EFFECTS OF EXERCISE AND DIETARY INTERVENTION ON METABOLIC SYNDROME MARKERS OF INACTIVE PREMENOPAUSAL WOMEN

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CERTIFICATE OF ORIGINALITY

I hereby declare that this submission is my own work and to the best of my knowledge it contains no materials previously published or written by another person, nor material which to a substantial extent has been accepted for the award of any other degree or diploma at UNSW or any other educational institution, except where due acknowledgement is made in the thesis. Any contribution made to the research by others, with whom I have worked at UNSW or elsewhere, is explicitly acknowledged in the thesis.

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ABSTRACT

The effects of exercise and dietary intervention on metabolic syndrome (Mets) markers of inactive premenopausal women were investigated. In Study I, early markers of MetS were examined in young, (21.2 ± 0.3 years), healthy but unfit women (*N* = 66) consuming a processed food diet. A second aim was to examine the relationship between ethnic influences (European versus Chinese) and early markers of MetS (e.g., hyperinsulinemia). Study II compared the hormonal and metabolic responses to steady state exercise (SSE) and high intensity interval exercise (HIIE) in untrained young women (*N* = 18, 21.7 ± 0.8 years). Women were further divided into high and low fasting insulin levels to determine if hyperinsulinemia influenced hormonal and metabolic response to SSE and HIIE. Study III examined the hormonal and metabolic response to a randomized controlled intervention named the **F**ish oil, **E**xercise and **M**editerranean diet (FEM) trial. The FEM trial was conducted with overweight, inactive young (23.5 ± 0.6 years) women (*N* = 56) for 12 weeks. Characteristics of a subset of women (*N* = 34, 23.5 ± 0.7 years) in the FEM trial, non-responders (NRES), who did not lose weight (≤ 1% loss in mass), were also examined.

Metabolic profiles were developed based on body composition, aerobic fitness, blood markers, diet, resting metabolic rate, medical history, blood pressure, and autonomic function. Body composition was measured by skinfolds and girths, bioimpedance (Tanita, Japan), and Dual Energy X-Ray Absorptiometry. Peak oxygen uptake was assessed using an open circuit spirometer, TrueMax 2400 Metabolic Cart. Venepuncture and cannulation techniques were used for collecting blood samples that were also centrifuged and frozen for later analysis.

In Study I young women who were physically unfit and consumed a processed food diet possessed high levels of fasting insulin, HOMA-IR (an insulin resistance

index), and C-reactive protein (CRP). Insulin, $p < .001$, and HOMA-IR, $p < .05$, were significantly greater in Chinese Australians compared to European Australians, whereas plasma CRP levels were significantly, $p < .05$, lower. Significant differences, $p < .05$, existed between the groups with the Chinese Australians possessing lower body composition indices. However, ethnic differences still existed for insulin, HOMA-IR, and CRP after adjusting for body composition. Both groups consumed significantly high protein relative to their body mass. Thus, it appears that hyperinsulinemia is one of the earliest markers of MetS in young inactive females of both European and Asian descent who are unfit and consume a high level of dietary protein.

In Study II, HIIE compared to SSE proved to be more effective at preventing an increase in insulin levels in the two hours after exercise. Resting respiratory quotient (RQ) was significantly lower, $p < .05$, following both HIIE and SSE, whereas plasma glycerol levels were higher, $p = .06$, suggesting greater lipolysis following HIIE. The women were divided by baseline fasting plasma insulin (> 9.98 µIU/ml) into high insulin (HI) and low insulin groups (LI). The fasting plasma human growth hormone levels of the HI women were significantly lower at baseline compared to that of LI women. Baseline RQ was correlated with baseline glycerol, $r = -.54$, $p < .05$. Insulin levels at one hour post HIIE was related to fasting plasma adrenocorticotropic hormone (ACTH) one hour post exercise, $r = .52$, $p < .05$. Fasting plasma leptin at one, $r = .56$, $p < .05$, and two, $r = .53$, $p < .05$, hours post exercise was associated with 2 hour post insulin levels. Interestingly, fasting ACTH was significantly elevated in the 2 hours post exercise in the HI women compared to LI. All diet data between the groups were similar and lipids were in the healthy range with no significant differences between the women possessing high or low fasting plasma insulin. Thus, young women who completed one session of short duration HIIE compared to SSE improved certain aspects of their

metabolic profile (e.g., reduced insulin levels) and enhanced their fat oxidation in the immediate two-hour exercise recovery period.

Following FEM (a 12-week multi-component lifestyle intervention) overweight women recorded significantly lower, $p < .05$, body composition (mass, fat mass, percent body fat, waist circumference), insulin, inflammation (CRP), blood pressure, and lipids. The improvements within the Mediterranean diet (Mediet) were related to the reductions in body weight, fat mass, and insulin. Consumption of saturated fats, legumes, meat, poultry, and egg were also significantly decreased, $p < .05$, following the trial. Adherence to the Mediet and fish oil consumption, measured through a Mediet score, was significantly increased, $p < .001$, and was associated with reduced levels of fat mass, $r = .43$, $p < .05$. Autonomic function (measured by power frequency analysis), aerobic fitness, and fat oxidation were all significantly, $p < .05$, enhanced. Therefore, an intervention incorporating fish oil consumption, HIIE, and Mediet significantly reduced body fat, fasting insulin, inflammatory markers, and some blood lipids.

Interestingly, some women did not lose fat mass following the FEM intervention, despite experiencing significant reductions in insulin, inflammation, waist circumference, blood pressure, and an increase in aerobic power. The major differences between those women who lost fat and those that did not was that the non-responders possessed significantly lower, $p < .05$, systolic blood pressure, lower resting heart rate, and a higher resting RQ.

In summary, in Study I, young, unfit women consuming a processed diet, demonstrated hyperinsulinemia and low grade inflammation. The high levels of fasting insulin suggest that these women are at a higher risk for developing MetS and type 2 diabetes. The results of Study II suggests that one bout of HIIE compared to a longer bout of SSE was more effective at preventing a rise in post-exercise insulin levels.

Finally, results of Study III indicate that a 12-week lifestyle intervention, encompassing HIIE, fish oil ingestion, and a Mediet positively influenced early MetS markers (e.g., hyperinsulinemia), aerobic and anaerobic fitness, low grade inflammation, and body composition in young women.

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[1] I was married in August 2008 and my maiden name is Sarah L Dien

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CHAPTER 1 GENERAL INTRODUCTION

1.1 Rationale

It is estimated that over one million Australians currently possess the cluster of health problems known as Metabolic Syndrome (MetS), whereas another million are undiagnosed (Zimmet, Magliano, Matsuzawa, Alberti, & Shaw, 2005b). In Australia, 29% of Australian adults over the age of 25 years have been diagnosed with MetS (Zimmet et al., 2005b), whereas in the USA it is estimated that 25% of adults have MetS (IDF, 2006; Tjonna, Lee, Rognmo, Stolen, Bye, Haram et al., 2008a). Within the age range of 20-25 years, the prevalence in women varies from 7% in France to 43% in Iran (Eckel, Grundy, & Zimmet, 2005). Factors causing Mets are complex but include a physically inactive lifestyle, an unhealthy diet made up of saturated fat and processed foods, and inherited influences (Eckel et al., 2005; Hu, Manson, Stampfer, Colditz, Liu, Solomon et al., 2001; Lacquemant, Vasseur, Lepretre, & Froguel, 2003; Matia Martin, Lecumberri Pascual, & Calle Pascual, 2007; Minich & Bland, 2008). MetS is considered a significant risk factor for heart disease (Ferrannini, 2006; IDF, 2006; Tjonna et al., 2008a) and type 2 diabetes (T2DM) (Martin, Warram, Krolewski, Bergman, Soeldner, & Kahn, 1992).

Clinical markers of MetS include obesity, dyslipidemia, hypertension, hyperinsulinemia, and high glucose levels (IDF, 2006). Ferrannini and Balkau (2006), have suggested that one of the earliest developments in the MetS is insulin resistance (IR). For example, over a 25-year period, a five-fold increased risk of developing T2DM in individuals with elevated fasting insulin was demonstrated (Martin et al., 1992). Thus, IR is considered one of the earliest markers of MetS and has been found in young adults free of other metabolic abnormalities such as dyslipidemia, high blood pressure, high fasting blood glucose levels, and obesity (Dvorak, DeNino, Ades, &

Poehlman, 1999). However, whether the early occurrence of IR is also accompanied by low fitness levels and an unhealthy diet (e.g., high levels of protein, processed foods, and low levels of anti-oxidants from fruit and vegetables) is undetermined. The contribution of physical inactivity and an unhealthy diet to the early development of IR is important to establish so that interventions such as regular exercise and healthy eating can be initiated.

Unfortunately, regular aerobic exercise such as walking and jogging has not resulted in significant reduction in MetS criteria such as body fat. The standard exercise prescription of 30 minutes of exercise on most days has lead to minimal fat loss (Ballor & Keesey, 1991). Ross and Janssen (2001) have suggested that a dose response relationship exists between exercise and fat loss with the more time spent exercising, the greater the loss. However, it has been shown that long-duration exercise programs have poor adherence rates and are unpopular among sedentary, overweight adults (Inelmen, Toffanello, Enzi, Gasparini, Miotto, Sergi et al., 2005). Thus, with the increasing rise in obesity rates in western countries an effective form of exercise for fat reduction is needed.

High intensity intermittent exercise (HIIE) is an alternative exercise protocol for fat loss that may help combat the current obesity epidemic. HIIE is short in duration and offers benefits to not only body composition but other MetS criteria such as hyperinsulinemia (Tjonna et al., 2008a; Tjonna, Stolen, Bye, Volden, Slordahl, Odegard et al., 2008b; Trapp, Chisholm, Freund, & Boutcher, 2008). From a hyperinsulinemia perspective it has not been determined if HIIE brings about a reduction in IR through an acute, chronic, or an acute/chronic combined effect. For example, insulin levels may go down immediately after HIIE but then rise to pre-exercise levels hours after the last bout of exercise. In contrast, chronic exposure to HIIE may bring about changes in the

insulin signalling pathways resulting in permanent increased in insulin sensitivity as long as participation in HIIE is continued.

An acute bout of aerobic or anaerobic exercise alters lipids, blood pressure, and certain hormones in the hours following (Kraemer, Hakkinen, Newton, McCormick, Nindl, Volek et al., 1998; Thompson, Crouse, Goodpaster, Kelley, Moyna, & Pescatello, 2001; Wideman, Weltman, Hartman, Veldhuis, & Weltman, 2002). Immediately after one hour of HIIE glycerol and lactate was elevated (Trapp, 2006). Also we have found that a reduction in RER in the 60 minutes following HIIE occurred indicating an elevation of fat oxidation (Trapp, 2006). Other hormonal and metabolic changes brought about by an acute bout of HIIE (e.g., insulin, leptin, ghrelin, cortisol, ACTH) appear to have been unexamined.

With regard to chronic HIIE, previous research in our laboratory has compared the effect of 15 weeks of steady state exercise (SSE) and HIIE on fat loss. The women in the HIIE program had significantly greater reductions in body fat and fasting insulin compared to the women on the SSE regimen (Trapp et al., 2008). Also a study examining MetS patients found that after 16 weeks of HIIE and SSE, insulin sensitivity and glucose were improved along with enhanced endothelial function, and decreased body mass in the HIIE group only (Tjonna et al., 2008a). Thus, there is evidence to suggest that exposure to chronic HIIE produces significant reductions in body composition, fasting insulin levels, and improved endothelial function.

Different HIIE protocols have also been investigated within a variety of healthy and diseased populations for metabolic and cardiovascular improvements (Babraj, Vollaard, Keast, Guppy, Cottrell, & Timmons, 2009; Boudou, Sobngwi, Mauvais-Jarvis, Vexiau, & Gautier, 2003; Gibala & McGee, 2008; Mourier, Gautier, De Kerviler, Bigard, Villette, Garnier et al., 1997; Talanian, Galloway, Heigenhauser,

Bonen, & Spriet, 2007; Tremblay, Simoneau, & Bouchard, 1994; Wisloff, Stoylen, Loennechen, Bruvold, Rognmo, Haram et al., 2007). Increased lipid utilization and muscular adaptations have been shown to occur after only two weeks of HIIE (Burgomaster, Hughes, Heigenhauser, Bradwell, & Gibala, 2005; Talanian et al., 2007). Following more prolonged HIIE training programs, reductions in fat mass, enhanced insulin sensitivity, and increased fitness have been demonstrated (Mourier et al., 1997; Tjonna et al., 2008a). Collectively, these results indicate that acute HIIE can decreased lipids and blood pressure and increase in glycerol levels during the immediate hours post exercise, whereas chronic exposure to HIIE can lead to reductions in body fat and decreases in IR. The influence of acute HIIE on insulin response, however, appears to be undetermined. Also the mechanisms underlying both acute and chronic effects of HIIE have not been identified.

It also has not been determined if the addition of diet modification to a HIIE program produces an enhanced synergistic effect on fat loss, IR, and metabolic health. For example, after eating a Mediterranean diet (Mediet), high in fibre, omega-3 fatty acids, fruits and vegetables, and low in red meat, saturated and trans fats, IR was reduced (Chrysohoou, Panagiotakos, Pitsavos, Das, & Stefanadis, 2004; Esposito, Marfella, Ciotola, Di Palo, Giugliano, Giugliano et al., 2004). After two years, weight decreased in the intervention group, whereas controls showed no change. In addition, after controlling for weight loss, inflammatory markers [interleukin-6 (IL-6) and C-reactive protein (CRP)] declined more in the intervention group and MetS prevalence was reduced by half (Esposito et al., 2004). Chrysohoou et al. (2004) also showed that participants whose diet resembled Mediterranean eating possessed lower CRP and IL-6, compared to those whose diets did not adhere to Mediterranean type eating.

With regard to cardiovascular health the positive effects of ω-3 fatty acids, natural antioxidants, ethanol, polyphenols, oleic acid, and folic acid, which are all basic nutrients of the Mediterranean diet, have also been demonstrated (de Lorgeril & Salen, 2006a). Olive oil's high monounsaturated fatty acid content has been shown to improve the lipid profile and reduce cardiovascular risk by lowering low density lipoprotein and increasing high density lipoprotein (de Lorgeril & Salen, 2006a). Monounsaturated fatty acid intake has also been related to lower plasma concentrations of glucose and insulin (de Lorgeril & Salen, 2006a). Also this diet has been shown to be cardioprotective for those not living in the Mediterranean regions (de Lorgeril, 1998).

The ingestion of ω-3 fatty acids in the form of fish oils also has beneficial effects on inflammatory responses, lipids, blood pressure, and vascular function (Engler & Engler, 2006). The effects of ω-3 fatty acids on decreasing inflammation could help to decrease the burden of the MetS (Carpentier, Portois, & Malaisse, 2006). With regards to fish oil's effect on blood lipids, the most consistent finding is a significant reduction in triglycerides (Aligeti, Gandhi, Braden, Rezk, & Elam, 2007; Harris, 1996, 1997; Howe, Clifton, & James, 1999). HDL cholesterol levels have been shown to be elevated with fish oil supplementation (Mori, 1999), whereas fish oil supplementation has also been shown to lower blood pressure (Engler, Engler, Kroetz, Boswell, Neeley, & Krassner, 1999).

Increased intakes or supplements of ω-3 fatty acids in fish oil may also improve insulin signalling defects, prevent alterations in glucose homeostasis, and decrease the risk of developing type 2 diabetes (Engler & Engler, 2006). This effect is probably mediated through a reduction in fatty acid accumulation in muscle and liver (Carpentier et al., 2006) and reduced hepatic triglyceride synthesis and release (Krebs, Browning, McLean, Rothwell, Mishra, Moore et al., 2006b). Fish oil supplementation had been

shown to decrease the insulin response to an oral glucose load due by increasing insulin sensitivity (Delarue, Couet, Cohen, Brechot, Antoine, & Lamisse, 1996). Thus, ω-3 polyunsaturated fatty acids present in fish oil may provide a valuable nutritional tool for preventing or diminishing the muscular insulin resistance that is associated with obesity.

It can be seen from the above that fish oil offers many metabolic benefits in reducing risks for the MetS probably through its effects on inflammation and insulin. It has also been shown that ω-3 fatty acids (ω-3 PUFA) ingestion results in lower glucose oxidation and higher fat oxidation (Davidson, 2006; Delarue et al., 1996), suggesting it could be of use in weight loss programs. For example, results of a randomized control trial showed that fish oil was effective in fat mass reduction when combined with exercise (Hill, Buckley, Murphy, & Howe, 2007). Authors suggested that results could be due to the vasodilatory effects of fish oil, improving blood flow and fatty acid delivery to muscle during exercise (Hill et al., 2007). Several features of the MetS may be improved by increased intakes of ω-3 PUFA (Carpentier et al., 2006). However, it has been suggested that fish oil supplementation should be part of a global approach that involves an adaptation of a Mediet and regular exercise to target MetS markers (Carpentier et al., 2006).

In summary, there is evidence to support the notion that IR is one of the earliest changes to occur with the development of MetS. However, if the development of IR in young female adults is also accompanied by physical inactivity and an unhealthy diet is undetermined. It has also been shown that chronic HIIE is a more effective exercise strategy for bringing about improvements in IR and fat loss. It has not been established, however, if these insulin changes are influenced by the acute effects of HIIE. For example, insulin levels may be reduced immediately after HIIE but may return to their pre-exercise level days after exercise. It has also been shown that ingesting a fruit and

vegetable diet such as the Mediterranean eating plan can reduce insulin resistance and low grade inflammation and can aid fat loss. The ingestion of ω-3 fatty acids in the form of fish oils has also produced similar affects to those found for the Mediterranean diet. However, the combined effects of HIIE, the Mediterranean diet, and fish oil ingestion on fat loss and metabolic health have not been examined. Consequently, the focus of this thesis will include: an examination of characteristics of the early development of insulin resistance; an investigation into the effects of both acute and chronic HIIE on Mets markers; and an examination of the synergistic effect of the combination of chronic HIIE, a Mediterranean diet, and regular fish oil ingestion on fat loss and IR.

1.2 Aims

The specific aims are:

1. To identify characteristics of women who develop early metabolic markers of MetS.
2. To establish if ethnicity, physical inactivity, and consuming an unhealthy diet are independently associated with the early appearance of MetS markers.
3. To assess if acute HIIE positively impacts on MetS early metabolic markers.
4. To establish if the metabolic response to acute HIIE is influenced by high and low levels of fasting insulin.
5. To examine the metabolic health response to a synergistic lifestyle intervention in young overweight women.
6. To identify characteristics of women who do and do not lose fat to a lifestyle intervention.

1.3 Hypotheses

It is hypothesized that:

1. An unhealthy diet, sedentary behavior, and high fasting insulin levels will be characteristic of young premenopausal women.
2. Body composition, regardless of ethnicity, will predict MetS markers in young premenopausal women.
3. HIIE, compared to SSE, will significantly improve MetS blood markers (hormones, proteins, and cytokines) in the immediate (two hours) post exercise phase.
4. The metabolic response to acute HIIE will be significantly influenced by high and low levels of fasting insulin.
5. Lifestyle modification (fish oil, Mediterranean diet, and HIIE) will significantly reduce early markers of MetS and decrease body fat in young premenopausal women.
6. The majority of but not all overweight women will lose a significant amount of body fat after participating in the lifestyle modification.

CHAPTER 2 LITERATURE REVIEW

2.1 Introduction

This review describes the ways in which insulin affects metabolism and specifically how insulin dysregulation could contribute to the development of MetS. Also examined are the effects of weight loss and exercise on metabolic dysfunction. The groups at risk for future disease, their lifestyle characteristics, and clinical markers are also discussed. Finally, lifestyle modification programs involving exercise and diet are discussed. These programs may help reduce the risk for complications later in life associated with metabolic dysfunction.

2.2 Metabolic Syndrome and Insulin Resistance

Metabolic syndrome (MetS) is becoming a worldwide epidemic. As previously mentioned, MetS affects 20-25% of the adult population globally (IDF, 2006). The syndrome is a clustering of complications that can lead to diabetes, heart disease, and hypertension. The International Diabetes Federation (IDF) along with other groups around the world set the criteria for the diagnosis of MetS. The criteria (see Figure 2.1 and 2.2 and Tables 2.1 and 2.2) are based on co-morbidities such as obesity, glucose intolerance, lipid abnormalities, and hypertension (Magliano, Shaw, & Zimmet, 2006).

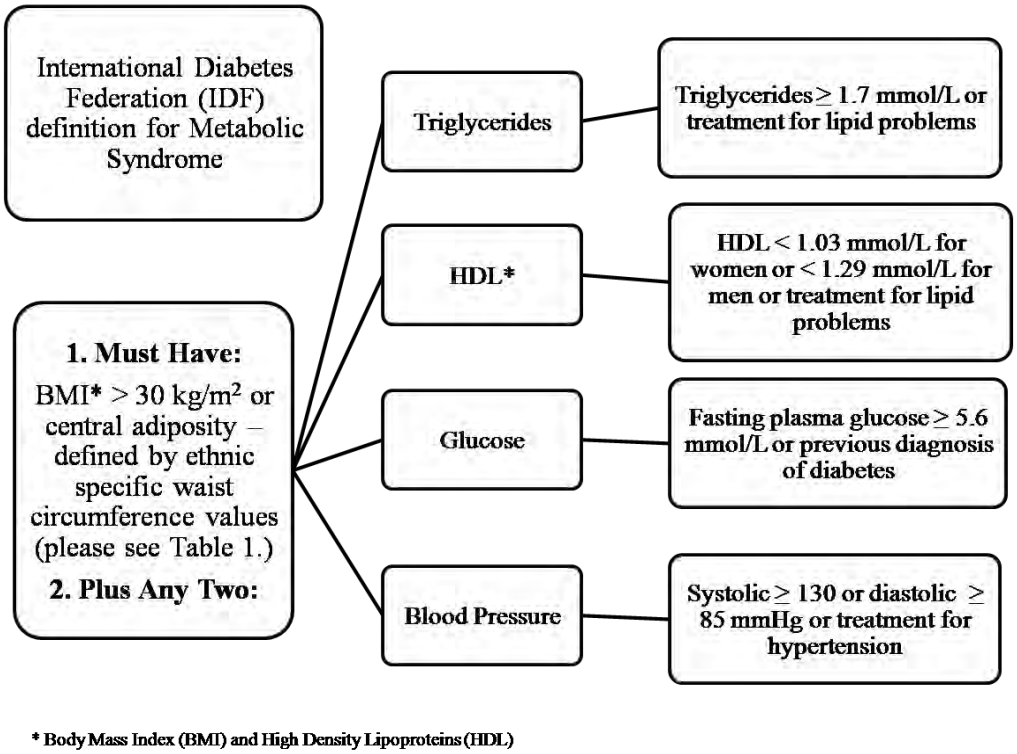


Figure 2.1 IDF definition for MetS (adapted from IDF, 2006).

Other groups such as the World Health Organization (WHO), European Group for the Study of Insulin Resistance (EGIR), and the Third Report of the National Cholesterol Educational Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATPIII) have differing criteria (see Figure 2.2) for diagnosing MetS (Zimmet et al., 2005b). The main focus of the IDF criteria is an increase in adiposity (BMI or waist circumference) first and then other metabolic perturbations to make up the syndrome (see Table 2.1).

Table 2.1 IDF Ethnic Specifications for Waist Circumference (Adapted from IDF, 2006)

Country/Ethnic Group	Male WC	Female WC
European	94 cm	80 cm
North American*	102 cm	88 cm
South Asians	90 cm	80 cm
Chinese	90 cm	80 cm
Japanese	90 cm	80 cm
Ethnic South and Central Americans**	90 cm	80 cm
Sub-Saharan Africans**	94 cm	80 cm
Eastern Mediterranean and Middle East (Arab) populations**	94 cm	80 cm
*ATPIII values are likely to continue to be used for clinical purposes ** Use other ethnic group recommendations until more specific data is available		

The criteria for MetS according to the guidelines set forth by ATPIII is based on adiposity or any of the metabolic measurements listed (see Figure 2.2), hence a person with low adiposity could still meet the criteria for MetS. Here in lies the problem, the metabolic measurements listed are not always the first presented disturbances within a homeostatic striving body. A precursor to the MetS criteria mentioned by the IDF or ADPIII could be hyperinsulinemia (Barnard, Roberts, Varon, & Berger, 1998) compensatory to IR.

Table 2.2 IDF Additional Metabolic Measurements for MetS (Adapted from IDF, 2006)

Physiological Abnormality	Measurement/Technique
Abnormal Body fat distribution	DEXA*, MRI*, CT*, leptin, or adiponectin
Atherogenic dyslipidemia	Apolipoprotein B or small LDL particles
Dysglycaemia	OGTT*
Insulin resistance	Fasting insulin/proinsulin levels, HOMA-IR*, insulin resistance by Bergman Minimal Model, fasting FFA*, FFA during OGTT or M value* from clamp
Vascular dysregulation	Endothelial dysfunction markers or microalbuminuria
Pro-inflammatory state	CRP*, TNF-α*, IL-6*, or adiponectin
Pro-thrombotic State	Fibrinolytic factors or clotting factors
Hormonal factors	Pituitary-Adrenal axis

*** Dual energy X-ray absortiometry (DEXA), magnetic resonance imaging (MRI), cat scan (CT), low density lipoproteins (LDL), homeostasis model assessment of insulin resistance (HOMA-IR), free fatty acid (FFA), oral glucose tolerance test (OGTT), average glucose infusion rate over a period of time from the start of the insulin infusion (M value), C-reactive protein (CRP), tumor necrosis factor-*alpha* (TNF-α), and interluekin-6 (IL-6)**

According to the most recent literature from the AUSDIAB study in Victoria, 30.7% of Australian adults have MetS as defined by the IDF criteria(Cameron, Welborn, Zimmet, Dunstan, Owen, Salmon et al., 2003). The prevalence of MetS in Australians is increasing along with obesity and diabetes rates. Early detection of the metabolic perturbations could help reduce the risk of disease and keep healthcare costs in Australia and worldwide at a minimum.

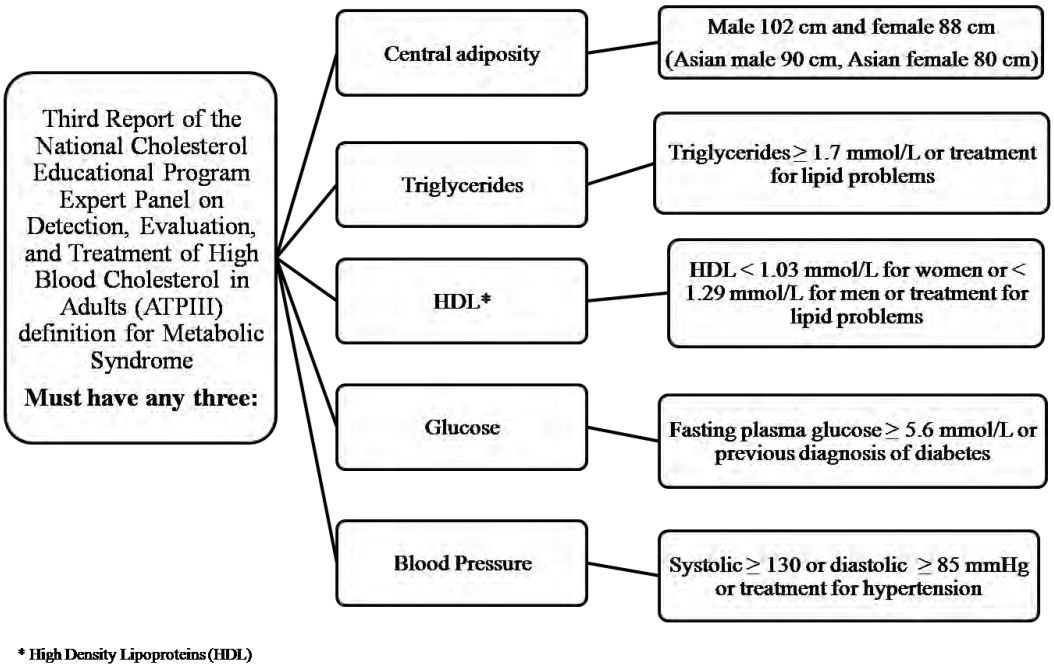


Figure 2.2 ATPIII definition for MetS (adapted from ATPIII, 2001).

The following section will review the multi-factorial pathogenesis of MetS along with at risk populations.

2.2.1 Lifestyle Characteristics

2.2.1.1 Obesity

The prevalence for overweight or obese individuals in Australia is 60% for both sexes (Zimmet, 2003). This represents an increase of 2.5 times the previously reported prevalence in the 1980's (Cameron et al., 2003). These alarming statistics are repeated in developing and developed countries (Cameron, Shaw, & Zimmet, 2004). MetS and obesity have been termed "twin epidemics" as they usually go hand in hand (Zimmet, 2005).

Not only in adults is obesity on the rise, in children the rate is increasing around the world together with adult populations. T2DM prevalence has increased in children alongside obesity in the USA (Mouraux & Dorchy, 2005) and high levels of insulin and

MetS have been recorded in obese children (Golley, Magarey, Steinbeck, Baur, & Daniels, 2006).

Obesity is associated with heart disease, hypertension, and diabetes. An excess of fat mass (overweight or obese) can be defined by body mass divided by height which is called the body mass index (BMI). Overweight is classified as having a BMI $\geq$ 25-29.9 kg/m^2 and obese, BMI $\geq$ 30 kg/m^2 (Cameron et al., 2003). Waist circumference is an indicator of visceral adiposity (Ross, Berentzen, Bradshaw, Janssen, Kahn, Katzmarzyk et al., 2008) and the World Health Organization (WHO) recognizes waist circumference as a marker for being overweight or obese. According to the WHO, men are considered overweight if the waist circumference is 94.0 - 101.9 cm and women with a waist circumference of 80.0 - 87.9 cm. An obese classification for men is a waist circumference of $\geq$ 102.0 cm and for women, a waist circumference of $\geq$ 88.0 cm (Cameron et al., 2003). Other measurements for obesity are: body fat percentage, total or visceral fat mass in kilograms, and waist-to-height (WHTR) that will be addressed along with methods to obtain these values.

Obesity is a chronically inflamed state in which adipose tissue reacts in relation to metabolic changes (Hotamisligil, 1999). Adipose tissue once thought to be only a depot for fat is a now considered an endocrine organ that releases cytokines and other regulators of fat metabolism. Positive changes in adiposity have been seen following diet and/or exercise interventions (Miller, Koceja, & Hamilton, 1997; Roberts & Barnard, 2005) which will be discussed in more detail later.

2.2.1.2 Physical inactivity and fitness

Living a sedentary lifestyle is associated with not only MetS (Biolo, Ciocchi, Stulle, Piccoli, Lorenzon, Dal Mas et al., 2005; Cameron et al., 2003; Dunstan, Salmon, Owen, Armstrong, Zimmet, Welborn et al., 2005; Zimmet, Alberti, & Shaw, 2001;

Zimmet & Thomas, 2003) but also diabetes (Lakka, Laaksonen, Lakka, Mannikko, Niskanen, Rauramaa et al., 2003) and an increased risk of hypertension, cancer, and coronary heart disease (CHD) (Blair & Brodney, 1999). It has been found that fitness levels were inversely associated with MetS (LaMonte, Ainsworth, & Durstine, 2005a). A study examining television (TV) viewing time and physical activity (PA) levels in Australian adults found an increased prevalence of MetS in those with higher amounts of TV and less hours per week of PA (Dunstan et al., 2005). Adequate amounts of daily exercise may reduce the risk or reverse the onset of MetS (Lakka et al., 2003). The physiological complications associated with less activity are: a decrease in muscle mass or atrophy, IR, poor fitness levels, and a reduced basal metabolic rate (BMR) (Biolo et al., 2005).

A reduced metabolic rate and poor physical fitness are related to atrophy of the muscle mass. Physically fit athletes with an increase in fat free mass compared to controls had larger mitochondria in the muscle leading to increased transport of oxidative properties and a higher BMR (Sestoft, 1980). The mitochondria are the energy producing organelles where adenosine triphosphate (ATP) is produced to power the body. With fewer or dysfunctional mitochondria the muscle has a decreased ability to function especially during high energy demand like exercise. When comparing lean to obese participants, the mitochondria in the obese were smaller and were more inefficient, less oxidative phosphorylation, ATP synthesis, and more heat production (Kelley, He, Menshikova, & Ritov, 2002). An individual's BMR can also be influenced by age (Poehlman, Goran, Gardner, Ades, Arciero, Katzman-Rooks et al., 1993), hormones (Goglia, Silvestri, & Lanni, 2002), genetic factors (Jacobson, Rankinen, Tremblay, Perusse, Chagnon, & Bouchard, 2006), energy output (Marra, Pasanisi, Montagnese, De Filippo, De Caprio, de Magistris et al., 2007), along with energy

intake, fat mass (Kempen, Saris, Kuipers, Glatz, & Van Der Vusse, 1998), and the thermogenesis mediated through the sympathetic nervous system (SNS) (Golan, Tal, Dror, Korzets, Vered, Weiss et al., 2002).

2.2.1.3 Diet

The typical westernized diet consists of excess saturated and trans fatty acid intake combined with refined grains and sugar and low amounts of fibre and omega-3 fatty acids (ω-3). Bazzano et al. (2005) has suggested that this type of diet is atherogenic (Bazzano, Serdula, & Liu, 2005). Studies have shown a diet deficient in ω-3, as seen in Westernized countries, can lead to major health problems such as: heart disease (Keys, Menotti, Karvonen, Aravanis, Blackburn, Buzina et al., 1986), cancer (Connor, 2000), and can indirectly affect body mass and insulin sensitivity (Simopoulos, 2006). Both ω-3 and omega-6 (ω-6) are polyunsaturated acids (PUFA). The ω-3 has three carbons between the double bond and omega terminal and the ω-6 is virtually the same but with 6 carbons instead of 3. Figure 2.3 shows the metabolic pathways for ω-3 and ω-6.

The ratio of ω-3 to ω-6 in the blood is a good marker of ω-3 deficiency with a lower ratio being healthier. A ratio of 1-2:1 is ideal although with today's processed foods and westernized diet, a healthy ratio would be 10-20:1 (Engler & Engler, 2006; Simopoulos, 2006) due to the lack of ω-3 and abundance of ω-6.

Trans fatty acids (TFA) found in fried foods, commercially baked goods, margarine, and vegetable shortening have been linked to negative changes in endothelial dysfunction, IR, inflammation and T2DM (Hu & Willett, 2002; Micha & Mozaffarian, 2008, 2009; Mozaffarian, 2006). TFA potentially act within the cell membrane (endothelial, adipose tissue and monocyte/macrophages) influencing the signalling pathways and has been shown to alter adipose gene expression (Micha & Mozaffarian,

2008, 2009; Mozaffarian, 2006).The role of diet and lower TFA intake in modifying cardiovascular risk is important for societies today with special consideration to a Mediterranean diet.

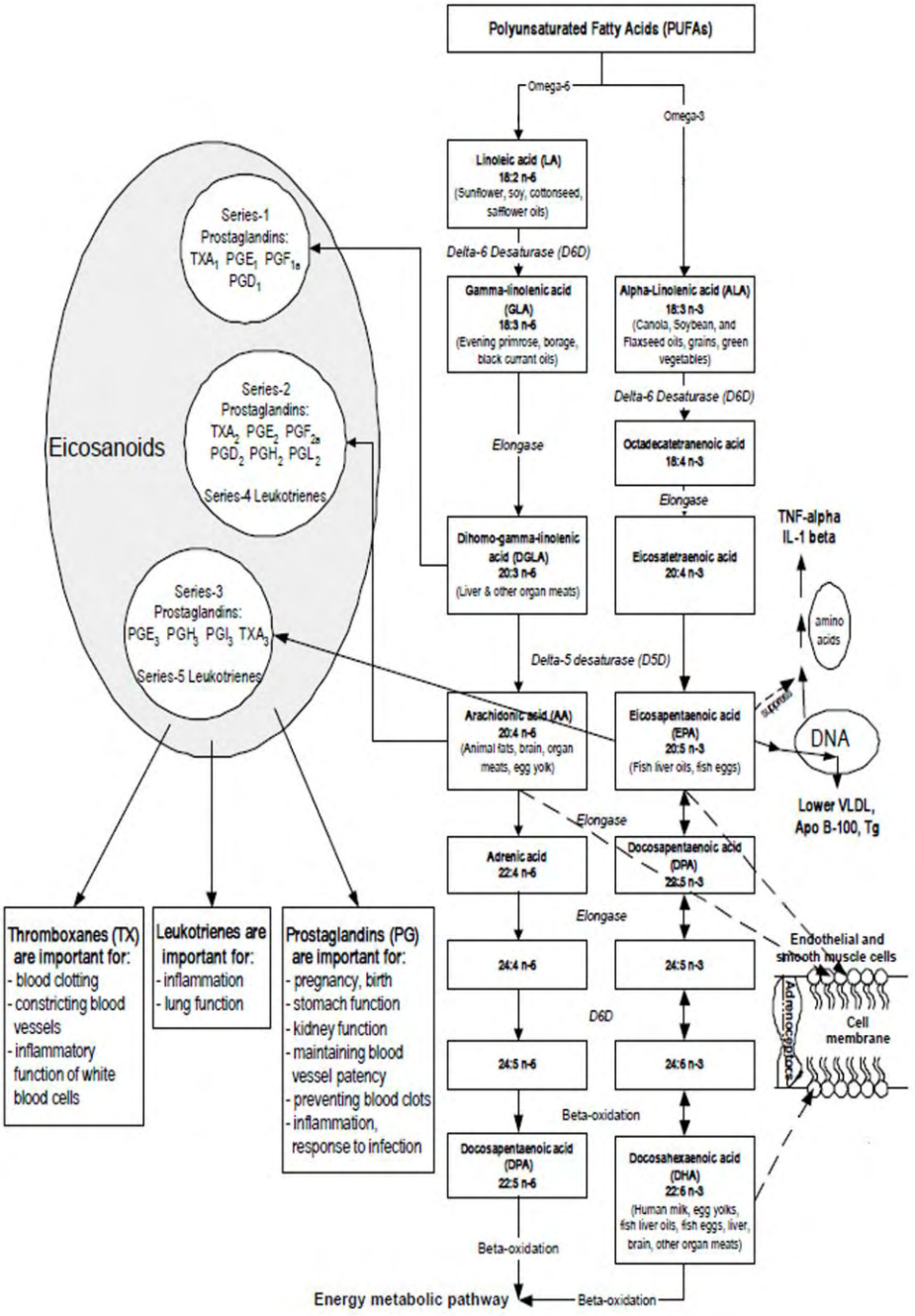


Figure 2.3 Omega Fatty Acid Metabolic Pathways (adapted from Balk et al, 2004).

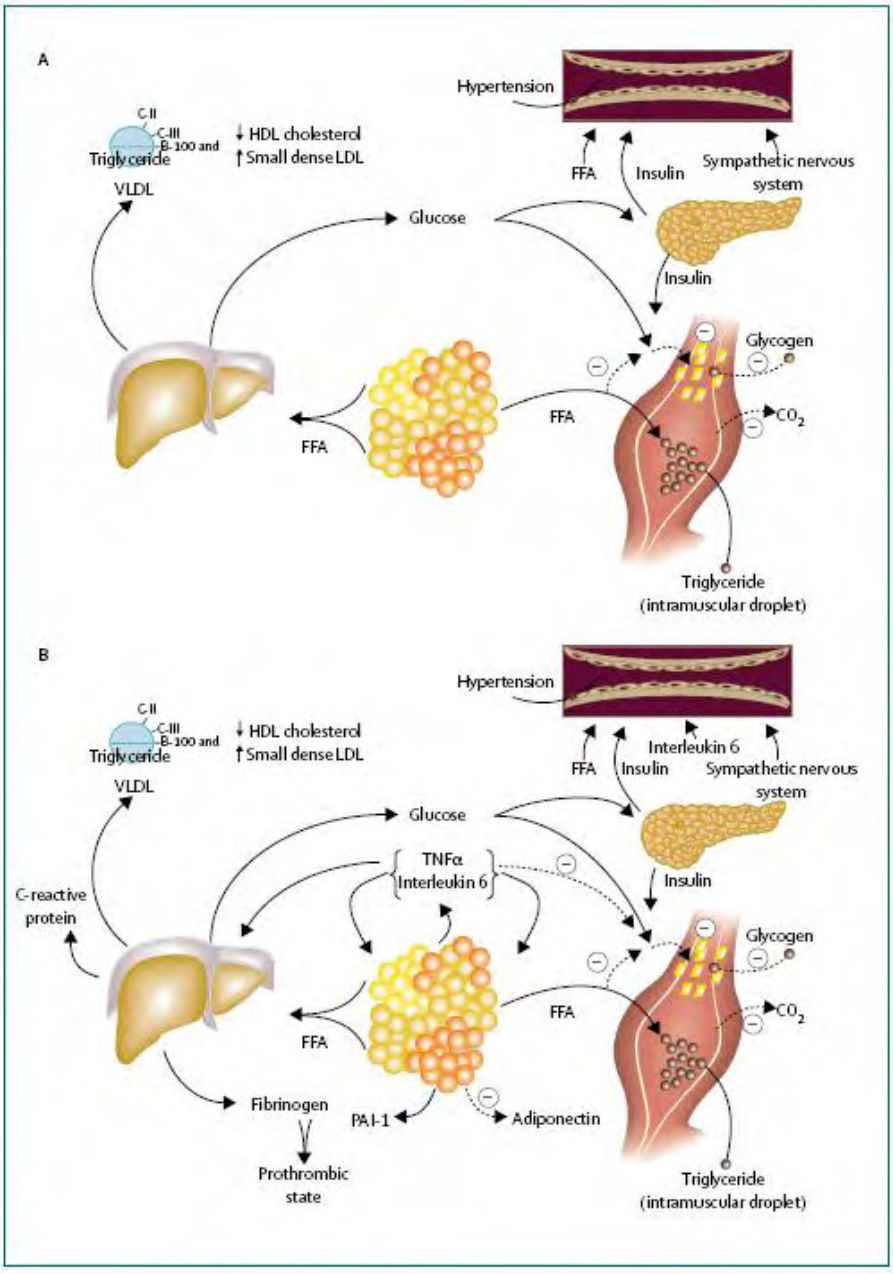
For people living in Crete (Kafatos, Diacatou, Voukiklaris, Nikolakakis, Vlachonikolis, Kounali et al., 1997; Moschandreas, Kafatos, Aravanis, Dontas, Menotti, & Kromhout, 2005; Voukiklaris, Kafatos, & Dontas, 1996) in the 1960's and the Greenland Eskimos (Bang & Dyerberg, 1975; Dyerberg, Bang, & Hjorne, 1975), the dietary intake of ω-3 eicosapentaenoic fatty acid (EPA) and docosahexaenoic fatty acid (DHA) or the consumption of a Mediet was associated with a lower incidence of disease compared to today.

Another aspect of a poor diet is a high glycaemic load. Glycaemic load (GL) or index (GI) is related to the total affect a meal has on glucose and is not related to the carbohydrate itself. In terms of glucose control, foods with a higher glycaemic index are absorbed quickly producing a higher insulin demand (Augustin, Franceschi, Jenkins, Kendall, & La Vecchia, 2002). Previous research in this area has associated high glycaemic diets with chronic disease such as diabetes (Salmeron, Ascherio, Rimm, Colditz, Spiegelman, Jenkins et al., 1997a; Salmeron, Manson, Stampfer, Colditz, Wing, & Willett, 1997b), cardiovascular disease (Liu, Willett, Stampfer, Hu, Franz, Sampson et al., 2000), and cancer (Augustin, Dal Maso, La Vecchia, Parpinel, Negri, Vaccarella et al., 2001). High GI foods tend to increase hunger, negatively impacting glucose storage and oxidation and lipolysis (Augustin et al., 2002). A low glycaemic Mediet with a more closely matched ω-3: ω-6 ratio could help western societies reduce the incidence of disease and could generate metabolic homeostasis (Berry, 1997; Connor, 2000; Friedberg, Janssen, Heine, & Grobbee, 1998; Waite, Lodge, Hart, Robertson, Badley, & Burton, 2008).

