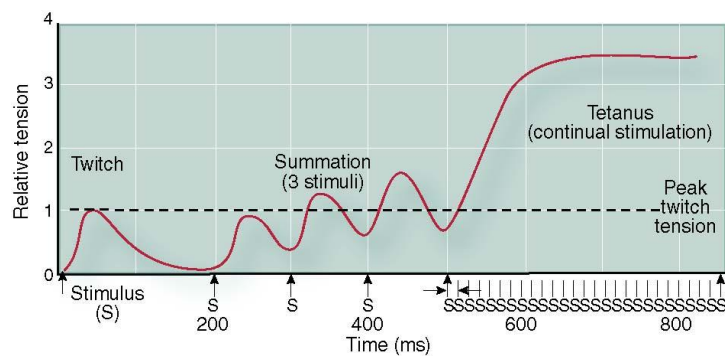


Figure 2.8 Increased frequency of stimulation of motor units initiates greater contractile response. G.A. Brooks, et al., 2005, *Exercise Physiology: Human bioenergetics and its applications*, 4th ed. (New York: McGraw-Hill), 388.

Chronic resistance training leads to structural adaptations in the muscle which increases the size of the muscle, this is known as hypertrophy. Hypertrophy occurs due to (i) an increase in the size of the existing muscle fibers (Figure 2.9) (ii) an increase in the number of muscle fibers known as hyperplasia, or (iii) a combination of both. Hyperplasia is the term given to the splitting of the original muscle fiber and the growth of each of these fibers to the size of the original parent fiber (McCall et al., 1996). Resistance training causes trauma or injury to the muscle fibers, which stimulates the activation of satellite cells. The satellite cells proliferate fuse to existing muscle fibers to repair them (hypertrophy) or fuse with each other to form a new myofibril (hypertrophy) (Hawke and Garry, 2001).

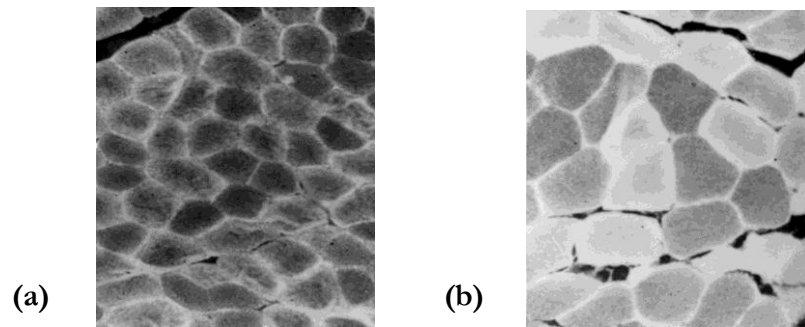


Figure 2.9 Microscopic view of muscle cross section of the vastus lateralis (a) before and (b) after 6 months of resistance training. Adapted from Wilmore, J.H., Costill, D.L., and Kenney W.L (2008) *Physiology of Sport and Exercise* (pg 207) Human Kinetics.

Hypertrophy is often initiated by the structural redevelopment following muscle damage (Figure 2.10). The eccentric phase of muscle contraction during resistance training is reported to be responsible for muscle damage, thus it is this type of contractions that is responsible for stimulating the increase in the cross sectional area of muscle (Shepstone et al., 2005). The muscle damage is evident 24-48 hours after the exercise bout and thus is termed delayed onset muscle soreness or DOMS (Evans and Cannon, 1991). In 1984, Armstrong proposed the following sequence of DOMS. Eccentric exercises causes high tension in the contractile-elastic system of skeletal muscle and results in damage to the physical structure of the muscle, and its cell membrane. This damage disturbs the calcium homeostasis in the injured fiber(s) leading to cell death that peaks at 48 hours post exercise. The products of macrophage activity in addition to intracellular contents e.g. histamines, kinnis, and K^+ , accumulate outside the cell. These substances stimulate free nerve endings in the muscle causing pain. Muscle strength is initially reduced in injured muscle fibers. This may be due to physical disruption of the muscle, failure of the excitation-contraction coupling process, and loss of contractile protein. In the days following an eccentric bout the muscle adapts, become stronger and more resilient to further damage (Warren et al., 2001).

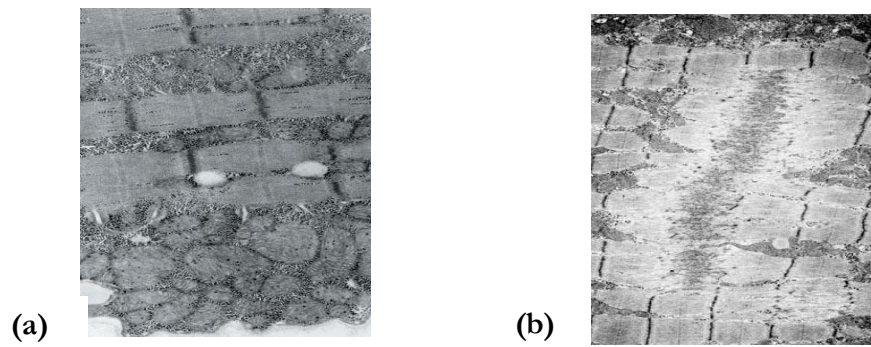


Figure 2.10 Electron micrograph showing normal arrangement of the actin and myosin filaments and Z-disk (a) before and (b) after exhaustive exercise. After exhaustive exercise there is moderate Z disk streaming and major disruption of the thick and thin filaments in a parallel group of sarcomeres as a result of the force of eccentric actions. Taken from From R.C. Hagerman et al., 1984, "Muscle damage in marathon runners," *Physician and Sportsmedicine* 12: 39-48.

2. 2. 6. Metabolic adaptations to resistance training

Resistance training improves insulin sensitivity and glucose tolerance (Fenicchia et al., 2004). One legged training studies report increased blood flow and glucose clearance in the exercise trained limb in comparison to the untrained control limb (Holten et al., 2004). The muscles of the trained limb exhibit an increase in GLUT4 content, an increase in the activity of glycogen synthase, and an increase in the expression and activity of various proteins involved in the insulin receptor and insulin signalling (Holten et al., 2004). Increased muscle mass resulting from resistance training may improve glycemic control (Eriksson et al., 1997) by increasing the storage space available for glucose. The area under the curve for glucose (Fenicchia et al., 2004) and insulin (Jones et al., 2009) have both been shown to decrease with resistance training.

Importantly, resistance training significantly increases lean tissue mass which has the strongest positive influence on and correlation with resting metabolic rate (Poehlman, 1989). Resting metabolic rate is the amount of energy that a person expends at rest. It represents 60-70% of an individuals total daily energy expenditure, with the remainder attributed to the thermic effect of food and physical activity (Levine et al., 2001). Increasing an individuals BMR and thus total daily energy expenditure, may contribute greatly to weight loss over time. Resistance training is also associated with augmented fat oxidation, an adaptation that improves insulin sensitivity in the obese population but also may help to reduce fat stores over time (Kirk et al., 2009).

Other health benefits associated with resistance training include maintenance of functional ability, prevents osteoporosis, sarcopenia, lower back pain, and other disabilities (Winett and Carpinelli, 2001).

The responses to exercise training thus far have focused predominantly on general physiological adaptations in addition to alterations in the structure and function of skeletal muscle. Exercise training also has important implications for the biology of tissues of metabolic importance which will be discussed in detail below.

2. 3. Tissues of metabolic importance and their biomarkers

The customary research approach to obesity has been to address individual organ systems and not the interaction between different systems that affect whole body function. Therefore, a scientific challenge would be to adopt an integrated physiological approach to disease monitoring and prevention. One strategy could be to identify “universal/general” biomarkers that integrate the responses to weight gain and responsiveness to lifestyle interventions. A biomarker can be defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (Atkinson et al. 2001). There is emerging evidence of an extensive communication network between all of the major organ systems whereby hormones released from the muscle, fat, gut, bone, brain, vasculature and liver have physiological influence on other organ systems (Jazet and Pijl, 2003, Pedersen, 2011, Tan and Bloom, 2013). The measurement of a cascade of one or a combination of these new proteins could provide a ‘real-time’ assessment of overall physiological function and allow for effective and individualised lifestyle intervention strategies. This section will review established and novel biomarkers that could become potential candidates for an integrated and personalised response to lifestyle intervention in obese individuals.

2. 3. 1. Adipose tissue as an endocrine organ

It is now widely accepted that adipose tissue is an important endocrine organ that not only functions to store energy but is also involved in the regulation of energy homeostasis and metabolism (Bays et al., 2008). Adipose tissue produces and secretes a number of hormones or adipokines that communicate with tissues of metabolic importance such as the liver, muscle and brain and play a role in the regulation of glucose and lipid metabolism

(Kahn and Flier, 2000), blood pressure, inflammation and atherosclerosis (Rabe et al., 2008). The level of adiposity of an individual greatly affects the production and circulating concentration of these adipokines (Cianflone et al., 2005). Obesity is a chronic inflammatory disorder (Wellen and Hotamisligil, 2005), which is characterised by the accumulation of macrophages in adipose tissue. As adipose tissue expands with obesity there is initial local inflammation induced by macrophage infiltration, which eventually becomes systemic low grade inflammation. By the time insulin resistance is present, most tissues in the body display activation of inflammatory signalling pathways (Murdolo and Smith, 2006). Macrophages infiltrate the adipose tissue and are involved in the production of proinflammatory factors such as cytokines, chemokines, and nitric oxide. This leads to further monocyte infiltration in adipose tissue, greater production of proinflammatory mediators, and the start of the acute phase response (Shi et al., 2006). When this response is activated the liver produces complement factors, cytokines, coagulation proteins, CRP and serum amyloid A among other agents, all of which are required for proper immune function (Gabay and Kushner, 1999). This response is normal and necessary for the control of acute inflammation, however, if this response is chronically activated, as with obesity, circulating concentrations of acute-phase reactants such as CRP are associated with biomarkers of metabolic disease. Thus the adipocytes in obese individuals exhibit altered metabolic and endocrine function, which leads to an increase in the production and secretion of proinflammatory adipokines and a reduction in the secretion of anti-inflammatory adipokines (Arner, 2001). These proinflammatory adipokines affect insulin sensitivity of the adipocytes in a paracrine fashion, and also affect insulin sensitivity of other tissues such as skeletal muscle and the liver in an endocrine fashion (Rosen and Spiegelman, 2006). Leptin and Adiponectin were two of the first hormones to be identified and investigated. Their role in metabolism led to the conclusion that cytokines may provide a link between obesity, insulin resistance and type 2 diabetes (Wellen and Hotamisligil, 2005). However, more than 300 adipokines have now been identified and the function of many of these chemical messengers is poorly understood. The remainder of this review will focus on some of the more established adipokines in addition to the novel biomarkers of insulin resistance that have recently been identified to better understand if they have a role in physiological consequences of fat accumulation and if they have the potential to respond to lifestyle intervention.

2. 3. 2. Established Biomarkers: Leptin and Adiponectin

2. 3. 2. 1. Leptin

Leptin was the first fat cell-derived hormone to be discovered. It was first identified in mice in 1950 at the Jackson Laboratory (Halaas et al., 1995). Mice homozygous for the ob mutation (ob/ob) ate ravenously and were massively obese. In 1994, the ob gene was isolated and the name given to this new hormone was Leptin (Halaas et al., 1995). Leptin is produced predominantly in the adipose tissue and plays a major role in the regulation of energy homeostasis by influencing food intake and energy expenditure. More specifically it acts on receptors in the hypothalamus of the brain to regulate appetite, hunger, metabolism and behaviour and thus body weight (Brennan and Mantzoros, 2006). It is considered to play a crucial role in defending against the excessive accumulation of body fat. Under normal conditions increased energy consumption and accumulation of body fat stores leads to elevated circulating concentrations of Leptin. Leptin ultimately suppresses appetite until fat is lost. In contrast, under conditions of starvation and loss of body fat, circulating concentrations of Leptin decrease resulting in an increase in food intake concomitant with a decrease in energy expenditure in an effort to conserve energy (Brennan and Mantzoros, 2006). It has been reported that Leptin concentrations also decrease in response to fasting or following very low calorie diets (Dubuc et al., 1998). Rare cases of complete Leptin deficiency due to mutations in the Leptin gene have been reported (Brennan and Mantzoros, 2006). Relative Leptin deficiency (lower levels than normal) has also been reported in individuals who have lipoatrophy and some forms of hypothalamic amenorrhea (Brennan and Mantzoros, 2006). These defects lead to a constant desire for food and consequently severe obesity (Brennan and Mantzoros, 2006). Research has shown that the administration of Leptin to these individuals, in replacement doses, normalises neuroendocrine, metabolic and immune function, but more clinical studies are required to determine its long term efficacy and safety (Brennan and Mantzoros, 2006).

In obese individuals, the enlarged adipose tissue releases larger quantities of Leptin. Although Leptin normally reduces appetite, these beneficial effects are not evident in obese individuals indicating that they are resistant to the action of Leptin (Considine et al., 1996). It is possible that chronically elevated serum Leptin in obesity may result in desensitization and resistance to the action of the hormone (Brennan and Mantzoros, 2006). Research has been conducted to investigate the potential role of Leptin administration in the treatment

of obesity (Heymsfield et al, 1999). Seventy three obese subjects were randomly assigned to one of two groups, Leptin or placebo. All subject self administrated recombinant human Leptin or the placebo subcutaneously once per day for 24 weeks. The Leptin dose was increased progressively over time. All subjects were also encouraged to walk for 20-30 minutes 3-5 times per week and adopt a calorie restriction of 500kcal per day. The caloric deficit should have lead to an average weight loss of 0.5kg per week for 24 weeks. The mean weight loss in the control group was 1.7kg which suggests that compliance to the dietary intervention was poor and thus the authors concluded that the effect of the diet in this study was minimal. The key finding was that there was a dose response relationship between Leptin administration and weight loss and fat mass loss. Weight loss at 4 weeks was 0.4 ± 2.0 kg in the placebo group and 1.9 ± 1.6 kg in the Leptin group. At 24 weeks this increased to 0.7 ± 5.4 kg in the placebo group and 7.1 ± 8.5 kg in the Leptin group. Fat loss accounted for greater than 95% of weight lost. Lowest energy intake was recorded when the highest dose of Leptin was being administered suggesting weight loss may be due to reduced food consumption. The authors presumed that weight loss in this group of subjects was related to increased central nervous system exposure to exogenous Leptin. However, there was wide variation between individuals in the amount of energy restricted and the 48 hour recall used to measure this is relatively insensitive. Adverse reactions occurred at the injection site in 71% of those administering the placebo and 62% of those administering Leptin. It appears from the results of this study that obese individuals with high endogenous levels of Leptin do not have absolute resistance to this hormone. High doses of Leptin may be required to provide enough of a stimulus for weight loss in this population. Further research is required to determine the potential role of Leptin in the treatment of obesity (Heymsfield et al, 1999).

2. 3. 2. 2. Adiponectin

Adiponectin is an insulin sensitizing, anti-inflammatory and anti-atherogenic hormone produced exclusively by the adipocytes. Weight loss significantly increases the expression of Adiponectin (Kopp et al., 2005) but weight gain suppresses its expression and thus its health promoting properties (Weyer et al., 2001). The circulating concentration of the hormone is inversely correlated with total body fat but the distribution of body fat also has an important influence. High levels of abdominal body fat are associated with a lower concentration of Adiponectin, but if body fat is mostly distributed in the lower body, the

circulating concentrations of Adiponectin isn't suppressed to the same extent independent of total body fat (Cnop et al., 2003).

Adiponectin is proposed to have a role in carbohydrate and fat metabolism. Adiponectin lowers circulating concentrations of FFA in addition to stimulating the oxidation of intramyocellular fat, leading to improvements in insulin signalling and insulin sensitivity (Carey et al., 1996). Adiponectin activated AMPK in the liver reduces the expression of enzymes involved in gluconeogenesis such as glucose-6-phosphatase and consequently hepatic glucose production (Trujillo and Scherer, 2005). It has been reported that Adiponectin may also act on the brain to decrease body weight. When the hormone was injected into the lateral cerebral ventricles of mice they experienced a dose dependent decrease in body weight. Food intake was not inhibited in these mice which suggest that the reduction in body weight occurred via increased energy expenditure (Qi et al., 2004). Circulating concentrations of Adiponectin are reduced in obese subjects but increase significantly after weight loss (Monzillo et al., 2003). Numerous studies have been carried out to investigate the effects of exercise on Adiponectin with and without weight loss. It appears that Adiponectin concentrations do not change after a single bout of exercise (Jamurtas et al., 2006) or exercise training (Yatagai et al., 2003, Hulver et al., 2002) in the absence of weight loss despite increases in insulin sensitivity and glucose tolerance. Circulating concentrations of Adiponectin are reported to increase following exercise training when weight and body composition are improved (Monzillo et al., 2003, Fatouros et al., 2005).

2. 3. 3. Novel biomarkers of insulin resistance

It is clear that the adipokines Leptin and Adiponectin play a crucial role in carbohydrate and lipid metabolism and in the maintenance of metabolic homeostasis. Novel adipokines, cytokines, hepatokines and myokines are continuing to emerge and their potential role in obesity and metabolic health is currently under investigation. Six novel biomarkers including Fibroblast Growth Factor 21 (FGF21), Chemerin, Omentin, Fetuin-A, Visfatin and Interleukin-13 (IL-13) have recently been identified. The tissues that produce these hormones and their primary metabolic function is depicted in Figure 2.11. To follow is a review of emerging biomarkers of insulin resistance and our current knowledge of their role in obesity in addition to their response to exercise training and lifestyle intervention.

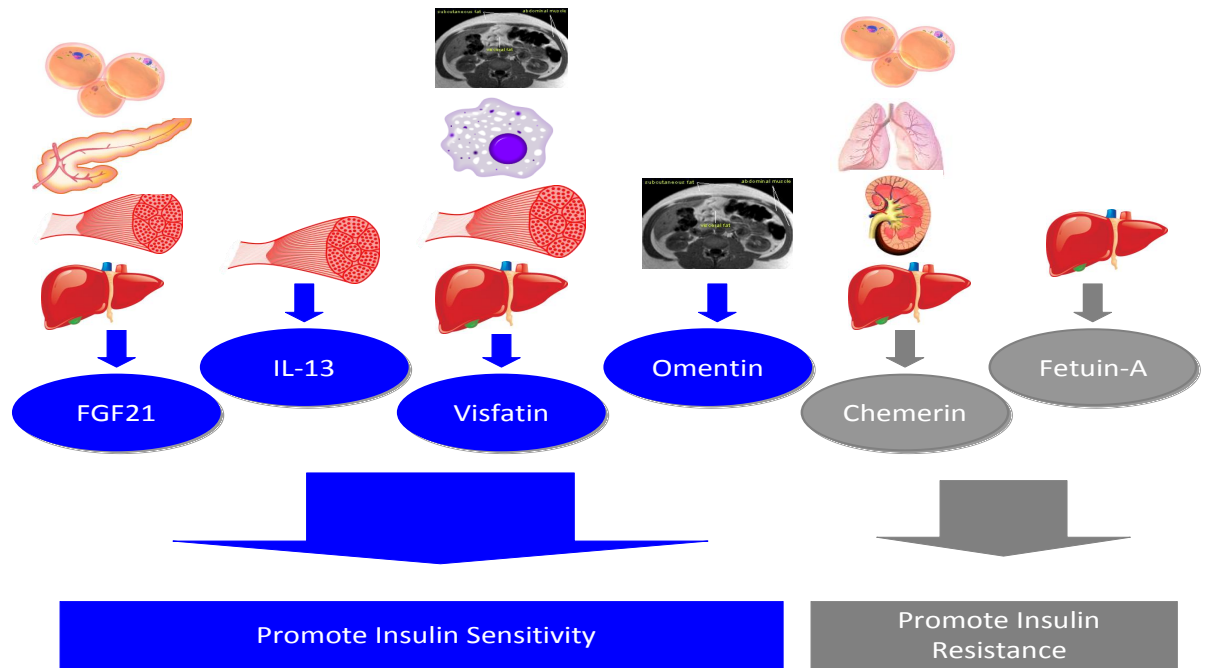


Figure 2.11 Summary of the origin of the novel biomarkers of insulin resistance and their primary metabolic function. The biomarkers are produced by a number of metabolically active tissues in the body. FGF21, IL-13, Visfatin and Omentin promote insulin sensitivity. Chemerin and Fetuin-A promote insulin resistance.

2. 3. 3. 1. Fibroblast Growth Factor 21

FGF21 is an endocrine hormone which is thought to enhance the action of insulin and thus play a role in improving glucose tolerance and lipid metabolism (Long and Kharitonov, 2011). FGF21 exerts many important metabolic effects that are initiated when the hormone binds to and activates its receptor, which is found on metabolically active organs such as the liver, white adipose tissue and the pancreas (Zhang et al., 2006). The hormone itself is expressed in the liver, but also in adipose tissue, skeletal muscle and the pancreas (Nishimura et al., 2000).

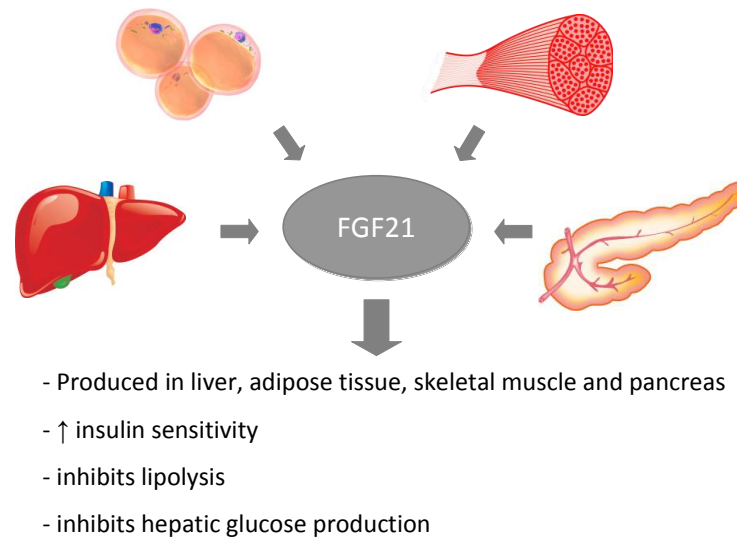


Figure 2.12 FGF21 is produced in the liver, adipose tissue, skeletal muscle and pancreas. It increases insulin sensitivity and stimulates the uptake of glucose into adipocytes and skeletal muscle. It also acts to inhibit hepatic glucose production and inhibit lipolysis.

Much of what is currently known about the beneficial effects of FGF21 has been derived from animal studies. These studies have reported that FGF21 increases insulin sensitivity by stimulating the translocation of GLUT1 thus increasing glucose uptake into the adipocytes, and also by decreasing glucose production by the liver (Coskun et al., 2008). This is an insulin independent action and is additive to the effects of insulin. FGF21 also helps to preserve *b*-cell function in diabetic mice and decreases the secretion of glucagon, which results in decreased hepatic glucose production and improved insulin sensitivity (Coskun et al., 2008). In fact, administration of FGF21 to diabetic mice causes a reduction in plasma glucose to near normal levels (Kharitonov et al., 2005), and improves the lipid profile by decreasing circulating concentrations of FFA's and TG's, in addition to increasing the oxidation of lipids (Kharitonov et al., 2005, Badman et al., 2007). FGF21 may have a therapeutic role in the treatment of obesity in ob/ob mice. Administration of the hormone to diet induced obese and ob/ob mice for two weeks resulted in a 20% reduction in mean body weight predominantly due to a reduction in adiposity. These mice did not decrease their calorie intake or increase their energy expenditure during this time and so the effects can be attributed to the administration of FGF21 alone. The mice showed increase energy expenditure, increase utilisation of fat, reduced liver fat content,

suppression of lipogenesis in the liver, and improved glycaemia and insulin resistance (Coskun et al., 2008).

In humans, the circulating concentration of FGF21 is elevated in obesity, insulin resistance, impaired glucose tolerance, type 2 diabetes, the metabolic syndrome and non-alcoholic fatty liver disease (Semba et al., 2012, Chavez et al., 2009). However, the expected positive effects of the hormone on glucose and lipid metabolism are absent in obesity and related conditions (Fisher et al., 2010). FGF21 concentrations are also elevated in situations of starvation (Mai et al., 2010), indicating that the hormone is responsive to extreme metabolic states. Positive correlations have been found between FGF21 and fat mass, liver fat, triglycerides, insulin, insulin resistance as measured by HOMA, area under the glucose curve, LDL cholesterol (Tynjismaa et al., 2011) and FFA's (Mai et al., 2009). FFA's have recently been proposed to be regulators of FGF21, as increased concentrations of FFA's promote increased concentrations of FGF21. This may in part explain the elevated circulating concentrations of FGF21 seen in obese and insulin resistant states, and also in starvation (Mai et al., 2010). To test this hypothesis, Mai et al, 2010 carried out a study to investigate the effects of moderate lipid infusion on serum FGF21 in lean healthy men. The lipid infusion replicated the physiological rise in FFA's seen in obesity. In this study FGF21 increased linearly with increases in FFA's (Mai et al., 2010). There also appears to be a significant relationship between FGF21 and cardiovascular disease in humans. Circulating concentrations of FGF21 are significantly higher in patients with CVD than in controls. If the CVD patients also have diabetes, hypertension or both, the circulating concentrations of FGF21 are even higher again. FGF21 is positively correlated with an adverse lipid profile in these patients (Lin et al., 2010).

However, there is some research to show that the beneficial metabolic effects of FGF21 reported in animal models are also evident in humans. FGF21 has been shown to enhance glucose uptake in skeletal muscle and inhibit lipolysis in adipocytes of humans both of which contribute to increased insulin sensitivity (Arner et al., 2008). Administration of antidiabetic drugs to patients with type 2 diabetes and subsequent improvements in insulin sensitivity results in a decrease in circulating FGF21. This suggests that improvements in insulin sensitivity affect the production of the FGF21 in humans (Li et al., 2009, Samson et al., 2011).

2.3.3.1.1. The effects of lifestyle intervention on circulating concentrations of FGF21

The research that has been conducted to date investigating the effects of lifestyle intervention on circulating concentrations of FGF21 has produced conflicting results. An acute bout of exercise appears to have no effect on serum FGF21 in the 4 hours following exercise (Cuevas-Ramos et al., 2012). The effects of exercise training on serum FGF21 is unclear. One study found that 2 weeks of daily exercise carried out by young, lean, healthy, sedentary women resulted in a significant increase in serum FGF21 but no change in anthropometric characteristics including body weight and body fat. The authors proposed that the exercise induced increase in FFA's may have been the main stimulus for increasing the serum concentrations of FGF21. They also proposed that FGF21 probably increases in obesity to counteract chronic exposure to elevated FFA's and lipotoxicity. Exercise may reinforce this compensatory pathway (Cuevas-Ramos et al., 2012).

In contrast, a concurrent exercise training study reported that the circulating concentration of FGF21 decreased moderately post intervention. Obese subjects in this study trained 5 days per week for 3 months, which consisted of 45 minutes of aerobic exercise working at 60-70% age-predicted heart rate maximum combined with 20 minutes of resistance training equating to 100kcal per session. Post intervention BMI, waist circumference, blood pressure, and TG levels decreased significantly, which was accompanied by a modest decrease in FGF21 levels (Yang et al., 2011).

Weight loss induced by bariatric surgery also yields conflicting results. Some studies show an increase in circulating FGF21 following substantial weight loss and this was accompanied with a significant improvement in insulin sensitivity and decreased hepatic fat content (Jansen et al., 2011). However, other studies report no change in circulating FGF21 despite significant reductions in body weight and insulin resistance, normalisation of lipid profile and resolution of the metabolic syndrome (Woelnerhanssen et al., 2011).

Similar controversies exist when investigating the effects of caloric restriction on circulating FGF21. Weight loss (~4kgs) induced by 6 months of calorie restriction significantly improved glucose and lipid metabolism but had no effect on serum FGF21. FGF21 was associated with markers of lipid metabolism and an estimate of abdominal adiposity (Mai et al., 2011). Although FGF21 expression does not increase with dietary restriction it does

increase during short term starvation in rodents. This protocol is a critical element in alternate day fasting and may possibly exert beneficial effects on serum FGF21 in humans (Mendelsohn, 2012).

The literature regarding the effects of diet, exercise, surgery and weight loss on circulating concentrations of FGF21 has produced conflicting results. Further research is required to determine the factors that regulate FGF21 and its response to intervention.

2. 3. 3. 2. Interleukin 13

IL-13 is an anti-inflammatory myokine, which is synthesized and released by skeletal muscle. It is proposed to suppress the secretion of inflammatory cytokines which in turn counteracts several cytokines linked to the development of insulin resistance and type 2 diabetes (Cannon and St Pierre, 1998). It is thought that myokine production may be altered in obese and insulin resistant states but there is limited information available on this. Myokines are reported to contribute to whole body metabolism by signaling directly to distant organs including the liver and adipose tissue and regulating metabolic processes in these tissues (Pedersen, 2011). Since skeletal muscle is one of the largest organs in the body, a modest change in the secretion of myokines may have substantial metabolic effects. Exercise is associated with increased secretion of several cytokines from skeletal muscle, which may promote a transient increase in glucose uptake and metabolism (Febbraio and Pedersen, 2005). IL-13 may prevent inflammation induced insulin resistance in adipose tissue indirectly due to a suppressive effect on macrophage activity, and thereby attenuate the release of pro-inflammatory cytokines (Febbraio and Pedersen, 2005).

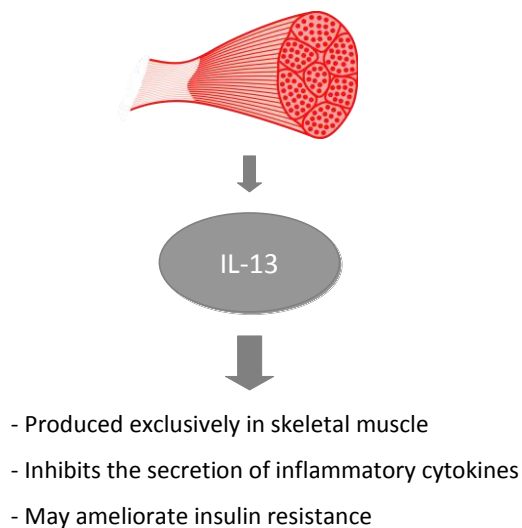


Figure 2.13 IL-13 is produced exclusively in skeletal muscle. It inhibits the secretion of inflammatory cytokines and thus counteracts several cytokines linked to the development of insulin resistance. It may regulate metabolic processes in other tissues including the liver and adipose tissue.

2. 3. 3. 2. 1 . Effects of lifestyle intervention on circulating concentrations of IL-13

There is very limited information available about the effects of lifestyle intervention on the production and secretion of IL-13. A single aerobic exercise session consisting of one hour of cycling at 70% $\dot{V}O_{2\max}$ improved the inflammatory response in sedentary women but there was no change in the circulating concentration of IL-13 (Garcia et al., 2011).

Peake et al. (2005) investigated the effects of different modes and intensity of aerobic exercise on plasma cytokine changes including IL-13. After 60 minutes of level treadmill running at 60% $\dot{V}O_{2\max}$ there was no effect on IL-13. The same was also true for 60 minutes of level treadmill running at 85% $\dot{V}O_{2\max}$, and 45 minutes of downhill running at 60% $\dot{V}O_{2\max}$ with a -10% gradient. It appears that a single bout of exercise has no effect on circulating concentrations of IL-13 but the effects of aerobic exercise training on circulating concentrations of IL-13 are unknown.

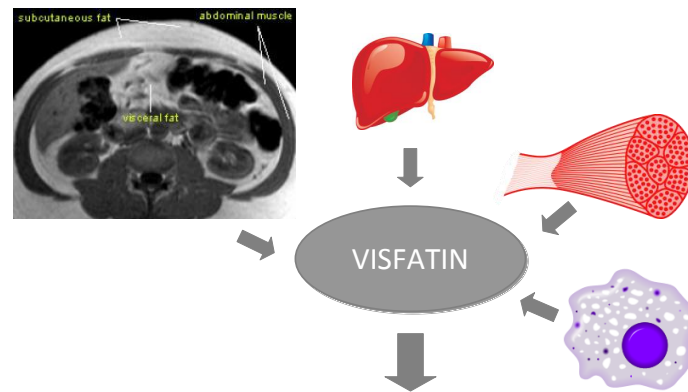
Resistance training has been shown to significantly increase the expression of IL-13 in skeletal muscle. Prokopchuk et al (2007) investigated the effects of 6 weeks of resistance

training on IL-13 expression. 24 collegiate males with resistance training experience ranging from 3 months to 5 years were divided into 2 groups. Group one (n=12) performed maximal contractions on the bench press (5 sets * 3 rep max) 3 days per week. Group 2 (n=12) performed 3 different workouts per week ranging from maximal contractions to submaximal contractions (30% 1RM) to body resistance loads. A muscle biopsy taken from the triceps brachii revealed that IL-13 expression increased significantly post intervention in both groups. This was the first time that IL-13 expression was shown to be up-regulated in skeletal muscle in response to strength training. Higher training loads (group one) produced a greater expression of IL-13 but this did not reach statistical significance between groups. The mechanisms behind this response remain to be established but the authors speculate that IL-13 is involved in muscle hypertrophy in addition to anti inflammatory damage control that occurs during strength training (Prokopchuk, 2007).

A recent personal communication suggests that IL-13 is particularly sensitive to freeze thaw cycles and that it may be necessary to measure using a sample that had not previously been thawed. This may explain some of the variance in the literature.

2. 3. 3. 3. Visfatin

Visfatin has been identified as a novel adipokine that is up regulated in visceral fat, hence the name (Fukuhara et al., 2005). It is also expressed in the liver, muscle and macrophages (Samal et al., 1994). Visfatin is reported to mimic the action of insulin and thus plays a role in increasing glucose uptake in adipose tissue and skeletal muscle (Fukuhara et al., 2005). The literature available to date suggests that Visfatin is involved in the pathogenesis of obesity, insulin resistance and type 2 diabetes, but controversy exists with regard to its role (Fukuhara et al., 2005).



- Produced predominantly in visceral fat but also in the liver, muscle and macrophages
- Mimics the actions of insulin
- ↑ glucose uptake in adipose tissue and skeletal muscle

Figure 2.14 Visfatin is produced predominantly in visceral adipose tissue but it also produced in the liver, skeletal muscle and macrophages. It is reported to mimic the action of insulin and plays a role in increasing glucose uptake in adipose tissue and skeletal muscle.

Some studies report that the circulating concentration of Visfatin is significantly increased in patients with insulin resistance and diabetes (Esteghamati et al., 2011), which is true in the presence (Chen et al., 2006) and absence (Dogru et al., 2007) of obesity. The increase in Visfatin concentration appears to become more pronounced with greater levels of glucose intolerance (Dogru et al., 2007). This indicates that there is a relationship between Visfatin and insulin sensitivity independent to that of adiposity, which is supported by other studies (Yildiz et al., 2010). However, the literature also reports no relationship between Visfatin and markers of insulin sensitivity such as fasting insulin, fasting plasma glucose or glucose infusion rate during the euglycaemic-hyperinsulinemic clamp (Berndt et al., 2005). It is proposed that obese, insulin resistant, and type 2 diabetic subjects who have elevated levels of Visfatin may be resistant to the action of the hormone, and the increased production of Visfatin may be a compensatory response to diminished insulin function in these individuals (Sun et al., 2007).

The relationship between Visfatin and obesity is even more controversial with some studies showing a positive relationship (Berndt et al., 2005), some showing a negative relationship (Pagano et al., 2006) and others showing no relationship (Dogru et al., 2007). Visfatin is

positively correlated with BMI and waist hip ratio in obese children and adolescents (Taskesen et al., 2012). A similar finding has been reported in adults (Olszanecka-Glinianowicz et al., 2011).

The inconsistency in the relationship between Visfatin and obesity and insulin resistance may be due to limitations in the detection of serum Visfatin by different immunoassays. The literature recognises that a variety of immunoassays are currently being used to quantify circulating Visfatin and they do not consistently yield comparable results. This finding must be considered when interpreting data from clinical studies (Korner et al., 2007).

2.3.3.3.1. The effects of lifestyle intervention on circulating concentrations of Visfatin

Changes in nutritional status such as over or under feeding, or changes in energy expenditure as with exercise, has significant effects on adipose tissue, gene expression and insulin sensitivity and may also effect on serum Visfatin.

The circulating concentration of Visfatin appears to be differentially influenced by an acute bout of exercise and exercise training. A single bout of high intensity exercise in lean fit men leads to an increase in plasma Visfatin immediately after the exercise bout. This occurred together with an increase in plasma glucose and insulin concentrations. After 45 minutes of recovery all of the physiological changes returned to baseline values. The authors concluded that plasma Visfatin may increase immediately after exercise to assist glucose uptake and glycogen restoration (Ghanbari-Niaki et al., 2010).

In contrast, 12 weeks of exercise training results in a significant decrease in the circulating concentration of Visfatin. Forty eight Korean women completed a 12 week concurrent training intervention where they exercised progressively for 5 days per week. The training sessions consisted of 45 minutes (~300kcal) of aerobic exercise working at 60-70% age predicted heart rate maximum, and 20 minutes (~100kcal) of resistance training. The resistance training programme began at 40% of heart rate maximum and progressed to 65-70% throughout the 12 weeks. Plasma Visfatin levels were elevated in the obese subjects at baseline compared to lean subjects. After 12 weeks of training, body weight, BMI, waist circumference, percent fat mass, blood pressure, fasting glucose levels, and insulin

resistance all decreased significantly in the subjects and this was accompanied by a significant decrease in plasma Visfatin (Choi et al., 2007). This study shows that exercise training in the presence of weight loss significantly reduces circulating Visfatin.

Twelve weeks of aerobic exercise training also resulted in a significant reduction in circulating Visfatin. The subjects in this study were obese (BMI $33.4 \pm 1.5 \text{ kg/m}^2$) at baseline and completed 5 training sessions per week for 12 weeks working at an intensity of 85% of heart rate maximum. After 12 weeks of training there was a significant increase in fitness, in addition to a significant reduction in body weight, visceral adipose tissue, subcutaneous adipose tissue, area under the glucose curve and area under the insulin curve. The decrease in circulating Visfatin was significantly correlated with the decrease in the area under the glucose and insulin curves. The authors concluded that the reduction in circulating Visfatin was most likely due to changes in body composition and weight (Haus et al., 2009). These findings are supported by other 12 week aerobic exercise training studies (Lee et al., 2010b).

The above studies show that the circulating concentration of Visfatin decreases in response to exercise induced favourable changes in physical and metabolic characteristics of obese individuals. This outcome is also evident in some diet induced weight loss interventions but not all. De Luis et al. (2008) reported that serum Visfatin decreased in obese subjects who lost weight following 3 months of a hypocaloric diet (de Luis et al., 2008). This finding is supported by some diet interventions (Lee et al., 2010a), but others report no change in circulating Visfatin despite a 4.4% reduction in body weight over a 2 month period (De Luis et al., 2010).

The effect of lifestyle intervention on the circulating concentration of Visfatin is unclear at present as is the mechanism behind any changes that do occur. A meta analysis of the literature available to date suggests that Visfatin concentration is increased in overweight and obese individuals, in addition to those with insulin resistance, type 2 diabetes, the metabolic syndrome and cardiovascular disease. For this reason the circulating levels of Visfatin may be a potential predictor of these metabolic conditions (Chang et al., 2011). Interventions that improve insulin resistance may improve the circulating concentration of this hormone.

2. 3. 3. 4. Omentin

Omentin is synthesised by visceral stromal vascular cells and not adipocytes. It is expressed abundantly in visceral adipose tissue and barely detectable in subcutaneous adipose tissue. Omentin is a secreted protein and is therefore detectable in serum (Yang et al., 2006). The circulating concentration of Omentin differs according to body weight and metabolic status (Akbarzadeh et al., 2012, Cai et al., 2009, Tan et al., 2010, Yan et al., 2011). Omentin is negatively correlated with HOMA-IR, body weight, body mass index, waist to hip ratio, triglycerides, and fasting insulin. Its expression is positively correlated with serum concentrations of HDL cholesterol and adiponectin (Cai et al., 2009). These findings are supported by other studies (Yan et al., 2011, Zhou et al., 2012).

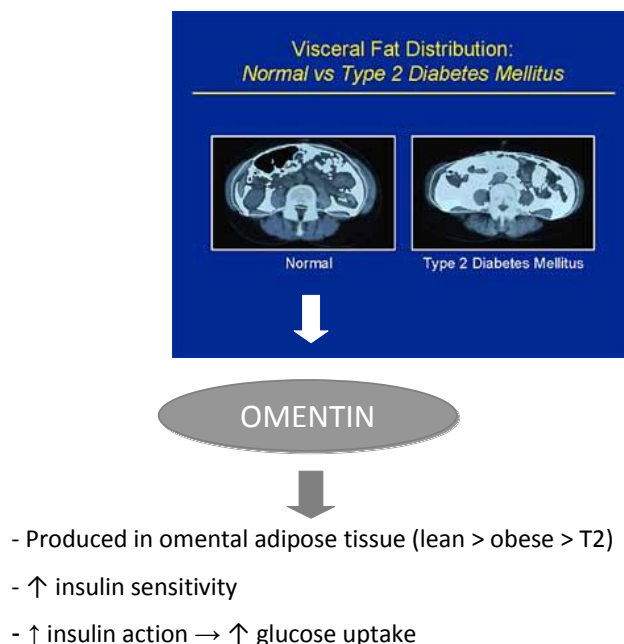


Figure 2.15 Omentin is expressed abundantly in visceral adipose tissue of lean individuals. It is reported to play a role in insulin sensitivity by enhancing the action of insulin. Its circulating concentration is suppressed in obese individuals, and even more so when obesity is combined with insulin resistance or T2DM.

Omentin appears to play a role in insulin sensitivity by enhancing the action of insulin. Addition of recombinant Omentin *in vitro* significantly increases insulin stimulated glucose uptake by ~47% in subcutaneous adipose tissue, and by ~30% in omental adipocytes (Yang et al., 2006). In contrast, administration of insulin and glucose to omental adipose

tissue significantly decreases the expression of Omentin in this tissue in a dose dependent manner (Tan et al., 2008). It is important to note that omentin enhances insulin action by augmenting insulin stimulated glucose transport but it does not cause glucose transport by itself. Omentin increases Akt phosphorylation transiently in the presence and absence of insulin. It may act in an autocrine and paracrine fashion to increase insulin sensitivity in visceral fat, but since it also circulates in the blood it may work in an endocrine fashion to increase insulin sensitivity and glucose metabolism in other tissues such as the liver, muscle and subcutaneous adipose tissue (Yang et al., 2006). This hormone is also involved in the regulation of vascular tone since it has been shown to vasodilate blood vessels (Tan et al., 2010, Zhou et al., 2012, Maenhaut and Van de Voorde, 2011).

The ability of Omentin to increase insulin sensitivity, as well as its anti-inflammatory and anti-atherogenic properties, means that it may have therapeutic effects on insulin resistance and the metabolic syndrome (Zhou et al., 2012). It could be a promising therapeutic target for this condition. Further research is required to determine the Omentin receptor, target tissue and signal transduction pathways as well as the exact physiological role of the hormone in glucose metabolism. (Rabe et al., 2008).

2.3.3.4.1. The effects of lifestyle intervention on circulating concentrations of Omentin

There is limited information available with regard to the effects of lifestyle intervention on serum Omentin. The research that is available suggests that the circulating concentration of Omentin increases in response to reductions in body weight and this is true for diet induced (Moreno-Navarrete et al., 2010) and exercise induced (Saremi et al., 2010a) weight loss.

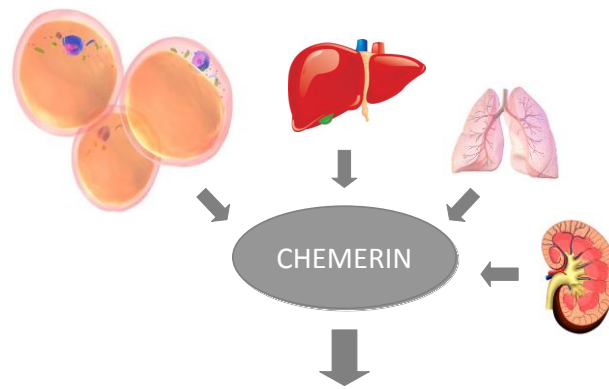
Caloric restriction of 500-1,000kcal per day for 4 months produced a weight loss of 0.5-1.0kg per week in obese individuals. Serum Omentin increased significantly post intervention in this group which was associated with a significant decrease in BMI, insulin resistance and fasting insulin (Moreno-Navarrete et al., 2010).

Twelve weeks of aerobic exercise training also significantly increased serum Omentin concentrations. Normal weight, over weight and obese subjects took part in a 12 week exercise intervention where they trained progressively 5 days per week for the 12 weeks.

The post intervention analysis revealed that there was a significant decrease in waist circumference, body fat, insulin resistance, fasting glucose, triglycerides, total cholesterol, LDL cholesterol, and systolic blood pressure in these individuals and this was accompanied by a significant increase in circulating Omentin. The change in Omentin was significantly correlated with changes in aerobic fitness, waist circumference and insulin resistance (Saremi et al., 2010a). The circulating concentration of Omentin increases with diet and exercise induced improvements in body weight and insulin resistance. Aerobic fitness may also influence the production of this hormone but further research is required.

2. 3. 3. 5. Chemerin

Chemerin has recently been identified as a novel adipokine and chemokine, which is expressed predominantly in the adipose tissue along with its receptor, chemokine-like receptor 1 (CMKLR1, or ChemR23), both of which have been found to play a key role in adipogenesis and adipocyte metabolism. It is also reported to be expressed in the lungs, liver and kidneys (Bozaoglu et al., 2007, Stejskal et al., 2008). Chemerin is a proinflammatory cytokine that activates immune cells by recruiting macrophages (Saremi et al., 2010b). Chemerin expression is increased in adipose tissue of obese subjects but not in the liver (Stejskal et al., 2008). It is possible that adipocytes of obese individuals are responsible for the elevated serum concentrations found in this population (Wang et al., 2009). Chemerin is positively correlated with BMI, insulin resistance, body fat, blood pressure, triglycerides and age (Bozaoglu et al., 2007). Research shows that Chemerin induces insulin resistance in skeletal muscle at the level of the insulin receptor substrate 1, protein kinase B, glycogen synthase 3 phosphorylation, and glucose uptake (Sell et al., 2009). These associations remain even after adjustment for age, gender and BMI. Chemerin is inversely associated with adiponectin (Chu et al., 2012) and it may be a valuable biomarker for the metabolic syndrome (Stejskal et al., 2008).



- Produced predominantly in adipose tissue, also produced in the liver, lungs and kidneys
- Pro inflammatory cytokine that promotes insulin resistance
- ↑ recruitment of macrophages into adipose tissue

Figure 2.16 Chemerin is produced predominantly in the adipose tissue of obese individuals but is also produced in the liver, lungs and kidney. It is a proinflammatory cytokine that promotes the recruitment of macrophages into adipose tissue which further stimulates the production of pro inflammatory cytokines.

2. 3. 3. 5. 1 . The effects of lifestyle intervention on circulating concentrations of Chemerin

Saremi et al (2010) carried out the first study to investigate the effects of aerobic exercise training on circulating concentrations of Chemerin. In this study, subjects engaged in aerobic exercise 5 days per week for 12 weeks. The duration and intensity of training was increased progressively from 15-20 minutes working at an intensity of 60-65% of heart rate maximum in week 1, to 45-50 minutes working at an intensity of 80-85% heart rate maximum in week 12. Dietary intake was monitored throughout the intervention to ensure that it did not change from baseline intake. The exercise training led to significant decreases in waist circumference, percent body fat, visceral fat, subcutaneous fat, fasting glucose, insulin resistance, triglycerides, total cholesterol, LDL cholesterol, systolic blood pressure and serum Chemerin. The primary finding of this study was that the change in Chemerin was significantly correlated with changes in visceral and subcutaneous fat, HOMA-IR, glucose, waist circumference and $\dot{V}O_{2peak}$ (Saremi et al., 2010b). Other 12 week aerobic exercise training studies support the finding that serum Chemerin concentrations decrease post intervention (Kim et al., 2013, Venojarvi et al., 2013).

Concurrent training studies also report reductions in circulating concentrations of Chemerin post intervention. This particular intervention was 6 months in duration and included 98 subjects across a wide range of age and BMI categories. Serum Chemerin was positively associated with total cholesterol, TG's, fasting insulin, HOMA-IR, systolic blood pressure and CRP independently of BMI. Multiple regression analysis revealed that Chemerin was an independent predictor of insulin resistance measured by HOMA-IR. The circulating concentration of Chemerin decreased significantly post intervention and this was associated with the change in HOMA-IR even after adjustment for waist circumference (Stefanov et al., 2013).

Exercise training without weight loss may also exert favourable effects on Chemerin induced insulin resistance. To test this hypothesis skeletal muscle cells were incubated in monocyte chemotactic protein (MCP)-1 and Chemerin, which induced insulin resistance by significantly reducing the insulin-stimulated phosphorylation of Akt. These cells were then contracted by applying electrical impulse stimulation. Contraction of the skeletal muscle cells resulted in increased glucose uptake in comparison to controls, and abrogation of the conditioned medium induced insulin resistance (Lambernd et al., 2012).

Chakaroun et al. (2011) investigated the effects of various weight loss interventions on serum Chemerin with the hypothesis that decreased body fat would lead to decreased concentrations of Chemerin. The interventions included a 12 week exercise intervention, a 6 month diet intervention, or a 12 month follow up post bariatric surgery. All interventions led to significant reductions in weight and Chemerin, and this was associated with improved glucose infusion rate. (Chakaroun et al., 2012).

All of the above findings suggest that improvements in physical characteristics, insulin resistance and glucose tolerance are associated with improvements in the circulating concentration of the pro-inflammatory cytokine Chemerin independently of the mechanism by which these improvements were achieved.

2. 3. 3. 6. Fetuin-A

Fetuin-A is a protein secreted mostly by hepatocytes in the liver, but also by the tongue and placenta. Circulating concentrations of Fetuin-A are elevated in individuals who are obese, and in those who have the metabolic syndrome, insulin resistance and impaired glucose tolerance. Fetuin-A has been shown to be an independent predictor of diabetes, and is associated with atherosclerosis (Brix et al., 2010).

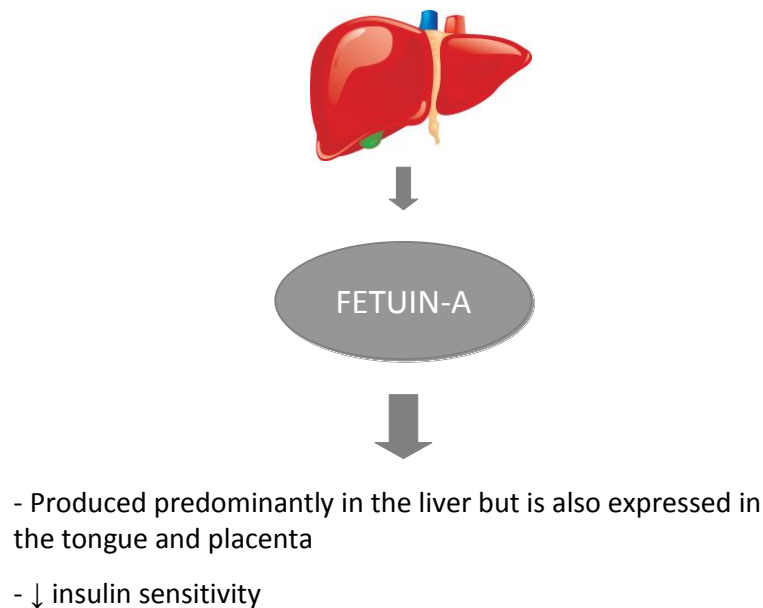


Figure 2.17 Fetuin-A is produced predominantly in the liver but is also thought to be expressed in the tongue and placenta. It is thought to promote insulin resistance.