2.2.2 Clinical Markers of Metabolic Syndrome

The lifestyle characteristics mentioned above together with pathophysiological markers may lead to MetS as described by Eckel et al. (2005) in the following diagram

(see Figure 2.4 A and B). The schematic representation (see Figure 2.4 A) involves the interaction between adipose tissue and the vessels, muscles, liver, and pancreas. When adipose tissue expands, or free fatty acids are in abundance, the liver secretes excess glucose and lipids (TG and VLDL). The rise in glucose will decrease the conversion of glucose to glycogen within the muscle, decrease the muscles sensitivity to insulin and increase the storage of intramuscular fats (Eckel et al., 2005). The increased production of glucose influences insulin secretion from the beta cells impacting blood pressure and ultimately the SNS. The second part of the representation (see Figure 2.4 B) portrays the same interactions mentioned above within an inflamed state. The secretion of Interluekin-6 (IL-6), C-Reactive protein (CRP), adiponectin, and other proinflammatory cytokines exacerbate the problems seen with adipose tissue expansion increasing the risk of hyperinsulinemia, IR, and eventually MetS (Eckel et al., 2005).



A: Free fatty acids (FFA), very low density lipoproteins (VLDL), high density lipoprotein (HDL), low density lipoproteins (LDL), and carbon dioxide (CO_2)
B: Tumor necrosis factor alpha (TNFα), plasminogen activator inhibitor-1 (PAI-1), and carbon dioxide (CO_2)

Figure 2.4 An overview of the underlying mechanisms of MetS (adapted from Eckel et al., 2005).

2.2.2.1 Glucose intolerance

Glucose anomalies coincide with risk of heart disease and diabetes. Impaired glucose tolerance (IGT) or abnormal responses to glucose is strongly associated with cardiovascular disease (CVD) and diabetes according to the IDF (Unwin, Shaw, Zimmet, & Alberti, 2002). Insulin's inability to control hepatic glucose uptake and to influence glucose in adipose tissue and skeletal muscle is the basis of IGT (Eckel, 2007). With an increase in adipose tissue, an increase in non esterified free fatty acids (NEFA) to the liver results in an increase in glucose production that will result in more demand for insulin control (Eckel et al., 2005). An early lifestyle intervention in people with IGT could normalize insulin action and restore euglycaemia (Eckel, 2007) reversing any early signs of disease.

2.2.2.2 Insulin resistance and hyperinsulinemia

Insulin is a major metabolic hormone secreted by the beta cells in the pancreas which regulates and controls cellular glucose metabolism (see Figure 2.5). Postprandially, the increase in insulin secretion controls the rate of glucose appearance in the blood and ultimately determines if fat or carbohydrate will be used or stored (Eckel et al., 2005). A chronic increase in insulin, leads to IR which is defined by Park et al. (2005) as a fasting insulin level $\geq$ 9.98 µIU/L or a Homeostasis Model Assessment (HOMA-IR) $\geq$ 2.56 (Park, Lee, Rhee, Jeon, Kim, Ryu et al., 2005b). HOMA-IR is a calculation that takes glucose and insulin levels into account and is considered a standardized measure for insulin resistance. HOMA-IR correlates ($r = -.61$, $P < .001$) with other methods of measuring IR or glucose intolerance, such as the "gold standard" glucose clamp technique (Lansang, Williams, & Carroll, 2001). HOMA-IR is based on the following equation:

HOMA-IR = [fasting insulin (µIU/ml) x fasting blood glucose (mmol/l)] / 22.5.

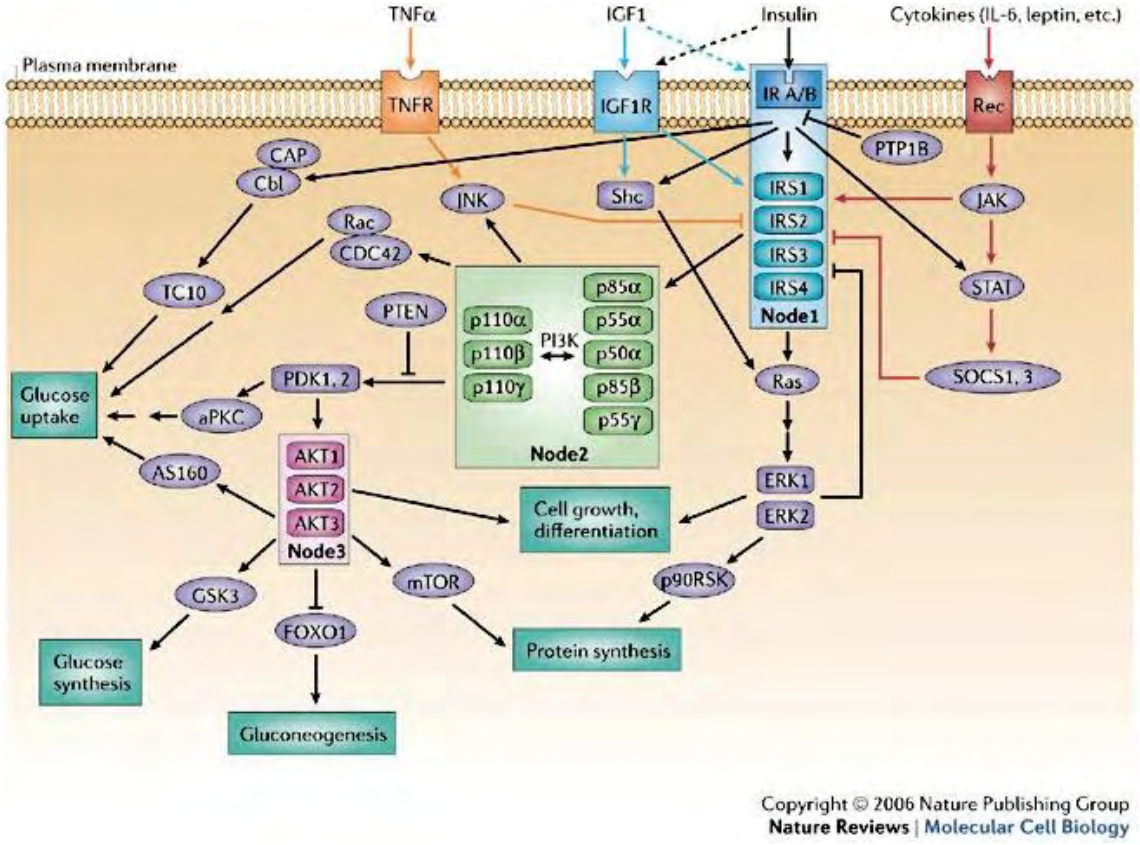


Figure 2.5 The Insulin Signalling Pathways (Taniguchi, Emanuelli, & Kahn, 2006).

IR could be the result of an increase in plasma free fatty acids (FFA) resulting from a failure of insulin to appropriately suppress lipolysis. Possibly, prolonged elevation of lipids within the circulation may impair beta cell function leading to insulin problems. The elevated levels of FFA could influence inflammation as discussed later on. Specifically, in muscle the increase in intramyocellular lipids (ceramides and diacylglycerol) can negatively impact insulin signalling resulting in IR (Eckel et al., 2005; Holland, Knotts, Chavez, Wang, Hoehn, & Summers, 2007). Chronic IR results in decreased adiponectin, elevated leptin, decreased sensitivity to SNS activation (Park, Lee, & Park, 2004), and deposition of hepatic fat (Petersen & Shulman, 2006). IR is seen in individuals with MetS (Eckel et al., 2005; Haffner, Valdez, Hazuda, Mitchell, Morales, & Stern, 1992; Lee, Critchley, Chan, Anderson, Thomas, Ko et al., 2000; Lin, Yang, Lee, Chen, Liu, Lin et al., 2006; Zimmet, 2005), diabetes (Eckel et al., 2005;

Lillioja, Mott, Spraul, Ferraro, Foley, Ravussin et al., 1993; Lim, Patel, & Lip, 2004; Lin, Lee, Jeng, Sheu, & Chen, 2004; Lowell & Shulman, 2005; Zimmet, Alberti, & Serrano Rios, 2005a), and obesity (Eckel et al., 2005; Lee et al., 2000; Lin et al., 2006; Petersen & Shulman, 2006; Zimmet, 2005). However, IR is considered one of the earliest mechanisms of MetS and has been found in young adults free of other metabolic abnormalities such as dyslipidemia, high blood pressure, high fasting blood glucose levels, and obesity (Dvorak et al., 1999). Lipolysis is regulated in part by insulin and lipoprotein lipase (LPL). Insulin stimulates LPL activity in adipose tissue and diminishes it in skeletal muscle (Kiens, Lithell, Mikines, & Richter, 1989). IR in the muscle is characterized with a decrease in the insulin stimulation of phosphoinositide 3 kinase (PI-3 kinase) leading to lower translocation of the glucose transporter 4 (Glut-4) to the sarcolemma. Insulin is regulated hormonally and by the SNS and has been shown to be affected by obesity, blood pressure, exercise, genetics, diet, sleep, stress, toxicity, and disease (Bjorntorp, 1999; Bjorntorp & Rosmond, 2000). Obese individuals have a blunted SNS response during fasting (Horowitz, Coppack, Paramore, Cryer, Zhao, & Klein, 1999) which affects insulin in the plasma and at the muscular level resulting in IR.

2.2.2.3 Lipid abnormalities

The IDF criteria for MetS regarding lipid abnormalities (see Figure 2.1) is low levels of the cholesterol, high density lipoproteins (HDL), and raised levels of triglycerol (TG) (IDF, 2006). The increases in visceral fat and NEFA to the liver are common in MetS and can be a burden on the metabolic system (Eckel, 2007). Due to this increase in NEFA an increase in very low density lipoproteins (VLDL) production occurs (Eckel, 2007). Changes to the HDL particle along with a decrease in the number of lipoproteins accompany an increase in TG. The HDL particle has been shown to have

protective cardiac benefits but with changes to the enzymatic make-up of HDL it can go from having a positive influence to a negative one (Ansell, Watson, Fogelman, Navab, & Fonarow, 2005). A newly modified lipoprotein will disappear quickly from the circulation (Chan, Barrett, & Watts, 2006) and has been shown to induce a pro-inflammatory state (Ansell et al., 2005). A study investigating male and female Chinese adults found those with elevated triglyceride values had an increased BMI and elevated insulin. The authors concluded that within this Asian population, hypertriglyceridemia is an independent risk factor for IR (Bao, Jia, Xiang, Chen, & Lu, 2001). An exercise program could bring about improvements in the lipid profile due to a change in the utilization of free fatty acids (FFA) and a reduction in inflammation.

2.2.2.4 Hypertension

Obesity and IR in non-diabetic populations have been shown to affect the vascular system leading to atherosclerosis (Poirier & Eckel, 2002). A study conducted in 1996 found that people in the obese or IR state had a blunted vasodilatory response to insulin due to endothelial resistance and dysfunction (Steinberg, Chaker, Leaming, Johnson, Brechtel, & Baron, 1996). Women with varying levels of MetS criteria were evaluated to look at contributing factors on multiple levels for the syndrome. Higher levels of blood pressure (BP) were related to obesity and not insulin in this ethnic group (Anderson, Critchley, Chan, Cockram, Lee, Thomas et al., 2001). The elevated BP may stem from the increase in fatty acids which influences vasoconstriction (Tripathy, Mohanty, Dhindsa, Syed, Ghanim, Aljada et al., 2003) through endothelial dysfunction, an increase in oxidant stress or vascular cell growth (Sarafidis & Bakris, 2007). Human and animal research has shown that high levels of plasma insulin and IR coincide with hypertension (Reaven, 1991; Zavaroni, Ardigo, Zuccarelli, Pacetti, Piatti, Monti et al., 2006) and could affect the vessel dilation through nitric oxide (Imazu,

2002). In an IR state, insulin does not have the capacity to promote vasodilation (Tooke & Hannemann, 2000). The question remains whether insulin is a cause or consequence for increased systolic and diastolic blood pressure.

2.2.2.5 Low grade inflammation

Low grade inflammation (pro-inflammatory cytokines) is associated with an increased risk of MetS (Hall, Watkins, Wright, Wenger, Kumanyika, Gavin et al., 2006; Zambon, Pauletto, & Crepaldi, 2005) and CHD (Festa, D'Agostino, Tracy, & Haffner, 2002; Timpson, Lawlor, Harbord, Gaunt, Day, Palmer et al., 2005). In obese states, increased IL-6, tumor necrosis factor-α (TNF-α), and CRP levels elicit a pro-inflammatory response possibly due to increased adiposity or FFA's releasing adipocytokines which impacts insulin signalling (see Figures 2.4 and 2.5). Support for this notion has come from Maachi et al. (2004) who showed that an increase in fat mass correlated with increases in inflammation (CRP, IL-6, and TNF-α) (Maachi, Pieroni, Bruckert, Jardel, Fellahi, Hainque et al., 2004). An accumulation of macrophages has been shown with increased adipose tissue and may aid in the development of low grade systemic inflammation (Sell & Eckel, 2007).

Also, fat cell size correlates with inflammatory markers (Bahceci, Gokalp, Bahceci, Tuzcu, Atmaca, & Arikan, 2007). Systemic inflammation was lower in "obese but metabolically healthy" individuals compared to "obese at risk for disease" women (Karelis, Faraj, Bastard, St-Pierre, Brochu, Prud'homme et al., 2005). The interesting finding with this study was that the "metabolically healthy" possessed significantly less low grade inflammation and visceral fat than the metabolically unhealthy. Accumulation of adiposity, specifically visceral fat, could be the reason for increased inflammation. An altered HDL particle, as mentioned previously, could promote other inflammatory cytokine levels. People who are systemically inflamed are at risk for

MetS whether or not they have the increased adiposity. With exercise, inflammation could decrease due to changes in visceral adiposity, adipose cell size, and increases in adiponectin.

2.2.2.6 Leptin and adiponectin

Individuals with obesity and/or IR have been shown to possess increased leptin (an appetite dampener hormone) and/or chronic increased leptin or leptin resistance (Blaak, Hul, Verdich, Stich, Martinez, Petersen et al., 2006; Dyck, 2005; Kougias, Chai, Lin, Yao, Lumsden, & Chen, 2005; McMurray & Hackney, 2005; Meier, 1996). Unger's hypothesis (Unger, 2003), "leptin resistance (LR) causes decreased insulin secretion leading to MetS" is controversial but more recently it has been shown that leptin and insulin have interrelated signalling pathways (Xu, Kaelin, Takeda, Akira, Schwartz, & Barsh, 2005). Leptin has also been shown to influence SNS activation (Egan, 2003) which could have implications for those that have LR. Adiponectin is an anti-inflammatory adipocytokine also involved in metabolic homeostasis and interacts at the hepatic and muscular level. Adiponectin promotes insulin sensitivity, fat oxidation, and glucose use by skeletal muscle (Bastard, Maachi, Lagathu, Kim, Caron, Vidal et al., 2006). Adiponectin levels are decreased in people with MetS (Matsuzawa, Funahashi, Kihara, & Shimomura, 2004), IR populations (Vettor, Milan, Rossato, & Federspil, 2005), and T2DM (Dyck, Heigenhauser, & Bruce, 2006; Meier & Gressner, 2004). Leptin, insulin, and adiponectin may interact together or one may cause a change in the other. Thus, individual differences in their levels and fat loss after exercise resulting in decreased leptin and an increase muscle oxidative capacity or adiponectin may influence MetS mechanisms.

2.2.2.7 Stress and the hypothalamic-pituitary-adrenal axis

The hypothalamic-pituitary-adrenal axis (HPA) regulates cortisol, a stress hormone, through a cascade of events (Figure 2.6) which can lead to visceral fat accumulation. The illustration portrays the cascade of events starting with stress, which activates corticotrophin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) leading to increased cortisol levels. Cortisol may impact fat accumulation in a variety of ways which is depicted by numbers in the diagram (see Figure 2.6). Bjorntorp (2001) explains that stress and an exacerbated HPA leads to chronic activation resulting in higher levels of cortisol. The elevation in cortisol leads to accumulation of visceral fat (see Figure 2.6, 1) possibly through stimulation of LPL, inhibition of TG movement (Bjorntorp, 2001), and maturation of the adipocyte (Nieuwenhuizen & Rutters, 2008). The negative feedback loop regulating cortisol may not function properly which could aggravate the problem even more causing an increase in the consumption of calorically dense foods (Abdel-Sayed, Binnert, Le, Bortolotti, Schneiter, & Tappy, 2008) (see Figure 2.6, 5), disrupting energy balance (see Figure 2.6, 3) , elevating leptin levels (see Figure 2.6, 4) (Nieuwenhuizen & Rutters, 2008), and affecting growth hormone (Bjorntorp, 1993) resulting in the storage of fat. Cortisol promotes insulin secretion and both can inhibit lipolysis resulting in fat accumulation and possibly metabolic abnormalities (Bjorntorp & Rosmond, 2000). Overweight girls have presented with low levels of growth hormone, IR, and elevated levels of adiposity and cortisol (Misra, Bredella, Tsai, Mendes, Miller, & Klibanski, 2008) together create a myriad of problems, one of which may stem from HPA dysregulation (Vicennati, Pasqui, Cavazza, Pagotto, & Pasquali, 2009) (see Figure 2.6, 2).

A recent study has shown that FFA may play a role in blunting HPA function (Coiro, Casti, Rubino, Manfredi, Maffei, Melani et al., 2007). Exercise and possibly diet

or a change in dietary habits could reduce stress through a reduction in cortisol or through the negative feedback loop with changes to insulin, leptin, growth hormone, FFA, and visceral fat (Davis, Galassetti, Wasserman, & Tate, 2000; Delarue, Matzinger, Binnert, Schneiter, Chiolero, & Tappy, 2003; Gomez-Pinilla, 2008; Innes, Vincent, & Taylor, 2007; Lee & Loke, 2005; Ma, Liu, & Ling, 2003; Tsatsoulis & Fountoulakis, 2006).

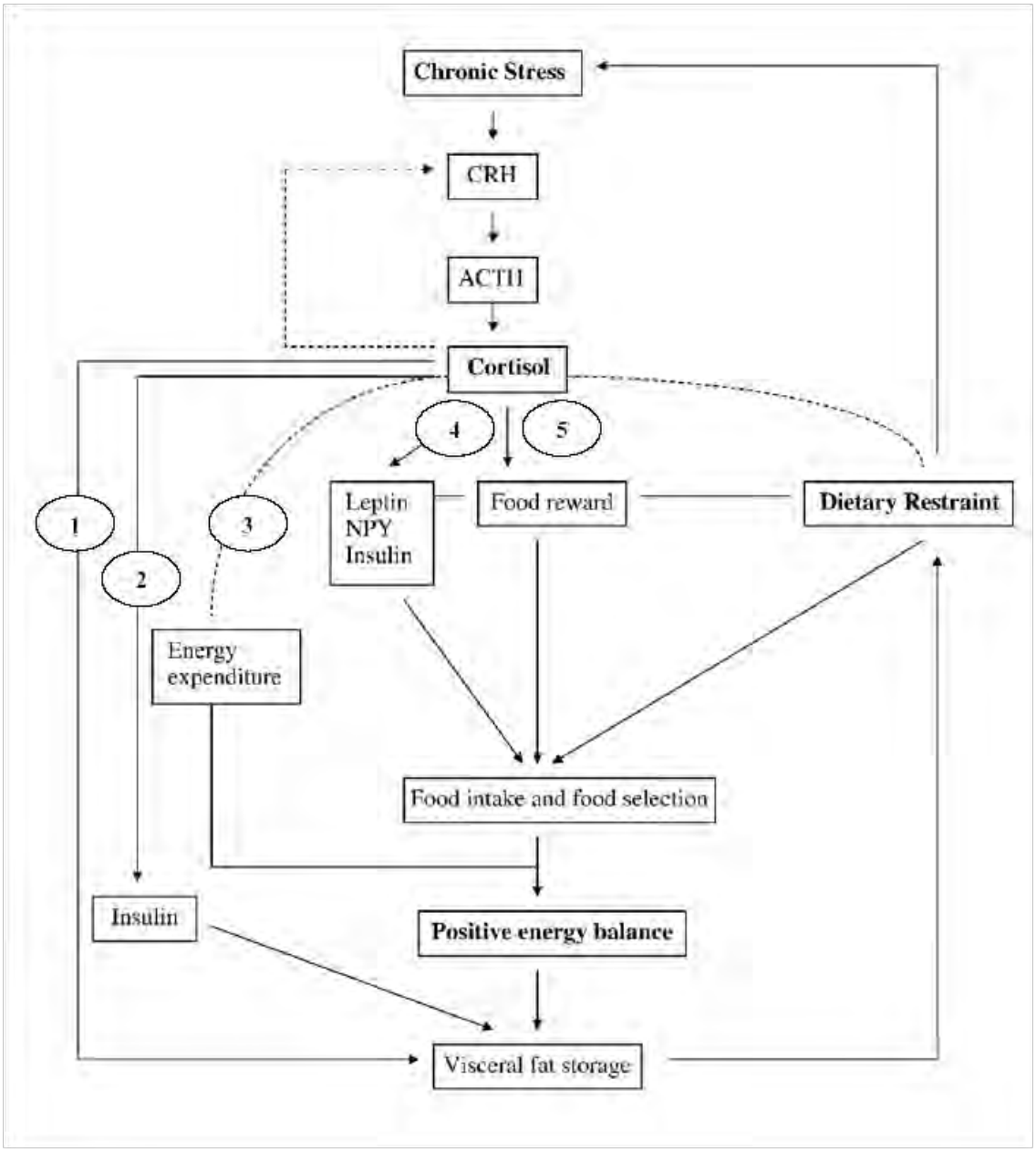


Figure 2.6 An overview of the underlying mechanisms of chronic stress and visceral fat accumulate on (adapted from Nieuwenhuizen et al., 2008).

2.2.2.8 Oxidation of fat or carbohydrate at rest

The increased rate of obesity could be due to, preferential carbohydrate (CHO) oxidation (Flatt, 1996), eating a high fat diet, being sedentary (Flatt, 1987), and a rise in adiposity levels to maintain fat oxidation rates (Astrup, Buemann, Western, Toubro, Raben, & Christensen, 1994). Women with increasing adiposity had a lower total respiratory quotient (RQ) inferring preferential fat burning (Schutz, Tremblay, Weinsier, & Nelson, 1992). "Metabolic inflexibility" is a term originating from Kelley and Mandarino (2000) referring to a person's capacity to switch the oxidation of lipid to carbohydrate following a change in insulin and glucose stimulation (Kelley & Mandarino, 2000). It could be due to genetics, adipose storage capacity within the adipose or muscular tissue, diet, or physical activity levels.

2.2.2.9 Autonomic function

Heart period variability (HPV) is the term used to describe the changes between consecutive heart beats and instantaneous consecutive heart beats (European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). HPV is an indirect assessment using power calculations to describe vagal tone and sympathetic drive. People with risk factors for MetS, including IR and obesity have reduced HPV (Egan, 2003). The assessment of high (HF) and low frequency (LF) spectral power and RR interval (heart rate) values along with other measurements make up what is known as HPV. The SNS may be distorted in an IR state based on the influence of insulin and leptin (Egan, 2003). Overfeeding is associated with increases in sympathetic and parasympathetic tone (Scheurink, Balkan, Strubbe, van Dijk, & Steffens, 1996). Altered autonomic activity, could have major implications on metabolic health (Lindmark, Wiklund, Bjerle, & Eriksson, 2003) and the occurrence is greater in

women with increased visceral adiposity (Egan, 2003). Vagal tone sensitivity could increase following exercise with changes to heart rate (HR) and the metabolic profile.

2.2.3 At Risk Populations

Various ethnic and racial groups have a higher prevalence of IR, obesity, inflammation or physical inactivity (Gu, Reynolds, Wu, Chen, Duan, Reynolds et al., 2005; Ho, Davy, Hickey, & Melby, 2005; Melby, Ho, Jeckel, Beal, Goran, & Donahoo, 2000) which puts them at an increased risk of MetS (Cameron et al., 2004). Criteria for ethnic populations, specifically for MetS, is controversial and more research is needed although the IDF have established cut off points for ethnic groups (IDF, 2006). Globalization has created more of a problem for those ethnic groups who have migrated to westernized societies as their metabolism may not react positively to the new environment.

2.2.3.1 Ethnic populations

It is known that the Asian population has an increased prevalence of T2DM and IR (Banerji, Faridi, Atluri, Chaiken, & Lebovitz, 1999; Potts & Simmons, 1994). Ehtisham et al. (2005) found dyslipidemia, IR, and excess body fat in a cohort of Asian adolescents compared to their European counterparts. The interesting finding with this study was that although there was IR and excess body fat, the BMI in this group was within a healthy range using the Europid BMI criteria. Asian BMI values have been mentioned in prior research although no specific values have been set by the IDF or other organizations. Use of the Asian BMI values from the World Health Organization (WHO, 2004) places people in categories according to "at risk" or "at increased risk". A BMI of 22-25 kg/m^2 and 26-31 kg/m^2 for an Asian person are considered "at risk" (WHO, 2004). The IDF does associate waist circumference differences between ethnic groups (see Table 2.1) but not BMI. According to Shiwaku et al. (2005), not all Asians

should be grouped together for BMI based on the ATPIII criteria. Mongolians typically have a higher BMI than the Japanese and Koreans (Shiwaku, Nogi, Kitajima, Anuurad, Enkhmaa, Yamasaki et al., 2005). More research and specific guidelines for BMI within the Asian and ethnic groups are needed. Migrants living in westernized countries are also at risk for MetS (McKeigue, Shah, & Marmot, 1991). In a study comparing Japanese men to their migrant counterparts living in the US, the dietary composition of fat, carbohydrate, protein, and total calories was higher in the men living in the US (Lands, Hamazaki, Yamazaki, Okuyama, Sakai, Goto et al., 1990). Dietary fat intake was regarded as the reason for discrepancies in insulin sensitivity between non-obese non diabetic Mexican and Hispanic Americans (Ho, Davy, Hickey, Summers, & Melby, 2002).

2.2.3.2 Responders versus non-responders

There are likely to be responders and non-responders in every exercise fat loss trial and reporting the overall effect as a mean change disguises the significant fat loss achieved by some participants. In a study by Donnelly et al. (2005) the weight loss response for women was more variable and was less than that reported for males (Donnelly & Smith, 2005). Although studies in this area have not typically reported individual responses and have not reported males and females separately, those studies that have, show a similar pattern of results (Andersson, Xu, Rebuffe-Scrive, Terning, Krotkiewski, & Bjorntorp, 1991; Donnelly, Hill, Jacobsen, Potteiger, Sullivan, Johnson et al., 2003). The gender difference discussed above has been present and absent (De Luis, Aller, Izaola, Gonzalez Sagrado, Conde, & Perez Castrillon, 2008; Stiegler & Cunliffe, 2006) in other interventions. Thus, it is reasonable to suggest that exercise fat loss programs are effective for producing a clinical decrease in fat (greater than 6% of body mass) for some but not all male and female participants (Donnelly & Smith,

2005). There are a range of program design factors that may explain why some studies have reported more weight loss than others. Mode, intensity, duration, and frequency of the exercise program vary considerably across studies and may contribute to the inconsistency of results and the presence of responders and non-responders. For example, non-responders may typically complete less exercise sessions and may have exercised at too low an exercise intensity (King, Caudwell, Hopkins, Byrne, Colley, Hills et al., 2007; Shaw, Gennat, O'Rourke, & Del Mar, 2006). However, a number of studies have controlled these factors but still find variability in weight loss after exercise (Stiegler & Cunliffe, 2006). Thus, it is likely that other individual factors that are behavioral, inherited, and physiological in origin also affect the fat loss response to exercise (Boutcher & Dunn, 2009).

2.2.4 Summary

In order to combat the growing epidemics of cardiovascular disease and T2DM a better understanding of the aetiology behind MetS and interventions designed to ameliorate the early mechanism driving the co-morbidities are needed. Dyslipidemia, obesity, hypertension, and glucose intolerance are all important outcomes in the diagnosis, but what is the early mechanism causing these MetS disturbances? Are environmental and behavioural factors including obesity, physical inactivity and diet along with hyperinsulinemia to blame for this global burden? The aim of this thesis is to identify the early mechanisms related to metabolic dysfunction and offer a lifestyle intervention targeting those early mechanisms.

2.3 Lifestyle Modification Program to Reduce the Rick of Metabolic Syndrome

The ATPIII (2005) report clearly states the first line of defence for any of the MetS risk factors should be lifestyle modification. Physical activity, diet, and weight loss are all considered an important first step for change (Sullivan, 2006).

2.3.1 Exercise

In 2002, the US Institute of Medicine (IOM) recommended that individuals trying to maintain body mass, not lose weight, should complete 60 minutes per day (420 minutes per week) of moderate intensity exercise. The recommendation is 120 more minutes a week than the American College of Sports Medicine (ACSM) stance from 2001. According to Donnelly et al. (2004), most people would not complete 200-300 minutes per week of activity (Donnelly, Smith, Jacobsen, Kirk, Dubose, Hyder et al., 2004). Those recommending exercise for fat loss suggest “longer duration” is favourable (Donnelly et al., 2004) as a dose response relationship with exercise and fat loss has been shown (Ross & Janssen, 2001). A large amount of time needs to be invested into an exercise program although the actual fat loss brought about by exercise

programs is usually less than expected and results are typically disappointing (Miller et al., 1997; Ross, Janssen, Dawson, Kungl, Kuk, Wong et al., 2004; Stiegler & Cunliffe, 2006).The dropout rate of an exercise intervention may be impacted by the number of days, duration of the intervention, a full time job (Inelmen et al., 2005) or other commitments. Based on the dose response relationship between exercise and fat or weight loss, the more time given to exercise presumably the better the results although overweight participants did not report they had extra free time (Inelmen et al., 2005). An optimal exercise duration (less time spent exercising per session) for weight loss and maintenance should be examined more closely.

2.3.1.1 Lifestyle characteristics and high intensity interval exercise

The scope of this thesis regarding exercise concentrates on high intensity interval exercise (HIIE). If HIIE hasn't been studied in a certain area previously mentioned in this thesis, the benefits derived from steady state exercise (SSE) will be explained. For a more thorough review of SSE or resistance training for disease prevention please see Roberts and Barnard (2005), Miller et al. (2006), or Shaw et al. (1997).

In the 70's research on the acute response to and during a HIIE bout found increased lipid utilization compared to SSE (Essen, Hagenfeldt, & Kaijser, 1977). Based on this research, different HIIE protocols have been studied within a variety of healthy and diseased populations (Boudou et al., 2003; Gibala & McGee, 2008; Mourier et al., 1997; Talanian et al., 2007; Tremblay et al., 1994; Wisloff et al., 2007). A summary of the effects of HIIE on metabolic and cardiovascular related variables is depicted in Table 2.3. In summary, HIIE impacts body composition through reductions in fat mass with small increases in fat free mass. Insulin and adipocytokines are positively influenced by the HIIE through muscular and signalling adaptations and through

alterations in adipose tissue. Fitness increases may be due to the muscular adaptations and reductions in blood pressure and some lipids due to lower inflammatory markers and alterations in FFA's have been investigated following HIIE.

Table 2.3 Effects of HIIE on Metabolic Health Variables in Adults

Parameter	Effect
Body Composition	
Fat mass	⇩(Tjonna et al., 2008b; Trapp et al., 2008; Tremblay et al., 1994)
Muscle mass in trunk	⇧ (Trapp et al., 2008)
Central abdominal/trunk fat	⇩(Boudou et al., 2003; Tjonna et al., 2008b; Trapp et al., 2008)
Glucose Metabolism	
Insulin sensitivity	⇧(Boudou et al., 2003; Mourier et al., 1997; Tjonna et al., 2008a; Tjonna et al., 2008b; Trapp et al., 2008)
Serum Adipokines	
Leptin	⇩(Trapp et al., 2008) ⇔(Boudou et al., 2003)
Adiponectin	⇔(Boudou et al., 2003) ⇧(Tjonna et al., 2008a; Tjonna et al., 2008b; Trapp et al., 2008)
C-reactive protein	⇔(Tjonna et al., 2008b)
Serum Lipids	
High density lipoprotein	⇧(Tjonna et al., 2008a)
Triglycerol	⇔(Tjonna et al., 2008a; Tjonna et al., 2008b)
Blood Pressure at Rest	
Systolic	⇩(Tjonna et al., 2008b)
Diastolic	⇩(Tjonna et al., 2008b)
Cardiovascular Disease	
Endothelial function	⇧(Schjerve, Tyldum, Tjonna, Stolen, Loennechen, Hansen et al., 2008; Tjonna et al., 2008a; Tjonna et al., 2008b; Wisloff et al., 2007)
Cardio-Respiratory Fitness	
$\dot{V}O_{2peak}$	⇧(Boudou et al., 2003; Laursen, Shing, Peake, Coombes, & Jenkins, 2002; Mourier et al., 1997; Perry, Heigenhauser, Bonen, & Spriet, 2008; Schjerve et al., 2008; Talanian et al., 2007; Tjonna et al., 2008a; Tjonna et al., 2008b; Trapp et al., 2008; Tremblay et al., 1994; Wisloff et al., 2007)

Note: ⇧ indicates increased; ⇩ decreased; ⇔negligible effect

2.3.1.1.1 Obesity and high intensity interval exercise

Tremblay et al. (1994) investigated HIIE and SSE and found that those in the HIIE group lost more fat compared to the SSE group (Tremblay et al., 1994). Not only did weight loss occur but the exercise duration was less for HIIE than SSE (Tremblay et al., 1994). Previous research in our laboratory compared SSE to HIIE using an acute bout of high intensity lasting 8 seconds with a 12 second recovery (20 minutes total) for 15 weeks. Fat mass significantly decreased in the HIIE compared to SSE (Trapp et al., 2008). Other studies have shown similar results with different sprint protocols, in different populations with MetS and diabetes (Boudou et al., 2003; Mourier et al., 1997;

Tjonna et al., 2008a; Tjonna et al., 2008b). In a study involving T2DM males, despite no significant change in body mass, abdominal adiposity decreased by 44% with an increase in muscle cross sectional area (Boudou et al., 2003). An earlier study in 1997 found a 48% reduction in visceral fat measured by MRI compared to 18% in subcutaneous following an exercise regimen consisting of SSE two days per week and HIIE one day a week for 8 weeks in T2DM (Mourier et al., 1997).

Lipolysis, the breakdown of fat, has been shown to increase after exercise training either due to less reliance on plasma glucose or muscle glycogen as a substrate or an increased sensitivity to catecholamine induced lipolysis (Brouns & van der Vusse, 1998; van Loon, Manders, Koopman, Kaastra, Stegen, Gijsen et al., 2005; van Loon, Thomason-Hughes, Constantin-Teodosiu, Koopman, Greenhaff, Hardie et al., 2005). Following HIIE training increases in muscle glycogen use along with replenishment may impact the changes in FFA and fat loss. With the increase in catecholamines and glycerol following a single bout of HIIE (Trapp, Chisholm, & Boutcher, 2007), lipolysis and fat oxidation increases (Perry et al., 2008) would partially explain fat loss after HIIE training.

2.3.1.1.2 Fitness and high intensity interval exercise

Evidence shows cardio-respiratory fitness levels increase following interval training for more than 4 weeks (Boudou et al., 2003; Edge, Bishop, Goodman, & Dawson, 2005; Laursen et al., 2002; Mourier et al., 1997; Perry et al., 2008; Rognmo, Hetland, Helgerud, Hoff, & Slordahl, 2004; Schjerve et al., 2008; Tjonna et al., 2008a; Tjonna et al., 2008b; Tremblay et al., 1994). Increases in $\dot{V}O_{2peak/max}$ following interval exercise have been shown despite the short time frame (Burgomaster et al., 2005; Helgerud, Hoydal, Wang, Karlsen, Berg, Bjerkaas et al., 2007; Mourier et al., 1997; Talanian et al., 2007; Wisloff et al., 2007). Within those studies and the short

time frame of two weeks, citrate synthase (CS), a muscular oxidative enzyme, increased showing early adaptations. For a more comprehensive review please see Ross (2001) in the area of muscular adaptations and oxidative capacity with sprint interval training.

2.3.1.1.3 Diet and high intensity interval exercise

Only one study has incorporated Mediet with exercise showing the benefits in the intervention group after completing the study compared to controls (Esposito, Pontillo, Di Palo, Giugliano, Masella, Marfella et al., 2003). In those studies encompassing a Mediet, the authors compare high carbohydrate to low carbohydrate but don't fully incorporate the Mediet (Roberts & Barnard, 2005). A comprehensive review by Roberts and Barnard (2005) examines in more detail the interventions integrating diet and endurance or resistance exercise (Roberts & Barnard, 2005).

2.3.1.1.4 Summary

After a HIIE intervention, a reduction in adiposity stems from an increase in lipolysis in the hours after the exercise session and an increased sensitivity to catecholamines impacting fat oxidation rates. The increases in oxidative enzymes within the muscle improve aerobic fitness.

2.3.1.2 Clinical markers and high intensity interval exercise

2.3.1.2.1 Glucose intolerance and high intensity interval exercise

In people with MetS (Tjonna et al., 2008a) and T2DM (Boudou et al., 2003; Mourier et al., 1997), HIIE improved metabolic complications associated with the syndrome. Specifically looking at glucose, positive changes were seen in adolescent boys and girls following 13 weeks of HIIE (Tjonna et al., 2008b) and in people with MetS, following 16 weeks of HIIE (Tjonna et al., 2008a). Immediately after exercise, glucose uptake to the muscle occurs to replenish depleted muscle glycogen either from a meal (Ivy, Katz, Cutler, Sherman, & Coyle, 1988) or stored fat (Kiens & Richter, 1998).

After HIIE training insulin sensitivity will most likely change allowing for better control of glucose and with a reduction in adipose tissue and NEFA to the liver.

2.3.1.2.2 Insulin resistance, hyperinsulinemia, and high intensity interval exercise

Trapp et al. (2008) showed significant changes to fasting insulin following fifteen weeks of HIIE within healthy young premenopausal women The study previously mentioned in adolescents (Tjonna et. al. 2008) found improvements to insulin and glucose regulation (Tjonna et al., 2008b). Insulin sensitivity was increased following two months of HIIE in two different studies of patients with T2DM, 58% and 46% improvements (Boudou et al., 2003; Mourier et al., 1997). A decrease in fasting insulin and a change to insulin sensitivity could be due to a change in the insulin signalling pathways peripherally and changes to insulin's action in adipose tissue and muscle. Greater fat oxidation, reduced intramuscular triglycerides, and mitochondrial changes all may play a role in the response to exercise by insulin. What sets HIIE apart from SSE is the reduction in fatty acid uptake in adipose tissue along with changes to insulin's action on lipogenesis. With lower FFA levels, inflammatory and glucose changes can occur (Eckel et al., 2005). Fatty acid binding proteins (FABP) have been shown to influence lipid metabolism and inflammation (Boord, Maeda, Makowski, Babaev, Fazio, Linton et al., 2004; Jeukendrup, Saris, & Wagenmakers, 1998). Exercise studies have found increases in FABP and transporters in as short as two weeks. Talanian et al. (2007) saw an increase in FABP after a short duration of HIIE in endurance athletes (Talanian et al., 2007). After 16 weeks of HIIE in people with MetS, the transport proteins for fatty acids in adipose tissue were lower. Thus, the authors concluded, after HIIE there was a decrease in adipose tissue of lipogenesis which is the conversion of glucose into TG or VLDL (Tjonna et al., 2008a). The patients with MetS

increased their insulin receptor phosphorylation both in the muscle and fat tissue (Tjonna et al., 2008a) verifying that insulin signalling did improve.

2.3.1.2.3 Lipid abnormalities and high intensity interval exercise

Only two studies show changes to HDL following HIIE and they are from the same laboratory, although the populations differed. One study found an increase of 0.11 mmol/L after HIIE training compared to a decrease of 0.09 mmol/L in the SSE training group (Tjonna et al., 2008b). HDL increased by 25% after 16 weeks of HIIE in Mets patients (Tjonna et al., 2008a). The other HIIE studies including one from our laboratory did not find changes in plasma lipids. Tjonna et al (2008) attributed the improvement in the adolescents HDL to a change in diet. The participants claimed to have more motivation to eat healthy when undergoing the intervention. One possible reason behind the improvements in HDL after HIIE could be an indirect effect of the reduction in adipose tissue, FFA's, or lower inflammatory markers after HIIE.

2.3.1.2.4 Hypertension and high intensity interval exercise

Blood pressure dropped in both MetS patients and adolescents following HIIE (Tjonna et al., 2008a; Tjonna et al., 2008b) although the drop was not significantly different to that of the SSE groups in both studies. Mean arterial pressure decreased significantly more in the HIIE compared to the SSE group of adolescents (Tjonna et al., 2008b). Nitric oxide was increased in both the studies mentioned and was said to result in flow mediated dilation which would lower blood pressure. Increased sensitivity to sympathetic activity is another possible mechanism in the reduction of blood pressure with exercise. After three weeks of exercise training, blood pressure, and HR were reduced along with sympathetic overactivity and all three markers were associated with a change in insulin (Kohno, Matsuoka, Takenaka, Miyake, Okuda, Nomura et al., 2000).

2.3.1.2.5 Low grade inflammation and high intensity interval exercise

No studies have looked at low grade inflammation and HIIE although some have looked at adiponectin, an anti-inflammatory marker (Boudou et al., 2003; Tjonna et al., 2008a; Tjonna et al., 2008b; Trapp et al., 2008). Inflammation and HIIE will be examined more closely in the next section. Following exercise other than HIIE, inflammation has been shown to decrease in different populations (Balagopal, George, Patton, Yarandi, Roberts, Bayne et al., 2005a; Esposito et al., 2003) (Kohut, McCann, Russell, Konopka, Cunnick, Franke et al., 2006) (Kim, Jung, & Kim, 2008). One study examining T2DM women, found that a 14-week intervention changed some but not all inflammatory markers (Giannopoulou, Fernhall, Carhart, Weinstock, Baynard, Figueroa et al., 2005). Monzillo et al. (2003) found decreases to inflammatory markers in obese IR people after 6 months of lifestyle intervention consisting of a hypocaloric diet and exercise (Monzillo, Hamdy, Horton, Ledbury, Mullooly, Jarema et al., 2003). After seven months of moderate aerobic exercise training, young obese Japanese women improved their metabolic profile by weight reduction, improved adipocytokines, and reduced inflammatory markers (Kondo, Kobayashi, & Murakami, 2006). The changes seen in inflammatory markers could be due to increases in adiponectin and anti-inflammatory cytokine or due to reductions in IR and lipid metabolism. Possibly an increase in FABP's (mentioned previously) would result in lower inflammatory markers after HIIE.

2.3.1.2.6 Leptin, adiponectin, and high intensity interval exercise

After 15 weeks of HIIE a decrease in leptin was seen but no change in adiponectin for young healthy women. The same outcome for adiponectin was seen following eight weeks HIIE in T2DM males, although leptin did not change either. This could possibly be due to the shorter training duration or the diabetic population

completing the study. A reduction in leptin has been seen following changes in fat mass or pathophysiology. Only two HIIE studies looked at leptin, one found a decrease with significant changes to body mass and fat mass (Trapp et al., 2008) and the other had no change in body mass but a reduction in abdominal adiposity which was in T2DM males (Boudou et al., 2003). In adolescents, adiponectin increased after thirteen weeks of HIIE (Tjonna et al., 2008b). Kondo et al. (2006) relates the changes in adiponectin to a loss of visceral adiposity and body mass although the intervention in this study was seven months of aerobic training with a small sample size (Kondo et al., 2006). The evidence supporting increases in adiponectin after exercise training are controversial and more research is needed, specifically for HIIE.

2.3.1.2.7 Stress, the hypothalamic-pituitary-adrenal axis, and high intensity interval exercise

Investigations of cortisol, stress, or the HPA axis after acute or chronic HIIE are unexplored with few studies looking at exercise training in general. Neuro-endocrine deterioration increases with age, although increased aerobic fitness in an elderly population was inversely associated with dysfunction (Traustadottir, Bosch, & Matt, 2005). Six weeks of exercise training showed adaptations to HPA function and maintenance of the negative feedback loop involving cortisol (Park, Chan, Li, Kiraly, Matthews, Vranic et al., 2005a). Exercise promotes FFA oxidation which also may play a role in the reduction of cortisol (Coiro et al., 2007). Acute exercise responses following ten minutes of high intensity exercise regarding cortisol, growth hormone, catecholamines, and adrenocorticotropic hormone (ACTH) have shown that all are increased above baseline immediately following the exercise bout (de Vries, Bernards, de Rooij, & Koppeschaar, 2000). Unpublished results from our laboratory indicate

increases in catecholamines in the first hour following HIIE (Trapp, 2006), whether the response is similar post HIIE for cortisol, ACTH and growth hormone is undetermined.

2.3.1.2.8 Resting oxidation of fat or carbohydrate and high intensity interval exercise

The magnitude of physical inactivity influences the amount of fat that is preferentially stored or utilized for fuel by the body. Talanian et al. (2007) compared fat oxidation rates during an endurance test and found a 36% increase after two weeks of HIIE. During the same test, whole blood glycerol was significantly higher after HIIE training compared to pre training. More research on RER at rest, before, or after HIIE training is needed. Previously in our laboratory, a single bout of HIIE showed increased levels of glycerol and catecholamines during and immediately following (Trapp et al., 2007). Interestingly, the glycerol values during exercise increased earlier in the trained than the untrained women. Not only does HIIE promote utilization of fat throughout the exercise session but with training an increased capacity to utilize more fat was noted (Perry et al., 2008). Although there are potential problems associated with glycerol measurements indicating fat use, it has been shown that glycerol is an accepted marker of lipolysis (Trapp et al., 2007).

An increase in muscular oxidative capacity may influence the oxidation rates of fat during exercise. Following HIIE, augmented mitochondrial enzymes, adiponectin levels and muscle buffering capacity have been confirmed (Burgomaster et al., 2005; Schjerve et al., 2008; Talanian et al., 2007; Tjonna et al., 2008a; Tjonna et al., 2008b).

2.3.1.2.9 Autonomic function and high intensity interval exercise

Few studies have investigated the improvements in autonomic function following HIIE. During and post aerobic exercise training, research has shown enhanced autonomic function, with high frequency power and RR interval changes

(Hottenrott, Hoos, & Esperer, 2006; Sandercock, Bromley, & Brodie, 2005; Tulppo, Hautala, Makikallio, Laukkanen, Nissila, Hughson et al., 2003). Animal studies show that with exercise HPV increases (Hull, Vanoli, Adamson, Verrier, Foreman, & Schwartz, 1994) and an improvement in the balance between vagal and sympathetic activity (La Rovere, Mortara, Sandrone, & Lombardi, 1992). Healthy older adults had improvements in HPV following exercise training compared to controls (Stein, Ehsani, Domitrovich, Kleiger, & Rottman, 1999). After a 30-week endurance training program, men had increased fitness and HPV (Seals & Chase, 1989). More recently in children, HPV was investigated after HIIE and no change in HPV was found (Gamelin, Baquet, Berthoin, Thevenet, Nourry, Nottin et al., 2009). HPV is reduced with advancing age (van Ravenswaaij-Arts, Kollee, Hopman, Stoelinga, & van Geijn, 1993) making it difficult to compare studies done in children due to the immaturity in autonomic control of the heart. More research is needed to determine if enhanced HPV in healthy young adults accompanies HIIE training.

2.3.1.2.10 Summary

Improvements in metabolic function following HIIE and diet in combination or separately are due to a reduction in insulin, glucose, inflammation, blood pressure, and body composition, with an increase in fitness. Alterations in lipids, cytokines, and to the oxidative properties within muscle have been demonstrated.

2.3.2 Diet

Diet plays a role in obesity, IR, and CHD. More specifically, a westernized diet consisting of processed or refined foods, high fructose corn syrup, saturated fat mostly from animal products, and an excess salt intake can increase the risk for cardiovascular disease and death (Cordain, Eaton, Miller, Mann, & Hill, 2002; Hu et al., 2001; Keys et al., 1986; Salmeron et al., 1997a; Salmeron et al., 1997b). Research has shown a

reduction in risk for metabolic dysfunction (Batsis, Nieto-Martinez, & Lopez-Jimenez, 2007; Carpentier et al., 2006; Esposito, Ciotola, & Giugliano, 2007; Matia Martin et al., 2007; Panagiotakos, Tzima, Pitsavos, Chrysohoou, Zampelas, Toussoulis et al., 2007; Steemburgo, Dall'Alba, Gross, & Azevedo, 2007; Tzima, Pitsavos, Panagiotakos, Skoumas, Zampelas, Chrysohoou et al., 2007), inflammation (Bullo, Casas-Agustench, Amigo-Correig, Aranceta, & Salas-Salvado, 2007; Dai, Miller, Bremner, Goldberg, Jones, Shallenberger et al., 2008; Esposito, Ciotola, & Giugliano, 2006), and cardiovascular disease (de Lorgeril & Salen, 2006b; de Lorgeril, Salen, Martin, Monjaud, Delaye, & Mamelle, 1999; Martin-Du Pan, 2003; von Schacky, 2004) with the consumption of a "traditional Mediterranean" diet (see Figure 2.7) (Esposito et al., 2004). The Mediterranean basin is culturally diverse, resulting in different eating habits based on the country or region. What is referred to in the literature as the traditional Mediet stems from the Seven Countries Study (Keys, 1980) and is a diet rich in fruits, vegetables, olive oil, ω -3 fatty acids, fibre, and wine with low amounts of red meat, saturated fats, and high fat dairy (de Lorgeril & Salen, 2006b) (see Figure 2.7). The diet has been shown to be cardioprotective in people not living in the Mediterranean area (de Lorgeril, 1998; Trichopoulou, Costacou, Bamia, & Trichopoulos, 2003).

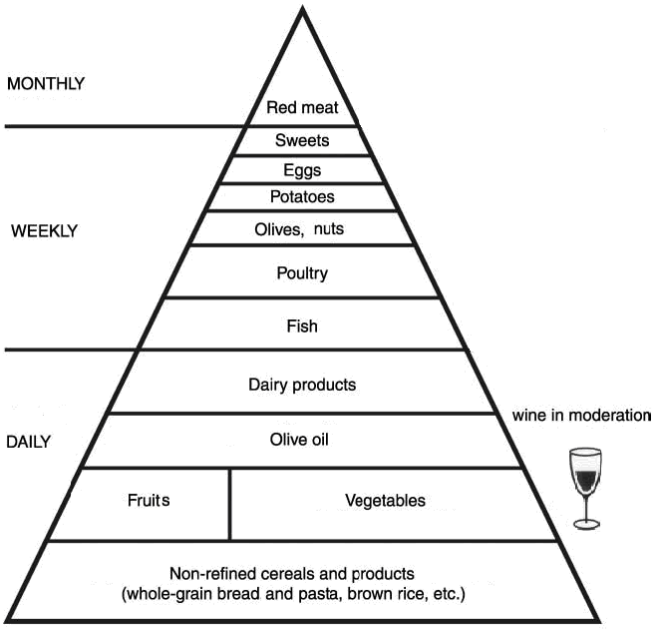


Figure 2.7 The Mediet pyramid (adapted from Willett et al., 1995).

Within the Mediet, the benefits derived from the individual aspects (fibre or vegetables) and their ingestion have been shown to minimize risk although the diet as a whole is what leads to lower inflammation (Papakonstantinou, Panagiotakos, Pitsavos, Chrysohoou, Zampelas, Skoumas et al., 2005), risk of developing the metabolic syndrome, and its associated low risk of CHD (de Lorgeril & Salen, 2006a; Martinez-Gonzalez & Sanchez-Villegas, 2004). The interactions of particular protective nutrients within the Mediet are thought to be the reasons for the protective benefit. Comparing all diets and cardiovascular health, the most effective dietary components in reducing cardiovascular risk are mono- and polyunsaturated fatty acids instead of trans fats, consumption of ω -3 fats and a diet high in fruits, vegetables, nuts, and whole grains and avoiding foods with a high glycemic load (GL) or index (Hu & Willett, 2002).

2.3.2.1 Obesity and diet

The evidence is equivocal regarding changes in body composition and ingestion of a Mediet. Esposito et al. (2004) found after two years, body mass decreased more in the group consuming the Mediet and exercise. A study comparing a low carbohydrate

non calorie restricted diet, low fat with restricted calorie diet, or a Mediet with restricted calories in moderately obese men and women found significant fat loss and metabolic changes after 24 months(Shai, Schwarzfuchs, Henkin, Shahar, Witkow, Greenberg et al., 2008). In women, greater weight loss was seen with the Mediet compared to the others but in men the Mediet was second to the low carbohydrate diet for weight change (Shai et al., 2008). Of note, with the above mentioned study, physical activity levels increased across the 24 month period. Another longitudinal study (5 years) conducted in Greece found that Mediet alone had no relation to BMI when controlling for energy intake (Trichopoulou, Naska, Orfanos, & Trichopoulos, 2005). The authors concluded that being physically inactive was the most likely reason for lack of change in BMI.

2.3.2.2 Clinical markers and diet

2.3.2.2.1 Glucose intolerance and diet

When looking at specific components of the Mediet that contributes to change, monounsaturated fatty acid (MUFA) intake is associated with lower plasma concentrations of glucose and insulin (de Lorgeril & Salen, 2006a). Glucose is significantly decreased in diabetic patients (Shai et al., 2008) on the Mediet and in obese premenopausal women (Esposito et al., 2003). Reduced glucose production following Mediet may stem from a change in either; FFA, adiposity, IS, or better insulin stimulated control of glucose.

2.3.2.2.2 Insulin resistance, hyperinsulinemia and diet

As mentioned previously, MUFA levels may also impact insulin (de Lorgeril & Salen, 2006a), along with increased fibre (Ludwig, Pereira, Kroenke, Hilner, Van Horn, Slattery et al., 1999), and a lower GL (Ludwig, 2003). Lower fibre intake in white women was associated with higher insulin (Ludwig et al., 1999), possibly due to fibre having a high glycaemic index which stimulates insulin secretion. A reduction in red

meat, a common aspect in the Mediet may lead to lower insulin levels, as studies have shown a positive association between red meat intake and insulin (Fung, Rimm, Spiegelman, Rifai, Tofler, Willett et al., 2001; Fung, Schulze, Manson, Willett, & Hu, 2004; Papakonstantinou et al., 2005). Studies have found beneficial effects on insulin with a higher intake of ω-3 in the diet or fish oil supplements (Ramel, Martinez, Kiely, Morais, Bandarra, & Thorsdottir, 2008). Of note, in an exercise and Mediet intervention trial, changes to adiponectin and FFA levels were associated with changes in insulin sensitivity (Esposito et al., 2003). Adiponectin, mentioned later on, and FFA level changes most likely reflect adipose tissue loss and a reduction in inflammatory markers.

2.3.2.2.3 Lipid abnormalities and diet

Positive changes in lipids are noted with the incorporation of a Mediet. The high MUFA found in olive oil alters the lipid profile and reduces cardiovascular risk by lowering LDL and increasing HDL (Hu & Willett, 2002). Another characteristic with the Mediet is a decrease in animal protein intake which has been shown to reduce LDL and TG, and improve HDL (Hu & Willett, 2002). As mentioned, direct changes to lipids can occur from the ingestion of a Mediet although indirectly lipids can be altered through reductions in inflammatory markers and FFA to the liver. Discussed later on are the benefits of fish oil on lipids.

2.3.2.2.4 Hypertension and diet

A reduction in blood pressure after Mediet could be indirectly altered by lower NEFA levels, reduced inflammatory markers, lower insulin, and changes to the SNS, or directly from an increase in fibre or ω -3 content. Use of a Mediet Score (MDS) has provided researchers with adherence information (Trichopoulou et al., 2003). A higher MDS is related to greater adherence to the Mediet. According to the EPIC study in Greece from 1994-1999 a higher MDS correlated to lower BP (Psaltopoulou, Naska,

Orfanos, Trichopoulos, Mountokalakis, & Trichopoulou, 2004). The authors found olive oil, fruits, and vegetables to be the most powerful aspects of the diet to reduce blood pressure. Specifically, antioxidants, potassium, magnesium, and calcium may all contribute to vascular health. Improvements in arterial stiffness following an increase in olive oil from the Mediet have been shown (Czernichow, Blacher, & Hercberg, 2004). Another potential beneficial component is the reduction in red meat or meat products and increase in ω -3. In white and black Americans, dietary fibre intake was inversely associated with both SBP and DBP in men and women but only in the whites (Ludwig et al., 1999). Fibre intake was inversely associated with BP (Ludwig et al., 1999) and cereal was positively associated with BP in the EPIC study. Of note, cereal usually contains salt and is a carbohydrate, known to be associated with BP (Psaltopoulou et al., 2004). The BP dropped in all three diets in the comparison study, the Mediet had the highest drop in SBP compared to the low carbohydrate and low fat diets (Shai et al., 2008). The Mediet has been shown to have beneficial effects on BP.

2.3.2.2.5 Low grade inflammation and diet

Systemic inflammation is positively affected by the Mediet. A higher MDS is related to lower levels of IL-6 (Dai et al., 2008). Lower levels of CRP also accompanied higher scores although the trend was not significant after adjustment (Dai et al., 2008). The Mediet compared to low carbohydrate and low fat diets had a more significant reduction in CRP after two years (Shai et al., 2008). After controlling for weight loss, IL-6, and CRP declined more in the Mediet group, for which the prevalence of the MetS was also reduced by half (Esposito et al., 2004). Inflammation improves with ingestion of particular elements with the Mediet. Arginine, ω -3, and fibre have been shown to lower levels of inflammation, IL-6, and CRP (Ma, Griffith, Chasan-Taber, Olendzki, Jackson, Stanek et al., 2006; Wells, Mainous, & Everett, 2005). No changes to

inflammatory markers in overweight IR women were seen after a double blind 12-week weight loss diet with supplemental long chain ω -3 poly unsaturated fatty acids, despite increased adiponectin levels (Krebs, Browning, McLean, Rothwell, Mishra, Moore et al., 2006a). This diet was a low fat-high carbohydrate diet with the least favourable change in CRP when comparing it to the low fat or Mediet (Shai et al., 2008). Elements of the Mediet or exercise bring about more positive changes in inflammatory markers. As previously mentioned with lipids, changes in inflammation may come directly from the diet, fish oil ingestion, or indirectly from changes to adiposity.

2.3.2.2.6 Leptin, adiponectin, and diet

Both leptin and adiponectin positively changed in the Mediet, low carbohydrate, and low fat with more pronounced improvements after the Mediet in diabetics (Shai et al., 2008). Leptin is most likely affected by fat loss either due to diet or exercise. Increased adiponectin may present with a decrease in IR, inflammation, or adiposity resulting in less NEFA circulation. In overweight IR women, adiponectin increased after a 12-week weight loss intervention with supplemental long chain ω -3 poly unsaturated fatty acids (Krebs et al., 2006a). Leptin, insulin, and adiponectin may interact together or one may cause a change in the other.

2.3.2.2.7 Stress, the Hypothalamic-Pituitary-Adrenal Axis, and Diet

The multifaceted problems with stress and diet are complex. The focus here is on the impact diet has on changes in HPA function and stress and not how the neuro-endocrine pathways affect energy balance. Please see Nieuwenhuizen et al. (2008) for a more thorough review of that area. Authors noted, after a 12-week calorie restricted diet in obese healthy women, weight loss had no impact on basal cortisol or HPA function (Ho, Keogh, Bornstein, Ehrhart-Bornstein, Lewis, Clifton et al., 2007). Mediet has been shown to be protective against HPA dysfunction in women (Garcia-Prieto, Tebar,

Nicolas, Larque, Zamora, & Garaulet, 2007). The authors concluded higher total fat and specifically saturated fat in the diet led to higher cortisol values which in turn affected HPA function. Mediet has been shown to lower FFA and with a lower intake of fat and saturated fat which in turn could reduce cortisol values (Garcia-Prieto et al., 2007).

2.3.2.2.8 Resting oxidation of fat or carbohydrate and diet

Diet may impact the oxidation of fat or carbohydrate at rest. Schutz et al. (1992) examined total RQ post weight loss from a dietary intervention and found that women exhibited a higher RQ at rest with a lower resting energy expenditure (REE) post weight loss. The overall REE and fat oxidation had decreased and CHO oxidation had increased (Schutz et al., 1992). Although the evidence is controversial, changes in fat mass may not be the direct (although strongly correlated) mechanism to changes in fat oxidation. Glucose tolerance and IR could be the key to understanding fat or CHO preference in overweight or obese individuals. More research involving fat and CHO oxidation after consumption of different diets is needed.

2.3.2.2.9 Autonomic function and diet

Alterations to sympathetic nervous system activity can be influenced by diet. A study investigating post menopausal women who were either vegetarian or omnivores found a higher LDL was associated with reduced HPV data (Fu, Yang, Lin, & Kuo, 2008). Another study comparing hypertensive obese women on an 11-day low or regular calorie diet found HPV data enhanced after the low calorie diet (Ashida, Ono, & Sugiyama, 2007). Diet has been shown to have some effect on HPV, although weight loss has been shown to have a more profound effect. Comparing weight loss through a surgical procedure and diet, the weight loss group had significantly enhanced HPV data (Karason, Molgaard, Wikstrand, & Sjostrom, 1999).

2.3.2.2.10 Summary

The cardio-protective Mediet high in fibre, fruits, vegetables, and low in saturated fats, refined foods and red meat positively impacts obesity and metabolic function. Similar modifications have been shown in body composition, glucose, insulin, hypertension, inflammation, cytokines, and stress after a Mediet compared to improvements seen with exercise. Increased MUFA and reductions in NEFA and red meat impact metabolic function through improved insulin signalling and sensitivity and healthier lipid profile, reduced LDL, increased HDL, and is antinflammatory (Bullo et al., 2007).

2.3.2.3 Glycaemic load

Studies show a protective effect from metabolic problems (Steyn, Mann, Bennett, Temple, Zimmet, Tuomilehto et al., 2004) and weight loss (Astrup, 2001) with ingestion of a low rather than high glycaemic meal. Studies report lower insulin demand, glucose, and C-peptide in the urine, indicating reduced insulin secretion, following 4 weeks of a low GL diet in healthy and diseased populations (Burke, Hartog, Heaton, & Hooper, 1982; Frost, Keogh, Smith, Akinsanya, & Leeds, 1996; Jenkins, Wolever, Collier, Ocana, Rao, Buckley et al., 1987). Ludwig et al. (1999) followed 3000 black and white men and women age 18-30 years and found those who ate a lower GL diet gained less weight over the study compared to individuals who ate a high GL diet (Ludwig et al., 1999). Higher GL foods tend to increase insulin levels, reducing the influence it has on glucose metabolism (see Figure 2.8) (Jenkins, Axelsen, Kendall, Augustin, Vuksan, & Smith, 2000). A low GL healthy Mediet eating plan with non-refined or processed foods should help normalize metabolic disturbances.

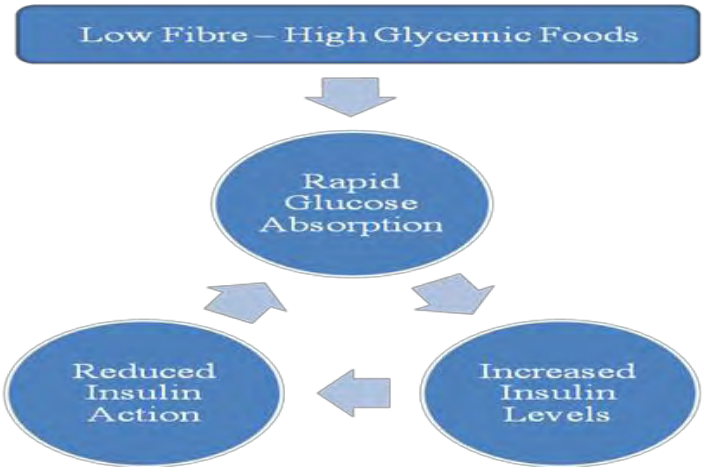


Figure 2.8 Schematic representation of insulin's response to high GL foods (adapted from Jenkins et al., 2000).

2.3.2.4 Fish oil supplementation

As mentioned previously, ω-3 within the diet can protect against risk for CHD, altering lipids, cardiac properties, vascular function, and inflammation (Engler & Engler, 2006). A variety of plant or vegetable oils along with fish contain ω-3 and ω-6 but the actual fatty acids are different. The omega's from plant sources are linoleic (LA) and alpha-linolenic (ALA), which convert into arachidonic (AA), eicosapentaenoic (EPA), and docosahexaenoic (DHA) acids (Balk, Chung, Lichtenstein, Chew, Kupelnick, Lawrence et al., 2004; Balk, Lichtenstein, Chung, Kupelnick, Chew, & Lau, 2006). The ratio of ω-3 to ω-6, as previously mentioned, is important and the amount of EPA and DHA from plant sources is small. Fish oil is a good source for EPA and DHA although fish don't actually produce the EPA and DHA they ingest them from other sources (Burdge & Wootton, 2002; Emken, Adlof, & Gulley, 1994; Pawlosky, Hibbeln, Lin, Goodson, Riggs, Sebring et al., 2003; Pawlosky, Hibbeln, Novotny, & Salem, 2001). Figure 2.3 demonstrates the pathways of EPA and DHA and their cardioprotective benefits. Cardiovascular and total mortality was reduced in a study comparing weekly fish intake and ω-3 supplement in men (Burr, Fehily, Gilbert, Rogers, Holliday, Sweetnam et al., 1989). Fish is a staple with the Mediet although

certain fish more than others carry the benefits. Oily fish have more EPA and DHA (see Table 2.4). Fish oil supplementation is an easy way to get the recommended daily dosage of EPA and DHA although the quality of the oil within the supplement differs among competing companies. Environmental toxins, low EPA and DHA per capsule, oil degradation, and farmed versus wild types of fish should all be considered when choosing a fish oil supplement. Improvements to insulin action, adipocyte size, and oxidation of glucose in muscle and beta cells have been recorded after fish oil consumption (Lombardo, Hein, & Chicco, 2007).

Table 2.4 Sources of Omega-3 Fatty Acids (Adapted from Kris-Etherton et al., 2002)

Fish	Amount (oz) **
Tuna	
Fresh	2.5 - 12
Light *	12
White *	4
Pink Salmon	2.5
Atlantic Salmon	
Farmed	1.5 - 2.5
Wild	2 - 3.5
Mackerel	2 - 8.5
Herring	1.5 - 2
Sardines	2 - 3
Cod	12.5 - 23
Pacific Oyster	2.5
Rainbow Trout	
Farmed	3
Wild	3.5
Lobster	7.5 - 42.5
Alaskan King Crab	8.5
Shrimp	11
Clam	12.5
Scallop	17.5

* Canned in water and drained
** Needed to provide EPA and DHA (approximately 1 g/d)
The amounts are rough estimates and variable depending on species, season, diet, packaging, and cooking methods

Another study in humans investigated the effects of fish oil with energy restriction on IR and found significant reductions in IR following the fish oil ingestion (Ramel et al., 2008). Increased ω-3 intake coincides with lower TG and LDL levels and raised HDL (Engler & Engler, 2006). Endothelial function and blood pressure are

improved after the intake of ω-3 along with reduced arterial wall thickness in the rat model (Engler & Engler, 2006). Recently, enhanced HPV markers, HF and LF, were shown to be associated with fish intake (Mozaffarian, Stein, Prineas, & Siscovick, 2008). Studies have shown positive changes to IS with fish oil (Krebs et al., 2006a; Lombardo et al., 2007; Ramel et al., 2008; Waite et al., 2008), most of the fish oil ingested is presented as polyunsaturated fatty acids (PUFA) without specific amounts of EPA or DHA. Studies should present the dosage in grams of both EPA and DHA as already discussed the ω-3:ω-6 ratio is an important factor in relation to diet.

2.4 Summary of Literature Review

This literature review has demonstrated that the complexities of metabolic function are immense but changes involving lifestyle behaviours such as physical inactivity and diet can alter the course of metabolic disease. Through the use of clinical markers, early signs for metabolic dysfunction can be caught and identification of "at risk" populations could prevent or delay the onset of problems associated with obesity and MetS. Exercise can alter metabolic pathways in the post exercise time period leading to greater rates of fat oxidation and increased sensitivity to catecholamines and insulin stimulation through hormonal and adipocytokine alteration. Consequently, a lifestyle modification program consisting of exercise (HIIE) and healthy eating (Mediet) has the potential to alter the clinical markers of MetS and help decrease obesity.

CHAPTER 3 INSULIN RESISTANCE IN YOUNG WOMEN (STUDY I)

3.1 Introduction

Several groups have called for identification of factors that initiate MetS. As previously mentioned, a key influence in the development of MetS is insulin resistance (IR). Hyperinsulinemia has been shown to be a major precursor of cardiac events, stroke, and myocardial infarction (Pyorala, Miettinen, Laakso, & Pyorala, 2000). Prior to hyperinsulinemia problems may occur in insulin regulation following a meal (Eckel et al., 2005). Whether young apparently healthy women who adopt a Western lifestyle (high fat, refined carbohydrate, energy dense diet and physical inactivity) also exhibit similar hyperinsulinemia is undetermined. Thus, identifying young individuals who are hyperinsulinemic and normalising their insulin levels through lifestyle interventions such as physical activity and healthy eating habits (ATPIII, 2001) may prevent later development of MetS.

This study was a cross sectional study evaluating the metabolic health of young premenopausal women. Participants were asked to complete a fitness test and diet diary, give a blood sample, and obtain a DEXA scan. Also, ethnic variation and insulin resistance is described in this group of women.

3.2 Methods (Study I)

Volunteer non-smoking, non-exercising but otherwise healthy young women (N = 66) aged 21.2 ± 0.3 years (see Table 3.1) were recruited from a university population. Participants signed an informed consent (see Appendix A) approved by the Human Ethics Committee at the University of New South Wales (HREC 03218, 05238, and 06293).

3.2.1 Participants

3.2.1.1 Participant data collection

Participants, who were advised to avoid strenuous activity and caffeine for 24 hours prior to testing, came into the laboratory after an overnight fast. All tests were completed at the same time of day (between 6:00 and 10:00 am) to avoid diurnal variation. Personal and familial medical history was collected to determine the use of tobacco products and drug use, birth weight, exercise history, and a complete diet diary recall (see Appendix B and C). Participants were asked to complete a Physical Activity Readiness Questionnaire (Par Q test, 1992) (Thomas, Reading, & Shephard, 1992) that screened for contraindications to exercise (see Appendix D).

3.2.2 Body Composition Assessment

Participants completed a Dual Energy X-Ray Absorptiometry (DEXA) scan using a Lunar Prodigy scanner (software version 7.51, GE Corporation, USA). Mass (M), fat mass (FM) along with fat free mass (FFM) in kilograms and total percent body fat (BF%) were measured for the whole body, arms, legs, and for the abdominal region. DEXA provides a measure of central adiposity. The percent trunk fat (%TrF) includes the area distal to the neck and superior to the pelvis without the limbs. Height (ht) was measured using a standard calibrated stadiometer, whereas body-mass index (BMI) was calculated by dividing mass by height squared (kg/m^2).

3.2.3 Blood Draw and Lipid Profile

A fasting blood sample was taken from an antecubital vein (either the cephalic or brachial). Whole blood was placed in EDTA vacutainers. Blood lipid profiles from whole blood [triglycerides (TG), total cholesterol (TC), low density lipoproteins (LDL), and high density lipoprotein (HDL) concentration] were quantified by automated enzymatic methods (Cholestech LDX, USA). The Friedewald equation (Friedewald,

Levy, & Fredrickson, 1972) was used to estimate very-low-density lipoprotein cholesterol (VLDL) concentration which was calculated by TG divided by 2.2. The remaining whole blood in EDTA tubes were spun immediately in a chilled centrifuge (Model Megafuge 1.0R, Heraeus, Germany) at 4^{o}C and stored at -86^{o}C for later analysis.

3.2.4 Aerobic Power Test

Aerobic power was assessed using a TrueMax 2400 Metabolic Cart (ParvoMedics Inc, USA). After a 3-minute warm up at 30 watts (W) with a set pedal frequency of 60 revolutions per minute (RPM), the initial load was set at 45 W and was increased 15 W every minute until voluntary cessation and/or pedal frequency could not be maintained. All $\dot{V}O_{2peak}$ sessions were performed on an electronically braked Monark cycle ergometer (Ergomedic 839E, Sweden) using a two-way breathing valve and nose clip (Hans Rudolph, USA). Temperature in the lab was maintained between 23-25°C and participants were asked to give a rating of perceived exertion (RPE) (Borg, 1982) every minute throughout the test. Due to the strenuous nature of the exercise session not all participants achieved the criteria for $\dot{V}O_{2max}$ (Schell & Leelarthaepin, 1994) $\dot{V}O_{2peak}$ was accepted as a measure of functional aerobic power for those participants. Heart rate (HR) was recorded during this session using a Polar S810I telemetry system (Polar, Finland).

3.2.5 Diet Analysis

All participants were asked to complete a diet diary record of food consumed on three separate days consisting of two week days and one weekend. The diets were analyzed using SERVE (SERVE Nutrition Management Systems, Professional Edition, version 5.1.002, 2004, Australia) and Foodworks (Foodworks 2007, version 5.00, Xyris Software, Australia) computer software.

3.2.6 Plasma Analysis and HOMA-IR Calculations

Plasma samples were analysed later in duplicate for insulin and C-reactive protein (CRP). Insulin and CRP were measured using commercially available immunoassay kits. The degree of enzymatic turnover of the substrate was determined by dual wavelength absorbance measurement at 450 and 620 nm for insulin (DSL 10-1600), and CRP (DSL10-42100). HOMA-IR, the insulin resistance index (Stern, Williams, Ferrannini, DeFronzo, Bogardus, & Stern, 2005) was calculated based on the following equation:

HOMA-IR = [fasting insulin (µIU/ml) x fasting blood glucose (mmol/l)] / 22.5.

3.2.7 Analysis

Data analysis was completed with the Statistical Package for Social Science for windows software (SPSS 15.1, USA). All results are expressed as mean and standard error (SE) for variables with normal distribution. A p value of $< .05$ was adopted. Due to skewness, some values were log transformed (Ln) for analysis. Student's independent *t*-tests were used to examine differences between the two ethnic groups with normal distribution. Correlation analyses (Pearson) determined associations between crude and transformed values. The influence of body composition on blood variables was controlled by linear regression.

3.3 Results

3.3.1 Overview

Sixty six women presented to the lab for a fitness test, blood sample, and for body composition measurements. The women also completed a medical history questionnaire, diet diary and a DEXA scan at a local hospital. The participant characteristics are presented initially. High fasting plasma insulin and CRP were found in young premenopausal women who were unfit and ingested high amounts of saturated

fat and protein relative to body weight. Finally, when the women were divided by ethnicity (European versus Chinese Australians) the Chinese women had significantly elevated fasting plasma insulin and lower levels of CRP and consumed a lower amount of saturated fat.

3.3.2 Participant Characteristics

The participants (N = 66) in the study were young, apparently healthy non-smoking women from the University community. The groups consisted of European women (n = 38) and Asian (Indonesian Chinese, Thai Chinese, and Malaysian Chinese) women (n = 28).

Table 3.1 shows participants' age, lipid, DEXA, fitness and metabolic parameters for the group as a whole and two ethnically different cohorts [European (EW) and Chinese (CW) women].

Table 3.1 Characteristics of Study I Participants

	All Women (N = 38)		European Women (EW) (n = 38)		Chinese Women (CW) (n = 28)	
	Mean	SE	Mean	SE	Mean	SE
Age (years)	21.2	0.3	21.4	0.4	20.9	0.4
Mass (kg)	67.1	1.5	71.5	2.1	61.1 *	1.5
Height (cm)	163.6	0.9	166.4	1.1	160.0 **	1.1
BMI (kg/m^2)	24.9	0.4	25.8	0.7	23.9 *	0.5
$\dot{V}O_{2peak}$ (L/min)	2.0	0.1	2.1	0.1	1.8 *	0.1
$\dot{V}O_{2peak}$ (ml/kg/min)	29.6	0.6	30.0	0.8	29.1	1.0
Waist circumference (cm)	77.7	1.2	81.5	1.6	72.8 **	0.8
Hip circumference (cm)	103.7	1.5	109.2	1.7	96.8 **	1.0
Waist to height ratio	0.47	0.01	0.49	0.01	0.45 *	0.01
Trunk fat (%)	38.1	1.1	39.0	1.6	37.0	1.5
Fat free mass (kg)	39.9	0.7	41.6	0.9	37.5 *	0.8
Body fat (%)	37.0	0.9	38.4	1.3	35.1	1.1
Fat mass (kg)	24.2	1.1	26.9	1.6	20.6 *	1.0
Fasting insulin (µIU/ml)	16.0	1.4	12.7	1.7	20.6 **	2.0
HOMA-IR	3.3	0.3	2.8	0.4	4.1 *	0.3
Fasting glucose (mmol/L)	4.7	0.1	4.7	0.1	4.6 *	0.1
Fasting CRP (ng/ml)	4.5	1.0	6.2	1.6	2.3 *	0.7
Triglycerol (mmol/L)	0.88	0.05	0.87	0.06	0.88	0.08
Total cholesterol (mmol/L)	4.1	0.1	3.9	0.1	4.4 *	0.1
HDL cholesterol (mmol/L)	1.4	0.0	1.4	0.0	1.5	0.1
LDL cholesterol (mmol/L)	2.3	0.1	2.2	0.1	2.5	0.1
VLDL cholesterol (mmol/L)	0.40	0.02	0.40	0.03	0.40	0.03

Mean and standard error (SE)
* Significant difference ($p < .05$)
** Significant difference ($p < .001$)
Note: Body mass index (BMI), homeostasis model assessment of insulin resistance (HOMA-IR), high density lipoproteins (HDL), estimated low density lipoproteins (LDL), and estimated very low density lipoproteins (VLDL)

3.3.3 Body Composition

For the group of women as a whole, their BMI = 25 kg/m^2 is considered overweight along with a body fat of 37%. The WC (77.7 ± 1.2 cm) did not meet the IDF MetS criteria cut off point which is 80 cm (IDF, 2006)

The CW were significantly smaller for most body composition indices: mass, $t(64) = 3.79$, $p < .001$, ht, $t(64) = 3.94$, $p < .001$, FM (see Figure 3.1), $t(58) = 3.38$, $p < .05$, FFM, $t(64) = 3.33$, $p < .05$, and BMI, $t(63) = 2.29$, $p < .05$. Fitness relative to body

weight was negatively correlated to most body composition indices and approached significance with fasting plasma insulin and HOMA-IR (see Appendices I).

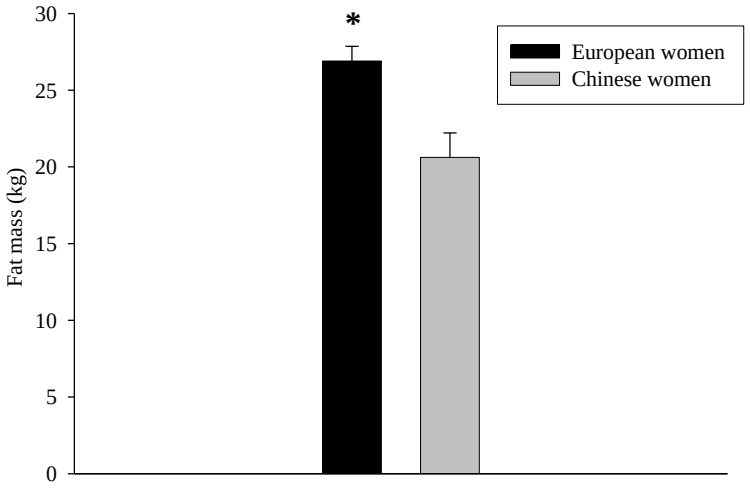


Figure 3.1 Fat mass in young women.

3.3.4 Aerobic Power

The cardio-respiratory fitness levels of both groups were similar (see Figure 3.2) and was average for physically inactive women of this age (Shvartz & Reibold, 1990). An inverse relationship between aerobic fitness and fasting plasma insulin approached significance, $r > 0.21$, $p = .09$, HOMA-IR, $r > 0.21$, $p = .09$, as did some lipids (TG, $r > 0.24$, $p = .06$, and VLDL, $r > 0.23$, $p = .06$,).

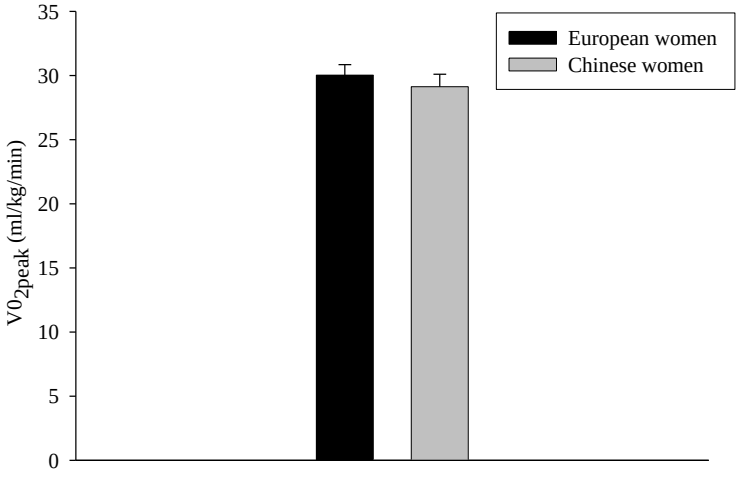


Figure 3.2 Fitness levels of young women.

3.3.5 Bloods Lipids, Insulin, C-Reactive Protein, and HOMA-IR

The mean fasting plasma insulin values for all women was 16.0 ± 1.4 µIU/ml whereas HOMA-IR was 3.3 ± 0.3. According to Park et al. (2005) these women are in an insulin resistant state (Park et al., 2005b). The mean CRP (4.5 ± 1.0 ng/ml) is also considered an elevated inflammatory marker (Haffner, 2006). The glucose and lipid values for the women were in the healthy range (ATPIII, 2001).

Glucose, TG, LDL, HDL, and VLDL were similar for both groups and TC was significantly, $t(64) = 2.35$, $p < .05$, greater in the CW (see Table 3.1). Fasting plasma insulin (see Figure 3.3), $t(63) = 3.93$, $p < .001$, and HOMA-IR, $t(62) = 3.63$, $p < .05$, were significantly elevated in CW compared to EW, whereas CRP (see Figure 3.4), $t(64) = 3.26$, $p < .05$, levels were significantly higher in EW compared to CW (see Table 3.1).

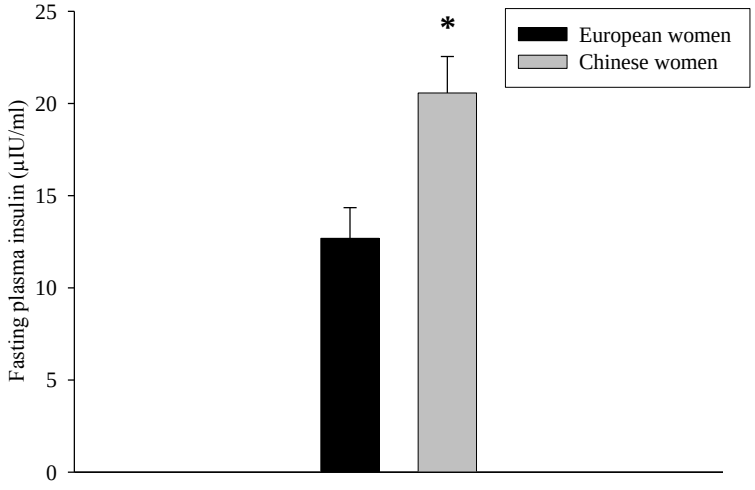


Figure 3.3 Fasting plasma insulin in young women.

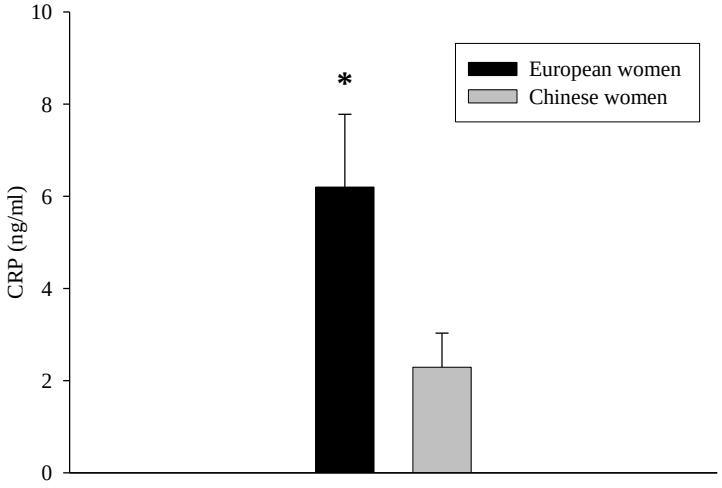


Figure 3.4 Fasting plasma CRP in young women.

Differences still existed for insulin, HOMA-IR, and CRP after the effect of body composition was controlled.

Table 3.2 Associations Between Body Composition and Whole Blood Glucose, Fasting Plasma CRP and Insulin

European and Chinese	Glucose (mmol/L)	Insulin (µIU/ml)	CRP (ng/ml)
Mass (kg)	0.24	0.18	0.34**
BMI (kg/m^2)	0.19	0.27*	0.40**
Trunk fat (%)	0.26*	0.28*	0.32*

European	Glucose (mmol/L)	Insulin (µIU/ml)	CRP (ng/ml)
Mass (kg)	0.19	0.60**	0.28
BMI (kg/m^2)	0.10	0.59*	0.29
Trunk fat (%)	0.17	0.61**	0.22

Chinese	Glucose (mmol/L)	Insulin (µIU/ml)	CRP (ng/ml)
Mass (kg)	0.19	- 0.16	0.13**
BMI (kg/m^2)	0.25	- 0.05	0.47**
Trunk fat (%)	0.36	- 0.34	0.45*

* Significant difference ($p < .05$)
** Significant difference ($p < .01$)
Note: C-reactive protein (CRP) and body mass index (BMI)

3.3.6 Dietary Intake

Table 3.3 shows participants' diet results. The CW consumed significantly higher protein relative to their body mass (see Figure 3.5), $t(56) = 2.40$, $p < .05$, and less percent saturated fat, $t(56) = -2.11$, $p < .05$, than EW.

Table 3.3 Study I Participant's Dietary Intake

	All women (N = 58)		European women (EW) (n = 31)		Chinese women (CW) (n = 27)	
	Mean	SE	Mean	SE	Mean	SE
Protein (g)	83.3	4.5	78.2	6.4	89.1	6.4
Fat (g)	60.5	4.9	64.8	7.9	55.7	5.3
Saturated fat (g)	25.6	1.5	28.1	2.5	22.7	1.5
Carbohydrate (g)	224.5	11.0	226.8	16.8	222.0	13.8
Protein (%)	23.0	0.7	21.8	1.1	24.3	0.9
Fat (%)	15.7	0.8	16.5	1.2	14.7	1.0
Saturated fat (%)	7.1	0.3	7.7	0.4	6.4 *	0.4
Carbohydrate (%)	61.4	1.1	61.7	1.6	60.9	1.4
Protein (g/kg)	1.3	0.1	1.1	0.1	1.5 *	0.1
Energy intake (kJ)	7889	356	8133	555	7608	428

Mean and standard error (SE)
* Significant difference ($p < .05$)

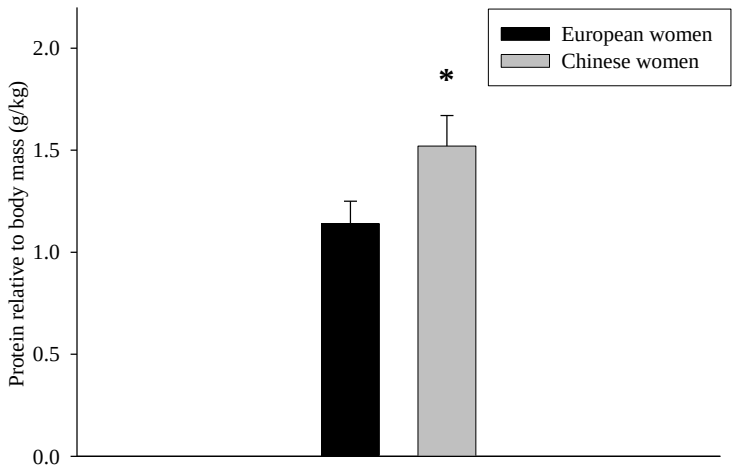


Figure 3.5 Protein relative to body mass intake in young women.