The circulating concentrations of Fetuin-A is associated with visceral adiposity (Ix et al., 2009), the accumulation of fat in the liver, and the metabolic syndrome (Stefan et al., 2006). Fatty liver is strongly associated with the metabolic syndrome and also strongly predicts type 2 diabetes (Shibata et al., 2007). Based on these findings it has been proposed that increased liver fat, as seen in obesity, is the link between obesity and elevated Fetuin-A levels in this population (Stefan et al., 2008). In support of this, Fetuin-A concentrations decrease linearly with decreases in liver fat (Stefan et al., 2008). Fetuin-A may play an important role in fatty liver induced type 2 diabetes (Stefan et al., 2008). It is reported to suppress the production of the insulin sensitizing hormone Adiponectin (Hennige et al., 2008). It has also been shown to exert pro-inflammatory effects and provoke cytokine expression in monocytes (Hennige et al., 2008), which infiltrate adipose tissue and

contribute to inflammation of the adipose tissue leading to the expression and secretion of other pro-inflammatory adipokines (Weisberg et al., 2003). However, not all obese individuals have fatty liver disease and elevated circulating Fetuin-A and so further investigation is required.

The mechanism behind the disproportionate association of Fetuin-A with lipids is not certain. The leading hypothesis is that Fetuin-A inhibits tyrosine kinase activity of the insulin receptor and this directly or indirectly inhibits insulin action. Insulin would normally act to suppress lipolysis in adipose tissue (Jensen et al., 1989), which may be negated by high concentrations of Fetuin-A and result in an influx of fatty acids from adipose tissue that increase VLDL production (Lewis et al., 1995) and HDL cholesterol (Brinton et al., 1991) but further research is required in this area (Ix et al., 2006).

Circulating Fetuin-A is elevated in insulin resistance and type 2 diabetes and appears to play a significant role in the development of both (Stefan et al., 2008). In an examination of a large random sample of subjects (2,500 of 27,548 subjects) from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study, Fetuin-A was reported to be an independent risk factor for type 2 diabetes. This finding remained even after adjustment for the established risk factors of this metabolic condition including age, body mass index, waist circumference, glucose, triglycerides, HDL cholesterol and A1c. These findings strongly suggest that Fetuin-A is an independent predictor for the risk of developing type 2 diabetes (Stefan et al., 2008).

2. 3. 3. 6. 1 . Effects of lifestyle intervention on circulating concentration of Fetuin-A

The research available to date investigating the effects of exercise training on serum Fetuin-A has reported varying results. Six weeks of aerobic exercise training in 14 non-diabetic obese women had no effect on Fetuin-A despite significant reductions in waist circumference and body fat mass (Schultes et al., 2010). Three months of concurrent training also had no effect on Fetuin-A in normal glucose tolerant obese women (n=40) despite significant reductions in BMI, waist circumference, blood pressure, and triglycerides post intervention (Yang et al., 2011).

Longer term exercise training may be required to positively influence serum Fetuin-A. Jenkins et al. (2011) investigated the effects of chronic exercise on serum Fetuin-A concentrations in young and older men with high activity levels compared to age and BMI matched less active controls. The investigators found that circulating Fetuin-A was significantly higher in the low activity groups (~20%) and was inversely related to fitness. The investigators also reported that Fetuin-A was related to cardiovascular and metabolic disease risk factors. Chronic exercise training exerted favourable effects on these risk factors, which was associated with the lower levels of Fetuin-A evident in the highly active groups (Jenkins et al., 2011).

There is no data currently available regarding the effects of short term dietary interventions on circulating concentrations of Fetuin-A. However, a longer term (2 year) diet study shows that serum Fetuin-A decreases significantly in obese adults following diet induced weight loss, and this trend continues even with weight regain. This may be due to either a delayed response to the initial weight loss or a continued benefit to adopting a healthier diet. The results suggest that Fetuin-A concentration may be influenced by factors other than just changes in body weight (Blüher et al, 2012). The finding that diet induced weight loss is associated with reduced circulating concentrations of Fetuin-A is supported by a 12 month study in obese children. In this study the reduction in Fetuin-A was significantly related to the change in HOMA-IR, waist circumference and blood pressure in this group (Reinehr and Roth, 2008).

There is no established pattern with regard to the effects of lifestyle intervention on the circulating concentration of Fetuin-A. This hormone is elevated in obese insulin resistant individuals and is also positively associated with NAFLD, diabetes and cardiovascular disease. Interventions that reduce adiposity and liver fat, and improve insulin resistance may decrease the production of Fetuin-A. Further research is required in this area.

2. 3. 3. 7. Biomarker Summary

It is clear that the circulating concentrations of the novel biomarkers are very much influenced by level of obesity and metabolic status. Research investigating the effects of exercise training and dietary restriction on these biomarkers has produced conflicting results but it appears that favourable alterations in body weight, body composition and metabolic health are associated with favourable changes in the production of these

biomarkers. The mechanisms underpinning these adaptations remain to be elucidated. Also, for the most part these biomarkers have been studied in isolation which may not be a true reflection of the integrated response from the body. Therefore there is a need to study the interaction between these biomarkers and their integrated response to lifestyle intervention. These biomarkers may prove to be useful independent predictors of metabolic status but further research is required.

2. 3. 4. Summary

Obesity is a serious global health issue which has currently reached epidemic proportions. When obesity is combined with low fitness levels the health consequences may be even more drastic (Kampert et al., 1996, Blair et al., 1989). It is widely acknowledged that diet and exercise interventions are effective in reducing obesity. The effectiveness of both has been compared in the literature but few isocaloric diet and exercise interventions exist making a direct comparison impossible. Also, there are a number fundamental differences in the response to both of these interventions. Firstly, exercise training alone significantly improves cardiovascular fitness which reduces the risk of all cause mortality in this population in a dose dependant manner (Blair et al., 1989, Kampert et al., 1996). Secondly, the changes that occur to body composition in response to both interventions are very different and can not be determined by a measurement of body weight. Weight loss post diet intervention is proportional to calories restricted but is often derived from a combination of reductions in fat and lean tissue mass. This is particularly true with very low calorie diets. Weight loss post exercise intervention is not proportional to calories expended in training because exercise training in previously sedentary individuals increases lean tissue mass. Importantly, weight loss post exercise intervention may be minimal but the changes that occur to body composition are often quite substantial. Finally, the literature reports that both interventions successfully improve metabolic health in this population but they may do so via different mechanisms. Their effect on the novel biomarkers of insulin resistance is not conclusive and limited information is available to date. Further research is required in this area.

**Chapter 3. The effects of isocaloric diet
and exercise interventions on body
composition and metabolic health in
obese individuals.**

3. 1. Rationale

Obesity is a serious global health problem which has reached epidemic proportions (WHO, 2009). The primary cause of obesity is a mismatch between energy intake and energy expenditure resulting in a surplus of energy, which is predominantly stored as fat in the adipose tissue. Chronic exposure to excess energy leads to an expansion of fat stores so that the person initially becomes overweight and eventually obese (Speakman, 2004). Diet and exercise interventions remain the cornerstone of treatment for obesity. Both interventions have been compared in the literature and the general consensus appears to lean towards diet being more effective when weight loss is the primary outcome measure (Franz et al., 2007). However, few isocaloric diet and exercise interventions exist making a direct comparison impossible. The caloric restriction most commonly prescribed in dietary interventions is 3,500kcal per week, which accumulates to a weight loss of ~0.39kgs per week when energy expenditure is controlled for (Franz et al., 2007). In contrast, the energy expenditure prescribed in many exercise interventions is approximately 1,000kcal per week (Donnelly et al., 2009), which yields a weight loss of ~0.11kgs per week controlling for energy intake. An additional challenge that exists when comparing the effectiveness of both interventions is that they are both associated with very different changes in body composition, which may have important implications for metabolic health. Exercise training reduces fat mass and simultaneously maintains or increases lean tissue mass especially in previously sedentary individuals (Stewart et al., 2005). For this reason net weight loss post exercise training is often minimal but the changes that occur to body composition can be quite substantial. In contrast, the weight loss achieved by caloric restriction is directly proportional to the amount of calories restricted, but it is often derived from reductions in fat mass concurrent with reductions in lean tissue mass (Franz et al., 2007). The differential changes in body composition in response to both interventions may also influence the production and thus circulating concentrations of novel biomarkers of insulin resistance. A comparison of isocaloric diet and exercise interventions is essential to determine their effects on the physical characteristics, metabolic characteristics and biomarker profile of obese individuals.

3. 2. Aim

The aim of this study was to compare the physiological responses and biomarker profiles of insulin resistance and body composition following an isocaloric diet or exercise interventions.

3. 3. Objective

- ❖ To assess changes in body composition, insulin resistance and aerobic fitness following a 12 week isocaloric dietary restriction or exercise intervention.
- ❖ To measure novel biomarkers responses to the intervention and create biomarker profiles.

3. 4. Hypothesis

Dietary restriction and exercise training will result in distinctive physiological and biological profiles following a 12 week intervention in obese individuals. Exercise training will confer more favourable changes on body composition than dietary restriction.

3. 5. Overview of Original Study Design

This was originally designed as a cross-over study following a successful European Foundation for the Study of Diabetes award to Prof. John Nolan in St. James's Hospital. In DCU, young (18-30yrs) and older (50-70yrs) obese but normal glucose tolerant individuals were to undertake a 3-month isocaloric diet or exercise intervention in random order. These subjects were matched for age and BMI with a group of young and old type 2 diabetic subjects recruited in St. James's Hospital. The original design is depicted in Figure 3.1. This study was a major undertaking as part of a PhD and the design was too complex to allow for meaningful comparisons. The data used in the analysis of this chapter was a subset of the overall cohort that allowed for unbiased group comparisons.

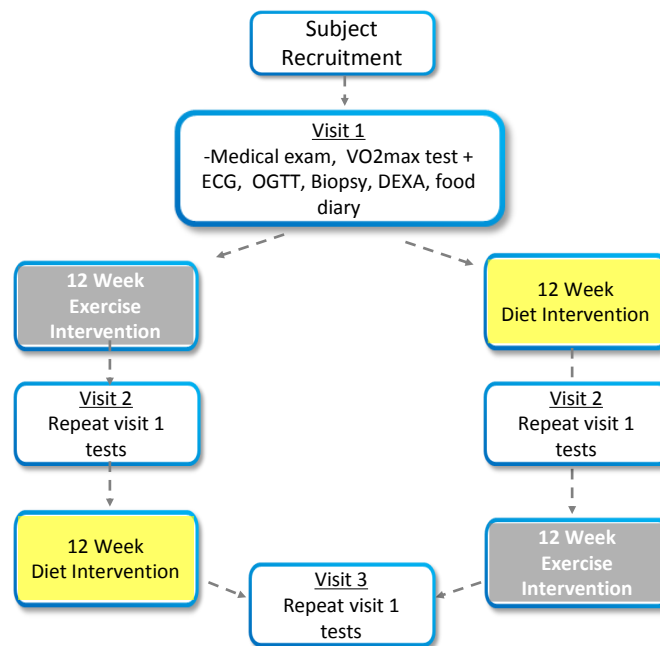


Figure 3.1 Overview of original study design which was decided before this PhD began.

3. 6. Overview of Actual Study Design

The physical and metabolic responses of obese subjects to a 12-week isocaloric diet restriction or exercise intervention were compared. The complexity of the analysis of the original cross over design and the interaction effects led us to exclude the second phase of the intervention. The complex nature of this original design also resulted in a smaller sample size than originally anticipated. While a larger number of subjects were recruited to the study, the data presented in this chapter, refers to those that successfully completed.

Subjects were recruited from staff and students in Dublin City University. Following a health screening and medical exam, subjects were randomly assigned to either a 12 week diet intervention or a 12 week exercise intervention. A number of tests were completed at baseline and repeated after the 12 week intervention. The tests and procedures are explained in detail below.

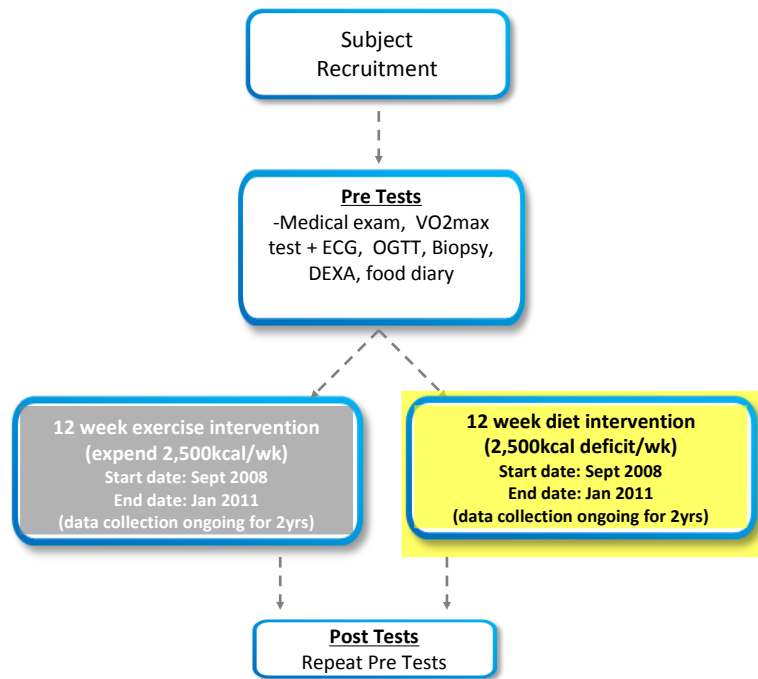


Figure 3.2 Overview of the actual study design that was conducted for this PhD thesis.

3. 7. Test Procedures

To follow is a detailed explanation of the tests outlined in Figure 3.2.

3. 7. 1. Subject recruitment

An email was sent to all staff and students in DCU briefly outlining the nature of the study (Appendix 3). All individuals who responded to the email received a copy of the plain language statement (Appendix 4), which explained the study in great detail. A private meeting was then arranged with each potential participant and all elements of the study were discussed in detail including the nature of the study, the tests involved, the interventions involved, and all risks and benefits associated with the study. The informed consent form (Appendix 5) was explained to the potential participant at this time and they were also given an opportunity to ask any questions they had. Potential participants were advised to re read the informed consent form in their own time and return a signed copy if they wished to take part in the study.

Upon receiving the informed consent form, each participant then completed a health history questionnaire (Appendix 6), which was reviewed by the study physician, and they

underwent a medical examination to confirm their suitability for the study. Subjects were excluded at this point if they had uncontrolled hypertension, unstable cardiovascular disease, hepatic or renal disease, active cancer, HIV infection, previous thyroid surgery or hyperthyroidism, chronic diarrhoea, were pregnant, or unable to stay in the same geographic location for the duration of the study. Twenty five subjects originally volunteered for the study. Of these individuals, one subject completed baseline testing only, two subjects dropped out of the study due to work commitments, three subjects dropped out of the study due to the development of medical conditions including appendicitis, pregnancy and stress induced high blood pressure (work related), and two subjects were non compliant with the interventions. The remaining seventeen subjects were randomly assigned to complete either a 12 week diet intervention or a 12 week exercise intervention in addition to a number of baseline and post intervention tests. One additional obese non diabetic subject was tested and trained in St James Hospital due to its proximity to their home, giving a total of eighteen subjects who completed the study. Ten subjects were randomly assigned to the exercise intervention of which 5 were male and 5 were female. Eight subjects were randomly assigned to the diet intervention of which 4 were male and 4 were female. This study was approved by the DCU Research Ethics Committee and conformed to the Declaration of Helsinki.

3. 7. 2. Anthropometric measurements

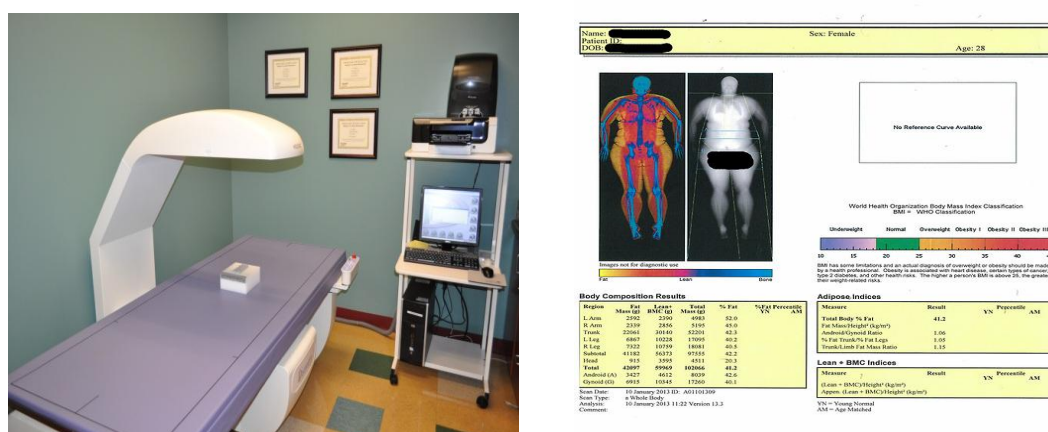
Height and body mass were measured to the nearest 0.1 cm and 0.1 kg respectively (SECA, Hamburg, Germany). Subjects were weighed barefoot and with minimal clothing. They were advised to keep their food and fluid intake similar on the day of the weight measurement. This measurement was taken at the same of the same day each week where possible.

3. 7. 3. Body composition analysis / DEXA Scan

Body composition was measured in the Exwell Medical Clinic, Ballymun, Dublin 9 using a Dual Energy X-Ray Absorptiometry (DEXA) scan. This technique is commonly used to measure bone mineral density but has become a standard technique for determining body composition (Putz et al, 2004; Azuma et al., 2007, Stefan N. et al, 2004). Subjects were required to sign an informed consent form (Appendix 7) prior to undertaking this scan due to exposure to a minimal amount of radiation. Subjects were instructed to remove shoes, socks, jewellery, as much clothing as possible, and lay flat on the bench with their eyes

closed or protected by goggles. The full body scan took 7 minutes after which a report was generated which detailed the body mass of the subject in addition to their percent body fat, percent lean tissue and percent bone mass (Figure 3.3). This technology was unavailable in the first year of data collection as it had been damaged by flooding. For this reason body composition analysis is only available for year 2 of data collection.

Note: The radiation exposure from a whole body DEXA is approximately 1.0-3.6 μSv (Njeh et al., 1999), in comparison to a standard chest X-ray of approximately 100 μSv . On an annual basis we are exposed to approximately 2400 μSv (Thorne MC, 2003). Therefore, the DEXA is equivalent to no more than 1-3 days of background exposure.



performed the warm up at 50W and the power output increased by 35W thereafter every three minutes.

All exercise tests took place under standard laboratory conditions (19-21°C, 40-55% relative humidity) and in the presence of the study physician. Expired oxygen, carbon dioxide, ventilatory volume, respiratory exchange ratios and $\dot{V}O_{2\max}$ were determined by indirect calorimetry using the Innocor (INN00010 – Innocor® CPX System). Systolic and diastolic blood pressure was measured by the physician at rest and during the last minute of each stage of exercise. Rate of perceived exertion was taken during the last ten seconds of each stage using the 6-20 point Borg scale (Appendix 8). Electrical activity of the heart was recorded at rest and at the end of each stage using 12 lead ECG. Heart rate was recorded in the last 10 seconds of each stage. Subjects exercised until reaching volitional fatigue. Oxygen uptake was deemed to have peaked if two or more of the following criteria were satisfied (i) plateau of oxygen consumption with increasing power output (increase of less than 2 ml/kg/min), (ii) heart rate within 10 beats of the subjects' age predicted maximum heart rate ($220\text{bpm} - \text{age in years}$) and (iii) respiratory exchange ratio >1.10 . $\dot{V}O_{2\max}$ was determined as the highest minute average recorded for oxygen uptake during the test.

3. 7. 5. Glucose Tolerance and Insulin Sensitivity

In order to rule out previously undiagnosed type 2 diabetes, impaired fasting glucose or impaired glucose tolerance, subjects underwent a standard 3 hour Oral Glucose Tolerance Test (OGTT) (Reinauer et al., 2002). In preparation for the test subjects were required to adhere to the following criteria (i) no exercise the day before the test (ii) consume adequate carbohydrate in the 3 days leading up to the test to avoid low glycogen levels (iii) refrain from taking medication the morning of the test (iv) fast for 10 hours prior to the test. On the morning of the test, the 75 g (113 ml) anhydrous glucose equivalent (Polycal; Nutricia Clinical, Trowbridge, United Kingdom) was consumed in 300 ml of water within 5 minutes. Blood samples were taken 10 minutes prior to consuming the glucose load, and also at 30, 60, 90, 120 and 180 minutes after ingesting the glucose load. Total area under glucose curve (AUCG) and insulin curve (AUCI) was determined by the trapezoidal method as presented in Equations 3.1 and 3.2 respectively (Tai, 1994, Psyrogiannis, 2003). HOMA-B (Equation 3.3) was used as an indicator of fasting insulin secretion (Song, 2007). The AUCG/AUCI index (Penesova, 2004), Matsuda model (Matsuda, 1999) and Stumvoll

model (Stumvoll, 2000) (Kanauchi, 2003) were also used as indices of insulin sensitivity (Equations 3.4, 3.5 and 3.6 respectively).

Equation 3.1 Area under the glucose curve

$$\text{AUCG} = 0.25 \times (\text{fasting glucose}) + 0.5 \times (\text{glucose at 30 minutes}) + 0.75 \times (\text{glucose at 60 minutes}) + 0.5 \times (\text{glucose at 120 minutes})$$

Equation 3.2 Area under the insulin curve

$$\text{AUCI} = 0.25 \times (\text{fasting insulin}) + 0.5 \times (\text{insulin value at 30 minutes}) + 0.75 \times (\text{insulin value at 60 minutes}) + 0.5 \times (\text{insulin value at 120 minutes})$$

Equation 3.3 HOMA-B

$$20 \times \text{fasting insulin } (\mu\text{IU/ml}) / \text{fasting glucose } (\text{mmol/ml}) - 3.5$$

Equation 3.4 Area under the glucose curve / area under the insulin curve

$$\text{AUCG (Equation 3.1)} / \text{AUCI (Equation 3.2)}$$

Equation 3.5 Matsuda index of insulin sensitivity

$$(10,000 / \text{square root of } [\text{fasting glucose} \times \text{fasting insulin}] \times [\text{mean glucose} \times \text{mean insulin during OGTT}])$$

Equation 3.6 Stumvoll index of insulin sensitivity

$$0.226 - (0.032 \times \text{BMI}) - (0.0000645 \times \text{Insulin at 120 minutes pmol/L}) - (0.00374 \times \text{Glucose at 90 minutes mmol/L})$$

3. 7. 6. Collection, handling and storage of Blood Samples

Prior to the OGTT subjects had a 20 or 22 GA indwelling cannula (BD Vialon™, Biomaterial, Spain) placed into a prominent forearm vein for blood sampling. Samples for glucose analysis were collected in grey top plasma tubes (BD Vacutainer®, 10 mg sodium fluoride, 8 mg potassium oxalate). Samples for insulin determination and other biomarkers were collected in red top serum tubes (BD Vacutainer®). Blood samples were collected 10

minutes before the oral glucose load and, 30, 60, 90, 120 and 180 minutes after the glucose load. Lines were flushed with saline solution after each blood draw. Approximately 2.5 ml of blood was evacuated as waste at each time point prior to the sample to be analysed being collected. The grey top vacutainers for glucose analysis were immediately stored on ice. The red top vacutainers were allowed to stand for 30 minutes at room temperature before centrifugation at 3000 r.p.m-1 (Dovio et al., 2007) for 15 min at 4°C. Following this process aliquots were stored at -80°C for further analysis.

3. 7. 7. Biochemical Analysis and Assays

Plasma glucose was measured using the glucose oxidase method (YSI 2300 Stat Plus, Yellow Springs, Ohio). Serum insulin was measured initially in St. James Hospital biochemistry laboratory using hospital using a commercially available fluoroimmunoassay.

FGF21 was measured using a commercially available ELISA kit (R&D Systems, UK), as was IL-13 (R&D Systems, UK), Chemerin (BioVendor, Germany), Omentin (BioVendor, Germany), Fetuin-A (R&D Systems, UK) and Visfatin (Phoenix Pharmaceuticals, Inc., CA).

Free Fatty Acids, plasma triglycerides, total cholesterol, LDL cholesterol and HDL cholesterol were measured with Randox reagents on the Randox-Daytona automated analyser using a spectro-photometric method (Randox, Antrim, Northern Ireland).

3. 7. 8. Muscle Biopsy

The muscle biopsies were performed by the study physician under sterile conditions. Skeletal muscle specimens were taken by muscle biopsy from the *m. vastus lateralis* under local anaesthesia. An area of skin, subcutaneous tissue, and fascia was anaesthetized with 2% w/v Lidocaine HCl and a small (0.5 cm) incision made. The biopsy needle was inserted into the muscle and approximately 100-200 mg of tissue removed using the percutaneous muscle biopsy technique with suction applied. Muscle samples were snap-frozen in liquid nitrogen and stored at -80°C until analysis. Muscle biopsies were taken in all subjects pre and post intervention. A fresh incision was made for each biopsy, at least 2 cm from a previous biopsy site. Subjects were provided with post biopsy care and treatment instructions (Appendix 12). The study investigator called all subjects on the evening of the day they had their biopsy and again the following morning to follow up. These biopsies are

currently being analysed as part of other projects and will not be reported as part of this PhD thesis.

3. 7. 9. Aerobic Exercise Prescription Using Metabolic Equations

A baseline $\dot{V}O_{2\max}$ test was conducted for all subjects. This data was used to individually prescribe aerobic exercise training for those who were randomly assigned to the exercise intervention. Subjects were required to exercise at 70% of their $\dot{V}O_{2\max}$. The ACSM metabolic equations (ACSM, 2010 pp158) were used to calculate the speed and grade on the treadmill, and the wattage on the cycle ergometer that corresponded to 70% of their $\dot{V}O_{2\max}$. These equations were also used to calculate the total exercise time required to expend 625kcal per session or 2,500kcal per week. A pilot study was conducted to confirm the accuracy and consistency of this approach (see 3.7.13). The subjects adapted to this workload quite quickly and so to ensure that the desired exercise intensity was maintained throughout the entire intervention, a $\dot{V}O_{2\max}$ test was conducted again in weeks 4 and 8 and the equations were recalculated to determine the new workloads for each subject.

Equation 3.7 ACSM Metabolic Equation for Walking

$$\dot{V}O_2 \text{ (ml/kg/min)} = 0.1 \text{ (speed in meters per min)} + 1.8 \text{ (speed in meters per min)} \\ \text{(grade in decimal form)} + 3.5$$

Equation 3.8 ACSM Metabolic Equation for Jogging

$$\dot{V}O_2 \text{ (ml/kg/min)} = 0.2 \text{ (speed in meters per min)} + 0.9 \text{ (speed in meters per min)} \\ \text{(grade in decimal form)} + 3.5$$

Equation 3.9 ACSM Metabolic Equation for cycling

$$\dot{V}O_2 \text{ (ml/kg/min)} = 7.0 + 1.8 \text{ (work rate in kgm/min)} / \text{(body weight in kgs)}$$

Equation 3.10 Calculation of Energy Expenditure from $\dot{V}O_2$

$$\text{To convert } \dot{V}O_2 \text{ (l/min) to kcal/min} = \dot{V}O_2 \text{ (l/min)} * (5\text{kcal/min})$$

3. 7. 10. Data collection / record sheets / monitoring of subjects

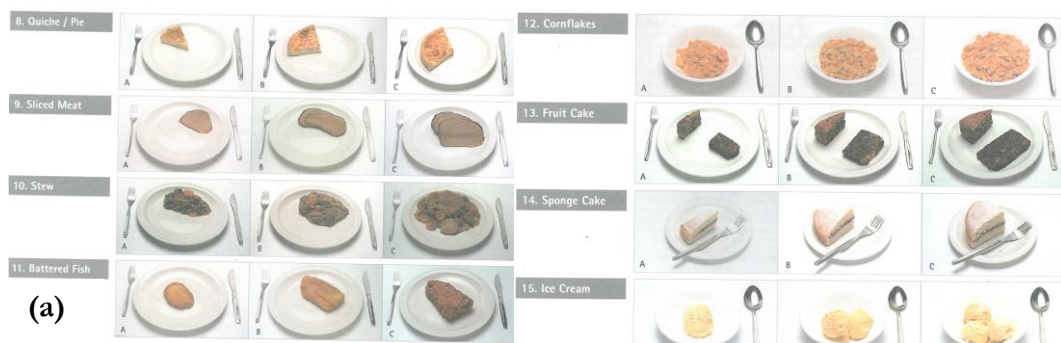
All exercise sessions for all subjects were monitored by the study investigator. Record sheets were devised for all subjects and the information recorded during the sessions included (i) heart rate, (ii) rate of perceived exertion, (iii) exercise intensity (speed, grade, level, RPM, Watts), and (iv) additional comments / events relevant to the particular exercise session.

3. 7. 11. Polar Heart Rate Monitors

When a special circumstance arose where a subject could not attend all 4 GYM based sessions per week, they were given a recordable polar heart rate monitor and a home based exercise session (based on walking or cycling) to complete to compensate for the missed GYM session. This monitor recorded heart rate throughout the entire session and this data was then downloaded by the study investigator and analysed to ensure the correct intensity and calorie expenditure was achieved

3. 7. 12. Food Diary Analysis

All subjects completed a 7 day food diary at baseline which was analysed by a clinical dietician in St. James Hospital. In order to simplify the completion of the food diary and improve adherence to this aspect of the intervention, subjects were not required to weigh their food intake. Instead, they estimated their intake based on pictures provided (Figure 3.4 (a)) and household measures. A sample one day food diary is also presented in Figure 3.4 (b). The clinical dietician converted these estimates into weights with the assistance of a food portion size converter reference manual (FSA, 2002). The weights were then entered into the WISP analysis package to determine food and nutrient intake. In the diet intervention, subjects also completed a 3 day food diary for analysis every two weeks. In the exercise intervention subjects completed a 3 day food diary every 4 weeks for analysis.



Day				Date	
Time slot	When	Where	With whom	Food/Drink description & preparation	Portion size
6am to 9am					
9am to 12 noon					
12 noon to 2pm					
2pm to 5pm					

If this is your last day, please fill in the checklist at the end of the booklet.

16

Time slot	When	Where	With whom	Food/Drink description Et preparation	Portion size
5pm to 8pm					
8pm to 10pm					
10pm to 6am					

17

- Space to write in recipe or ingredients of made up dishes or take-away food if not already described

Please describe any vitamins, minerals or other food supplements you may have taken

Brand	Name (in full)	Number of pills, capsules, teaspoons

(b)

--	--	--

Figure 3.4 (a) demonstrates the method used to determine food portion size, (b) is a sample one day food diary. *Nelson, M., Atkinson, M., and Myer, J. (1997). Food Portion Sizes : A Photographic Atlas. MAFF publications.*

3. 7. 13. Pilot Investigations

In order to validate the methodology for this intervention, 4 weeks of pilot work was carried out prior to subject recruitment. Two individuals consented to take part in this pilot work. A $\dot{V}O_{2\max}$ test was conducted at baseline for both subjects. The ACSM equations were used to determine the speed and grade on the treadmill and the wattage on the bike that the subjects needed to work at to exercise at an intensity of 70% $\dot{V}O_{2\max}$. These training sessions took place in the human performance laboratory in the school of health and human performance in Dublin City University where indirect calorimetry was also used to calculate the energy expenditure derived from the exercise bouts. This data was compared to the estimated energy expenditure calculated using the ACSM metabolic equations. The energy expenditure derived from actual and estimated measurements was comparable confirming that the ACSM equations could be used to prescribe exercise at the correct intensity and caloric expenditure for the obese subjects. The subjects completed 4 exercise sessions per week for 4 weeks to investigate how quickly they adapted to exercise training and when programme updates would be required. Pilot work was also carried out on procedures including the OGTT, muscle biopsy and DEXA scan prior to subject recruitment. A number of ELISA kits were also run at this time to ensure that the investigator was proficient in this technique prior to the analysis of the novel biomarkers of insulin resistance.

3. 8. Exercise intervention study design

On the first day of testing subjects completed a $\dot{V}O_{2\max}$ test with ECG on a cycle ergometer, as per detailed protocol in section They were also given a 7 day food diary to complete and return to the clinical dietician in St. James Hospital who performed the analysis. On the second day of testing they underwent a muscle biopsy and a 3 hour OGTT. This was followed by a DEXA scan in the ExWell medical clinic, Ballymun, to determine body composition. Please refer back to section 3.7 for details of these test procedures.

For the exercise intervention, subjects trained in the fitness centre in Dublin City University 4 days per week for 12 weeks, working at an intensity of 70% $\dot{V}O_{2\max}$ and expending 625kcal per session. The ACSM metabolic equations were used to determine the speed, grade, and length of time that the subjects would need to walk on the treadmill for

and the level and revolutions per minute that they would need to cycle for to expend 625kcal per session. This was verified by the pilot work that was carried out prior to subject recruitment which is detailed in section 3.7.13. The subjects level of fitness, determined by the $\dot{V}O_{2\max}$ test, in addition to their current weight, influenced the total time required to expend 625kcal. The average exercise session duration was 60-75 minutes. $\dot{V}O_{2\max}$ testing was repeated for all subjects in weeks 4 and 8. The ACSM metabolic equations were recalculated using the new fitness levels and body weights and programmes for all subjects were updated to ensure that the desired energy expenditure and intensity of exercise was continuously achieved. To include variety and enhance motivation to train, other aerobic exercise equipment were incorporated into the sessions in week 6 including the cross trainer and the rowing machine. There are no metabolic equations for these pieces of equipment and so the subjects were given a target heart rate to work at on these pieces of equipment that corresponded to 70% $\dot{V}O_{2\max}$. The subjects also completed a 3 day food diary in week 4 and week 8, which was submitted to the clinical dietician and analysed to ensure that dietary intake did not change from baseline throughout the intervention. All subjects were weighed by the study investigator each week at the same time of the day and under the same conditions as much as possible. All training session for all subjects were supervised by the study investigator for the 2 years of data collection. The baseline tests were repeated upon completion of the intervention.

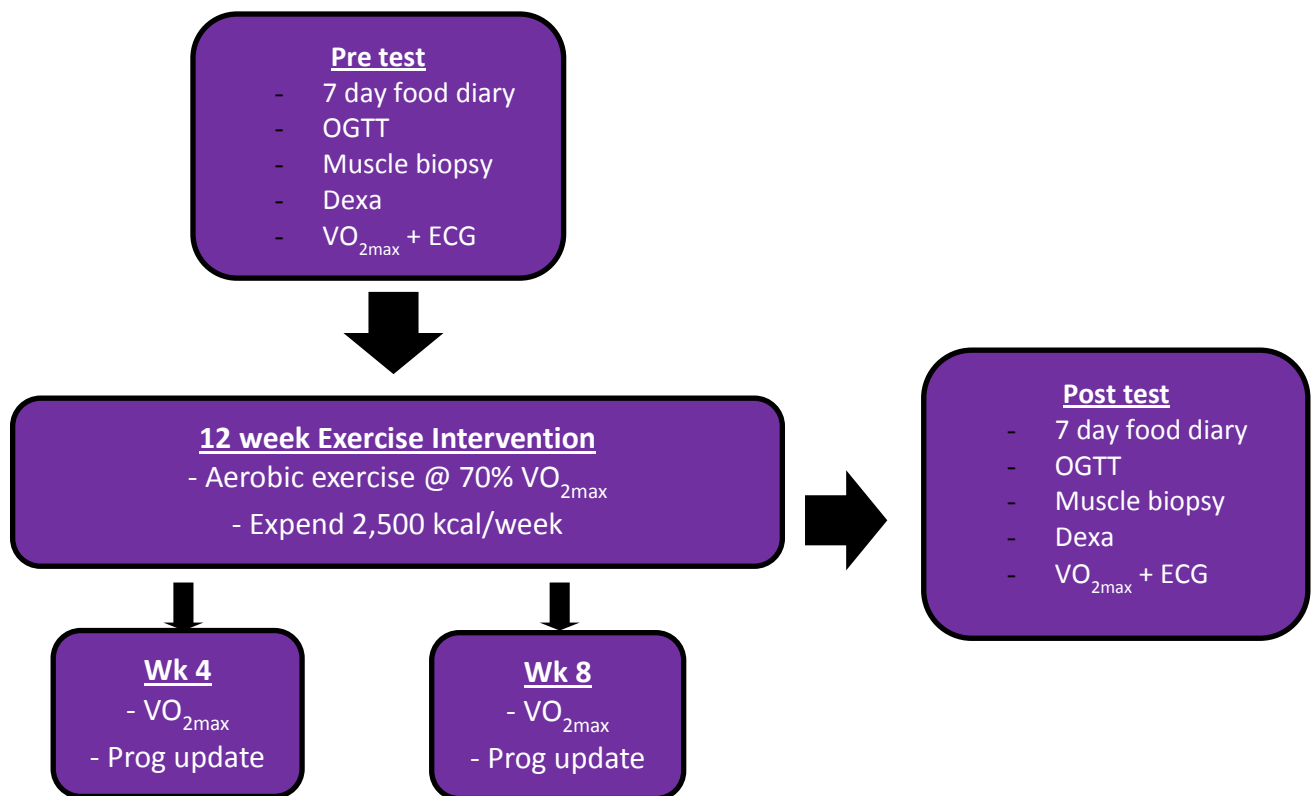


Figure 3.5 Schematic representation of the exercise intervention

3. 9. Diet intervention study design

The method used to recruit subjects to the dietary arm of this intervention was the same as that used for the exercise arm of the intervention. In order to avoid repetition the reader is asked to please refer to section 3.7.1 for details on recruitment. The tests and procedures outlined in figure 3.4 are common with the exercise intervention and so to avoid repetition the reader is asked to refer to sections 3.7.2 to 3.7.8 and 3.7.12. At the beginning of the diet intervention, the subjects attended St. James Hospital for a consultation with the clinical dietician associated with the study. During this time the analysis from their baseline 7 day food diary was discussed. The subjects were instructed to adopt a lower fat diet with a caloric deficit of ~365kcal per day or 2,500kcal per week. To help them achieve this the clinical dietician taught the subjects how to read food labels so that they could determine the calorie content and fat content of their portion sizes and calculate their total calorie intake each day. This was supported with handouts. The dietician also gave them extensive handouts which detailed the calorie and fat content of many common foods including a variety of fruits, vegetables, meats, carbohydrates and alcohol. The clinical dietician also

provided on going support in the form of weekly emails and phone calls to the subjects. In order to monitor compliance to the dietary intervention a 3 day food diary was completed by each subject every 2 weeks and submitted to the clinical dietician for analysis. Also to monitor compliance, the study investigator weighed all of the subjects every week at the same time of the day and under the same conditions. The weight loss target was 0.35kg every week. If the weight loss target was achieved the subjects were deemed to be compliant. If the weight loss target was either not achieved or was too great the clinical dietician arranged a phone based consultation with the subject to discuss compliance, give advice and modified their diet as necessary.

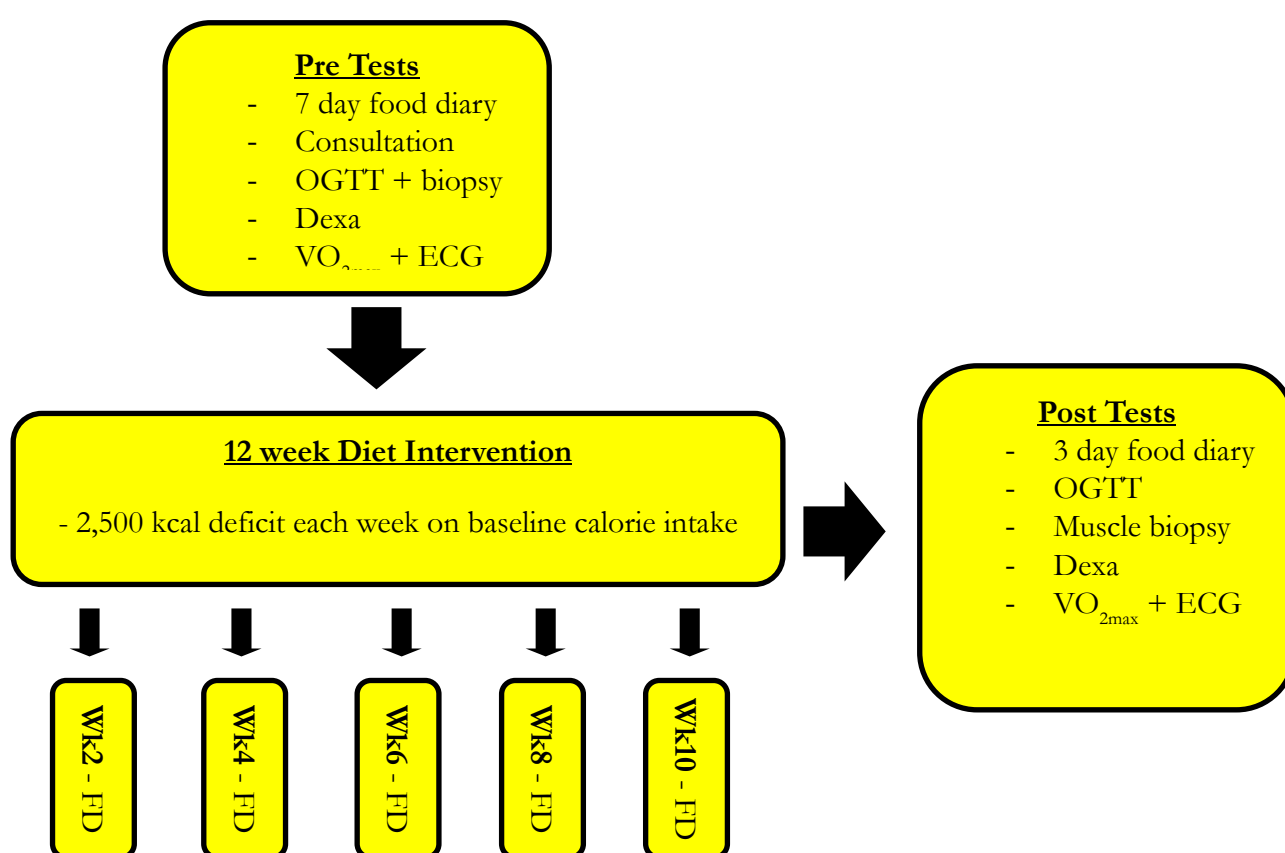


Figure 3.6 Schematic representation of diet intervention

3. 10. Statistical Analyses

Experimental data is presented as mean \pm standard deviation. Data was evaluated using the Sigmaplot 12 statistical package (Systat Software, Inc. Chicago, IL). Statistical significance was set as $p \leq 0.05$. To follow is a description of the statistical tests that were used in the analysis of the data in this chapter.

3. 10. 1. Dependent t-test

A paired or dependent samples t-test was used to compare mean values of a dependent variable pre and post intervention for the same group. Statistical significance was set at $p \leq 0.05$.

3. 10. 2. Independent t-test

An unpaired or independent samples t-test was used to compare the change (mean change) in a dependent variable in one group to the change (mean change) in the same dependent variable in another group post intervention. Statistical significance was set at $p \leq 0.05$.

3. 10. 3. Pearson correlation

Pearson correlations were used to measure the strength of association between two variables. The correlation coefficient is represented by r . An r value of 1.0 indicates a perfect positive relationship between two variables. An r value of -1.0 indicates a perfect negative relationship between two variables. Statistical significance was set at $p \leq 0.05$.

3. 10. 4. Stepwise linear regression (backwards stepwise regression)

Stepwise linear regression is used to predict the value in one variable from the value in one or more other variables. This test can determine the independent variable (s) that best predict the dependent variable. For the analysis in this chapter, a backwards stepwise regression test was used to identify the change in the independent variable (s) that best predicted the change in the dependent variable post intervention. Prior to performing backwards regression, pearson regression analysis was run to identify the independent variables that were significantly correlated with the dependent variable. These independent variables were entered into the backwards regression model and were eliminated one by one until the independent variable (s) that best predict the change in the dependent variable were identified. Multicollinearity exists when independent variables are highly correlated ($r = .9$ and above). To avoid multicollinearity only one of the related variables were entered into the regression analysis. An R^2 of 1.0 indicates a perfect prediction model. Statistical significance was set at $p \leq 0.05$.

3. 11. Results

The data was analysed in a number of sequential steps. Firstly, a comparison of the physical and metabolic raw data was performed. Secondly, we studied a number of novel biomarkers that were identified as mainly, though not exclusively, secreted from adipose tissue to determine if the diet and exercise interventions differentially regulated their circulating concentration. Finally, physiological and biological changes associated with the diet and exercise interventions were identified using a linear regression analysis to establish the relationship between changes in variables and then by backward stepwise regression analysis to identify those that best predicted the changes.

All subjects who took part in this study were obese at baseline and had been inactive for a minimum of 6 months prior to enrolling in the study. Inactive was defined as not meeting the ACSM's recommendations for health which is a minimum of 150 minutes of moderate intensity physical activity per week (Donnelly, Blair et al. 2009). The baseline values for the physical characteristics, metabolic characteristics, and biomarker profile are presented in table 3.1, 3.2 and 3.3 respectively. Intervention groups were matched for age, weight and BMI but there were still some baseline differences, despite randomisation, in fat mass ($p=0.03$), AUCI ($p=0.01$), AUCG/AUCI ($p=0.002$), Matsuda ($p=0.01$) and Omentin ($p=0.04$). The differences in insulin were most surprising and despite having analysed them a number of times, in different centres, the difference can only be explained by random variation.

3. 11. 1. Physical Characteristics

Following both interventions there was a significant reduction in body weight ($p=0.01$), BMI ($p=0.01$) and fat mass (kg) ($p=0.05$). An unpaired t-test revealed that there was no difference between groups in the percent change in these variables. Although the weight lost in each group was similar, it is important to note that the exercise group achieved a 12.2% reduction in fat mass compared to a 5.8% reduction in the diet group. There was an 11% or 3kg increase in lean tissue following the exercise intervention but there were no statistically significant changes in either group, or between groups post intervention. Total percent body fat and total percent lean tissue determined by the DEXA scan did not change significantly in either group post intervention. In all future analysis, fat mass (kg) and lean tissue mass (kg) are used instead of percent body fat and percent lean tissue in order to avoid multicollinearity. Aerobic fitness, measured by $\dot{V}O_{2\max}$ (ml/kg/min)

increased significantly post exercise intervention ($p<0.001$) but did not change post diet intervention ($p=0.22$). The smaller than anticipated sample size limited the depth of statistical analysis and meant a paired samples t-test was used to examine the extent of changes following the intervention rather than the preferred option of a 2-way ANOVA.

Table 3.1 Pre and post values for the physical characteristics of the subjects in the exercise and diet group. The percent change in each variable that occurred post intervention is also presented.

Variable	Exercise		Diet		% change	
	Pre	Post	Pre	Post	Exercise	Diet
	(n=10)		(n=8)			
Age (yr)	45 \pm 14		45 \pm 16			
Weight (kg)	98.6 \pm 20.7	96.0 \pm 20.0*	99.3 \pm 15.5	96.3 \pm 15.3*	-2.6 \pm 2.5	-3.1 \pm 2.9
BMI (kg/m ²)	34.8 \pm 5.2	33.9 \pm 5.3*	32.7 \pm 4.0	31.7 \pm 4.1*	-2.6 \pm 2.5	-3.1 \pm 2.9
Fat mass (kg)	46.8 \pm 21.9	40.9 \pm 19.1*	45. \pm 6.5	43.0 \pm 5.2*	-12.2 \pm 11.0	-5.8 \pm 5.1
% body fat	47.0 \pm 13.6	42.3 \pm 12.0 [†]	46.2 \pm 5.9	44.4 \pm 5.11	-9.8 \pm 9.1	-3.7 \pm 4.4
Lean tissue (kg)	47.6 \pm 12.6	50.8 \pm 11.1	52.1 \pm 13.1	52.5 \pm 12.3	8.6 \pm 14.2	1.4 \pm 6.3
% Lean Tissue	50.3 \pm 13.3	54.8 \pm 11.6 [†]	51.5 \pm 6.0	53.3 \pm 5.5	11.0 \pm 12.6	3.6 \pm 5.1
$\dot{V}O_{2max}$ (ml/kg/min)	22.7 \pm 5.6	29.3 \pm 8.5*	21.8 \pm 6.1	23.5 \pm 6.3	29.3 \pm 20.7	10.1 \pm 20.8
$\dot{V}O_{2max}$ (L/min)	2.3 \pm 0.7	2.9 \pm 0.8*	2.2 \pm 0.9	2.3 \pm 0.9	28.3 \pm 18.3	8.3 \pm 21.6

Data is presented as mean \pm standard deviation. * $p\leq 0.05$ represents a significant change in the variable post intervention. Note: n=5 for fat mass, % body fat, lean tissue mass and % lean tissue in both groups. [†] $p\leq 0.09$ is trending towards a significant change post intervention.

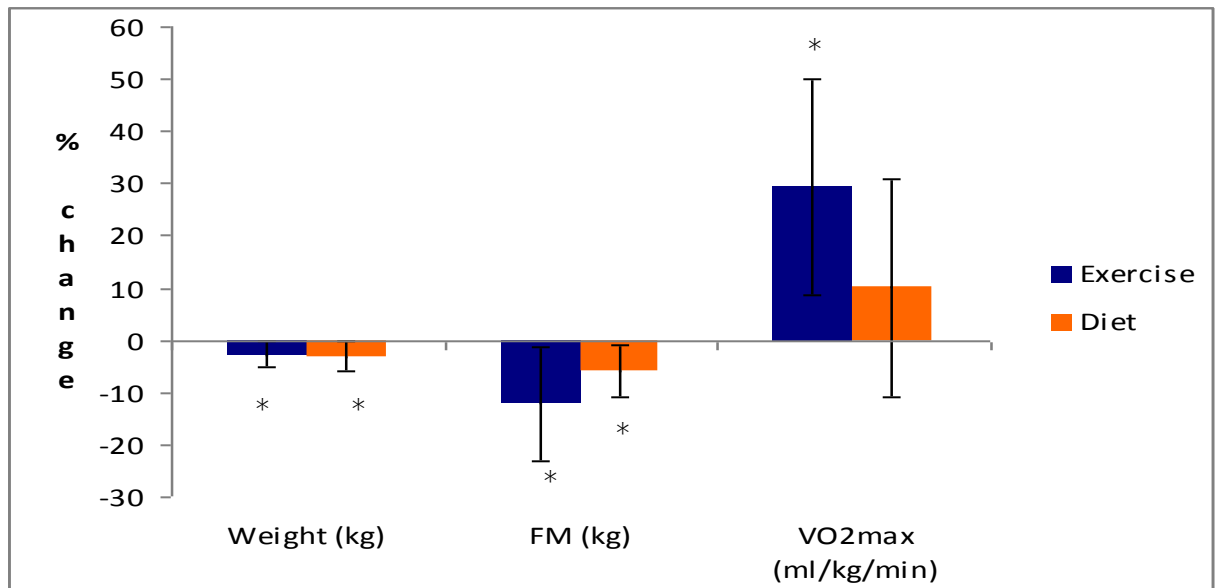


Figure 3.7 Percent change in weight (kg), fat mass (kg) and fitness ($\dot{V}O_{2max}$ ml/kg/min) post intervention presented as mean \pm SD. * $p \leq 0.05$ represents a significant change in the variable post intervention.

3. 11. 2. Metabolic characteristics

The changes that occurred in the metabolic characteristics post intervention are presented in Table 3.2. There was a significant decrease in insulin secretion and increased insulin sensitivity in the exercise group but not in the diet group. In the exercise group, this was evident as a significant reduction in AUCI ($p=0.01$) and HOMA-B ($p=0.04$), in addition to a significant increase in the AUCG/AUCI index ($p=0.02$). These findings are supported by trending changes in the Stumvoll ($p=0.06$) and Matsuda ($p=0.08$) predictors of insulin sensitivity. Following the diet intervention, there was no change in AUCG ($p=0.71$), AUCI ($p=0.12$), AUCG/AUCI ($p=0.10$), HOMA-B ($p=0.18$), Stumvoll ($p=0.71$), or Matsuda ($p=0.54$).

The lipid profile of the subjects was also measured pre and post intervention. These values in addition to the percent change that occurred to the lipids are presented in Table 3.2. There was no significant change in the lipid profile of the subjects following the diet or exercise intervention.

Table 3.2 Pre and post values for the metabolic characteristics of the subjects in the exercise and diet group. The percent change that occurred to each of the variables post intervention is also presented.

Variable	Exercise		Diet		% change	
	Pre	Post	Pre	Post	Exercise	Diet
	(n=10)		(n=8)			
AUCG	1092 ± 189	109 ± 197	1166 ± 157	1142 ± 135	1.1 ± 14.8	-0.9 ± 14.6
AUCI	21020 ± 8850	15373±9031*	9332 ± 6274	7494 ± 4666	-27.4 ± 21.3	-12.4 ± 26.0
AUCG /AUCI	0.06 ± 0.02	0.09 ± 0.05 *	0.17 ± 0.09	0.19 ± 0.08	50.3 ± 46.3	19.0 ± 25.2
HOMA-B	58.3 ± 25.8	51.5 ± 28.5 *	35.6 ± 24.9	23.8 ± 13.0	-14.1 ± 17.9	-14.9 ± 39.4
Stumvoll	0.136 ± 0.043	0.125±0.031‡	0.118 ± 0.015	0.116 ± 0.013	-7.02 ± 7.53	-0.7 ± 7.0
Matsuda	7.7 ± 3.5	10.2 ± 4.7‡	18.1 ± 10.0	19.6 ± 7.9	34.8 ± 49.1	21.2 ± 44.3
FFA's	0.60 ± 0.12	0.62 ± 0.16	0.60 ± 0.30	0.51 ± 0.17	4.52 ± 19.42	4.51 ± 55.60
TG's	1.77 ± 1.07	1.40 ± 0.66	1.25 ± 0.31	1.16 ± 0.30	-10.1 ± 32.6	-6.6 ± 11.2
TC	5.11 ± 1.64	5.00 ± 1.54	5.20 ± 0.41	5.20 ± 0.35	1.96 ± 31.15	0.19 ± 6.56
LDL	3.52 ± 1.49	3.21 ± 1.23	3.18 ± 0.74	3.41 ± 0.50	-1.4 ± 32.9	10.1 ± 18.7
HDL	1.08 ± 0.20	1.08 ± 0.31	1.31 ± 0.37	1.23 ± 0.22	4.8 ± 26.8	-3.6 ± 15.3

Data is presented as mean ± standard deviation. *p≤0.05 represents significant change pre and post intervention. ‡p≤0.09 is trending towards a significant change post intervention. Note: AUCG = Area Under the Glucose Curve, AUCI = Area Under the Insulin Curve, AUCG/AUCI = Area Under the Glucose Curve / Area Under the Insulin Curve (index of insulin sensitivity), HOMA-B is an index of fasting insulin secretion, Stumvoll and Matsuda are indexes of insulin sensitivity, FFA's = Free Fatty Acids, TG's = Triglycerides, TC = Total Cholesterol, LDL = Low Density Lipoprotein Cholesterol, HDL = High Density Lipoprotein Cholesterol.

An unpaired samples t-test revealed that there was no difference between groups in the percent change that occurred to AUCI ($p=0.21$), AUCG/AUCI ($p=0.11$), or HOMA-B ($p=0.96$) post intervention.

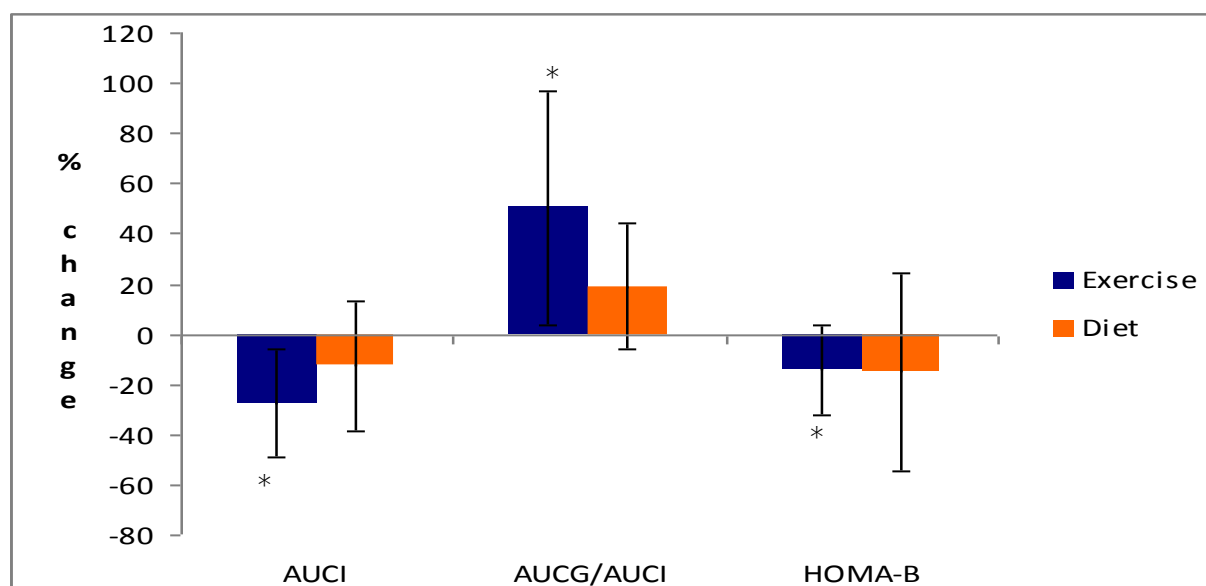


Figure 3.8 Percent change in the area under the insulin curve (AUCI), the area under the glucose curve / area under the insulin curve (AUCG/AUCI) and HOMA-B which is a measure of fasting insulin secretion post intervention in the exercise and diet group. Data is presented as mean percent change \pm standard deviation. * $p \leq 0.05$ represents a significant change post intervention.

3. 11. 3. Biomarker Profile

A number of novel biomarkers related to insulin resistance and metabolic health were measured pre and post intervention. The circulating concentrations of these biomarkers as well as the percent change that occurred to the concentrations of these biomarkers post intervention are presented in Table 3.3. There was a lot of inter-individual variation for all of the biomarkers and the paired samples t-tests showed that there was no significant change in the circulating concentration of FGF21 ($p=0.99$), IL-13 ($p=0.95$), Chemerin ($p=0.33$), Omentin ($p=0.87$) or Visfatin ($p=0.86$) post exercise intervention, but there was a significant reduction in Fetuin-A ($p=0.02$). Following the diet intervention, there was no change in any of the biomarkers but there was a trend toward a change in Omentin ($p=0.06$). The percent change in Chemerin was significantly greater following the diet than the exercise intervention ($p=0.05$). While the individual variation makes it difficult to

determine if changes in these biomarkers have any role, there is some evidence of biomarker specific responses to diet or exercise. Therefore, the next stage was to conduct correlation and regression analysis of the data to examine association between the variables.

Table 3.3 Pre and post values for the novel biomarkers. The percent changes that occurred post intervention are also presented.

Variable	Exercise		Diet		% change	
	Pre	Post	Pre	Post	Exercise	Diet
	(n=10)		(n=8)			
FGF21	222.3 ± 124.1	223.0 ± 79.0	382.8 ± 241.7	256.4 ± 118.7	28.5 ± 85.9	-1.1 ± 81.4
IL-13	106.6 ± 33.4	105.8 ± 30.4	98.3 ± 19.9	94.1 ± 13.5	3.1 ± 23.8	-3.2 ± 9.4
Chemerin	224.1 ± 39.0	216.2 ± 38.4	278.0 ± 106.6	216.4 ± 65.1	-3.2 ± 11.9	-19.2 ± 16.2
Omentin	279.0 ± 133.9	271.5 ± 118.8	424.9 ± 114.6	374.3 ± 134.8 [‡]	7.1 ± 37.0	-12.4 ± 13.9
Visfatin	16.0 ± 4.3	16.2 ± 5.6	14.4 ± 2.9	14.9 ± 3.1	0.2 ± 3.4	6.0 ± 23.7
Fetuin-A	578.5 ± 141.2	464.9 ± 76.1*	561.5 ± 78.0	530.31 ± 163.5	-19.8 ± 13.1	-4.1 ± 32.1

Data is presented as mean ± standard deviation. *p≤0.05 represents a significant change post intervention. [‡]p≤0.09 is trending towards a significant change post intervention.

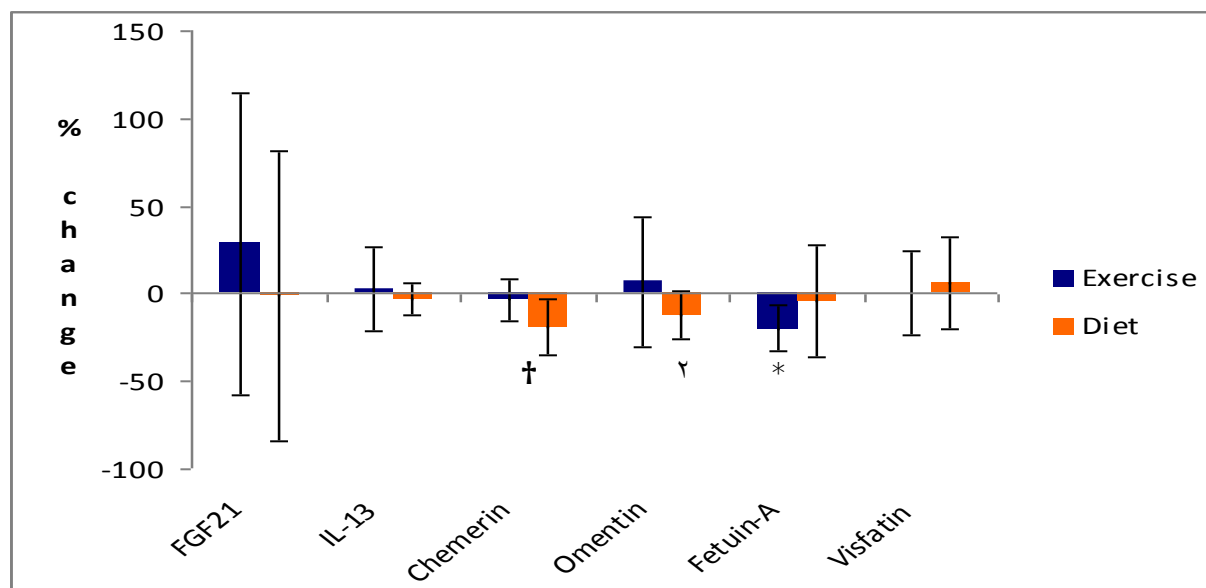


Figure 3.9 Percent change in the biomarkers post intervention. Data is presented as mean \pm SD. * $p \leq 0.05$ represents a significant change post intervention, † $p \leq 0.09$ represents a trend towards a significant change post intervention. ‡ $p \leq 0.05$ represents a significant difference between groups in the percent change the occurred to the biomarker post intervention.

3. 11. 4. Pearson correlation and backwards stepwise regression analysis

The pearson correlation analysis for the physical characteristics, as presented in Table 3.4. showed that the percent change in weight following the exercise and diet interventions did not relate to any of the metabolic adaptations and it was only correlated with the percent change in BMI and the Matsuda insulin sensitivity index for the diet intervention.

On the other hand, the percent change in fat mass appeared to correlate better with metabolic adaptations following the exercise intervention and was positively related to the percent change in AUCG and total cholesterol, with a trend for HOMA-B. In the diet group, the percent change in fat mass was only significantly correlated with the percent change in another predictor of insulin sensitivity, Stumvoll.

The percent change in fitness following the exercise intervention was significantly positively correlated with indicators of insulin sensitivity and lipid metabolism including the percent change in Matsuda, AUCG, AUCI, HOMA-B, triglycerides, total cholesterol, and LDL cholesterol, with a trending relation with the AUCG/AUCI index. In the diet group,

the percent change that occurred to fitness, although not statistically significant, was significantly correlated with the percent change in fat mass and FFA's, with a trending relation with HOMA-B.

The change that occurred in percent lean tissue (%) was correlated with the percent change in IL-13 ($p=0.03$) in the exercise group.

Backwards stepwise regression was not carried out on the physical characteristics because the percent change that occurred to these variables post intervention was directly predicted by the actual physical exercise training that was carried out, in addition to the calories expended during the exercise sessions or the calories restricted during the dietary intervention.

Table 3.4 Summary of pearson correlations for the physical characteristics for the exercise and diet groups.

Independent Variables	<u>Weight (kg)</u>		<u>Fat Mass (kg)</u>		<u>Fitness (ml/kg/min)</u>	
	Exercise	Diet	Exercise	Diet	Exercise	Diet
Body Mass Index		r=1.000 * p<0.001				
Fat Mass						r=0.979 * p=0.004
AUCG			r=0.873 * p=0.05		r=-0.665 * p=0.05	
AUCI					r=-0.813 * p=0.01	
AUCG/AUCI					r=0.635 † p=0.07	
HOMA-B			r=0.869 † p=0.06		r=-0.755 * p=0.01	r=0.690 † p=0.06
Stumvoll				r=-0.907 * p=0.03		
Matsuda		r=-0.700 * p=0.05			r=0.841 * p=0.001	
TC			r=0.952 * p=0.05		r=-0.789 * p=0.02	
TG's					r=-0.708 * p=0.05	
LDL					r=-0.655 * p=0.04	
FFA's						r=0.925 * p=0.003

r represents the strength of the correlation between two variables, *p≤ 0.05 is significant.

†p≤0.09 is trending towards significance. Note: AUCG = Area Under the Glucose Curve, AUCI = Area Under the Insulin Curve, AUCG/AUCI = Area Under the Glucose Curve / Area Under the Insulin Curve (index of insulin sensitivity), HOMA-B is an index of fasting insulin secretion, Stumvoll and Matsuda are indexes of insulin sensitivity, FFA's = Free Fatty Acids, TG's = Triglycerides, TC = Total Cholesterol, LDL = Low Density Lipoprotein Cholesterol.

Pearson correlations were run to investigate whether the change that occurred to AUCI, HOMA-B and AUCG/AUCI post intervention, was related to, or associated with, the change that occurred to any of the other measured variables. This data is presented in Table 3.5. In the exercise group, the percent change in AUCI was significantly positively correlated with the percent change in TG's and total cholesterol, and inversely correlated with the percent change in $\dot{V}O_{2\max}$ (ml/kg/min). These variables were entered into a backwards stepwise regression model, which eliminates each independent variable one by one until it ultimately determines the independent variable that most significantly predicts the percent change in the dependent variable. In this case the percent change in AUCI was most significantly predicted by the percent change in TG's, which accounted for 70.6% of the variance in AUCI post intervention. Further backwards regression analysis revealed that the percent change in fitness best predicted the percent change in TG's. This data is presented in Table 3.6.

The percent change in AUCG/AUCI was inversely correlated with the percent change in TG's and LDL cholesterol. It was trending towards a positive correlation with $\dot{V}O_{2\max}$ (ml/kg/min). Backwards stepwise regression revealed that the percent change in TG's was the most significant contributor to the percent change in AUCG/AUCI and accounted for 64.9% of the variance as presented in Table 3.6. The percent change in fitness once again best predicted the percent change in TG's as shown in Table 3.6.

The percent change in HOMA-B was positively correlated with the percent change in FFA's and AUCG. It was inversely correlated with the percent change in $\dot{V}O_{2\max}$ (ml/kg/min). When backwards stepwise regression was performed, it revealed that the percent change in HOMA-B was most significantly predicted by the percent change in fitness, which accounted for 50.2% of the variance in HOMA-B post intervention as presented in Table 3.6.

With regard to the diet intervention, the percent change in AUCI was only correlated with the percent change in AUCG. The percent change in AUCG/AUCI was only correlated with the percent change in total cholesterol. The percent change in HOMA-B was only correlated with the percent change in FFA's and so backwards regression could not be run for these variables.

Table 3.5 Summary of pearson correlations for the metabolic characteristics in the exercise and diet groups.

Independent Variables	AUCI		AUCG/AUCI		HOMA-B	
	Exercise	Diet	Exercise	Diet	Exercise	Diet
$\dot{V}O_{2\max}$ (ml/kg/min)	r=-0.813 * p=0.01		r=0.635 † p=0.07		r=-0.755 * p=0.01	
AUCG		R=0.806 * p=0.02			r=0.735 * p=0.03	
TG's	r=0.840 * p=0.01		r=-0.806 * p=0.02			
FFA's					r=0.660 * p=0.05	r=0.799 * p=0.03
TC	r=0.802 * p=0.02			r=0.835 * p=0.02		
LDL			r=-0.761 * p=0.03			

r represents the strength of the correlation between two variables, * $p \leq 0.05$ represents a significant correlation between two variables. † $p \leq 0.09$ represents a trend towards a significant relationship. Note: AUCI = Area Under the Insulin Curve, AUCG/AUCI = Area Under the Glucose Curve / Area Under the Insulin Curve, HOMA-B is a measure of fasting insulin secretion, AUCG = Area Under the Glucose Curve, FFA's = Free Fatty Acids, TG's = Triglycerides, TC = Total Cholesterol, LDL = Low Density Lipoprotein Cholesterol.

Table 3.6 Backwards stepwise regression analysis to predict the percent change in AUCI, AUCG/AUCI, and HOMA-B in the exercise group post intervention.

Dependant variable	Indep. variables in model	Coef.	Std. Coeff.	Std. Error	F-to-Remove	P
% change in AUCI (R ² =0.706)	Constant	-23.53		4.748		
	% change in TG's	0.565	0.840	0.149	14.43	0.009
% change in AUCG/AUCI (R ² =0.649)	Constant	45.44		10.740		
	% change in TG's	-1.114	-0.806	0.334	11.11	0.016
% change in HOMA-B (R ² =0.891)	Constant	-2.196		5.124		
	% change in $\dot{V}O_{2max}$ (ml/kg/min)	-0.700	-0.944	0.141	24.61	0.016
% change in TG's (R ² =0.502)	Constant	21.798		15.678		
	% change in $\dot{V}O_{2max}$ (ml/kg/min)	-1.091	-0.708	0.444	6.068	0.049

R² represents the extent to which the change in the independent variable predicts the percent change in the dependent variable. $p \leq 0.05$ is significant. Note: AUCI = Area Under the Insulin Curve, AUCG/AUCI = Area Under the Glucose Curve / Area Under the Insulin Curve (index of insulin sensitivity), HOMA-B is an index of fasting insulin secretion, TG's = Triglycerides, $\dot{V}O_{2max}$ = aerobic fitness.

Pearson correlations were run on the biomarkers to investigate if the percent change that occurred to these biomarkers post exercise or post diet, was significantly correlated with the percent change in any other variable. This data is presented in Table 3.7.

Table 3.7 Summary of pearson correlations between the percent change in the biomarkers and the percent change in all other measured variables for both the exercise and diet group.

Variable	Group	Correlations with Independent Variables		
% change in FGF21	Ex	Not correlated with the percent change in any other measured variable		
	Diet	TC ($r=0.767$, $p=0.04$) *	HDL ($r=0.799$, $p=0.03$) *	
% change in IL-13	Ex	Lean Tissue (kg) ($r=0.907$, $p=0.03$) *		
	Diet	Visfatin ($r=0.934$, $p=0.07$) †		
% change in Chemerin	Ex	$\dot{V}O_{2max}$ (L/min) ($r=-0.716$, $p=0.05$) *		
	Diet	Not correlated with the percent change in any other measured variable		
% change in Omentin	Ex	Stumvoll ($r=-0.666$, $p=0.07$) †		
	Diet	LDL ($r=-0.851$, $p=0.02$) *	IL-13 ($r=0.835$, $p=0.03$) *	
% change in Visfatin	Ex	Not correlated with the percent change in any other measured variable		
	Diet	IL-13 ($r=0.934$, $p=0.07$) †	Delta % LT ($r=0.967$, $p=0.03$) *	Delta % BF ($r=-0.968$, $p=0.03$) *
% change in Fetuin-A	Ex	FFA's ($r=0.684$, $p=0.08$) †		
	Diet	Not correlated with the percent change in any other measured variable		

r represents the strength of the correlation between variables. * $p \leq 0.05$ is significant. † $p \leq 0.09$ is trending towards significance. Note: TC = Total Cholesterol, HDL = High Density Lipoprotein Cholesterol, LDL = Low Density Lipoprotein Cholesterol, Delta % LT = the actual change in % lean tissue, Delta % BF = the actual change in % body fat, FFA's = Free Fatty Acids.

Backwards stepwise regression requires numerous significantly correlated independent variables to be entered into the model to determine those that best predict that change in the dependent variable. The number of significant correlations with the biomarkers that were identified by the pearson correlation analysis was limited. When these variables were entered into the backwards regression models they only accounted for approximately 10% of the variance in each particular biomarker in the exercise and diet group. In an attempt to better understand the variables that predicted the change in the biomarkers in both groups post intervention, backwards regression was run using the variables in Table 3.7 in addition to various combinations of other physical and metabolic variables. These combinations were determined based on the relationships that were identified in the literature.

This backwards regression analysis presented in Table 3.8 revealed a number of similarities and differences between groups in terms of the independent variables that best predicted the percent change in the biomarkers post intervention. In the exercise group the percent change in lean tissue mass and fitness accounted for 93% of the variance in FGF21. In the diet group the percent change in HDL cholesterol accounted for 63.8% of the variance in FGF21.

The percent change in IL-13 was best predicted by the percent change in lean tissue mass in the exercise group, which accounted for 82.4% of the variance, compared to the percent change in Visfatin, which accounted for 87.2% of the variance in the diet group post intervention.

The single best predictor of the percent change in Chemerin post exercise intervention was the percent change in fitness, which accounted for 93.4% of the variance. Interestingly no measured variable predicted the percent change in Chemerin in the diet group.

Indicators of insulin sensitivity predicted the percent change in Omentin in both groups post intervention. The percent change in Stumvoll accounted for 44.3% of the variance in Omentin in the exercise group. The percent change in AUCI accounted for 94% of the variance in Omentin in the diet group.

None of the measured variables predicted the percent change in Visfatin in the exercise group. The percent change in IL-13 was the best single predictor of the percent change in Visfatin in the diet group, accounting for 87.2% of the variance post intervention.

Finally, 42% of the variance in Fetuin-A in the exercise group was predicted by the percent change in FFA's. However none of the measured variables predicted the percent change in Fetuin-A post intervention in the diet group.

Table 3.8 Backwards stepwise regression analysis to predict the percent change in the biomarkers in the exercise and diet groups post intervention.

Study	Dependant variable	Indep. variables in model	Coef.	Std. Coeff.	Std. Error	F-to-Remove	P
% change in FGF21	Exercise (R ² =0.930)	Constant	1.553		8.055		
		% change in LT (kgs)	-1.599	-0.911	0.344	21.65	0.043
		% change in $\dot{V}O_{2max}$ (ml/kg/min)	0.820	0.693	0.231	12.558	0.071
	Diet (R ² =0.638)	Constant	14.293		20.924		
		% change in HDL	4.251	0.799	1.432	8.815	0.031
% change in IL-13	Ex (R ² =0.824)	Constant	1.082		2.357		
		% change in LT (kgs)	0.299	0.907	0.080	14.003	0.033
	Diet (R ² =0.872)	Constant	-6.768		2.606		
		% change in Visfatin	0.389	0.934	0.106	13.619	0.066
		Note: the % change in LT perfectly predicted the percent change in IL-13					

% change in Chemerin	Ex (R ² =0.934)	Constant	35.59		8.081		
		% change in $\dot{V}O_{2\max}$ (L/min)	-1.190	-0.967	0.223	28.42	0.033
	Diet	None of the measured variables predicted the percent change in Chemerin post intervention in the diet group					
% change in Omentin	Ex (R ² =0.443)	Constant	-16.41		15.044		
		% change in Stumvoll	-3.107	-0.666	1.422	4.777	0.072
	Diet (R ² =0.940)	Constant	-4.401		1.434		
		% change AUCI	0.701	0.970	0.125	31.418	0.03
% change in Visfatin	Ex	None of the measured variables predicted the percent change in Visfatin in the exercise group post intervention					
	Diet (R ² =0.872)	Constant	16.884		5.312		
		% change in IL-13	2.239	0.934	0.607	13.619	0.066
% change in Fetuin-A	Ex (R ² =0.420)	Constant	-21.551		3.896		
		% change in FFA's	0.409	0.648	0.196	4.349	0.082
	Diet	None of the measured variables predicted the percent change in Fetuin-A post intervention in the diet group					

R² represents the extent to which the change in the independent variable predicts the percent change in the dependent variable. p≤0.05 is significant. p≤0.09 is trending towards significance. Note: LT = Lean Tissue, $\dot{V}O_{2\max}$ = aerobic fitness, AUCI = Area Under the Insulin Curve, FFA's = Free Fatty Acids.

3. 12. Discussion

The main findings of the current study were that both the diet and the exercise intervention were equally effective in reducing body weight and body fat mass, but the exercise group showed a trend towards greater improvements in body composition. Fitness and metabolic profile improved significantly in the exercise group only, with the increase in fitness accounting for the improvements in metabolic health in this group. Importantly the percent change in body weight did not relate to any of the metabolic adaptations post intervention in either group, but the percent change in fat mass did. The results also indicate that the biomarkers of insulin resistance may be differentially regulated by exercise and diet. Fetuin-A was the only biomarker to decrease significantly post intervention, which occurred in the exercise group, but there was a trend towards a change in Omentin in the diet group. The changes that did occur to the other biomarkers in both groups, although non significant, were associated with improvements in lean tissue and fitness and metabolic profile so they may be useful independent predictors in larger scale studies.

Exercise training and/or dietary restriction continues to be the focus of weight loss interventions for obese individuals. Numerous studies have been conducted to investigate the effects of diet or exercise on weight loss and metabolic health in this population, but a review of this literature shows varying degrees of effectiveness (Franz et al., 2007). The difficulty in reviewing this data is that the designs underpinning the exercise interventions often differ greatly in terms of the number of exercise session per week, the intensity of exercise, the mode of exercise used in the intervention, and the total duration of the study (Franz et al., 2007). A similar picture is true for the diet interventions that have been reported in the literature since there is extensive variation between studies with regard to the amount of calories restricted, in addition to the nutritional composition of the diets (Franz et al., 2007). It is unclear whether one type of intervention is superior to the other in terms of the magnitude of metabolic benefits that they produce in this population.

The purpose of this study was to compare the effectiveness of an isocaloric diet or exercise intervention on body composition and metabolic health in an obese population. The results show that both interventions were successful in reducing body weight and body fat mass. It is worthy to note that the actual change in fat mass was a 12% or 5.9kg reduction in the exercise group compared to a 6% or 2.8kg reduction in the diet group, but the difference between groups did not reach statistical significance because of the small

sample size. The literature supports the finding that 12 weeks of diet or exercise successfully reduces body weight and body fat mass in obese individuals, but there is large variability in the magnitude of change due to differences that exist in the exercise and dietary protocols. The main criteria for the dietary intervention in this current study, was to adopt a lower fat diet with a total calorie deficit of approximately 350kcal per day. This yielded an average weight loss of 3.1kgs or 3.1% of total body weight, and an average fat loss of 2.8kgs or 5.9% of total body fat. One other study in the literature that used a similar calorie deficit and intervention duration, reported a similar weight loss of 2.7%, but an average fat loss of 8.2% (Lee et al., 2013a). The difference in fat loss between the two studies may be a reflection of the small sample size for body composition in this current study. A review of short term dietary interventions surmises that there is a dose response relationship between the amount of fat restricted in the diet, and the amount of weight loss achieved. A modest 10% reduction in daily dietary fat intake in obese individuals, similar to the guidelines in the present study, produces a 4-5kg reduction in body weight (Astrup et al., 2002), which is a similar reduction to that found in the present study. The literature also indicates that in general, weight loss and fat loss results may be augmented when the protein content of the diet is greater than that of a regular balanced diet, in addition to modest decreases in total fat intake (Noakes et al., 2005, Clifton et al., 2009, Lee et al., 2009).

A review of short term (<16 weeks) exercise training studies indicates that the amount of body weight and fat mass lost is directly related to the amount of calories expended in training. The studies with an energy expenditure of 1,100kcal per week obtained an average weight loss of 0.06kg per week, and an average fat mass loss of 0.06kg per week. Exercise interventions based on an energy expenditure of 2,200kcal per week obtained an average weight loss of 0.18kg, and an average fat mass loss of 0.21kg per week (Ross and Janssen, 2001). In the higher energy expenditure interventions this yielded an estimated weight loss of 2.2kgs over a 12 week period concomitant with a 2.5kg reduction in fat mass. The energy expenditure in the current study was 2,500kcal per week which yielded an average weight loss of 2.6kgs and an average fat loss of 5.9kgs in 12 weeks or a 12% reduction in fat mass from baseline values. The difference in the magnitude of fat loss between this current study and the studies included in the review may be due to differences in the intensity of exercise training or the mode of aerobic exercise used.

There was no statistical change in lean tissue mass in either group post intervention but there was a tendency for increased lean tissue in the exercise group compared to the diet group. The exercise group exhibited a 3.2kg or 8.6% increase in lean tissue mass compared to a 0.45kg or 1.36% increase in the diet group. This is clinically important because weight loss, particularly when achieved by caloric restriction alone, can be associated with a decline in fat free mass, and consequently a decline in resting metabolic rate, which is a major contributor to total daily energy expenditure. This may ultimately hinder the progress of weight loss in the future in addition to having deleterious effects on carbohydrate and lipid metabolism in this population (Stiegler and Cunliffe, 2006). Weight loss through calorie restriction alone has been reported to reduce metabolic rate in the long term (Martin et al., 2007), and so exercise training may be important to prevent a decline in lean tissue and metabolic rate during a period of weight loss (Broeder et al., 1992). The small sample size for body composition measurements in both groups may mask the extent of the actual changes that occurred post intervention.

Clearly weight loss can be achieved by both interventions but their effects on other aspects of health must also be considered. It is interesting to note that the change in total body weight in both groups was not correlated with the change in any of the metabolic variables. The change in fat mass however was significantly correlated with improvements in fasting insulin secretion, insulin sensitivity and glucose tolerance in the exercise group, and Stumvoll, a predictor of insulin sensitivity, in the diet group. Also, the change in lean tissue was significantly correlated with improvements in the insulin sensitizing hormone IL-13 in the exercise group, and TG's and LDL in the diet group. These are important findings and worth some consideration since the primary outcome measure of many lifestyle interventions is weight loss, when in fact it is more appropriate to focus changes in body composition due to its relationship with metabolic health in this population. It has been well established that fat mass, visceral fat mass in particular, is associated with metabolic dysfunction in obese individuals (Capurso and Capurso, 2012). It has also been well established that lean tissue mass is highly metabolically active and confers a range of metabolic benefits for the obese population (Ebeling et al., 1993). Weight loss induced by lifestyle intervention should ideally be derived from fat loss only. A simple measurement of body weight can not possibly give an insight into the composition of weight lost. It is therefore essential for researchers and clinicians to shift their focus from weight loss as an outcome measure for these individuals, to changes in body composition. The results of the

present study indicate that favourable changes in body composition must be a primary outcome measure in interventions of this nature.

Interestingly, the metabolic profile of the subjects improved significantly in the exercise group but not in the diet group, despite statistically similar reductions in weight, BMI and fat mass. This was evident as a significant decrease in HOMA-B and AUCI, and an increase in AUCG/AUCI. The best single predictor for the percent change in AUCI in the exercise group was the percent change in TG's, which accounted for 70.6% of the variance in AUCI Table 3.6. The percent change in TG's also accounted for 64.9% of the variance in AUCG/AUCI post intervention Table 4.6. Further analysis revealed that the percent change in fitness was the best single predictor of the percent change in TG's Table 3.6. The percent change in fitness was also the best single predictor of the reduction in HOMA-B in this group, which accounted for 89.1% of the variance in HOMA-B Table 3.6. Fitness improved significantly in the exercise group post intervention, but did not change in the diet group, which may account for the lack of improvement in metabolic profile of the subjects in the diet group. It could also possibly be related to the fact that the insulin values were significantly greater in the exercise group than in the diet group at baseline. The increase in fitness in the aerobic group was highly correlated with improvements in insulin secretion and insulin sensitivity as mentioned above, but it was also correlated with an improved lipid profile in these subjects. This is a clinically significant finding given the positive relationship between lipid accumulation and the development of insulin resistance in obese individuals (Belfort et al., 2005, Petersen and Shulman, 2006). The results indicate that exercise training and increased fitness, independent of weight loss, improves metabolic profile in obese individuals.

The positive effects of exercise on the lipid profile and metabolic health of this population have been widely reported. O'Leary et al. 2006 carried out a 12 week aerobic exercise intervention in obese elderly individuals and reported that exercise training significantly reduced visceral fat and the availability of fat in circulation, in addition to improving glucose metabolism. Exercise training and fitness was also associated with the reversal of insulin resistance in these subjects (O'Leary et al., 2006). Other 12 week aerobic exercise training studies have found that exercise training significantly improves insulin sensitivity and glucose metabolism (Kadoglou et al., 2012), which is true in the presence (O'Leary et al., 2006) and absence (Nassis et al., 2005) of weight loss and fat mass loss. Even an acute

bout of exercise has been shown to enhance insulin sensitivity (O'Gorman et al., 2006). This is particularly evident in one legged training studies where glucose uptake increases in the exercising leg during and after the training bout, but not in the non exercising leg of the same individual (Richter et al., 1989). In fact as exercise intensity increases in the exercising limb, so too does the magnitude of glucose uptake (Wahren et al., 1971).

It appears from the results of the current study that improvements in insulin secretion and insulin sensitivity were due to exercise induced improvements in lipid profile. It is likely that exercise training reduced the availability of lipids by reducing total body fat mass, but it may also have improved the oxidative capacity of skeletal muscle leading to improvements in lipid oxidation, thus correcting the mitochondrial dysfunction that is often present in this population (Kelley et al., 2002). These findings emphasise the importance of aerobic fitness in improving metabolic health. This is further supported by the enhanced fat oxidation and insulin sensitivity that is evident in athletes (Ebeling et al., 1993). It is therefore necessary to focus on fitness as an outcome measure of interventions of this nature, in addition to changes in body composition.

Although there were no significant metabolic adaptations to the diet intervention in this current study, calorie restriction is proposed to improve insulin sensitivity. This is true even in the initial stages of calorie restriction before there is any major effect on obesity (Arciero et al., 1999). A 10 day low calorie diet consisting of 50% of the calories required to maintain energy balance, reduced insulin concentrations during hyperglycemia by 40% in obese individuals (Arciero et al., 1999). A 12 week diet intervention with similar caloric restriction to this current study revealed a 2.7% decrease in body weight of the obese women, which was associated with a significant decrease in insulin resistance measured by HOMA-IR (Lee et al., 2013a). Diet interventions using various nutrient compositions have also proved to enhance insulin sensitivity. Noakes et al. 2005 investigated the effects of two different types of diet on metabolic status. An energy restricted high protein/low carbohydrate diet, and a high carbohydrate/low protein diet both produced a weight loss of 7.3kgs in a 12 week period and this was associated with a decrease in fasting insulin (Noakes et al., 2005). The mechanism responsible for the diet induced increase in insulin sensitivity is proposed to be as follows. In the immediate response to reduced calorie intake and reduced glucose levels, the restricted individual starts to break down muscle glycogen stores. Over an extended period of time as the glycogen stores become depleted, the body

must rely on fat for energy and as a consequence of this, reduction in fat mass occurs (Barzilai and Gabriely, 2001). The liver is thought to up regulate the expression of enzymes involved in gluconeogenesis and suppresses the expression of the enzymes involved in glycolysis (Dhahbi et al., 1999). The liver also produces ketones from the breakdown of fat and protein, which help to meet energy demands and so blood glucose remains lower than baseline values during this phase (Greene et al., 2001). The beta cells of the pancreas sense lower glucose levels and produce less insulin. Insulin levels remain lower than that at baseline, thus improving insulin sensitivity (Dhahbi et al., 2001). The results of this study confirm that diet and exercise interventions are both important in the fight against obesity, but increasing physical activity in this population is likely to have a greater impact on metabolic health mediated by increased fitness and possibly better changes in body composition.

The alterations in adiposity that occur with calorie restriction and exercise training may have additional important benefits for obese individuals. In recent years, adipose tissue has been identified as an important endocrine organ that produces and secretes a number of adipokines involved in glucose and lipid metabolism (Jazet et al., 2003). Some of these adipokines, Omentin for example, promote insulin sensitivity and their circulating concentration is down regulated in obesity (de Souza Batista et al., 2007). Other adipokines, including FGF21 and Visfatin, enhance insulin sensitivity but their circulating concentration is elevated in obesity without the beneficial effects, possibly showing resistance to the action of the hormones. The overproduction of these hormones may be in compensation for the poor action of insulin (Fisher et al., 2010, Beltowski, 2006). There are a number of other adipokines that promote insulin resistance, such as Chemerin, whose circulating concentration increases with increases in fat mass (Catalan et al., 2011). Research shows that the serum concentration of these biomarkers is very much related to the physical and metabolic characteristics of obese individuals, and they are generally improved with improvements in body composition and metabolic status (Brix et al., 2010, Cuevas-Ramos et al., 2012, Moreno-Navarrete et al., 2010, Chakaroun et al., 2012, Choi et al., 2007). These cytokines in addition to IL-13 and Fetuin-A were analysed pre and post intervention in both groups. The reduction in fat mass that occurred in the exercise and diet group may have altered the expression and secretion of these biomarkers, which in turn may have influenced or been associated with the metabolic health of the subjects. It is

possible that the circulating concentration of these hormones could be a useful indicator of metabolic status in the obese population.

The lack of significant change in the biomarkers in either group post intervention may be due to the large variability that existed between subjects in the baseline concentrations of the hormones. The large variability in the individual response (Figure 3.9) to caloric restriction and exercise training may also be a factor. Many of these novel cytokines are produced in adipose tissue but they are also produced in other tissues throughout the body such as skeletal muscle and the liver, and so changes in fat mass may not be the only regulator of their circulating concentration. It may also be the case that the biomarkers are differentially regulated by diet or exercise.

The significant decrease in Fetuin-A in the exercise group is supported by some but not all studies in the literature. Some aerobic training studies have found no change in this hormone post intervention despite significant reductions in waist circumference and body fat content (Schultes et al., 2010). Other concurrent training studies incorporating aerobic and resistance exercise have also found no change in Fetuin-A post intervention (Yang et al., 2011). However, weight loss induced by a combination of diet and exercise resulted in a decrease in circulating Fetuin-A (Reinehr and Roth, 2008). Also, a study comparing young and older high and low active men reported that Fetuin-A was inversely related to fitness and concluded that regular exercise may be important in maintaining low levels of Fetuin-A (Jenkins et al., 2011). It is difficult to determine the mechanism behind the effects of exercise training on Fetuin-A. In the current study, the percent change in FFA's best predicted the percent change in Fetuin-A but this did not reach significance ($p=0.08$). The reduction in the circulating concentrations of this hormone is likely explained by the significant reduction in fat mass combined with the significant increase in fitness that occurred in this group, since an equal reduction in fat mass in the diet group had no effect on serum concentrations. The literature has reported that the circulating concentration of this insulin resistance promoting hormone is greater in obese individuals, particularly those with high liver fat content (Ix and Sharma, 2010). The liver fat content of the subjects in the present study was not measured but obesity is associated with excess fat in the liver (Tominaga et al., 1995), primarily because excess availability of fatty acids in addition to the presence of insulin resistance fuels the synthesis of triglycerides in the liver (Reinehr and Roth, 2008, Ix and Sharma, 2010). Excess liver fat has been proposed to stimulate the

production of Fetuin-A (Ix and Sharma, 2010). The results of this study suggest that exercise training reduced total fat mass, which may possibly also have reduced fat mass in the liver, and this in turn may have suppressed the production of Fetuin-A resulting in lower circulating concentrations of this hepatokine post intervention. The results also suggest that Fetuin-A may be an indicator of liver fat mass in obese individuals.

There was no significant change in the circulating concentration of the other biomarkers in the exercise group, but the changes that did occur provided an insight into the possible mechanisms by which they may be regulated, and their potential relationship with metabolic health. The exercise induced increase in lean tissue mass and aerobic fitness accounted for 93% of the variance in FGF21, 82.4% of the variance in IL-13, and 93.4% of the variance in Chemerin post intervention. These adaptations are specific to exercise training and show the potential role of exercise in the regulation of the production of these biomarkers. IL-13 is an anti-inflammatory insulin sensitizing cytokine that is secreted exclusively by skeletal muscle (Cannon and St Pierre, 1998). Its circulating concentration increased by 3% after exercise training, which was predicted by gains in lean tissue. This suggests that the exercise induced increase in lean tissue, although not significant, augmented the production of IL-13. Therefore, modes of exercise that promote greater gains in lean tissue, resistance training for example, may lead to more significant increases in the production of this hormone, which may in turn contribute to improved insulin sensitivity. In contrast, the circulating concentration of IL-13 decreased by 3% in the diet group, and this was mostly accounted for by the percent change in Visfatin, possibly demonstrating a regulatory role between both of these hormones. FGF21, an insulin sensitizing hormone, is also produced in skeletal muscle, in addition to the liver, adipose tissue and thymus (Zhang et al., 2008). Its circulating concentration increased by 28% in the exercise group compared to a 1% reduction in the diet group. The increase in fitness and lean tissue in the exercise group predicted the increase in FGF21. Exercise training that promotes greater increases in muscle mass may also stimulate greater production of this hormone. Chemerin is a proinflammatory cytokine predominantly secreted from adipose tissue, but is also produced in the lungs, liver and kidney (Goralski et al., 2007). Its circulating concentration decreased by 19% in the diet group which was significantly greater than the 3% reduction in the exercise group. Surprisingly no measured variable was found to predict the change in Chemerin in the diet group, whereas increased fitness accounted for the change in Chemerin in the exercise group. Many 12 week aerobic and /

or resistance training studies have reported a decrease in serum Chemerin post intervention, independent of change in body mass or body fat mass (Chakaroun et al., 2012, Venojarvi et al., 2013). This suggests that Chemerin is influenced by muscle contraction and not just changes in body weight and body fat. However, it is likely that the change in Chemerin in the diet group was partly due to the reductions in fat mass but must also be influenced by other variables since the reductions in fat mass was similar in both groups.

Visfatin is another insulin sensitizing hormone produced in skeletal muscle. It is also produced in the liver and macrophages but is predominantly released from visceral adipose tissue (Dogru et al., 2007). Controversy exists in the literature regarding Visfatin concentrations and its association with obesity and metabolic disease (Chang et al., 2011). Visfatin did not change in the exercise group but increased by 6% in the diet group post intervention. An overview of the current literature suggests that circulating concentrations of Visfatin should decrease post lifestyle intervention, thus ameliorating the resistance to the insulin sensitizing action of this hormone. This is true following 12 weeks of aerobic exercise training intervention (Choi et al., 2007, Haus et al., 2009, Lee et al., 2010b), and caloric restriction (Kovacikova et al., 2008, de Luis et al., 2008). In all of these studies the reduction in Visfatin was correlated with markers of insulin sensitivity independent of weight loss. Other interventions have found no relationship between fat mass and Visfatin but have found an inverse relationship between Visfatin and lean tissue mass suggesting that lean tissue could influence its circulating concentrations (Agueda et al., 2012). In fact 12 weeks of concurrent exercise training incorporating aerobic and resistance training (300kcal/day aerobic, and 100kcal/day resistance training / 60mins per day) significantly reduced circulating Visfatin (Choi et al., 2007). Lean tissue mass was not measured in the aforementioned study but it is known to increase with resistance training. In the present study, the percent change in Visfatin in the diet group was best predicted by the percent change in the myokine IL-13, which further supports the hypothesis that lean tissue mass might regulate the production of Visfatin. Thus exercise training that augments gains in lean tissue mass might also be associated with significant reductions in Visfatin.

The percent change in Omentin in both groups was influenced by the percent change in insulin sensitivity. Omentin is a cytokine secreted exclusively from visceral stromal vascular cells and is reported to enhance the action of insulin by augmenting

glucose uptake (Moreno-Navarrete et al., 2010). Interestingly its circulating concentration increased by 7% in the exercise group, which was best predicted by the percent change in the Stumvoll index of insulin sensitivity, but its circulating concentrations decreased by 12% in the diet group and this was predicted by the percent change in AUCI. The circulating concentration of Omentin is reduced in obese individuals compared to lean insulin sensitive individuals, and further reductions are evident in obese individuals with insulin resistance or type 2 diabetes (Akbarzadeh et al., 2012, An et al., 2012). Other 12 week aerobic exercise studies reported an increase in circulating Omentin post training, and this was significantly inversely correlated with improvements in insulin resistance, glucose tolerance, waist circumference and fitness (Saremi et al., 2010a). Dietary interventions using a caloric restriction of 500-1,000kcal per day less than RMR also reported a significant increase in Omentin post intervention and this was associated with a significant reduction in BMI in addition to a significant improvement in insulin sensitivity (Moreno-Navarrete et al., 2010). Since body weight and body fat mass improved to a similar degree in both groups, it appears that the additional improvements in the metabolic status of the subjects in the exercise group was associated with the increase in Omentin in this group.

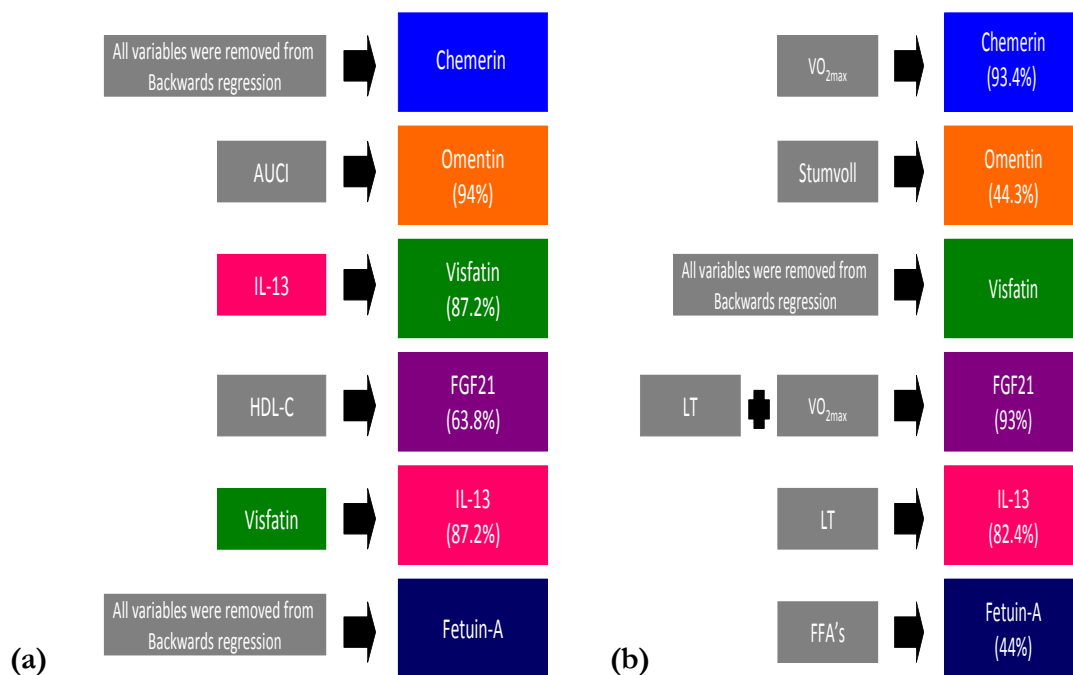


Figure 3.10 Schematic illustrating the percent change in the independent variables that best predict the percent change in the biomarkers post (a) diet intervention and (b) exercise intervention. Note: AUCI = Area Under the Insulin Curve, HDL-C = High Density Protein Cholesterol, LT = Lean Tissue, FFA's = Free Fatty Acids.

The response of the novel biomarkers to exercise training and caloric restriction is very interesting. Both interventions produced a similar energy deficit per week, but it appears that both modes of intervention differentially regulated the production and circulating concentration of the biomarkers. It is clear from the results that improvements in fitness, lean tissue mass, and insulin sensitivity accounted for the variance in many of the biomarkers which highlights the role of exercise training in the regulation of the biomarkers, and also suggests that greater improvements in lean tissue, such as that seen with resistance training, may lead to more substantial alterations in the circulating concentration of the biomarkers.

3. 13. Study limitations

The smaller than anticipated sample size in both groups limited the depth of the statistical analysis and meant that t-tests were used instead of the preferred option of a 2 way ANOVA. The small sample size for body composition limited the depth and interpretation of statistical analysis. A final limitation was the statistical difference in the baseline insulin values. Higher baseline insulin values in the exercise group may account for some of the metabolic differences between both groups, although the literature in addition to the research presented above consistently shows that both acute and chronic exercise improves insulin sensitivity in obese individuals.

3. 14. Conclusions and future recommendations

The literature suggests that both caloric restriction and exercise training are beneficial in reducing body weight and body fat mass in obese individuals (Franz et al., 2007). The extensive variation in the design of diet and exercise interventions makes it difficult to determine whether one intervention is superior to the other with regard to improving body composition and metabolic health of obese individuals. A comparison of an isocaloric diet and exercise intervention in this study revealed that both interventions were statistically equally successful in reducing body weight and body fat mass in these subjects. However, exercise training may lead to greater improvements in metabolic health mediated by increases in fitness and possibly greater improvements in body composition. It appears that exercise and diet interventions differentially regulate the circulating concentration of the novel biomarkers of insulin resistance. Interestingly, improvements in insulin sensitivity, fitness, and lean tissue mass significantly influenced the circulating concentration of many

of the biomarkers. It is possible that exercise training that significantly increases lean tissue mass, such as resistance training, may also significantly favourably affect the circulating concentration of the biomarkers. Future studies must investigate the effects of combined aerobic and resistance training on the metabolic health of these individuals. This type of training, also known as concurrent training, may provide all of the improvements in body composition, aerobic fitness, and insulin sensitivity evident in the aerobic group in the current study, but may additionally improve lean tissue mass, potentially resulting in a more substantial impact on the circulating concentrations of the biomarkers and improved metabolic health.

**Chapter 4. The impact of concurrent
exercise training incorporating an
eccentric component on energy
expenditure and metabolism in obese
individuals.**

4. 1. Rationale

Concurrent training has emerged in the literature as a potential weight loss therapy for obese individuals. A review of the acute and chronic effects of concurrent training in obese individuals concludes that this type of training enhances energy expenditure both during and after the training sessions, and also positively alters body composition in these individuals (Vilaca et al., 2011). This may contribute to greater weight loss in the obese population than either aerobic or resistance training alone (Vilaca et al., 2011).