Table 3.2 shows correlations between metabolic markers and body composition for all women and for EW and CW. Fasting insulin, glucose, and CRP were

significantly correlated, $r > 0.26$, $p < .05$, with percent trunk fat showing that those with increased adiposity possessed more metabolic perturbation.

Protein (g) per body mass (kg) was significantly inversely associated, $r = -.30$, $p < .05$, with CRP and percent protein was positively associated, $r = -.36$, $p < .05$, with insulin (see Figure 3.6) and HOMA-IR.

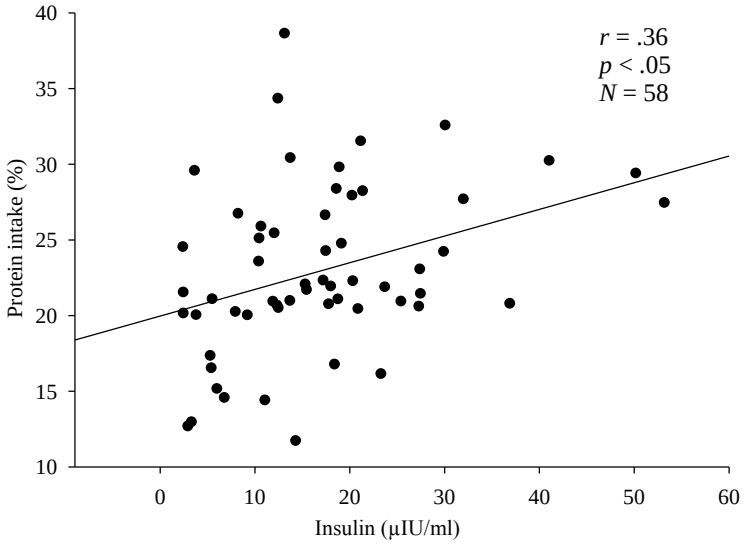


Figure 3.6 Correlation between fasting plasma insulin and percent protein intake in young women.

3.4 Discussion

The major findings for Study I were young (21.2 ± 0.3 yr) healthy premenopausal women who were unfit and overweight (24.9 ± 0.4 kg/m^2) possessed high fasting plasma insulin and CRP levels. The dietary protein intake relative to the body mass of the women was elevated according to guidelines proposed by the ATPII. These results support Hypothesis 1 (see page 9) that states that young sedentary premenopausal women consuming a westernized diet would possess elevated fasting plasma insulin. The aerobic fitness for the women was negatively correlated to a number of MetS markers, body composition, insulin, HOMA-IR, and some lipids. Although hyperinsulinemia was influenced by ethnicity and the Chinese women were significantly lighter in body mass with a lower BMI their body fatness did not

significantly contribute to the elevated fasting plasma insulin. Thus, these results do not support Hypothesis 2 (see page 9). The MetS criteria does not stipulate aberrant diet, insulin, CRP, or fitness levels as initial markers for the syndrome, although these factors are likely to significantly influence the development of MetS.

3.4.1 Insulin, HOMA-IR, and C-Reactive Protein in Young Women

Fasting plasma insulin levels (16.0 µIU/ml) were in the hyperinsulinemic range (> 9.98 µIU/ml) as proposed previously (Park et al., 2005b). IR has been shown to precede glucose abnormalities (Caballero, 2005) and is associated with impaired fat oxidation (Blaak et al., 2006). Thus, these data further confirm that IR is among the earliest MetS markers (Ferrannini & Balkau, 2002; Martin et al., 1992; Pyorala et al., 2000). Visceral adiposity has been previously shown to predict IR (Wahrenberg, Hertel, Leijonhufvud, Persson, Toft, & Arner, 2005). The women in the current study did not meet the IDF criteria for Mets which is based on a high BMI or waist circumference > 80 cm, inferring a modest amount of visceral adiposity. Disregarding visceral adiposity, the unhealthy diet and lack of physical daily activity may account or contribute to the hyperinsulinemia found in these women.

High CRP levels have been found to accompany hyperinsulinemia in American middle-aged women (LaMonte et al., 2005a). The women in this study had high CRP levels (4.5 ng/ml) despite being young. CRP was positively correlated to TG, VLDL, and body composition measures (mass, FM, %BF, %TrBF) but did not have an association with hyperinsulinemia.

3.4.2 Cardiorespiratory Fitness in Young Women

The sedentary women investigated had average aerobic fitness levels compared to other women their age. Despite being sedentary, fitness levels were inversely related to

insulin, HOMA-IR, triglycerol, and VLDL. These results may imply that increased fitness leads to lower risk of MetS due to a lowering of insulin, HOMA-IR and lipids.

3.4.3 Dietary Intake in Young Women

The women consumed high levels of saturated fat, carbohydrate, and protein relative to their body mass. All lipids were within the healthy range. Interestingly, fasting insulin and HOMA-IR were both significantly correlated with percent dietary protein. Thus, protein intake appeared to influence fasting insulin more so than any body composition factor. The ATPIII report recommends that protein intake should be approximately 15% of total calories (ATPIII, 2001). Protein intake was 23.0% of total intake. Thus, this cohort of young premenopausal women consumed significantly more protein than that recommended by ATPIII. It has been shown that greater ingestion of protein is associated with IR (Papakonstantinou et al., 2005) and T2DM (Fung et al., 2004; Gannon & Nuttall, 2004). Consequently, excessive dietary protein intake could place these young women at increased risk for IR.

3.4.4 Ethnic Variation within Metabolic Variables

An interesting finding was the disparity in metabolic variables measured when comparing the Chinese and European women. CW compared to EW had significantly higher levels of fasting plasma insulin and lower levels of CRP (see Table 3.1). The EW compared to CW were heavier and possessed greater body fat and BMI. Nevertheless, differences in body composition did not significantly influence the higher insulin and lower CRP levels of EW and CW. CW's insulin levels were 38% greater than that of the EW (see Table 3.1). These results support prior research that has shown that hyperinsulinemia was characteristic of middle-aged Chinese immigrant women (Unwin, Harland, White, Bhopal, Winocour, Stephenson et al., 1997).

However, CW women, despite being hyperinsulinemic, had low levels of CRP. The women in the American study were older and of African American, Caucasian, and Native American descent. CRP concentrations in pre and peri-menopausal Chinese and Japanese women have been found to be significantly lower compared to those of African Americans, Hispanics, and Caucasians even after controlling for BMI (Kelley-Hedgepeth, Lloyd-Jones, Colvin, Matthews, Johnston, Sowers et al., 2008). Thus, CRP levels appear to be influenced by ethnicity. However, in Asian populations it has been shown that even with lower overall CRP concentrations, those with relatively higher levels of CRP were still at a heightened risk for MetS and diabetes. In the present study, after controlling for percent trunk fat and BMI, CRP still remained significantly lower in the CW compared to EW.

The CW consumed significantly more protein, relative to body mass, less saturated fat in their diet, and had significantly higher TC than EW. Thus, healthy but unfit young women of Chinese descent displayed elevated levels of fasting insulin but low levels of CRP. Protein intake was 21.8% of total intake for the EW group and 24.3% for CW (see Table 3.3). Relative to body mass there was a significant increase (25%) in the amount of protein consumed in the CW compared to EW.

3.4.5 Trunk Fat in Young Women

In the current study percent trunk fat had a positive, significant correlation with insulin and HOMA-IR levels in the EW and with CRP in the CW. It has been shown in lean and obese men and women that trunk fat (subcutaneous and visceral fat) accumulation contributes to IR (Goodpaster, Thaete, Simoneau, & Kelley, 1997). Despite the strong association, ethnicity remained an independent predictor of insulin, HOMA-IR, and CRP in this cohort of young women. These results are similar to previous investigations that have shown that trunk fat, reflected by waist circumference,

was a significant predictor for insulin, glucose, HDL, and TG in middle-aged Chinese women (Lear, Chen, Frohlich, & Birmingham, 2002; Patel, Unwin, Bhopal, White, Harland, Ayis et al., 1999). As the Chinese women in the present study were much younger than the middle-aged women of the Lear et al. (Lear et al., 2002) and Patel et al. (Patel et al., 1999) studies, early identification and treatment of hyperinsulinemia may help reduce the development of MetS at an older age. Lack of a relationship between fasting plasma insulin and waist circumference suggests that other variables such as diet and fitness make a greater contribution to hyperinsulinemia in this cohort of women.

3.4.6 Summary

High fasting plasma insulin, CRP, protein intake relative to body weight, and saturated fat consumption were characteristics found in a physically inactive population of young premenopausal women. All the data shown here are within the accepted ranges except for fasting plasma insulin and CRP. Although the current guidelines do not put these women in an "at risk" category nor do they meet the criteria for MetS, these results suggest that they are in a 'pre-MetS' state. Without early intervention these unfit young women may go on to develop MetS and/or diabetes.

Despite the BMI for an Asian population being in the "increasing but acceptable risk" category, their elevated plasma insulin and living a sedentary lifestyle are not considered by the current guidelines to be "at risk" for MetS. However, contrary to the current criteria, the women are at risk because of their elevated HOMA-IR scores, fasting insulin levels (Park et al., 2005b), and sedentary behaviours (Carnethon, Gidding, Nehgme, Sidney, Jacobs, & Liu, 2003).

Early markers (diet, fitness, and insulin) for later development of MetS should be considered when evaluating the health status of individuals, especially ethnic groups.

Future studies should look into "early warnings" for younger and ethnic populations in order to ameliorate these abnormalities before they propagate.

CHAPTER 4 ACUTE RESPONSE TO EXERCISE (STUDY II)

4.1 Introduction

Participants were required to perform an acute exercise session consisting of high intensity interval exercise (HIIE) and steady state exercise (SSE) on two separate occasions. These exercise protocols were compared in the 2 hours after to assess the acute response in blood markers associated with MetS (hormones, proteins, cytokines and chemical compounds). The women were further divided into high and low baseline insulin groups to determine if fasting insulin impacts the response to the type of exercise performed or the two hours of recovery after exercise. It is undetermined if HIIE compared to SSE will have a greater impact on blood markers in the immediate (two hours) post exercise phase or if HIIE will have a different impact on women with varying levels of baseline insulin.

4.2 Methods (Study II)

4.2.1 Participants

Volunteer, premenopausal recreationally active but untrained women (*N*= 18) between the ages of 18 and 30 years (see Table 4.1) were recruited from a university population. After all risks and procedures were explained in accordance with university policy, participants gave written consent. The informed consent (see Appendix A) was approved by the Human Ethics Committee at the University of New South Wales (HREC 05238).

4.2.1.1 Baseline participant data collection

Participants, who were advised to avoid strenuous activity and caffeine for 24 hours prior to testing, came into the laboratory after an overnight fast on three occasions. All tests were done at the same time of day (between 6:00 and 10:00 am) to avoid diurnal variation and during the follicular phase of the menstrual cycle on both

exercise days. Personal and familial medical history information was collected consisting of: use of tobacco products and medication, birth weight, exercise history, and a complete diet diary recall (see Appendix C). Participants were asked to fill in a Physical Activity Readiness Questionnaire (Par Q test) (Thomas et al., 1992) which helped screen for contraindications to exercise (see Appendix D).

4.2.2 Body Composition Assessment

Body composition data were measured during the initial session. Body composition measurements included: height (ht), mass (M), percent body fat (%BFtanita), and percent body fat (%BFISAK), based on the nine site anthropometrics from the International Society for the Advancement of Kinanthropometry (ISAK) protocol (Norton & Olds, 1996). The ISAK program include the following skinfolds and girths: waist circumference (WC), hip circumference (HC), upper arm circumference (UAC), calf circumference (CC), biceps, triceps, subscapular, mid axilla, abdominal, iliac crest, supraspinale, thigh, calf skinfolds, and two bone breadths (femur and humerus epicondyles). WC was measured between the lower costal border (last rib) and the iliac crest at the narrowest point and HC was measured at the widest part around the hips and gluteals (usually same level as symphysis pubis). Calibrated Harpenden skinfold calipers (Harpenden, United Kingdom) were used for all measurements in triplicate (TEM <1%) by skilled research staff. All anthropometric data was analysed using Lifesize computer software (Norton & Olds, 1996). Body-mass index (BMI) was calculated by dividing mass by height squared (kg/m^2).

4.2.3 Blood Draw and Lipid Profile

A 20 gauge IV cannula (BD Insyte Autoguard) was inserted in an antecubital vein and participants were given a 30-minute rest period in a reclined position before taking baseline measurements. Fasting blood samples were collected in 10 ml EDTA

tubes at baseline, in the two minutes of each exercise session and at 15-minute intervals for the next 2 hours. Blood lipid profiles were obtained in the same manner as described in Chapter 3. Blood was also collected at all the same intervals as the EDTA tube in 5 ml sodium fluoride for glucose and lactate analysis on a YSI analyser (Yellowsprings, USA).

4.2.4 Aerobic Power Test

Aerobic power was assessed using the same procedures described previously in Chapter 3. HR_{peak} was determined as the last average HR value for the highest load obtained during the $\dot{V}O_{2peak}$ test.

4.2.5 Blood Pressure and Heart Rate

Resting blood pressure (BP) was recorded on the initial day in the lab and again on the morning before exercise as a baseline measure. On the initial day blood pressure was assessed manually. On the HIIE and SSE days blood pressure was measured using a Colin Jentow monitor (Model 7000, Colin Medical, Japan). HR was recorded during this session using a Polar S810I telemetry system (Polar, Finland).

4.2.6 Exercise Sessions

Participants were required to perform two exercise sessions, one week apart (see Figure 4.1). The counterbalanced exercise sessions consisted of one HIIE session for 20 minutes and a SSE session for 30 minutes. The HIIE workload was set at 65-70 % of $\dot{V}O_{2peak}$ with a cadence between 100 and 130 RPM for 8 seconds and recovery (12 seconds) was performed at the same amount of resistance but at a cadence of 30 RPM. The 8:12 second sprint ratio allows for three sprints each minute or 60 sprints in the 20 minute workout. The women were instructed to keep their intensity at a level to commensurate with an average respiratory exchange ratio (RER) of 0.92. The SSE workload was set at 65-70 % of $\dot{V}O_{2peak}$. All sessions were supervised and performed

on a Monark cycle ergometer, with HR and oxygen consumption recorded continuously. The participants conducted a 5-minute warm-up and cool-down on the bike prior to each exercise session. All cycling data included: RPM and RPE recorded at 1-minute intervals throughout the 20-minute HIIE and at 5-minute intervals for SSE. To make it easier on participants to keep up with the timing (8:12), the HIIE was set to a pre-recorded CD counting down each sprint in a 3-2-1 fashion. Encouragement was given during both sessions to motivate participants throughout exercise.

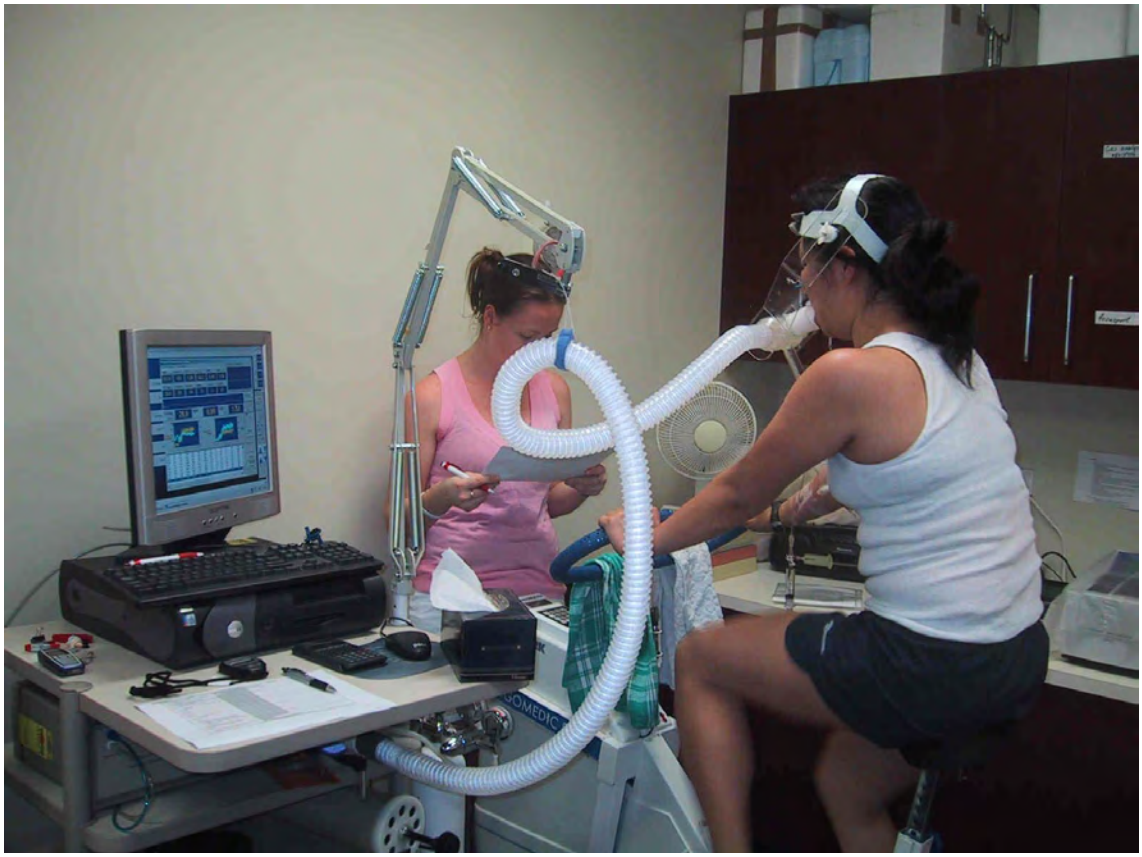


Figure 4.1 Participant on the bike ergometer performing HIIE.

4.2.7 Baseline and Post Exercise Monitoring

On each of the exercise occasions the women were asked to complete 2 hours of post exercise (PE) monitoring. All baseline and PE values [blood pressure, bloods, resting respiratory quotient[2] (RQ) and HR] were measured with the participant seated in a reclined chair in a quiet environment within the lab. After insertion of the cannula, the

[2] Respiratory quotient (RQ) represents the ratio of fat and carbohydrate oxidation at rest and respiratory exchange ratio (RER) represents the same ratio during exercise.

women were asked to relax (not sleep) in the chair for 30 minutes prior to the first set of measurements. Gas analysis using a TrueMax 2400 Metabolic Cart (ParvoMedics Inc, USA) was continuous throughout the exercise session using a two-way breathing valve and nose clip (Hans Rudolph). Immediately following the exercise cool down participants were seated in an adjacent chair for the first 15 minutes of the PE phase. The women had the option to use the restroom and were wheeled into the restroom using a wheelchair after the initial 15 minutes of PE. The duration of PE was resumed for a total of 115 minutes with participants relaxing in the reclined chair. Temperature in the lab was maintained between 24-25 °C.

4.2.8 Diet Analysis

All dietary analysis was completed following the same protocol explained in Chapter 3. Women were asked not to change their dietary habits while participating in the study.

4.2.9 Plasma Analysis and HOMA-IR Calculations

Frozen plasma samples were analysed later in duplicate for insulin, cortisol, ACTH, human growth hormone, glycerol, and leptin (see Figure 4.2). All blood markers (plasma) with the exception of lactate and glucose (whole blood analysis) were measured using commercially available sandwich immunoassay kits. The degree of enzymatic turnover of the substrate was determined by dual wavelength absorbance measurement at 450 and 620 nm for insulin (DSL 10-1600), cortisol (DSL-10-2000), ACTH (DSL-10-5100), human growth hormone (DSL-10-1900), leptin (DSL-10-23100), and 540 nm for glycerol (Sigma FG0100). HOMA-IR was calculated using the same equation mentioned in Chapter 3.

Figure 4.2 Analysis of fasting blood samples.

4.2.10 Analysis

Data analysis was completed with the Statistical Package for Social Science for windows software (SPSS 15.1, USA). All results are expressed as mean and standard error (SE) for variables with normal distribution. A *p* value of < .05 was adopted. Due to skewness, some values were log transformed (Ln) for analysis. The groups were divided by insulin levels and a value of > 9.98 µIU/ml as proposed by Park et al. (2005) was considered high insulin. High insulin (HI) and low insulin (LI) groups were established based on baseline insulin values. A Students independent *t*-test, and one-way or two-way repeated measures ANOVA were used to test for differences between HIIE and SSE within all the women and between the HI and LI groups. The Mauchly sphericity test was used to test for homogeneity of covariance for within subject factors. The Greenhouse-Geisser test was used to correct for non-homogenous values. To compare all non-parametric values, a Friedman test or Wilcoxon signed-rank test was performed on pre, 1 hour and 2 hours post variables. Pearson correlation analysis was used to determine associations between all variables on crude and log transformed

values. Spearman's rank order correlation was performed on values that remained skewed after log transformation.

4.3 Results

4.3.1 Overview

The results reported in this section are from pre and two hours post SSE and HIIE testing. Participant characteristics and baseline data are reported first followed by data collected at 1 hour and 2 hours post either SSE or HIIE. Following the data from the blood samples, differences in RQ, HR, and RPE following both types of exercise is described. Diet information is provided with information regarding food intake.

4.3.2 Participant Characteristics

The participants (N= 18) in the study were young, apparently healthy overweight women from the University and surrounding communities. The group was comprised of Anglo women (n = 13), Asian women (n = 4), and one Fijian woman. The HI group consisted of Anglo women (n = 4), Asian women (n = 3), and a Fijian woman, whereas the low insulin women were Anglo women (n = 9) and one Asian woman.

4.3.3 Baseline Variables

Table 4.1 shows participants' age, lipid, body composition, diet, metabolic, and fitness parameters for all (N = 18) women and for both high (n = 8) and low (n = 10) insulin groups. Correlations were performed on a number of other baseline variables (see Appendix J). The most interesting relationship at baseline was between fasting plasma glycerol which was negatively associated with RQ, r = - .54, p < .05 (see Figure 4.3).

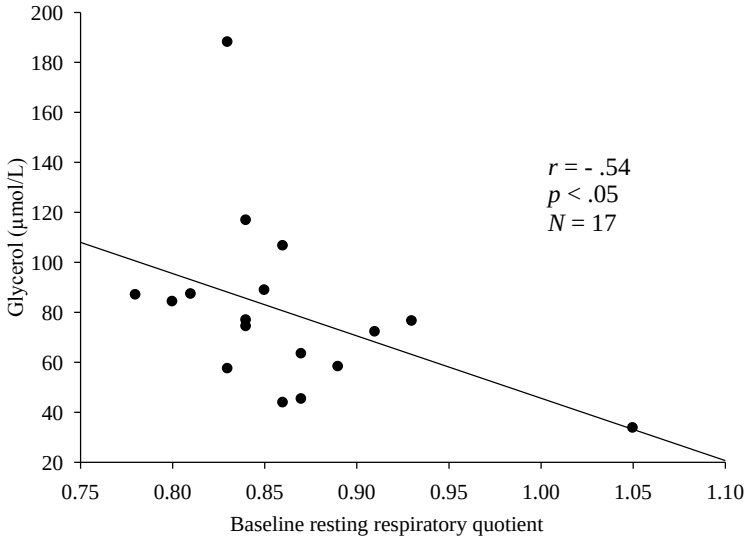


Figure 4.3 Correlation between baseline glycerol and baseline RQ.

4.3.3.1 Differences between groups at baseline

There were significant differences in body mass, $t(16) = 2.38$, $p < .05$, BMI, $t(16) = 2.50$, $p < .05$, and fitness relative to body mass, $t(16) = 3.20$, $p < .05$, at baseline between HI and LI (see Table 4.1).

The dietary intake for the two groups differed in total kilojoules, $t(12) = 3.07$, $p < .05$, protein, $t(12) = 3.03$, $p < .05$, total fat, $t(12) = 2.77$, $p < .05$, and monounsaturated fat, $t(12) = 3.20$, $p < .05$ in grams, cholesterol, $t(12) = 3.33$, $p < .05$, and monounsaturated fat, $t(12) = 3.15$, $p < .05$ expressed as a percentage of the diet (see Table 4.1).

Table 4.1 Characteristics of Study II Participants

	ALL (n = 18)		High Insulin (n = 8)		Low Insulin (n = 10)	
	Mean	SE	Mean	SE	Mean	SE
Age (years)	21.7	0.8	23.1	1.5	20.6	0.7
$\dot{V}O_{2peak}$ (L/min)	2.4	0.1	2.3	0.1	2.5	0.1
$\dot{V}O_{2peak}$ (ml/kg/min)	36.2	1.5	31.8	2.4	39.7 *	1.1
SBP (mmHg)	113.1	1.3	114.8	2.6	111.7	1.3
DBP (mmHg)	72.3	1.3	74.6	2.4	70.5	1.0
Height (cm)	167.7	1.4	167.1	2.4	168.1	1.9
Mass (kg)	67.0	2.2	72.3	3.0	62.7 *	2.6
BMI (kg/m^2)	23.9	0.9	26.0	1.2	22.2 *	0.9
Waist circumference (cm)	75.6	1.6	78.7	2.6	73.0	1.8
Hip circumference (cm)	102.5	1.8	106.3	2.5	99.4	2.2
Body fat (%) by ISAK	31.8	1.5	35.2	2.4	29.4	1.7
Body fat (%) by Tanita	29.5	1.7	33.0	2.2	26.6	2.1
Respiratory quotient	0.86	0.01	0.87	0.03	0.85	0.01
Triglycerol (mmol/L)	0.82	0.06	0.88	0.11	0.77	0.06
Total cholesterol (mmol/L)	4.0	0.2	4.0	0.3	4.0	0.3
HDL cholesterol (mmol/L)	1.2	0.1	1.3	0.1	1.2	0.1
LDL cholesterol (mmol/L)	2.4	0.2	2.2	0.3	2.5	0.2
VLDL cholesterol (mmol/L)	0.37	0.03	0.39	0.05	0.36	0.03
Total energy (kJ)$	7368	679	9545	1094	6159 *	565
Carbohydrate (g)$	205.9	18.1	262.4	37.1	174.6	10.2
Protein (g)$	74.2	8.7	102.0	14.3	58.8 *	7.3
Total fat (g)$	61.4	6.3	80.4	8.5	50.9 *	6.4
Saturated fat (g)$	21.4	1.9	26.0	2.5	18.9	2.3
Polyunsaturated fat (g)$	11.4	1.3	14.2	2.1	9.8	1.5
Monounsaturated fat (g)$	23.1	2.7	32.0	3.4	18.1 *	2.6
Cholesterol (g)$	176.4	30.6	279.0	48.5	119.4 *	24.0
Fibre (g)$	21.7	2.3	26.4	5.0	19.1	2.1
Iron (mg)$	12.7	2.7	18.4	7.2	9.5	0.9
Saturated fat (%)$	39.1	1.4	36.4	0.8	40.6	2.0
Polyunsaturated fat (%)$	20.4	1.3	19.6	1.2	20.9	1.9
Monounsaturated fat (%)$	40.5	1.2	44.4	0.9	38.3 *	1.3

Mean and standard error (SE)
* Significant difference ($p < .05$)
** Significant difference ($p < .001$)
$ n = 14 for diet data
Note: Systolic blood pressure (SBP), diastolic blood pressure (DBP), body mass index (BMI), International Society for the Advancement of Kinanthropometry (ISAK), high density lipoproteins (HDL), estimated low density lipoproteins (LDL), and estimated very low density lipoproteins (VLDL)

Table 4.2 shows participants' insulin, glucose, HOMA-IR, cortisol, lactate, ACTH, growth hormone, glycerol, and leptin for both HI and LI groups. At baseline

there were significant differences for insulin, $t(16) = 9.03$, $p < .001$, HOMA-IR, $t(8) = 6.26$, $p < .001$, glucose, $t(16) = 2.64$, $p < .05$, ACTH, $t(16) = 2.55$, $p < .05$, growth hormone, $t(16) = 2.57$, $p < .05$, and leptin, $t(16) = 2.69$, $p < .05$ between the HI and LI groups (see Table 4.2).

Table 4.2 Study II Participant's Blood Characteristics

	ALL (n = 18)		High Insulin (n = 8)		Low Insulin (n = 10)	
	Mean	SE	Mean	SE	Mean	SE
Fasting insulin (µIU/ml)	9.9	1.7	16.5	1.9	4.7 **	0.5
Fasting glucose (mmol/L)	4.2	0.1	4.4	0.1	4.1 *	0.1
HOMA-IR	1.9	0.3	3.2	0.4	0.8 **	0.1
Fasting cortisol (µg/ml)	43.4	4.2	46.0	8.5	41.5	4.3
Fasting lactate (mmol/L)	0.62	0.03	0.62	0.06	0.61	0.04
Fasting adrenocorticotropic hormone (pg/ml)	47.6	20.2	84.6	42.8	18.1 *	5.0
Fasting growth hormone (ng/ml)	4.3	0.7	2.6	0.9	5.7 *	0.8
Fasting glycerol (µmol/L)	79.9	8.6	70.6	8.9	88.3	14.1
Fasting leptin (ng/ml)	18.2	4.2	27.2	8.4	11.0 *	1.7

Mean and standard error (SE)
* Significant difference ($p < .05$)
** Significant difference ($p < .001$)
Note: Homeostasis model assessment of insulin resistance (HOMA-IR)

4.3.4 Response in Blood Markers following Exercise

All fasting blood markers (see Table 4.3) were measured on the day of exercise at baseline, one hour, and two hours post exercise (SSE or HIIE).

Table 4.3 Blood Variables at Baseline, One, and Two Hours Post Exercise (HIIE or SSE)

	HIIE						SSE					
	Baseline		1 Hour		2 Hours		Baseline		1 Hour		2 Hours	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Fasting insulin (mIU/mL)	9.46	1.59	10.24	1.68	9.00	1.59	10.35*	1.94	10.73*	1.67	11.17*	1.81
Fasting glucose (mmol/L)	4.15	0.11	4.21	0.08	4.20	0.06	4.25	0.11	4.32	0.07	4.30	0.06
HOMA-IR	1.80	0.31	1.78	0.34	1.74	0.36	2.00	0.39	2.05	0.31	2.09†	0.42
Fasting cortisol (µg/mL)	43.08**	4.58	36.35**	4.49	29.30**	4.51	42.34**	4.24	33.69**	3.30	24.69**	2.49
Fasting lactate (mmol/L)	0.61**	0.05	1.09†**	0.21	0.70**	0.09	0.62*	0.04	0.74*	0.04	0.61*	0.05
Fasting adrenocorticotropic hormone (pg/mL)	40.79	14.59	51.70	16.48	68.99	29.68	54.49	26.12	46.70	16.02	88.30	33.37
Fasting growth hormone (ng/mL)	4.39**	0.85	0.73**	0.14	0.16**	0.04	4.29**	0.71	0.77**	0.35	0.48**	0.22
Fasting glycerol (µmol/L)	76.35*	7.28	79.00*	7.67	97.65*	6.01	83.51	13.32	93.61	19.17	95.13	9.13
Fasting leptin (ng/mL)	18.50**	4.58	16.84**	4.63	17.20**	4.53	17.91**	3.83	15.71**	3.80	15.84**	3.80

Mean and standard error (SE)
† Significant difference ($p < 0.05$) between HIIE and SSE
**Significant difference ($p < 0.001$) across time
*Significant difference ($p < 0.05$) across time
Note: Homeostasis model assessment of insulin resistance (HOMA-IR)

4.3.4.1 Response in insulin, glucose, and HOMA-IR following exercise

Following HIIE insulin was reduced non-significantly whereas after SSE insulin increased linearly with a significant increase[3], $p < .05$, across time (see Figure 4.4, A). There were no significant changes in glucose (see Figure 4.4, B) or HOMA-IR (see Figure 4.4, C) across time following either SSE or HIIE and no significant difference existed between SSE or HIIE. When comparing the HI and LI groups, insulin[4], $p < .05$, and HOMA-IR, $p < .05$, had a significantly different response in the 2 hours after HIIE compared to SSE[5] (see Figure 4.4, D and F). The insulin following HIIE in the HI women was lower at 2 hours after exercise compared to baseline and higher after 2 hours following SSE. The LI women reacted similarly following both types of exercise with no significant change at 2 hours compared to baseline. There were no significant differences in the responses for the HI and LI women following HIIE or SSE in regard to glucose.

[3] Friedman test, $\chi^2(2, N = 18) = 1.58$
[4] Wilcoxon signed-rank test, insulin 2-hour Z = 5.07, HOMA-IR 2-hour Z = 4.27
[5] Wilcoxon signed-rank test, insulin 2-hour Z = 5.99, HOMA-IR 1-hour Z = 5.47 and 2-hour Z = 5.76

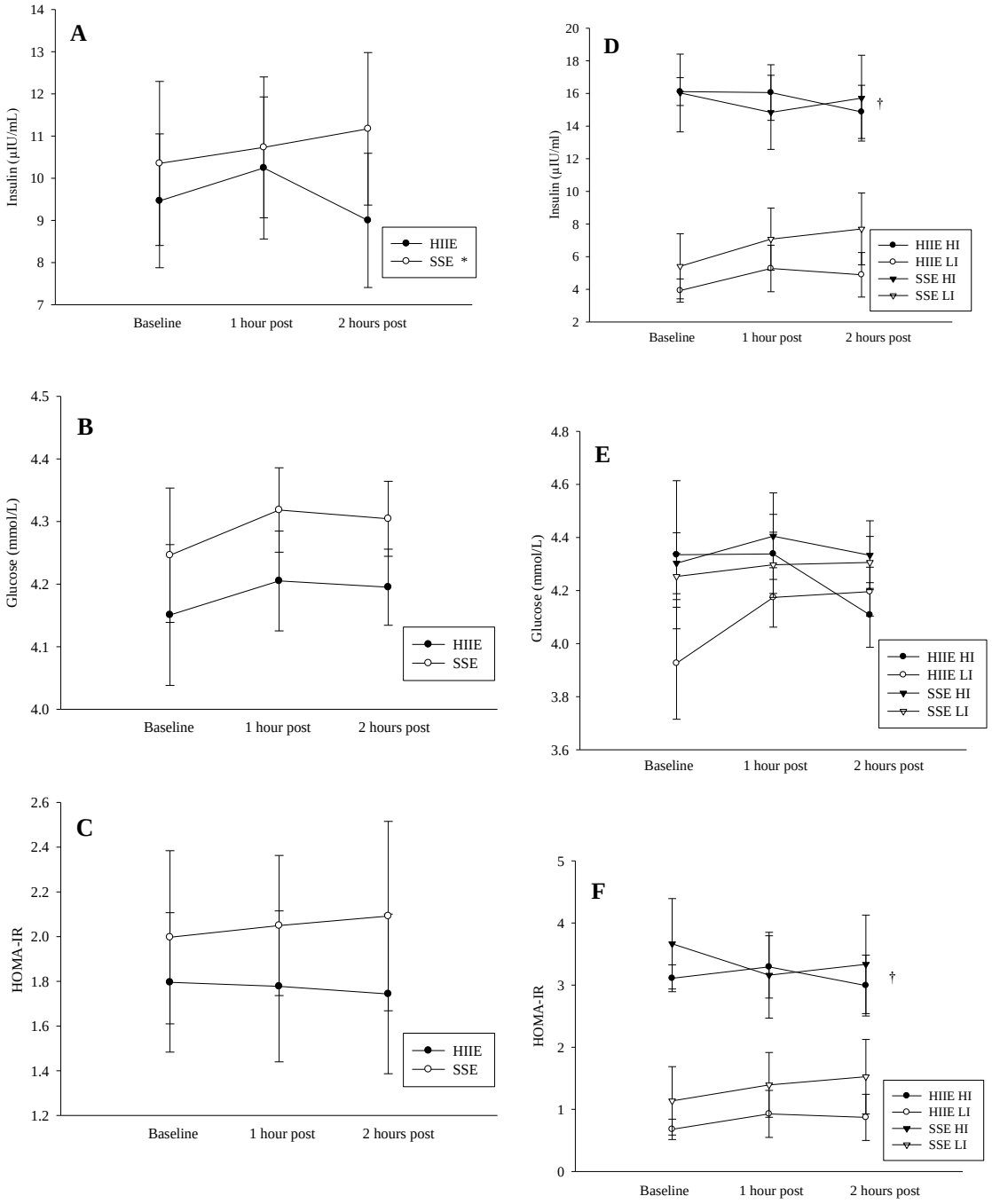


Figure 4.4 (A-F) Response in insulin, HOMA-IR, and whole blood glucose following exercise (HIIE and SSE) in all women and between HI and LI women (*, $p < .05$, difference across time and †, $p < .05$, different response between the types of exercise).

4.3.4.2 Response in leptin following exercise

Leptin decreased significantly post HIIE and SSE, $F(2, 30) = 22.73$, $p < .001$, but was not significantly different between the types of exercise (see Figure 4.5 A). Leptin was significantly different across time when compared between HI and LI, $F(2, 28) = 22.73$, $p < .05$, women (see Figure 4.5 B). There was no difference between the type of exercise and HI and LI women.

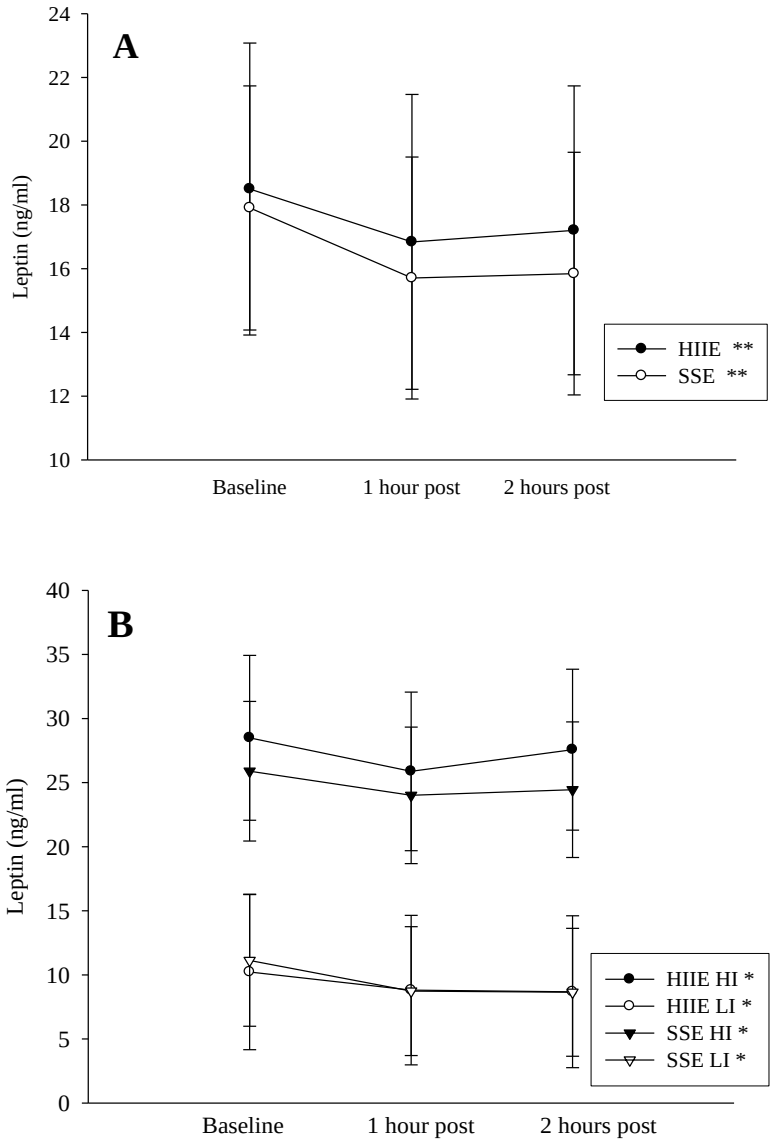


Figure 4.5 (A, B) Response in leptin following exercise (HIIE and SSE) in all women and between HI and LI women (*, $p < .05$, **, $p < .001$).

4.3.4.3 Response in lactate following exercise

Lactate was significantly elevated[6], $p < .05$, after HIIE compared to SSE in the first hour post exercise but not in the second (see Figure 4.6 A). A significant across time interaction was seen following both HIIE[7], $p = .05$, and SSE, $p < .05$. After SSE, a significant response in lactate[8], $p < .05$, was found at the 2 hour measurement between HI and LI (see Figure 4.6 B).

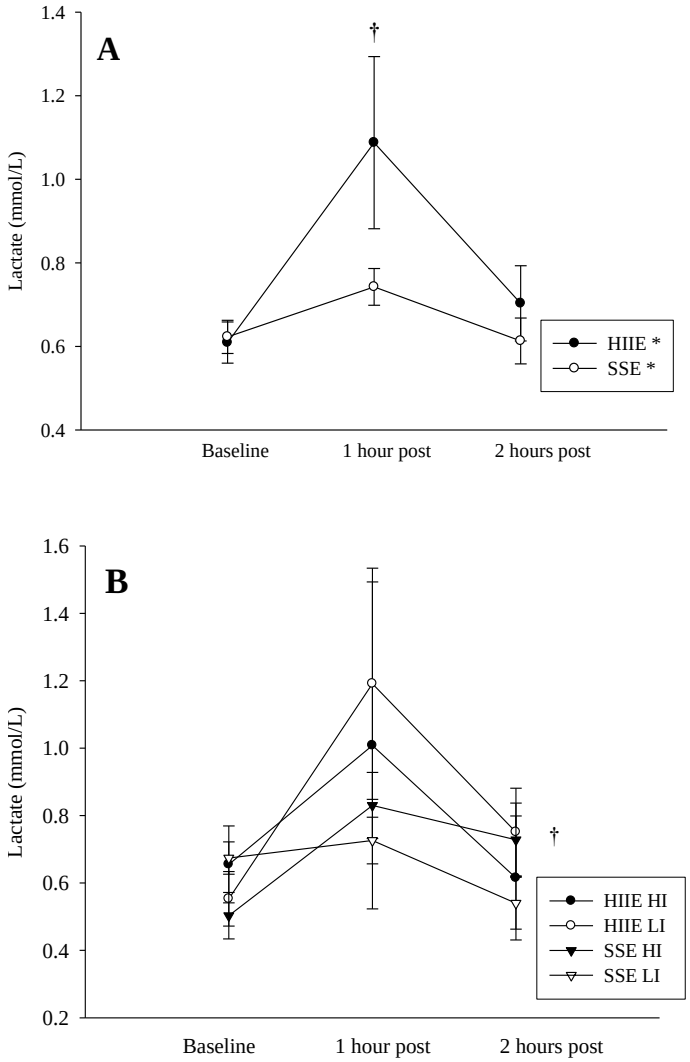


Figure 4.6 (A, B) Response in whole blood lactate following exercise (HIIE and SSE) in all women and between HI and LI women (*, $p < .05$, difference across time and †, $p < .05$, different response between the types of exercise).

[6] Wilcoxon signed-rank test, Z = 2.55
[7] Friedman test, HIIE $\chi^2(2, N = 14) = 13.29$ and SSE $\chi^2(2, N = 15) = 8.13$
[8] Wilcoxon signed-rank test, Z = 7.27

4.3.4.4 Response in glycerol following exercise

Glycerol significantly increased after both exercise sessions, $F(1, 14) = 5.20$, $p = .05$, (see Figure 4.7 A). There were no significant differences at baseline, one hour, or two hours post exercise between SSE and HIIE or between HI and LI groups (see Figure 4.7 B).