The vast majority of the literature to date focuses on the combination of aerobic exercise and regular resistance training, which incorporates an eccentric and concentric component. However, a greater contribution of eccentric exercise may prove to be a novel way of augmenting energy expenditure and fat oxidation thus potentially augmenting loss of fat mass in these individuals. Eccentric exercise reduces cardiovascular stress and perceived exertion but can illicit the same rate of energy expenditure as concentric contraction, which may also improve adherence (Minetti et al., 2002). The muscle damage caused by eccentric exercise is quite extensive and is energy expensive during the repair period which may contribute to increased energy expenditure and increased metabolic rate in the hours and days post training (Welle and Nair, 1990). Resting metabolic rate has been shown to be elevated at 24 and 48 hours following a bout of eccentric resistance exercise, and this elevation appears to be greater in sedentary untrained individuals than in trained individuals (Dolezal et al., 2000). Also, following a bout of eccentric exercise the muscle adapts and becomes stronger and more resilient to damage (Friden et al., 1983). Over time this may allow the individual to perform more work and thus expend more energy in the same time frame thus expending more calories during training sessions. A small number of studies have been conducted to investigate the effects of concurrent training incorporating an eccentric resistance component on energy expenditure and fat oxidation, but these are mainly acute studies (Burt et al., 2013, Dolezal et al., 2000, Paschalis et al., 2010). There is little research available to show the effects of chronic eccentric training on energy expenditure and fat oxidation in obese individuals.

4. 2. Aim

The aim of this study was to investigate the effects of concurrent training on fat oxidation, energy expenditure and metabolic health in obese individuals, and to determine whether an eccentric component leads to augmented fat loss and health benefits in this population.

4. 3. Objective

- ❖ To measure the impact of eccentric exercise, as part of an aerobic and resistance training intervention, on body composition and insulin resistance in obese individuals.
- ❖ To identify a biomarker profile of concurrent exercise training.

4. 4. Hypothesis

Both interventions will result in distinctive physiological and biological profiles following 10 weeks of exercise training but eccentric exercise will confer more favourable changes resting metabolic rate and fat oxidation in obese subjects.

4. 5. Overview of Original Study Design

Subjects were recruited from staff and students in DCU. Following the initial screening and medical examination, eligible participants were randomly assigned to begin either a 10-12 week exercise training programme consisting of (i) concurrent exercise training intervention incorporating aerobic exercise and regular resistance training (REG) or (ii) aerobic exercise and eccentric resistance training (ECC). A number of tests were carried out at baseline and repeated after 12 weeks of training.

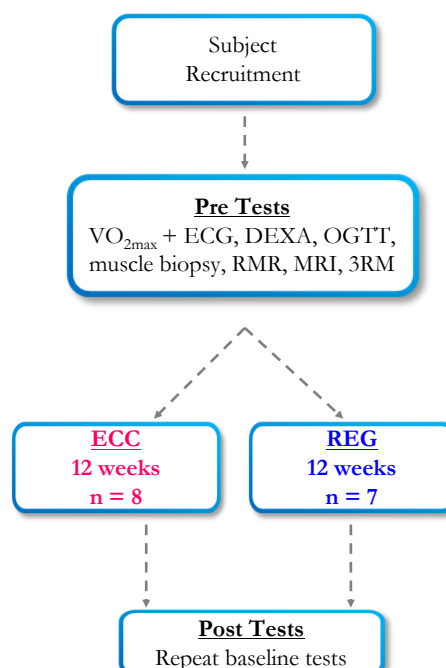


Figure 4.1 Overview of Original Study Design

4. 6. Test Procedures

Some of the tests that were carried out in this intervention were the same of those already outlined in chapter 3. Please refer to section 3.7.2 for details of anthropometric assessment, 3.7.3 for details of the DEXA scan and body composition assessment, 3.7.5 for details of the measurement and assessment of glucose tolerance and insulin sensitivity, 3.7.6 for details regarding the collection of blood samples, 3.7.7 for details of biochemical analysis and assays, and 3.7.8 for details regarding the muscle biopsy. Aerobic exercise prescription using the ACSM metabolic equations is detailed in section 3.7.9. Data collection and monitoring of the subjects exercise sessions is details in section 3.7.10. Additional tests were performed that were unique to this intervention are explained in detail to follow.

4. 6. 1. Subject Recruitment

An email was sent to all staff and students in DCU briefly outlining the nature of the study (Appendix 9). All individuals who responded to the email received a copy of the plain language statement (Appendix 10), which explained the study in great detail. A private meeting was then arranged with each potential participant and all elements of the study were discussed in detail including the nature of the study, the tests involved, the interventions involved, and all risks and benefits associated with the study. The informed consent form (Appendix 11) was explained to the potential participant at this time and they were also given an opportunity to ask any questions they had. Potential participants were advised to re read the informed consent form in their own time and return a signed copy if they wished to take part in the study.

Upon receiving the informed consent form, each participant then completed a health history questionnaire (Appendix 6), which was reviewed by the study physician, and they underwent a medical examination to confirm their suitability for the study. Subjects were excluded at this point if they had uncontrolled hypertension, unstable cardiovascular disease, hepatic or renal disease, active cancer, HIV infection, previous thyroid surgery or hyperthyroidism, chronic diarrhoea, were pregnant, or unable to stay in the same geographic location for the duration of the study.

Of the twenty five subjects who volunteered for the study, one subject was excluded after baseline screening due to the detection of T2DM during the OGTT and the detection of resting hypertension. This subject was referred to St. James Hospital for further tests. Two

subjects completed baseline testing in addition to 10 week exercise intervention but could not be contacted for the final testing period. Four subjects averaged 2 training sessions per week and so were excluded for non compliance. One subject moved to France in week 5 of the study. Finally, 2 subjects dropped out of the training study after 6 weeks due to work commitments. Of the remaining 15 subjects, 7 were randomly assigned to the 'ECC' training programme and 8 were randomly assigned to 'REG' training programme. The study was approved by the Dublin City University Research Ethics Committee and conformed to the Declaration of Helsinki.

4. 6. 2. $\dot{V}O_{2max}$ with Electrocardiogram (ECG)

For this intervention all subjects performed an incremental exercise test to volitional fatigue on a treadmill (HaB h/p/cosmos quasar) to determine $\dot{V}O_{2max}$. The data collection for this study occurred alongside the final phase of data collection for intervention 1, which was on going for two years, and so availability of equipment dictated that the treadmills be used for testing in this intervention. Both male and female subjects underwent a 3 minute incremental protocol, but the speed and grade for the warm up, as well as the speed and grade for the stage increments were greater for males than for females. Briefly all subjects started with a walking warm up of 6.5km/hr. Every 3 minutes the speed was increased by 1km/hr for all subjects until they reached their maximum walking speed. After this time the grade was increased by 2% every 3 minutes. If the subject continued to exercise for longer than 12 minutes, the grade remained the same but the speed was increased to jogging pace.

All exercise tests took place under standard laboratory conditions (19-21°C, 40-55% relative humidity) and in the presence of the study physician. Expired oxygen, carbon dioxide, ventilatory volume, respiratory exchange ratios and $\dot{V}O_{2max}$ were determined by indirect calorimetry the Sormedics metabolic system (Sormedics Vmax 229, Sormedics Corp., Yorba Linda CA). Systolic and diastolic blood pressure was measured by the physician at rest and during the last minute of each stage of exercise. Rate of perceived exertion was taken during the last ten seconds of each stage using the 6-20 point Borg scale (Appendix 8). Electrical activity of the heart was recorded at rest and at the end of each stage using 12 lead ECG. Heart rate was recorded in the last 10 seconds of each stage. Subjects exercised until reaching volitional fatigue. Oxygen uptake was deemed to have peaked if two or more of the following criteria were satisfied (i) plateau of oxygen

consumption with increasing power output (increase of less than 2 ml/kg/min), (ii) heart rate within 10 beats of the subjects' age predicted maximum heart rate ($220\text{bpm} - \text{age in years}$) and (iii) respiratory exchange ratio >1.10 . $\dot{V}O_{2\text{max}}$ was determined as the highest minute average recorded for oxygen uptake during the test.

4. 6. 3. Resting Metabolic Rate (RMR)

To determine energy expenditure and fat oxidation at rest, subjects underwent an RMR test in the human performance laboratory, DCU. Subjects reported to the laboratory after an overnight fast and they rested in a quiet, dark room for 60 minutes. During the last 30 minutes a metabolic hood was placed over the subjects head and $\dot{V}O_2$, ventilation, respiratory exchange ratio, fraction of inspired oxygen and carbondioxide, and fraction of expired oxygen and carbondioxide was measured by indirect calorimetry using the sensormedics metabolic system (Sensormedics Vmax 229, Sensormedics Corp., Yorba Linda CA). The hood was ventilated by 50 litres of air per minute. The subject was monitored throughout the test to ensure that steady state was reached for at least 15 minutes. RMR was calculated for each subject using the Weir equation (Weir, 1949). This test was conducted on the ECC group and the REG group only.



Figure 4.2 Resting metabolic rate procedure

Equation 11 The Weir equation to estimate energy expenditure per minute

$$((1.1 * RQ) + 3.9) * \dot{V}O \text{ (l/min)}$$

4. 6. 4. Magnetic Resonance Imaging (MRI) Scan

An MRI scan was used to determine any exercise induced change to the total cross sectional area of the thigh (total CSA), the total cross sectional area of fat in the thigh (fat CSA), and the total cross sectional area of muscle in the thigh (muscle CSA). This scan was carried out in Cappagh Hospital, Finglas, Dublin. All subjects were transported to and from the hospital by the study investigator. The subjects completed a screening form in the reception area which was revised by a radiological nurse prior to the scan. The subjects were then guided to a private room, instructed to remove clothing, metal piercings and jewellery, and wear a gown for the scan. The entire process took 20 minutes per subject. The output from the scan was an image of the cross sectional area of the thigh (Figure 4.4). Total CSA, fat CSA and muscle CSA of both thighs for all subjects pre and post intervention was manually calculated by the study investigator. These areas were traced individually using specialized image analysis software (Image J version 1.34) and the total area of each of these components was computed.

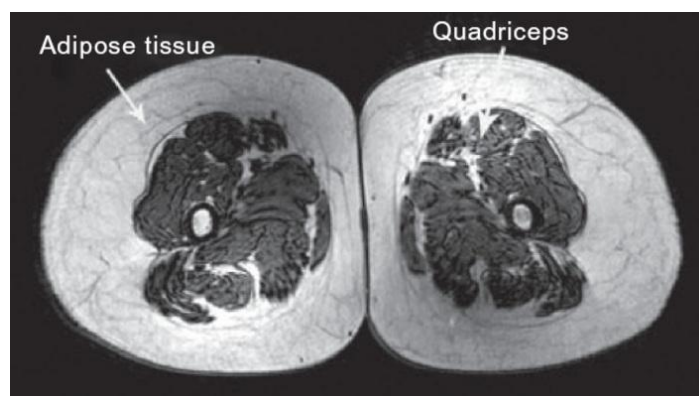


Figure 4.3 Sample image output from the MRI scan. The total CSA, fat CSA and muscle CSA was traced from the scan and calculated manually using Image J analysis software.

4. 6. 5. Protocol for 3 Repetition Maximum Testing (3RM)

1. Instruct the individual to warm up with a light resistance that easily allows 5-10 repetitions of the exercise.
2. Provide 1 minute rest.
3. Estimate a warm up load that allows the individual to complete 5-7 repetitions of the exercise.
4. Provide 2 minutes rest.

5. Estimate a near maximum load that allows the individual to complete 4-5 repetitions of the exercise.
6. Provide 2-4 minutes rest.
7. Increase the load to maximum
 - If the individual can lift >3 repetitions, repeat steps 6 and 7 until the 3RM is determined
 - Continue increasing or decreasing the load until the individual can complete only 3 repetitions with correct execution. Ideally this is achieved within 5 testing sets.
 - This procedure was conducted for the (1) Chest Press (Figure 4.6b) (2) Lat Pull Down (Figure 4.6c) and (3) Leg Extension (Figure 4.6a) exercises at baseline for all subjects in the REG and ECC groups.

4. 6. 6. Resistance Training Programme Design and Update

Once the 3RM was determined for all 3 exercises for all subjects, their 1RM was estimated using a conversion table (Figure 4.5). The 1RM is the preferred test to conduct but due to the subjects in this study being obese and untrained it was decided to perform the less aggressive 3RM test and use the conversion tables to convert their 3RM score to an estimated 1RM.

Once the 1RM's were determined, the ECC group completed 6 sets of 10 repetitions of all 3 exercises working at an intensity of 110% of their 1RM. The REG group completed 3 sets of 10 repetitions of all 3 exercises working at an intensity of 80% of their 1RM.

The 3RM test is very time consuming and was impractical to repeat with all subjects throughout the intervention. When the subjects adapted to training, which was evident by them being able to perform 12 repetitions of any exercise with appropriate technique, the load was increased by 2.5kgs. This process occurred continuously over the 10 week period in both groups to ensure continued adaptation and progression.

Table 18.8 Estimating 1RM and Training Loads													
Max reps (RM)	1	2	3	4	5	6	7	8	9	10	12	15	
%1RM	100	95	93	90	87	85	83	80	77	75	67	65	
Load (lb or kg)	10	10	9	9	9	9	8	8	8	8	7	7	
	20	19	19	18	17	17	17	16	15	15	13	13	
	30	29	28	27	26	26	25	24	23	23	20	20	
	40	38	37	36	35	34	33	32	31	30	27	26	
	50	48	47	45	44	43	42	40	39	38	34	33	
	60	57	56	54	52	51	50	48	46	45	40	39	
	70	67	65	63	61	60	58	56	54	53	47	46	
	80	76	74	72	70	68	66	64	62	60	54	52	
	90	86	84	81	78	77	75	72	69	68	60	59	
	100	95	93	90	87	85	83	80	77	75	67	65	
	110	105	102	99	96	94	91	88	85	83	74	72	
	120	114	112	108	104	102	100	96	92	90	80	78	
	130	124	121	117	113	111	108	104	100	98	87	85	
	140	133	130	126	122	119	116	112	108	105	94	91	
	150	143	140	135	131	128	125	120	116	113	101	98	
	160	152	149	144	139	136	133	128	123	120	107	104	
	170	162	158	153	148	145	141	136	131	128	114	111	
	180	171	167	162	157	153	149	144	139	135	121	117	
	190	181	177	171	165	162	158	152	146	143	127	124	
	200	190	186	180	174	170	166	160	154	150	134	130	
	210	200	195	189	183	179	174	168	162	158	141	137	
	220	209	205	198	191	187	183	176	169	165	147	143	
	230	219	214	207	200	196	191	184	177	173	154	150	
	240	228	223	216	209	204	199	192	185	180	161	156	
	250	238	233	225	218	213	208	200	193	188	168	163	
	260	247	242	234	226	221	206	208	200	195	174	169	
	270	257	251	243	235	230	224	216	208	203	181	176	
	280	266	260	252	244	238	232	224	216	210	188	182	
	290	276	270	261	252	247	241	232	223	218	194	189	

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Figure 4.4 This table was adapted from Baechle, T.R., and Earle, R.W. (2000). *Essentials of Strength Training and Conditioning*, Human Kinetics.

4. 6. 7. Spotter Protocol for the Eccentric Resistance Exercises

Two spotters were required to perform the concentric phase for all exercises for all subjects in the ECC group. In the leg extension exercise (Figure 4.6a) the spotters lifted the weight from the starting position (1) to the end position (2) and the subject lowered it slowly and in a controlled manner back to the starting position (1). In the chest press exercise (Figure 4.6b) the spotters pushed the weight forward from the starting position (2) until the subjects arms were fully expended (1) and the subject then lowered the weight back to the starting position (2) in a slow and controlled manner. For the lat pull down (Figure 4.6c), the spotters pulled the weight from the starting position (1) towards the ground until the subjects hands (and the bar) were in line with their chest (2). The subject then returned the weight to the starting position (1) in a slow and controlled manner.

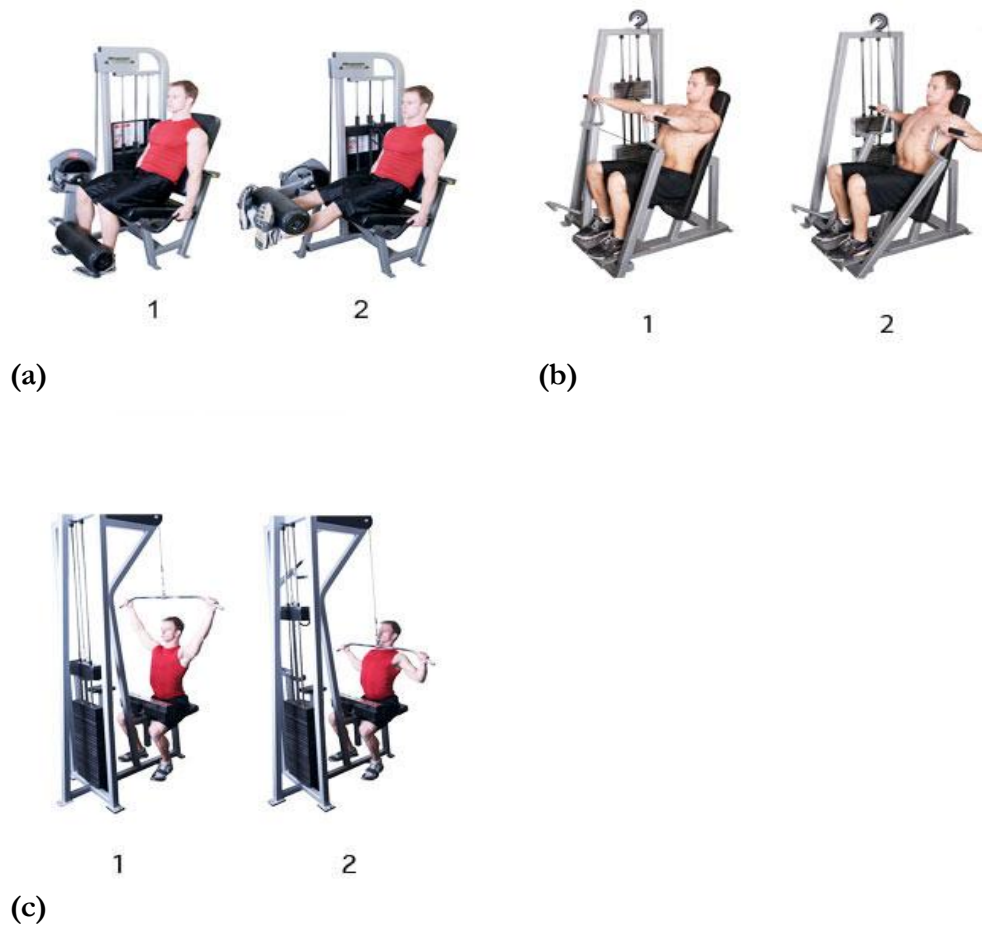


Figure 4.5 (a) The leg extension exercise, (b) the chest press exercise, and (c) the lat pull down exercise.

4. 6. 8. Pilot Investigations

In order to validate the methodology for this study, 4 weeks of pilot investigations were carried out. Four subjects consented to take part in a 4 week training programme. Each subject underwent a $\dot{V}O_{2\max}$ test at baseline test in addition to a 3RM test on the leg extension, lat pull down and chest press exercises. Two subjects completed 4 weeks of the ECC interventions detailed in section 4.7.17, and two subjects completed 4 weeks of the REG intervention detailed in section 4.7.18. The study investigator and another trained instructor completed the concentric phase of the leg extension, lat pull down and chest press exercise for the subjects in the ECC group.

4. 6. 9. Details of the ECC study design

The ECC study design is depicted in Figure 4.6. Eight subjects were randomly assigned to the ECC group. The pre tests included a $\dot{V}O_{2\max}$ test with ECG on day one. The subjects underwent an RMR test, a muscle biopsy, and an OGTT on day two. On a third day the subjects had their body composition analysed in the ExWell Medical Clinic, Ballymun, using a DEXA scan, and this was followed by a visit to Cappagh Hospital for an MRI scan of their thighs. The 3RM test was completed in the fitness centre in DCU as part of their first day of training.

All subjects completed 4 exercise sessions per week for 10 weeks, which were supervised by the study investigator. Two of the weekly sessions consisted of 60 minutes of aerobic exercise working at 70% $\dot{V}O_{2\max}$. The other two training sessions consisted of 15 minutes of aerobic exercise followed by 45 minutes of eccentric resistance training. In each of the resistance training sessions, the subjects completed 6 sets of 10 repetitions of the chest press, lat pull down and leg extension exercises, which totalled 180 repetitions per session, or 360 repetitions per week. Each subject was instructed on correct technique for the resistance exercises and monitored throughout their session. Two individuals (spotters) performed the concentric phase of the repetition (lift or push phase of the exercise), as outlined in Figure 4.6. Once this phase was complete the subjects assumed the correct position and returned the weight to the starting position in a slow and controlled manner. Sixty seconds recovery time was allowed between sets. Only 4 lifters / spotters were available for this study and so while one subject was resting between sets the spotters moved on to the next subject to begin their set. Tightly controlled scheduling of subjects and timing of sets was required in each session. The resistance element of the programme was continuously updated as progressed as outlined in section 4.7.14.

$\dot{V}O_{2\max}$ testing was repeated in week 5 for all subjects to determine their new fitness levels and update their training programmes using the ACSM's metabolic equations. This ensured that the subject exercised at the desired intensity for the duration of the intervention and that they continued to adapt accordingly. Body weight was also recorded for all subjects every week. The baseline tests were repeated in week 10.

4. 6. 10. REG Intervention Study Design

The study design for the regular (REG) exercise group is depicted in Figure 4.6. Seven subjects were randomly assigned to the REG group. The tests that were completed at baseline were the same as those completed in the ECC intervention. Two of the weekly sessions consisted of 60 minutes of aerobic exercise working at 70% $\dot{V}O_{2\max}$. The other two training sessions consisted of 45 minutes of aerobic exercise working at 70% $\dot{V}O_{2\max}$ followed by 15 minutes of regular resistance training incorporating a concentric and eccentric component.

The weight for the chest press, lat pull down and leg extension was set at 80% of their 1RM, which was estimated from their 3RM scores. In each session, the subjects completed 3 sets of 10 repetitions of the 3 exercises, which totalled 180 repetitions per session (counting concentric and eccentric movements), or 360 repetitions per week, which matched the repetitions performed by the ECC group. Each subject was instructed on correct technique for the resistance exercises and monitored throughout their session. The resistance was increased by 2.5kgs each time the subject could perform 12 repetitions of any exercise.

$\dot{V}O_{2\max}$ testing was repeated in week 5 for all subjects to determine their new fitness levels and update their training programmes using the ACSM's metabolic equations. This ensured that the subject exercised at the desired intensity for the duration of the intervention and that they continued to adapt accordingly. Body weight was also recorded for all subjects every week. The baseline tests were repeated on completion of the 10 week intervention.

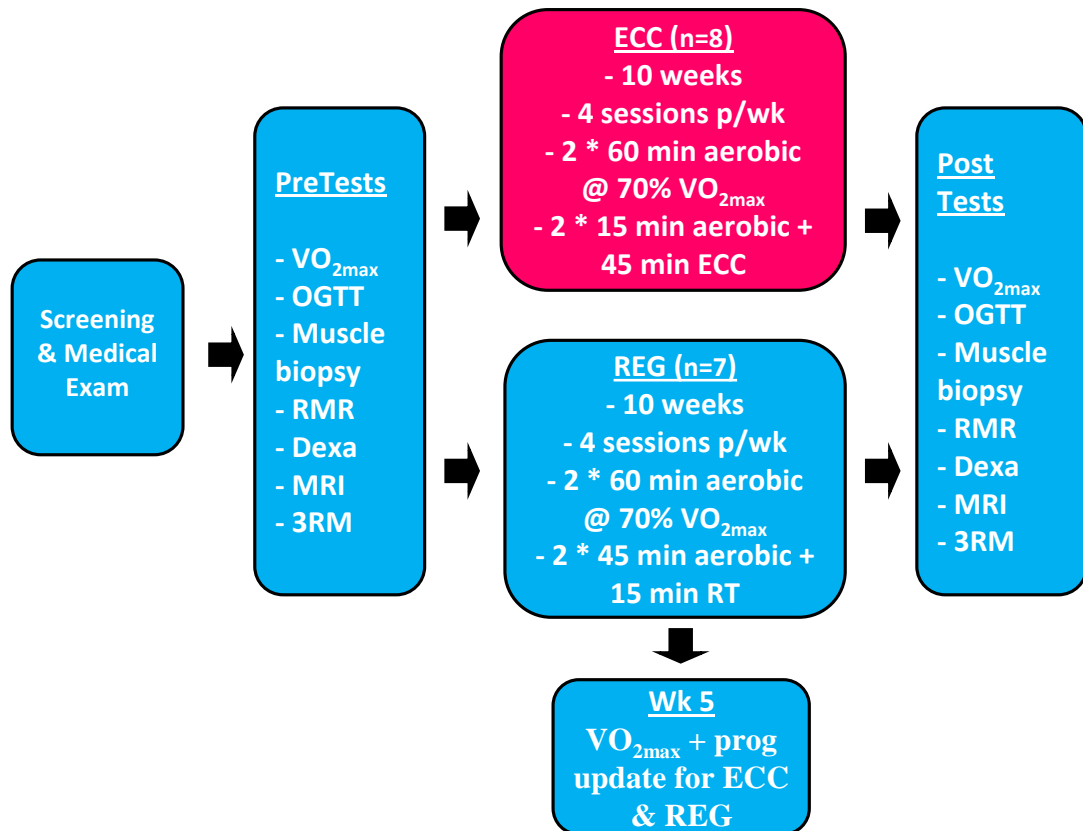


Figure 4.6 Schematic representation of the ECC and REG interventions

4. 7. Statistical Analyses

Experimental data is presented as mean \pm standard deviation. Data was evaluated using the Sigmaplot 12 statistical package (Systat Software, Inc. Chicago, IL). Statistical significance was set as $p \leq 0.05$. The statistical tests used to perform the analysis of the data in this chapter included a dependent t-test, an independent t-test, pearson correlation analysis and backwards stepwise regression. These tests are explained in detail in sections 3.10.1, 3.10.2, 3.10.3, and 3.10.4 respectively.

4. 8. Results

The data is analysed in a number of sequential steps. Firstly, a comparison of the physical and metabolic raw data was performed. Secondly, we studied a number of novel biomarkers that were identified as mainly, though not exclusively, secreted from adipose tissue to determine if the diet and exercise interventions differentially regulated their circulating concentration. Finally, physiological and biological changes associated with the diet and exercise interventions were identified using a linear regression analysis to establish

the relationship between changes in variables and then by backward stepwise regression analysis to identify those that best predicted the changes.

All subjects who took part in this study were obese at baseline and had been sedentary for a minimum of 6 months prior to enrolling in the study. The baseline values for the physical characteristics, metabolic characteristics, and biomarker profile are presented in Table 4.1, Table 4.2 and Table 4.3 respectively. Intervention groups were matched at baseline for physical and metabolic characteristics.

4. 8. 1. Physical Characteristics

The smaller than anticipated sample size limited the power of statistical analysis and meant a paired samples t-test was used to examine the extent of changes following the intervention rather than the preferred option of a 2-way ANOVA. Following both interventions there was a significant reduction in percent body fat ($p=0.05$ for ECC, $p=0.01$ for REG), and a significant increase in percent lean tissue ($p=0.05$ for ECC, $p=0.01$ for REG). Fat mass (kg) was significantly reduced in the REG group ($p=0.03$) but not in the ECC group ($p=0.11$). In contrast, lean tissue mass (kg) increased significantly in the ECC group ($p=0.04$) but not in the REG group ($p=0.09$). In all future analysis in this chapter, fat mass (kg) and lean tissue mass (kg) are used instead of percent body fat and percent lean tissue in order to avoid multicollinearity. Aerobic fitness ($\dot{V}O_{2\max}$ ml/kg/min) improved significantly in the ECC group ($p<0.001$) but not in the REG group ($p=0.26$). There was no change in weight or BMI in either group, but the REG group trended towards a change in both ($p=0.07$ for weight, $p=0.07$ for BMI). The weight loss achieved post intervention was 0.4kg in the ECC group compared to 3.3kg in the REG group. Fat mass was reduced by 2.2kg in the ECC group compared to 4.2kg in the REG group. An unpaired samples t-test confirmed that there was no difference between groups in the changes that occurred to any of the variables post intervention.

Muscle strength measured by the 3RM test increased significantly in both groups for all 3 resistance exercises. The strength gains achieved post intervention were significantly greater in the ECC group compared to the REG group for the lat pull down ($p=0.01$), chest press ($p<0.001$), and leg extension ($p<0.001$) exercises. An analysis of the MRI scans showed that the cross sectional area of fat in the thigh decreased significantly in the REG group ($p=0.03$) but not in the ECC group ($p=0.35$). The total cross sectional area of the thigh

also decreased significantly in the REG group ($p=0.05$) but not in the ECC group ($p=0.26$). There was no change in the cross sectional area of muscle in the thigh in either group ($p=0.74$ for ECC, $p=0.75$ for REG) post intervention.

Table 4.1 Pre and post values for the physical characteristics of the subjects in the REG and ECC groups. The percent change that occurred in each variable post intervention is also presented.

Variable	ECC		REG		% change	
	Pre	Post	Pre	Post	ECC	REG
	(n=7)		(n=8)			
Age (yr)	29 ± 10		36 ± 9			
Weight (kg)	83.3± 12.1	82.9 ± 12.9	97.7± 22.1	94.4±20.3 [‡]	-0.6 ± 2.8	-3.1 ± 3.8
BMI (kg/m ²)	30.0 ± 3.5	29.8 ± 4.1	33.7 ± 4.8	32.6 ± 4.4 [‡]	-0.6 ± 2.8	-3.1 ± 3.8
Fat mass (kg)	39.3 ± 11.6	37.1 ± 12.6	45.4 ± 15.3	41.2 ± 14.6*	-6.2 ± 8.1	-9.3 ± 8.4
% body fat	46.6 ± 8.3	44.0 ± 9.3*	46.0 ± 8.7	43.2 ± 9.4*	-5.8 ± 6.1	-6.5 ± 5.47
Lean tissue (kg)	41.4 ± 5.9	43.2 ± 6.8*	49.5 ± 12.8	50.4 ± 12.7 [‡]	4.2 ± 4.5	2.0 ± 3.2
% Lean Tissue	50.3 ± 8.1	52.9 ± 9.1*	51.0 ± 8.5	53.8 ± 9.2*	4.9 ± 5.8	5.4 ± 4.6
$\dot{V}O_{2max}$ (ml/kg/min)	31.8 ± 5.3	35.0 ± 5.9*	29.6 ± 6.3	31.6 ± 6.1	10.1 ± 5.5	9.0 ± 17.1
$\dot{V}O_{2max}$ (L/min)	2.7 ± 0.5	2.9 ± 0.5 *	2.9 ± 0.9	3.0 ± 0.9	8.7 ± 6.2	7.1 ± 11.4
3RM LPD (kgs)	51.8 ± 8.8	90.0± 12.4*	52.8 ± 17.7	62.8 ± 19.3*	75.1±14.5 [†]	19.8 ± 4.1
3RM CP (kgs)	40.4 ± 8.1	70.7± 11.7*	46.6 ± 19.3	56.9 ± 21.3*	77.8 ± 27.7 [†]	24.1 ± 7.7
3RM LE (kgs)	85.4 ± 22.2	124.3±20.1*	84.1 ± 34.9	96.9 ± 37.6*	51.2 ± 30.7 [†]	17.1 ± 9.2
Muscle CSA	7395 ± 1100	7352 ± 975	8813 ± 1443	8847 ± 1430	-0.2 ± 6.1	0.5 ± 4.7
Fat CSA	9598 ± 3651	9380 ± 3958	11005 ± 4229	10028 ± 4449 *	-3.0 ± 8.0	-9.6 ± 14.8
Thigh CSA	17350 ± 3275	17189 ± 3548	20297 ± 4393	19355 ± 4547 *	-1.7 ± 4.5	-4.7 ± 8.7

Data is presented as mean ± standard deviation. *p≤0.05 represents a significant change post intervention. [†]p<0.05 represents a significant difference between groups in the percent change that occurred to the variable post intervention. [‡]p≤0.09 represents a trend towards a change post intervention. Note: BMI = Body Mass Index, $\dot{V}O_{2max}$ = aerobic fitness, 3RM LPD = 3 Repetition Maximum on the Lat Pull Down, 3RM CP = 3 Repetition Maximum on the Chest Press, 3RM LE = 3 Repetition Maximum, Muscle CSA = Muscle Cross

Sectional Area, Fat CSA = Fat Cross Sectional Area, Thigh CSA = Total Cross Sectional Area of the Thigh.

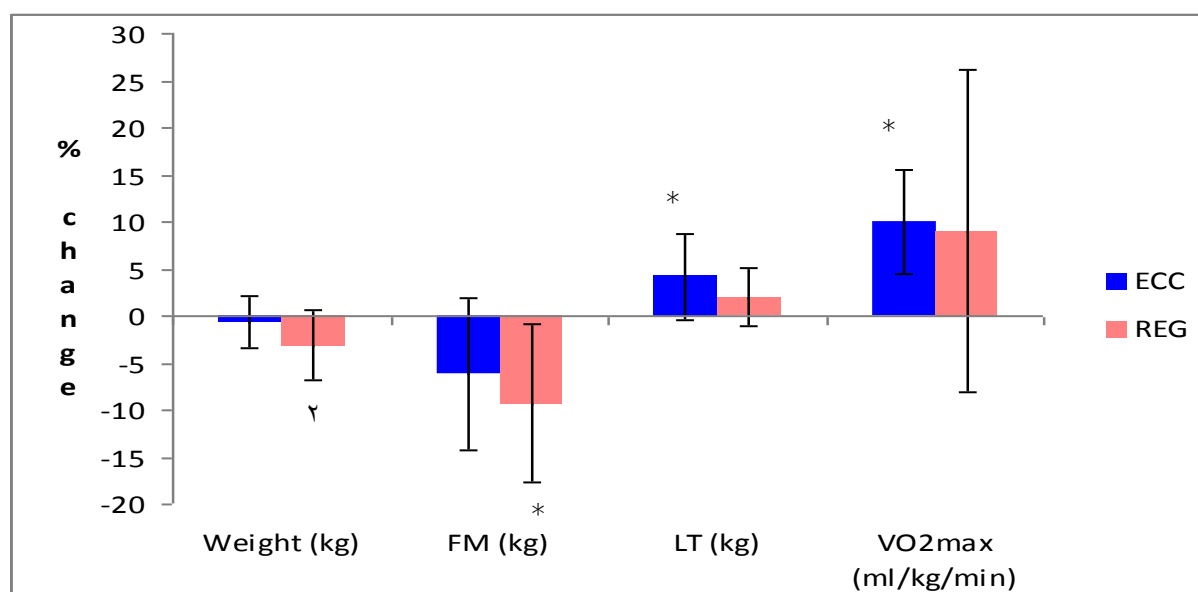


Figure 4.7 Percent change in weight (kg), fat mass (kg) and fitness ($\dot{V}O_{2max}$ ml/kg/min) post intervention presented as mean \pm SD. * $p \leq 0.05$ represents a significant change post intervention. † $p \leq 0.09$ represents a trend towards a significant change post intervention.

4. 8. 2. Metabolic characteristics

The metabolic adaptations to training are presented in Table 4.2 for both groups. There was no change in AUCG, AUCI, AUCG/AUCI or HOMA-B in either group post intervention. The Stumvoll and Matsuda indices of insulin sensitivity also did not change in either group, but the REG group experienced a 23% increase in the Matsuda predictor of insulin sensitivity compared to a 14% increase in the ECC group. There was no change in RMR in either group but it increased by 6.9% in the ECC group compared to 2.9% in the REG group. There was a significant increase in fat oxidation in both groups represented by a significant reduction in RQ ($p=0.01$ for ECC, $p<0.001$ for REG) post intervention.

The lipid profile of the subjects was also measured pre and post intervention. These values in addition to the percent change that occurred to the lipids are presented in Table 4.2. There was no significant change in the lipid profile of the subjects in the ECC or REG group post intervention. Also there was no difference between groups in the percent change that occurred to the metabolic variables.

Table 4.2 Pre and post values for the metabolic characteristics of the subjects in the ECC and REG groups. The percent change that occurred to each of the variables post intervention is also presented.

Variable	ECC		REG		% change	
	Pre	Post	Pre	Post	ECC	REG
	(n=7)		(n=8)			
AUCG	1081 ± 232	1052 ± 126	1089 ± 260	1033 ± 200	-0.4 ± 16.1	-3.3 ± 18.7
AUCI	10531 ± 6742	9541 ± 6698	8844 ± 4064	8101 ± 4229	-8.5 ± 14.4	-9.1 ± 23.5
AUCG/AUCI	0.14 ± 0.08	0.16 ± 0.11	0.17 ± 0.14	0.19 ± 0.18	10.5 ± 20.0	10.9 ± 24.8
HOMA-B	45.2 ± 2.7	40.2 ± 24.4	39.9 ± 30.8	28.6 ± 11.2	-6.4 ± 28.5	-8.9 ± 97.6
Stumvoll	0.128 ± 0.014	0.131 ± 0.018	0.116 ± 0.024	0.117 ± 0.022	3.7 ± 13.7	1.7 ± 11.4
Matsuda	18.1 ± 15.8	18.4 ± 12.7	20.3 ± 16.5	26.7 ± 32.6	14.3 ± 19.6	23.7 ± 52.8
RMR	1409 ± 133	1508 ± 211	1640 ± 433	1670 ± 401	7.0 ± 9.7	2.9 ± 8.9
RQ	0.89 ± 0.04	0.82 ± 0.02*	0.88 ± 0.06	0.79 ± 0.03*	-7.6 ± 5.3	-9.3 ± 5.2
FFA's	0.51 ± 0.23	0.47 ± 0.27	0.54 ± 0.10	0.50 ± 0.184	7.7 ± 77.9	-7.0 ± 32.3
TG's	1.04 ± 0.37	1.17 ± 0.49	1.31 ± 0.98	1.33 ± 0.96	25.5 ± 32.5	-11.9 ± 46.8
TC	4.99 ± 0.81	4.78 ± 1.10	4.91 ± 1.92	5.23 ± 0.95	4.4 ± 18.4	-8.9 ± 32.0
LDL	2.69 ± 0.33	2.52 ± 0.44	3.13 ± 1.71	3.22 ± 0.57	2.9 ± 12.2	26.1 ± 57.7
HDL	1.38 ± 0.38	1.55 ± 0.51	1.08 ± 0.24	1.30 ± 0.42	1.1 ± 15.7	30.3 ± 76.5

Data is presented as mean ± standard deviation. *p≤0.05 represents a significant change post intervention. Note: AUCG = Area Under the Glucose Curve, AUCI = Area Under the Insulin Curve, AUCG/AUCI = Area Under the Glucose Curve / Area Under the Insulin Curve (index of insulin sensitivity), HOMA-B is an index of fasting insulin secretion, Stumvoll and Matsuda are indexes of insulin sensitivity, RMR = Resting Metabolic Rate, RQ = Respiratory Quotient, FFA's = Free Fatty Acids, TG's = Triglycerides, TC = Total Cholesterol, LDL = Low Density Lipoprotein Cholesterol, HDL = High Density Lipoprotein Cholesterol.

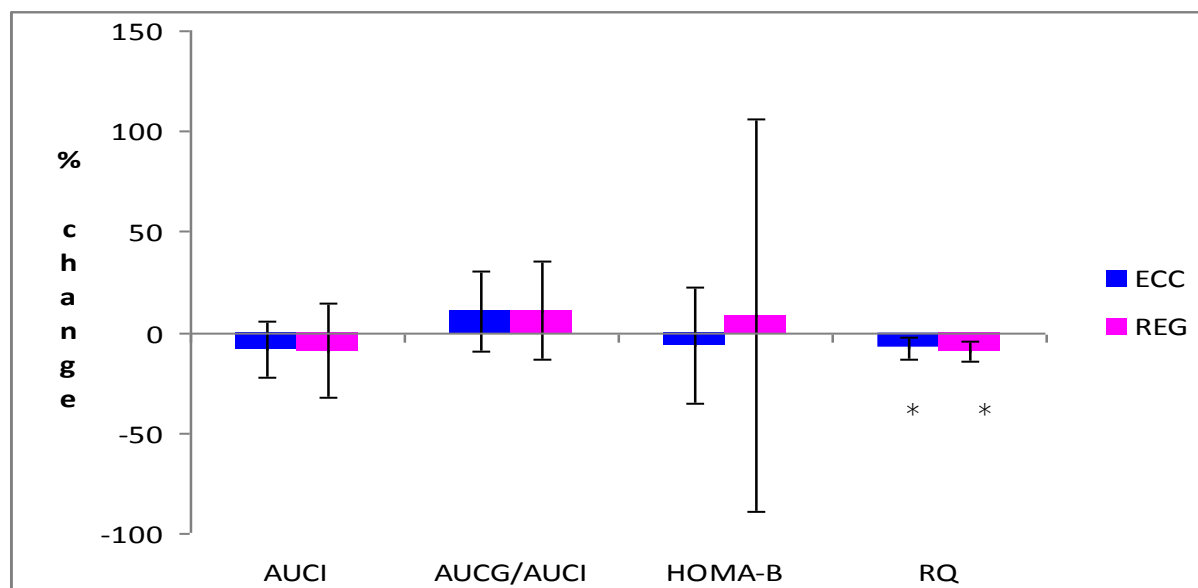


Figure 4.8 Percent change in the area under the insulin curve (AUCI), the area under the glucose curve / the area under the insulin curve (AUCG/AUCI), fasting insulin secretion (HOMA-B) and respiratory quotient (RQ) post intervention in the ECC and REG groups. Data is presented as mean percent change \pm standard deviation. * $p \leq 0.05$ represents a significant change post intervention.

4. 8. 3. Biomarker Profile

A number of novel biomarkers related to insulin resistance and metabolic health were measured pre and post intervention. The circulating concentrations of these biomarkers in addition to the percent change that occurred to the concentration of these biomarkers post intervention are presented in Table 4.3. The individual variation in the baseline values of the biomarkers was quite large and the same was true for the individual response to exercise training as presented in Table 4.3. Following the ECC intervention, there was a significant decrease in the circulating concentration of Visfatin ($p=0.05$). There was no change in circulating concentrations of FGF21 ($p=0.82$), IL-13 ($p=0.87$), Chemerin ($p=0.34$) or Omentin ($p=0.77$). There was no change in the circulating concentration of FGF21 ($p=0.38$), IL-13 ($p=0.95$), Chemerin ($p=0.76$), Omentin ($p=0.23$) or Visfatin ($p=0.64$) in the REG group post intervention. While the individual variation makes it difficult to determine if changes in these biomarkers have any role, there is some evidence of biomarker specific responses to different types of exercise training. Therefore, the next stage was to conduct correlation and regression analysis of the data to examine association between the variables.

Table 4.3 Pre and post values for the novel biomarkers in the ECC and REG groups. The percent changes that occurred to the biomarkers post intervention are also presented.

Variable	ECC		REG		% change	
	Pre	Post	Pre	Post	ECC	REG
	(n=7)		(n=8)			
FGF21	303.7 ± 129.7	324.9 ± 241.8	383.4 ± 108.3	326.4 ± 131.1	12.3 ± 68.1	-9.8 ± 39.6
IL-13	152.3 ± 36.6	154.9 ± 23.4	213.2 ± 175.4	214.1 ± 153.9	7.9 ± 30.8	5.2 ± 22.7
Chemerin	234.2 ± 35.2	196.2 ± 64.9	194.0 ± 53.1	183.3 ± 54.1	-11.4 ± 40.7	2.9 ± 41.6
Omentin	378.4 ± 183.5	397.0 ± 90.9	374.6 ± 153.0	442.1 ± 156.8	21.0 ± 55.9	25.7 ± 56.1
Visfatin	18.5 ± 4.5	16.8 ± 3.6 *	16.5 ± 3.3	15.9 ± 4.1	-8.1 ± 8.2	-2.9 ± 22.9

Data is presented as mean ± standard deviation. * $p \leq 0.05$ represents a significant change post intervention.

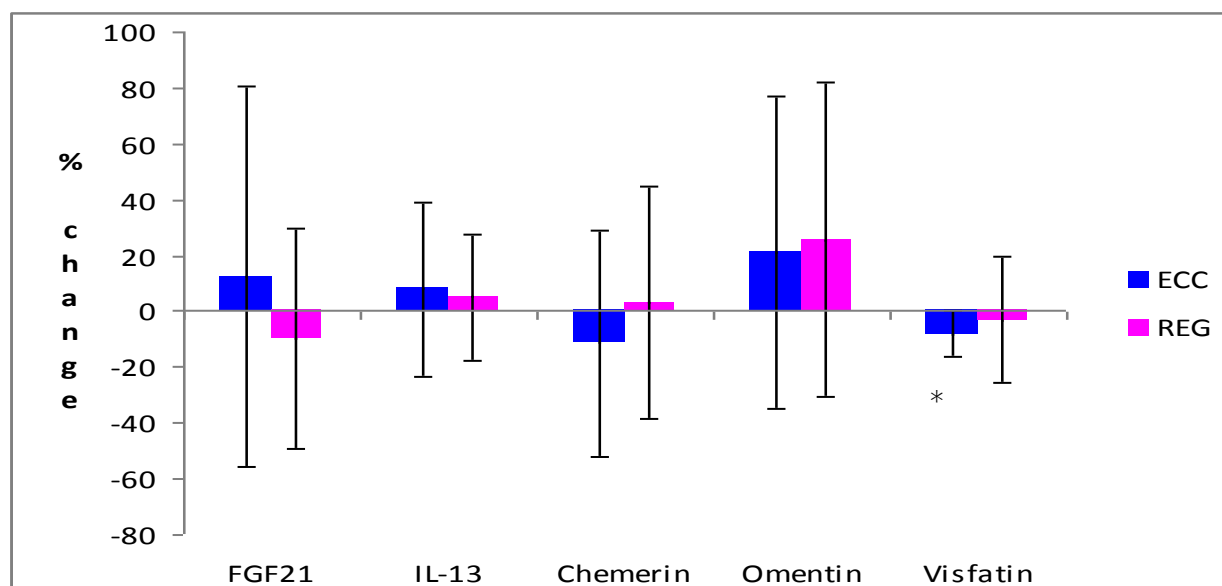


Figure 4.9 Percent change in the biomarkers post intervention in both groups. Data is presented as mean ± SD. * $p \leq 0.05$ represents a significant change post intervention.

4. 8. 4. Pearson correlations and backwards stepwise regression analysis

The pearson correlation analysis for the physical characteristics, as presented in Table 4.4, show that the percent change in weight in both groups did not relate to any of the metabolic adaptations, but was significantly correlated with the percent change in IL-13 and trending towards a correlation with the percent change in FGF21, Chemerin and LDL cholesterol in the ECC group. In the REG group, the percent change in weight was trending towards a correlation with the percent change in RMR.


On the other hand, the percent change in fat mass appeared to correlate better with metabolic adaptations in the REG group, which was positively related to the percent change in AUCI, in addition to the percent change in circulating Chemerin. The percent change in fat mass in the ECC group did not reach significance post intervention and was not correlated with the percent change in any other variable.

In the ECC group, the percent change in lean tissue was positively correlated with adaptations in insulin secretion and insulin sensitivity represented by AUCI. It was inversely correlated with the percent change in circulating Omentin. In addition, it was trending towards an inverse correlation with circulating FGF21. In the REG group, the percent change in lean tissue mass, although not significant, was positively correlated with strength gained in the chest press exercise, and trending towards a correlation with increased fat oxidation represented by a reduction in RQ.

The percent change in fitness following the ECC intervention was significantly positively correlated with the Matsuda indicator of insulin sensitivity and trending towards a correlation with AUCG. In the REG group, the non significant percent change in fitness, was significantly correlated with the percent change in circulating concentrations of IL-13.

Backwards stepwise regression was not run for the physical characteristics because all changes that occurred to body composition, fitness and strength were a direct result of the actual physical training in addition to the energy expended in the exercise sessions.

Table 4.4 Pearson correlations for weight, body composition and fitness in the ECC and REG group.

Variable	% change weight (kgs)		% change FM (kgs)		% change LT (kgs)		% change  O _{2max} (ml/kg/min)	
	ECC	REG	ECC	REG	ECC	REG	ECC	REG
% chg. RMR	r=0.723 [‡] p=0.07							
% chg. AUCG							r=0.801 [‡] p=0.06	
% chg. Matsuda							r=-0.99* p=0.001	
% chg. AUCI				r=0.755* p=0.05	r=0.827* p=0.04			
% chg. Omentin					r=-0.80* p=0.03			
% chg. FGF21		r=-0.68 [‡] p=0.06			r=-0.73 [‡] P=0.06			
% chg. Chemerin		r=0.681 [‡] p=0.09		r=0.702* p=0.05				
% chg. IL-13		r=0.823* p=0.01						r=- 0.73* p=0.01
% chg. LDL		r=0.721 [‡] p=0.07						
% chg. CP						r=0.781* p=0.02		
% chg. RQ						r=0.636 [‡] p=0.09		

r represents the strength of the relationship between two variables. *p≤0.05 is significant.

[‡]p≤0.09 is trending towards significance. Note: FM = Fat Mass, LT = Lean Tissue, RMR = Resting Metabolic Rate, AUCG = Area Under the Glucose Curve, AUCI = Area Under the Insulin Curve, FGF21 = Fibroblast Growth Factor 21, IL-13 = Interleukin-13, LDL = Low Density Lipoprotein Cholesterol, CP = Chest Press, RQ = Respiratory Quotient.

The pearson correlations for the percent change in AUCI, HOMA-B, RQ and RMR in both groups is presented in Table 4.5. The percent change in AUCI in the ECC group was significantly positively correlated with the percent change in lean tissue mass and significantly inversely correlated with the percent change in Visfatin. It was trending towards being positively correlated with Chemerin, and inversely correlated with FGF21. In contrast, the percent change in AUCI in the REG group was significantly correlated with the percent change in fat mass and the fat derived hormone Omentin.

The percent change in HOMA-B was positively correlated with the percent change in Chemerin in the ECC group, and with the percent change in lean tissue mass, RQ and strength gained in the chest press exercise in the REG group.

The percent change in RQ was correlated with metabolic adaptations in both groups. It was trending towards a correlation with the percent change in the Matsuda predictor of insulin sensitivity in the ECC group, and significantly correlated with the percent change in HOMA-B in the REG group. Additionally in the REG group, it was correlated with the percent change in RMR and inversely correlated with the percent change in serum Visfatin.

In the ECC group, the percent change in RMR was trending towards a correlation with body weight only. While in the REG group, the percent change in RMR was inversely correlated with the lipid profile of the subjects including total cholesterol, LDL cholesterol and HDL cholesterol, in addition to being positively correlated with fat oxidation or RQ. It was also positively correlated with the percent change in the Stumvoll index of insulin sensitivity, the percent change in the cross sectional area of muscle in the thigh. It was inversely correlated with the percent change in the circulating concentrations of Omentin and Visfatin.

Table 4.5 Pearson correlations for the percent change in AUCI, HOMA-B, RQ and RMR post intervention in the ECC and REG group.

Variable	% change AUCI		% change HOMA-B		% change RQ		% change RMR	
	ECC	REG	ECC	REG	ECC	REG	ECC	REG
% change weight							R=0.723 [†] P=0.07	
% change LT	r=0.827* p=0.04			r=0.850* p=0.01				
% change FGF21	r=-0.791 p=0.06 [†]							
% change Chemerin	r=0.773 [†] p=0.07		r=0.760* r=0.05					
% change Visfatin	r=-0.82* p=0.04					r=-0.65* p=0.01		r=-0.90* p=0.002
% change in BF		r=0.73* p=0.05						
% change Omentin		r=-0.77* p=0.03						r=-0.665 p=0.07 [†]
% change in RQ				r=0.856* p=0.01				r=0.712* p=0.04
% change CP				r=0.873* p=0.001				
% change RMR						r=0.712* p=0.05		
% change HOMA-B						r=0.856* p=0.01		
% change Matsuda					r=0.858 p=0.06 [†]			
% change Stumvoll								r=0.727* p=0.04
% change Muscle CSA								r=0.874* p=0.002
% change TC								r=-0.97* p=0.03
% change HDL								r=-0.95* p=0.05
% change								r=-0.97*

LDL								p=0.03
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r represents the strength of the relationship between two variables. * $p \leq 0.05$ is significant.

$\dagger p \leq 0.09$ is trending towards significance. Note: AUCI = Area Under the Curve for Insulin, HOMA-B = fasting insulin secretion, RQ = Respiratory Quotient, RMR = Resting Metabolic Rate, LT = Lean Tissue, BF = Body Fat, CP = Chest Press, Muscle CSA = Cross Sectional Area of Muscle, TC = Total Cholesterol, HDL = High Density Lipoprotein Cholesterol, LDL = Low Density Lipoprotein Cholesterol.

The backwards regression analysis for AUCI, HOMA-B, RQ and RMR in both groups is presented in Table 4.6. The results of this analysis showed that 99.9% of the variance in AUCI was predicted by the percent change in FGF21, Chemerin and Visfatin in the ECC group. In the REG group, 59% of the variance in AUCI was predicted by the percent change in Omentin.

Backwards stepwise regression was not run for HOMA-B in the ECC group as this was only correlated with one variable. Simple linear regression revealed that the percent change in Chemerin accounted for 57.8% of the percent change in HOMA-B in this group. In the REG group, the percent change in lean tissue and RQ accounted for 88.9% of the variance in HOMA-B post intervention.

Backwards stepwise regression was not run for RQ in the ECC group as this was only correlated with one variable. Simple linear regression revealed that the percent change in Matsuda accounted for 73.6% of the variance in RQ. In the REG group, the percent change in RMR and HOMA-B accounted for 94% of the variance in RQ.

In the ECC group, the percent change in strength gained in the chest press predicted 85.5% of the variance in RMR post intervention. In the REG group, the percent change in the cross sectional area of muscle in the thigh, in addition to the percent change in Omentin, predicted 90.5% of the change in RMR post intervention.

Table 4.6 Backwards stepwise regression analysis to predict the change in AUCI, AUCG/AUCI, and HOMA-B post exercise intervention.

Study	Dependant variable	Indep. variables in model	Coef.	Std. Coeff.	Std. Error	F-to-Remove	P
% change AUCI	ECC (R ² =0.999)	Constant	-13.380		0.403		
		% chg. FGF21	-0.073	-0.358	0.005	213.25	0.005
		% chg. Chemerin	0.162	0.435	0.008	370.03	0.003
		% chg. Visfatin	-0.886	-0.462	0.046	371.50	0.003
	REG (R ² =0.592)	Constant	-0.769		6.390		
		% chg. Omentin	-0.323	-0.769	0.109	8.703	0.026
% change HOMA-B	ECC	Note: Backwards regression could not be performed for HOMA-B in the ECC group. Simple linear regression revealed that the percent change in Chemerin accounted for 57.8% of the variance in HOMA-B post intervention					
	REG (R ² =0.889)	Constant	69.683		44.63		
		% change LT	15.867	0.514	5.948	7.117	0.044
		% change RQ	9.982	0.529	3.636	7.538	0.041
% change RQ	ECC	Note: Backwards regression could not be performed for RQ in the ECC group. Simple linear regression revealed that the percent change in Matsuda accounted for 73.6% of the variance in RQ post intervention					
	REG (R ² =0.940)	Constant	-10.448		0.560		
		%change RMR	0.297	0.483	0.067	17.390	0.09
		% change HOMA-B	0.037	0.697	0.006	36.196	0.002
% change RMR	ECC (R ² =0.855)	Constant	32.055		4.868		
		% change Chest press	-0.323	-0.925	0.059	29.492	0.003
	REG (R ² =0.905)	Constant	3.886		1.332		
		% change Omentin	-0.064	-0.402	0.023	7.398	0.042
		% change Muscle CSA	1.395	0.729	0.283	24.303	0.004

R² represents the extent to which the change in the independent variable predicted the change in the dependent variable. p≤0.05 is significant. Note: AUCI = Area Under the Curve for Insulin, HOMA-B = fasting insulin secretion, RQ = Respiratory Quotient, LT = Lean Tissue, RMR = Resting Metabolic Rate, HOMA-B = Fasting Insulin Secretion, Muscle CSA = Cross Sectional Area of Muscle.

The pearson correlations for the percent change in the biomarkers post intervention in both groups are presented in Table 4.7.

Table 4.7 Pearson correlations for selected biomarkers for the ECC and REG groups

Variable	Group	Correlations with the % change in Independent Variables		
% change in FGF21	ECC	LT ($r=-0.730$, $p=0.06$) †	Omentin ($r=0.805$, $p=0.03$) *	AUCI ($r=-0.791$, $p=0.06$) †
	REG	Weight ($r=-0.682$, $p=0.06$) †	Stumvoll ($r=0.731$, $p=0.04$) *	
% change in IL-13	ECC	Chemerin ($r=-0.821$, $p=0.05$) *	TG's ($r=-0.961$, $p=0.04$) *	
	REG	Weight ($r=0.823$, $p=0.01$) *	$\dot{V}O_{2max}$ (ml/kg/min) ($r=-0.730$, $p=0.04$) *	Matsuda ($r=-0.707$, $p=0.05$) *
% change in Chemerin	ECC	IL-13 ($r=-0.821$, $p=0.05$) *	HOMA-B ($r=0.760$, $p=0.05$) *	AUCI ($r=0.773$, $p=0.07$) †
	REG	Fat Mass ($r=0.755$, $p=0.05$) *	LDL ($r=0.855$, $p=0.03$) *	
% change in Omentin	ECC	Lean Tissue ($r=-0.802$, $p=0.03$) *	FGF21 ($r=0.804$, $p=0.03$) *	Lat Pull Down ($r=-0.777$, $p=0.04$) *
	REG	AUCG ($r=-0.749$, $p=0.05$) *	RMR ($r=-0.665$, $p=0.07$) †	
% change in Visfatin	ECC	AUCI ($r=-0.822$, $p=0.05$) *	LDL ($r=0.927$, $p=0.07$) †	
	REG	RQ ($r=-0.858$, $p=0.01$) *	RMR ($r=-0.904$, $p=0.002$) *	Muscle CSA ($r=-0.790$, $p=0.02$) *

r represents the strength of the correlation between variables. * $p \leq 0.05$ is significant.

† $p \leq 0.09$ is trending towards significance. Note: LT = Lean Tissue, AUCI = Area Under the

Curve for Insulin, TG = Triglycerides, $\dot{V}O_{2max}$ = aerobic fitness, HOMA-B = Fasting Insulin Secretion, LDL = Low Density Lipoprotein Cholesterol, AUCG = Area Under the Curve for Glucose, RMR = Resting Metabolic Rate, RQ = Respiratory Quotient, Muscle CSA = Cross Sectional Area of Muscle.

The backwards regression analysis for the biomarkers is presented in Table 4.8. This analysis revealed that the percent change in FGF21 was mostly predicted by the percent change in Omentin in the ECC group, which accounted for 70% of the variance in FGF21. In the REG group the percent change in the Stumvoll predictor of insulin sensitivity accounted for 53.4% of the variance in FGF21 post intervention.

The percent change in IL-13 was mostly predicted by the percent change in TG's in the ECC group, which accounted for 92.4% of the variance in IL-13. The percent change in weight and fitness accounted for 86.3% of the variance in IL-13 in the REG group.

In the ECC group, the percent change in IL-13 predicted 67.4% of the percent change in Chemerin, while the percent change in LDL cholesterol accounted for 73% of the variance in Chemerin in the REG group.

The best single predictor of the percent change in Omentin in the ECC group was the percent change in FGF21, which accounted for 64.6% of the variance in Omentin. The percent change in AUCG accounted for 56.1% of the variance in Omentin in the REG group.

The percent change in RQ and RMR predicted 91% of the variance in Visfatin in the ECC group. The percent change in AUCI accounted for 67.6% of the variance in Visfatin in the REG group.

Table 4.8 Backwards stepwise regression analysis to predict the change in the biomarkers post ECC intervention and REG intervention.

Study	Dependant variable	Indep. variables in model	Coef.	Std. Coeff.	Std. Error	F-to-Remove	P
% change in FGF21	ECC (R ² =0.702)	Constant	-15.667		18.473		
		% change Omentin	0.958	0.838	0.312	9.424	0.037
	REG (R ² =0.534)	Constant	-14.196		10.443		
		% change Stumvoll	2.546	0.731	0.970	6.886	0.039
% change in IL-13	ECC (R ² =0.924)	Constant	39.371		7.350		
		% change in TG's	-0.957	-0.961	0.194	24.401	0.039
	REG (R ² =0.863)	Constant	22.349		4.713		
		% change weight	3.724	0.629	1.074	12.021	0.018
		% change fitness	-0.627	-0.472	0.241	6.772	0.048
% change in Chemerin	ECC (R ² =0.674)	Constant	1.142		11.752		
		% change in IL-13	-1.158	-0.821	0.402	8.289	0.045
	REG (R ² =0.730)	Constant	-17.245		12.435		
		%change LDL	0.638	0.855	0.194	10.830	0.030
% change in Omentin	ECC (R ² =0.646)	Constant	12.895		14.022		
		% change in FGF21	0.659	0.804	0.218	9.129	0.029
	REG (R ² =0.561)	Constant	22.457		16.473		
		% change in AUCG	-2.367	-0.749	0.937	6.381	0.053
% change in Visfatin	ECC (R ² =0.676)	Constant	-10.066		2.311		
		% change in AUCI	-0.429	-0.822	0.148	8.365	0.044
	REG (R ² =0.910)	Constant	-16.451		9.375		
		% change in RQ	-1.927	-0.435	0.844	5.208	0.071
		% change in RMR	-1.520	-0.594	0.488	9.701	0.026

R² represents the extent to which the change in the independent variable predicts the change in the dependent variable. p≤0.05 is significant. Note: TG's = Triglycerides, IL-13 = Interleukin-13, LDL = Lipoprotein Cholesterol, AUCG = Area Under the Curve for Insulin, AUCG = Area Under the Curve for Insulin, RQ = Respiratory Quotient, RMR = Resting Metabolic Rate.

4. 9. Discussion

The main findings of this study were that the changes that occurred to body composition were highly specific to the mode of exercise undertaken, and not simply due to total energy expenditure which was matched in both interventions. The ECC group demonstrated significant gains in fitness and lean tissue mass, while the REG group displayed significant reductions in fat mass. There was no change in insulin secretion or insulin sensitivity in either group post intervention, however both groups demonstrated a significantly greater capacity to oxidise fat post intervention, which has important implications for insulin sensitivity and possibly augmented reductions in fat mass in the long term in this population. Muscle strength improved significantly in both groups which confer numerous health benefits for these subjects, and the strength gains were significantly greater in the ECC group compared to the REG group for all resistance exercises. There was no significant change in RMR post intervention, but the modest increase that occurred in the ECC group may still have important benefits for longer term weight loss. Finally, there was no change in the circulating concentrations of the novel biomarkers of insulin resistance in either group, with the exception of Visfatin which decreased significantly in the ECC group post intervention. In this study it appears that the production of the biomarkers is differentially influenced by the mode of exercise training. The biomarkers themselves appear to be inter-dependent, and markers of metabolic health are also associated with the changes in the biomarkers. The biomarkers may be a useful indicator of metabolic health in larger scale studies.

Concurrent training has emerged in the literature as an interesting therapy for obese individuals. A review of the acute and chronic effects of concurrent training in obese individuals concludes that this type of training enhances energy expenditure both during and after the training sessions, and also positively alters body composition in these individuals (Vilaca et al., 2011). This may contribute to greater weight loss in the obese population than either aerobic or resistance training alone (Vilaca et al., 2011). Concurrent training incorporating an eccentric component may augment energy expenditure and fat oxidation in obese individuals and thus may potentially lead to greater loss of fat mass and greater improvements in metabolic health (Burt et al., 2013, Dolezal et al., 2000, Paschalis et al., 2010). Many of the studies incorporating eccentric exercise are acute bouts, little is known about the effects of chronic eccentric exercise training.

In the present study, the changes that occurred to the physical characteristics of the subjects post intervention appeared to be highly influenced by the training mode undertaken. The 3RM scores for the lat pull down, chest press, and leg extension resistance exercises increased significantly in both groups post intervention. These results were expected and indicate that the subjects responded as expected to the training stimulus (Guilhem et al., 2010, Reeves et al., 2009, Roig et al., 2009). This increase in strength is clinically important because it confers numerous health benefits for these individuals. It is proven to contribute to the maintenance of functional ability that would otherwise decline with age, prevent osteoporosis by increasing bone density, prevent sarcopenia or muscle wasting that is also evident with age, and improve lower back pain among other age and inactivity related disabilities (Winett and Carpinelli, 2001).

For many years the strength gains that accompany resistance training were mostly attributed to hypertrophy, or increased size of the trained muscles. While there is a relationship between muscle size and muscle strength, this theory does not explain the increased strength that is evident with resistance training in the absence of hypertrophy. Research shows that the neuromuscular system is very responsive to resistance training. The largest strength gains occur within the first 8-10 weeks of training and for the most part these gains are attributed to neural adaptations (Staron et al., 1994). Longer term strength gains occur as a result of adaptations to the physical structure of the muscle including hypertrophy (Staron et al., 1994). The neural adaptations to resistance training are numerous and include improved synchronization of motor units, which improves the rate of force development and the capability of the muscle to exert steady forces (Duchateau and Enoka, 2002). There may be increased recruitment of additional motor units, which would also lead to greater force production (Duchateau and Enoka, 2002). An increase in the frequency of stimulation of the muscle fibers has also been observed, which ultimately results in a state of tetanus and peak force development in the muscle fibers (Enoka, 1997). Other adaptations include reduced autogenic inhibition allowing the working muscles to produce greater amounts of force and reach greater levels of strength before they are inhibited (Aagaard et al., 2000). Finally, a reduction in coactivation of the antagonist muscles have been observed allowing for greater strength generation in the agonist or working muscles (Enoka, 1997). These neural adaptations explain the increase in strength in both groups, even in the absence of a significant increase in lean tissue mass in the REG group.

The literature reports that strength can increase by as much as 25-100% above baseline values in untrained subjects. The average increase in the 3RM scores in the ECC group was 68.1%, which was significantly greater than 20.3% observed in the REG group. The increase in strength in the ECC group was accompanied by a significant increase in lean tissue mass post intervention. This indicates that hypertrophy occurred in the ECC group in addition to the neuromuscular adaptations, possibly accounting for the magnitude of the change in strength in this group.

The eccentric component of resistance training is reported to be the most important factor responsible for the increase in the cross sectional area of muscle following training (Shepstone et al., 2005). A number of studies have reported greater hypertrophy with eccentric training compared to concentric training or combination training (Shepstone et al., 2005). The results are augmented further when the eccentric movements are performed with high velocity (Shepstone et al., 2005). Eccentric exercise is thought to stimulate hypertrophy in the muscles being used because it causes extensive damage to these muscles compared to concentric actions, and muscle damage is the precursor to hypertrophy. The muscle damage creates muscle soreness which peaks at 24-48 hours after the eccentric exercise bout and this is termed delayed onset muscle soreness or DOMS (Miles and Clarkson, 1994). In 1984, Armstrong proposed the following sequence of DOMS. Eccentric exercises causes high tension in the contractile-elastic system of skeletal muscle and results in damage to the physical structure of the muscle, and its cell membrane. This damage disturbs the calcium homeostasis in the injured fiber(s) leading to cell death that peaks at 48 hours post exercise. The products of macrophage activity and intracellular contents e.g. histamines, kinnis, and K^+ , accumulate outside the cell. These substances stimulate free nerve endings in the muscle causing pain. Muscle strength is initially reduced in injured muscle fibers. This may be due to physical disruption of the muscle, failure of the excitation-contraction coupling process, and loss of contractile protein. In the days following an eccentric bout the muscle adapts, become stronger and more resilient to further damage (Warren et al., 2001).

The increase in strength and size following eccentric training may have important implications for the obese population. The ability to lift a greater amount of weight over time means that work rate, and thus energy expenditure, increases per session over time.

The increase in lean tissue may also increase resting metabolic rate (Dolezal and Potteiger, 1998), which accounts for 60-75% of total daily energy expenditure, thus a small increase in RMR can have a substantial effect on a persons total daily energy expenditure (Broeder et al., 1992). These adaptations may accumulate to greater energy expenditure and weight loss in obese individuals in the long term, above that induced by regular concurrent training. Resistance training is also proposed to augment fat oxidation. This is attributed to increased energy expenditure and improved body composition, in addition to enhanced lipolysis in abdominal subcutaneous adipose tissue (Ormsbee et al., 2007). Since there is a causative relationship between fat accumulation and insulin resistance (Petersen and Shulman, 2002, Shulman, 2000, Yu et al., 2002), increased fat oxidation may have significant implications for improving insulin sensitivity and metabolic health in obese individuals.

There was no statistically significant change in RMR in either group post intervention in the present study. Lean tissue mass increased significantly in the ECC group, but it may not have been sufficient to stimulate a significant elevation in RMR. There was an increase in percent lean tissue in the REG group but no significant change in lean tissue mass which might explain the lack of change in RMR in this group. Is it possible that an interference effect occurred between the aerobic training and the strength training in the REG group. The larger volume of aerobic training in the REG intervention may have negated the gains in lean tissue that generally result from resistance training. Babcock et al. (2012), reported that the physiological environment created by endurance training appears to negatively affect the satellite cells usual response to resistance training, ultimately compromising muscle fiber growth post training (Babcock et al., 2012). The exact mechanism behind this interference effect is unclear and research continues to be carried out in this area. This finding is supported by the fact that many studies investigating the effects of endurance training on RMR have reported no change post intervention (Tagliaferro et al., 1986, Bingham et al., 1989, Tremblay et al., 1990). The sequence of training may also have affected muscle growth in the REG group. In the present study and on 2 days per week, 45 minutes of aerobic exercise was performed first, followed by 15 minutes of resistance training. Cadore et al. (2012) suggest that strength training immediately prior to endurance training has no influence on maximal endurance power adaptation, but aerobic exercise immediately prior to strength training reduces the force that can be produced per unit of muscle mass thus influencing the quality of the training

session and the magnitude of adaptation that could potentially occur from resistance training (Cadore et al., 2012). It is possible that a greater volume of resistance training is required as part of a concurrent training programme. The REG intervention in the current study incorporated only two 15 minutes bouts of resistance training per week. A more intense resistance training component may be necessary to ensure that participants obtain the reported benefits of fat loss that are associated with aerobic exercise and resistance training, in addition to the increases in metabolically active lean tissue and RMR that are associated with resistance training (Byrne and Wilmore, 2001).

Although RMR did not change significantly in either group post intervention, it is clinically important to note that RMR increased by an average of 100kcal per day in the ECC group. Hill et al (2003) estimated that an “energy gap” of only 100kcal per day may over time be responsible for the weight gain in 90% of the American population. Therefore, a reduction or elimination of this energy gap could prevent or reduce body fat gain in the majority of American people (Hill et al., 2003). An increase in energy expenditure of 100kcal per day in the subjects in the present study equates to 700kcal per week or approximately 1lb (3500kcal) of body fat in 35 days. Over time, and with continued training, this could accumulate to significant fat loss in this population. The small numbers in this study may mask the implications of these findings.

Importantly, there was a significant decrease in RQ in both groups post intervention confirming that the subjects had a greater ability to oxidise fat for fuel after a period of exercise training. This finding has also been reported in the literature even after an acute bout of resistance training (Ormsbee et al., 2007). This is a key finding with clinical significance because there is substantial evidence to show that elevated concentrations of intracellular lipids in obese individuals contribute to the development of insulin resistance by inhibiting insulin stimulated glucose transport into target tissues (Petersen and Shulman, 2002, Shulman, 2000, Yu et al., 2002). The increase in fat oxidation may be due in part to a training induced conversion of type IIx muscle fibers to type IIa muscle fibers which have greater oxidative properties (Staron et al., 1990). It may also be due to the adaptations that occur in response to the aerobic endurance training that formed part of each intervention. Endurance training is known to increase the number and function of type I fibers in skeletal muscle, which have a high oxidative capacity and a greater ability to oxidise fat for fuel (Costill et al., 1976). This is particularly evident in

highly trained endurance athletes, who paradoxically have elevated intra muscular lipid content, but their skeletal muscle is markedly insulin sensitive, has a very high oxidative capacity and thus a high fat turnover (Goodpaster et al., 2001). Given the adverse relationship between fat accumulation and insulin resistance in obese sedentary individuals, an increased ability to oxidise fat may be important in the prevention or amelioration of insulin resistance in the obese population. It may also contribute to greater loss of fat mass over time.

Surprisingly there was no change in body weight or BMI in either group post intervention. Subjects were instructed to maintain their pre intervention energy intake for the duration of the study. If this was achieved then the energy expended in the exercise sessions should have been sufficient to cause a reduction in fat mass and body weight in this previously sedentary group, as it did in the aerobic exercise group in chapter 3 of this thesis. In the present study, weight loss may have been offset by gains in lean tissue that were acquired with resistance training. This may be particularly true for the ECC group who experienced a significant increase in percent lean tissue and lean tissue mass. This group also achieved a significant reduction in percent body fat and a non statistically significant decrease in fat mass. This finding is supported by other studies (Dolezal and Potteiger, 1998). The REG group achieved a significant reduction in percent body fat and fat mass in addition to a significant increase in percent lean tissue but a non statistically significant increase in lean tissue mass. These changes in body composition may have ultimately resulted in no net change in body weight or BMI. A more detailed discussion of the similarities and differences between the outcome measures in the 3 exercise interventions will be discussed in detail in the next chapter. An alternative explanation is that it is quite possible that either consciously or subconsciously the subjects in this study partly compensated for the energy expended in the weekly exercise training sessions. Other studies have reported that when compliance to supervised exercise training interventions is near perfect, which it was in the present study, the effectiveness of exercise may be undermined by compensatory responses that negate the energy deficit caused by exercise training (King et al., 2008). Compensatory responses include increased energy intake (Stubbs et al., 2004) and / or reduced daily physical activity and energy expenditure in non exercising time (Donnelly et al., 2003). The latter may be an attempt to recover from the exercise session. Other compensatory responses include a reduction in RMR. Weight loss in obese individuals, particularly substantial weight loss, has been associated with decreases

in RMR, even when exercise is the mode of intervention (Leibel et al., 1995). RMR was maintained in this study and so this theory is not relevant to this particular situation.

The finding that fat mass reduced in the REG group after 10 weeks of training, and not in the ECC group, was surprising but percent body fat did decrease significantly in both groups after training. Both groups completed 4 hours of exercise training per week. Within the 4 hours of training per week, the REG group performed two 15 minute bouts of resistance training, where as the ECC group performed two 45 minutes bouts of resistance training. The remainder of the exercise time consisted of aerobic exercise. It is possible that the aerobic exercise stimulus in the ECC group was not sufficient to induce significant fat loss. The literature suggests that aerobic training is superior to resistance training with regard to decreasing fat mass, but resistance training is superior to aerobic exercise in terms of increasing lean tissue mass (Dolezal and Potteiger, 1998). This would explain the greater reduction in fat mass found in the REG group, and the greater increase in lean tissue mass found in the ECC group. The combination of both modes of training provide all of these benefits just to a lesser extent than either mode of exercise alone (Dolezal and Potteiger, 1998). It is possible that a better balance between aerobic exercise and resistance training in both groups is required to achieve significant reductions in fat mass concomitant with significant gains in lean tissue mass.

Interestingly, there was no change in insulin secretion or insulin sensitivity in either group post intervention. This was a surprising finding that lies in direct contrast to the metabolic adaptations that occurred in response to the exercise intervention in study one of this thesis. A comparison of the metabolic adaptations to each of the three exercise interventions will be discussed in detail in the chapter to follow. Given the well documented inverse relationship between insulin sensitivity and body weight and body fat mass (Carey et al., 1996, Redinger, 2007), the lack of improvement in the metabolic status of the subjects in the present study might be explained by the fact that there was no significant change in body weight in the REG group, and no significant change in body weight or body fat mass in the ECC group post. A limited number of studies have reported no change in insulin sensitivity with exercise training even in the presence of significant reductions in body weight and body fat mass (Short et al., 2003). However this is very unlikely because the literature overwhelmingly and consistently supports the fact that exercise training even in the absence of weight loss improves insulin sensitivity (Holloszy,

2005). Some theories have been proposed as to why insulin sensitivity may not improve with exercise training. A greater duration, intensity or frequency of exercise may be required in some individuals to improve insulin sensitivity (Short et al., 2003). The beneficial effects of exercise on insulin sensitivity may be lost quicker in some individuals and so the timing of the post training OGTT could be important (Short et al., 2003). Insulin mediated trafficking of GLUT4 may be impaired in some individuals but the mechanism behind this remains to be elucidated (Short et al., 2003). Specific to the ECC group, a single bout of eccentric resistance training has been reported to impair insulin sensitivity immediately after the training session and so the timing of the post intervention OGTT could again be crucial. This is thought to be due to the muscle damage that occurs with this type of training. The muscle damage may impair insulin stimulation of IRS-1, PI3 kinase and Akt kinase, which may inhibit insulin stimulated glucose disposal (Marcus et al., 2009, Kirwan et al., 1992, Del Aguila et al., 2000). Study one of this PhD thesis established a positive causative relationship between aerobic fitness and insulin sensitivity, in addition to an inverse relationship between fat mass and insulin sensitivity. It is possible that greater improvements in fat mass and fitness were required in the REG and ECC group to result in significant improvements in the metabolic status of these subjects.

Although insulin secretion, insulin sensitivity and the indices of insulin sensitivity did not change significantly post intervention, the non significant changes that did occur appeared to play a role in the increase in fat oxidation that was evident in both groups post intervention. The percent change in RQ in the ECC group was positively correlated with the percent change in the Matsuda index of insulin sensitivity ($r=0.858$, $p=0.06$). Also, 94% of the percent change in RQ in the REG group was predicted by the percent change in RMR and the percent change in HOMA-B. These findings indicate that even small improvements in insulin secretion and insulin sensitivity can have important implications for overall metabolic health in this population.

The novel biomarkers of insulin resistance that were referred to in study one of this thesis were also analysed pre and post the ECC and REG interventions in an attempt to further investigate the possible relationships between the biomarkers and the physical and metabolic characteristics. It appeared from the analysis of the results in study one that significant increases in insulin sensitivity, fitness, and lean tissue mass influenced the production of these hormones. The gains in lean tissue mass did not reach statistical

significance in the aerobic exercise group and so it is possible that a significant increase in lean tissue mass, such as that seen in the ECC group, might have a significant impact on the circulating concentration of many of the biomarkers. This may be particularly true for the biomarkers that are secreted from lean tissue such as FGF21, IL-13 and Visfatin. The circulating concentration of Visfatin decreased significantly in the ECC group. There was no significant change in the circulating concentration of the other biomarkers in either group post intervention. However, the non statistically significant changes that did occur to the biomarkers in each group yielded some very interesting findings.

The results of the present study indicate that different modes of exercise training may differentially regulate the circulating concentrations of the biomarkers, in addition to the relationship between the biomarkers and all other physical and metabolic variables. With the ECC group, the percent change in FGF21 and Omentin was positively correlated with the percent change in each other. In fact the percent change in Omentin accounted for 70% of the percent change in FGF21, and the percent change in FGF21 accounted for 64.6% of the percent change in Omentin. This suggests a regulatory role between both of these biomarkers. The pearson correlation analysis revealed that the percent change in IL-13 and Chemerin were inversely correlated with the percent change in each other. The backwards regression analysis showed that the percent change in IL-13 accounted for 67.4% of the percent change in Chemerin, and the percent change in TG's accounted for 92.4% of the variance in IL-13. These findings indicate that Chemerin and IL-13 may have a role in the regulation of each other. The correlations that appear in the ECC group are not evident in the REG group, which suggests that the biomarkers may be differentially regulated by various modes of exercise training. However, some of the results of the REG intervention including no change in fitness or lean tissue mass after 10 weeks of exercise training were not typical or expected and may mask the changes that should occur to the biomarkers in response to this type of exercise training.

The main differences in the outcome measures between both groups were that fitness and lean tissue mass increased in the ECC group only, where as fat mass decreased in the REG group only. In addition, the strength gains achieved in the ECC group post intervention were significantly greater than those achieved by the REG group. The final difference in outcome measures between groups was that Visfatin decreased significantly in the ECC

group but not in the REG group post intervention. The percent change in Visfatin in the ECC group was correlated with the percent change in lean tissue mass, which also actually predicted its variance in study one of this thesis. In the current study, the single best predictor of the percent decrease in circulating Visfatin was the percent decrease in AUCI, which accounted for 67.7% of the variance in Visfatin post intervention. Further analysis revealed that 99.9% of the percent change in AUCI was attributed to the percent change in FGF21, Chemerin and Visfatin. These are important findings because they show the changes that occurred to the biomarker profile of the subjects post intervention almost completely accounted for the improvements in insulin secretion and insulin sensitivity in these subjects. In turn, the decrease in AUCI mostly accounted for the significant reduction in circulating Visfatin in these subjects. These results indicate a possible cyclical relationship between the biomarkers and insulin secretion and insulin sensitivity. It appears that the increase in fitness and lean tissue mass in the ECC group was responsible for the changes that occurred to the biomarkers and metabolic status in these subjects. However, it is unclear whether the improvements in insulin secretion and insulin sensitivity regulate the secretion of the biomarkers, or if changes in the circulating concentrations of the biomarkers regulate the improvement in insulin secretion and insulin sensitivity. It is also possible that both of these responses occur simultaneously. Interestingly in the REG group, none of the biomarkers accounted for the percent change in each other. The change in Stumvoll, AUCG and AUCI were the best predictors of the change in FGF21, Omentin and Visfatin respectively supporting the relationship between the biomarkers and insulin sensitivity and glucose tolerance. The percent change in weight and fitness were the best predictors of the change in IL-13. Finally, the percent change in LDL was the best predictor of the percent change in Chemerin.

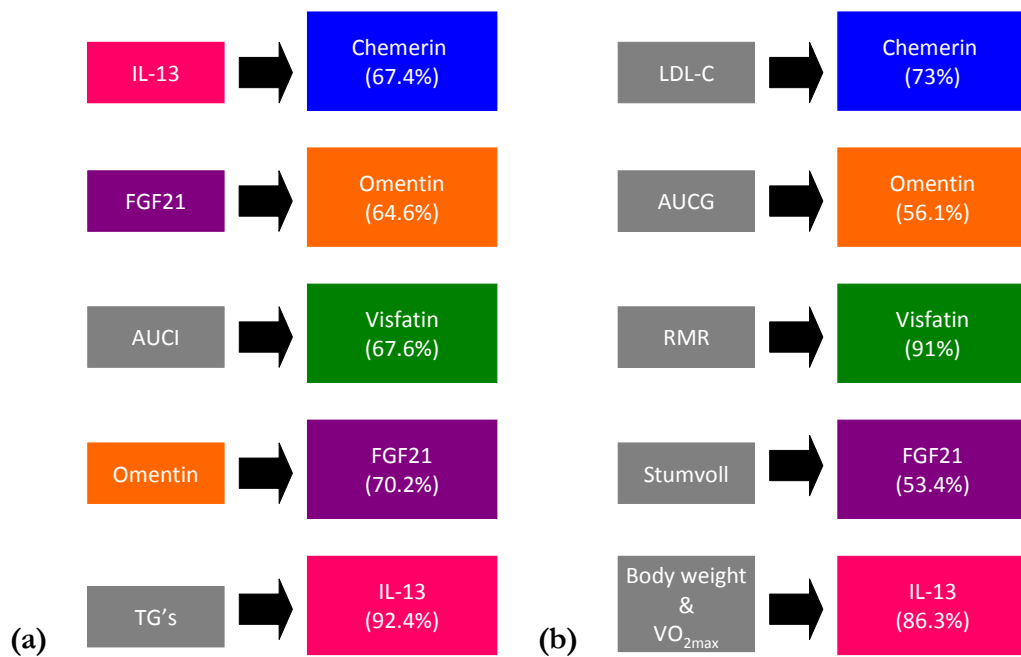


Figure 4.10 Schematic illustrating the percent change in the independent variables that best predicted the percent change in the biomarkers post (a) ECC and (b) REG interventions. Note: IL-13 = Interleukin-13, FGF21 = Fibroblast Growth Factor 21, TG's = Triglycerides, LDL-C = Low Density Lipoprotein Cholesterol, AUCG = Area Under the Curve for Glucose, RMR = Resting Metabolic Rate.

4. 10. Limitations

The smaller than anticipated sample size in both groups limited the dept of the statistical analysis which meant that t-tests were used instead of the preferred option of a 2 way ANOVA. Also, Fetuin-A data was not available for the subjects in either group due to a technical error that occurred in the final stages of the analysis of those ELISA kits.

The ECC intervention was very time intensive to operate. It was very physically demanding for the small number of spotters / lifters and could not be sustained long terms despite its effectiveness. The strength of the spotters could be a limiting factor in longer term studies. Also, the improvements in eccentric ability over time were quite large and so a limiting factor for one subject was that the weight available on the leg extension was less than that required. It would be quite difficult to administer this intervention on a population based level without the development of specialised equipment to complete the concentric phase of contraction.

4. 11. Summary, conclusion and future recommendations

It appears that the changes that occurred to body composition, metabolic health, and the biomarker profile in the obese individuals in this study were specific to the mode of exercise undertaken. The concurrent intervention incorporating an eccentric component (ECC) showed promising potential to augment fat mass loss and improve metabolic health in the longer term in obese subjects. This was evident by the significant increase in fitness, lean tissue mass and fat oxidation, and the clinically important but non statistically significant increase in RMR post intervention. The potential regulatory relationship between fitness, lean tissue mass and the biomarkers identified in study one was further supported in this ECC group with a significant decrease in circulating Visfatin.

However, there are practical implications for conducting this type of intervention. Eccentric resistance training is extremely demanding of time and human resources. Two lifters were required per subject to complete the concentric phase of every repetition, which in this study equated to 180 lifts per subject, two days per week, for 10 weeks. Therefore there was a total of 2,520 lifts per week for the 7 subjects in the ECC group, which was very challenging with a small team of 4 lifters including the study investigator. It would be very difficult to implement this type of intervention on a larger scale. Also, subjects can tolerate much higher loads eccentrically than they can with regular resistance training and so in many cases, particularly with the leg extension exercise, the lifters were required to lift or push loads ranging from 150-250kgs with every repetition. This was achieved in the current study but in the longer term as subjects continue to adapt to training, greater loads would need to be lifted to ensure the stimulus is sufficient to maintain the desired intensity and induce the appropriate adaptations to training. The strength of the lifters could be a limiting factor in this situation. It may not be practical to continue with this type of intervention in the long term. For this mode of training to be available routinely for obese individuals and on a large scale, specialised equipment must be designed specifically for this purpose.

The results of the REG intervention were surprising and unexpected, particularly the findings that there were no changes in fitness or lean tissue mass after 10 weeks of exercise training. Also there was no change in insulin secretion or insulin sensitivity despite progressive exercise training, and a significant reduction in fat mass, both of which should

improve insulin sensitivity. For this reason it is difficult to interpret the effects that this type of intervention should have on the novel biomarkers. However, the results did support a link between the biomarkers and metabolic health in obese individuals. The significant reduction in fat mass in the REG group supports the findings of the aerobic exercise intervention in study one of this thesis, indicating that aerobic exercise is important for reducing fat mass in this population. It is possible that the volume of resistance training in this intervention was insufficient to stimulate the expected changes in lean tissue mass, although significant improvements in fat oxidation were observed indicating that the subjects did respond in some way to the training stimulus. Future concurrent training studies incorporating a greater volume and intensity of regular resistance exercises may provide all of the benefits associated with aerobic exercise and resistance training, which may lead to greater improvements in energy expenditure, fat mass loss, and metabolic health of obese individuals.

Chapter 5. Integration of results from studies 1 and 2 and secondary analysis.

5. 1. Rationale

It is clear from chapters 3 and 4 of this PhD thesis that exercise training favourably influences the physical and metabolic characteristics of obese individuals, and plays a role in the regulation of the circulating concentration of the novel biomarkers of insulin resistance. The adaptations that occur may be specific to the mode of exercise undertaken. One limitation that exists when interpreting the results of the three exercise interventions is that the number of subjects in each intervention was smaller than originally anticipated which limited the depth of the statistical analysis and the interpretation of the findings. Also, there was wide variation in the baseline values for insulin and the novel biomarkers of insulin resistance, in addition to the individual response to exercise training. In an attempt to address the issue of the small sample size in the 3 exercise interventions, the subject data from the aerobic exercise group, the ECC group and the REG group was combined and a pre post analysis was carried out. These interventions did not use exactly the same mode of exercise training but it was broadly similar in terms of the frequency of training per week, the intensity of the aerobic exercise, the duration of the session and the energy expenditure derived from each bout. Combining the data increased the power thus enhancing the dept of statistical analysis which may enhance the interpretation of the findings.

5. 2. Aim

The aim of this analysis was to increase statistical power and investigate the effects of 10-12 weeks of exercise training on body composition, metabolic health, and novel biomarkers of insulin resistance in obese subjects.

5. 3. Objectives

To measure the impact of exercise training on body composition and metabolic health in obese individuals.

To investigate the relationship between body composition, insulin sensitivity and the circulating concentrations of the novel biomarkers of insulin resistance.

5. 4. Hypothesis

Exercise training will significantly reduce fat mass, increase lean tissue mass and improve insulin sensitivity in obese subjects.

Improved body composition and fitness will predict the improvements in insulin sensitivity. There will be a cyclical relationship between the biomarker profile of the obese subjects and their metabolic health.

5. 5. Study Design

Twenty five subjects were included in this analysis. 10 of these subjects completed the aerobic exercise intervention detailed in chapter 3, 7 completed the eccentric intervention detailed in chapter 4, and 8 completed the aerobic and resistance training intervention detailed in chapter 4. Please refer to the relevant chapters for a detailed explanation of these 3 interventions. A pre post analysis was carried out to determine the effects of 10-12 weeks of exercise training on body composition, insulin sensitivity, and the novel biomarkers of insulin resistance in obese subjects.

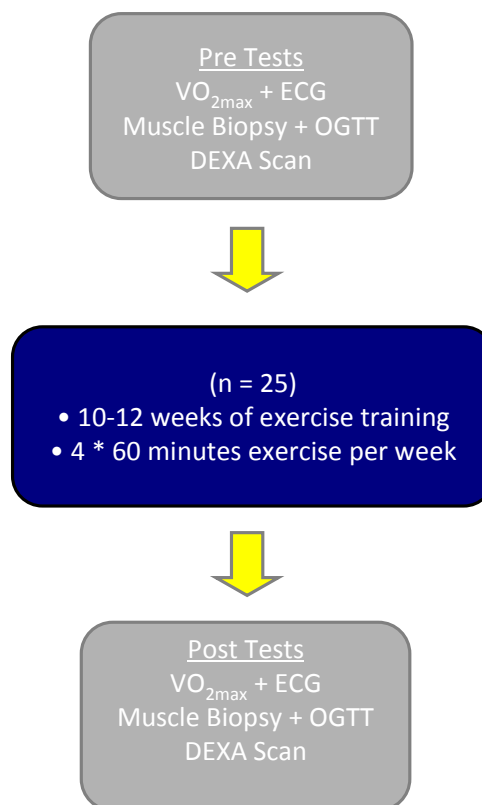


Figure 5.1 Schematic representation of study design.

5. 6. Methods

Please refer to chapters 3 and 4 for a detailed explanation of the tests and procedures outlined in Figure 5.1.

5. 7. Statistical analysis

Experimental data is presented as mean \pm standard deviation. Data was evaluated using the Sigmaplot 12 statistical package (Systat Software, Inc. Chicago, IL). Statistical significance was set as $p \leq 0.05$. The statistical tests used to perform the analysis of the data in this chapter included a dependent t-test, pearson correlation analysis and backwards stepwise regression. These tests are explained in detail in sections 3.10.1, 3.10.3, and 3.10.4 respectively.

Best subsets regression was also used in this analysis. Best Subsets Regression is a technique for selecting variables in a multiple linear regression by systematically searching through different combinations of independent variables and selecting the subsets of variables that best contribute to predicting the dependent variable. It is important to avoid multicollinearity and so where two independent variables are highly correlated only one of those variables can be entered into the best subsets regression model. The output from this analysis details all possible prediction models ranging from the lowest prediction to the highest prediction. The predictive ability of the model is reflected by the R^2 value which is a measure of how well the model describes the data. The larger the R^2 value, the better the model predicts the dependent variable. The R^2 value does not take in to account the number of variables used in the equation. More variables result in higher R^2 values and so it is necessary to interpret the data and choose the model with the lowest number of independent variables that best predict the dependent variable.

Note: There is a subtle difference between a backwards stepwise regression and best subsets regression. Backwards stepwise regression is used when a number of independent variables are known to predict a dependent variable and you are interested in systematically removing the independent variables to determine those that best predict the dependent variable. In contrast, best subsets regression is best used when you are not sure which independent variables best predict the dependent variables and want to consider various possible combinations to determine that independent variables that best predict the dependent variable (Sigmaplot 12, Systat Software Inc., 2012).

5. 8. Results

All 25 subjects included in this study were obese at baseline and had been sedentary for a minimum of 6 months prior to enrolment.

5. 8. 1. Physical Characteristics

The pre and post values for the physical characteristics in addition to the percent change that occurred to these variables after exercise training are presented in Table 5.1. A paired samples t-test revealed that there was a significant reduction in weight ($p=0.003$), BMI ($p=0.003$), fat mass ($p=0.001$) and percent body fat ($p=0.001$) post intervention. This was accompanied by a significant increase in lean tissue mass ($p=0.022$), percent lean tissue ($p=0.001$) and fitness ($\dot{V}O_{2\max}$ ml/kg/min) ($p<0.001$).

Table 5.1 All measured physical characteristics pre and post intervention in addition to the percent change that occurred to each variable.

	Pre	Post	% change
	(n=25)		
Age (yr)	38 \pm 13		
Weight (kg)	94.1 \pm 19.7	91.8 \pm 18.6 *	-2.3 \pm 3.1
BMI (kg/m ²)	33.07 \pm 4.90	32.32 \pm 4.79 *	-2.30 \pm 3.13
Fat mass (kg)	43.61 \pm 15.53	39.70 \pm 14.46 *	-8.92 \pm 8.82
% body fat	46.47 \pm 9.41	43.28 \pm 9.53 *	-7.08 \pm 6.55
Lean tissue (kg)	46.19 \pm 10.89	47.98 \pm 10.61 *	4.44 \pm 7.72
% lean tissue	50.59 \pm 9.20	53.72 \pm 9.28 *	6.63 \pm 7.67
$\dot{V}O_{2\max}$ (ml/kg/min)	27.47 \pm 6.84	31.64 \pm 7.19 *	17.43 \pm 18.77
$\dot{V}O_{2\max}$ (L/min)	2.56 \pm 0.75	2.9 \pm 0.75 *	16.01 \pm 16.67

Data is presented as mean \pm standard deviation. * $p \leq 0.05$ represents a significant change post intervention. Note: BMI = Body Mass Index, $\dot{V}O_{2\max}$ aerobic fitness.

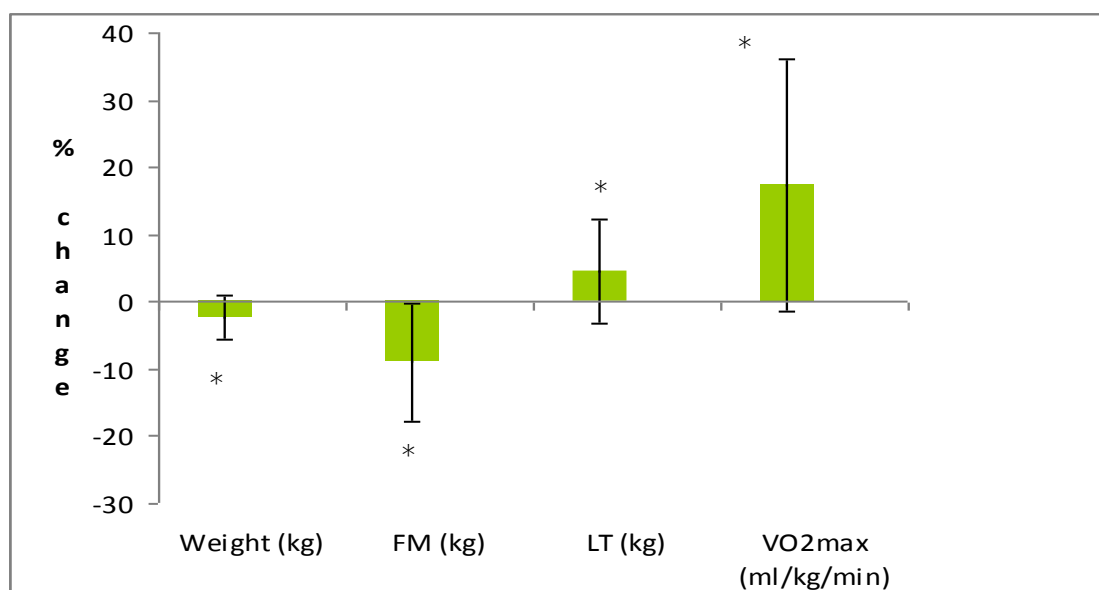


Figure 5.2 Percent change in weight (kg), fat mass (kg), lean tissue mass (kg) and aerobic fitness ($\dot{V}O_{2\max}$ ml/kg/min) post intervention. Data is presented as the mean percent change \pm standard deviation. * $p \leq 0.05$ represents a significant change post intervention.

5. 8. 2. Metabolic characteristics

The baseline and post intervention values for the metabolic characteristics are presented in Table 5.2, in addition to the percent change that occurred to these variables post intervention. A paired samples t-test identified a significant reduction in HOMA-B ($p=0.04$) which was accompanied by a significant increase in the AUCG/AUCI index ($p=0.03$). AUCI decreased significantly ($p=0.01$) but there was no change in AUCG ($p=0.52$) or the Stumvoll ($p=0.133$) and Matsuda ($p=0.166$) indexes of insulin sensitivity.

The lipid profile of the subjects was also measured pre and post intervention. These results in addition to the percent change that occurred to the lipids after exercise training is presented under in Table 5.2. There was no change in free fatty acids ($p=0.93$), triglycerides ($p=0.57$), total cholesterol ($p=0.65$), LDL cholesterol ($p=0.82$) or HDL cholesterol ($p=0.22$) post intervention. Pearson correlation and backwards stepwise regression analysis was not carried out for these variables.

Table 5.2 All measured metabolic characteristics pre and post intervention in addition to the percent change that occurred to each variable.

	Pre	Post	% change
	(n=25)		
AUCG	1088.43 ± 216.73	1064.33 ± 177.34	-0.73 ± 15.77
AUCI	14049 ± 8785	11322 ± 7543	-16.09 ± 21.75 *
AUCG/AUCI	0.117 ± 0.099	0.144 ± 0.127	26.21 ± 38.15 *
HOMA-B	48.70 ± 26.91	40.99 ± 24.25	-4.60 ± 56.55 *
Stumvoll	0.127 ± 0.030	0.124 ± 0.025	-1.186 ± 11.315
Matsuda	14.80 ± 13.50	18.07 ± 20.86	26.09 ± 44.59
FFA's	0.56 ± 0.15	0.54 ± 0.21	4.52 ± 19.42
TG's	1.43 ± 0.92	1.32 ± 0.72	-3.27 ± 39.39
TC	5.01 ± 1.52	5.04 ± 1.21	-1.52 ± 29.23
LDL	3.18 ± 1.37	3.05 ± 0.90	9.65 ± 41.55
HDL	1.15 ± 0.28	1.27 ± 0.42	13.41 ± 49.48

Data is presented as mean ± standard deviation. * $p \leq 0.05$ represents a significant change in the variable post intervention. Note: AUCG = Area Under the Glucose Curve, AUCI = Area Under the Insulin Curve, AUCG/AUCI = Area Under the Glucose Curve / Area Under the Insulin Curve (index of insulin sensitivity), HOMA-B is an index of fasting insulin secretion, Stumvoll and Matsuda are indexes of insulin sensitivity, FFA's = Free Fatty Acids, TG's = Triglycerides, TC = Total Cholesterol, LDL = Low Density Lipoprotein Cholesterol, HDL = High Density Lipoprotein Cholesterol.

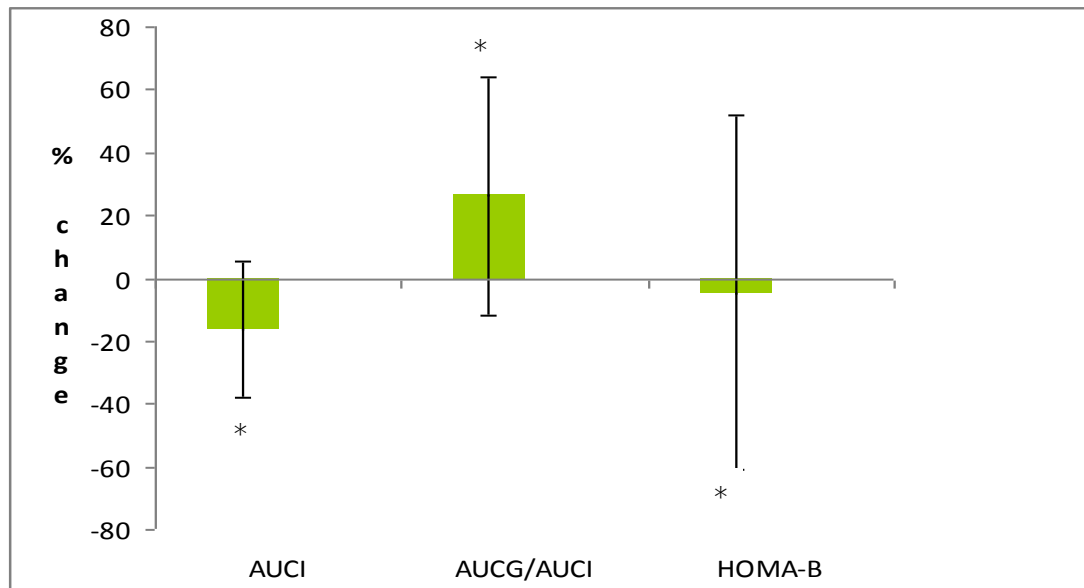


Figure 5.3 Percent change in the area under the curve for insulin (AUCI), the area under the curve for glucose / the area under the curve for insulin (AUCG/AUCI), and fasting insulin secretion (HOMA-B) post intervention. Data is presented as the mean percent change \pm standard deviation. * $p \leq 0.05$ represents a significant change post intervention.

5. 8. 3. Novel Biomarkers of Insulin Resistance

The circulating concentration of the selected biomarkers pre and post intervention is presented in Table 5.3. The percent change that occurred in the biomarkers post intervention is also included. A paired samples t-test revealed that there was a significant decrease in Fetuin-A post intervention ($p=0.02$). However, Fetuin-A was only measured in a subgroup of the subjects ($n=9$) because of a technical error that occurred during the analysis of the other Fetuin-A kits containing samples for the remainder of the subjects. There was no change in the circulating concentration of FGF21 ($p=0.77$), IL-13 ($p=0.87$), Chemerin ($p=0.35$), Omentin ($p=0.45$), or Visfatin ($p=0.58$) post intervention.

Table 5.3 All measured biomarkers pre and post intervention in addition to the percent change that occurred to each variable.