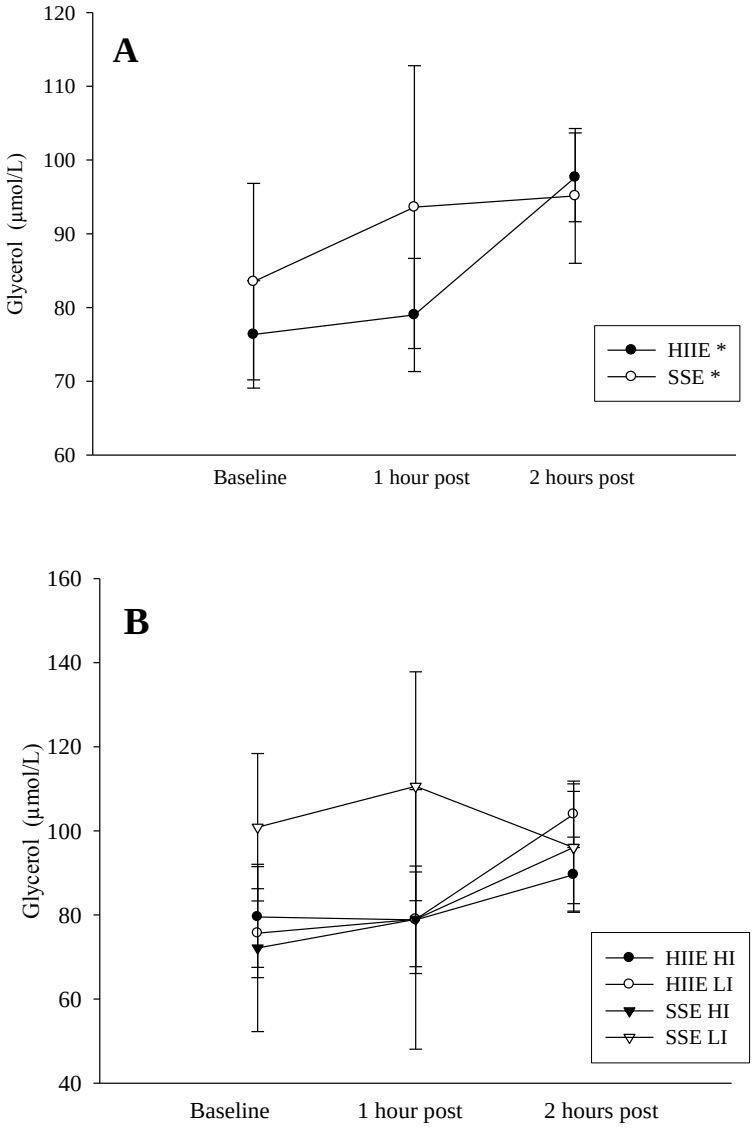


Figure 4.7 (A, B) Response in glycerol following exercise (HIIE and SSE) in all women and between HI and LI women (*, $p < .05$).

4.3.4.5 Response in human growth hormone following exercise

Human growth hormone decreased significantly following both types of exercise, $F(2, 16) = 71.69$, $p < .001$, with no significant differences between SSE and

HIIE (see Figure 4.8 A). A significant difference was found between HI and LI women following both types of exercise, $F(2, 16) = 6.04$, $p < .05$, although no significant difference existed between HI and LI for SSE or HIIE (see Figure 4.8 B).

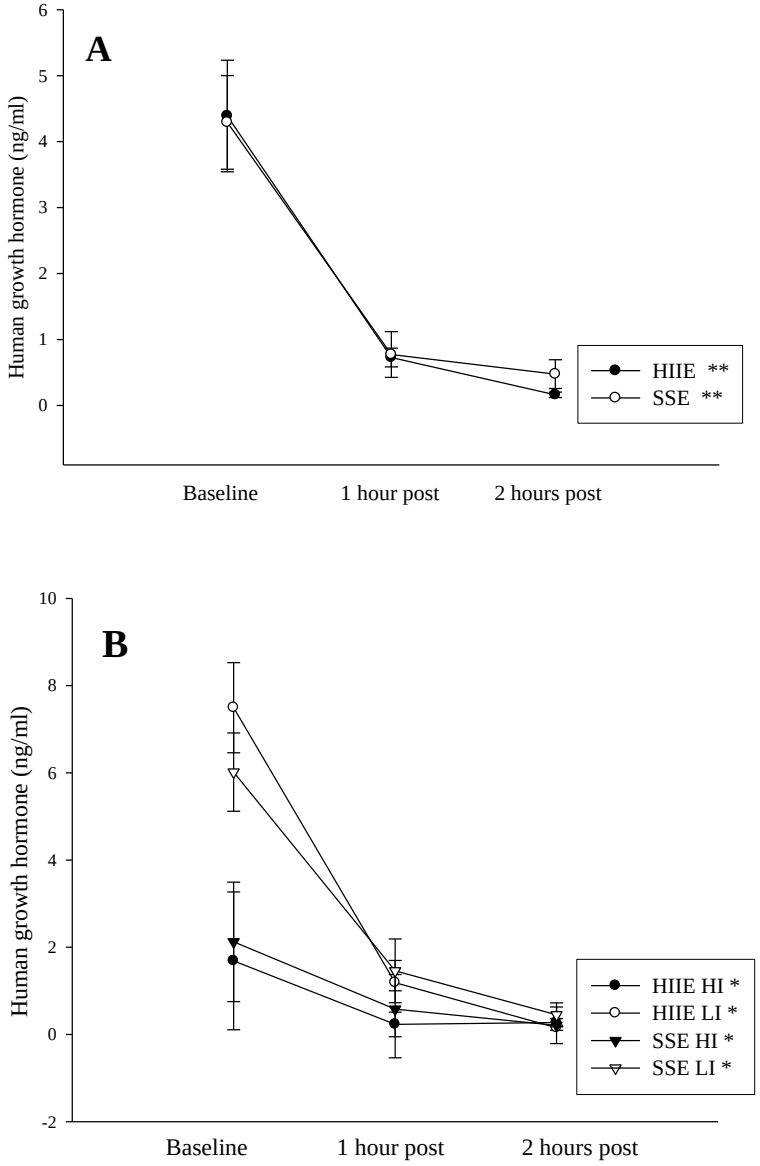


Figure 4.8 (A, B) Response in human growth hormone following exercise (HIIE and SSE) in all women and between HI and LI women (*, $p < .05$, **, $p < .001$).

4.3.4.6 Response in adrenocorticotropic hormone following exercise

ACTH increased in the two hours post exercise although not significantly and there was not a significant change post exercise between the types of exercise (see

Figure 4.9 A). One and two hours after exercise ACTH increased significantly in the HI women, $F(2, 30) = 3.57$, $p < .05$ compared to LI with no differences between the type of exercise in relation to the HI or LI women (see Figure 4.9 B).

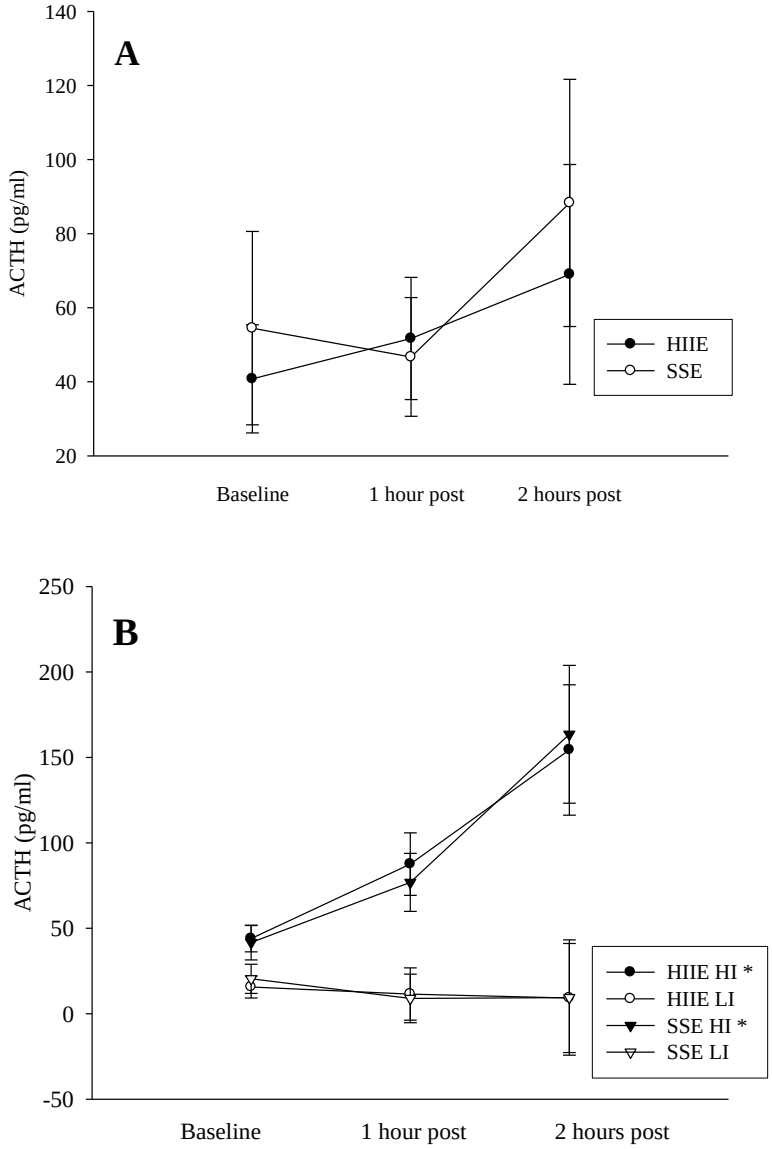


Figure 4.9 (A, B) Response in ACTH following exercise (HIIE and SSE) in all women and between HI and LI women (*, $p < .05$).

4.3.4.7 Response in cortisol following exercise

A significant decrease in cortisol was seen in the two hours post both HIIE and SSE, $F(1, 14) = 13.85$, $p < .05$, (see Figure 4.10 A). The differences between SSE and

HIIE were not significant at any time point. Cortisol was not significantly different between HI and LI women(see Figure 4.10 B).

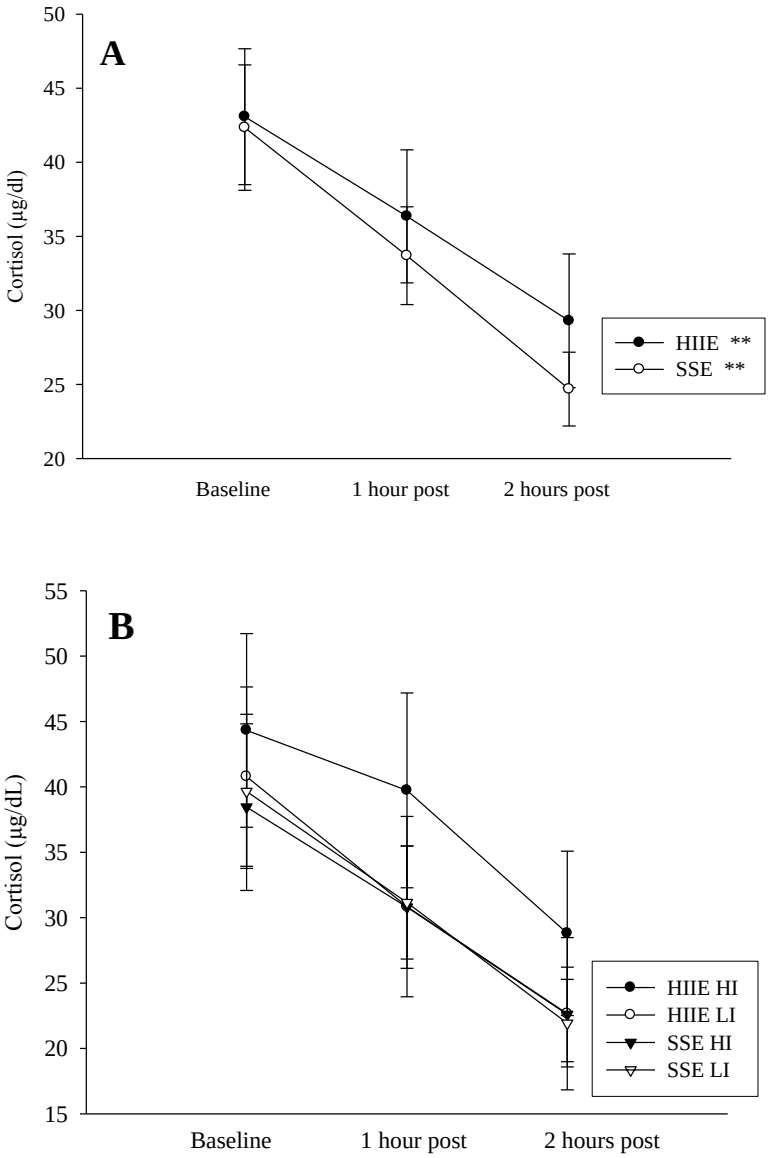


Figure 4.10 (A, B) Response in cortisol following exercise (HIIE and SSE) in all women and between HI and LI women (**, $p <$ .001).

4.3.5 Response in Heart Rate following Exercise

The resting HR was significantly elevated across time for both HIIE and SSE, $F(1, 23) = 12.17$, $p = .001$, (see Figure 4.11 A). The resting HR response after SSE and

HIIE was similar and there were no significant differences between the HI and LI groups (see Figure 4.11 B).

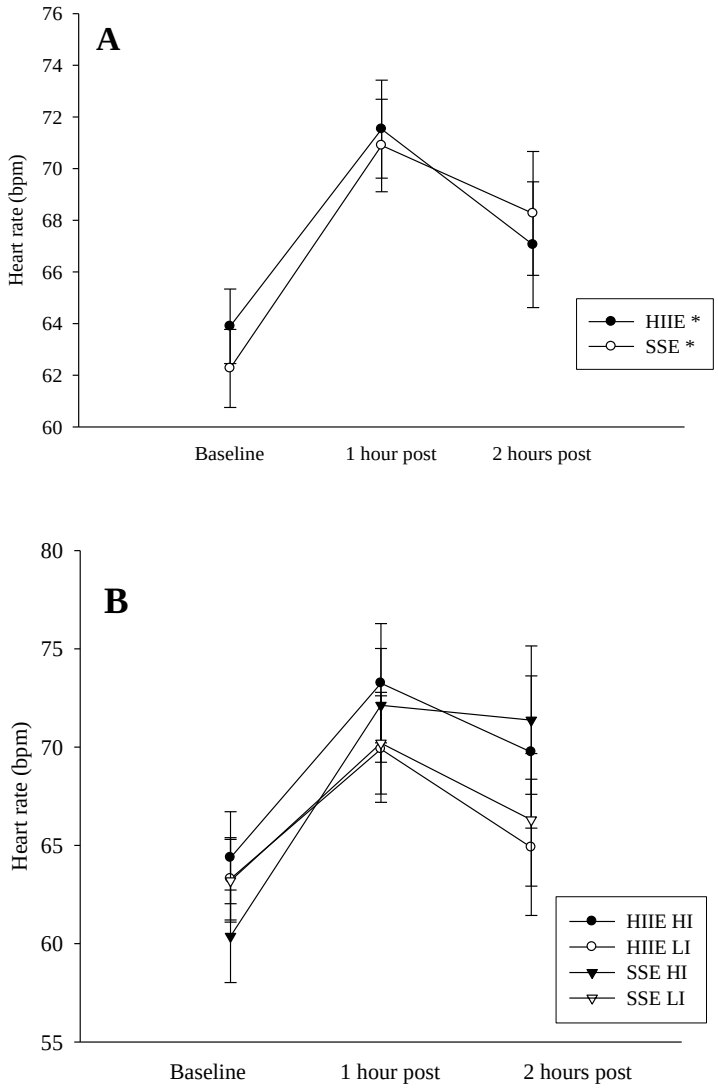


Figure 4.11 (A, B) Response in resting HR following exercise (HIIE and SSE) in all women and between HI and LI women (*, $p < .05$).

4.3.6 Response in Rating of Perceived Exertion following Exercise

The RPE of the women was significantly different for HIIE and SSE, $F(2, 23) = 56.11$, $p < .001$, across time (see Figure 4.12 A) but not between the two types of exercise. Between HI and LI women there were no significant differences (see Figure 4.12 B).

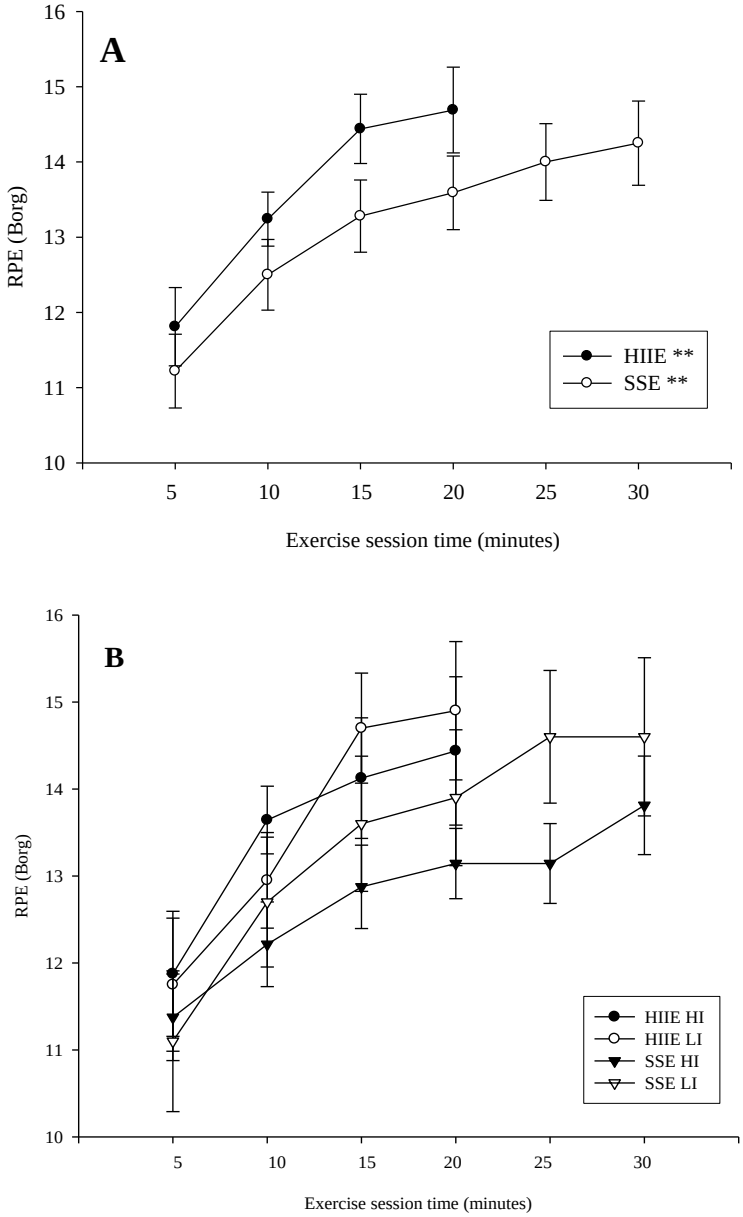


Figure 4.12 (A, B) Response in RPE during exercise (HIIE and SSE) in all women and between HI and LI women (**, $p < .001$).

4.3.7 Response in Respiratory Quotient following Exercise

RQ decreased significantly following both HIIE[9], $p = .001$, and SSE, $p < .05$, with no significant differences between SSE and HIIE (see Figure 4.13 A) or between the groups (see Figure 4.13 B).

[9] Friedman test, HIIE $\chi^2(2, N = 18) = 14.94$ and SSE $\chi^2(2, N = 18) = 8.83$

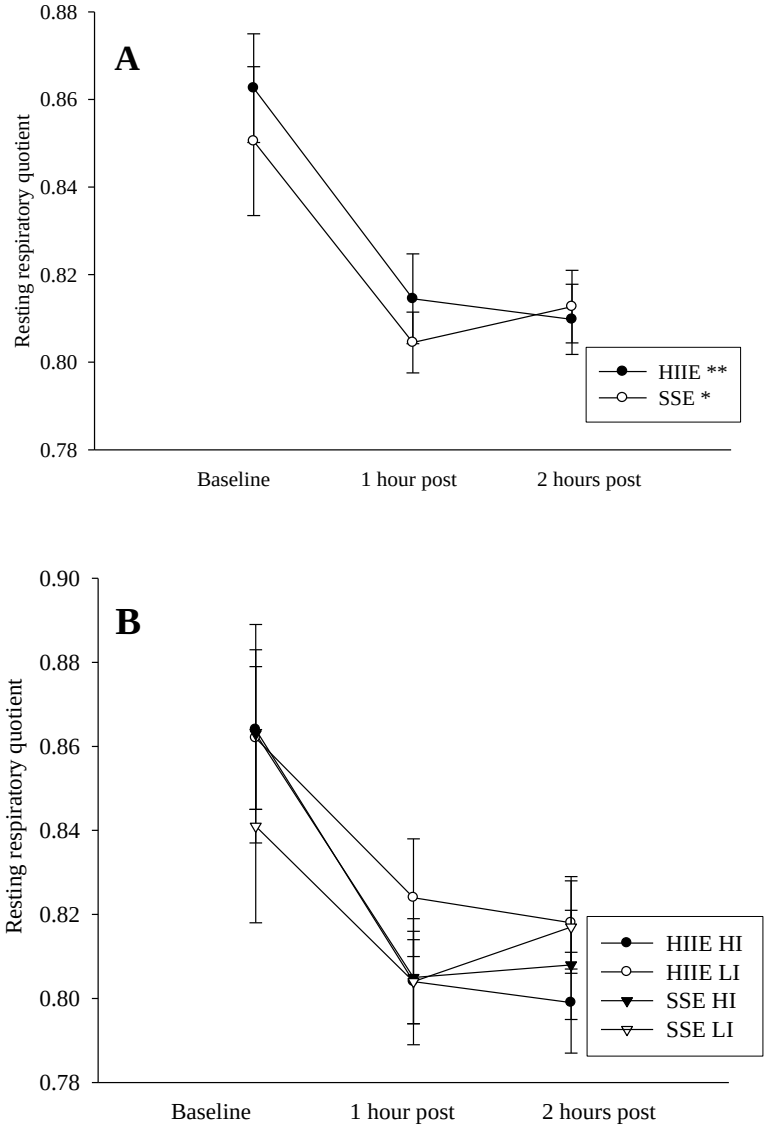


Figure 4.13 (A, B) Response in resting RQ following exercise (HIIE and SSE) in all women and between HI and LI women (*, $p <$ .05, **, $p <$ .001).

4.4 Discussion

The major findings of Study II were that significantly elevated glycerol and a reduction in RQ, in the immediate 2 hours following HIIE were found. The HIIE was shorter in duration than SSE although it produced higher lactate levels during and in the first hour after exercise. Fasting baseline insulin was significantly correlated to other baseline blood variables and fasting plasma glycerol was associated with baseline RQ. ACTH and leptin were correlated to insulin in the post HIIE period. Thus, young women who completed an acute bout of HIIE improved their early MetS markers

(insulin, HOMA-IR) and enhanced their fat oxidation in the hours after HIIE which is in agreement with Hypothesis 3 (see page 9).

When the women were divided by fasting insulin levels, defined by Park et al. (2005), significant differences at baseline for human growth hormone existed. Insulin and HOMA-IR responded differently in the 2 hours after exercise in the HI women with a reduction following HIIE and an increase following SSE (see Figure 4.4 D and E). An interesting finding involving ACTH and the HI and LI groups was the ACTH response following exercise (see Figure 4.9 B). Disregarding HIIE and SSE the HI women had elevated ACTH levels at 2 hours post exercise. All diet results for the groups were similar (see Table 4.1) and lipids were in the healthy range with no significant differences between the high and low insulin women. Therefore, in support of Hypothesis 4 (see page 9) young hyperinsulinemic women had a significantly different metabolic response (insulin and HOMA-IR) in the two hours following HIIE compared to SSE. There was partial support for Hypothesis 3 (see page 9) as some MetS markers changed after HIIE.

4.4.1 Lipids in Hyperinsulinemic and Non-Hyperinsulinemic Women

All the lipids measured in this study were within the normal range according to the ATPIII report (ATPIII, 2001). There was a trend in HI women to have a more unfavourable lipid profile with elevated levels of TG and VLDL but the differences were not statistically significant.

4.4.2 Body Composition in Hyperinsulinemic and Non-Hyperinsulinemic Women

Body composition was significantly different based on baseline insulin levels. The HI women had higher body composition measures and correlation analysis revealed a positive association with leptin and a negative association with lactate and growth hormone (see Appendix J). Previous studies have shown leptin is related to adiposity

(Benini, Camilloni, Scordato, Lezzi, Savia, Oriani et al., 2001; Couillard, Mauriege, Prud'homme, Nadeau, Tremblay, Bouchard et al., 1997; Doucet, St-Pierre, Almeras, Mauriege, Despres, Richard et al., 2000; Lofgren, Herron, West, Zern, Brownbill, Ilich et al., 2005; McConway, Johnson, Kelly, Griffin, Smith, & Wallace, 2000; Morio, Gachon, Boirie, Rousset, Gachon, Beaufrere et al., 1999; Tai, Lau, Ho, Fok, & Tan, 2000) specifically visceral fat (Tai et al., 2000). Obesity inhibits growth hormone secretion at rest (Weltman, Weltman, Watson Winfield, Frick, Patrie, Kok et al., 2008) and is associated with high levels of visceral adiposity (Misra et al., 2008). Lactate in the present cohort of women was positively associated with some of the body composition measurements which is consistent with prior research in obese and non-obese women (Chen, Varasteh, & Reaven, 1993). The present lactate levels were low although similar to that of the non obese glucose tolerant women (Chen et al., 1993). Increased sympathetic nerve activity at the muscular levels, which has been shown to accompany obesity, may increase resting lactate levels within the muscle (Scherrer, Randin, Tappy, Vollenweider, Jequier, & Nicod, 1994). Another possible reason for a relationship between lactate and body composition is altered glycolysis leading to excess lactic acid (Mullerova, Matejkova, Rusavy, & Muller, 1998).

Interestingly, BMI was associated with percent intake of monounsaturated fat and body mass was inversely associated to polyunsaturated fat intake in the diet. Prior studies in overweight populations determined fat intake rather than overall energy intake impacted body fatness (Doucet, Almeras, White, Despres, Bouchard, & Tremblay, 1998; Miller, Niederpruem, Wallace, & Lindeman, 1994). Therefore, previously reported polyunsaturated fat intake was unrelated to adiposity in men (Doucet et al., 1998) but was related in the present cohort of women.

4.4.3 Response in Insulin, Glucose, and HOMA-IR following Exercise

According to the hyperinsulinemic cut off set forth by Park et al. (2005), the HI women are considered IR. The mean insulin level in the HI group was 72% higher than the LI women. Baseline insulin levels were negatively correlated to baseline growth hormone with a trend in ACTH and positively correlated to baseline glucose in the women. Previous studies in women have shown a similar relationship between insulin and glucose (Mendoza-Nunez, Garcia-Sanchez, Sanchez-Rodriguez, Galvan-Duarte, & Fonseca-Yerena, 2002; Ryan & Elahi, 1996), and between insulin, growth hormone, and ACTH (Bjorntorp & Rosmond, 2000).

Low cardio-respiratory fitness or sedentary habits and hyperinsulinemia are precursors to MetS (Carroll & Dudfield, 2004) and the present fitness levels of the HI women are considered average (Shvartz & Reibold, 1990) for their age which could increase their risk of MetS in the future.

HIIE compared to SSE proved to be more effective at preventing an increase in insulin levels in the two hours after exercise. The responses in insulin and HOMA-IR following HIIE were significantly different when HI and LI women were compared. Similar to SSE, a heavy bout of resistance exercise did not result in immediate changes to insulin levels in the thirty minutes following exercise (Kraemer et al., 1998).

4.4.4 Response in Adrenocorticotropic and Human Growth Hormones following Exercise

As discussed in the literature review (see Figure 2.6), alterations in the HPA axis downstream affect ACTH secretion leading to fat storage (Nieuwenhuizen & Rutters, 2008). ACTH was elevated and growth hormone was depressed at baseline in the HI group. Hypereactivity of the HPA with increasing body fat (Weaver, Kopelman, McLoughlin, Forsling, & Grossman, 1993) may develop from reduced sensitivity to the

HPA, mental and physiological stress, or increased FFA (Lindmark, Lonn, Wiklund, Tufvesson, Olsson, & Eriksson, 2005) leading to IR. A study in mice found ACTH affected insulin and glucose release, leading to hyperinsulinemia and hyperglycemia (Bailey & Flatt, 1987).

In the post exercise recovery period ACTH increased non-significantly in all women due to the high intensity of both exercise sessions and this effect has been shown previously with heavy resistance training (Kraemer et al., 1998). In healthy active and inactive males ACTH is reduced after exercise although the time of exercise was relatively short (Duclos, Corcuff, Rashedi, Fougere, & Manier, 1997; Elias, Wilson, Pandian, Chune, Utsumi, Kayaleh et al., 1991; Lehmann, Knizia, Gastmann, Petersen, Khalaf, Bauer et al., 1993). When comparing HI and LI, ACTH was significantly elevated following both exercise sessions but only in the HI women. Due to an increase in catecholamines during HIIE (Trapp et al., 2007), ACTH secretion may be elevated (Conte-Devolx, Oliver, Rey, Guillaume, Castanas, Giraud et al., 1985). The prolonged elevation in ACTH, despite reductions in cortisol in the 2 hours post exercise, could be due to an inefficient feedback loop between cortisol and glucocorticoid receptors which controls HPA axis activity and an increased clearance of cortisol (Vicennati & Pasquali, 2000). Adipose tissue influences cortisol turnover and secretion impacting ACTH production and stimulation of the adrenal cortex (Fossati & Fontaine, 1993). Prior research in obese individuals illustrates a relationship between adiposity, IR, and HPA dysfunction (Duclos, Marquez Pereira, Barat, Gatta, & Roger, 2005). Insulin crosses the blood brain barrier and may impose a reaction on the HPA axis (Vicennati & Pasquali, 2000). Chronic insulin stimulation or dysfunction of the negative feedback loop with cortisol may impact sensitivity of the HPA axis leading to alterations in ACTH.

Human growth hormone significantly decreased in the 2 hours post exercise in both groups after both types of exercise (Figure 4.8 B) which has also been established in younger and older adults and was found to influence lipolysis (Wee, Charlton, Simpson, Jackson, Shojaee-Moradie, Stolinski et al., 2005). Human growth hormone was significantly depressed at baseline in the HI women compared to LI which has been previously found in diabetics (Hubinger, Franzen, & Gries, 1987; Lee, Chan, Yeung, Chow, Lau, Ko et al., 1999).

4.4.5 Response in Resting Respiratory Quotient, Glycerol, and Lactate following Exercise

The significant reduction in RQ following both SSE and HIIE represents an increase in fat oxidation (see Figure 4.13 A) and HIIE (6%) had a significantly greater reduction from baseline to the 2nd hour compared to SSE (4%). Glycerol was negatively associated with RQ at baseline and significantly increased after both sessions further promoting fat oxidation and lipolysis. Glycerol represents the breakdown of triglyceride. Similar findings involving lipolysis have been found in the post exercise recovery period following 60 minutes of steady state at 60% of $\dot{V}O_{2max}$ exercise recovery in young males (Mulla, Simonsen, & Bulow, 2000). Lipolysis from that study decreased in the first hour following exercise but increased in the second hour which was similar to the current findings after HIIE. In the second hour post HIIE, lactate returned to near baseline levels, glycerol increased, and insulin decreased which probably influenced lipolysis.

A characteristic of Study II was the large variability of some of the blood markers. This reflects the natural variability found in hormones such as cortisol and insulin. Another characteristic of the study was the women involved in the study were untrained and were performing HIIE for the first time. Thus, their response after

exposure to chronic HIIE is likely to be dissimilar to that reported for a single bout in Study II. For example, it has been shown previously that trained female cyclists exhibit a very different glycerol response to HIIE (Trapp et al., 2007).

4.4.6 Summary

Young apparently healthy premenopausal women performed two exercise bouts, HIIE and SSE followed by 2 hours of post exercise monitoring. HIIE compared to SSE proved to be more effective at preventing an increase in insulin levels in the two hours after exercise. Following HIIE RQ was significantly lower and the increase in glycerol came close to reaching significance following HIIE and not SSE, implying greater lipolysis. The women were divided by fasting insulin and the initial insulin levels were significantly associated with baseline glucose, human growth hormone, and approached significance with ACTH. Baseline RQ was correlated with baseline glycerol, glucose, and lactate levels. The insulin level at one hour post HIIE was related to ACTH and leptin. All diet data between the groups were similar and lipids were in the healthy range with no significant differences between the groups. Thus, young premenopausal women who completed one session of short duration HIIE did not increase insulin and HOMA-IR in contrast to an elevation in the SSE. Enhanced fat oxidation occurred in the immediate two hour recovery period after both HIIE and SSE.

CHAPTER 5

FISH OIL, EXERCISE, AND MEDITERRANEAN DIET (STUDY III)

5.1 Introduction

The **F**ish oil, **E**xercise and **M**editerranean diet study (FEM) is a randomized controlled trial involving previously sedentary overweight and obese women recruited from a university and surrounding community. Using peak heart rate (HR_{peak}), determined by a fitness test, the women randomized to the intervention arm of the study were required to perform HIIE at 85 % HR_{peak} for 20 minutes, three times per week, for 12 weeks. Pre- and posttesting for body composition, resting metabolic rate, fitness, diet, autonomic function, diet, and bloods were carried out. The women in the study were asked to consume fish oil on a daily basis and eat a Mediterranean style diet.

5.2 Methods (Study III)

5.2.1 Participants

Volunteer, premenopausal, recreationally active but untrained overweight women between the ages of 18 and 35 years (see Table 5.2) were recruited from a university population. After all risks and procedures were explained in accordance with university policy, inclusion and exclusion criteria was explained (see Appendix E), and participants gave written consent. The informed consent (see Appendix A) was approved by the Human Ethics Committee at the University of New South Wales (HREC 06293).

5.2.1.1. Baseline participant data collection

All pre- and posttests and the questionnaires for FEM (see Appendix F) were completed under the protocol as depicted in Chapters 3 and 4 unless otherwise stated.

5.2.1.2 Randomization

Randomization was completed on the initial visit by picking a small piece of paper labelled control or exercise out of an envelope. Participants were divided into two groups; a control group (CON) who were required to keep their normal daily habits and routines consistent for the 12 weeks of the study (see Figure 5.1). The second group of participants (FEM) were asked to complete 12 weeks of the intervention. FEM women completed 48 sessions in total and the CON women completed 10 sessions in the lab for pre, post, and during training measurements and (FEM) exercise sessions.

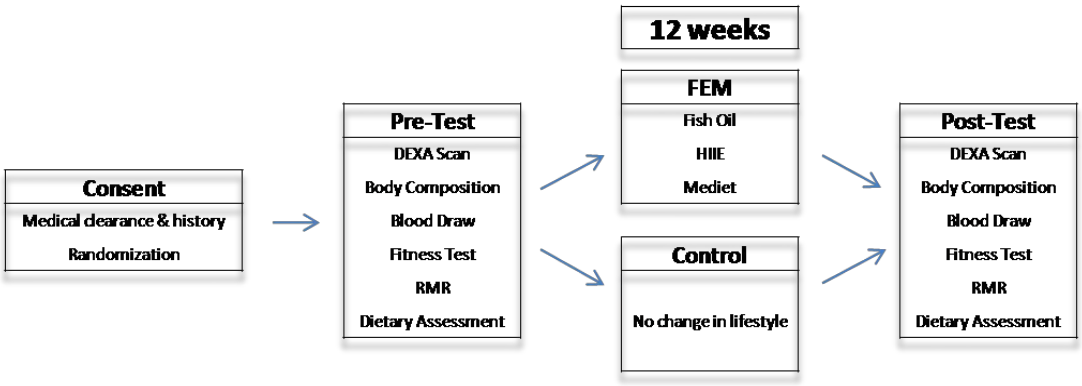


Figure 5.1 FEM protocol.

5.2.2 Body Composition Assessment

Fasting women reported to the lab every week for body composition measurements. BMI, WC, and HC were obtained using the equations and protocols followed in Chapter 4. Waist-to-height ratio (WHTR) was determined by dividing WC by height.

5.2.3. Dual Energy X-Ray Absorptiometry

The same protocol as described in Chapter 3 was followed for FEM women although participants were asked to report to the nuclear medicine unit in the fasted state with no liquid 2 hours prior for standardization purposes.

5.2.4 Resting Metabolic Rate Assessment

For RMR assessment, the fasting women relaxed in a reclined chair for 15 minutes while resting heart rate (HR) and gas analysis was measured using a metabolic cart TrueMax 2400 Metabolic Cart (ParvoMedics Inc, USA) for fifteen minutes. Participants were asked not to sleep or conduct any paced breathing techniques during the sampling period.

5.2.5 Autonomic Function Assessment

HR at rest (during RMR testing) was recorded using a Polar S810I telemetry system (Polar, Finland). Pulse pressure (PP), mean arterial pressure (MAP), and rate pressure product (RPP) were calculated using the following equations; PP = SBP – DBP, MAP is approximately DBP ⅓(SBP – DBP), and RPP = HR x SBP.
High frequency power (HF power) and low frequency power (LF power) of resting heart period variability (HPV) were recorded based on a previous protocols (Pagani, Lombardi, Guzzetti, Rimoldi, Furlan, Pizzinelli et al., 1986; Park, 2008). Based on the RR interval variability, two major frequency components, (LF power; 0.04-0.149 Hz) and (HF power; 0.15-0.4 Hz), were defined and power was summed and normalised. The normalised units were obtained by dividing each frequency power by the difference between total power (TP; 0-0.4 Hz) and very LF power (0-0.039 Hz), and multiplying by 100. A ratio of (LF/HF) was calculated in order to derive a quantitative indication of sympathetic/parasympathetic balance, found previously by Pagani et al. (1986).

5.2.6 Blood Draw and Lipid Profile

Fasting blood was drawn at baseline and in week twelve. Samples (300 ml) were collected (see Figure 5.2) and stored in the same manner described in Chapter 3.

Figure 5.2 Fasting blood sample collection by way of venepuncture.

5.2.7 Aerobic Power Test

Aerobic power was assessed using the same procedures describes in Chapter 3. During the $\dot{V}O_{2peak}$ test, respiratory exchange ratio (RER) was monitored throughout for changes in fat oxidation at a given load pre and post intervention. HR_{peak} was determined as the last average HR value for the highest load obtained during the $\dot{V}O_{2peak}$ test.

5.2.8 Intervention Exercise Sessions

Participants, who were randomly assigned to FEM, performed a HIIE session (see Figure 5.3) for 20 minutes using the same sprint interval exercise as described in Chapter 4. The HIIE workload was set at 80-85 % of the individuals HR_{peak} with a cadence between 100 and 130 RPM for 8 seconds and recovery (12 seconds) at the same amount of resistance but at a cadence of 30 RPM. The participants were instructed to keep their intensity at a level to ensure the average HR for exercise fell within their individual HR_{peak} and was increased when participants HR during HIIE fell below their HR_{peak} range. All sessions were supervised and performed on a Monark cycle ergometer, with HR being recorded continuously. The participants conducted a 5-minute warm-up and cool-down on the bike prior to each exercise session. All cycling data included: RPM and RPE recorded at 5-minute intervals. To make it easier on participants to keep up with the timing, the HIIE was set to a pre-recorded compact disc

counting down each sprint in a 3-2-1 fashion. Encouragement was given during both sessions to motivate participants throughout exercise.

Figure 5.3 Participants performing a HIIE session.

5.2.9 Diet Analysis and Fish Oil

All participants were asked to complete a pre and post diet diary recall of food consumed on three separate days consisting of two week days and one weekend. All dietary analysis was completed following the same protocol explained in Chapter 3. Women assigned to the study eating plan, consisting of a low glycemic Mediterranean

style diet were asked to complete a food diary throughout the 12-week intervention. Weekly food frequency questionnaires and food diaries were recorded (see Appendix G). A fish oil capsule intake form was used by participants to ensure all capsules were consumed (see Appendix H). Three capsules (see Figure 5.4) per day were ingested, for 12 weeks containing 550 mg of EPA/DHA per 1100 mg capsule (YourHealth Group, Manly).

Figure 5.4 Fish oil supplement.

The FEM women met with research staff to go over the healthy eating plan for the study prior to commencing the program. Participants (FEM) were given recipes, advice, and nutritional information along with a Mediterranean pyramid (see Figure 2.7). After the initial diet sessions, FEM women were given more dietary guidelines throughout the study (every 3 weeks) along with feedback from their previous one-day diet diary and ways to progress towards the recommended diet. Use of a Mediet Score (MDS) provided researchers with adherence information on a scale of zero to nine, zero represented the least adherent to a Mediet and nine represented total adherence to a Mediet (Trichopoulou et al., 2003). Table 5.1 is an example of the categorical breakdown of the dietary components in order to calculate the MDS for participants. Scoring was based on the median values calculated based on mean scores for all women in FEM.

Table 5.1 Example of the Breakdown of Dietary Components for Calculation of Mediet Score (Adapted from Trichopoulou et al., 2003)

	Median (g/day)	Score	
Diet components		Above	Below
Beneficial components			
Vegetables	500	1	0
Legumes	7	1	0
Fruits and nuts	360	1	0
Cereals	140	1	0
Monounsaturated : saturated lipid ratio	1.7	1	0
Detrimental components			
Meat, poultry, and eggs	90	0	1
Dairy products	190	0	1
Alcohol consumption (ethanol)	5-25	0	0

5.2.10 Plasma Analysis and HOMA-IR Calculations

Frozen plasma samples were later analysed in duplicate. Insulin, CRP, and IL-6 were all measured using commercially available sandwich immunoassay kits. The degree of enzymatic turnover of the substrate was determined by dual wavelength absorbance measurement at 450 and 620 nm for insulin (DSL 10-1600) and 492 and 620 nm for IL-6 (R&D HS600B). HOMA-IR, the insulin resistance index (Stern et al., 2005) was calculated using, The Homeostasis Model Assessment (version 2.0, available online from http://www.dtu.ox.ac.uk) (Levy, Matthews, & Hermans, 1998) derives ß-cell dysfunction, insulin sensitivity (IS), and calculates HOMA-IR.

5.2.11 Lactate during Exercise Test

A subset of FEM women (n = 12) provided a finger prick sample of blood for lactate analysis using a Lactate Pro analyser (Arkray, Japan) at three time points before and during a HIIE session. Resting fasting lactate was measured before the start of the exercise session in week one and then again in weeks six and twelve. Two more samples were measured around minutes 10 and 20 of the exercise session using either the same finger on the other side or an alternate finger.

5.2.13 Analysis

Data analysis was completed with the Statistical Package for Social Science for windows software (SPSS 15.1, USA). Student's *t*-tests were used to examine differences between the two groups at baseline and based on the delta score between pre- and posttesting. All results are expressed as mean and standard error (SE) for variables with normal distribution. A p value of $< .05$ was adopted. Due to skewness, some values were log transformed (Ln) for analysis. If values were still skewed after log transformation, a Mann-Whitney U test was performed to test for significant difference. Pearson correlation analysis was used to determine associations between all variables on crude and log transformed values. Spearman's rank order correlation was performed on values that remained skewed after log transformation. All results are expressed as mean and standard error (SE). A p value of $p < .05$ was considered significant.

5.3 Results

5.3.1 Overview

The results reported in this section are from the FEM intervention trial. Participant characteristics and baseline data pre-intervention are initially reported with changes post-intervention following. Changes in body composition and aerobic power are reported first followed by changes in blood markers which are broken down by hormones, lipids, and inflammatory markers. Following the data from the blood samples, blood pressure and autonomic changes are described as are changes to HR and the response to the HIIE measured through HR and RPE. Diet information is provided with information regarding adherence to the Mediet and changes in food intake. Finally, a summary of all the results is presented. Adherence to the intervention was 90% in the randomized FEM group. All (n = 29) FEM women completed 36 exercise sessions and were allowed make up sessions if one was missed leading to high compliance. The

MDS verified conformity to the Mediet and a fish oil intake form was signed by all participants.

5.3.2 Participant Characteristics

The participants (N = 56) in the study were young, apparently healthy overweight women from the University and surrounding communities. The group consisted of Anglo women (n = 25), Chinese (Indonesian Chinese, Thai Chinese, and Malaysian Chinese) women (n = 16), Korean women (n = 4), Indian women (n = 5), Phillipino women (n = 2), and other (Portuguese, Mauritus, African and Laos) women (n = 4). For the FEM intervention group a 90 % adherence rate was noted (see Figure 5.5).