	Pre	Post	% change
	(n=25)		
FGF21	296.81 \pm 134.92	284.62 \pm 156.85	11.69 \pm 68.20
IL-13	155.46 \pm 110.42	156.22 \pm 99.19	5.05 \pm 24.30
Chemerin	218.82 \pm 44.07	200.11 \pm 51.82	-3.88 \pm 32.41
Omentin	339.85 \pm 156.40	364.98 \pm 142.68	17.78 \pm 48.54
Visfatin	16.90 \pm 4.17	16.23 \pm 4.44	-3.42 \pm 14.16
Fetuin-A	578.49 \pm 141.23	464.89 \pm 76.12	-19.79 \pm 13.08 *

Data is presented as mean \pm standard deviation. * $p \leq 0.05$ represents a significant change post intervention. Note: n=9 for the Fetuin-A data (aerobic exercise subjects only)

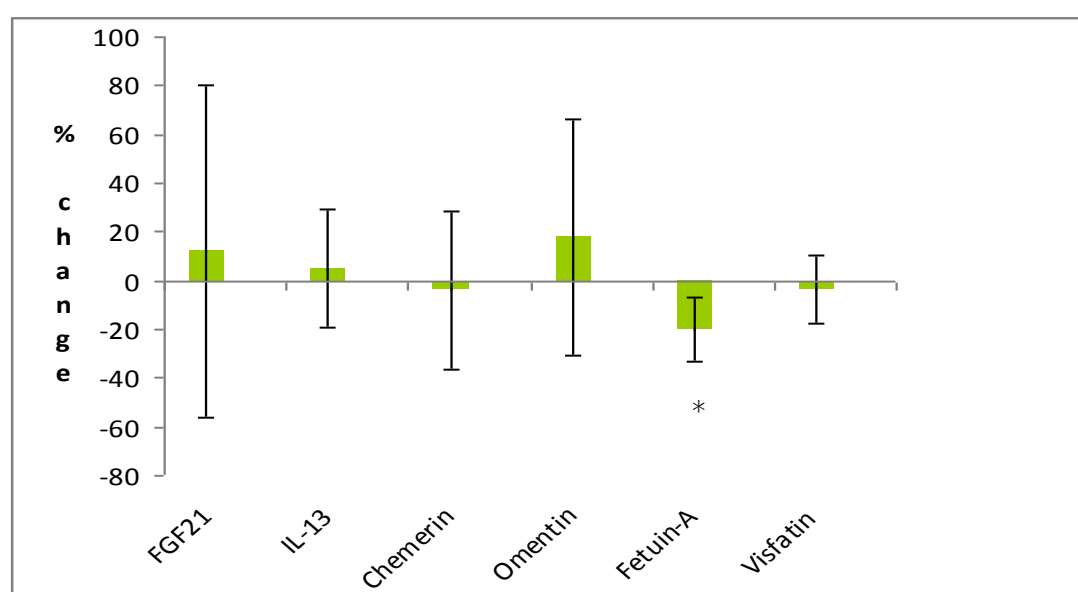


Figure 5.4 Percent change in selected biomarkers post intervention. Data is presented as the mean percent change \pm standard deviation from the mean. * $p \leq 0.05$ represents a significant change post intervention.

5. 8. 4. Pearson correlation analysis for the physical characteristics

Pearson correlations revealed that the percent change in weight post intervention was significantly positively correlated with the percent change in AUCI ($r=0.433$, $p=0.04$), and

LDL cholesterol ($r=0.478$, $p=0.04$), and significantly inversely correlated with the percent change in the Matsuda index of insulin sensitivity ($r=-0.431$, $p=0.05$).

The percent change in fat mass was significantly positively correlated with the percent change in AUCI ($r=0.524$, $p=0.02$) as shown in Figure 5.5, and significantly inversely correlated with the percent change in Matsuda ($r=-0.547$, $p=0.02$) and $\dot{V}O_{2\max}$ (ml/kg/min) ($r=-0.433$, $p=0.05$), the latter relationship is also presented in Figure 5.5.

The percent change in lean tissue mass (kg) post intervention was not correlated with the percent change in any other variable. The percent change in fitness ($\dot{V}O_{2\max}$ ml/kg/min) was significantly positively correlated with the percent change in the AUCG/AUCI index ($r=0.662$, $p<0.001$). It was significantly inversely related to the percent change in fat mass ($r=-0.443$, $p=0.05$), AUCI ($r=-0.665$, $p<0.001$), LDL cholesterol ($r=-0.491$, $p=0.03$) and trending towards an inverse correlation with HOMA-B ($r=-0.377$, $p=0.06$).

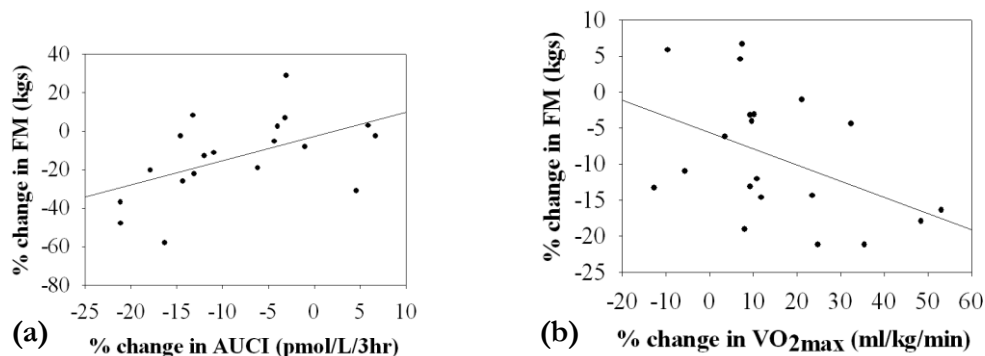


Figure 5.5 (a) Depicts the positive relationship between the percent change in fat mass (kgs) and the percent change in the area under the curve for insulin (AUCI) post intervention ($r=0.524$, $p=0.02$). **(b)** Depicts the inverse relationship between the percent change in fat mass (kgs) and the percent change in aerobic fitness ($\dot{V}O_{2\max}$ ml/kg/min) post intervention ($r=0.443$, $p=0.05$).

Backwards stepwise regression was not run on the physical characteristics because the percent change that occurred to these variables post intervention was completely due to the actual exercise training that was undertaken, in addition to the calories expended during the exercise sessions.

5. 8. 5. Pearson correlation and backwards stepwise regression analysis for the metabolic characteristics

Pearson correlations revealed that the percent change in AUCI was significantly positively correlated with the percent change in weight ($r=0.433$, $p=0.04$), BMI ($r=0.434$, $p=0.04$), fat mass (kg) ($r=0.524$, $p=0.02$), serum Chemerin ($r=0.469$, $p=0.03$), HDL cholesterol ($r=0.557$, $p=0.02$), and LDL ($r=0.693$, $p<0.001$). It was significantly inversely correlated with the percent change in Omentin ($r=-0.453$, $p=0.003$) and fitness ($r=-0.665$, $p<0.001$). Backwards stepwise regression presented in Table 5.4 illustrates that the percent change in AUCI was most significantly predicted by the percent change in fitness ($\dot{V}O_{2\max}$ ml/kg/min) and HDL cholesterol, which accounted for 74.7% of the variance in AUCI post intervention.

The percent change in AUCG/AUCI was significantly positively correlated with the percent change in fitness ($\dot{V}O_{2\max}$ ml/kg/min) ($r=0.662$, $p<0.001$), and significantly inversely correlated with the percent change in LDL cholesterol ($r=-0.574$, $p=0.01$). Backwards stepwise regression presented in Table 5.4 shows that the percent change in AUCG/AUCI was most significantly predicted by the percent change in fitness, which accounted for 74.7% of the variance in AUCG/AUCI post intervention.

Following exercise training, the percent change in HOMA-B was trending towards a significant inverse correlation with the percent change in fitness ($\dot{V}O_{2\max}$ ml/kg/min) ($r=-0.377$, $p=0.06$). It was significantly inversely correlated with the percent change in the circulating concentration of Visfatin ($r=-0.506$, $p=0.01$), and positively correlated with the percent change in FFA's ($r=0.689$, $p=0.04$). Backwards stepwise regression revealed that the percent change in fitness accounted for 71.6% of the variance in HOMA-B post intervention. This data is presented in Table 5.4.

Table 5.4 Backwards stepwise regression analysis showing the independent variables that best predict the change in the dependent variables AUCI, AUCG/AUCI, and HOMA-B post exercise training.

Dependant variable	Indep. variables in model	Coef.	Std. Coeff.	Std. Error	F-to-Remove	P
AUCI (R ² =0.740)	Constant	-6.313		5.216		
	% change in $\dot{V}O_{2\max}$ ml/kg/min	-0.803	-0.646	0.181	19.593	<0.001
	% change in HDL	0.199	0.417	0.0696	8.165	0.013
AUCG/AUCI (R ² =0.747)	Constant	4.261		10.812		
	% change in $\dot{V}O_{2\max}$ ml/kg/min	1.399	0.660	0.398	12.337	0.003
HOMA-B (R ² =0.716)	Constant	8.787		7.324		
	% change in $\dot{V}O_{2\max}$ ml/kg/min	-0.807	-0.846	0.207	15.112	0.008

R² represents the extent to which the percent change in the independent variable (s) contribute to the percent change in the dependent variable. p≤0.05 is significant. Note: AUCI = Area Under the Curve for Insulin, AUCG/AUCI = area under the curve for glucose / area under the curve for insulin, HOMA-B = fasting insulin secretion, HDL = High Density Lipoprotein Cholesterol, = $\dot{V}O_{2\max}$ aerobic fitness.

5. 8. 6. Pearson correlation and backwards stepwise regression analysis for the biomarkers.

Pearson correlations for all of the biomarkers are presented in Table 5.5. The percent change in IL-13 was not correlated with the percent change in any other variable post intervention but was trending towards a positive correlation with the percent change in body weight (p=0.07). The percent change in Fetuin-A was not significantly correlated with the percent change in any other variable post intervention but was trending towards a correlation with the percent change in free fatty acids (p=0.08).

Table 5.5 Pearson correlations for selected biomarkers.

	% change in FGF21	% change Chemerin	% change Omentin	% change Fetuin-A	% change in Visfatin	% change in IL-13
% change in FGF21		r=-0.438 * p=0.04				
% change in Chemerin	r=-0.438 * p=0.04		r=-0.563 * p=0.01			
% change in Omentin		r=-0.563 * p=0.01				
% change in AUCG			r=-0.618 * p=0.003		r=-0.407 p=0.70	
% change in AUCI		r=0.469 * p=0.03	r=-0.453 * p=0.03			
% change in Matsuda			r=0.409 † p=0.07		r=0.507 * p=0.02	
% change in Stumvoll					r=-0.440 * p=0.04	
% change in HOMA- B					r=-0.506 p=0.01 *	
% change in LDL	r=-0.424 † p=0.07	r=0.643 * p=0.004				
% change in HDL		r=0.526 * p=0.03				
% change in FFA's				r=0.648† p=0.08		
% change In weight						r=0.391† p=0.07

r represents the strength of the correlation between two variables. * $p \leq 0.05$ is significant.

† $p \leq 0.09$ is trending towards significance. Note: AUCG = Area Under the Curve for Glucose, AUCI = Area Under the Curve for Insulin, HOMA-B = Fasting Insulin Secretion, LDL = Low Density Lipoprotein Cholesterol, HDL = High Density Lipoprotein Cholesterol, FFA's = Free Fatty Acids, FGF21 = Fibroblast Growth Factor 21, IL-13 = Interleukin-13.

The change that occurred to IL-13 post intervention was not significantly correlated with the percent change in any other variable, thus backwards stepwise regression was run using a combination of variables identified in the literature as having an important influence on the circulating concentration of this biomarker. The only independent variable that was not removed from the model was body weight. The change in body weight predicted 22% of the change in IL-13 post intervention.

The percent change in FGF21 also was not significantly correlated with the percent change in any other variable post intervention. A combination of variables identified in the literature as having an important influence on the production of FGF21 was entered into the backwards stepwise regression. The only independent variable that was not removed from the model was Chemerin. The percent change in Chemerin accounted for 19.2% of the variance in FGF21 post intervention.

Only 9 data points were available for Fetuin-A, which represented the subjects who undertook the aerobic exercise intervention. Backwards stepwise regression was conducted on Fetuin-A in study one of this PhD thesis and these results are included in the current chapter presented in Table 5.6 below. The findings show that 42% of the variance in Fetuin-A in the aerobic exercise group was predicted by the percent change in FFA's. This data analysis can not be applied to the entire group in the current study.

The percent change in Chemerin was mostly predicted by the percent change in Omentin and AUCI, which accounted for 55.4% of the variance in Chemerin post intervention. Similarly, when backwards regression analysis was performed on Omentin, the percent change in Omentin was mostly predicted by the percent change in Chemerin and AUCG, which accounted for 61% of the variance in Omentin post intervention. This data is presented in Table 5.6.

Finally, Table 5.6 shows that the percent change in Visfatin post intervention was mostly predicted by the percent change in AUCG and HOMA-B, which accounted for 49.4% of the variance in Visfatin.

Table 5.6 Backwards stepwise regression analysis illustrating the percent change in the independent variable (s) that best predicted the percent change in the dependent variables post intervention.

Dependant variable	Indep. variables in model	Coef.	Std. Coeff.	Std. Error	F-to-Remove	P
% change in FGF21 (R ² =0.192)	Constant	-1.345		9.972		
	% change in Chemerin	-0.681	-0.438	0.313	4.749	0.041
% change in Chemerin (R ² =0.554)	Constant	15.338		6.230		
	% change in Omentin	-0.289	-0.495	0.106	7.400	0.015
	% change in AUCI	0.499	0.372	0.244	4.181	0.057
% change in Omentin (R ² =0.610)	Constant	19.008		7.642		
	% change in Chemerin	-0.875	-0.511	0.275	10.147	0.005
	% change in AUCG	-1.437	-0.446	0.517	7.734	0.013
% change in Visfatin (R ² =0.494)	Constant	-1.563		2.357		
	% change in AUCG	-0.309	-0.360	0.149	4.278	0.054
	% change in HOMA-B	-0.126	-0.559	0.039	10.333	0.005
% change in Fetuin-A (R ² =0.420)	Constant	-21.551		3.896		
	% change in FFA's	0.409	0.648	0.196	4.349	0.082
% change in IL-13 (R ² =0.220)	Constant	11.90		6.036		
	% change in weight	3.575	0.469	1.546	5.346	0.03

R² represents the extent to which the percent change in the independent variable predicted the percent change in the dependent variable. $p \leq 0.05$ is significant. Note: FGF21 = Fibroblast Growth Factor 21, AUCI = Area Under the Curve for Insulin, AUCG = Area Under the Curve for Glucose, HOMA-B = Fasting Insulin Secretion, FFA's = Free Fatty Acids, IL-13 = Interleukin-13.

The backwards regression analysis presented in Table 5.6 identified independent variables that predicted only 19-61% of the variance in any biomarker post intervention. In an attempt to identify other variables that might influence the production of the biomarkers, best subsets regression analysis was performed. This analysis looks at every possible combination of selected variables and identifies all possible models ranging from the smallest to the largest predictive ability. The output from this analysis revealed that combining the variables originally identified in the pearson correlation analysis presented in Table 5.5, with other combinations of measured variables deemed to be important by the literature, had very little additional impact on the predictive ability of the backwards regression models presented in Table 5.6. Also, the only independent variables to significantly contribute to the change in any particular biomarkers post intervention were those that were originally identified in the backwards stepwise regression in Table 5.6.

5. 9. Discussion

There are 3 key findings to the current analysis. Firstly, exercise training significantly improved body weight, all components of body composition, and fitness in obese, previously sedentary subjects. The 3 individual interventions demonstrated adaptations in the physical characteristics that were specific to the mode of exercise undertaken including greater reductions in body weight and body fat when aerobic exercise was the predominant mode of training (aerobic and REG groups), and greater increases in lean tissue mass when a greater volume of resistance training was combined with aerobic exercise (REG and ECC groups). The current analysis supports these findings and suggests that with general exercise and with a greater sample size all physical characteristics respond favourably and significantly to training. Secondly, insulin secretion and insulin sensitivity improved significantly post training which was evident as a significant reduction in HOMA-B and AUCI in addition to a significant increase in the AUCG/AUCI index. These findings support those of the aerobic intervention. HOMA-B, AUCI and the AUCG/AUCI index also improved in the ECC and REG groups post training and although these improvements did not reach statistical significance, fat oxidation improved significantly which has important implications for metabolic health in obese individuals. The larger number of subjects in the current analysis shows that exercise training improves insulin sensitivity and insulin secretion in obese previously sedentary subjects. These findings add further support to the previous chapters of this thesis and the current literature that exercise training improves metabolic health in obese individuals. Importantly, the current analysis identified that increased aerobic fitness post training was the single best predictor of the improvements in metabolic health in these subjects, supporting the findings of the aerobic exercise intervention. In the ECC and REG group that improvements in metabolic health was mostly attributed to improvements in the circulating concentrations of the

biomarkers suggesting that they also play a role, but overall and with greater numbers of subjects increased fitness was the best predictor of metabolic health. Finally, the results of the 3 individual interventions in addition to the current analysis indicate a cyclical relationship between metabolic health and the circulating concentrations of the biomarkers as opposed to a causative relationship. It also appears that the biomarkers play a role in the regulation of each other.

Ten to twelve weeks of exercise training is associated with significant improvements in body weight, body composition and aerobic fitness in obese individuals. Overall there was a 2.3% or 2.2kg reduction in body weight, which is supported by a review of numerous other training studies of similar duration and energy expenditure (Ross and Janssen, 2001). The subjects in the current group expended approximately 2,500kcal per week which equated to approximately 25,000 - 30,000kcal over 10-12 weeks. The subjects were required to maintain their pre intervention energy intake and so the total caloric deficit caused by exercise training should have accumulated to a total weight loss of approximately 2.8 - 3.3kg post intervention since there are approximately 9,000kcal in 1kg of body fat. However, this simplistic calculation does not take into account the changes that occurred to body composition as a result of physical training. The subjects in the current analysis achieved an 8.9% or 3.9kg reduction in fat mass, which is greater than a 2.5kg reduction reported in a review of exercise training studies of similar duration and energy expenditure (Ross and Janssen, 2001). This difference may be due to variations in the mode and intensity of exercise training in the present study. The loss in fat mass was accompanied by a 4.4% or 1.8kg increase in lean tissue mass, in addition to a 17.4% increase in aerobic fitness. These findings were an expected consequence of exercise training and are supported by numerous other training studies in the literature (O'Leary et al., 2006, Haus et al., 2009, Saremi et al., 2010a).

The adaptations that occurred to the physical characteristics in response to exercise training in the current analysis support those identified in the aerobic, REG and ECC groups detailed in chapters 3 and 4 adding support to the less powered interventions. In the aerobic exercise intervention, body weight, BMI, fat mass and fitness all improved significantly. Lean tissue mass increased by $8.6 \pm 14.2\%$ and although this did not reach statistical significance the change in percent lean tissue trended towards significance ($p=0.09$). Body composition data was only available for 5 subjects in the aerobic intervention and the current analysis suggests that greater power in the aerobic intervention may have led to significant increases in lean tissue mass post intervention. The adaptations that occurred to the physical characteristics of the subjects in the ECC and REG groups appeared to be very specific to the mode of exercise training undertaken. There were greater reductions in fat mass in the REG group ($-9.3 \pm 8.4\%$) compared to the ECC group ($-6.2 \pm 8.1\%$). The difference between groups, although not statistically significant, was

possibly due to the greater contribution of aerobic exercise in the REG intervention. In contrast, there were greater gains in lean tissue mass in the ECC group ($4.2 \pm 4.5\%$) compared to the REG group ($2.0 \pm 3.2\%$). The difference between groups, although not significant, was most likely due to the greater volume of resistance training in the ECC group (45mins twice per week) compared to the REG group (15mins twice per week), in addition to the fact that muscle damage such as that evident post eccentric training often stimulates hypertrophy (Shepstone et al., 2005). The greater reductions in fat mass combined with smaller increases in lean tissue mass in the REG group explains why body weight and BMI trended towards a decrease ($p=0.07$) in the REG group but did not change in the ECC group post intervention. However, in general and when the data from all 3 interventions are combined it is clear that exercise training is associated with significant weight loss in addition to significant improvements in body composition.

Aerobic fitness increased significantly in the aerobic exercise group and in the ECC group post intervention but surprisingly did not change in the REG group. The average increase in fitness was $22.7 \pm 5.6\%$, $10.1 \pm 5.5\%$ and $9.0 \pm 17.1\%$ in the aerobic, ECC and REG groups respectively. These findings clearly indicate that the greatest gains in fitness were evident following aerobic exercise training only. The average change in fitness was similar between the ECC and REG groups but the large standard deviation in the REG group might explain why the increase in fitness in this group did not reach statistical significance. It also highlights the potential for wide variation in individual response to exercise training which may have masked the true changes in aerobic fitness in the REG group. When all data is combined and the sample size is larger it is clear that exercise training significantly increases aerobic fitness. The physical characteristics of the obese subjects in the 3 individual interventions demonstrated adaptations that were specific to the mode of exercise undertaken but overall it is clear from the current analysis that exercise training significantly improves the physical characteristics of obese subjects including body weight, body composition and aerobic fitness. Any deviations from these findings are most likely explained by the varying modes of exercise in addition to small sample sizes.

The metabolic profile of the subjects improved significantly following exercise training. This was evident as a significant reduction in insulin secretion and insulin sensitivity measured by HOMA-B, AUCI and the AUCG/AUCI index. These findings support those of the aerobic exercise intervention and are well documented by numerous other exercise training studies (Duncan et al., 2003, Hayashi et al., 1997, Holloszy, 2005). HOMA-B ($-6.4 \pm 28.5\%$ in ECC and $-8.9 \pm 97.6\%$ in REG), AUCI ($-8.5 \pm 14.4\%$ in ECC and $-9.1 \pm 23.5\%$ in REG), and the AUCG/AUCI index (10.5 ± 20.0 in ECC and $10.9 \pm 24.8\%$ in REG) all improved in the ECC and REG groups post intervention but did not reach statistical significance. This was an unexpected finding given the well established relationship between exercise training, improved body composition and insulin sensitivity

(Carey et al., 1996, Redinger, 2007) and possible explanations for these findings are detailed in Chapter 4. Importantly, both groups demonstrated a significant increase in fat oxidation post intervention which has important implications for enhanced insulin sensitivity in an obese population (Petersen and Shulman, 2002, Shulman, 2000, Yu et al., 2002). These adaptations confirm that the subjects did respond metabolically to exercise training, and the findings from the current analysis suggest that the other measures of insulin sensitivity may have increased significantly with a greater sample size. Similar to the findings of the 3 individual interventions, the Stumvoll and Matsuda indices of insulin sensitivity did not change significantly following exercise training demonstrating that a greater sample size still had no effect on these predictive models of insulin sensitivity. This may be due to the large variability that existed between the subjects in their baseline fasting insulin values, in addition to the variability in their insulin response to a glucose load. These findings suggest that the use of the Stumvoll and Matsuda models alone may not be the most comprehensive predictors of insulin sensitivity in an obese population, and may need to be supported by other indicators. Finally, the circulating concentration of the lipids did not change in any of the 3 interventions post training, or in the current analysis even with a larger sample size. However, RQ did improve significantly following the ECC and REG intervention demonstrating that fat oxidation improved in these groups with exercise training which in the longer term could ameliorate insulin resistance. The subjects in the aerobic group did not undergo an RMR and so data pertaining to fat oxidation is not available. These findings indicate that it is important to measure fat oxidation in this population to show improvements in fuel utilization and metabolism that may not be evident in measures of circulating lipids.

In the current analysis, the improvement in the metabolic health of the subjects post intervention was highly correlated with the favourable changes that occurred to their physical characteristics. The reduction in body weight and body fat mass were both significantly positively correlated with the reduction in AUCI. The increase in aerobic fitness in particular was associated with improvements in all indices of metabolic health with the exception of the Stumvoll predictor of insulin sensitivity. In fact, the increase in fitness was the single best predictor of the improvements in insulin secretion and insulin sensitivity in these subjects. The backwards regression analysis revealed that the percent increase in fitness accounted for 71.7% of the reduction in HOMA-B, 74.7% of the increase in the AUCG/AUCI index of insulin sensitivity, and 74.7% of the decrease in AUCI post intervention (Table 5.4). These results support those of the aerobic exercise intervention, which also revealed that the percent change in fitness best predicted the favourable percent change in HOMA-B, AUCI and the AUCG/AUCI index (Table 3.6). For the ECC and REG group, the best predictors of the change in these measures were the percent changes that occurred to the circulating concentration of the biomarkers in addition to the percent increase in lean tissue mass (Table 4.6). All 3 interventions led to

improvements in these variables and it is possible that the improvements were regulated by the differential changes in body composition in response to different modes of training. Overall, when all data is combined, it is clear that aerobic fitness is the best predictor of insulin resistance and insulin sensitivity in an obese population. There is an abundance of literature to support this finding (Duncan et al., 2003) which is true in the presence (Saremi et al., 2010b) and absence (Nassis et al., 2005) of weight loss. Exercise training has even been reported to reverse insulin resistance in overweight subjects (O'Leary et al., 2006). As little as one bout of exercise has been shown to enhance insulin sensitivity in this population (O'Gorman et al., 2006). These results illustrate the crucial role of exercise and aerobic fitness in improving the metabolic health of obese individuals, and support the recommendation that aerobic fitness must be a primary outcome measure in interventions of this nature.

The literature that is currently available suggests that significant improvements in body composition, fitness and insulin sensitivity lead to significant favourable changes in the circulating concentration of the novel biomarkers of insulin resistance (Brix et al., 2010, Cuevas-Ramos et al., 2012, Moreno-Navarrete et al., 2010, Chakaroun et al., 2012, Choi et al., 2007). The results of the current analysis confirms that exercise training significantly improves body composition, fitness and insulin sensitivity, but there was no significant change in the circulating concentration of any of the biomarkers. The large variation in the individual response of the biomarkers to exercise training suggests that larger scale studies may be required to determine the effects of exercise on the biomarkers. Chapters 3 and 4 of this thesis also suggest that the circulating concentration of the biomarkers may be differentially regulated by different modes of exercise training since Fetuin-A decreased significantly following aerobic exercise only, and Visfatin increased significantly following the ECC intervention only.

Although the serum concentration of the biomarkers did not change significantly post intervention, the changes that did occur provide further support to the results of the previous studies, along with some additional interesting findings. The pearson correlations in the current analysis revealed that there was no relationship between the percent change in any of the biomarkers and the percent change in any of the physical characteristics. Instead, the percent change in the circulating concentrations of the biomarkers was significantly correlated with the percent change in each other, the lipids, and various parameters of insulin secretion and insulin sensitivity. The backwards regression analysis in the current chapter showed that the best predictor of the percent change in Chemerin was the percent change in Omentin and AUCI, which accounted for 44.5% of the variance in Chemerin. The literature supports a significant inverse relationship between the pro-inflammatory cytokine Chemerin and the anti-inflammatory cytokine Omentin (El-Mesallamy et al., 2011). It has been suggested that the adipocytes of obese individuals are

responsible for the elevated concentration of Chemerin (Stejskal et al., 2008), and that Chemerin induces insulin resistance at the level of the insulin receptor, thus inhibiting glucose uptake (Lambernd et al., 2012). The results of the current study suggest that exercise induced improvements in fat mass and thus circulating Omentin, down regulated the production of Chemerin by 3.9% in this population. Other exercise training interventions of similar duration support these findings (Saremi et al., 2010b, Chakaroun et al., 2011). However, exercise training even in the absence of weight loss has also been shown to down regulate the expression of Chemerin mediated by the contraction induced increase in glucose uptake (Lambernd et al., 2012). The percent change in Chemerin was best predicted by the percent change in aerobic fitness (93.4%) in the aerobic intervention, the percent change in IL-13 (67.4%) in the ECC group and the percent change in LDL (73%) in the REG group. Taking all findings into consideration it is likely that the production of Chemerin is not simply regulated by one factor but rather is influenced by a combination of factors including fitness, body composition, insulin sensitivity, and the circulating concentrations of other biomarkers.

The best predictor of the percent change in Omentin was the percent change in Chemerin and AUCG, which accounted for 61% of the variance in Omentin post intervention. This supports the previous finding that Chemerin and Omentin regulate the production of each other (El-Mesallamy et al., 2011). Both biomarkers are produced in fat mass so it is possible that changes in fat mass alter their expression, but the results also suggest a regulatory relationship between them. The literature reports that Omentin may have a role in improving insulin sensitivity. In fact administration of recombinant Omentin has been shown to increase insulin stimulated glucose uptake by 47% in adipose tissue and 30% in omental adipose tissue by enhancing insulin action and thus improving glucose uptake (Yang et al., 2006). The literature suggests that Omentin plays a role in the regulation of blood glucose levels but the results of this current study suggest that improvements in insulin sensitivity and glucose tolerance also regulate the production of Omentin, indicating a possible cyclical relationship between Omentin and Chemerin, in addition to Omentin and glucose tolerance. These findings support those of the aerobic and REG interventions which reported that the best predictor of the percent change in Omentin was the percent change in Stumvoll (44.3%) and AUCG (67.6%) respectively. Additionally, the ECC group identified the percent change in FGF21 (64.6%) to be the best predictor of the percent change in Omentin adding further support to the finding that Omentin is also regulated by other biomarkers of insulin resistance.

The percent change in Visfatin was mostly predicted by the percent change in AUCG and HOMA-B, which accounted for 49.4% of the variance in Visfatin post intervention. Visfatin is a multi functioning protein that acts as a hormone, cytokine and enzyme. The enzyme version of Visfatin is known as Nampt and it is this enzyme that is essential for

beta cell functioning (Rongvaux et al., 2002). The results of the present study suggest that the reduction in fasting insulin secretion following exercise training may down regulate the production of Visfatin. Visfatin is reported to improve insulin sensitivity but its circulating concentration increases with greater levels of glucose intolerance (Dogru et al., 2007). This is possibly a compensatory response to diminished insulin function (Sun et al., 2007). Thus improvements in insulin sensitivity and glucose tolerance post training in the present study may have resulted in the suppression of Visfatin and thus a reduction in the circulating concentration of this hormone. These findings support those of the ECC group which indicated that the percent change in AUCG predicted 67.6% of the variance in Visfatin. They also support the findings of the REG group which revealed that the percent change in RQ and RMR predicted 91% of the variance in Visfatin. The reduction in RQ represents improved fat metabolism which has important implications for insulin sensitivity and glucose tolerance. No variable best predicted the percent change in Visfatin in the aerobic group. Taken together, it appears that improvements in glucose tolerance and insulin sensitivity regulate the production of Visfatin. Since Visfatin functions to enhance insulin sensitivity it is likely that there is a cyclical relationship between insulin sensitivity and Visfatin.

Backwards stepwise regression was run on FGF21 in the current analysis but surprisingly all combinations of variables were rejected and removed from the models with the exception of Chemerin which predicted 19.2% of the change in FGF21 post intervention. The percent change in FGF21 was best predicted by the percent change in LT and aerobic fitness (93%) in the aerobic group, the percent change in Omentin (70.2%) in the ECC group, and the percent change in Stumvoll (53.4%) in the REG group. Backwards regression was also run on IL-13 in the current analysis but similar to FGF21 all combinations of variables were rejected and removed from the models. The exception was the change in body weight which predicted 22% of the change in IL-13 post intervention. The percent change in IL-13 was best predicted by the percent change in LT (82.4%) in the aerobic group, the percent change in TG's (92.4%) in the ECC group, and the percent change in body weight and aerobic fitness (86.3%) in the REG group. Taking all findings into account it appears that the production of FGF21 and IL-13 are not regulated by one factor but may be influenced by a combination of factors including fitness, body composition, insulin resistance and other biomarkers.

Overall, the findings of the current analysis add support to those of chapters 3 and 4 by suggesting that the regulation of the circulating concentration of the biomarkers is not simplistic. Their production seems to be influenced by each other in addition to improvements in body composition, insulin sensitivity and glucose tolerance. It also appears that they may be differentially regulated by different modes of intervention possibly mediated by the differential changes in body composition.

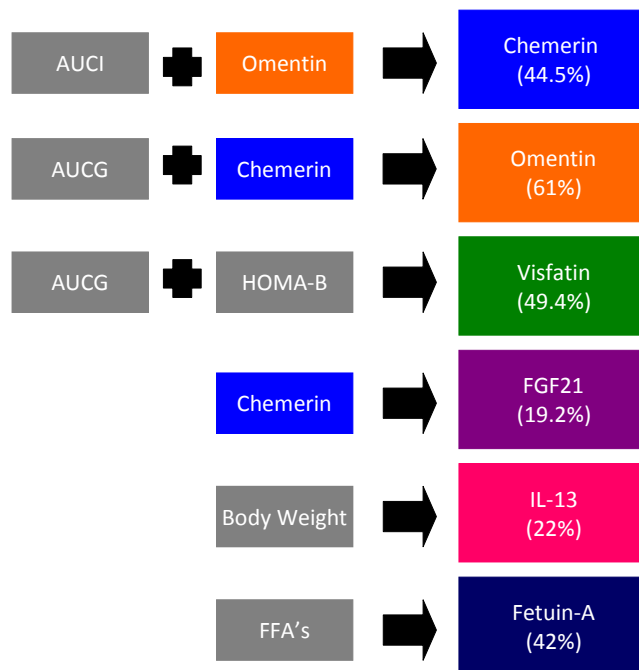


Figure 5.6 Schematic summarising the percent change in the independent variables that predict the percent change in each biomarker post intervention in the current analysis. This clearly shows the cyclical relationship between the biomarkers in addition to the influence of insulin sensitivity and glucose tolerance on the production of the biomarkers.

5. 10. Limitations

Fetuin-A was only measured in a subgroup of this population and so could not be included in the analysis using a larger sample size. There was a slight difference in intervention duration between groups with the aerobic exercise intervention consisting of 12 weeks of training but the ECC and REG interventions consisted of 10 weeks. This could not be avoided with the latter interventions due to end of term examinations but must be noted when considering this analysis. The mode of exercise in each group was slightly different but the interventions were broadly similar with regard to the number of training sessions per week, the intensity of the aerobic exercise, and the duration and energy expenditure of each training session.

5. 11. Summary and Conclusion

This chapter clearly shows that 10-12 weeks of exercise training significantly reduces body weight, BMI, and body fat mass in obese subjects in addition to significantly increasing lean tissue mass and aerobic fitness. Exercise training also significantly improves insulin secretion and insulin sensitivity which is evident as a significant reduction in HOMA-B and AUCI and a significant increase in the AUCG/AUCI index. These physical and metabolic adaptations to exercise training are expected but were not all evident in all 3 individual exercise interventions possibly due to small sample size. The individual interventions also suggested that physical adaptations are specific to the mode of intervention undertaken.

The Stumvoll and Matsuda indices of insulin sensitivity did not change following any of the 3 individual exercise interventions or with the greater sample size in the current analysis. These findings suggest that these indices may not be the most comprehensive indicator of metabolic health in obese individuals and it is important to use other measures to support them including HOMA-B and the AUCG/AUCI index. The lipid profile of the subjects also did not change in the current analysis but the improvements in fat oxidation in the ECC and REG groups indicate that fat oxidation is an important measurement to obtain in exercise training interventions to get a true reflection of improvements in lipid metabolism and fuel utilization in this population.

Finally, there was no change in the circulating concentration of the biomarkers in the current analysis. It appears that the regulation of the biomarkers is complex and can not be attributed to a change in any one variable. Many of novel biomarkers are derived from adipose tissue but they are also produced in various other tissues of metabolic importance and the relative contribution of these tissues to the circulating concentration of the biomarkers remains unknown. It is likely that their production is regulated by changes in a combination of variables post exercise training. The literature reports that the biomarkers have a regulatory role in metabolic health but the results of the 3 individual interventions in addition to the current analysis strongly indicate a cyclical relationship between metabolic health and the circulating concentrations of the biomarkers as opposed to a causative relationship. It also appears that the biomarkers play a role in the regulation of each other. The large individual variation in the response of the biomarkers to exercise training suggests that these may be important indicators of metabolic health in larger scale studies. Additional mechanistic studies are required to determine their regulation.

Secondary Analysis

5. 12. Rationale

Lifestyle interventions remain the primary therapy for the treatment of obesity. Both diet and exercise interventions have been reported to successfully produce weight loss in this population, but there is extensive variation in the design of these interventions, making it difficult to compare them and determine which might be the most successful. The purpose of this analysis was to compare the four isocaloric interventions discussed in Chapters 3 and 4 and identify which intervention or combination of interventions was associated with the greatest improvements in body composition and metabolic health in obese subjects. Three of the interventions are those that are typically reported in the literature; diet, aerobic exercise, and aerobic exercise combined with resistance training. The final intervention takes a novel approach to exercise training and energy expenditure and consists of aerobic exercise combined with eccentric resistance training.

5. 13. Aim

The aim of this analysis was to identify the key physiological and biological adaptations to each type of intervention and use these to determine how to maximise the benefits gained by obese individuals from a lifestyle intervention.

5. 14. Objective

To compare the effects of 4 isocaloric interventions on body composition, insulin sensitivity, and the circulating concentrations of the novel biomarkers of insulin resistance.

To compute the most effective combination of dietary restriction and physical activity based on improvements in body composition and changes in biomarker profile in obese subjects.

5. 15. Hypothesis

Dietary restriction and all modes of exercise training lead to distinctive physiological and biological profiles post intervention.

A combination of dietary restriction, aerobic exercise and resistance training will be necessary to maximise the impact on physiological variables and biomarkers of insulin resistance.

5. 16. Study Design

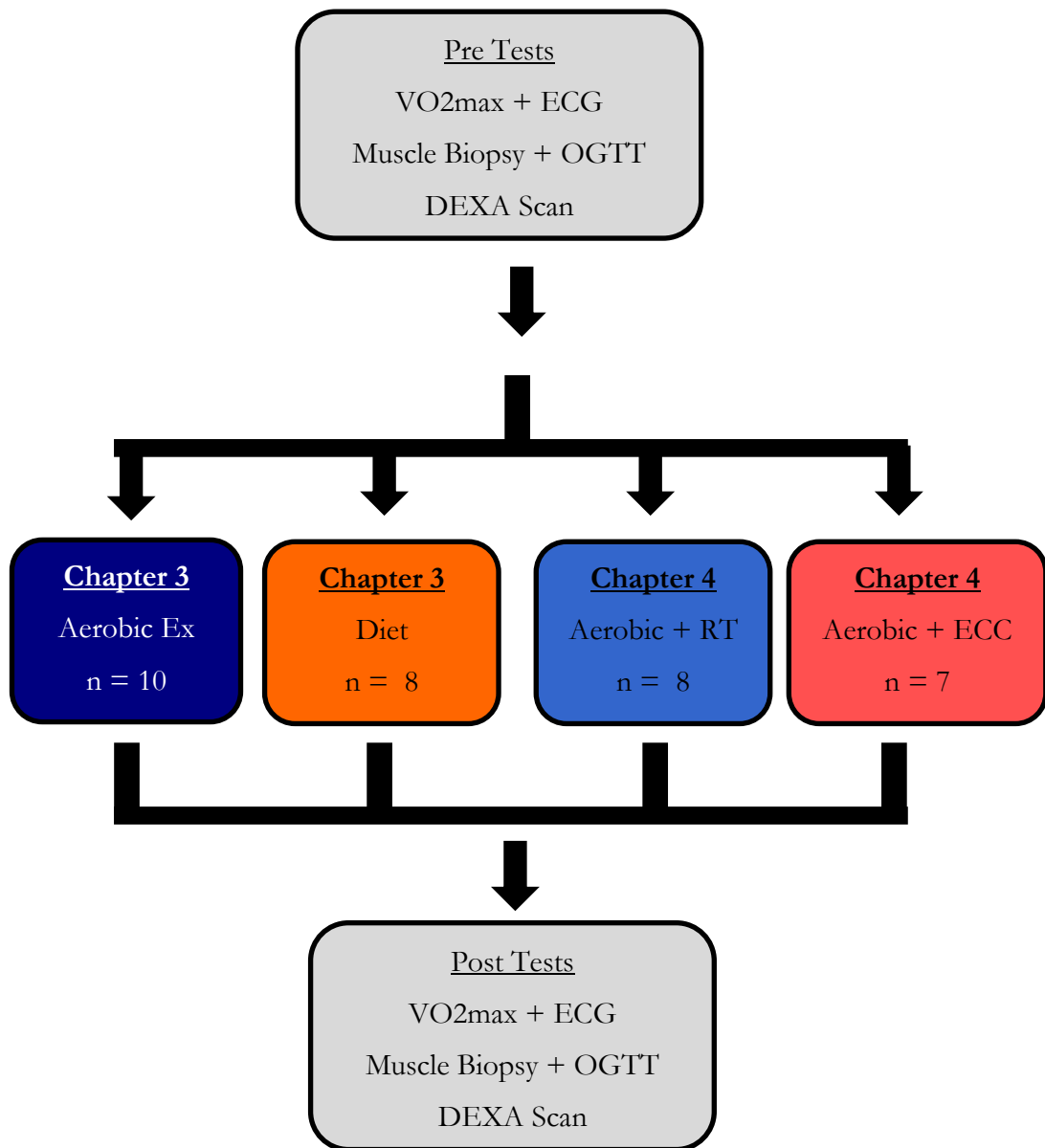


Figure 5.7 Schematic representation of study design

5. 17. Methods

Please refer to chapters 3 and 4 for a detailed description of the tests and procedures and individual study designs outlined in Figure 5.1.

5. 18. Statistical Analysis

A mixed-between-within ANOVA was used to compare the impact of the four different interventions on all measured variables. A summary of these results is presented in Table 5.3, Table 5.6 and Table 5.9.

The first step of this analysis investigates the interaction effect between the type of intervention and time. In particular this analysis investigates whether the change in the dependent variable over time was the same in all four groups. If $p > 0.05$ for the Wilks Lambda test, the change in the variable over time is the same in all four groups and so no difference exists between them. If $p \leq 0.05$ for the Wilks Lambda test, the change that occurred in the variable over time is different between groups and further analysis is required to determine where the differences are, and thus which intervention is associated with the greatest change in that particular variable.

If the change in the variable over time is significantly different between groups, the main effect for time examines the effect of time on the variable within each of the four interventions. If $p \leq 0.05$ for the Wilks Lambda test for this step, this indicates that there is a significant change in the dependent variable post intervention. The partial eta squared value specified the effect size of this result, where .01=small effect, .06=moderate effect, .14=large effect, and any value greater than .14 indicates a very large effect size. A post Hoc test is then used to determine the intervention(s) that this result applies to.

Finally, the between interventions analysis determines whether there is any difference between interventions with regard to their effect on a particular variable. If $p \leq 0.05$ for the between intervention Wilks Lambda test, then there was a difference between interventions. The partial eta squared value confirms the effect size of the change, and a post Hoc test establishes the intervention(s) that this result applies to.

The effect of time on all of the measured variables has already been documented in detail in chapter 3 and 4 of this thesis. Therefore the current chapter will focus primarily on the main effect between interventions analysis in order to determine the intervention(s) that had the greatest favourable impact on body composition and metabolic health in these individuals.

5. 19. Results

The findings of the individual interventions have been presented in detail in chapters 3 and 4. The current results section focuses on a comparison between the 4 groups in the percent change that occurred to the physical characteristics, the metabolic characteristics, and the novel biomarkers of insulin resistance post intervention. The purpose of this analysis is to identify the key physiological and metabolic adaptations to each type of intervention so that this information may be used in the design of future interventions to maximise the benefits gains by obese individuals to a lifestyle intervention.

5. 19. 1. Physical Characteristics

The percent change that occurred to the physical characteristics post all 4 interventions is presented in Table 5.7. The mixed-between within analysis for these physical characteristics is presented in Table 5.8. There was a significant decrease in weight and BMI in the diet and aerobic exercise group post intervention, but there was no difference between groups in the amount of weight lost ($p = 0.36$) or in the reduction in BMI ($p = 0.27$). There was a significant decrease in fat mass in the diet, aerobic exercise, and REG groups, but there was no difference between groups in the amount of fat mass lost ($p = 0.85$). Lean tissue mass increased significantly in the ECC group only but there was no difference between groups in the amount of lean tissue gained ($p = 0.42$). Following exercise training there was a significant increase in fitness in the aerobic and ECC groups. The between intervention analysis revealed that there was a significant difference between these two groups ($p = 0.01$) with quite a large effect size (partial eta squared .330). Post Hoc analysis identified the greatest improvement in fitness to be in the aerobic exercise group ($p = 0.012$).

Table 5.7 Percent change in physical characteristics in all groups post intervention

	Aerobic % change (n=8)	Diet % change (n=10)	ECC % change (n=7)	REG % change (n=8)
Weight (kg)	-2.6 ± 2.5 *	-3.1 ± 2.9 *	-0.6 ± 2.8	-3.1 ± 3.8 †
BMI (kg/m ²)	-2.62 ± 2.46 *	-3.08 ± 2.89 *	-0.60 ± 2.79	-3.09 ± 3.81 †
Fat mass (kg)	-12.15 ± 11.04 *	-5.82 ± 5.05 *	-6.21 ± 8.08	-9.29 ± 8.4 *
% body fat	-9.8 ± 9.1 †	-3.7 ± 4.4	-5.8 ± 6.1 *	-6.5 ± 5.47 *
Lean tissue (kg)	8.62 ± 14.18	1.36 ± 6.34	4.21 ± 4.54 *	2.02 ± 3.16 †
% lean tissue	11.0 ± 12.6 †	3.6 ± 5.1	4.9 ± 5.8 *	5.4 ± 4.6 *
$\dot{V}O_{2\max}$ (ml/kg/min)	29.33 ± 20.70 *	10.14 ± 20.81	10.06 ± 5.54 *	9.02 ± 17.11
$\dot{V}O_{2\max}$ (L/min)	28.30 ± 18.28 *	8.26 ± 21.58	8.69 ± 6.21 *	7.06 ± 11.42

Data is presented as mean ± standard deviation. *p≤0.05 represents a significant change in the variable post intervention. †p<0.09 represents a trend towards a significant change post intervention.

Table 5.8 Summary of mixed-between-within analysis for the physical characteristics

Variable	<u>Interaction Effect</u>		<u>Main Effect for Time Within Intervention</u>		<u>Main Effect Between Interventions</u>	
	<i>P</i>	partial eta squared	<i>P</i>	partial eta squared	<i>P</i>	partial eta squared
Weight	0.28	0.123	<0.001 *	0.402	0.36	0.104
BMI	0.28	0.122	<0.001 *	0.395	0.27	0.126
FM (kgs)	0.40	0.128	<0.001 *	0.526	0.85	0.037
LT (kgs)	0.37	0.136	0.009 *	0.286	0.42	0.112
$\dot{V}O_{2\max}$ (ml)	0.06	0.226	<0.001 *	0.440	0.01 *	0.330
$\dot{V}O_{2\max}$ (l)	0.01 *	0.334	<0.001 *	0.429	0.39	0.097

*p≤0.05 is significant. partial eta squared 0.01=small effect size, 0.06 moderate effect size, 0.14 large effect size, >0.14 very large effect size. Note: BMI = Body Mass Index, FM = Fat Mass, LT = Lean Tissue, $\dot{V}O_{2\max}$ = aerobic fitness.

5. 19. 2. Metabolic Characteristics

The percent change that occurred to the metabolic characteristics post all 4 interventions is presented in Table 5.9. Insulin sensitivity and insulin secretion improved significantly in the aerobic group only post intervention. This was evident as a significant decrease in AUCI and HOMA-B in addition to a significant increase in the ACUG/AUCI index of insulin sensitivity. The mixed-between-within analysis presented in Table 5.10 showed that there was a significant difference between groups in the change that occurred to AUCI post intervention ($p < 0.001$), which had a large effect size (partial eta squared .522). Post hoc analysis revealed that the change in AUCI was greatest in the aerobic exercise group post intervention. However, since the interaction effect between group and time was significant for AUCI ($p < 0.001$), these results must be interpreted with caution. This finding may be influenced by the fact that the baseline AUCI values were higher in this group than in any other group. However, the literature consistently reports that exercise training even in the absence of weight loss improves insulin sensitivity (Holloszy, 2005). There was no significant difference between groups in the change that occurred to the AUCG/AUCI index ($p = 0.13$) or HOMA-B ($p = 0.10$) post intervention.

There was no change in AUCG, or the Stumvoll and Matsuda indices of insulin sensitivity in any group post intervention as outlined in chapters 3 and 4. The between groups analysis showed the change that occurred to AUCG ($p = 0.95$), Stumvoll ($p = 0.51$) or Matsuda ($p = 0.23$) was similar between groups.

There was no change in the lipid profile of the subjects. The main effect of time in the current analysis showed that there was no change in FFA's ($p = 0.46$), TG's ($p = 0.82$), Total cholesterol ($p = 0.68$), LDL cholesterol ($p = 0.84$) or HDL cholesterol ($p = 0.48$) in any group post intervention. The main effect between interventions analysis revealed that there was no difference between groups in the change that occurred to FFA's ($p = 0.32$), TG's ($p = 0.66$), total cholesterol ($p = 0.95$), LDL cholesterol ($p = 0.65$) or HDL cholesterol ($p = 0.15$) post intervention.

Table 5.9 Percent change in the metabolic characteristics in all 4 groups post intervention.

	Aerobic	Diet	ECC	REG
	% change	% change	% change	% change
	(n=8)	(n=10)	(n=7)	(n=8)
AUCG	1.08 ± 14.82	-0.92 ± 14.63	-0.43 ± 16.15	-3.30 ± 18.65
AUCI	-27.40 ± 21.26*	-12.40 ± 25.95	-8.52 ± 14.28	-9.05 ± 23.53
AUCG/AUCI	50.33 ± 46.29*	19.01 ± 25.23	10.46 ± 19.96	10.91 ± 24.75
HOMA-B	-14.12 ± 17.86*	-14.91 ± 39.42	-6.40 ± 28.50	8.88 ± 97.56
Stumvoll	-7.02 ± 7.53	-0.67 ± 7.04	3.69 ± 13.68	1.72 ± 11.36
Matsuda	34.79 ± 49.09	21.16 ± 44.31	14.33 ± 19.64	23.66 ± 52.79
FFA's	4.52 ± 19.42	4.51 ± 55.60	7.70 ± 77.93	-6.96 ± 32.30
TG's	-10.09 ± 32.63	-6.60 ± 11.19	25.48 ± 32.46	-11.91 ± 46.75
TC	1.96 ± 31.15	0.19 ± 6.56	4.39 ± 18.44	-8.89 ± 32.03
LDL	-1.39 ± 32.88	10.14 ± 18.74	2.87 ± 12.17	26.14 ± 57.73
HDL	4.80 ± 26.75	-3.62 ± 15.29	1.06 ± 15.71	30.32 ± 76.50

Data is presented as mean ± standard deviation. *p≤0.05 represents a significant change post intervention. Note: AUCG = Area Under the Glucose Curve, AUCI = Area Under the Insulin Curve, AUCG/AUCI = Area Under the Glucose Curve / Area Under the Insulin Curve (index of insulin sensitivity), HOMA-B is an index of fasting insulin secretion, Stumvoll and Matsuda are indexes of insulin sensitivity, FFA's = Free Fatty Acids, TG's = Triglycerides, TC = Total Cholesterol, LDL = Low Density Lipoprotein Cholesterol, HDL = High Density Lipoprotein Cholesterol.