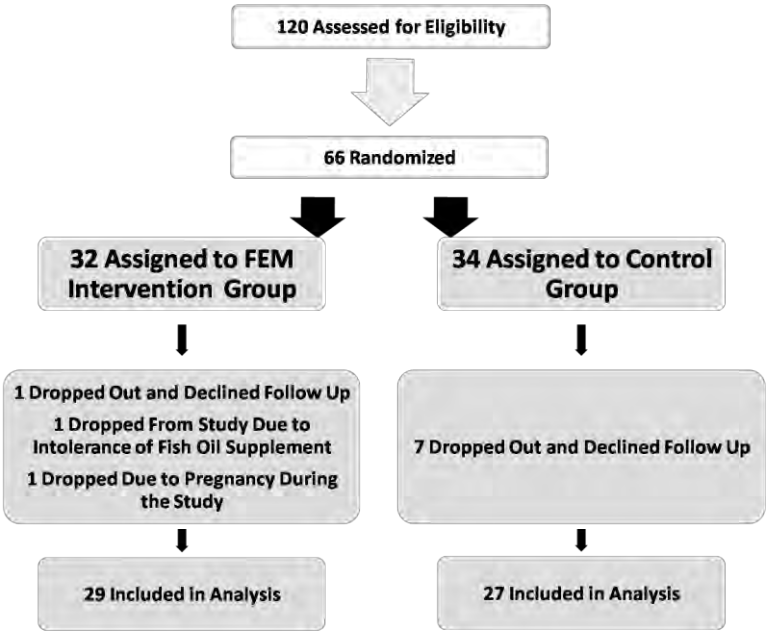


Figure 5.5 Randomization for FEM.

5.3.3 Baseline Variables

Table 5.2 shows participants' age, lipid, DEXA, aerobic fitness, and metabolic parameters. There was no significant difference between any of the baseline characteristics.

Table 5.2 Characteristics of Study III Participants

	FEM (n =29)				CON (n =27)			
	PRE		POST		PRE		POST	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Age (years)	23.7	0.8			23.3	0.9		
Mass (kg)	73.2	2.4	71.8*	2.3	70.8	1.8	71.6	2.0
Height (cm)	162.1	1.4			163.6	1.6		
BMI (kg/m^2)	28.0	0.7	27.3	0.5	26.8	0.4	27.0	0.5
$\dot{V}O_{2peak}$ (L/min)	1.9	0.1	2.2**	0.1	2.0	0.1	1.9	0.1
$\dot{V}O_{2peak}$ (ml/kg/min)	26.0	0.7	30.5**	0.7	27.6	1.2	27.0	1.0
SBP (mmHg)	113	2	107*	1	109	2	111	1
DBP (mmHg)	65	2	61	2	64	1	63	1
Resting respiratory quotient	0.89	0.01	0.90	0.01	0.93	0.02	0.90	0.02
Resting heart rate (bpm)	67	2	64	2	67	2	67	2
Peak heart rate (bpm)	183	1	184	2	176	5	180	2
Pulse pressure (mmHg)	48.2	1.4	45.5*	1.5	45.9	1.5	48.0	1.3
Mean arterial pressure (mmHg)	81.0	1.6	76.2*	1.4	78.8	1.3	79.0	1.1
Resting metabolic rate	1559	45	1642	44	1575	69	1584	71
Fat mass (kg)	30.6	1.5	29.0**	1.5	27.6	1.3	28.4	1.4
Fat free mass (kg)	40.0	1.0	40.2	1.0	40.5	1.0	40.5	1.0
Body fat by DEXA (%)	42.9	0.8	41.4**	0.9	40.2	1.1	40.8	1.2
Waist circumference (cm)	79.4	1.6	75.9*	1.5	76.7	0.9	76.2	1.0
Hip circumference (cm)	103.9	1.6	100.1*	1.3	102.7	1.5	102.2	1.6
Waist-to-height-ratio	0.49	0.01	0.47*	0.01	0.47	0.01	0.47	0.01
Fasting insulin (µIU/ml)	17.4	1.8	13.2*	1.1	14.5	1.2	14.0	1.3
HOMA-IR	2.2	0.2	1.8	0.1	1.8	0.1	1.7	0.2
Beta cell dysfunction	185.8	15.1	146.9*	10.7	159.4	11.8	154.6	10.6
Insulin sensitivity	57.8	6.4	66.1	5.1	69.2	7.7	68.9	6.0
Fasting glucose (mmol/L)	4.8	0.1	5.0	0.1	4.8	0.1	4.9	0.1
Fasting interluekin-6 (pg/ml)	1.5	0.2	1.0**	0.1	1.2	0.2	1.3	0.2
Triglycerol (mmol/L)	0.89	0.06	0.70*	0.06	0.93	0.09	1.04	0.14
Total cholesterol (mmol/L)	4.5	0.1	4.3	0.1	4.6	0.2	4.7	0.2
HDL cholesterol (mmol/L)	1.4	0.1	1.3	0.1	1.4	0.1	1.5	0.1
LDL cholesterol (mmol/L)	2.8	0.1	2.6	0.1	2.7	0.2	2.8	0.2
VLDL cholesterol (mmol/L)	0.41	0.03	0.32*	0.03	0.43	0.04	0.47	0.06

Mean and standard error (SE)
**Significant difference ($p < 0.001$) between FEM and CON
*Significant difference ($p < 0.05$) between FEM and CON
Note: Body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), homeostasis model assessment of insulin resistance (HOMA-IR), high density lipoproteins (HDL), estimated low density lipoproteins (LDL), and estimated very low density lipoproteins (VLDL)

5.3.4 Response in Body Composition following the Intervention

Significant differences between the FEM and CON women were seen post intervention for almost all body composition indices: mass, $t(54) = 3.28$, $p < .05$ (see

Figure 5.6 A), %BF, $t(54) = 3.72$, $p < .001$, FM, $t(54) = 3.88$, $p < .001$ (see Figure 5.6 B),WC, $t(54) = 3.15$, $p < .05$ (see Figure 5.6 C), HC, $t(49) = 3.60$, $p < .05$, and WHTR, $t(54) = 3.16$, $p < .05$. FFM was not significantly increased although there was a trend in the FEM women for an increase after the intervention. The reduction in FM was associated with improvements in body composition WC, $r = .53$, $p < .001$, HC, $r = .66$, $p < .001$, and WHTR, $r = .51$, $p < .001$), IL-6, $r = .40$, $p < .05$, high, $r = -.32$, $p < .05$ and low, $r = -.39$, $p < .05$, frequency power, SBP, $r = .46$, $p < .001$, DBP, $r = .35$, $p < .05$, MAP, $r = .44$, $p < .05$, MDS, $r = .43$, $p < .05$, PP, $r = .28$, $p < .05$, and fitness, $r = .41$, $p < .05$. The FEM women reduced their body mass by 2% and fat mass by 6%.

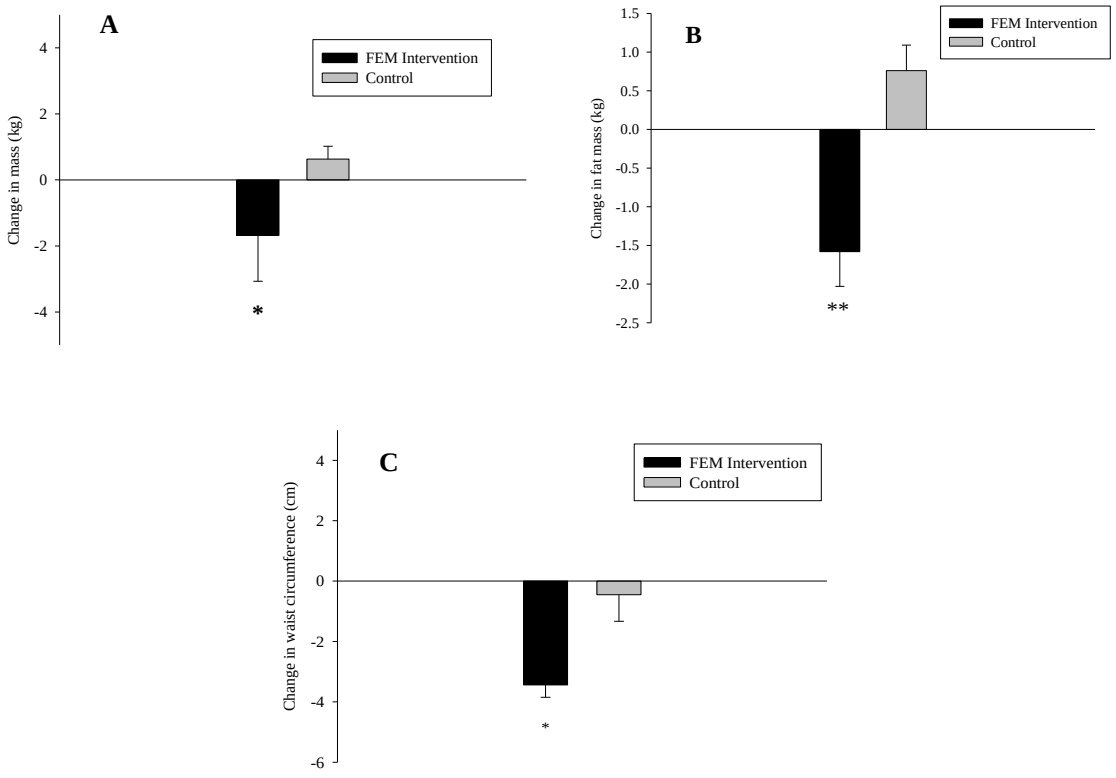


Figure 5.6 (A-C) Response in body composition measures (mass, fat mass, and waist circumference) following FEM (*, $p < .05$, **, $p < .001$).

5.3.5 Response in Aerobic Power following the Intervention

Aerobic power was significantly higher in the FEM group, $\dot{V}O_{2peak}$ (L/min), $t(54) = 7.05$, $p < .001$ (see Figure 5.7), and $\dot{V}O_{2peak}$ (ml/kg/min), $t(54) = 8.55$, $p < .001$. The FEM women improved 14% from baseline. No significant changes in the CON

women regarding fitness occurred. HR_{peak} between groups at baseline was not significantly different. An inverse relationship between the increase in $\dot{V}O_{2peak}$ (L/min) and the reductions in IL-6, $r = -.36$, $p < .05$, PP, $r = -.27$, $p < .05$, SBP, $r = -.32$, $p < .05$, TG, $r = -.29$, $p < .05$), and VLDL, $r = -.36$, $p < .05$, following FEM existed.

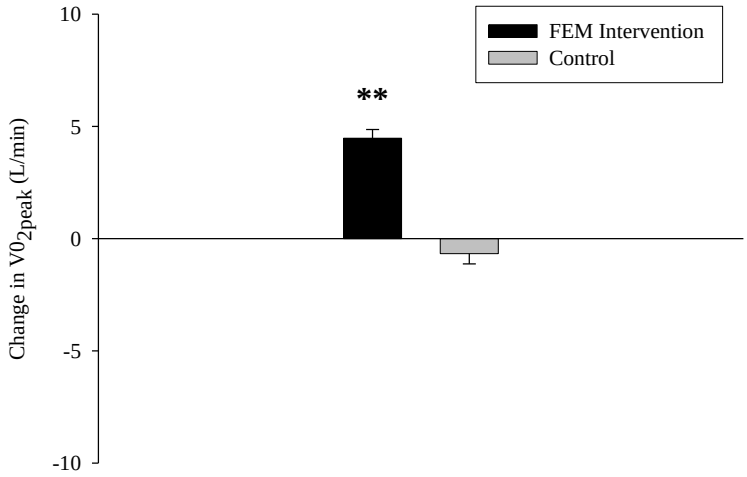


Figure 5.7 Response in $\dot{V}O_{2peak}$ following FEM (**, $p < .001$).

5.3.6 Response in Blood Markers following the Intervention

5.3.6.1 Response in insulin following the intervention

Fasting plasma insulin, $t(47) = 2.12$, $p < .05$, was significantly decreased in FEM compared to CON (see Figure 5.8). The FEM women had a 25% decrease in fasting plasma insulin after the intervention. The decrease in insulin in the FEM group was positively correlated to a reduction in WC, $r = .30$, $p < .05$, WHTR, $r = .31$, $p < .05$, beta cell dysfunction, $r = .65$, $p < .001$, SBP, $r = .42$, $p < .05$, DBP, $r = .38$, $p < .05$, MAP, $r = .43$, $p < .05$, glucose, $r = .30$, $p < .05$, VLDL, $r = .30$, $p < .05$, protein intake, $r = .29$, $p < .05$, saturated fat, $r = .33$, $p < .05$, and cholesterol intake, $r = .39$, $p < .01$. The association between the reduction in insulin and FM approached significance, $r = .27$, $p = .06$, following the intervention.

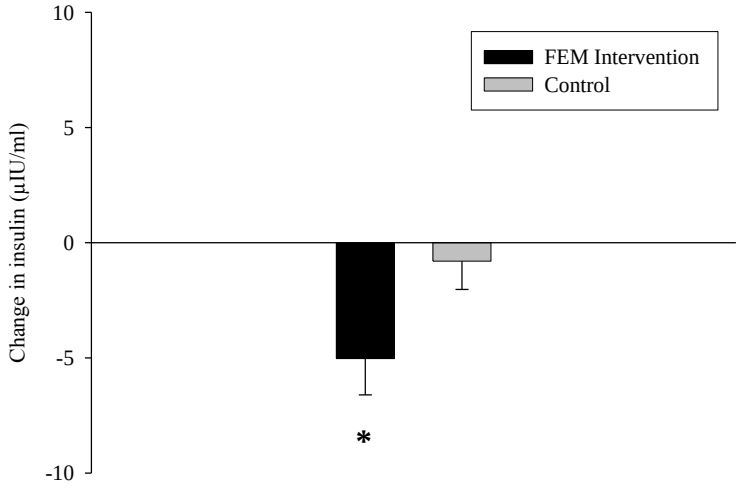


Figure 5.8 Response in fasting plasma insulin following FEM (*, $p < .05$).

5.3.6.2 Response in glucose, HOMA-IR, insulin sensitivity, and beta cell dysfunction following the intervention

Glucose, HOMA-IR, and IS did not change significantly between groups (see Table 5.2), however the FEM women approached a significant difference in the reduction (24%) of HOMA-IR, $p = .10$ compared to CON (4%). A significant decrease, $t(50) = 2.07$, $p < .05$, in beta cell dysfunction was seen in the FEM women compared to CON (see Table 5.2). The decrease in HOMA-IR was correlated to the same variables as insulin except WC and WHTR following the FEM trial.

5.3.6.3 Response in interluekin-6 following the intervention

Fasting plasma IL-6, $t(44) = 5.97$, $p < .001$, significantly decreased in FEM (33%) compared to CON (see Figure 5.9).

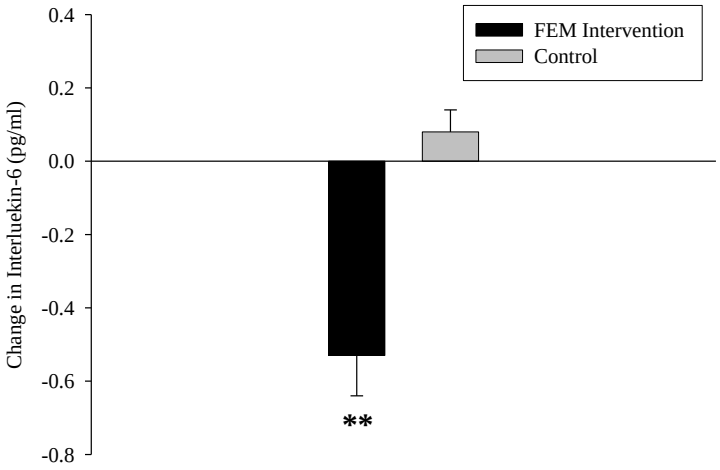


Figure 5.9 Response in fasting plasma IL-6 following FEM (**, $p <$ .001).

5.3.6.4 Response in lipids following the intervention

In the FEM women, TG[10], $p < .05$, and VLDL[11], $p < .05$, were significantly decreased compared to CON (see Figure 5.10 A and B). TC and LDL were also lowered due to the intervention but there were no significant differences between groups. HDL decreased slightly in the FEM women and increased slightly in the CON women. TC/HDL decreased in both groups but differences were not significant. TG/HDL decreased in FEM but not in CON and approached significance (see Table 5.2).

[10] Wilcoxon signed-rank test, TG, Z = 2.34
[11] Wilcoxon signed-rank test, VLDL, Z = 2.24

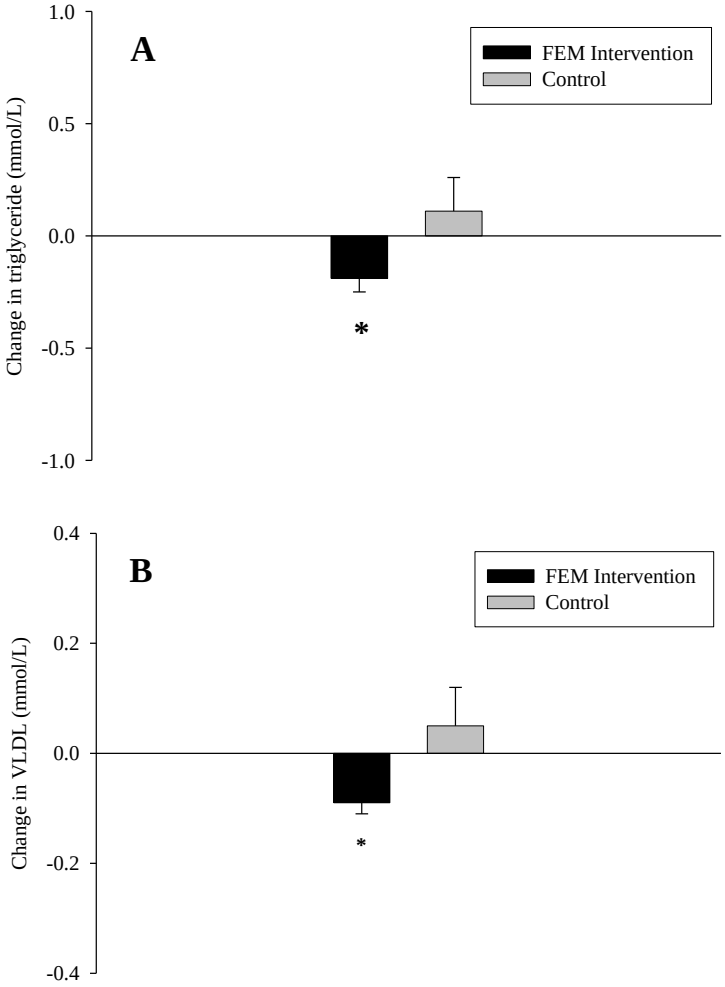


Figure 5.10 (A, B) Response in lipids (triglycerol and VLDL) following FEM (*, $p < .05$).

5.3.7 Acute Blood Lactate, Ergometer Load and Revolutions Per Minute

Blood lactate in a subset of the women participating in FEM ($n = 12$) was significantly increased from baseline to the end of the two HIIE sessions, $F(2, 20) = 36.02$, $p < .001$, but did not change pre and post intervention (see Figure 5.11).

In the same sample of women, there was no change in RPM across the 20 minutes of HIIE although the RPM increased significantly between the first and twelfth week of FEM , $F(1, 11) = 53.44$, $p < .001$, and the load on the cycle ergometer increased (1.01 to 1.12 W) significantly throughout the trial, $t(28) = 4.22$, $p < .001$.

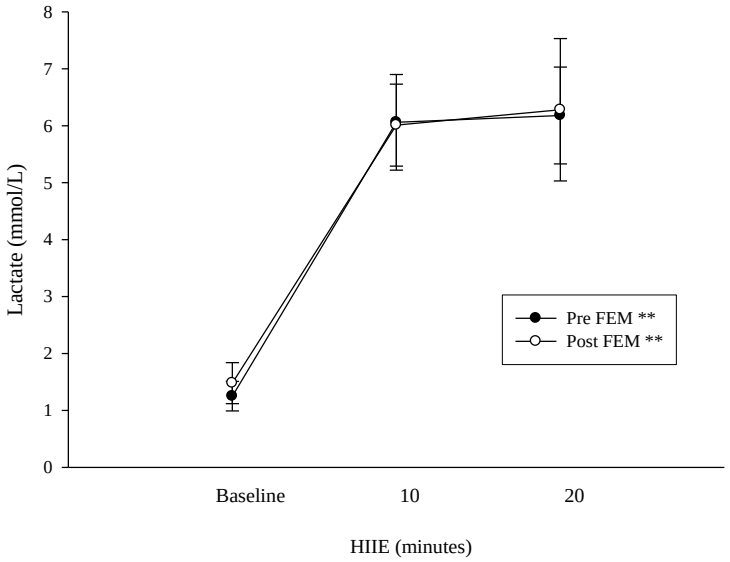


Figure 5.11 Response in lactate during a HIIE session (**, $p < .001$).

5.3.8 Response in Autonomic Function following the Intervention

5.3.8.1 Response in blood pressure, mean arterial pressure, and pulse pressure following the intervention

Significantly lower BP was seen in the women who completed the intervention. Systolic, $t(54) = 3.68$, $p < .05$ (see Figure 5.12 A), and diastolic BP, $t(54) = 2.00$, $p = .05$, decreased in FEM, whereas there was no change in CON. PP, $t(54) = 2.55$, $p < .05$, and MAP, $t(54) = 2.98$, $p < .05$, also decreased significantly in FEM (see Figure 5.12 B and Table 5.2). A relationship between the reduction in SBP and the enhanced HF power, $r = -.36$, $p < .05$, and lower resting HR, $r = .36$, $p < .05$, beta cell dysfunction, $r = .29$, $p < .05$, saturated fat, $r = .30$, $p < .05$, %BF, $r = .40$, $p < .05$), WHTR, $r = .36$, $p < .01$, and WC, $r = .39$, $p < .05$ was evident following the intervention. Fitness increases in absolute terms were inversely associated with SBP, $r = -.32$, $p < .05$.

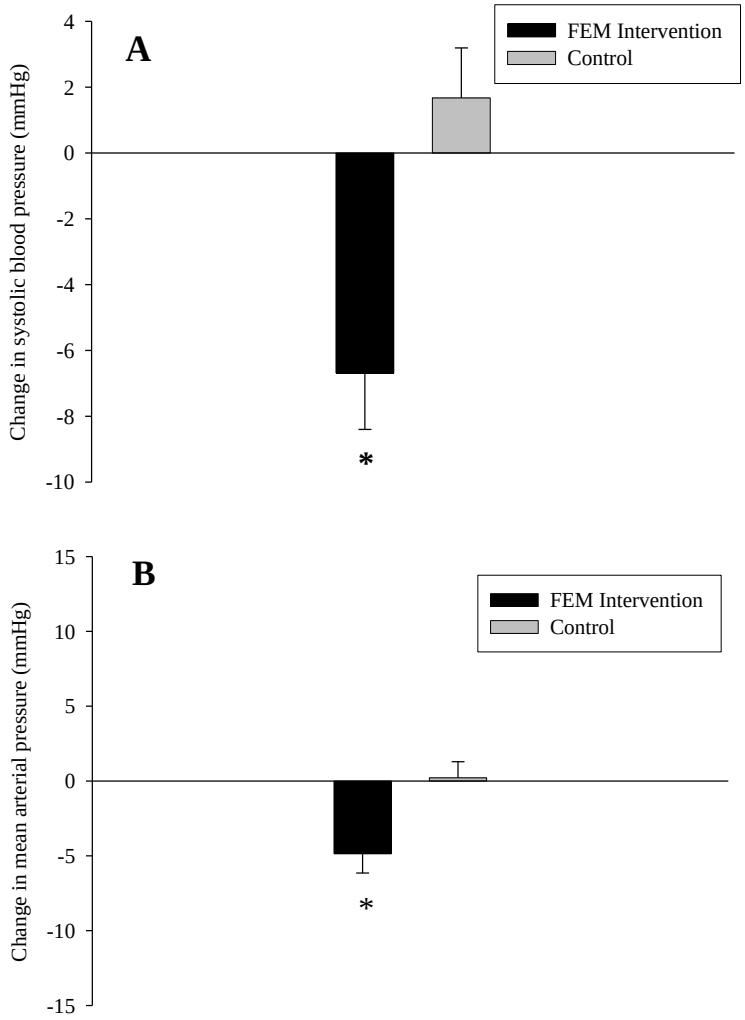


Figure 5.12 (A, B) Response in BP and MAP following FEM (*, $p < .05$).

5.3.8.2 Response in high and low frequency power following the intervention

LF power, $t(46) = 2.64$, $p < .05$, and HF power, $t(46) = 3.07$, $p < .05$, (see Figure 5.13 A and B) of resting HPV was significantly increased in the FEM women.

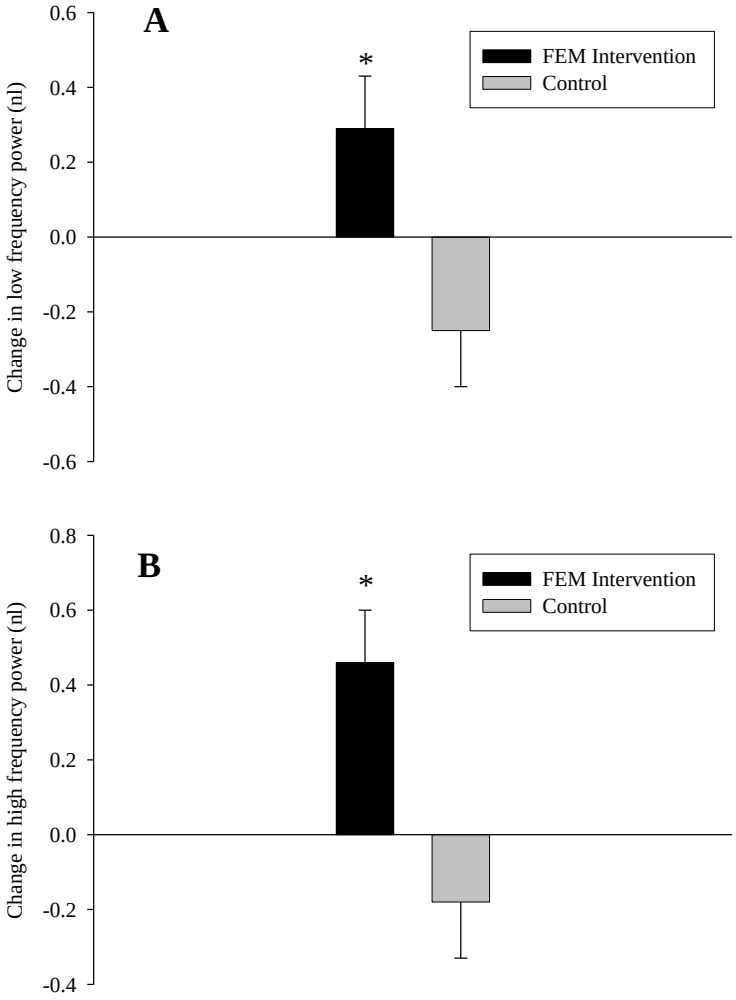


Figure 5.13 (A, B) Response in autonomic function (high and low frequency power) following FEM.

5.3.9 Response in Heart Rate to Exercise

5.3.9.1 Response in heart rate to a high intensity interval exercise session

The average HR during a HIIE session was (155.5 ± 1.3) which corresponds to 85% of HR_{peak}. There was not a significant difference in resting HR after the intervention compared to CON although there was a trend for a decreased HR in the FEM women.

5.3.9.2. Response in heart rate to an aerobic power test

Comparing the HR values during the post fitness test for FEM and CON (see Figure 5.14 A & B) women after the intervention, the FEM women had significantly lower HR values at 105 W, $t(51) = 3.14$, $p < .05$, and 120 W, $t(48) = 3.59$, $p = .05$.

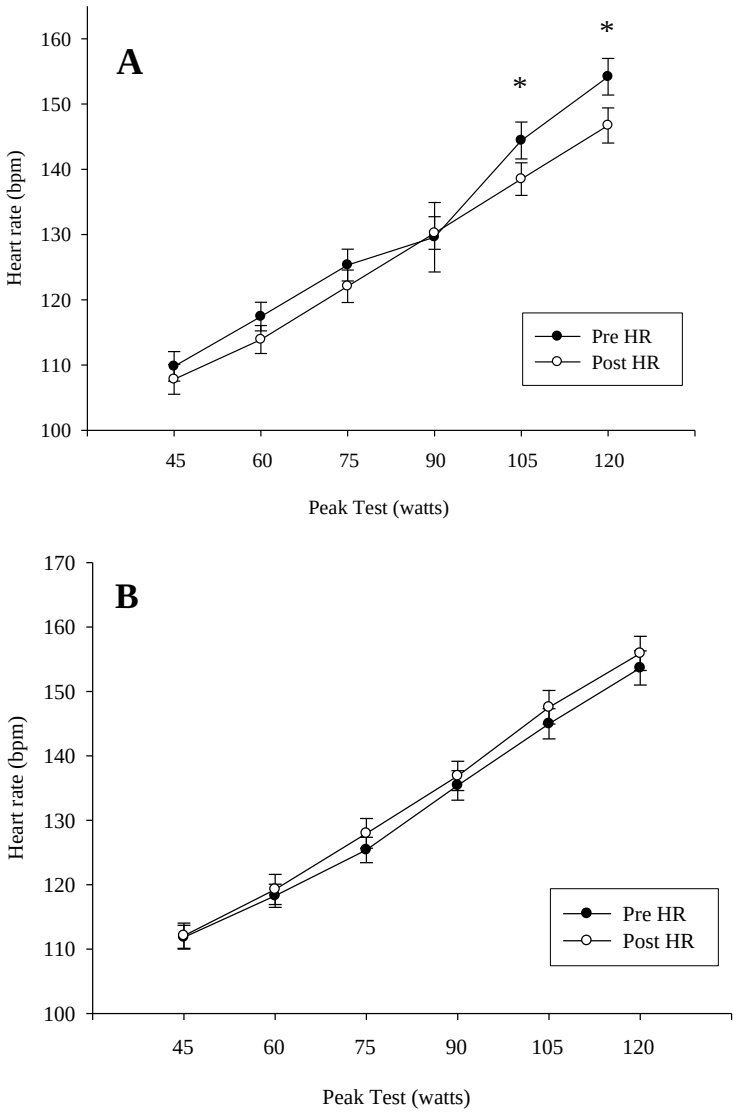


Figure 5.14 (A, B) Response in heart rate, (A) intervention women and (B) controls, during the peak test following FEM (*, $p < .05$).

5.3.10 Response in Rating of Perceived Exertion to an Exercise Session

The overall change in pre and post RPE was significantly different between FEM and CON, $t(6) = 3.74$, $p < .05$, (see Figure 5.15) during the fitness tests for all intensities.

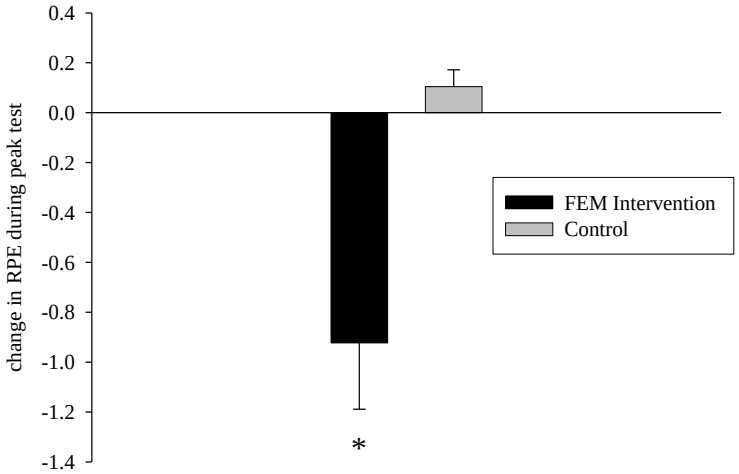


Figure 5.15 RPE during the peak test following FEM (*, $p < .05$).

5.3.11 Response in Respiratory Exchange Ratio during an Aerobic Power Test

During the post fitness test the FEM women had a lower RER (see Figure 5.16 A) at almost all levels of intensity 60 W, $t(53) = 3.41$, $p = .001$, 75 W, $t(53) = 4.17$, $p < .001$, 90 W, $t(53) = 5.06$, $p < .001$, 105 W, $t(53) = 5.10$, $p < .001$, and 120 W, $t(51) = 4.85$, $p < .001$ compared to CON (see Figure 5.16 B).

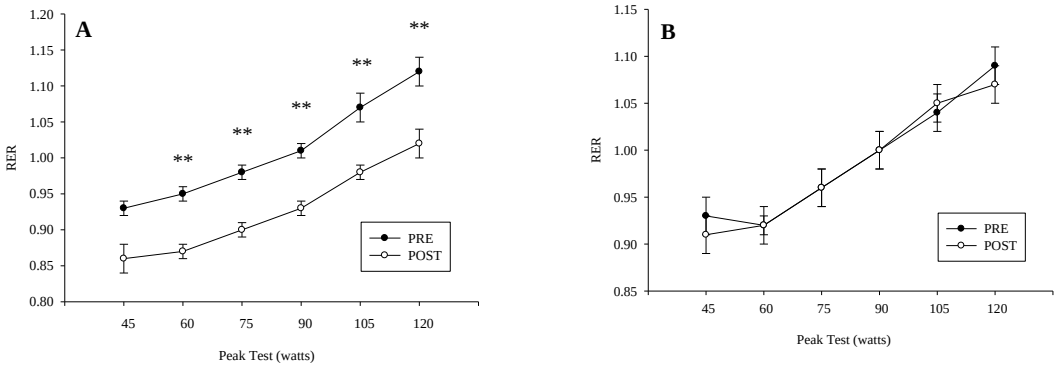


Figure 5.16 (A, B) RER, (A) intervention women and (B) controls, during the peak test following FEM (**, $p < .001$).

5.3.12 Dietary Intake following the Intervention

There were no significant differences at baseline for any of the dietary intake variables (see Table 5.3).

Table 5.3 Study III Participant's Dietary Intake

	FEM (*n* =29)				CON (*n* =27)			
	PRE		POST		PRE		POST	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Total energy (kJ)	6809	299	5780*	263	6826	395	6801	360
Protein (g)	68.1	4.0	65.6	3.8	66.9	4.1	69.9	3.2
Total fat (g)	58.8	3.8	46.9*	3.9	61.9	5.3	62.4	4.9
Saturated fat (g)	21.3	1.6	15.0*	1.2	22.4	1.9	23.3	2.1
Polyunsaturated fat (g)	9.5	0.7	8.6	0.8	10.4	1.0	9.6	0.8
Monounsaturated fat (g)	22.3	1.6	18.3*	2.4	23.4	2.7	23.9	2.2
Cholesterol (g)	227.2	23.3	188.8*	21.1	214.4	19.6	252.6	20.2
Carbohydrate (g)	189.8	8.5	156.3	8.3	196.1	13.8	181.4	9.9
Fibre (g)	20.1	1.0	22.7	1.8	18.6	1.6	16.9	1.2
Iron (mg)	10.3	0.6	10.8	0.9	9.6	0.7	9.4	0.5
Protein (%)	17.3	0.7	20.1	0.9	16.9	0.7	18.2	1.0
Fat (%)	32.2	1.2	30.1	1.7	35.6	2.8	32.9	1.4
Carbohydrate (%)	48.7	1.5	47.5	1.8	48.4	1.9	45.7	1.7

Mean and standard error (SE)
*Significant difference ($p < 0.05$) between FEM and CON

5.3.12.1 Dietary changes following the intervention

The FEM women had significant reductions in total kilojoules, $t(54) = 2.20$, $p < .05$, fat, $t(54) = 2.11$, $p < .05$, saturated fat, $t(54) = 2.94$, $p < .05$ (see Figure 5.17 A), monounsaturated fat, $t(54) = 2.04$, $p < .05$, and cholesterol, $t(54) = 2.10$, $p < .05$ compared to controls (see Figure 5.17 B, and Table 5.3).

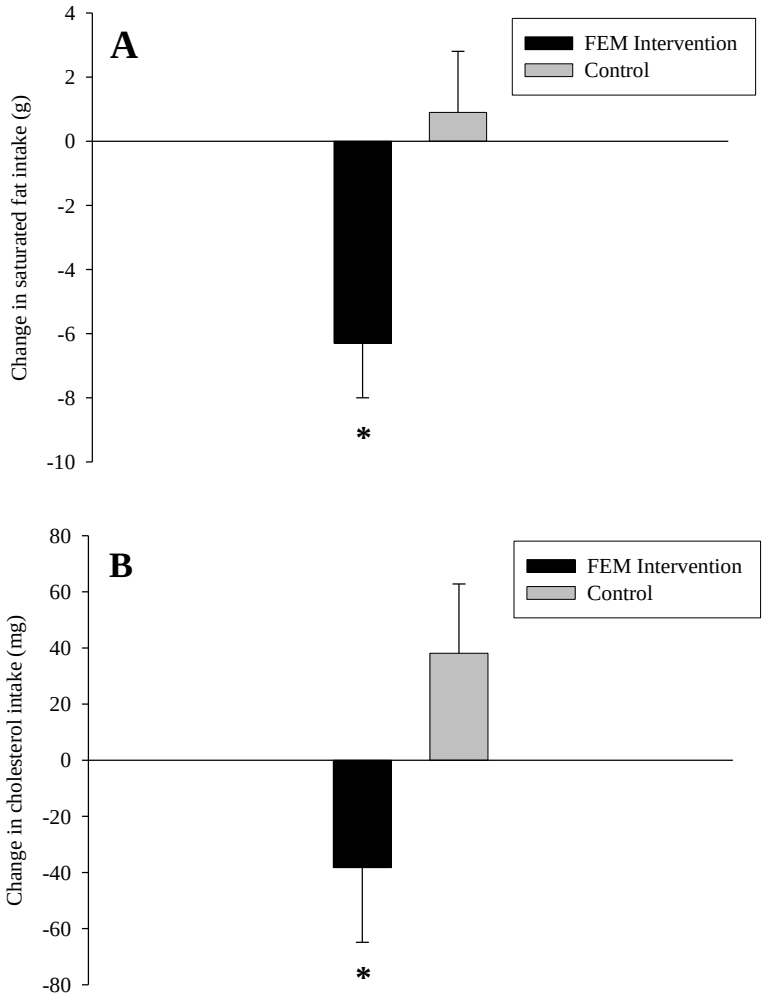


Figure 5.17 (A, B) Changes in dietary intake (saturated fat and cholesterol) following FEM (*, $p < .05$).

5.3.12.2 Mediet score following the intervention

There was a positive significant increase in the MDS score, $t(28) = 4.00$, $p < .001$, for the FEM women which was positively associated with weight loss, $r = .48$, $p < .01$. The score increased 22% from baseline and was significantly correlated to a reduction in FM, (see Figure 5.18). Fruit and nut, vegetable, and legume consumption increased, meat, poultry and egg (MPE) and dairy decreased and cereal did not change (see Table 5.4).

Table 5.4 Study III Participant's Mediet Score Components

	FEM (n =29)			
	PRE		POST	
	Mean	SE	Mean	SE
Cereal (g/day)	220.1	13.8	219.5	14.6
Dairy (g/day)	112.8	18.2	97.5	14.9
Ethanol (g/day)	4.3	1.9	5.0	2.0
Fats (g/day)	6.1	1.6	6.8	1.3
Fruit and Nut (g/day)	183.5	27.0	237.3	30.9
Legumes (g/day)	21.9	8.4	39.5	7.3
Meat (g/day)	115.8	14.1	80.1	10.1
Vegetable (g/day)	181.4	20.3	209.9	15.4

Mean and standard error (SE)

Only legume, $t(28) = 2.15$, $p < .05$, and MPE , $t(28) = 2.92$, $p < .05$, were significantly different from baseline.

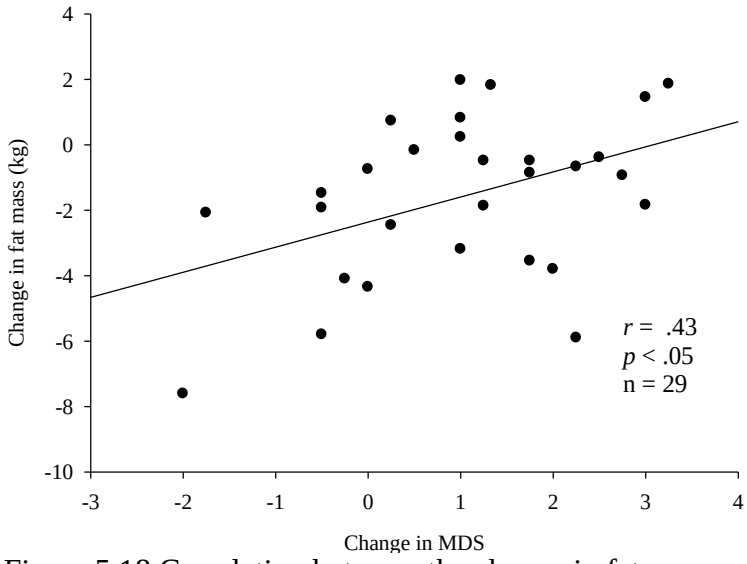


Figure 5.18 Correlation between the change in fat mass and the change in MDS following FEM.

Baseline legume ingestion was negatively correlated to fasting plasma insulin, $r = -.46$, $p < .05$, at baseline. MPE consumption at baseline was positively associated with pre-intervention fasting LDL, $r = .40$, $p < .05$, TC, $r = .39$, $p < .05$, and PP, $r = .48$, $p < .05$. The decrease in MPE, $r = .45$, $p < .05$ and increase in legume, $r = .43$, $p < .05$ intake were positively associated with reduction in FM. The reductions found in cholesterol, $r = .42$, $p < .05$ and saturated fat, $r = .41$, $p < .05$ were positively correlated

to the decrease in MPE ingestion and the increase in legumes was associated with a lower WHTR, $r = .38$, $p < .05$, in FEM women.

5.4 Discussion

After the intervention, the FEM women reduced their body composition, insulin, inflammation, BP, and lipid levels compared to CON. Increased HPV, aerobic fitness, and fat oxidation (RER) following FEM was found in the steady state stage of the peak oxygen uptake test. The intervention women altered their dietary intake, conforming more to a Mediet, as confirmed through an increase in MDS and a significant reduction in saturated fat. Thus, a 12-week intervention combining HIIE, fish oil ingestion, and Mediet improved metabolic hormones and inflammatory markers and reduced fat mass in overweight women agreeing with Hypothesis 5 (page 9). Studies investigating the individual components of FEM are numerous as presented in the literature review. The focus of the current trial (FEM), however, was to combine multiple lifestyle components into an intervention to examine synergistic outcomes. Thus, it was predicted that a multi-faceted intervention would result in metabolic, cardiovascular, and autonomic improvements in overweight and obese young women. Throughout the following discussion regarding the FEM trial, the synergistic effects from the combination of ingestion of fish oil, Mediet, and exercise will be discussed.

5.4.1 Response in Body Composition following the Intervention

The women were overweight (BMI = 28 kg/m^2) at baseline and following the intervention a reduction in fat mass by 6% (clinically significant) and a 2% decrease in body mass was observed. These results support prior findings involving HIIE (Trapp et al., 2008; Tremblay et al., 1994). Previous studies from our laboratory found a 12% decrease in fat mass after a 15-week program in women with a lower BMI (24.4 kg/m^2) (Trapp et al., 2008). Reductions in body weight or fat mass may be a consequence of a

number of variables (reviewed in detail by Boutcher and Dunn, 2009) with greater reductions evident in some individuals (responders) more than others (non-responders). Examination of responders (RES) and non-responders (NRES) based on a loss in body mass ≤ 1% following FEM is presented in Chapter 6.

The reduction in FM was associated with improvements in body composition, inflammatory markers, autonomic function, Mediet, fitness, and a trend in fasting plasma insulin. Therefore, the combination of HIIE and Mediet with fish oil ingestion reduced body fat in overweight women.