Table 5.10 Summary of mixed-between-within analysis for the metabolic characteristics

Variable	<u>Interaction Effect</u>		<u>Main Effect for Time</u> <u>Within Intervention</u>		<u>Main Effect Between</u> <u>Interventions</u>	
	<i>P</i>	partial eta squared	<i>P</i>	partial eta squared	<i>P</i>	partial eta squared
AUCG	0.95	0.013	0.45	0.022	0.95	0.013
AUCI	<0.001 *	0.642	0.05 *	0.132	<0.001 *	0.522
AUCG/I	0.94	0.015	0.002 *	0.307	0.13	0.188
HOMA-B	0.86	0.025	0.01 *	0.194	0.10	0.193
Matsuda	0.69	0.052	0.16	0.072	0.23	0.144
Stumvoll	0.14	0.173	0.42	0.023	0.51	0.079
FFA's	0.88	0.024	0.46	0.021	0.32	0.119
TG's	0.26	0.163	0.82	0.002	0.66	0.069
TC	0.93	0.020	0.68	0.008	0.95	0.015
LDL	0.77	0.049	0.84	0.002	0.65	0.070
HDL	0.53	0.094	0.48	0.023	0.15	0.210

* $p \leq 0.05$ is significant. partial eta squared 0.01=small effect size, 0.06 moderate effect size, 0.14 large effect size, >0.14 very large effect size. Note: AUCG = Area Under the Glucose Curve, AUCI = Area Under the Insulin Curve, AUCG/AUCI = Area Under the Glucose Curve / Area Under the Insulin Curve (index of insulin sensitivity), HOMA-B is an index of fasting insulin secretion, Stumvoll and Matsuda are indexes of insulin sensitivity, FFA's = Free Fatty Acids, TG's = Triglycerides, TC = Total Cholesterol, LDL = Low Density Lipoprotein Cholesterol, HDL = High Density Lipoprotein Cholesterol.

5. 19. 3. Novel biomarkers of insulin resistance

The percent change in the circulating concentration of the novel biomarkers post intervention is presented in Table 5.11. A summary of the mixed between within analysis for these characteristics is presented in Table 5.12. The main effect for time analysis demonstrated that there was no change in any group in the circulating concentration of FGF21 ($p=0.22$), IL-13 ($p=0.96$), Omentin ($p=0.77$), or Visfatin ($p=0.49$) post intervention, but Fetuin-A was trending towards a change ($p=0.08$) in the aerobic group. There was a significant change for Chemerin over time ($p=0.04$) in the diet group. The main effect between interventions analysis revealed that there was no difference between

groups in the change that occurred to FGF21 ($p=0.14$), IL-13 ($p=0.07$), Chemerin ($p=0.17$), Omentin ($p=0.11$), Fetuin-A ($p=0.61$) or Visfatin ($p=0.66$) post intervention.

Table 5.11 Percent change in biomarkers in all groups post intervention

	Aerobic % change (n=8)	Diet % change (n=10)	ECC % change (n=7)	REG % change (n=8)
FGF21	28.46 \pm 85.85	-1.10 \pm 81.38	12.32 \pm 68.12	-9.83 \pm 39.56
IL-13	3.06 \pm 23.82	-3.22 \pm 9.43	7.85 \pm 30.84	5.20 \pm 22.72
Chemerin	-3.23 \pm 11.88	-19.22 \pm 16.23	-11.44 \pm 40.73	2.94 \pm 41.60
Omentin	7.06 \pm 36.98	-12.42 \pm 13.85	21.02 \pm 55.87	25.67 \pm 56.13
Visfatin	0.21 \pm 3.37	6.04 \pm 23.65	-8.12 \pm 8.17	-2.94 \pm 22.89
Fetuin-A	-19.79 \pm 13.08*	-4.05 \pm 32.11		

Data is presented as mean \pm standard deviation. * $p \leq 0.05$ represents a significant change post intervention.

Table 5.12 Summary of mixed-between-within analysis for the biomarkers

Variable	<u>Interaction Effect</u>		<u>Main Effect for Time</u>		<u>Main Effect Between</u>	
	<i>P</i>	partial eta squared	<u>Within Intervention</u>		<u>Interventions</u>	
			<i>P</i>	partial eta squared	<i>P</i>	partial eta squared
FGF21	0.42	0.094	0.22	0.052	0.14	0.176
IL-13	0.99	0.005	0.96	0.000	0.07	0.224
Chemerin	0.46	0.094	0.04 *	0.158	0.17	0.173
Omentin	0.39	0.104	0.77	0.003	0.11	0.200
Visfatin	0.64	0.065	0.49	0.019	0.66	0.062
Fetuin-A	0.30	0.077	0.08 †	0.205	0.61	0.019

* $p \leq 0.05$ is significant. † represents a trend towards significance. partial eta squared 0.01=small effect size, 0.06 moderate effect size, 0.14 large effect size, >0.14 very large effect size.

5. 20. Discussion

There are two elements to this discussion. Firstly, the key findings of the diet, aerobic exercise, REG and ECC interventions will be briefly reviewed. Secondly, the key characteristics identified in each intervention will be compared to investigate the intervention or combination of interventions associated with the most favourable changes in body composition and metabolic health in an obese population.

5. 20. 1. Review of key findings from the aerobic, diet, ECC and REG interventions

Chapters 3 and 4 have discussed in detail all of the unique physical and metabolic adaptations that occur following each of the four interventions and so to follow is a brief review of the key findings of these interventions. Isocaloric aerobic exercise and diet interventions yield significant and similar reductions in body weight and BMI in an obese population, but aerobic exercise training is associated with greater reductions in fat mass and greater increases in lean tissue mass in these subjects although the difference between groups did not reach statistical significance. All exercise interventions improve body composition but the adaptations that occur to fat mass and lean tissue mass are specific to the mode of training undertaken. There are greater reductions in fat mass when aerobic exercise is the predominant mode of training (aerobic and REG groups). Conversely there are greater increases in lean tissue mass when resistance training is incorporated into the programme, particularly eccentric resistance training (ECC group). Body weight did not decrease significantly in the ECC and REG groups after 10 weeks of exercise training but the changes that occurred to body composition, which have important implications for metabolic health of obese individuals, support the recommendation that body composition must be a primary outcome measure in lifestyle interventions as opposed to body weight.

Insulin secretion and insulin sensitivity improved significantly following 12 weeks of aerobic exercise training but not caloric restriction despite significant reductions in body weight and body fat mass in this group. In the aerobic groups this was evident as a significant decrease in AUCI and HOMA-B in addition to a significant increase in the AUCG/AUCI index of insulin sensitivity. Importantly, the increase in aerobic fitness post intervention was the single best predictor of the improvements in metabolic status of these obese subjects. Insulin secretion and insulin sensitivity did not improve significantly in the ECC and REG groups post intervention which was a surprising finding since the literature consistently shows that exercise training improves insulin sensitivity even after a single bout (Wahren, Felig et al. 1971) and in the absence of weight loss (Duncan, Perri et al. 2003). However, the favourable changes that did occur to AUCI, HOMA-B and the

AUCG/AUCI index after training were predicted by the favourable change in body composition and the circulating concentrations of the novel biomarkers of insulin resistance. The improvements in these biomarkers suggest amelioration of insulin resistance in these groups. Both groups also demonstrated significant improvements in fat oxidation at rest after exercise training. This is a key finding with clinical significance because there is substantial evidence to show that elevated concentrations of intracellular lipids in obese individuals contribute to the development of insulin resistance by inhibiting insulin stimulated glucose transport into target tissues (Petersen and Shulman, 2002, Shulman, 2000, Yu et al., 2002). Increased fat oxidation post intervention may lead to improved metabolic health in this population in the longer term. Fat oxidation was not measured in the aerobic exercise or diet group but the results of the concurrent interventions suggest that this measure should be taken in obese individuals to assess changes in metabolic flexibility post intervention. Finally, although RMR did not change post ECC or REG intervention, it is clinically important to note that RMR increased by an average of 100kcal per day in the ECC group. An increase in energy expenditure of 100kcal per day in these obese subjects equates to 700kcal per week or approximately 1lb (3500kcal) of body fat in 35 days. Over time, and with continued training, this could accumulate to significant fat loss in this population. The results of the 4 interventions suggest that the adaptations that occur to the metabolic characteristics of obese subjects post intervention are specific to the mode of intervention undertaken. The small numbers in the exercise interventions may mask some of the metabolic adaptations that occurred but the results of the individual studies in addition to chapter 5 where all exercise data was combined, clearly indicate that exercise training improves insulin secretion and insulin sensitivity in obese subjects.

Finally, it appears that the regulation of the biomarkers is complex and not attributed to a change in any single variable post intervention. Their production seems to be differentially regulated by each of the 4 interventions which is evident by the fact that the circulating concentration of Fetuin-A, Omentin and Visfatin only significantly decreased in the aerobic, diet and ECC groups respectively. In the aerobic group the changes that occurred to the biomarkers post intervention were mostly predicted by increases in fitness and lean tissue mass. In the diet, ECC and REG groups, the change that occurred to the circulating concentration of the biomarkers was best predicted by the improvements in insulin sensitivity in addition to improvements in the circulating

concentration of other biomarkers of insulin resistance. The findings of all 4 interventions indicate that there is a cyclical, as opposed to a causative, relationship between metabolic health and the circulating concentration of the biomarkers. Also, the production of the biomarkers appears to be regulated by each other. The small numbers of subjects in these interventions in addition to the large variation in the baseline values of the biomarkers, and the large variation in their individual response to exercise training indicates that the response of these biomarkers to exercise could be highly specific to an individual. It is possible that a much greater number of subjects are required to determine the effects of exercise training on their circulating concentrations, but even with greater numbers it may still be difficult to identify for certain the factors that are responsible for regulating the biomarkers. Additional mechanistic studies are required to determine their regulation.

5. 20. 2. A comparison of the physiological and metabolic adaptations to each of the four interventions

This section of the discussion focuses on the mixed-between-within analysis presented in Tables 5.8, 5.10 and 5.12. The primary aim of this analysis was to compare the changes that occurred in all variables following each intervention and identify the intervention or combination of interventions associated with the most favourable changes to body composition, insulin secretion, insulin sensitivity, and the novel biomarkers of insulin resistance. There was no difference between interventions in their ability to reduce body weight ($p=0.28$), BMI ($p=0.28$) or body fat mass ($p=0.40$), or increase lean tissue mass ($p=0.37$). The clinically significant changes that were evident indicate that the diet and REG group were matched for weight loss with both groups achieving a 3.1% reduction in body weight compared to 2.6% in the aerobic group and 0.6% in the ECC group. Aerobic exercise training was associated with the greatest reduction in fat mass. This group achieve a 12% reduction compared to a 9.3%, 6.2% and 5.8% reduction in the REG, ECC, and diet group respectively. The aerobic group also achieved greater gains in lean tissue mass which increased by 8.6%, compared to 4.2%, 2%, 1.4% in the ECC, REG and diet group respectively. It was expected that the greatest gains in lean tissue mass would be evident in the ECC group but the average body weight of this group at baseline (83.3 ± 12.1 kgs) was lower than all other groups (98.6 ± 20.7 kgs, 99.3 ± 15.5 kgs, and 97.7 ± 22.1 kgs in the aerobic, diet and REG groups respectively), as was the average lean tissue mass of the ECC group at baseline (41.42 ± 5.93 kgs compared to 47.58 ± 12.58 kgs, 52.08 ± 13.09 kgs and 49.48 ± 12.84 kgs in the aerobic, diet and REG groups respectively). This may possibly have contributed to the findings that the percent increase in lean tissue mass was greatest in the aerobic group post intervention. Taking all findings into consideration it appears that 12

weeks of aerobic exercise lead to the greatest improvements in body composition in this population, although this did not reach statistical significance.

There was a significant interaction effect for fitness ($\dot{V}O_{2\max}$ l/min) ($p=0.01$) and the effect size was quite large (partial eta squared = 0.334). This indicates that the change in fitness over time was different in the four groups. A change was identified in the aerobic and ECC group over time but further analysis revealed that there was no difference between groups in the percent change in fitness ($p=0.39$). The actual percent increase in fitness was 29% in the aerobic group, 10.1% in the diet and ECC groups, and 9% in the REG group, demonstrating that aerobic exercise training is associated with the best clinically significant increase in fitness. This is an important finding given the positive relationship between fitness and metabolic health that has been established in this thesis and in the literature (O'Leary, Marchetti et al. 2006; Kadoglou, Vrabas et al. 2012). The 10% increase in fitness in the diet group could be attributed to familiarisation of the $\dot{V}O_{2\max}$ test but also indicates that these subjects subconsciously increased their daily activity levels since they did not take part in structured exercise sessions.

There was no difference between groups in the change that occurred metabolic variables including AUCG ($p=0.95$), AUCG/AUCI ($p=0.94$), HOMA-B ($p=0.86$), Stumvoll ($p=0.14$) and Matsuda ($p=0.69$). There was a significant interaction effect for AUCI ($p<0.001$) with a large effect size (partial eta squared 0.642). The post hoc analysis revealed that the greatest reduction in AUCI occurred in the aerobic exercise group ($p<0.001$) which also had a large effect size (partial eta squared 0.522). This is reflected by a 27.4% reduction in AUCI following aerobic exercise training compared to a 12.4%, 9.05%, 8.5% reduction following the diet, REG and ECC interventions respectively. These findings suggest that aerobic exercise training is associated with the best improvements in insulin secretion and insulin resistance in an obese population but this conclusion must be interpreted with caution since it may be influenced by the fact that the baseline values for insulin in this group were significantly greater than the baseline values in any other group. However, the literature consistently reports that both acute exercise (Wahren, Felig et al. 1971) and chronic aerobic exercise training (Nassis, Papantakou et al. 2005) improves insulin sensitivity in this population, even in the absence of weight loss (Duncan, Perri et al. 2003). These findings also indicate that although insulin secretion and insulin sensitivity did not improve significantly in the diet, ECC and REG groups post intervention, the percent change that did occur suggest important clinical implications for the metabolic health of these subjects.

There was no difference between interventions in the change that occurred to the circulating concentration of FGF21 ($p=0.42$), IL-13 ($p=0.99$), Chemerin ($p=0.46$),

Omentin ($p=0.39$), Visfatin ($p=0.64$), or Fetuin-A ($p=0.30$). The analysis from the previous two chapters of this thesis suggests that the biomarkers respond differently to different modes of intervention and are influenced by improvements in fitness, body composition and the circulating concentration of other biomarkers. Possible explanations for the non significant changes in the biomarkers include the smaller than anticipated sample size in each group, the individual variation in baseline values of the biomarkers, and the wide variation in the individual response to each intervention. It is clear that the production of the biomarkers is complex and not regulated by one variable but respond to an integration of physical and metabolic adaptations to lifestyle intervention. The biomarkers may be more useful independent predictors of metabolic health in larger scale studies.

5. 21. Limitations

The small sample sizes in each group may limit the depth of statistical analysis. Also, this analysis could only compare variables that were measured in all 4 groups, which meant that fat oxidation, resting metabolic rate and Fetuin-A could not be included in the analysis. One final limitation was the difference between groups in intervention duration, 12 weeks for the aerobic and diet groups compared to 10 weeks for the ECC and REG groups.

5. 22. Conclusion

In obese subjects, the physical characteristics, metabolic characteristics and novel biomarkers of insulin resistance are differentially regulated by aerobic exercise training, caloric restriction, concurrent training and concurrent training with an eccentric component. All 4 groups display unique positive adaptations post intervention. When the effectiveness of all interventions is compared there is no difference between groups in their ability to improve body weight or body composition in the obese subjects. One key finding is that aerobic exercise leads to the greatest increase in aerobic fitness and is associated with improvements in insulin secretion, insulin sensitivity and the circulating concentration of the novel biomarkers of insulin resistance. The subjects in the aerobic exercise group were also identified as having the greatest improvement in insulin secretion and insulin sensitivity which was reflected by the greatest reduction in AUCI post intervention. However, this may be due to the fact that the baseline insulin values were significantly higher in this group than in all other groups and so this outcome must be interpreted with caution. There was no difference between groups in the change that occurred to the lipid profile of the subjects post intervention or the other models of insulin sensitivity. This

finding was also true for the circulating concentration of the novel biomarkers of insulin resistance but this may be due to the small numbers of subjects in each group in addition to the large variation in the baseline values of the biomarkers and the large variation in their individual response to diet and different modes of exercise training. It is possible that a much greater number of subjects are required to determine the effects of caloric restriction and exercise training on their circulating concentrations, but even with greater numbers it may still be difficult to identify for certain the factors that are responsible for regulating the biomarkers. Additional mechanistic studies are required to determine their regulation. The key findings to date suggest that obese subjects would obtain maximum physiological and biological benefit from a combined lifestyle intervention.

Chapter 6. Thesis Summary, Conclusion and Future Recommendations

6. 1. Thesis Summary and Main Findings

When all exercise interventions are considered in isolation, the different modes of training differentially affect body composition, insulin sensitivity and the novel biomarkers of insulin resistance as discussed above. However, when all exercise data is combined (Chapter 5) it is clear that exercise training improves body weight, BMI, and all elements of body composition, insulin secretion, and insulin sensitivity. The improvements in metabolic health are solely attributed to increased aerobic fitness.

When the effectiveness of the 4 isocaloric interventions is compared (Chapter 5) there is no difference between groups in their ability to improve physical or metabolic characteristics with the exception of aerobic fitness which improved the most in the subjects in the aerobic exercise group. Additionally, the aerobic exercise group was identified as having the greatest improvements in insulin sensitivity and insulin secretion measured by AUCI. However, this may be due to the fact that the baseline insulin values were significantly higher in this group than in all other groups and so this outcome must be interpreted with caution.

The novel biomarkers of insulin resistance are differentially regulated by diet and exercise and also with different modes of exercise training. The pearson correlation and backwards regression analysis consistently indicate that there is a cyclical, as opposed to a causative, relationship between metabolic health and the circulating concentration of the biomarkers. It also appears that the production of these biomarkers is regulated in some part by each other. The wide inter individual variation in baseline concentrations of the biomarkers in addition and their response to intervention means that these biomarkers might be better indicators of metabolic health in larger scale studies.

6. 2. Recommendations for revised lifestyle intervention

The key characteristics of each of the 4 interventions suggest that obese individuals could obtain maximal physiological and biological benefit from a combined intervention. The findings so far indicate that aerobic exercise training leads to the most clinically significant improvements in body fat mass and fitness. The improvement in fitness is the best predictor of improvements in metabolic health in this population, and when combined with reductions in fat mass favourably influences the production of the novel biomarkers of insulin resistance, particularly those that are derived from adipose tissue. Thus aerobic exercise should form the foundation of exercise prescription for this population. The addition of resistance training is important for the development of lean tissue mass and improving fat oxidation and resting metabolic rate. These adaptations may enhance weight

loss and insulin sensitivity in the longer term and may also improve the circulating concentration of the myokines. The improvement in fat oxidation and resting metabolic rate may be augmented with eccentric resistance training, however since no statistically significant difference was found between the REG group and the ECC group in the change that occurred to these variable, from a practical perspective regular resistance training is recommended but of greater volume and intensity than that used in the REG intervention. Finally, the addition of moderate caloric restriction (500kcal/day) to the exercise prescription may augment fat mass loss and weight loss in these individuals which in turn may augment the metabolic benefits associated with lifestyle intervention. Augmented weight loss may increase motivation and improve adherence to the programme thus ameliorating one of major difficulties with these types of interventions (Franz, VanWormer et al. 2007).

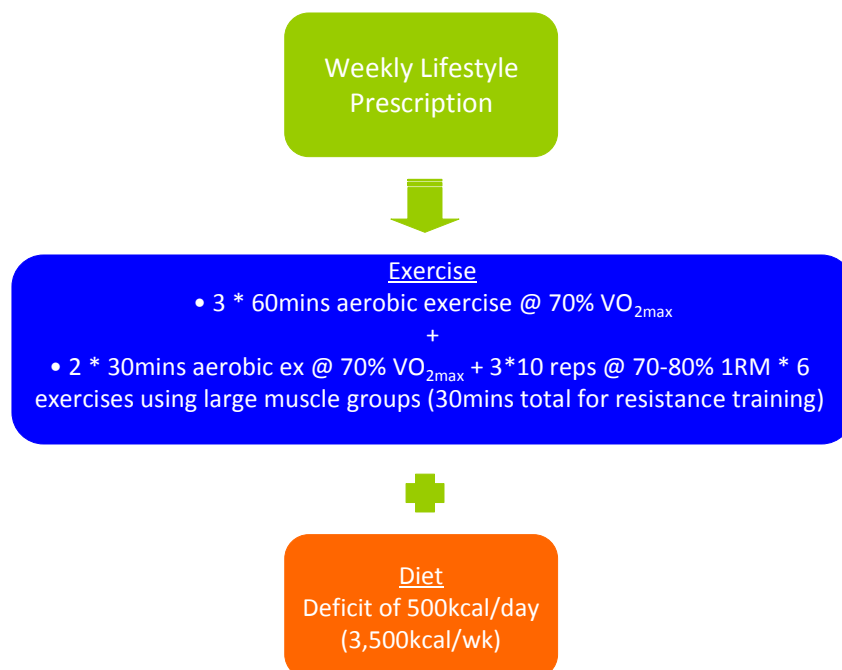


Figure 6.1 Recommended weekly prescription for lifestyle intervention for obese population.

6. 3. Practical application of these recommendations for free living adults / population based obesity interventions

Short term interventions are reported to successfully reduce obesity but one of the well documented difficulties in weight loss programmes is poor long term adherence to the programme and in many cases weight regain (Figure 6.2) (Foster, Wadden et al. 1997).

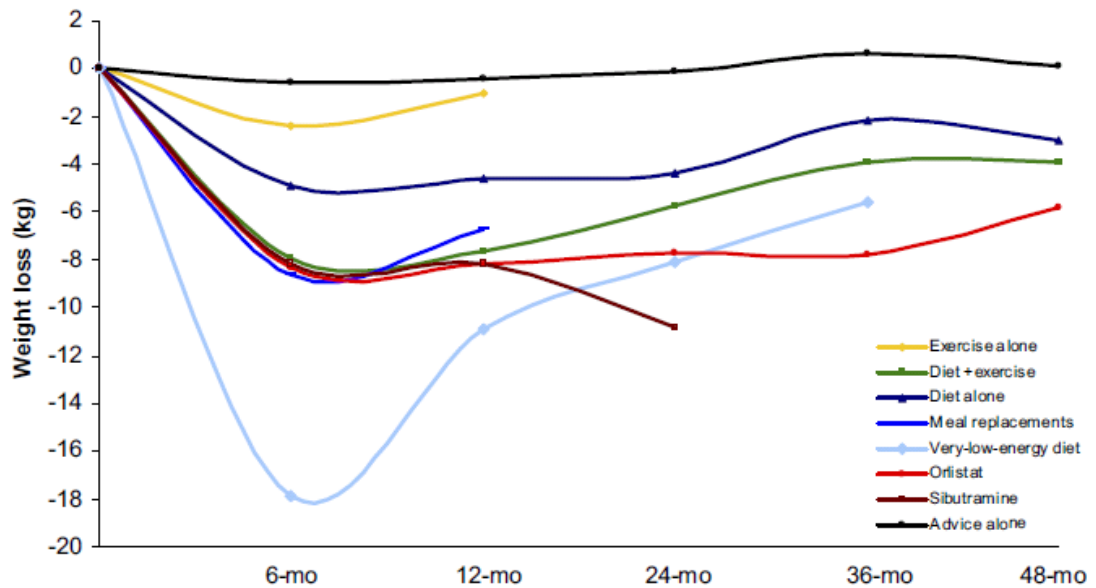


Figure 6.2 Average weight loss of subjects completing a minimum 1 year weight management intervention; based on a review of 80 studies (n=26,455; 18,199 completers (69%), Taken from (Franz, VanWormer et al. 2007).

There is an urgent need to devise a lifestyle intervention that is effective and can be sustained in the long term. To follow, in no particular order, are a number of recommendations that must be considered when implementing population based weight loss programmes which may help to improve adherence and success of outcome measures. These recommendations are presented in Figure 6.3.

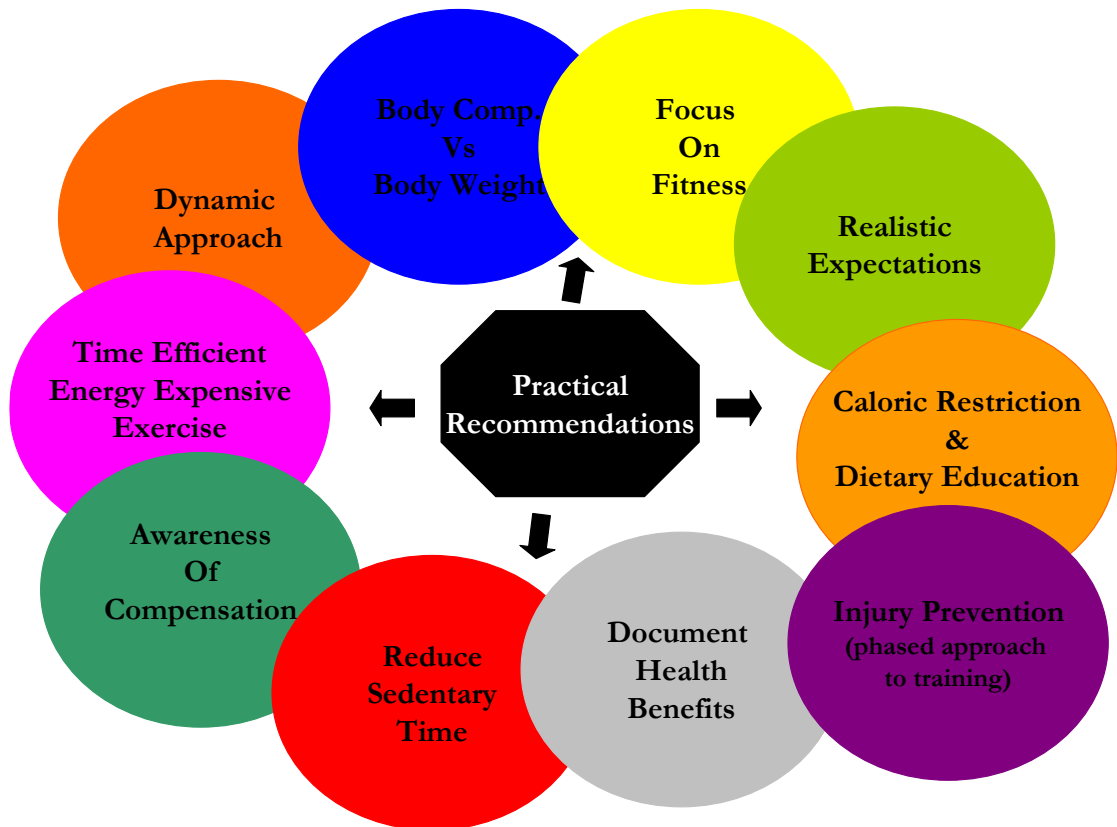


Figure 6.3 Practical elements to consider when devising and delivering community based lifestyle intervention programmes.

6. 3. 1. Realistic Expectations

To improve adherence it is necessary to understand the factors that contribute to poor attrition rates. One explanation that has been proposed is the mismatch between participants expected weight loss and actual weight loss outcomes (Foster, Wadden et al. 1997). The 2001/2002 National Health and Nutrition Examination Survey reported that almost 50% of adults were trying to lose weight in the previous 12 months (Weiss, Galuska et al. 2006). It is likely that this figure has increased over the past 10 years with the growing epidemic of obesity. Yet a review of various types of diet and exercise weight loss interventions has reported that the attrition rate across all interventions was approximately 31% regardless of the length of intervention (Franz, VanWormer et al. 2007). Many of these individuals reported disappointment with their weight loss outcomes (Foster, Wadden et al. 1997). Women taking part in a weight loss programme reported a desired reduction in body weight of 32%. Forty seven percent of women were disappointed with a 16kg weight loss in 48 weeks and deemed the intervention to be unsuccessful (Foster, Wadden et al. 1997). This perception may be affecting attrition rates. To improve perceived success and thus adherence it is necessary to educate participants to set realistic weight loss outcomes. In relation to exercise training interventions it is essential to highlight the energy

expenditure derived from an exercise training session and the energy expenditure actually required to cause substantial weight loss. Many participants engaging in a research based lifestyle programme are required to complete up to 5 training session (5 hours of exercise) per week. This a substantial increase in physical activity for a previously sedentary individual but only equates to a maximum of ~3,500kcal per week, which would yield a reduction in body fat mass of 1lb per week if energy intake is controlled at pre intervention values. Individuals aiming to augment these results must undertake greater volumes of activity and/ or restrict energy intake. It is important to educate participants so that they understand the energy equivalent of exercise training and the effort that is required to achieve substantial weight loss thorough exercise training.

6. 3. 2. Focus on body composition as a primary outcome measure as opposed to body weight

Significant changes occur to body composition in response to exercise training. Previously inactive muscles undergo hypertrophy with training and so the weight of lean tissue mass in kilograms increases. This occurs alongside significant reductions in fat mass but the net weight loss is often minimal. Participants must be educated with regard to these changes and the effect that these adaptations have on net weight change. It is important to quantify improvements in fat mass and lean tissue in order to give a true indication of intervention success so that participants do not become discouraged with small changes in body weight. This may help to improve adherence. The primary outcome measure of obesity interventions must be body composition as opposed to body weight. Participants should be given body fat targets to achieve and the health benefits associated with these changes must be communicated to the participant.

6. 3. 3. Focus on fitness as a primary outcome measure

There is a strong, independent, graded, and inverse relationship between physical fitness and all cause mortality (Blair, Kohl et al. 1989; Kampert, Blair et al. 1996). Low fitness confers the same risk of mortality to an individual as that of actually having diabetes or cardiovascular disease (Wei, Kampert et al. 1999). In contrast, individuals with high fitness are protected against mortality regardless of their BMI, waist circumference and percent body fat. In support of this one of the key findings of Chapter 6 of this thesis was that improved fitness was solely responsible for the improvements in metabolic health in this obese population. For this reason fitness must be a primary outcome measure of intervention for the obese population (McAuley, Artero et al. 2012) and this can only be achieved through exercise training.

6. 3. 4. Incorporate mild caloric restriction and dietary education

Obesity programmes must incorporate education relating to the nutrient composition and caloric content of convenience foods compared to healthy food. Food diary analysis frequently reveals that obese individuals consume high fat, high sugar, high processed, calorie dense foods. Daily calorie intake is often significantly greater than that actually required by the individual to meet energy needs. It is impossible for moderate amounts of exercise training to compensate for this type of dietary intake. Also, any exercise training that is carried out is very quickly compensated for by poor food choices after the exercise bout. Sixty minutes of moderate intensity exercise yields an energy expenditure of approximately 500-600kcal. However, consumption of as little as 5-6 chocolate biscuits or 2 standard chocolate bars or a slice of pizza above daily energy requirements is sufficient to counteract the energy expended in the training session. The best approach is to combine exercise training with a mild caloric deficit (500kcal per day) and education with regard to appropriate food choices. A combined approach will lead to greater reductions in body fat mass in a shorter period of time which may improve motivation and enhance adherence to the programme.

6. 3. 5. Injury prevention - phased approach to training

It is often the case the previously sedentary individuals progress from doing no exercise in their typical week to completing 4-5 training sessions per week as part of a weight loss programme without a transition phase. This type of approach places extensive overload on a deconditioned individual and may result in musculoskeletal injury. An injury at an early stage of training is an unnecessary obstacle and if severe enough, or perceived to be severe enough, could potentially stop the individual participation in exercise. It is necessary to adopt a phased approach to exercise training starting with 2 sessions per week and progressing to 4-5 or daily if possible. During the initial few weeks when the volume of exercise training is low, particular attention and time should be given to changing dietary habits so that weight loss occurs during this time. Dietary modification should also occur in a progressive manner.

6. 3. 6. Document the health benefits associated with exercise training and lifestyle intervention (possible collaboration between exercise specialists and physicians)

Effort should be made to facilitate the participants understanding of the health benefits associated with exercise training and a healthy lifestyle including the favourable metabolic and cardiovascular adaptations that occur. This could simply be in the form of education, but could be more comprehensive with collaboration between exercise specialists and

physicians. It would be progressive to have blood samples taken at the beginning a lifestyle programme in addition to various time points throughout the year to evaluate changes in parameters such as glucose levels, insulin, lipids, and blood pressure. Favourable changes to these parameters may assist with adherence rates particularly during times of less than desired weight loss or weight plateau. There are of course time, resource and financial implications to this approach but it would be interesting to observe the effects of incorporating a clinical element to weight loss programmes run outside of the research setting.

6. 3. 7. Reduce sedentary time

Daily energy expenditure can be categorised into exercise or non exercise activity thermogenesis (NEAT). NEAT encompasses energy expended in the day excluding structured exercise sessions and significantly contributes to total daily energy expenditure since we are awake for approximately 16 hours per day (Matthews, Chen et al. 2008). Mathews et al (2008) reported that American adults spend on average 7.7 hours of their waking day engaging in sedentary behaviours such as TV watching (Matthews, Chen et al. 2008). Reducing sedentary time or substituting it with more active tasks throughout the day would augment total energy expenditure. Over time this may accumulate to substantial energy deficits and enhanced weight loss. This may also reduce the time required for structured exercise training.

6. 3. 8. Awareness of compensation

Even within tightly controlled weight loss interventions there is wide variation between subjects in the amount of weight lost. One possible explanation is that either consciously or subconsciously the subjects compensate for energy expenditure and negate the energy deficit caused by exercise training (King, Hopkins et al. 2008). Compensatory responses include increased energy intake (Stubbs, Hughes et al. 2004) and / or reduced daily physical activity and energy expenditure in non exercising time (Donnelly, Hill et al. 2003). The latter may be in an attempt to recover from fatigue associated with exercise training. It is crucial to highlight this issue so that participants are aware of compensation and the implications this has for the effectiveness of a lifestyle intervention.

6. 3. 9. Time efficient but energy expensive exercise training modes

It is essential to promote time efficient but energy expensive modes of exercise training. An example of this is home based functional circuit training. Home based activities eliminate travel time associated with GYM based exercise training. The exercises chosen for the circuit should alternate between aerobic and resistance. The results of this PhD suggest that

the mixture of training modes would facilitate reductions in fat mass and simultaneous increases in lean tissue mass. The resistance exercises should be functional and incorporate a number of muscles simultaneously e.g. squat, plank, press ups, as opposed to exercises that isolate a muscle group e.g. bicep curl. There are three key advantages to this type of training (i) every exercise recruits a large number of muscles in the body simultaneously which may improve functionality in every day living (ii) energy expenditure in a given time period may be greater than that achieved by traditional resistance training in a GYM which isolates particular muscles per exercise, (iii) functional training engages the core muscles to stabilise the body as it performs the exercise in contrast to machine based resistance training which support the body during the exercise thus reducing the requirements on the core stabilisers. Finally, high intensity interval training is another type of training that could be investigated for use in this population.

6. 3. 10. Dynamic approach to lifestyle intervention and the treatment of obesity

In order to study the response of obese individuals to a lifestyle intervention in a research setting, these interventions must be tightly controlled, monitored and supervised for their duration. For the participant who has enrolled in the study, the intervention is a priority for that time period and they have signed a consent form committing to completing the intervention and not deviating from the experimental design. The reality of long term weight loss in free living adults is quite different. They are not as intensely monitored, their lifestyles are not as controlled, and lifestyle change is not their only priority. The practicality of long term weight loss is that obstacles arise every day that must be overcome in order to prevent weight regain and drop out. Examples of these obstacles include lack of social support, will power, planning, organisation, general knowledge of food, general knowledge of effective exercise training, acquiring minor injuries due to continuous heavy loading on a deconditioned musculoskeletal system, stressful periods in their lives when healthy food preparation and exercise training are not a priority, and plateaus in weight loss despite continued exercise training, which significantly diminishes motivation. The reality of long term population based weight loss interventions is that they require a very dynamic approach. The professional delivering the programme must be knowledgeable, skilled and flexible enough to adapt to all of these obstacles including injury and illness, and alter lifestyle prescription accordingly. With the increasing prevalence of clinical conditions, they must be able to adapt lifestyle interventions for special populations including individuals with CVD, T2DM and a range of other clinical conditions. They must be able to continuously apply sufficient overload and recovery so that the participant continues to adapt and progress over a long period of time. A varied programme is also essential to avoid boredom. They must have excellent communication skills so that they can adapt to all of the different personalities that they come in contact with. It is essential for the

participant to trust the professional so that they will adhere to the programme and stick with it through tough times. Participants must be treated individually even within a group setting. There is a need for these experts to deliver such programmes in order for them to be effective in the long term.

6. 4. Thesis Limitations

With regard to intervention 1, the original study was a cross-over design but the complexity of the analysis and the interaction effects led us to exclude the second phase of the intervention. The complex nature of this original design also resulted in a smaller sample size than originally anticipated. For intervention 2, the original study design was a 12 week intervention. However, due to the time required to conduct the extensive baseline testing, the intervention was reduced to 10 weeks to ensure that the post tests could be completed prior to end of semester. This meant that intervention 1 and 2 were not exactly matched for intervention duration which must be taken into account when comparing the effectiveness of each of them. The time commitment required for the extensive testing procedures and training in the ECC and REG interventions also resulted in a smaller sample size than originally recruited. The small sample size affected the dept of statistical analysis. The final limitations was that the DEXA scan was damaged by flooding in year one of data collection which means that body composition analysis is only available for subjects recruited in year 2 of data collection. This also limits the dept of statistical analysis.

6. 5. Thesis Conclusion

The physiological and biological adaptations that occur in response to lifestyle intervention are specific to the mode of intervention undertaken. Diet, aerobic exercise, concurrent training and concurrent training incorporating eccentric exercise all display unique characteristics which when combined could maximise physiological and biological benefits for obese individuals. The key findings of this thesis suggest that body composition as opposed to body weight must be a primary outcome measure for lifestyle intervention due to its relationship with metabolic health in this population. Also obese previously sedentary individuals who undertake an exercise training programme often display significant changes in body composition despite modest changes in body weight which when measured may help to improve adherence to lifestyle intervention. Aerobic fitness must also be a key outcome measure as it has been shown to best predict the improvements in insulin secretion and insulin sensitivity in obese individuals (chapters 3 and 5). The biomarkers

may be useful indicators of metabolic health as they have also been shown to predict the improvements in insulin secretion and insulin sensitivity (chapters 3 and 4). A consistent finding throughout this thesis is that the regulation of the biomarkers is complex and influenced by a combination of variables including changes to body composition, metabolic status and each other. They in turn influence metabolic health demonstrating a cyclical relationship between the circulating concentration of the biomarkers and insulin sensitivity. The large variability in individual response to intervention indicates that larger scale mechanistic studies are required to determine the true impact of lifestyle intervention on their production.

Translating research findings into effective public health programmes has not been successful and changes in the scientific approach to lifestyle intervention is required. A combination of aerobic exercise, resistance training and caloric restriction with education will maximise the physiological and biological benefits for obese individuals as outlined in Figure 6.1. This combined intervention will lead to augmented weight loss and fat loss in addition to gains in lean tissue mass and aerobic fitness. These adaptations in turn will influence metabolic health and the circulating concentration of the novel biomarkers of insulin resistance. Other considerations that must also be taken into account to improve adherence and success include greater volumes of exercise training or reducing sedentary time, taking a dynamic approach to intervention, educating the individual to set realistic goals, and broadening the participants focus from physical changes to health benefits associated with lifestyle intervention.

6. 6. Recommendations for Future Research

Each of the 4 interventions of this thesis displayed key adaptations that were specific to the mode of intervention undertaken. It would be interesting to administer the revised lifestyle intervention outlined in Figure 6.1 to investigate if the combined intervention would yield all of the physiological and biological benefits associated with the individual interventions. The energy expenditure derived from the exercise training in the combined intervention should equate to ~3,500kcal per week, in addition to a caloric deficit of 3,500kcal per week. In comparison to the individual interventions outline in this thesis, this is almost a 3 fold increase in energy deficit per week which should augment the changes to the physical and metabolic characteristics of the obese subjects which may have a greater impact on the circulating concentration of the novel biomarkers of insulin resistance.

It would also be interesting to conduct a community based weight loss intervention incorporating the considerations outlined in Figure 6.3. This could be used for comparison to the controlled study design recommended in Figure 6.1 to determine how effectively research studies translate to population based interventions. Finally, other modes of novel exercise could be studied to determine their effectiveness in reducing obesity including (i) functional training and (ii) high intensity interval training.

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Appendices

MANAGEMENT PERSPECTIVE

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Exercise and Type 2 diabetes: the metabolic benefits and challenges

Diane Cooper¹ & Donal J O'Gorman^{1,2}



Practice Points

- Despite the known benefits of exercise there is a need to develop a personalized approach to optimize the therapeutic effects of exercise prescription. This should cater for the duration of diabetes, glycemic control, lifestyle habits, body fat, initial fitness and complications.
- Reducing sedentary time could be an important alternative to formalized exercise prescription. This takes the focus away from specific intensity, duration and frequency recommendations. The development of accurate monitors to objectively measure spontaneous physical activity and energy expenditure are required to create personalized daily recommendations.
- Diabetes specific exercise programs need to be more widely available and accessible. One of the major barriers is the assessment of cardiovascular risk prior to and during an exercise program. A validated, reliable, submaximal assessment should be developed to increase mass participation in appropriate programs.
- The development of a community based exercise specialist qualification in Europe would increase the availability and effectiveness of programs as well as optimize the impact on patient health.

SUMMARY Regular exercise is known to decrease the risk of all-cause mortality, improve insulin sensitivity and decrease blood glucose in patients with Type 2 diabetes. These improvements have been associated with increased skeletal muscle glucose disposal and a decreased hepatic glucose production. In recent times exercise has been linked to improved cognitive function and a reduced risk of dementia. In addition, the changes in hepatic gene expression are very rapid and suggest a direct impact of exercise. Therefore, the benefits of exercise may be more wide ranging than previously believed. Despite the known benefits it has been a challenge to influence policy makers to incorporate exercise into the management of Type 2 diabetes. This is partly owing to a lack of randomized controlled trials to determine the optimal exercise prescription and the variability in study design. In order to affect an increase in daily physical activity there is a need to develop new screening and monitoring tools to provide individual guidelines.

¹Athlone Institute of Technology, Athlone, Ireland

²Centre for Preventive Medicine, School of Health & Human Performance, Dublin City University, Dublin 9, Ireland

³Author for correspondence: Tel: +353 1700 8060; Fax: +353 1700 8888; donal.ogorman@dcu.ie

The second half of the 20th century witnessed a dramatic increase in the incidence of Type 2 diabetes. It is anticipated that the worldwide prevalence of diabetes will be 300 million by 2025, an exponential increase on the 30 million estimated by the WHO in 1985 [1,2]. During this brief period of time there have been tremendous technological developments that have dramatically changed our lifestyles. In particular it has become clear that decreased daily physical activity is significantly impacting on metabolic regulation. It has been argued that the human genome has evolved to support physical activity and that muscle contraction stimulates the expression of genes responsible for glucose transport, lipid oxidation and mitochondrial biogenesis. The absence of a mechanical stimulus, by exercise, not only decreases energy expenditure but also the expression of these metabolic genes. The consequences of these changes can be directly related to the defects leading to metabolic dysfunction, obesity and Type 2 diabetes [3]. In this context exercise not only provides a valuable preventive and therapeutic strategy for Type 2 diabetes but also a unique model to study the mechanisms of metabolic disease.

Etiology of Type 2 diabetes

The development of Type 2 diabetes is associated with a gradual deterioration in insulin action and insulin secretion [4–6]. A decrease in insulin-stimulated glucose disposal as well as increased hepatic glucose production and adipose tissue lipolysis are the consequences of these metabolic alterations. Normal glycemia can be maintained if the pancreatic β -cells compensate by increasing insulin secretion, but those who develop impaired glucose tolerance (IGT) or Type 2 diabetes fail to produce an adequate insulin response [5–8]. The net outcome of insulin resistance and insulin secretory dysfunction is an increase in the fasting and postprandial glucose concentrations.

A common characteristic of this multitissue metabolic dysfunction is the accumulation of intracellular lipid and/or a decrease in lipid turnover [9]. This is a result of chronic energy imbalance that leads to an increase in adipose tissue mass and circulating nonesterified fatty acids (NEFA) through *de novo* lipogenesis and decreased insulin suppression of lipolysis [10–12]. A short-term increase in circulating NEFAs by intralipid infusion results in a decrease in

whole-body glucose disposal [13–15], an increase in gluconeogenesis [16,17] and a decreased capacity to suppress glycogenolysis, leading to increased hepatic glucose production [17,18]. In addition, elevated NEFAs impair glucose-stimulated insulin secretion in IGT and Type 2 diabetes but not those with normal glucose tolerance [17,19]. However, these metabolic disturbances do not occur when circulating NEFAs are suppressed by acipimox [20,21].

The accumulation of intracellular lipid may not be attributed solely to excess lipid availability but also a decrease in the capacity for substrate oxidation. The number and size of mitochondria are decreased in the skeletal muscle of patients with Type 2 diabetes and long chain fatty acid transport across the mitochondrial membrane is decreased [22]. The nonoxidative metabolism of intracellular triglycerides and their subsequent cytosolic accumulation leads to the generation of lipid intermediaries such as diacylglycerol and ceramides. Both of these have been shown to activate intracellular signaling cascades and impair insulin signaling [9].

Other factors have also been shown to influence insulin sensitivity including adipokines, myokines and inflammation markers to name but a few [23]. A review of these areas is beyond the scope of this article but an emerging area worthy of mention is the potential contribution of neurological impairment to the development of diabetes. There is evidence that dementia is more prevalent in patients with Type 2 diabetes indicating a relationship between metabolism and nervous system function [24]. Koch *et al.* have shown, using whole body and peripheral insulin receptor knockout mice, that central insulin action is involved in the regulation of glucose metabolism and white adipose tissue mass [25]. Tschritter *et al.* have subsequently shown that the cerebrocortical response to hyperinsulinemia is reduced in overweight insulin resistant subjects [26]. There is also evidence that circulating lipids can be sensed by the hypothalamus, and given the importance of this gland to the regulation of energy balance, it is plausible that neuronal insulin resistance may contribute to the development and progression of Type 2 diabetes [27]. It remains to be determined if metabolic dysfunction in cells of the nervous system contributes to the development of Type 2 diabetes and to what extent diabetes contributes to impairment of nervous system function.

Exercise & Type 2 diabetes

Physical inactivity is recognized as an independent risk factor for more than 25 chronic diseases, including Type 2 diabetes [3], and is defined as all movement accumulated during the day. For the purposes of this article, exercise is defined as any structured activity designed to investigate clinical or physiological responses. A low level of physical fitness, as a result of physical inactivity, is associated with a twofold adjusted risk for all-cause mortality in men with Type 2 diabetes [28]. The relationship between fitness and mortality has also been shown in healthy and obese adults [29–31], while other data shows an increased prevalence of cardiovascular risk factors in obese adults and adolescents compared with lean subjects [32]. These associations persist after adjustment for BMI, waist circumference and body fat [30,31]. However, some studies report changes in daily physical activity rather than changes in aerobic fitness or strength. Physical activity is usually quantified by questionnaire or pedometer/accelerometer. While these measures are easier to implement, the subjective reporting by questionnaire and the crude estimates of activity have made it more difficult to interpret the impact of activity on metabolic health. If daily physical activity is to be used to assess the risk of developing Type 2 diabetes or to predict all-cause mortality, then more objective and accurate assessment tools need to be developed. In addition, these tools need to reflect biologically relevant outcomes such as energy expenditure, as will be discussed later.

A meta-analysis of structured exercise training on physical fitness report an 11% increase in maximal oxygen uptake in patients with Type 2 diabetes compared with a 1% decrease in non-exercise controls [33]. On average patients exercised for 49 min, 3.4 times per week for 20 weeks with a broad range of exercise intensities. The analysis revealed that studies adopting higher exercise intensities tended to produce greater increases in physical fitness. Exercise training is known to improve insulin sensitivity [34], glucose tolerance [35] and glycemic control [33] while decreasing fasting insulin concentrations [36]. There are also additional lipid lowering [37], antihypertensive [38], vascular [39] and body mass benefits [40] associated with chronic exercise training. Therefore, exercise has the potential not only to be an effective treatment for Type 2 diabetes but also to reduce the morbidity and mortality associated with the disease.

The benefits of exercise for patients with Type 2 diabetes**■ Glycemic control & insulin sensitivity**

There have been a number of recently published randomized controlled trials assessing the impact of exercise/lifestyle intervention on glycemic control [41–44]. The Italian Diabetes and Exercise Study (IDES) [41] and the Look Action for Health in Diabetes (AHEAD) trial [43] compare standard care with a lifestyle intervention combining exercise and dietary restriction. The IDES found a -0.42% reduction in glycosylated hemoglobin (HbA1c) after 12 months [41] and the Look AHEAD trial report a similar decrease (-0.36%) after 4 years [43]. The benefits of aerobic exercise, resistance training and combined aerobic and resistance exercise on glycemic control has been investigated by the Diabetes Aerobic and Resistance Exercise (DARE) [44] and Health Benefits of Aerobic and Resistance Training in Individuals with Type 2 Diabetes (HART-D) [42] studies. Both studies found that combined aerobic and resistance exercise improved glycemic control. The DARE study found that aerobic exercise and resistance training alone also improved glycemic control but the HART-D study only found an improvement with aerobic training in patients with an initial HbA1c greater than 7%.

Given the importance of exercise training in the management of Type 2 diabetes, the literature contains relatively few randomized controlled trials and there is a high degree of variability in the mode, duration and intensity of exercise training. Despite these difficulties the results from meta-analyses and systematic reviews consistently report a reduction in glycosylated hemoglobin. Boule *et al.* examined 12 aerobic training studies and two resistance training studies that compared an exercise training group to a non-exercise control group [33]. The aerobic training groups exercised 3.4 ± 0.9 times per week for 18 ± 15 weeks. The resistance exercise groups trained 2.5 ± 0.4 times per week (2.5 ± 0.7 sets of 10.0 ± 0.7 exercises with 13 ± 7 repetitions) for 15 ± 10 weeks. The weighted mean post intervention HbA1c was -0.66% lower in the trained versus control groups. There was no significant reduction in body mass following any of these interventions indicating that HbA1c can be improved by exercise independent of weight loss.

These results are similar to those of Thomas *et al.* who report a 0.6% reduction in HbA1c in their meta-analysis of 13 randomized

controlled trials involving 361 participants [45]. The trials lasted from 8 weeks to 1 year and comprised resistance exercise, aerobic exercise or both. The analysis also demonstrated that HbA1c had decreased 0.8% in exercise trials less than 3 months and 0.7% following interventions less than 6 months in duration. Short-duration trials often have more supervised sessions and individual prescriptions and may therefore influence the outcome of these trials. It may also be that compliance is greater in short-term interventions but more difficult with increasing duration.

The improvement in glycemic control is attributed to improved glucose tolerance and insulin sensitivity following exercise training. Holloszy *et al.* normalized or improved glucose tolerance in a group of patients with Type 2 diabetes or those with IGT following a 12-month exercise training program [35]. Perseghin *et al.* have shown improved insulin sensitivity in the insulin resistant offspring of Type 2 diabetes parents after a 6 week aerobic training program [46], while others have shown that 7 days [47,48] or even a single bout of exercise [49–52] improved glucose tolerance and insulin sensitivity in healthy and insulin-resistant individuals. These changes, especially in response to acute or short-term exercise are independent of improvements in oxygen consumption, blood flow or weight reduction indicating that local changes regulate the improvements in glucose uptake and utilization. The training related benefits of exercise can also diminish relatively quickly. Hortobagyi *et al.* reported decreased insulin sensitivity in exercise trained endurance athletes following 14 days of training cessation [53]. Heath *et al.* found a similar result in active men and women following 10 days of detraining, but found that glucose tolerance could be restored following an acute bout of exercise [54].

■ Skeletal muscle glucose disposal & gene expression

The synergistic effect of exercise and insulin on whole body glucose disposal led to the speculation that exercise and insulin may have distinct mechanisms promoting glucose transport [55]. Skeletal muscle accounts for 80% of insulin-mediated glucose disposal and has been the focus of mechanistic studies. Insulin-stimulated and contraction-mediated glucose transport requires the translocation of GLUT-4-containing vesicles to the plasma membrane but the signaling cascades are distinct [56]. Insulin binds

to the α -subunit of its receptor and initiates β -subunit autophosphorylation, IRS-1 docking and increased PI3K activity. Activation of Akt and AS160 are required for GLUT-4 translocation but the distal or final steps have yet to be identified.

In healthy skeletal muscle insulin receptor and IRS-1 phosphorylation [57,58] as well as PI3K activity [57–60] are either unchanged or decreased immediately following exercise. Muscle contraction initiates a distinct set of cellular signaling cascades related to calcium flux (CaMKII), ATP turnover (AMPK) and physiological stress (p38 MAPK). These cascades have been shown to independently increase glucose transport and they can be differentially activated by the intensity of exercise [61]. Therefore, glucose disposal is regulated by nutrient- and contraction-mediated cellular events and emphasize the importance of exercise as a stimulator of glucose transport.

Exercise training is associated with a positive relationship between insulin-stimulated glucose disposal and PI3K activity in healthy skeletal muscle [34,62]. However, acute exercise [63] and short-term training [64] increase insulin-mediated glucose disposal in Type 2 diabetic muscle, independent of PI3K activity. The most important role of exercise in the treatment of Type 2 diabetes may be the regulation of gene and protein expression in skeletal muscle as GLUT-4 protein content, as well as other metabolic regulators, increase following exercise [3,64,65].

The contraction-mediated signaling cascades not only stimulate GLUT-4 translocation but also regulate metabolic gene expression and mitochondrial biogenesis [66,67]. Exercise training also leads to mitochondrial biogenesis including an increase in the number and size of mitochondria, increased Krebs cycle, β -oxidation and oxidative phosphorylation enzyme activity and increased uncoupling proteins and antioxidant production [68–71]. In conjunction with increased myoglobin and hexokinase activity, decreased phosphofructokinase and lactate dehydrogenase activity [68], cellular substrate utilization shifts toward lipid oxidation at rest and during submaximal exercise with decreased lactate production [72]. These data support the contention by Booth *et al.* that the maintenance of normal metabolic function requires skeletal muscle contraction mediated gene expression [3].

The increased oxidative capacity of the muscle cell following exercise training could partly explain the improved insulin sensitivity in

subjects with Type 2 diabetes [73], despite a failure to improve insulin-stimulated intracellular signaling. Intramyocellular lipid (IMCL) is an important substrate during exercise and its proximity to the skeletal muscle mitochondria makes it an ideal source of energy. Interestingly, well-trained athletes, as well as obese and Type 2 diabetic subjects, have increased IMCL content [74]. The structural characteristics and distribution are similar, which make it unlikely that the quantity of IMCL is responsible for insulin resistance [75]. The mechanism responsible has yet to be determined but it has been speculated that the turnover in IMCL content associated with each exercise session, may be an important factor [76]. The reduced mitochondrial function associated with inactivity is reversible [77,78]. A lifestyle modification involving weight loss and increased exercise training in patients with Type 2 diabetes led to an increase in both mitochondrial content and functional capacity as well as improvements in insulin sensitivity [77]. It is not yet clearly understood how exercise increases mitochondrial biogenesis but recently epigenetic modifications and miRNAs have been shown to be important regulators of gene expression. It remains to be determined if exercise, or inactivity, play a role in the regulation of these processes.

The emerging benefits of exercise to patients with Type 2 diabetes

■ Exercise & the liver

The impact of aerobic exercise on hepatic glucose production has been well described [34,79]. These studies demonstrate that, for the same circulating insulin concentration during a hyperinsulinemic-euglycemic clamp, endogenous glucose production rates are lower in exercise-trained subjects. The reduction in hepatic glucose production has been attributed to enhanced insulin suppression of glycogenolysis and gluconeogenesis. Ropelle *et al.* recently performed an insulin tolerance test 8 hours after prolonged aerobic exercise in diet-induced obese rats [80]. They found improved insulin signaling associated with increased liver glycogen, and insulin-stimulated phosphorylation of Akt/protein kinase B and its downstream target FOXO1. In addition, the protein content of gluconeogenic enzymes and the interaction of the metabolic gene coactivator PGC-1 α with FOXO1, which is associated with gluconeogenesis, were reduced. Collectively, these results suggest that the liver plays a direct role in the metabolic improvements attributed to

aerobic exercise and provide a mechanism for the suppression of endogenous glucose production following exercise.

Another recent study by Hoene *et al.*, provides evidence that the hepatic response is similar but much more rapid than skeletal muscle [81]. They found that a 60 min session of aerobic exercise rapidly induces the mRNA of gluconeogenic enzymes and genes associated with lipid oxidation including *PDK4* and *PPARGC1*. This was accompanied by an increase in AMPK phosphorylation and IRS-2 protein content. Following glucose stimulation the tyrosine phosphorylation of IRS-1 and Akt were greater in the exercised animals. The reported increase in gluconeogenic enzyme mRNA reported in this study appears to contradict the findings of Ropelle *et al.* but the timing and stimuli are very different [80]. The immediate response to exercise is often characterized by a gene expression signature reflective of the physiological stress. During exercise the stimulus is for increased glucose production to maintain plasma glucose, but the adaptation to exercise improves insulin signaling and metabolic flexibility and facilitates a more efficient processing of substrate.

The enhanced metabolic functioning following exercise may also be related to changes in intrahepatic lipid content or turnover. In a cross-sectional study of 138 overweight and obese subjects Haufe *et al.* recently reported that aerobic fitness was positively correlated with insulin sensitivity but inversely related to intrahepatic, visceral and total lipid content [82]. The relationship between fitness and insulin sensitivity was maintained after adjusting for visceral and total fat but was no longer evident following adjustment for intrahepatic lipid. These authors suggest that the positive link between fitness and insulin sensitivity is mediated by hepatic lipid content. There is evidence to support a modest reduction of intrahepatic lipid content following exercise training in Type 2 diabetic patients, although there is a need for randomized controlled trials to provide definitive evidence [83]. We have also shown that insulin sensitivity can be increased following short-term exercise training in the absence of increased aerobic fitness or altered body composition [64]. These findings raise a question about the emphasis placed on total and visceral adiposity in the context of adaptations to exercise training, and suggest that a change in intrahepatic lipid may, in fact, be more relevant. Ideally, a decrease in total and

visceral adiposity is desired but a reduction in intrahepatic lipid may confer the same metabolic benefits. If this were the case it may be more advantageous to design an exercise prescription that would optimize the metabolic response in the liver and not necessarily focus on reducing total or visceral adiposity. There is increasing evidence for a direct impact of exercise on hepatic metabolic regulation but further investigations are required. A growing body of literature also supports the role of tissue–tissue cross-talk and communication. Hennige *et al.* have shown that enforced activation of protein kinase C signaling in skeletal muscle causes fatty liver, physical inactivity and brain insulin resistance [84]. Therefore, the primary and secondary causes of metabolic dysfunction are poorly understood but this exciting area of research is likely to reveal a much more comprehensive interaction between tissues.

■ Exercise & the brain

There is a growing body of evidence to support the role of physical activity in the prevention and management of cognitive impairment. Given the increased prevalence of Alzheimer's disease and dementia in Type 2 diabetes, there is a need for randomized controlled trials to investigate the impact of physical activity on cognitive function in these patients. In elderly men and women without cognitive impairment, a greater baseline level of physical activity [85–87] or physical fitness [88] is associated with a lower incidence of cognitive dysfunction. Barnes *et al.* found that a higher baseline aerobic fitness was associated with preservation of global cognitive function, attention, verbal memory and fluency in healthy older adults [88]. Etgen *et al.* followed 3903 participants without cognitive impairment at baseline for 2 years [85]. At follow-up 207 (5.9%) of participants had developed incident cognitive impairment. They report a lower odds ratio for those who exercised one to two times per week (0.57, $p = 0.01$) and ≥ 3 times per week (0.54, $p = 0.005$).

Physical activity has also been associated with a reduction in incident dementia and the relative risk of Alzheimer's disease [89,90]. Podewils *et al.* found 480 incident cases of dementia in 3375 men and women, free from dementia at baseline, following a 5.4 year follow-up [90]. Participants involved in ≥ 4 physical activities per week had a relative risk of 0.51 compared with those who engaged in ≤ 1 activity per week.

Larson *et al.* found the incident rate of dementia was 19.7 per 1000 person years for those who exercise less than three times per week compared with 13.0 per 1000 person years for those exercising ≥ 3 times per week [89]. Lautenschlager *et al.* found a modest improvement in cognitive function after conducting a randomized trial to investigate the effect of 6-month aerobic exercise intervention on cognitive function in subjects with memory problems [91].

The mechanism of exercise-mediated maintenance or enhancement of cognitive function is difficult to investigate but should form an important part of exercise research in the coming decade. Aerobic fitness is associated with reduced brain atrophy [92] and preserved medial temporal lobe volume [93] in patients with Alzheimer's disease. Colcombe *et al.* reported an increase in gray matter volume in the frontal and temporal cortex as well as anterior white matter in older adults who had been randomly assigned to an aerobic exercise intervention compared with a stretching control group [94]. It has also been proposed that exercise may preserve neuronal plasticity and increase synapses and dendritic receptors following injury [95]. Other potential benefits of exercise include an increase in cerebral blood flow [96,97] and influences on endocrine and immune responses [97].

These changes have been investigated in animal models and a number of neurotrophic, growth and signaling molecules have been identified. The expression and function of BDNF has been linked with behavioral improvements while IGF-1 and VEGF have been linked with angiogenesis and neurogenesis [98]. In the context of diabetes it is also noteworthy that exercise improves insulin action in the brain. Flores *et al.* found increased insulin and leptin sensitivity in the hypothalamus of male wistar rats following acute exercise [99]. These changes were associated with reduced food intake and provide a possible mechanism for exercise-mediated appetite suppression.

Exercise prescription

In spite of the evidence supporting a positive role for exercise in the treatment of Type 2 diabetes, there is still uncertainty regarding the optimal exercise prescription. It has been difficult to convince policy makers to fund exercise prescription programs to treat Type 2 diabetes, as they do with dietary or medical interventions, despite the known clinical and socioeconomic benefits.

There are many reasons why this may be the case and it is important that the key issues are debated. The evidence base for developing exercise programs has been strengthened by recently published randomized controlled trials [41–43]. This information should be used to lobby and advocate for diabetes-specific exercise programs. A second issue is that exercise and physical activity specialists often blame others for the lack of specific programs but fail to address the differences in opinion and approach of those implementing exercise research studies and programs. One approach has been to identify the lowest amount of exercise that elicits a positive clinical response, while the other has been to devise an exercise prescription for optimal therapeutic responses. These divergent approaches have led to large variations in study design, exercise intensity and frequency as well as research outcomes. It is reasonable to advocate for either of these approaches so long as the outcomes are quantified and appropriately translated into recommendations. The reality is that no one set of recommendations will be suitable for all patients with Type 2 diabetes. A debate on these issues is warranted and a number of issues should be considered.

In the first instance it is important to clearly define the purpose of the exercise program. This is dependent on a number of factors including glycemic control, the presence of complications, the duration of diabetes and the age of the patient. In otherwise healthy patients the primary goal of an exercise program, for patients with Type 2 diabetes, should be to improve insulin sensitivity and glycemic control. A secondary goal for these subjects could be to reduce body fat mass, which is also beneficial to insulin sensitivity. Glycemic control has been linked with exercise capacity in a cross-sectional study [100] and exercise training has been shown to reduce HbA1c in subjects treated with oral medications and those who are long standing insulin treated [46,101]. However, most studies report on a patient cohort with an average HbA1c between 7 and 8% [41–44]. The DARE [44] and HART-D [42] studies provide a good example as the former found that aerobic, resistance and combined training improved glycemic control while the HART-D found that only combined exercise lowered HbA1c. A subgroup analysis of the HART-D subjects found that aerobic training significantly lowered HbA1c in those who had an initial value greater than 7%. Therefore, it is arguable that exercise recommendations should also consider the baseline glycemic control.

However, it is possible that other factors such as the exercise stimulus, the duration of diabetes, gender and ethnic balance, and the management of medications could also influence the outcomes. The added difficulty in studying poorly controlled subjects with Type 2 diabetes is that they are more likely to have exercise-limiting complications. Therefore, the exercise prescription has to be modified, usually by decreasing the exercise intensity or limiting the type of exercise. These changes are necessary to ensure that secondary complications associated with high intensity exercise do not further the damage caused by retinopathy or peripheral neuropathy. As exercise, especially resistance training, increases systolic blood pressure it is possible that small vessels may hemorrhage during such activities. This does not mean exercise is ineffective in these populations but the magnitude of the response will be reduced.

The current exercise recommendations do not cater for the variety of factors that impact on a patient with diabetes. The most recent American Diabetes Association and American College of Sports Medicine guidelines recommend that individuals with Type 2 diabetes engage in at least 150 min of moderate intensity (40–60% of VO_2max) aerobic activity per week and suggest that additional benefits may be obtained from more vigorous intensity aerobic activity (>60% of VO_2max) [102]. In the absence of contraindications, resistance training at least twice but ideally three times per week should also be performed. The resistance training sessions should consist of at least five to ten exercises targeting all of the major muscle groups. A minimum of one set, but as many as three to four sets of ten to 15 repetitions to near fatigue should be performed. Over time the resistance should be increased so that only eight to ten repetitions can be performed. A combination of aerobic and resistance training three times per week may be of greater benefit to individuals with Type 2 diabetes than either type of training alone in terms of blood glucose control [42]. As the benefits of an acute exercise session on insulin sensitivity are relatively short, it is recommended that exercise sessions should not be separated by more than two consecutive rest days. These guidelines are very similar to those for the general healthy population and, as minimal recommendations, do not address the specific issues related to diabetes. Praet and van Loon have made a significant contribution

to exercise prescription guidelines with their review article [103]. They offer guidelines and recommendations for patients based on the duration of diabetes, the initial fitness level of the patient, their BMI and the length of time they have been training. In addition, they integrate aerobic, resistance and interval exercise recommendations. This template is more comprehensive and should form the basis for developing a detailed exercise prescription.

The vast majority of research studies investigating the effect of exercise on patients with Type 2 diabetes are not designed to optimize exercise recommendations. This is acknowledged by the authors of meta-analyses who highlight the diverse range of exercise frequency, intensity and duration in published articles [33,45]. A recent review of the impact of training modalities on clinical outcomes found that there were no studies investigating the impact of:

- Training session volume/duration;
- Training frequency;
- High intensity interval exercise in patients with Type 2 diabetes [104].

Therefore, many of the guiding principles for exercise prescription are derived as secondary outcomes from research studies. A good example of this is the recommendation that exercise be conducted on nonconsecutive days. This recommendation is based on the impact of an acute exercise session on insulin sensitivity. Unlike other treatment modalities, exercise can improve insulin sensitivity after a single session [46,49,63]. This effect is transient and lasts for 12–40 h following exercise [54,105]. While this has raised very interesting mechanistic questions it also suggests that patients with Type 2 diabetes should exercise at least every second day to maintain and continue to improve insulin sensitivity.

An important challenge in devising an exercise prescription for patients with Type 2 diabetes is assessing cardiovascular risk. In most cases patients with Type 2 diabetes are recommended to have a stress test prior to commencing an exercise program. While it is important to minimize risk to the patient this guideline is not practical and potentially limits the therapeutic use of exercise. In the absence of a stress test clinicians are likely to adopt a more cautious approach and not an optimal exercise prescription for patients. It is important that a screening tool and/or exercise test be developed to

assess and monitor patients with Type 2 diabetes in nonclinical settings. This could possibly include a submaximal exercise test with an upper threshold for diastolic and systolic blood pressure. It should also be possible to create an algorithm to predict risk during an exercise test based on heart rate, breathing rate, diabetes duration, glycemic control, and history of cardiovascular disease.

The current exercise recommendations for patients with Type 2 diabetes have been derived from research focused on determining the minimal intensity and duration of exercise to improve glycemic control. However, it appears that the minimal recommendations may not be sufficient to prevent weight gain. As most patients with Type 2 diabetes are overweight or obese, evidence suggests that they may gain weight over time even by adhering to the guidelines. The International Association for the Study of Obesity [106] and the Institute of Medicine [107,108] have concluded that a daily energy expenditure equivalent to approximately 500 kcal is necessary to prevent weight gain. The current recommendations would achieve approximately half of this target and suggest that total daily energy expenditure rather than the intensity and duration of a single-exercise session may achieve the best results. The recent evidence suggesting that physical inactivity may be a better determinant of disease risk would also support this view and further research in this area may significantly change our current views on the role of exercise prescription in the management of Type 2 diabetes [109].

The emphasis on exercise recommendations is being challenged by emerging evidence of a link between sedentary behaviors and health reviewed in reference [110]. The amount of sedentary time is reported to be at least 7.7 h per day or 55% of the measured time from the 2003 to 2004 National Health and Nutrition Examination Survey (NHANES) study [111]. Sedentary time, as determined by accelerometry or self-reported television viewing, is associated with increased fasting insulin [112], decreased glucose tolerance [113], markers of insulin resistance and the metabolic syndrome [114–117]. Recent evidence has shown that a decrease in muscle mass results from the activation of an atrophic signalling cascade and is not merely a reversal of the hypertrophic adaptation to training. Similarly, it is possible that sedentary time promotes cellular and biochemical changes that have a negative

impact on physiological processes and ultimately health. However, it remains to be determined if these associations are independent of physical activity. It is possible that the current recommendations do not provide a sufficient stimulus to overcome the much greater sedentary time and that a greater emphasis needs to be placed on decreasing sedentary time as a strategy to enhance health.

Another argument to reduce the emphasis on exercise prescription is the issue of adherence. It has been reported that long-term adherence to exercise programs can vary from 10 to 80% [117]. A variety of factors may be responsible including time, motivation, readiness to change or adequate support structures. Large-scale exercise studies are very expensive to implement and require a high degree of supervision. The variance in findings from exercise training studies is partly related to the experimental design and verification of exercise adherence [33,45]. The greatest physiological benefits are often reported in studies where exercise sessions are supervised and monitored [33,45]. However, 'real life' application of exercise will require a novel approach and use a combined approach with decreasing physical inactivity. Given the greater benefits resulting from short term, supervised, exercise programs and the sustained benefits reported from lifestyle interventions [43], a periodically structured program (e.g., 3 months per year) may be sufficient to improve glycemic control if daily physical activity is increased. Alternatively, exercise referral programs that have a high degree of variety, social integration and enjoyment could be partly used to meet daily recommendations. The next big step may involve a joining of the two approaches where decreasing sedentary time is supplemented by exercise training sessions to achieve the overall goal of maintaining and improving health.

Conclusion

There have been significant advances in our understanding of the contribution of exercise to the prevention and treatment of Type 2 diabetes but significant challenges remain. In the pathophysiology of Type 2 diabetes, future research should focus on understanding the interaction between the CNS and peripheral metabolism. It will also be important to better understand the exercise training adaptation in noncontracting tissues such as the liver and brain and how miRNA and epigenetic modifications are

influenced by acute and chronic exercise. In a practical and applied setting future research should focus on inactivity physiology and determine if a combination of decreasing sedentary time in conjunction with a prescribed exercise program would produce better adherence and clinical outcomes. Alternatively, daily energy expenditure targets could be used but this will require the development of monitors with greater accuracy and reliability. Specifically addressing exercise prescription there is a need for more studies to determine the impact of exercise mode and intensity on glycemic control. Finally, the future rests in researchers working together to provide novel and varied ways of increasing daily physical activity.

Future perspective

The next decade holds potential for exercise and physical activity research. In practical and applied research the focus is likely to shift from identifying minimal exercise recommendations that confer health-related benefits toward a decrease in sedentary time. The investigation of inactivity physiology will provide an alternative perspective on metabolic regulation and should complement behavior research designed to increase physical activity compliance. The role of prescribed exercise, while still playing an important role in diabetes treatment, will become a more valuable model of disease pathophysiology. In addition to contraction-mediated glucose transport and gene expression, the metabolic regulation of noncontracting tissues such as the liver and brain, following exercise, will provide valuable information on the development and progression of diabetes. It will also be necessary to determine if these changes are direct or secondary effects owing to tissue secreted proteins (e.g., myokines, adipokines). This research on tissue-tissue communication will also identify biomarkers that are responsive to exercise and could be used to predict the onset and progression of diabetes.

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Appendix A2: Published Abstract

Agneiszka Pazderska², Diane Cooper¹, Declan O'Hanlon², Noelle Collura², Shah Syed², Krzysztof Wanic², Fiona Lithander², Prof. John Nolan², Donal O'Gorman¹. 12 weeks aerobic exercise improves intrinsic muscle mitochondrial function in patients with type 2 diabetes. Irish Endocrine Society Meeting, Dublin, Ireland, September 2010.
Dublin City University, Dublin¹, St. James Hospital, Dublin²

Title

12 weeks aerobic exercise improves intrinsic muscle mitochondrial function in patients with type 2 diabetes.

Abstract

Exercise is known to increase maximal oxygen uptake ($\text{VO}_2 \text{ max}$) and improve insulin resistance in patients with type 2 diabetes. Several recent studies have shown that exercise enhances in vivo mitochondrial function. However, it remains unclear whether this improvement is due to an increase in skeletal muscle mitochondrial mass or in intrinsic mitochondrial function. The aim of this study was to assess changes in electron transport capacity in mitochondria isolated from muscle biopsies from patients after an exercise programme.

Six sedentary men with type 2 diabetes (age 41 ± 11.5 ; HbA1c $8.3 \pm 1.7\%$; BMI 34.4 ± 6.7) participated in a 12 weeks aerobic exercise programme consisting of four supervised sessions/week at $70\% \text{VO}_2 \text{ max}$. Muscle biopsies from vastus lateralis were obtained before and after the intervention. High resolution respirometry was used to measure oxygen flux capacity in isolated skeletal muscle mitochondria.

T-test was used for statistical analysis.

$\text{VO}_2 \text{ max}$ improved following the intervention (baseline $\text{VO}_2 \text{ max}$: 2.65 L/min, post exercise: 2.99 L/min; $p=0.012$). Following training, significant increases were observed in oxygen fluxes, expressed per milligram of mitochondrial protein ($\text{pmolO}_2/\text{s}/\text{mg protein}$), stimulated by parallel electron input from complexes I and II in the presence of pyruvate+malate and succinate (188.21 vs. 420.96; $p=0.024$). Similarly, significant increases

were observed in oxygen fluxes in the presence of ADP (506.61 vs. 1527.15; $p=0.007$), as well as in response to uncoupling by FCCP (572.40 protein vs. 1645.18; $p=0.013$).

12 weeks aerobic exercise training leads to improvements in several components of intrinsic mitochondrial function in patients with type 2 diabetes

Appendix A3: Recruitment email for intervention 1 (Chapter 3)

Hello all,

The School of Health and Human Performance at DCU are conducting a research study in January 2010 to investigate how an exercise programme and a dietary programme influences body weight, body fat and the use of energy by the muscles. This project is in conjunction with Prof. John Nolan at St. James's Hospital, Dublin who is looking at similar changes in patients with type 2 diabetes.

This is a 6-month study divided into a 3-month exercise programme and a 3-month dietary programme. The order of these will be randomly assigned. A number of measurements will be made before the study and at the end of each 3-month phase. These include fitness testing on a bicycle, a test for diabetes and a muscle biopsy.

We are looking for males and females aged 18-30 and aged 50-70 years who are overweight and interested in participating in a weight loss programme.

If you would like to hear more about this study or would consider participating, please contact Diane Cooper (Tel: 7008472; e-mail: (diane.cooper2@mail.dcu.ie))

Looking forward to hearing from you.

Kind regards,

Diane.

Appendix A4: Plain Language Statement for intervention 1 (Chapter 3)

Cellular mechanisms of insulin resistance and exercise resistance in early onset type 2 diabetes.

Principal investigator:

Prof. John Nolan	-	jnolan@stjames.ie	01-4162488
Dr. Donal O’Gorman	-	donal.ogorman@dcu.ie	01-7008060

Metabolic Research Unit, St. James’s Hospital Dublin and School of Health and Human Performance, Dublin City University.

This leaflet is intended to explain what the study involves. A number of different procedures will be carried out over a period of about 6 months; from the time consent is taken up until the last tests are carried out. This study will involve three months diet intervention and three months exercise intervention.

The first visit will involve a full medical history and physical examination by a doctor. You will need to be fasting from the night before this visit. Please bring any medications that you are currently taking, to this first visit. We will ask for information about allergies, family medical history and your past medical history, if any. We will weigh you, check your height and then estimate your total body fat composition. Waist, thigh and hip measurements will be taken. An ECG (electrocardiogram, a painless test that takes a few minutes) will be performed to check your heart. You will be asked to give a urine sample to check your kidney function. A 3-hour oral glucose tolerance test will be performed to test your glucose, insulin and lipid levels. A blood sample will also be taken for DNA analysis for the purpose of identifying specific genes that may be associated with diabetes or obesity.

At Visit 2 an MRI scan will be performed. This scan, which is painless, is based on magnetic excitation resulting in signals that can be computed into graphs or images. This will allow us to calculate your body composition, as well as the distribution of muscle and fat. On the same day, you will have an exercise test known as a VO₂max measurement. This test usually takes no more than half an hour. We measure your breathing, and how

your heart rate and blood pressure change while you exercise on an exercise bicycle in our exercise laboratory. You will wear a comfortable facemask and a blood pressure cuff on your arm. The test gives us important information about how well your heart and lungs are working, and estimates your level of physical fitness before your exercise program begins.

At Visit 3 a muscle biopsy will be performed. This procedure involves giving a small injection of local anaesthetic initially, which will anesthetize and numb the area that is to be biopsied. A very small tissue sample from your leg, on the outside of the thigh, will then be taken. The biopsied area may feel slightly uncomfortable for a short period of time after the biopsy. We will clean and dress the site afterwards with a small plaster. There is a very small (< 1:200) risk of local bleeding and infection associated with this procedure, but by using appropriate techniques during this procedure the risk is minimised. The muscle sample will be analysed in various ways to calculate how glucose and other fuels are being used to produce energy in your body.

Following these experimental procedures, you will initially be randomised (by chance) to begin the study either with 3 months of monitored *dietary intervention* or monitored *exercise intervention*:

Dietary intervention

If you are randomised initially to the dietary intervention, this will involve a low fat diet at 2500 calories less than your usual daily diet each week. You will be instructed to maintain a food diary and will be reviewed by a dietician on a 2 weekly basis.

Exercise intervention

If you are randomised initially to the exercise intervention, you will exercise for 1 hour, 4 times per week in the GYM at DCU. This exercise programme will be equivalent to 2500 kcal of energy used per week. It is very important that you attend all of the exercise sessions. The exercise session will be divided into five minutes warm-up, sixty minutes of aerobic exercise and then a five-minute warm down. During the exercise training, we will monitor your heart rate, and blood pressure as before. This will enable us to calculate the correct level of exercise intensity for you. We will also check your fitness level every 4 weeks and change the exercise programme to reflect your improvement in fitness.

We cannot emphasise enough that attendance for the exercise training and compliance with diet is crucial as this is what the study is all about – to see if exercise and diet helps insulin to work better in your body, and to try to find out how it does so. This is the part of the study that requires the most commitment from you and from us, the investigators.

At the end of the first 3 months of intervention (either dietary or exercise), you will have your body weight and blood pressure measured and VO_2 max test repeated. On the following days, you will have the MRI scan, oral glucose tolerance test and muscle biopsy repeated to reassess your response to the above intervention. Similarly, in the following final 3 months, you will be changed over to the other intervention programme (either dietary or exercise). You will have the same measurements and procedures repeated at the end of the study (body weight, blood pressure, MRI scan, VO_2 max test, oral glucose tolerance test and muscle biopsy).

Thank you for reading this information leaflet, please note that it is intended to complement the other information you have received, and we would be delighted to discuss any queries you might have at any time.

Risks to Participants: Exercise does carry a risk of injury, such as a pulled muscle, muscle soreness or in extreme cases abnormal heart rhythm, heart attack or death.

Benefits of participation: After completing the study you will be given a report of your results which will include feedback on the changes, if any, that occur during the research study.

Your confidentiality will be guarded:

- All information we gather will be stored in a secure filing cabinet. The results of the study will be used for a postgraduate project and may be published in academic journals. You will not be identified, as your information will be presented as part of a group. Confidentiality of information provided can only be protected within the limitations of the law. It is possible for data to be subject to subpoena, freedom of information claim or mandated reporting by some professions.
- You may withdraw from the research study at any point. There will be no penalty for withdrawing before all stages of the Research Study have been completed.

Project funding

This project is funded by a grant awarded by the European Foundation for the Study of Diabetes.

If you have concerns about this study and wish to contact an independent person, please contact: The Secretary, Dublin City University Research Ethics Committee, c/o Office of the Vice-President for Research, Dublin City University, Dublin 9. Tel 01-7008000

**If you require further information or are interested in taking part in the study please
contact Diane Cooper on 01 7008472, 087 2388748 or email
diane.cooper2@mail.dcu.ie**

Appendix A 5: Informed Consent Form for Intervention 1 (Chapter 3)

INFORMED CONSENT FORM FOR CLINICAL RESEARCH STUDY

Title of project : Cellular mechanisms of insulin resistance and exercise resistance in early onset type 2 diabetes.

Principal Investigators:

- Prof. John J. Nolan, Metabolic Research Unit, St. James's Hospital, Dublin
- Dr. Donal O'Gorman, School of Health and Human Performance, Dublin City University.

I, _____ understand that I have been asked to participate in a study at the Department of Endocrinology at St James's Hospital and at the School of Health and Human Performance at Dublin City University to determine the influence of exercise and dietary intervention on insulin resistance and muscle metabolism. To determine if I am eligible to participate in the study, I will visit the Metabolic Research Unit in the Department of Endocrinology for screening, including having an oral glucose tolerance test performed. On my second visit, I will have an MRI scan performed to assess my intra-abdominal fat content. I will also have an exercise test called a VO₂ max test to assess my fitness level. Following that, on a different day, I will come in for a muscle biopsy performed by the study physicians. In the next 6 months, I will be randomly assigned to a 3 monthly period of:

- 1) Supervised dietary intervention; which will involve a low fat diet (at 2500 calories less than my usual diet each week).

AND

- 2) Supervised exercise intervention; which will involve 4 sessions a week, equivalent to 2500 kcal of energy used.

During the exercise element I will have my fitness measured every 4 weeks and my blood pressure and heart rate will be monitored during exercise. After each 3 monthly period I

will have each of the baseline tests (oral glucose tolerance test, MRI scan, VO₂ max test, and muscle biopsy) repeated.

Benefits: At the conclusion of the project, I may obtain information about my own exercise capacity. I will also benefit from having a specialised dietary input in this programme. Involvement in this project is designed to improve my physical fitness and to help me lose weight.

Discomforts and Risks:

Blood Draw : The insertion and placement of the catheter should be minimally painful, a slight ache may be felt and a small bruise may appear on the arm. There is also a small risk of infection, but by using the appropriate techniques, this risk is minimal.

VO₂ Max test : Some risks are associated with this test. As subjects approach a maximal effort, they may experience episodes of transient light-headedness, chest discomfort, leg cramps, occasional irregular heartbeats, and abnormal blood pressure response. The risk of coronary event although minor (approximately one occurrence per 15,000 tests) does exist. Even though a regular exercise session may involve some hyperventilation; the aim of the test is to achieve a maximum effort.

Exercise training : Exercise training may lead to muscle tightness, soreness, fatigue, and rarely a pulled muscle. An exercise physiologist and physician will closely monitor the exercise training session.

Muscle biopsy: There will be some minor discomfort during the muscle biopsy procedure, which will be performed under local anaesthesia. There is a small (< 1:200) risk of local bleeding and infection. Due to the local anaesthesia administered, temporary numbness of the skin near the biopsy site may occur. By using appropriate techniques during these procedures the risks outlined above are minimised.

MRI scan of the abdomen: This scan involves a very powerful magnetic field, and therefore any participants with implanted metal devices (such as a pacemaker or prosthetic device) will be excluded from having the scan performed. All jewellery (including dentures and reading glasses) will need to be removed prior to the scan. The scan is painless, however

some people experience a slight claustrophobic sensation while the scan is being performed (which may last for 15 minutes).

Exclusion from participation: I understand that my doctors have told me that I cannot participate in this study if I have had any evidence of heart, liver, gastrointestinal or kidney disease, or if my blood sugar or blood pressure is not well controlled. If I suffer from claustrophobia, I can choose not to have the MRI scan performed.

Alternative treatment: I do not have to take part in this study to be treated. Other treatments are available and the research doctor has discussed this with me.

Confidentiality and Right to Ask Questions: All records associated with my participation in the study will be subject to the usual confidentiality standards applicable to medical records, and in the event of any publication resulting from the research, no personally identifiable information will be disclosed. Confidentiality of information provided can only be protected within the limitations of the law. It is possible for data to be subject to subpoena, freedom of information claim or mandated reporting by some professions.

I have been given the opportunity to ask any questions I may have, and all such questions or enquiries have been answered to my satisfaction. I have been provided with a detailed patient information leaflet explaining each of the stages in the study. If I have any further questions about the study, or my participation in it, I may contact any of the study investigators on a 24 hour basis.

St. James's Hospital

Prof John J. Nolan 01-4162488 W

Nicole Burns 086-1942705 M

Dr Shabahat Shah 086-6039220 M

Dr Krzysztof Wanic

Dublin City University

Dr. Donal O’Gorman 01-7008060

Ms. Diane Cooper 01-7008472

Dr. Davide Susta

Compensation:

I understand that in the event of injury resulting from research, the doctors are covered by standard medical malpractice insurance. No other compensation is available.

Voluntary Participation: I understand that my participation in this study is voluntary, and that I may withdraw from this study at any time by notifying the investigator. My withdrawal from the study, or my refusal to participate, will in no way affect my care or access to medical services. I understand that the trial has ethical approval from St. James’s Hospital and Dublin City University and this is to certify that I consent to participate as a volunteer in this programme of investigation. I understand that I will receive a signed copy of this consent form.

Project funding

I understand that this project is funded by a grant awarded by the European Foundation for the Study of Diabetes.

Volunteer’s signature

Date

Name (Print)

I, the undersigned, have defined and explained the studies involved to the above volunteer.

Investigator's signature

Date

Name (Print)

Witness' Signature

Date

Name

Appendix A6: Medical Questionnaire



Cellular Mechanisms of Insulin and Exercise Resistance in Early Onset Type 2
Diabetes

DATE

CONTACT DETAILS)

Last name:

First name:

Date of birth: Age

Address:

Mobile Work:

Home:

Email Address:

Next of kin

Name Contact tel:

Relationship to you

MEDICAL HISTORY (PHYSICIAN ADMINISTERED)

1. Do you suffer from any of the following (tick box)?	Yes	No
a) High blood pressure (hypertension)	<input type="checkbox"/>	<input type="checkbox"/>
b) Angina i.e. chest pain, neck pain, jaw pain, arm pain or undue breathless on exertion (such as walking fast or walking up a hill)	<input type="checkbox"/>	<input type="checkbox"/>
c) Heart disease of any sort e.g. heart attack blocked blood vessels to the heart abnormal heart rhythm		<input type="checkbox"/> <input type="checkbox"/>
d) Peripheral vascular disease e.g. intermittent claudication (calf pain on walking) stroke	<input type="checkbox"/>	<input type="checkbox"/>
e) Elevated blood cholesterol or triglycerides	<input type="checkbox"/>	<input type="checkbox"/>
f) Diabetes		<input type="checkbox"/> <input type="checkbox"/>

2. Have you ever had any of the following (tick box)?	Yes	No
a) A heart attack	<input type="checkbox"/>	<input type="checkbox"/>
b) Heart surgery	<input type="checkbox"/>	<input type="checkbox"/>
c) An angiogram	<input type="checkbox"/>	<input type="checkbox"/>
d) Insertion of a stent		<input type="checkbox"/> <input type="checkbox"/>
e) Treatment of an irregular heart beat	<input type="checkbox"/>	<input type="checkbox"/>
f) A blackout (loss of consciousness)	<input type="checkbox"/>	<input type="checkbox"/>

**3. Please list any other medical conditions you suffer from
at present or have suffered from in the past**

1. _____
2. _____
3. _____
4. _____

4. List any medications which you are now taking

5. Your family history

Do any of your first degree relatives (parents, brothers, sisters) suffer from any of the following (tick box if yes)?

	Yes	No
a) heart disease	<input type="checkbox"/>	<input type="checkbox"/>
b) high blood pressure	<input type="checkbox"/>	<input type="checkbox"/>
c) diabetes	<input type="checkbox"/>	<input type="checkbox"/>

Has any first degree relative of yours died from heart disease? Yes ☐ No ☐

6. Alcohol / Cigarettes

Do you consume alcohol regularly? Yes ☐ No ☐

If yes, how many units per week? _____

Do you smoke? Yes ☐ No ☐

If yes, how many cigarettes a day? _____

7. Your Exercise Pattern

Do you take part in regular exercise of physical activity ? Yes ☐ No ☐

If yes, give details (how often per week, duration per session)

PHYSICAL EXAMINATION

Blood Pressure ____ / ____ Pulse ____

GENERAL APPEARANCE

Subject looks: Healthy ____ Not healthy ____ Very ill ____

SUMMARY FINDINGS

	Nothing Abnormal Found	Details if Abnormal
HEAD AND NECK	<input type="checkbox"/>	<hr/> <hr/>
CHEST AND LUNGS	<input type="checkbox"/>	<hr/> <hr/>
HEART	<input type="checkbox"/>	<hr/> <hr/>

ABDOMEN

☐

EXTREMITIES

☐

NEUROMUSCULAR

☐

RESTING ECG

Descriptive Analysis:

Rate: _____bpm

Rhythm: _____

Arrhythmias _____

Clinical Impression:

According to the medical history and physical exam, does subject qualify for this research study?

Yes ☐

No ☐

Comments:

Physician's signature

(Date)

Appendix A7: Informed Consent Form for DEXA Scan



DUBLIN CITY UNIVERSITY Informed Consent Form for DEXA Scan

Title:

Cellular mechanisms of insulin resistance and exercise resistance in early onset type 2 diabetes.

Investigators:

Dr. Donal O’Gorman

Prof. John Nolan

Ms. Diane Cooper

This consent form is in addition to the detailed consent form that has already been signed by the participant for the above study. A DEXA scan was not included in the original consent form.

Potential risks to participants from involvement in the Research Study:

I understand that DEXA Scanning involves exposure to a small amount of x-ray radiation, ~2% of that used in a traditional x-ray scan. This is a routine assessment.

Participant – please complete the following (Circle Yes or No for each question)

Do you understand all the information provided here? Yes/No

Have you had an opportunity to ask questions and discuss this study?

Yes/No

Have you received satisfactory answers to all your questions? Yes/No

Signature:

I have read and understood the information in this form. My questions and concerns have been answered by the researchers, and I have a copy of this consent form. Therefore, I consent to undergo a DEXA scan.

Participants Signature:_____

Name in Block Capitals: _____

Witness: _____

Date: _____

Appendix A8: 6-20 Point Borg Scale

Rating of Perceived Exertion (RPE)	
6	No exertion at all
7	Extremely light
8	
9	Very light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard (heavy)
16	
17	Very hard
18	
19	Extremely hard
20	Maximal exertion

Appendix A9: Recruitment email for intervention 2 (Chapter 4)

Hi All,

The School of Health and Human Performance at DCU are conducting a research study in January 2010 to investigate how body weight, body fat and the use of energy by the muscles is influenced by different types of exercise training. We want to conduct a 3-month exercise training programme using two different approaches. For the first 4-weeks all participants will exercise for 4x 1 hour sessions consisting of aerobic exercise, like walking and cycling, as well as approximately 15-minutes of strength exercises. For the last 8 weeks some participants will continue with this programme while others will change the resistance exercise. Instead they will only perform half of the strength exercise, ie they will only let the weight down, but not lift it.

A number of measurements will be made before the study and at the end of each 3-month phase. These include fitness testing, an MRI of the arm and leg, a measure of energy use by the body, a measure of how well the body reacts to insulin, measurement of body fat and a muscle biopsy.

We are looking for males and females aged 18-50 years who are overweight and interested in participating in a weight loss programme. **If you would like to hear more about this study or would consider participating, please contact Diane Cooper (Tel: 7008472; e-mail: diane.cooper2@mail.dcu.ie)**

Kind regards,

Diane.

Appendix A10: Plain Language Statement for Intervention 2 (Chapter 4)

Plain Language Statement

Title: The impact of eccentric exercise training on energy expenditure and metabolism in obese participants.

Principal investigators:

Dr. Donal O’Gorman donal.ogorman@dcu.ie 01-7008060

Dr. Davide Susta davide.susta@dcu.ie

Other investigator:

Ms. Diane Cooper - diane.cooper2@mail.dcu.ie 01-7008472

What is the study about?

Regular exercise has many important benefits for health including weight loss as well as the prevention of diabetes and heart disease. One of the key roles of exercise is to increase the amount of energy we use during the day. This study compares a traditional exercise programme, combining some aerobic (walking) exercise with some strength exercises, to a programme that has a similar amount of aerobic exercise but modifies the strength exercises. We wish to determine if the modified exercise programme is a more effective way of increasing energy used by the body.

What will be involved in the study?

The training study will involve 4x1-hr sessions of exercise each week for 12-weeks. Each of these sessions will involve 45-mins of aerobic exercise (walking on a treadmill, cycling on a stationary bicycle, etc) and 15-mins of strength exercises (lifting a weight with your arms and legs). All participants in the study will follow the same exercise programme for the first 4-weeks. At that time one group will continue with the same exercise programme and the other will have a modified programme for the strength exercises. This group will only perform half of the exercise. For example, if you were doing a bench press (see picture below), you will not lift the weight but you will have to lower it slowly back to your chest. Two people, standing next to you, will take the weight and lift it back up for you to lower it again. Otherwise the programme remains the same.



A number of tests will be carried out before and after the 12-week training programme. The energy your body uses at rest will be measured in the lab by lying down for 1-hr and having the air you breathe out analysed for oxygen and carbon dioxide. Your aerobic fitness and strength will be determined before you start and every 4-weeks during the programme. In order for us to examine the changes that have occurred in the muscle we will take a scan of your arm and leg to measure the amount of muscle you have. We will also take a sample of muscle from your leg so that we can measure the size and number of individual muscle fibres. The amount of fat in your body will be measured by scanning it with a DEXA analyser.

Exercise does carry a risk of injury, such as a pulled muscle, muscle soreness or in extreme cases abnormal heart rhythm, heart attack or death. The benefits of being involved in this study include a detailed assessment of your fitness, your response to exercise and your energy use. All information we gather will be stored in a secure filing cabinet. The results of the study will be used for a postgraduate thesis and may be published in academic journals. You will not be identified, as your information will be presented as part of a group. Confidentiality of information provided can only be protected within the limitations of the law. It is possible for data to be subject to subpoena, freedom of information claim or mandated reporting by some professions. All data will be destroyed after 5 years. Your participation in this research project is voluntary and you may withdraw your consent at any time.

This study is being funded by a research training grant for Ms. Diane Cooper from the Irish Research Council for Science, Engineering and Technology.

If you have concerns about this study and wish to contact an independent person, please contact:

The Secretary, Dublin City University Research Ethics Committee, c/o Office of the Vice-President for Research, Dublin City University, Dublin 9. Tel 01-7008000

Appendix A11: Informed Consent Form for Intervention 2 (Chapter 4)

Informed Consent Form

Title: The impact of eccentric exercise training on energy expenditure and metabolism in obese participants

Principal investigators: Dr. Donal O’Gorman, Dr. Davide Susta
School of Health and Human Performance

Other investigators: Ms. Diane Cooper

Purpose: The purpose of this study is to determine if the amount of weight and body fat lost in response to exercise training could be improved by including strength exercises that accelerate the changes in muscle.

This is what will happen during the research study

1. I will have the purpose of the study, each of the steps involved and the risks of participating in the study explained to me. I will have the opportunity to ask any questions and if I am happy with the answers I will:
 - a. Provide written informed consent for participation in the research project.
 - b. I will then complete a medical history form, which will ask questions about my general health, personal and family health history, smoking, exercise, and dietary habits.
2. Pre- and Post test evaluation
 - a. I will have a number of measurements of my body size and shape. Firstly, my blood pressure will be measured. I will have an electrocardiogram (ECG) done to check my heart. This is a painless, 5 minute procedure that involves lying down and having adhesive pads attached to my arms, legs and chest. My height and weight will be measured in light clothing, and without shoes.
 - b. I understand that any of these procedures or tests may be waived at the discretion of the doctor for the following reasons: (i) I have completed the same or similar step in the past 6 months as part of another research

- protocol at the School of Health and Human Performance; (ii) It has been determined that I am not eligible to participate in this research project; and thus completion of the entire screening process will not be necessary.
- c. The amount of fat and muscle in my body will be measured in two ways. My body fat will be measured using a DEXA scanner. This involves lying on a flat table for about 10-mins. During this time my body will be scanned. The picture taken will be used to determine the amount of fat. I will also have an MRI of my arm and leg. This is also a painless procedure where an image is taken that can be used to measure the size of the muscle. These pictures will be used to see if the amount of muscle in my body increased and the amount of fat decreased following exercise.
 - d. Resting metabolic rate. I will rest by lying down on a bed for 1-hr. The bed will be located in a well-ventilated private semi-darkened room. After 30-mins I will have a clear plastic hood placed over my head. I will still be able to breathe normally as 50 litres of air is drawn through every minute. The air I breathe will be analysed to determine the amount of energy I use at rest.
 - e. On one morning I will come to the lab between 0800h and 0900h after an overnight fast, with only water taken for the previous 10 hours. After resting for 15 minutes, I will have a muscle biopsy taken from my thigh. For the biopsy I will have the area anaesthetised with local anaesthetic, then a small 0.5 cm incision will be made in the skin and a needle inserted briefly into the muscle. A small piece of muscle, less than 0.15 of a gram, will be taken from my leg. The incision is pulled close with sterile strips and my leg will be wrapped snugly with an elastic bandage to maintain pressure. Before I leave I will be given contact information and supplies to change the dressing around the biopsy sites. A muscle biopsy will be taken before the exercise training and following 12 weeks of exercise.
 - f. After the biopsy my metabolism and the way my body uses glucose will be determined. I will have a small tube placed in a vein of my left and right arm. After some baseline blood samples are taken I will have be given some glucose through one tube over 1-min. Blood samples will be taken from the other tube every 2-minutes for the next 10-mins. During the next 10-mins two other blood samples are taken. Then I will be given a small amount of

insulin and blood samples will be taken at 10-,20-,30-, 50-,70- and 160-min after this. The test will take 3-hrs.

3. Exercise capacity and determination of exercise intensity

- a. I will undergo an exercise test designed to measure my fitness, and to evaluate my current physical condition. I understand that I will walk on a treadmill, with the speed or slope getting more difficult every 2 minutes until, fatigue, breathlessness, chest pain and/or symptoms that indicate to the doctor or myself that I should stop exercise. To assess my fitness I will have a mouthpiece similar to a snorkel in my mouth to measure the amount of air I breathe in and out.
- b. For the strength exercises I will be asked to lift a comfortable weight 10 times. The amount of weight will increase gradually every 5-mins until I cannot lift it 3-times. This maximum weight will be used to determine the weight I will lift during the exercise training.

4. Exercise training

- a. I will undertake 4 exercise sessions each week in DCU Sport. Each of these sessions will involve 45-mins of aerobic exercise, like walking on a treadmill, and 15-mins of strength exercises. I understand that all participants will undertake the same programme for the first 4- weeks.
- b. At that time I will be asked to either (i) continue with the same programme or (ii) modify the strength exercises so that I am only lowering the weight and not lifting it. I understand that, if I am part of this group, the weight I lower will be slightly heavier than what I was previously lifting but two people will lift the weight for me and I will lower it slowly.
- c. My fitness on the treadmill and my strength tests will be measured every 4-weeks during the exercise training programme to monitor my performance and to adjust the training programme.

Sometimes there are side effects from performing exercise tests. These side effects are often called risks, and for this project, the risks are:

1. Exercise testing carries with it a very small risk of exercise induced asthma, abnormal heart rhythms, heart attack, or death in less than one in 30,000 patients. The risk of sudden death during exercise for healthy men is 1:15000-18000. Because I will be asked to give a maximum effort, I may experience some muscle soreness in my arms and legs or nausea following the maximal exercise test.
2. I understand that taking the blood sample should be minimally painful but a slight ache may be felt and a small bruise may appear on my arm. There is also a small risk of infection, but by using the appropriate techniques this risk is minimal.
3. I understand that following the glucose injection I may feel flushed with slight nausea for a couple of minutes.
4. I understand that lowering the weight is strenuous and my muscles may feel stiff and sore for a few days after the first few sessions.

There may be benefits from my participation in this study. These are:

1. I will receive a copy of my personal results, body fat and fitness measurements and energy use during exercise.
2. I understand that no other benefits have been promised me.

Participant – please complete the following (Circle Yes or No for each question)

I have read the Plain Language Statement	Yes/No
I understand the information provided	Yes/No
I have had an opportunity to ask questions and discuss this study	Yes/No
I have received satisfactory answers to all my questions	Yes/No

My confidentiality will be guarded:

Dublin City University will protect all the information about me, and my part in this study, within the limitations of the law. My identity or personal information will not be revealed or published. All records associated with my participation in the study will be subject to the usual confidentiality standards applicable to medical records. In addition, the study findings may be presented at scientific meetings and published in a scientific journal and/or as part of a postgraduate thesis, but my identity will not be divulged and only presented as part of a group.

If I have questions about the research project, I am free to call Dr. Donal O’Gorman at 01-7008060.

Taking part in this study is my decision.

I understand that my participation in this study is voluntary and that I may withdraw my consent at any time by notifying any of the investigators. I may also request that my data and samples be removed from the database or storage and destroyed. My withdrawal from this study, or my refusal to participate, will in no way affect my relationship with Dublin City University or my entitlements as a student or staff member. I understand that my participation in this research may be terminated by the investigator without regard to my consent if I am unable or unwilling to comply with the guidelines and procedures explained to me.

This study is being funded by a research training grant for Ms. Diane Cooper from the Irish Research Council for Science, Engineering and Technology.

Signature:

I have read and understood the information in this form. My questions and concerns have been answered by the researchers, and I have a copy of this consent form. Therefore, I consent to take part in this research project

Participants Signature: _____

Name in Block Capitals: _____

Witness: _____

Date: _____

Acknowledgement

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Appendix A12: Post-Biopsy Care and Treatment Instructions

Information for subject:

1. You will be given a supply kit containing some steri-strips, sterile gauze, cotton wool, alcohol wipes, bandage and tegaderm transparent dressing. Inspect the biopsy site regularly.
2. Keep the biopsy incision dry for at least one full day. Do not get it wet in a shower, bathtub, jacuzzi, whirlpool, or go swimming during this time. Use the transparent dressing over the site if you are having a shower.
3. Check the incision several times each day. Change the bandage when necessary with the bandages supplied.
4. Do not permit sweat to get in the incision. This means no saunas, and no vigorous exercise for at least two full days.
5. Do not perform heavy lifting with the legs for at least two full days. When you do return to lifting, begin the training session gradually with light weights.
6. Be sure to have contact with the research team the day after the biopsy and report any unusual feelings or responses since the biopsy.
7. If the incision is painful, take some paracetamol as directed by the manufacturer, apply an ice pack, and elevate the leg. It is not recommended to take Aspirin or Advil since these may allow unnecessary bleeding or bruising.
8. Inspect the biopsy area for swelling, red appearance or infection. If you notice anything unusual please contact the research team.