A limitation of this study when examining changes in post intervention body composition is sensitivity to the menstrual cycle. When a woman completed the trial, all posttesting was conducted in the days immediately following, with a minimum of 24 hours between the last session and the first posttest. Due to variation in the menstrual cycle between participants, and adhering to the 12-week protocol, the women were not all measured during the same phase of the cycle (follicular, luteal or menstruation) and some variability in water weight gain should be considered.

5.4.2 Response in Aerobic Power following the Intervention

Improvements in fitness have been shown to be associated with decreased MetS risk in asymptomatic women (LaMonte, Barlow, Jurca, Kampert, Church, & Blair, 2005b). The FEM women did not meet the criteria for MetS at baseline but would still be considered at risk due to their low fitness levels. Aerobic fitness levels increased by 14% in the intervention group but the CON women showed no change (Figure 5.7). Aerobic training studies have shown smaller increases in a similar cohort of women following longer protocols (Jakicic, Marcus, Gallagher, Napolitano, & Lang, 2003; Okura, Nakata, Ohkawara, Numao, Katayama, Matsuo et al., 2007). Prior research

conducted in our laboratory has shown larger fitness increases although the study was 3 weeks longer and was conducted with normal weight individuals (Trapp et al., 2008).

The possibility of a "learning curve" for participants performing the peak test exists although the data does not support this notion for either FEM or CON women. For example, HR_{peak} did not change from pre- to posttest for either group. Reductions in resting HR, suggesting an increase in fitness on the posttest, were found for FEM women although the decrease was not significant. A lower HR at the higher levels of the peak test was significantly different in the FEM women. A decrease of 5 bpm at 105 W and 8 bpm at 120 W was recorded in the intervention group which was not the case in the control group.

The lack of change in lactate with increasing work load and RPM measured during two HIIE session from week 3 to week 12 is an indicant of anaerobic fitness improvement. RPE decreased significantly in the FEM women and on the post peak test, for each individual load, a reduction was noted in the FEM women.

5.4.3 Response in Metabolic Syndrome Blood Markers and Lipids following the Intervention

Insulin (25%) decreased significantly (Figure 5.8) following the intervention and was similar to the reduction (31%) demonstrated by Trapp et al. (2008) with a 15-week intervention using the same sprint protocol. The drop in BP and FM was associated with the reduction in insulin in this group of women which has been found previously following weight loss and exercise interventions (Dengel, Hagberg, Pratley, Rogus, & Goldberg, 1998). The decrease in insulin may be the result of alterations in the signalling pathways and glucose metabolism enhancing sensitivity to insulin (Koval, Maezono, Patti, Pendergrass, DeFronzo, & Mandarino, 1999), lower inflammatory markers (Kadoglou, Iliadis, Angelopoulou, Perrea, Ampatzidis, Liapis et al., 2007),

changes to adipocytokines and hormones (Abete, Parra, Crujeiras, Goyenechea, & Martinez, 2008; Balagopal, George, Yarandi, Funanage, & Bayne, 2005b), or adipocyte morphology (He, Goodpaster, & Kelley, 2004).

The intervention produced an anti-inflammatory response after twelve weeks in the FEM group. The 33% reduction in IL-6 following HIIE, Mediet, and fish oil is similar to results of a study performed in T2DM, obese, and lean men for 12 weeks involving 60 minutes of exercise daily with a 55%, 17%, and 32% reduction in IL-6 (Dekker, Lee, Hudson, Kilpatrick, Graham, Ross et al., 2007). Also a 25% reduction was found in adolescents following 45-minute sessions, 3 days a week, for 12 weeks (Balagopal et al., 2005a). Referring back to Figure 2.4, it can be seen that IL-6 and other inflammatory markers influence metabolism through action on the vascular system, liver, pancreas, adipose tissue, and muscle (Eckel et al., 2005).

A reduction in all lipids after the FEM intervention was documented although only VLDL and triglycerol were significantly lower compared to CON (Table 5.2). After 15 weeks of HIIE and an unaltered diet, lipids did not change (Trapp, 2006). Contradictory to the findings by Tjonna et al. (2008) involving HDL after a HIIE intervention (Tjonna et al., 2008a; Tjonna et al., 2008b), HDL decreased in this cohort of women although not significantly. The findings for HDL and lipids following a HIIE have only been demonstrated by Tjonna et al. (2008). Thus, without the Mediet or fish oils in combination with HIIE, alterations in the lipid profile may not have occured after a 12-week HIIE training regimen.

5.4.4 Response in Autonomic Function following the Intervention

The synergistic effect from the exercise training, fish oil ingestion, and Mediet in the FEM women most likely contributed to the lowering of BP and MAP (Bond, Stephens, Adams, Vaccaro, Demeersman, Williams et al., 2002; Dickinson, Mason,

Nicolson, Campbell, Beyer, Cook et al., 2006) and increased HF and LF power. The reductions in BP were significantly associated with changes to insulin, IS, and HOMA-IR. Interestingly, in the FEM women, the difference in MAP was significantly correlated to the change in iron intake (see Figure 5.19). Prior studies involving HIIE have recorded decreases in BP and MAP (Tjonna et al., 2008a; Tjonna et al., 2008b). The reason behind the association of lower iron intake and the reduction in BP could involve inflammation or the non-haem component of iron (Tzoulaki, Brown, Chan, Van Horn, Ueshima, Zhao et al., 2008). Iron has been shown to increase the production of inflammatory markers and oxidative stress (Mendes, Arruda, Siqueira, Ito, & Silva, 2009) and a reduction could result in an anti-inflammatory effect and lowered BP or MAP.

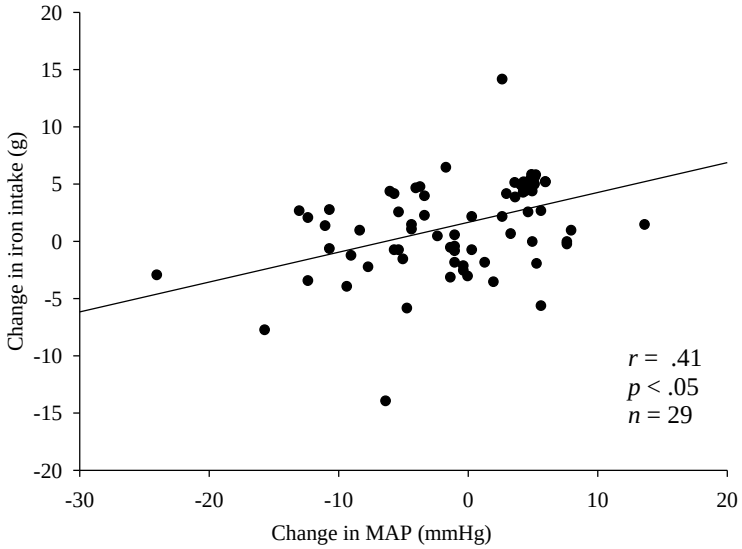


Figure 5.19 Correlation between the change in iron intake and the change in MAP following FEM.

Significantly increased high and low frequency power (indicators of autonomic control of the cardiovascular system) was measured following the intervention (Figure 5.13 A and B) which was not found following 15 weeks of HIIE without dietary modification and consumption of fish oil (Park, 2008). Previously, autonomic modulation following exercise has been found (Amano, Kanda, Ue, & Moritani, 2001;

Cooke & Carter, 2005; Gutin, Owens, Slavens, Riggs, & Treiber, 1997; Tulppo et al., 2003). The changes following FEM may be due to electrophysiological changes to the heart (Mozaffarian et al., 2008) as post intervention resting HR was lower.

5.4.5 Response in Resting Respiratory Quotient following the Intervention

The lower RER during the peak test, following FEM, suggests increased fat oxidation for a given work load. Increased whole body fat oxidation was demonstrated after only 2 weeks of HIIE in young women (Talanian et al., 2007). Improvements in muscle mitochondrial content and efficiency [increased citrate synthase (Burgomaster et al., 2005; Talanian et al., 2007) and PGC-1α (Schjerve et al., 2008; Tjonna et al., 2008a)], increased adiponectin (Tjonna et al., 2008a; Tjonna et al., 2008b), and alterations in the function of the sarcoplasmic reticulum involving calcium ion reuptake (Tjonna et al., 2008a) following HIIE may influence fat oxidation rates. Fat receptors responding to elevations in catecholamines induce fat utilization. Increased catecholamines throughout a single HIIE exercise bout and in the immediate recovery period (Trapp, 2006) may alter fat utilization through an influence on fat receptors. Insulin reduction, or an increase in FFA availability, may be the reason underlying the lower RER following FEM. The lowered RER may be influenced by the loss in FM as an increase in FFA, despite similar RER for a given exercise session, has been shown in previously obese women (Ezell, Geiselman, Anderson, Dowdy, Womble, Greenway et al., 1999). The lower RER found in the FEM intervention group appears to represent a "switch" in substrate utilization at the lower intensities of the post peak test.

5.4.6 Dietary Changes and the Mediet Score following the Intervention

Total kilojoules (15%), total fat (20%), saturated fat (30%), monounsaturated fat (18%), and cholesterol intake (17%) decreased significantly compared to controls. These results have been previously demonstrated following a two-year Mediet (calorie

reduction) and physical activity intervention in an obese population of premenopausal healthy women (Esposito et al., 2003). Specifically, fruit and nut, vegetable, fats ratio, and legume consumption increased, and MPE and dairy decreased contributing to a significant increase (22%) in the MDS score representing adherence to the FEM Mediet. Adherence to a Mediet following FEM was associated with an improved FM, similar to the improved BMI following a 3-month dietary intervention in men and women (Vincent-Baudry, Defoort, Gerber, Bernard, Verger, Helal et al., 2005). The MDS system incorporated in the FEM investigation (medians used) may have strengthened the association as no relation to BMI in either men or women was found in a large Greek epidemiological study (Trichopoulou et al., 2005).

Baseline fasting plasma insulin was negatively correlated to legume ingestion at baseline and both were significantly altered following FEM. The baseline MPE consumption for all women (FEM and CON) was positively associated with pre-intervention fasting LDL, TC, and PP and a significant reduction in MPE intake for the FEM women was found post intervention which was related to the reduction in FM. The reductions found in cholesterol and saturated fat were positively correlated to the decrease in MPE ingestion and the increase in legumes was associated with a lower WHTR in FEM women. Thus, specific alterations within the dietary components of Mediet (less MPE and greater legume intake) favourably impacted metabolic variables, lipids, and body composition following the 12-week intervention.

5.4.7 Summary

Following a 12-week multi-component intervention (FEM) reductions in body composition, insulin, inflammation, BP, and lipids were demonstrated. Mediet improvements resulted in an increase in legumes and a reduction of saturated fat and meat, poultry and egg intake. An increase in MDS, heart period variability, aerobic

fitness, and fat oxidation during the peak test aft FEM was found. Therefore, an intervention trial incorporating fish oil consumption, HIIE and Mediet (FEM) was successful at improving a number of MetS markers in young premenopausal, overweight women.

CHAPTER 6

RESPONDERS AND NON-RESPONDERS

6.1 Introduction

After completion of the FEM trial (see Chapter 5) a subset of the women participating was investigated. A majority of the participants responded (RES) to the intervention with a loss in body mass. RES were compared to the women who completed the intervention but did not respond (NRES) with a loss in body mass ≤ 1%. All exercise sessions were supervised and heart rate (HR) and load were recorded for all sessions. All women investigated attended 100% of the HIIE sessions with reductions in inflammatory markers, insulin, and blood pressure. All women had an increase in aerobic fitness despite being a RES or NRES.

6.2 Methods

6.2.1 Participants

Only the women (N = 34) partaking in the intervention were studied and divided into two groups, responders (RES) and non-responders (NRES) to a lifestyle intervention involving HIIE and diet modification. Some of the control participants (n = 5) went on to complete the intervention, so the number of participants is larger in this group than reported in the previous chapter. The same intervention protocol and methods as described in Chapter 5 were implemented.

6.2.2 Analysis

Data analysis was completed with the Statistical Package for Social Science for windows software (SPSS 15.1, USA). Student's *t*-tests were used to examine differences between the two groups at baseline and on the delta score between pre- and posttesting. The groups were divided by those that lost ≤ 1% body mass (see Figure 6.1). All results are expressed as mean and standard error (SE) for variables with normal

distribution. A *p* value of < .05 was adopted. Due to skewness, some values were log transformed (Ln) for analysis. Pearson correlation analysis was used to determine associations between all variables on crude and log transformed values. Spearman's rank order correlation was performed on values that remained skewed after log transformation.

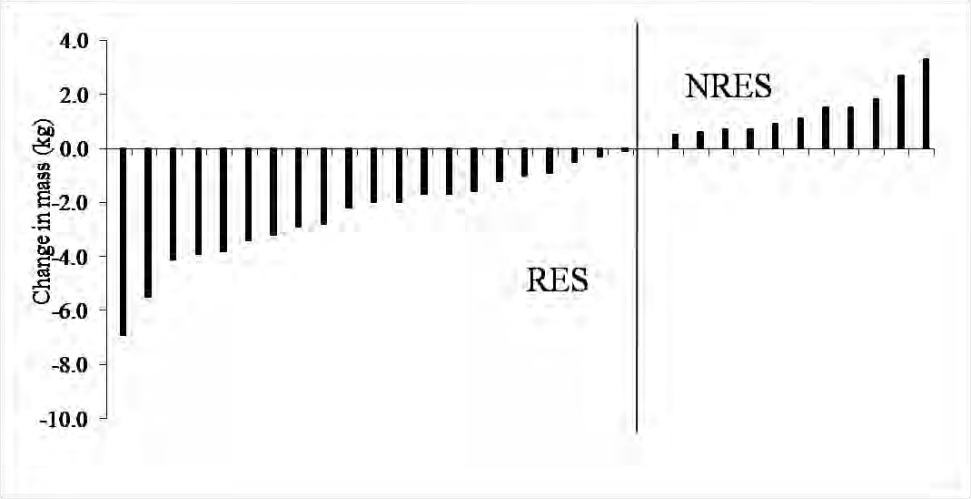


Figure 6.1 Responders and non-responders based on the change in mass (≤ 1%) following FEM.

6.3 Results

6.3.1 Overview

The following section reports results obtained from the FEM trial involving responders and non-responders to the intervention. Participant characteristics and baseline data pre-intervention are initially reported with changes post-intervention following. Changes in body composition, lipids, and blood pressure following the trial are reported. The differences between groups at baseline are noted as are the responses to HIIE measured through HR and RPE. Diet information is provided with information regarding adherence to the Mediet and changes in food intake. Finally, a summary of all the results is presented. Only intervention women were compared and adherence to the intervention was 100% for all exercise sessions. Participants were allowed to make up a

session if one was missed leading to high compliance. The MDS verified conformity to the Mediet and a fish oil intake form was signed by all participants.

6.3.2 Participant Characteristics

The participants (N = 34) in the study were young, apparently healthy overweight women from the University and surrounding communities. The group consisted of Anglo women (n = 15), Chinese (Indonesian Chinese, Thai Chinese, and Malaysian Chinese) women (n = 10), Korean women (n = 2), Indian women (n = 3), Phillipino women (n = 2), and other (Portuguese and Mauritus) women (n = 2).

The RES and NRES groups consisted of Anglo women (RES n = 9, NRES n = 6), Chinese (Indonesian Chinese, Thai Chinese, and Malaysian Chinese) women (RES n = 5, NRES n = 5), Korean women (RES n = 2, NRES n = 0), Indian women (RES n = 3, NRES n = 0), Phillipino women (RES n = 1, NRES n = 1), and other (Portuguese and Mauritus) women (RES n = 0, NRES n = 2).

6.3.3 Baseline Variables

Table 6.1 shows participants' age, lipid, DEXA, fitness, and metabolic parameters. The NRES had significantly higher WTHR, $t(32) = -2.07$, $p < .05$, HDL, $t(32) = 3.05$, $p < .05$, and resting respiratory quotient (RQ), $t(32) = 2.70$, $p < .05$ (see Table 6.1). A lower SBP, $t(32) = -2.28$, $p < .05$, and resting HR, $t(32) = -2.51$, $p < .05$, was also seen in the RES women compared to NRES (see Table 6.1).

Table 6.1 Characteristics of Responders and Non-Responders

	Responders RES (n =21)				Non-Responders NRES (n =13)			
	PRE		POST		PRE		POST	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Age (years)	24	1			22	1		
Mass (kg)	74.4	2.8	71.6††	2.8	70.5	3.3	71.7	3.3
Height (cm)	162.2	1.8			162.5	1.9		
BMI (kg/m^2)	27.8	0.7	26.7††	0.7	26.4	0.8	26.8	0.8
$\dot{V}O_{2peak}$ (L/min)	1.9	0.1	2.2	0.1	1.9	0.1	2.2	0.1
$\dot{V}O_{2peak}$ (ml/kg/min)	25.6	0.8	30.2	0.8	27.6	0.9	31.4	1.0
SBP (mmHg)	116	2	107†	2	109*	2	107	2
DBP (mmHg)	67	2	63	2	62	3	59	3
Respiratory quotient	0.87	0.01	0.88	0.01	0.93*	0.02	0.92	0.01
Resting heart rate (bpm)	70	2	67	2	64*	1	62	2
Peak heart rate (bpm)	183	2	184	2	183	2	184	2
Pulse pressure (mmHg)	49.4	1.6	44.2†	1.5	46.5	1.6	47.6	2.5
Mean arterial pressure (mmHg)	83.3	1.7	77.7	1.5	77.8	2.6	75.3	2.3
Resting metabolic rate	1563	52	1649	61	1554	72	1575	48
Fat mass (kg)	31.5	1.7	28.8††	1.7	28.6	2.4	29.2	2.5
Fat free mass (kg)	40.3	1.3	40.2	1.2	39.3	1.4	39.8	1.3
Body fat by DEXA (%)	43.5	0.9	41.2††	1.0	41.6	1.5	41.7	1.6
Waist circumference (cm)	80.6	1.8	76.4††	1.7	75.5	2.3	74.1	2.2
Hip circumference (cm)	105.1	1.5	100.1†	1.5	102.6	3.0	101.1	2.4
Waist-to-height-ratio	0.50	0.01	0.47††	0.01	0.46*	0.01	0.46	0.01
Fasting insulin (µIU/ml)	17.5	2.0	14.1	1.0	20.3	5.1	18.1	5.2
HOMA-IR	2.3	0.3	1.7	0.1	2.3	0.5	2.1	0.5
Beta cell dysfunction	174.9	14.8	138.6	7.1	189.7	26.5	160.6	21.1
Insulin sensitivity	56.5	7.2	63.6	4.4	65.7	11.0	70.1	10.6
Fasting glucose (mmol/L)	4.9	0.1	5.0	0.1	4.7	0.1	4.8	0.1
Fasting interleukin-6 (pg/mL)	1.6	0.2	1.1	0.2	1.5	0.3	1.1	0.2
Triglycerol (mmol/L)	0.92	0.07	0.77	0.08	0.82	0.08	0.62	0.05
Total cholesterol (mmol/L)	4.3	0.1	4.3	0.1	4.6	0.2	4.4	0.2
HDL cholesterol (mmol/L)	1.3	0.0	1.2	0.0	1.6*	0.1	1.6†	0.1
LDL cholesterol (mmol/L)	2.7	0.1	2.7	0.2	2.8	0.2	2.5	0.2
VLDL cholesterol (mmol/L)	0.42	0.03	0.35	0.03	0.37	0.04	0.28	0.02

Mean and standard error (SE)
* Significant difference ($p < 0.05$) between RES and NRES at baseline
†† Significant difference ($p < 0.001$) between RES and NRES following FEM
† Significant difference ($p < 0.05$) between RES and NRES following FEM
Note: Body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), homeostasis model assessment of insulin resistance (HOMA-IR), high density lipoproteins (HDL), estimated low density lipoproteins (LDL), and estimated very low density lipoproteins (VLDL)

6.3.4 Body Composition in Responders and Non-Responders

Significant differences between the RES and NRES women were seen post intervention for the majority of body composition indices. The RES had a greater change in the following variables: mass, $t(31) = 7.78$, $p < .001$ (see Figure 6.2 A), BF%, $t(32) = 3.28$, $p < .05$, BMI, $t(31) = 7.75$, $p < .001$, WC, $t(32) = 4.01$ $p < .001$ (see Figure 6.2 B), HC, $t(32) = 2.80$, $p < .05$, WHTR, $t(32) = 4.28$, $p < .001$, and FM, $t(32) = 5.14$, $p < .001$ (see Figure 6.2 C). FFM was not significantly different between RES and NRES following the trial.

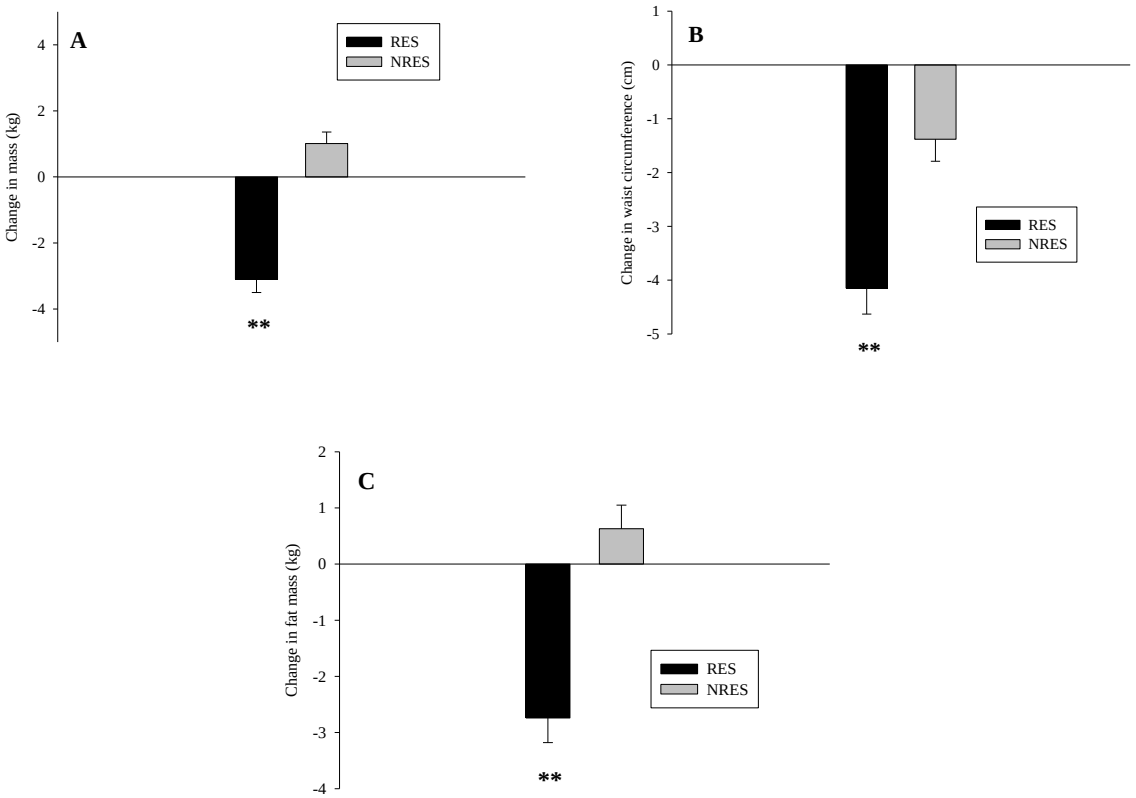


Figure 6.2 (A-C) Response in body composition measures (mass, waist circumference, and fat mass) in responders and non-responders (**, $p < .001$).

6.3.5 Aerobic Power in Responders and Non-Responders

There was no significant difference in aerobic power (see Figure 6.3) before or after the intervention for both groups of women.

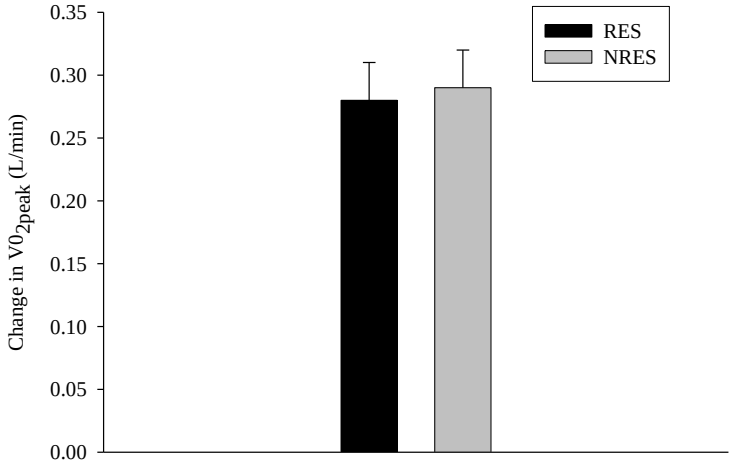


Figure 6.3 Response in $\dot{V}O_{2peak}$ in responders and non-responders.

6.3.6 Blood Markers Response in Responders and Non-Responders Following the Intervention

No blood markers (see Figure 6.4 A and B) measured were significantly different between the NRES and RES women except lipids. Despite the difference in the reduction in insulin levels (RES 24%, NRES 8%) it was not significant, $t(26) = 1.40$, $p = .17$.

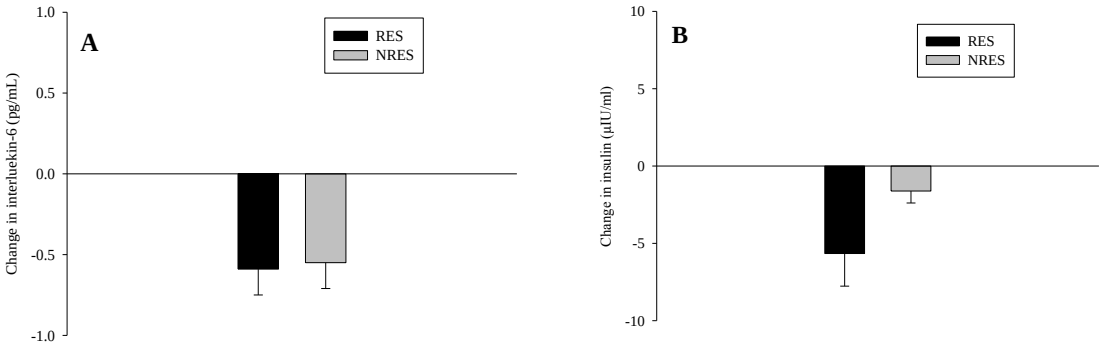


Figure 6.4 (A, B) Response in fasting plasma IL-6 and insulin in responders and non-responders.

A significant relationship existed between the change in insulin after FEM and the change in glucose, $r = .42$, $p < .05$.

6.3.7 Autonomic Function Response in Responders and Non-Responders following the Intervention

Systolic blood pressure, $t(32) = 2.43$, $p < .05$, decreased in both groups with a significantly greater change in the RES women (see Figure 6.5).

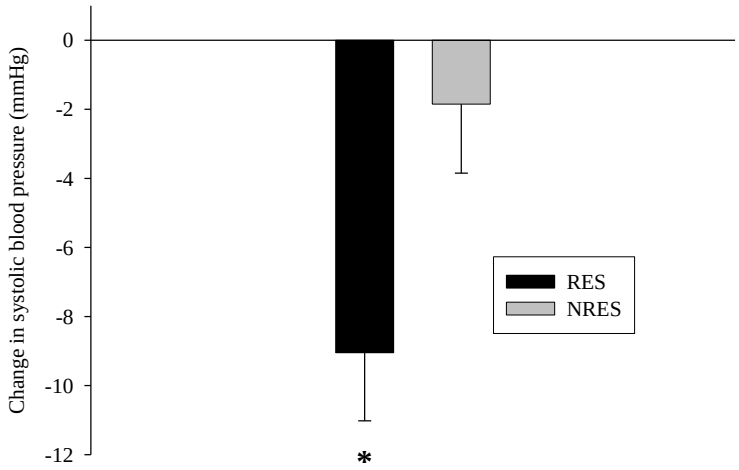


Figure 6.5 Response in SBP in responders and non-responders (*, $p < .05$).

There was a significant correlation between the changes in SBP, $r = .59$, $p < .001$, and DBP, $r = .63$, $p < .001$, and insulin after FEM.

6.3.8 Heart Rate Response in Responders and Non-Responders following the Intervention

Baseline resting HR was significantly higher in the RES women compared to NRES. There was no significant difference between the HR_{peak} and mean HR across all the HIIE sessions for both groups of women. The mean HR was 157 bpm for NRES and 155 bpm for RES which corresponds to 85% of their HR_{peak}. Following the trial there was no significant difference in HR_{peak} or resting HR.

6.3.9 Respiratory Exchange Ratio during the Aerobic Power Test in Responders and Non-Responders following the Intervention

During the post fitness test both NRES, $F(3, 37) = 24.62$, $p < .001$, and RES, $F(3, 64) = 53.77$, $p < .001$, (see Figure 6.6 A) had a lower RER across time (see Figure 6.6 B) and the RER was lower in RES throughout the test compared to NRES with a significantly lower RER at 45 W .

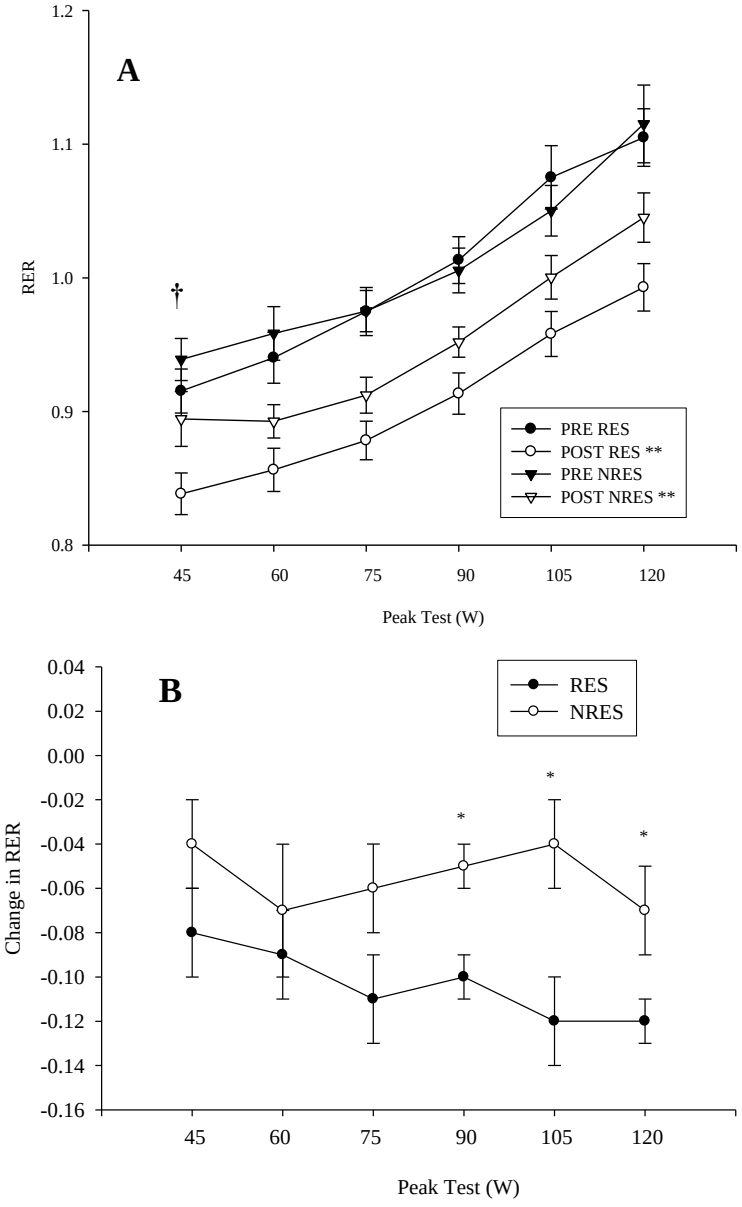


Figure 6.6 (A, B) RER during the peak test in responders and non-responders (*, $p < .05$, and **, $p < .001$ for differences across time, and †, $p < .05$, for the difference at a certain intensity).

6.3.10 Dietary Intake Changes in Responders and Non-Responders

6.3.10.1 Dietary intake changes in responders and non-responders following the intervention

Dietary intake between the groups was similar pre- and post intervention (see Table 6.2). A positive trend was found in iron intake with the NRES eating more iron, $t(31) = 1.94$, $p = .06$, after the trial.

Table 6.2 Responder's and Non-Responder's Dietary Intake

	Responders (n =21)				Non-Responders (n =13)			
	PRE		POST		PRE		POST	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Total kJ	7001	409	5904	305	6729	304	5759	414
PRO grams	69.0	4.9	65.4	4.1	66.9	5.3	64.0	6.3
FAT grams	63.0	5.6	46.1	5.3	55.6	3.5	46.3	3.9
SATFAT grams	22.8	2.2	14.9	1.6	20.2	1.6	15.5	2.3
POLYFAT grams	9.7	0.9	7.7	0.8	9.4	1.0	9.8	1.6
MONOFAT grams	24.6	2.6	18.6	3.2	21.2	1.3	15.8	1.8
CHOL mg	263.5	31.3	210.5	30.1	190.4	28.3	164.0	27.2
CHO grams	185.6	12.1	164.9	9.7	195.0	8.8	161.9	17.2
SUGAR grams	74.7	7.5	66.6	5.5	85.4	9.4	69.2	10.4
FIBRE grams	19.9	1.5	22.1	1.7	19.4	1.2	24.2	3.3
IRON mg	10.7	0.6	10.4	0.7	9.3	0.8	11.8	1.8
PRO percent	17.2	0.8	19.6	0.9	17.0	1.0	19.9	1.8
FAT percent	33.3	1.6	28.7	2.2	31.1	1.3	30.5	2.2
CHO percent	46.2	2.1	49.1	2.5	50.3	1.7	48.9	2.5

Mean and standard error (SE)

Baseline fat intake expressed as a percentage of the diet and baseline insulin were significantly correlated, $r = .45$, $p < .05$, (see Figure 6.7) in all women.

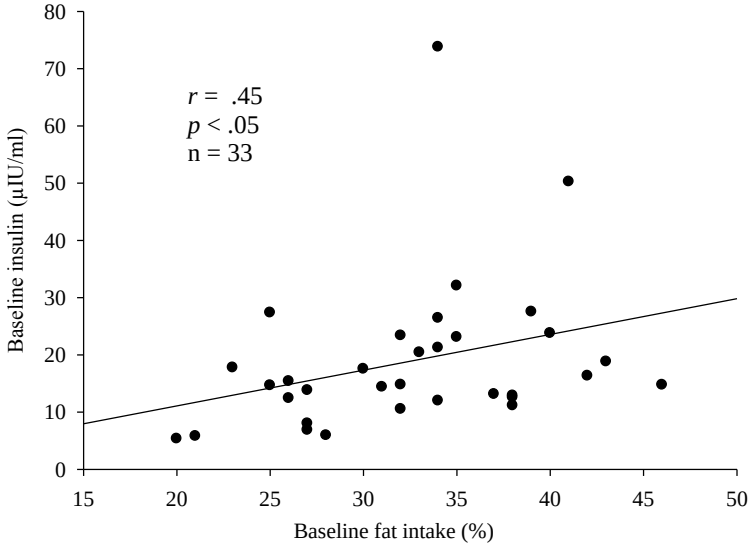


Figure 6.7 Correlation between the baseline insulin and baseline fat intake in responders and non-responders.

The amount of cholesterol ingested at baseline was correlated to the change in insulin, $r = -.39$, $p < .05$, (see Figure 6.8) after FEM.

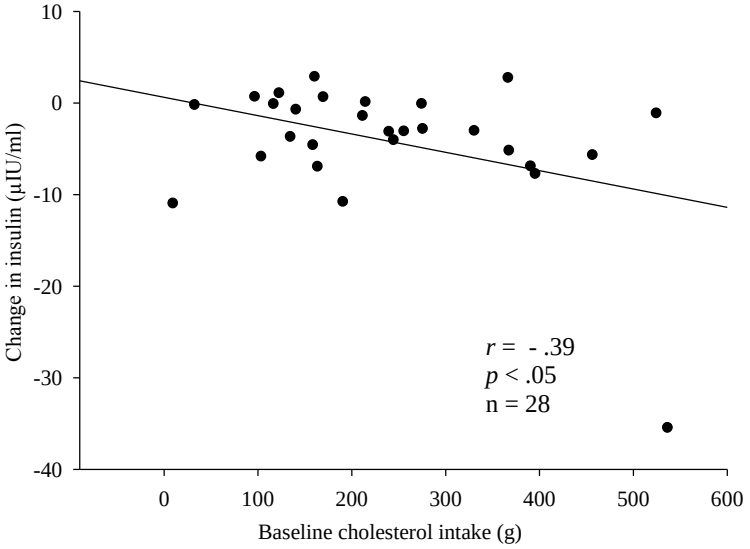


Figure 6.8 Correlation between the change in insulin and the change in cholesterol in responders and non-responders.

6.3.10.2 Mediet score in responders and non-responders following the intervention

A lower consumption of meat approached significance $t(32) = 2.01$, $p = .05$, in the RES compared to NRES women after twelve weeks. No significant differences were apparent for all the other components measured within the MDS (see Table 6.3). Interestingly, cereal and ethanol consumption increased in the NRES whereas in the

RES they decreased although not significant in response to the intervention (see Table 6.3).

Table 6.3 Responder's and Non-Responder's Mediet Score Components

	Responders (n =21)				Non-Responders (n =12)			
	PRE		POST		PRE		POST	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Cereal (g/day)	213.4	17.7	210.1	17.8	207.9	22.2	229.4	24.3
Dairy (g/day)	120.6	29.9	99.5	21.6	123.5	23.6	110.1	23.7
Ethanol (g/day)	7.2	3.2	5.7	2.6	3.8	2.4	4.5	2.3
Fats (g/day)	4.0	1.4	5.3	1.4	8.0	3.1	7.6	2.1
Fruit and Nut (g/day)	179.6	31.6	236.2	28.8	198.9	38.6	268.0	59.5
Legumes (g/day)	26.6	11.4	34.6	8.2	6.5	3.9	40.2	11.7
Meat (g/day)	135.3	15.4	79.8	12.4	91.1	25.5	80.8	16.1
Vegetable (g/day)	193.7	25.1	236.7	24.1	147.3	24.9	198.0	25.1

Mean and standard error (SE)

The ingestion of legumes at baseline was positively associated with baseline glucose, r = .40, p < .05, and inversely associated with fasting plasma insulin at baseline, r = - .40, p < .05, and the increase in IS, r = .59, p < .001, following the intervention. A reduction in FM following the trial was indicative of a higher MDS post intervention, r = .40, p < .05, (see Figure 6.9), and inversely associated with vegetable, r = - .35, p < .05, and meat, poultry and egg (MPE), r = .39, p < .05, intake.

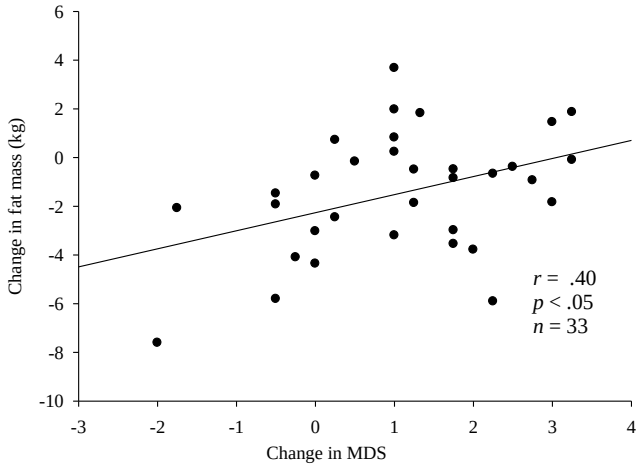


Figure 6.9 Correlation between the change in fat mass and the change in MDS in responders and non-responders.

6.4 Discussion

Following the intervention, all women had reduced their insulin, inflammation, BP, lipids, and a few but not all body composition indices. Mass, FM, and %BF increased in 38% (NRES) of the women involved in Study III (FEM). Improved aerobic fitness and fat oxidation indirectly measured through RER after FEM was recorded in the peak fitness test. The intervention women altered their dietary intake, conforming more to a Mediet, as confirmed through an increase in MDS and a significant reduction in saturated fat. In agreement with Hypothesis 6 (page 9), a 12-week intervention combining HIIE, fish oil ingestion and Mediet improved the metabolic profile but failed to reduce fat mass in 38% of the women.

6.4.1 Baseline Variables

The NRES women had significantly higher WTHR, HDL, RQ, and lower SBP, and resting HR prior to starting the FEM intervention. High RQ levels are an indirect measure of carbohydrate oxidation at rest and have predicted weight loss in the past (Marra, Scalfi, Covino, Esposito-Del Puente, & Contaldo, 1998). An elevated RQ is also associated with weight gain (Flatt, 1995; Marra, Scalfi, Contaldo, & Pasanisi, 2004; Zurlo, Lillioja, Esposito-Del Puente, Nyomba, Raz, Saad et al., 1990), low sympathetic activity (Snitker, Tataranni, & Ravussin, 1998), and is not significantly different based on WHTR, gynoid, or android obesity (Schutz & Tremblay, 1992). The present NRES findings are in accordance with the results from Marra et al. (1998) although their participants were not overweight (BMI of 24.7 kg/m^2). Elevated glycogen stores leading to preferential carbohydrate utilization, high fat intake, and mitochondrial dysfunction in relation to oxidation or physical inactivity promote higher RQ levels (Marra et al., 2004).

Based on the findings from Study II a significant decrease in RQ following one HIIE session and increase in glycerol implies an increase in lipolysis. Although the NRES women didn't reduce fat mass a shift in RER towards greater fat oxidation was seen in the peak test following FEM (see Figure 6.6). Although a more pronounced change was noted in RES women, the NRES women did have a significant reduction in RER (an increase in fat oxidation) following the 12-week intervention.

HDL improvements were noted following 3 months of HIIE in adolescents (Tjonna et al., 2008b) and the baseline values were comparable to the present RES women but lower than that of the NRES women. The same research group investigated MetS patients following the same HIIE protocol and found an increase in HDL post intervention (Tjonna et al., 2008a) although the reported baseline levels were 50 % lower than the baseline levels reported for the FEM RES women and almost two thirds lower than the NRES women. The HDL levels at baseline were significantly elevated in the NRES women and continued to stay elevated after the 12-week intervention compared to RES.

The low resting HR and BP of the NRES suggests low sympathetic tone. For example, low sympathetic tone is associated with low resting HR (Grassi, Vailati, Bertinieri, Seravalle, Stella, Dell'Oro et al., 1998). Sympathetic outflow is altered in healthy populations by modified stroke volume and cardiac output (Charkoudian, Joyner, Johnson, Eisenach, Dietz, & Wallin, 2005) which may be the reason for the lower BP in the NRES. Improvements in vagal balance and sympathetic activity have been found with weight loss after dietary intervention (Ashida et al., 2007) and exercise training (Pober, Braun, & Freedson, 2004).

6.4.2 Response in Body Composition and Resting Metabolic Rate in Responders and Non-Responders following the Intervention

These previously sedentary premenopausal young women were overweight (BMI = 27.1 kg/m^2). Fat mass was reduced by 9% (6% clinically significant) in the RES women with a 4% decrease in mass (Figure 6.2 A-C). The NRES women had an increase in fat mass by 2%, fat free mass increased by 1%, leading to an overall 2% increase in body mass from baseline. The losses presented here for RES (loss of body mass 2.8 kg and 2.7 fat mass kg) are similar to findings involving RES (loss of body mass 2.9 kg and 3.9 fat mass kg) after 15 weeks of HIIE (Trapp, 2006). Although the value to determine responding women in that study was > 1.5 kg body mass lost the participants were of smaller stature with lower body composition values compared to FEM.

Both groups reduced WC, HC, and WHTR although the RES women had significantly larger reductions following the intervention. The lowering of some of the body composition measures despite no fat mass or body mass loss suggests possible adipose tissue and muscle morphological changes (He et al., 2004) after the 12-week regimen. Lack of weight loss but a reduction in waist circumference has been shown following a 14-week exercise program in premenopausal women (Ross et al., 2004).

The same cautions regarding post intervention body composition measurements and the menstrual cycle should be applied. When participants completed the trial, all posttesting was conducted in the days immediately following with a minimum of 24 hours between the last session and the first posttest. Being a 12-week trial, the women were not all measured during the same phase of the menstrual cycle (follicular, luteal, or menstruation) and some variability in water weight gain should be considered.

RMR increased in both groups following FEM although they were not significantly different (Table 5.2) which is similar to findings by Potteiger, et al. (2008) following a 9- month moderate intensity exercise programs. In that study, a increase in

RMR but not resting fat oxidation was seen in overweight women despite reductions in mass and percent body fat (Potteiger, Kirk, Jacobsen, & Donnelly, 2008) similar to FEM. The elevations found following FEM were 5% for RES and 1% for NRES. Despite this small change in RMR for RES, post exercise increases across a 12-week (HIIE) intervention may bring about greater fat oxidation in RES compared to NRES. Other studies using differing exercise modalities have found increases in RMR after exercise. For example, following an acute bout of heavy resistance exercise (90 minute protocol), trained males had an elevated RMR in the 24 hours following (Melby, Scholl, Edwards, & Bullough, 1993). Also following shorter duration moderate intensity resistance training, RMR in the immediate hour following, was elevated (although only slightly) and was not dissimilar to the increase in RMR found after steady state exercise (Melby, Tincknell, & Schmidt, 1992). The elevations in RMR found in the FEM women were due to chronic metabolic rate improvements but the NRES women had less of an increase in RMR which may have impacted on their fat oxidation following each HIIE session, and ultimately, to their overall metabolic rate, leading to lower loses or even gains in fat mass.

6.4.3 Blood Markers and Aerobic Fitness Response in Responders and Non-Responders following the Intervention

Both NRES and RES women had improvements in aerobic power and reductions in insulin and IL-6 with no significant differences between groups. Despite these metabolic changes in the NRES women, fat mass, and body mass increased in this cohort. Fasting insulin decreased in both groups although a more pronounced reduction (with no significant difference) was found in NRES. A reduction in fasting insulin predicted weight loss after a 24-week insulin suppression protocol in obese children (Velasquez-Mieyer, Cowan, Arheart, Buffington, Spencer, Connelly et al., 2003). The

non-responding children had a lower reduction in insulin area under the curve and their food choices and cravings for carbohydrate were less altered than that of the high responders. The authors interpretation of the non-responders focused on the influence of insulin on hyperphagia, which amplifies carbohydrate cravings (Brandes, 1977). A non-significant higher carbohydrate intake was noted in the NRES women compared to RES. The higher carbohydrate intake of the NRES was positively correlated with RQ recorded at baseline.

6.4.4 Autonomic Function Response in Responders and Non-Responders following the Intervention

Similar to the women in the FEM trial, obese middle-aged men and women following 3 months aerobic (60 minutes) exercise, involving 3 sessions per week (De Luis et al., 2008), had reductions in BP although the reductions were only significantly different in the group recording a fat loss (responders). Inflammatory markers were reduced, although not significantly, and fitness only slightly increased without significance in the non-responding obese patients. Thus, minor deviations from baseline in inflammation, hormonal levels, and fitness after the FEM intervention may be the reason for the diminished BP in NRES.

6.4.5 Dietary Intake Changes and the Mediet Score in Responders and Non-Responders following the Intervention

No significant differences in the dietary intake were noted between the RES and NRES women. The consumption of fat expressed as a percent of diet and cholesterol and legumes at baseline all impacted fasting plasma insulin levels at baseline and following the intervention.

The RES compared to NRES women consumed lower amounts of MPE (approaching significance) although they had a similar MDS following FEM. Decreased

FM post intervention was inversely associated with MPE intake at baseline and a higher MDS and greater reduction in MPE consumption was indicative of a lower FM after the trial. A study investigating the dietary intake of rural Chinese adults revealed that the incidence of meat intake was inversely associated with legume, plant, and vegetable intake (Campbell, Parpia, & Chen, 1998) despite having a 10% lower animal product intake than US adults.

The improvement in MDS was greater in the NRES (31%) compared to RES (21%) women probably due to a lower but non-significant MDS at baseline. No significant differences were found for any other MDS components measured.

Interestingly, the elevated cereal and ethanol consumption in conjunction with a lower reduction in meat intake in NRES following the intervention may have influenced the small reduction in BP. An epidemiological study of Greeks ingesting a Mediet found meat, ethanol, and cereal were all inversely associated with BP (Psaltopoulou et al., 2004).

The fats ratio in the RES group increased non-significantly indicating monounsaturated fat consumption increased and saturated fats decreased. The NRES group had a lower fats ratio following the trial due to reductions in both monounsaturated and saturated fats. Despite the reductions in monounsaturated and saturated fats, the polyunsaturated fat intake increased non-significantly.

Previously mentioned (Chapter 5) fasting plasma insulin was inversely related to legume intake at baseline for FEM. The same association within this cohort was found. Interestingly, those women with a higher baseline legume consumption had a greater FM, glucose, and insulin reduction and a larger improvement in MDS after twelve weeks.

6.4.6 Summary

Despite reductions in insulin, inflammation, waist circumference, RER during exercise, BP, and an increase in aerobic power, the NRES women gained body mass and fat mass following the FEM intervention. However, reductions (23-44%) in all cause mortality with increases in fitness, regardless of weight loss, has been shown (Gaesser, 1999). NRES women possessed at baseline, significantly lower BP and HR inferring dysfunction of the HPA. Baseline RQ compared to RES was elevated and biased toward carbohydrate oxidation. Similar to the outcomes involving the FEM women, dietary intake was related to fat loss and insulin changes in these women.

The metabolically healthy obese (MHO) phenotype was found in 20-30% of obese populations and is shown to be similar to lean women in terms of metabolic profile but not body composition (Karelis et al., 2005). A study comparing MHO Korean women compared to women of a similar body composition but abnormal metabolic lipids and inflammatory markers found that MHO women lost weight after completing a dietary intervention but they did not have the same improvements in metabolic and inflammatory states (Shin, Hyun, Kim, Kim, Jang, & Lee, 2006). Although the response was not the same (no change in inflammatory in MHO versus reductions in NRES) in the present study involving RES and NRES women, the phenotype concept may be a factor to explain why some women did not respond following FEM. A point worth mentioning regarding the MHO intervention is that the authors noted that the weight loss due to the dietary intervention (without exercise) in the MHO women did not affect their inflammatory or lipid profile. The NRES and RES women all had reductions in inflammation and lipids following FEM due to the combined effect of the fish oil, exercise, and Mediet.

The duration of the program (12 weeks) may have brought about individual responses in body composition. A longer duration intervention may result in greater reductions in mass or fat mass eventually in NRES women. More research focusing on the individual response to fat loss is needed to further investigate why not all women respond when adhering to multi-component lifestyle intervention.

CHAPTER 7 GENERAL CONCLUSIONS AND FUTURE RESEARCH

Three studies were conducted to investigate the effects of acute and chronic HIIE and Mediet on MetS markers in young premenopausal women. In Study I, early markers of MetS in sixty six young, premenopausal women were examined. A second aim was to investigate ethnic influences on MetS by comparing hormonal and metabolic markers of Europid and Chinese Australians. Study II investigated the hormonal and protein response of young women two hours after HIIE and SSE. Women were also divided into high and low fasting insulin groups to determine if hyperinsulinaemia influenced the hormonal and metabolic response to HIIE and SSE. Finally, Study III was a multifactorial, randomized control intervention incorporating fish oil, HIIE, and Mediet and was designed to examine the body composition and metabolic response of young overweight women to a 12-week intervention.

This general discussion will firstly summarise the major findings, then outline the implications and importance of the results, and finally will make overall conclusions and suggestions for future research.

7.1 Study I

The major findings of Study I were that elevated levels of fasting insulin in women were accompanied by elevated protein consumption, low aerobic fitness, and high CRP levels. Body composition did not significantly contribute to any of these variables. Thus, a westernized diet high in protein and a physically inactive lifestyle may predispose young women to MetS. Both groups consumed high dietary protein relative to their body mass although the Chinese women possessed the higher protein intake. Although the fasting insulin level of the Chinese women was significantly higher than that of the Europid women their CRP levels was significantly lower. Also, it has been previously reported that Chinese women living in the United Kingdom showed a

higher prevalence of glucose intolerance compared with Europeans, despite possessing a significantly lower BMI (Unwin et al., 1997). Similar to these findings the Chinese compared to Europid women in the present study also possessed a lower BMI. Thus, this ethnic group might be more prone to develop metabolic abnormalities at a lower level of BMI. Therefore, it appears that hyperinsulinemia can occur in Australian Chinese women without the presence of low grade inflammation or a high BMI.

For both groups of women fasting insulin was the earliest marker of MetS. Thus, inactive females of both Europid and Asian descent who were physically unfit and consumed a high level of dietary protein exhibited insulin resistance at a young age (21.2±0.3 years). Genetic and environmental factors including "Westernisation" of diets (e.g., increase in consumptions of animal protein, animal fats, and processed carbohydrates) and lifestyle factors (e.g., less physical activity through increased use of automobiles, etc) might play an important role in bringing out this susceptibility to metabolic disorders.

The results of Study I indicate that hyperinsulinemia is common in young women who are inactive and who consume a westernized diet. Results also indicate that possessing a Chinese ethnicity and an unhealthy lifestyle may further enhance the risk of MetS development. The implications of these results are that young inactive women, regardless of ethnicity, should be targeted for engagement in physical activity and healthy eating.

7.2 Study II

Study II examined the acute effect of HIIE and SSE on insulin and metabolic response. The women were also divided by baseline fasting plasma insulin (> 9.98 μIU/ml) into high insulin and low insulin groups. It was found that HIIE resulted in a small decrease in insulin and HOMA-IR in the immediate two hours post exercise,

whereas insulin levels were slightly increased after SSE. RQ significantly decreased following both types of exercise regardless of hyperinsulinemic status suggesting enhanced fat oxidation and lipolysis after exercise. An interesting finding was the blunted fasting human growth hormone response in the hyperinsulinemic women and the significant increase in ACTH of these women following one bout of SSE and HIIE.

The results of Study II indicate that exercise of an anaerobic or aerobic nature resulted in enhanced fat oxidation in the two-hour post exercise period. Results also indicate that possessing hyperinsulinemia was associated with blunted growth hormone and elevated ACTH response at baseline and after exercise. The implications of these results are that moderately hard exercise is likely to be important for fat reduction because of the enhanced post-exercise fat oxidation that occurred. That HIIE was only performed for 20 minutes compared to 30 minutes of SSE suggests that HIIE may be a more efficient form of exercise for weight reduction for those with busy schedules.

7.2 Study III

The major findings from Study III were a significant reduction in insulin, IL-6, autonomic function (BP, MAP, PP, and power frequency), lipids, and body composition, and a significant improvement in cardio-respiratory fitness and fat oxidation rates following the 12-week lifestyle intervention. Therefore, an intervention incorporating fish oil consumption, HIIE, and Mediet significantly reduced body fat, fasting insulin, inflammatory markers, and some blood lipids. Both aerobic power and anaerobic capacity also were significantly improved after the intervention. Mediet adherence increased significantly and was positively associated with reduction in insulin and body composition. Previous research has confirmed that HIIE, Mediet, and fish oil consumption all have positive effects on MetS markers. However, this study appears to be the first to examine the synergy of these three interventions.

The results of Study III indicate that a 12-week multi-factorial intervention can significantly reduce a number of MetS markers and improve metabolic health. The high adherence rates to this randomized controlled trial also confirms that this form of intervention can be successfully carried out by overweight, young women. The implications of these results are that a number of Mets markers can be reversed with participation in an exercise-based lifestyle intervention.

Despite improvements in insulin, IL-6, autonomic function, lipids cardio-respiratory fitness, fat oxidation, and waist circumference, fat mass either increased or did not change following the intervention for 38% of the women. The major differences between those women who lost fat and those that did not was that the non-responders possessed significantly lower SBP, lower resting HR, and a higher RQ. Although RQ did not significantly change after the intervention, exercise RER was lowered. The lower RER suggests that these women had an increased ability to oxidise more fat during aerobic exercise. Thus, for these overweight women, enhanced oxidation at rest may only occur with more exposure to the intervention.

7.4 Limitations and Suggestions for Future Research

The limitations of Study I were that the great majority of participants were University students. Thus, results cannot be generalized to young women in the general community. For example, these students are likely to possess greater academic achievement and more years studying in tertiary institutions. A study that included non-students from a variety of settings would help determine if the prevalent hyperinsulinemia within Study I is also present in the non-student population. The results from Study I should be interpreted with caution there was a relatively small sample size. That all women lived in an urban Australian environment restricts the application of these results to urban dwellers. Lastly, the use of fasting plasma insulin

and HOMA-IR as measures of insulin resistance are generally accepted but are not as definitive as the gold standard, euglycemic clamp. Studies using greater number of participants from both country and urban settings encompassing the euglycemic clamp technique are needed to confirm these findings.

In Study II untrained women performed a HIIE exercise bout for the first time. The hormonal and metabolic response to HIIE is likely to be different after exposure to multiple HIIE sessions. The large variability amongst hormones and protein before and after exercise may be due to individual differences in factors such as training status, pulsatile secretion and muscle fibre type. Study II examined the effects of HIIE and SSE on metabolic and hormonal varaiables and did not attempt to identify underlying mechanisms. Thus, muscle biopsies and more invasive techniques should be utilized to further elucidate these mechanisms.

Another possible limitation for Study II relies in the day of exercise baseline measurements. Resting RQ was measured in the minutes prior to exercise, thirty minutes following cannulation and resting in the recline chair and may not reflect a true resting condition. The post exercise RQ values may not reflect true substrate oxidation due to the restoration of H+ ions generated during HIIE, more CO2 is blown off during exercise and during early recovery from exercise.

Study III's design (a multifactorial synergistic intervention) does not allow identification of the contribution of each of the individual components of the intervention (HIIE, Mediet, fish oil). Thus, it is not possible to separately identify the impact of each component on metabolic health. However, previous research has shown that each individual component in the FEM trial positively influences metabolic and cardiovascular health. Consequently, the results of Study III represent the synergistic effect of combining fish oil, Mediet, and HIIE. Study III also examined the effects of

the lifestyle intervention and did not attempt to identify underlying mechanisms. Therefore, muscle biopsies, CT scans, a metabolic kitchen, and use of more invasive techniques should be utilized to further elucidate these mechanisms. The women who participanted in Study III did not meet the criteria for MetS but were considered overweight based on BMI. Study III was focused primarily on risk markers associated with MetS and the metabolic health response to a synergistic lifestyle intervention in young overweight women, had the participants been more cardiometabolically impaired the results may have been greater.

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APPENDICES

Appendix A
(Study I, II, and III UNSW consent forms)

THE UNIVERSITY OF NEW SOUTH WALES

Approval No (03218)

THE UNIVERSITY OF NEW SOUTH WALES

PARTICIPANT INFORMATION STATEMENT AND CONSENT FORM

Training Intensity Study

You, _____ (name of participant) are invited to participate in a training study examining the effects of different training loads on fat loss. We, Associate Professor S. Boutcher and E. Gail Trapp, hope to determine the optimal exercise protocol for inducing fat loss. You were selected as a possible participant in this study because you are a female under the age of 30 years and not currently exercising.

If you decide to participate, we will get you to exercise on a stationary bicycle before you commence training and after 15 weeks of training. On each of these occasions, you will be breathing through a mouthpiece so that we can measure the amount of oxygen you breathe in and the amount of carbon dioxide you breathe out. A small needle (venous catheter) will be placed in a vein in the back of your hand or forearm so that we can take a small amount of blood for testing at regular intervals. A heart rate monitor will be strapped around your chest to measure heart rate and your blood pressure will be taken regularly. Before the first test and at the end of the training period, body composition measures will be taken. Body composition measures include DXA, bioimpedance, skinfold, girth, mass and height measures. During the initial and final tests you will be asked to cycle at ever increasing workloads until you reach your maximum exercise capacity. This research study involves exposure to a very small amount of radiation. As part of every day living every one is exposed to naturally occurring background radiation and receives a dose of about 2-3 milliSieverts (mSv) each year. The effective dose from this study is about 0.001 mSv. At this dose level no harmful effects of radiation have been demonstrated and the risk is negligible.

Have you have participated in other research studies involving extra x-rays or nuclear medicine tests that were not part of your normal treatment in the last five years? If YES please inform the study coordinator of the details of these involvements are there is a radiation dose limit for volunteers of 10 mSv per five year period.

The total time involvement for you for the testing procedures should be about three to four hours. This will involve two separate sessions of approximately one to two hours at the University. You will also be asked to visit St. Vincent's Hospital Nuclear Medicine Department for DXA scans which will give us information about your body composition and bone density.

There may be some discomfort associated with the exercise if you are unaccustomed to regular exercise but this is likely to be minimal and you will recover rapidly. If you are not used to bicycling you may find you have delayed onset muscle soreness for a day or two after the exercise bout, but this resolves quickly. There should be little discomfort associated with the insertion and/or withdrawal of the needle and we have found little discomfort to be associated with exercising with a needle inserted in the hand or forearm. Most individuals rapidly adjust to breathing through a mouthpiece. DXA measures involve lying on a bed while the machine scans your body. There is no discomfort associated with this procedure.

The training will involve three to four sessions per week of cycle training. There will be two training groups and one control group, one of which you will be assigned to on the toss of a coin (random selection). One training regime will involve high intensity, intermittent work periods separated by low intensity rest periods with a work to rest ratio of 8s:12s. The total exercise time per session will start at 20 min. and progress over the training period to a total of 40 min. The other training regime involves moderate intensity continuous exercise that will start at 20 minutes per session and progress to a total of 40 min. These time allocations include a 10 minute warm up. It is suggested that after you have completed the session you do five minutes of cool down followed by stretching to reduce the likelihood of post exercise soreness. Please do not do any additional physical activity (other than your normal daily activities) for the period of the study. Subjects allocated to the control group will be asked to maintain their normal daily activity patterns until after the study is complete, at which time we will give you an exercise program if you so desire.

You will also be asked to complete a food intake diary in which you record the quantity and type of food you eat each day. Random days will be selected for this purpose. We ask that you do not change your normal food intake over the period of this study to ensure that any changes are a result of the exercise program and not because you have changed your eating habits.

There are few risks associated with exercise for adult females below the age of 30 years. The most likely outcome is post exercise soreness but this may not occur. There is a small risk of bruising associated with the withdrawal of blood but all care will be taken to ensure this does not happen. Radiation exposure associated with DXA is limited.

Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission or except as required by law. If you give us your permission by signing this document, we plan to publish the results in scientific journals. In any publication, information will be provided in such a way that you cannot be identified.

Complaints may be directed to the Ethics Secretariat, The University of New South Wales, SYDNEY 2052 AUSTRALIA (phone 9385 4234, fax 9385 6648, email ethics.sec@unsw.edu.au).

Your decision whether or not to participate will not prejudice your future relations with The University of New South Wales. If you decide to participate, you are free to withdraw your consent and to discontinue participation at any time without prejudice.

If you have any questions, please feel free to ask us. If you have any additional questions later, Gail Trapp on 0403 336 488 or email (etrapp@bigpond.net.au), will be happy to answer them.

You will be given a copy of this form to keep.

THE UNIVERSITY OF NEW SOUTH WALES

PARTICIPANT INFORMATION STATEMENT AND CONSENT FORM (continued)
Training Intensity Study

You are making a decision whether or not to participate. Your signature indicates that, having read the information provided above, you have decided to participate.

……	**……..**
Signature of Research Participant	Signature of Witness
……	**……..**
(Please PRINT name)	(Please PRINT name)
……	**……..**
Date	Nature of Witness

……
Signature(s) of Investigator(s)

……..
Please PRINT Name

REVOCATION OF CONSENT

Training Intensity Study

I hereby wish to **WITHDRAW** my consent to participate in the research proposal described above and understand that such withdrawal **WILL NOT** jeopardise any treatment or my relationship with The University of New South Wales.

....

Signature Date

.....

Please PRINT Name

The section for Revocation of Consent should be forwarded to:

Associate Professor Steve Boutcher
School of Physiology and Pharmacology
Faculty of Medicine
UNSW
Kensington NSW 2052

THE UNIVERSITY OF NEW SOUTH WALES

Approval No (HREC 05238)

THE UNIVERSITY OF NEW SOUTH WALES

PARTICIPANT INFORMATION STATEMENT AND CONSENT FORM

Exercise Intensity Study

You, _____ (name of participant) are invited to participate in an exercise study examining the effects of high intensity short interval exercise on fat loss. We, Associate Professor S. Boutcher and Sarah Dien, hope to determine the optimal exercise protocol for inducing fat loss. You were selected as a possible participant in this study because you are a female under the age of 30 years and currently exercising minimally.

If you decide to participate, we will get you to exercise on a stationary bicycle for three sessions in total. On each of these occasions, you will be breathing through a mouthpiece/mask so that we can measure the amount of oxygen you breathe in and the amount of carbon dioxide you breathe out. A small needle (venous catheter) will be placed in a vein in the back of your hand or forearm so that we can take a small amount of blood for testing at regular intervals. A heart rate monitor will be strapped around your chest to measure heart rate and your blood pressure will be taken before you start the study. Before the first test, body composition measures will be taken. Body composition measures include bioimpedance, skinfold, girth, mass and height measures. We will also look at blood flow and arterial stiffness before and after exercise. During the initial and test you will be asked to cycle at ever increasing workloads until you reach your maximum exercise capacity.

The total time involvement for you for the testing procedures should be about three hours or less for each session. This will involve three separate sessions of approximately three hours or less at the University.

There may be some discomfort associated with the exercise if you are unaccustomed to regular exercise but this is likely to be minimal and you will recover rapidly. If you are not used to bicycling you may find you have delayed onset muscle soreness for a day or two after the exercise bout, but this resolves quickly. There should be little discomfort associated with the insertion and/or withdrawal of the catheter and we have found little discomfort to be associated with exercising and a catheter inserted in the hand or forearm. Most individuals rapidly adjust to breathing through a mouthpiece/mask. During the first session you will be orientated on the stationary bike and mask/mouthpiece.

One exercise regime will involve high intensity, intermittent work periods separated by low intensity rest periods with a work to rest ratio of 8s:12s. The total exercise time for that session is 20 minutes. The other exercise regime involves moderate intensity continuous exercise lasting for 30 minutes. These time allocations do not include a 10 minute warm up. It is suggested that after you have completed the session you do five minutes of cool down followed by stretching to reduce the likelihood of post exercise soreness. Please do not do any additional physical activity (other than your normal daily activities) for the period of the study. Following both exercise sessions, we would like to monitor you for two hours to see what happens to your breathing pattern, hormones and other metabolic parameters associated with exercise.

You will also be asked to complete a food intake diary in which you record the quantity and type of food you eat each day. Random days will be selected for this purpose. We ask that you do not change your normal food intake over the period of this study to ensure that any changes are a result of the exercise program and not because you have changed your eating habits.

There are few risks associated with exercise for adult females below the age of 30 years. The most likely outcome is post exercise soreness but this may not occur. There is a small risk of bruising associated with the withdrawal of blood but all care will be taken to ensure this does not happen.

Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission or except as required by law. If you give us your permission by signing this document, we plan to publish the results in scientific journals. In any publication, information will be provided in such a way that you cannot be identified.

Complaints may be directed to the Ethics Secretariat, The University of New South Wales, SYDNEY 2052 AUSTRALIA (phone 9385 4234, fax 9385 6648, email ethics.sec@unsw.edu.au).

Your decision whether or not to participate will not prejudice your future relations with The University of New South Wales. If you decide to participate, you are free to withdraw your consent and to discontinue participation at any time without prejudice.

If you have any questions, please feel free to ask us. For questions please contact Sarah Dien, at sarah.dien@student.unsw.edu.au or call her at 0425237612.

You will be given a copy of this form to keep.

THE UNIVERSITY OF NEW SOUTH WALES

PARTICIPANT INFORMATION STATEMENT AND CONSENT FORM (continued)
Exercise Intensity Study

You are making a decision whether or not to participate. Your signature indicates that, having read the information provided above, you have decided to participate.

……	**……..**
Signature of Research Participant	Signature of Witness
……	**……..**
(Please PRINT name)	(Please PRINT name)
……	**……..**
Date	Nature of Witness

……
Signature(s) of Investigator(s)

……..
Please PRINT Name

REVOCATION OF CONSENT
Exercise Intensity Study

I hereby wish to **WITHDRAW** my consent to participate in the research proposal described above and understand that such withdrawal **WILL NOT** jeopardise any treatment or my relationship with The University of New South Wales.

....
Signature Date

.....
Please PRINT Name

The section for Revocation of Consent should be forwarded to:
Associate Professor Steve Boutcher
School of Medical Sciences
Faculty of Medicine
UNSW
Kensington NSW 2052

THE UNIVERSITY OF NEW SOUTH WALES

Approval No (HREC 06293)

THE UNIVERSITY OF NEW SOUTH WALES

PARTICIPANT INFORMATION STATEMENT AND CONSENT FORM

Exercise Intensity Study

You, _____ (name of participant) are invited to participate in an lifestyle change study examining the effects of high intensity short interval exercise training and lifestyle change on fat loss. We, Associate Professor S. Boutcher and Sarah Dien, hope to determine the optimal lifestyle protocol for inducing fat loss. You were selected as a possible participant in this study because you are a female under the age of 35 years and not currently exercising.

If you decide to participate, we will get you to exercise on a stationary bicycle for 36 sessions in total lasting 12 weeks. On the first and last of these occasions, you will be breathing through a mouthpiece so that we can measure the amount of oxygen you breathe in and the amount of carbon dioxide you breathe out. During the initial and final tests you will be asked to cycle at ever increasing workloads until you reach your maximum exercise capacity. A small needle will be placed in a vein in the back of your hand or forearm so that we can take a small amount of fasting blood for testing. A heart rate monitor will be strapped around your chest to measure heart rate. The first and last sessions in the lab will include the following; girths of the waist and hips, a fasting blood sample and resting metabolic rate measurements (the biochemical processes of your body at rest) along with a visit to the study nutritionist. At these sessions we will monitor your muscle blood flow through non-invasive measurements and we will look at how stiff your arteries are using a sensor to detect your pulse in the wrist. We will also examine your blood pressure. Two sessions will involve obtaining a dual-energy x-ray absorptiometry (DEXA) scan off site of the University campus. This scan will measure percent body fat.

The time involvement for you for the testing procedures should be about 35 minutes for each session three times a week for exercise, not including the initial and final sessions, the nutritionist and blood sample sessions or the DEXA sessions. The initial and final sessions should be about one hour, the DEXA sessions will take about 20 minutes and the nutritionist/fasting blood sessions should take about 15 minutes and will happen at three other times throughout the 12 weeks of training.

The exercise regime will involve high intensity, intermittent work periods separated by low intensity rest periods. The session includes short sprints for 8 seconds followed by 12 seconds of turning over the pedals and resting. The total exercise time for that session is 20 minutes. Please do not do any additional physical activity (other than your normal daily activities) for the period of the study.

You will also be asked to complete a food intake diary in which you record the quantity and type of food you eat each day. Random days will be selected for this purpose. Our nutritionist will go over all the diet details with you on your initial visit.

We will be randomly assigning a fish oil supplement to half the study's subjects. If you receive the supplement you will be taking 3 capsules per day with 600-mg of fish oil in each capsule (total = 1.8 g).

We ask that you inform us of any changes to your study diet, normal daily physical habits, or to your health. **If for some reason you are unable to complete the trial we ask that you return all unused fish oil and diet diary to the research staff.**

There are few risks associated with exercise for adult females below the age of 35 years. The most likely outcome is post exercise soreness but this may not occur. There is a small risk of bruising associated with the insertion of the needle but all care will be taken to ensure this does not happen. In case of a medical emergency we have nominated Karen Gibson MD PhD.

There may be some discomfort associated with the exercise if you are unaccustomed to regular exercise but this is likely to be minimal and you will recover rapidly. If you are not used to cycling you may find you have delayed onset muscle soreness for a day or two after the exercise bout, but this resolves quickly. There should be little discomfort associated with the insertion and/or withdrawal of the needle. There may be some discomfort associated with the mouthpiece although most individuals rapidly adjust. There should be minimal or no discomfort associated with the blood flow or arterial stiffness equipment. There are very few side effects associated with fish oil supplementation. Some side effects with high doses include a fishy taste in the mouth and gastric upset. However, the dosage administered in this study is well below those that have been associated with gastric upset.

Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission or except as required by law. If you give us your permission by signing this document, we plan to publish the results in scientific journals. In any publication, information will be provided in such a way that you cannot be identified.

Complaints may be directed to the Ethics Secretariat, The University of New South Wales, SYDNEY 2052 AUSTRALIA (phone 9385 4234, fax 9385 6648, email ethics.sec@unsw.edu.au).

Your decision whether or not to participate will not prejudice your future relations with The University of New South Wales. If you decide to participate, you are free to withdraw your consent and to discontinue participation at any time without prejudice.

If you have any questions, please feel free to ask us. For questions please contact Sarah Dien at:

Health and Exercise Science
LGO2G Wallace Wurth Building
The University of New South Wales
Sydney, NSW, 2052
Australia
Tel +61-2-9385-8710
Fax +61-2-9385-1551

You will be given a copy of this form to keep.

THE UNIVERSITY OF NEW SOUTH WALES

PARTICIPANT INFORMATION STATEMENT AND CONSENT FORM (continued)
Exercise Intensity Study

You are making a decision whether or not to participate. Your signature indicates that, having read the information provided above, you have decided to participate.

……	**……..**
Signature of Research Participant	Signature of Witness
……	**……..**
(Please PRINT name)	(Please PRINT name)
……	**……..**
Date	Nature of Witness

……
Signature(s) of Investigator(s)

……..
Please PRINT Name

REVOCATION OF CONSENT
Exercise Intensity Study

I hereby wish to **WITHDRAW** my consent to participate in the research proposal described above and understand that such withdrawal **WILL NOT** jeopardise any treatment or my relationship with The University of New South Wales.

....
Signature Date

.....
Please PRINT Name

The section for Revocation of Consent should be forwarded to:
Associate Professor Steve Boutcher
School of Medical sciences
Faculty of Medicine
UNSW
Kensington NSW 2052

Appendix B

(Personal and familial medical history questionnaire)

Research Questionnaire -- ID_____

1. How often do you exercise?
2. What do you do for exercise?
3. What time of day do you exercise?
4. Have you ever exercised on a stationary bike or tried a spinning class before?
5. Have you tried to lose weight or been on a diet in the last year?
6. Has your weight fluctuated by more than 2kg throughout your life?
7. What was your birth weight?
8. Does anyone in your family have diabetes, if so who?
9. Have you ever checked your insulin/blood sugar levels?
10. Would you be willing to be contacted about the results from this study?
11. Would you be interested in Sarah contacting you if you are eligible for other research studies in our department in the next year?
12. Can we take photos of you while we are performing the research , to be used for presentations?
13. Is it ok for students to help out with the research while we are testing you?
14. Are you currently on any medication, if so what and how much?
15. Are you currently taking any supplements or vitamins?
16. Have you ever smoked cigarettes? If so, for how long and are you a current smoker?
17. What is your ethnicity?
18. Contact info & Date of birth
    a. Phone
    b. Email
    c. DOB

Appendix C
(Diet Diary for Studies, I and II)

Dietary Intake Instructions
3 weekdays and 1 weekend day

Try to be as specific as possible!!!!!!!

- when eating fruit or veggies, try to figure out if it is small, medium or large and what type
- if you are eating a muesli bar or something coated in chocolate or yogurt make sure to put that down, briefly describe contents
- do you know if the food you are eating was cooked in oil or fat?
- if you are eating cheese, what type and how much?
- for meats try to say white meat, dark meat, thigh, breast, cutlet, etc…
    - also think about how it was cooked, fried, grilled, baked
    - with or without skin
- write down flavours of yogurt, ice cream, etc. . .
- think of things in amounts, grams, teaspoons, cups, etc. . .
- don't forget liquids, alcohol, soda, **water**, etc. . .
- did you put sugar or sweet-n-low in your coffee or tea
    - what size is your coffee or tea?

The main message is
be specific
and
write down anything and everything!!!!!

NAME

DATE

DAY OF THE WEEK

Time	Meal	Food Item	Serving
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
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am/pm			

Appendix D
(Physical Activity Readiness Questionnaire)

Name_____

Par-Q
Pre Exercise Screening Test

1. Has your doctor ever said you have heart trouble?	**Yes**	**No**
2. Do you frequently have pains in your heart and chest?	**Yes**	**No**
3. Do you often feel faint or have spells of severe dizziness?	**Yes**	**No**
4. Has a doctor ever said your blood pressure was high?	**Yes**	**No**
5. Has your doctor ever told you that you have a bone or joint problem such as arthritis that has been aggravated by exercise or might be made worse with exercise?	**Yes**	**No**
6. Is there a good physical reason not mentioned here why you should not follow an activity program even if you wanted to?	**Yes**	**No**
7. Are you over the age of 65 and not accustomed to vigorous exercise?	**Yes**	**No**

If you answered ***yes*** to one or more questions:

If you have not recently done so consult with your personal physician by telephone or in person **BEFORE** increasing your physical activity and/or taking a fitness appraisal. Tell your physician what questions you answered YES to PAR-Q or present your PAR-Q copy.

After medical evaluation seek advice from your physician as to your suitability for:

√ Unrestricted physical activity starting off easily and progressing gradually.
√ Restricted or supervised activity to meet your specific needs at least on an initial basis. Check in your community for special programs or services.

If you answered ***no*** to all questions:

√ If you answered the PAR-Q accurately, you have reasonable assurance of your present suitability for exercise testing.
√ Postpone the testing if you have a temporary minor illness such as a common cold.

Signed_____ Name_____

Date_____

Appendix E
(FEM exclusion criteria)

FEM
Fish Oil, **E**xercise and **M**editerranean Diet

The FEM trial is a study looking at fat loss and metabolic changes in women after 12 weeks of a lifestyle modification. Please see below at the eligibility criteria and study design.

- We are looking for:
  - Non-smoking participants between the ages of 18-35 years with a BMI between 25-35 kg/m^2 or between 23-33 kg/m^2 for Asian women
    - BMI calculation:
      - take your height and convert it to metres (m^2)
      - calculate the square of your height in metres
      - take your weight and divide it by your height in metres squared (kg/m^2)
      - if the number is between 25-35 or 23-33 for Asian women, you are eligible for the study and should contact us

- You will be asked to do the following at UNSW:
  - 12 weeks of exercise training, fish oil consumption and participate in a healthy eating plan following a Mediterranean style diet.
    - 36 sessions in total on a bike doing High Intensity Interval Exercise (HIIE) at UNSW (3 days per week)
      - 20 minutes of HIIE short sprints for 8 seconds followed by 12 seconds of turning over the pedals

- Pre and Post measurements
  - VO2max (measure of fitness levels)
  - Girths (wait circumference and other body composition measurements)
  - Blood analysis (lipids, insulin, glucose and other metabolic markers)
  - resting metabolic rate
  - blood flow measurements
  - arterial stiffness measurements
  - DEXA and/or CT scans
  - diet visits

If you meet the criteria and are interested in participating please contact,

Sarah at 02 9385 8710 or at the following email address:
sarah.dien@student.unsw.edu.au

Exclusion Criteria for FEM

- **sedentary or somewhat sedentary (<2-2.5 hours a week of physical activity)**

- **having any of the following:**
  - **diabetes**
  - **impaired glucose tolerance (fasting plasma glucose levels above 110 mg/dL [6.0 mmol/L])**
  - **hypertension (blood pressure >140/90 mm Hg)**
  - **cardiovascular disease**
  - **psychiatric problems**
  - **history of alcohol abuse (intake of >500 g/wk in the last year)**
  - **current or recent (in the past 3 years) smoking**
  - **certain medication use**
  - **symptoms of chronic or current infection**
  - **a chronic inflammatory condition**
  - **any thyroid condition**
  - **liver disease or malignancy**
  - **pregnant or become pregnant during the study**

Appendix F
(FEM Pre- and post questionnaires)

FEM PRE Participant Questionnaire

DATE:	
ID (Office use): VEIN:	
First Name:	Surname:
DOB:	Age:
Address:	
Tel (H):	Mobile number:
Email:	
Participant Information	
Your country of birth:	
Mother's country of birth:	Father's country of birth:
If you were born overseas, Date of Arrival:	
Your Ethnicity:	
Birth weight:_____ kg (please find out beforehand)	
Were you breast or formula fed as a baby?	
Are you a student or in the working world?	
Do you live on campus (UNSW) or off?	
What is your dominant arm? right left	
Do you have any existing and past medical conditions? Y N	
Please specify:	
Have you had any sort of infection recently? Y N	
Please specify:	
Are you currently taking any medication? Y N	
Please specify: e.g. oral contraceptives, beta-blockers	
Are you currently taking any supplements or vitamins? Y N	
Please specify: e.g. multi-vitamin, phytonutrients	
Have you ever smoked before? Y N	
If yes, currently or in the past and when in the past?	
How many years and/or months were you smoking?	
How many cups of tea and/or coffee do you consume per day?	

❑ none at all ❑ 1 cup ❑ 2-3 ❑ 4-5 ❑ > 5	
Do you have any allergies?	
Do you have a regular menstrual cycle (30-45 days)?	
Do you exercise regularly? Y N	
What type of exercise do you do?	
What time of day do you exercise? morning noon evening/night	
How many times a week do you exercise?	
How many hours do you typically sleep at night?	
When you wake up are your refreshed or tired? refreshed tired	
What time do you go to sleep?	
What is the quality of your sleep? good ok bad	
Have you ever suffered from sleep deprivation or lack of sleep?	
Have you recently been on a diet or tried to lose weight?	
Has your weight fluctuated more than 2 (kg) in the last 6 months?	
Have you ever checked the following before and if so what was your reading? Insulin Y N Last Reading: Blood Pressure Y N Last Reading: Glucose Y N Last Reading:	
Do/did any members of your family suffer from a medial condition? (especially Type II diabetes, hypertension, high cholesterol)	
Condition(s):	Relation to you:
This Study	
How did you hear about this study? ❑Blitz ❑friends ❑email ❑lecture announcements ❑media/TV/newspaper	
Can we take photos of you during testing? The photos may be used in a poster presentation of this study. Y N	
Is it ok with you that students help out during your testing session? Y N	
Would you like to participate in other studies in the future for our department? Y N	

FEM POST Participant Questionnaire

DATE: ID (Office use):
Have you had any sort of infection while in the study? Y N
Please specify (what & when):
Did you smoke during the study? Y N If yes, how much?
How many cups of tea and/or coffee did you consume per day? ❑ none at all ❑ 1 cup ❑ 2-3 ❑ 4-5 ❑ > 5
What is the TOTAL length of your menstrual cycle in days? (Ex. 30)
Currently where are you in your menstrual cycle? ❑ Menstruation phase ❑ Follicular phase (first half of cycle after menstruation) ❑ Luteal phase (second half of cycle after menstruation)
How many hours did you typically sleep at night during the study?
When you woke up were you refreshed or tired? refreshed tired
What time did you typically go to sleep?
What was the quality of your sleep? good ok bad
Did you suffer from sleep deprivation or lack of sleep?
Will you continue to exercise after the study?
Did you enjoy the exercise component of the study? Y N
Please specify:
Rate the intensity of all the exercise sessions? ❑ Very Hard ❑ Hard ❑ Somewhat Hard ❑ Fairly Light ❑ Very Light
Did you enjoy the Mediterranean diet? Y N
Please specify:
Will you continue the Mediterranean diet after the study?
What foods will you go back to or start eating now that the study is over?
Please specify:
Did you enjoy the fish oil? Y N
Please specify:
Will you continue to take fish oil supplements after the study?
Is there anything we can change to make the study better or more enjoyable?
Please specify:
Would you like to participate in other studies for our department? Y N
Has any of your contact details changed since the start of the study? Y N If yes, please write the new details on the back of this form.

CONTROL POST Participant Questionnaire

DATE: ID (Office use):
Have you had any sort of infection while in the study? Y N
Please specify (what & when):
Did you smoke during the study? Y N If yes, how much?
How many cups of tea and/or coffee did you consume per day? ❑ none at all ❑ 1 cup ❑ 2-3 ❑ 4-5 ❑ > 5
What is the TOTAL length of your menstrual cycle in days? (Ex. 30)
Currently where are you in your menstrual cycle? ❑ Menstruation phase ❑ Follicular phase (first half of cycle after menstruation) ❑ Luteal phase (second half of cycle after menstruation)
How many hours did you typically sleep at night during the study?
When you woke up were you refreshed or tired? refreshed tired
What time did you typically go to sleep?
What was the quality of your sleep? good ok bad
Did you suffer from sleep deprivation or lack of sleep?
Is there anything we can change to make the study better or more enjoyable?
Please specify:
Would you like to participate in other studies for our department? Y N
Has any of your contact details changed since the start of the study? Y N If yes, please write the new details on the back of this form.

Appendix G

(FEM diet diary, food frequency questionnaire and Mediterranean diet information)

Instructions for Food Diary

Try to be as specific as possible!!!!!!!

- Please read through the EXAMPLE entry
- If you have dishes that you don't know the name of, try to describe the contents, take note of the quantity and the way it is cooked. For example, a stir-fry with hokkien noodles (approximately 2 cups), beef (less than ½ cup), capsicum, onion, cabbage and shallots, cooked in vegetable oil and seasoned with hoi-sin sauce.
- Try to record your food intake on days that are closest to what your normal diet is like.
- Try to estimate the size of the servings as best as you can. Think: teaspoons, tablespoons, cups, grams, mL etc.
- When eating fruit or veggies, try to figure out if it is small, medium or large and what type
- If you are eating a muesli bar or something coated in chocolate or yogurt make sure to put that down, briefly describe contents
- Do you know if the food you are eating was cooked in oil or fat?
- If you are eating cheese, what type and how much?
- For meats try to say white meat, dark meat, thigh, breast, cutlet, etc…
    - also think about how it was cooked, fried, grilled, baked
    - with or without skin
- Write down flavours of yogurt, ice cream, etc. . .
- Think of things in amounts, grams, teaspoons, cups, etc. . .
- Don't forget liquids, alcohol, soda, **water**, etc. . .
- Don't forget lollies, chocolates, cookies, traditional sweets, etc…
- Did you put sugar or sweet-n-low in your coffee or tea
    - What size is your coffee or tea?

The main message is
be specific
and
write down anything and everything!!!!!

EXAMPLE

NAME Faith

DATE 21/05/06

DAY OF THE WEEK Saturday

Time	Meal	Food Item	Serving
8:30 am/~~pm~~	Breakfast	Wonton, filled with pork (10g each) x 12	120g
am/pm		Water	300 mL
am/pm		Vitamin C	1 tablet
11:30 am/~~pm~~	Morning Tea	Bagel (wheat) small, toasted	1
am/pm		Strawberry jam	2 teaspoons
1:00 ~~am~~/pm	Lunch	Chicken skewers (breast and thigh)	10 pieces
am/pm		Satay sauce	1/3 cup
am/pm		Rice, white (cooked)	½ cup
am/pm		Water	300 mL
am/pm		Violet crumble bar 50g	1
3:45 ~~am~~/pm	Snack	Pretzels (small bag)	20 g
7:30 ~~am~~/pm	Dinner	Beef stir fry with black bean sauce	200 g
am/pm		Chinese broccoli (gai lum) cooked with olive oil	1 cup (160mL)
am/pm		Oyster sauce	2 tablespoons
am/pm		Green salad	1 cup
am/pm		Salad dressing (French low fat)	2 tablespoons
am/pm		Rice, white (cooked)	1 cup
am/pm		Strongbow cider x 2	375 mL x2
am/pm		Chocolate ice cream	½ cup

Day 1
(Weekday)

Food Diary

NAME

DATE

DAY OF THE WEEK

Time	Meal	Food Item	Serving
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			

Day 2
(Weekday)

Food Diary

NAME

DATE

DAY OF THE WEEK

Time	Meal	Food Item	Serving
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			

Day 3
(Weekend day)

Food Diary

NAME

DATE

DAY OF THE WEEK

Time	Meal	Food Item	Serving
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			
am/pm			

What is the Mediterranean Diet?

- abundance of plant foods
    - vegetables
    - fresh and dried fruits (watch the sugar and preservatives with dried fruit)
    - whole-grain cereals
    - nuts and legumes
- olive oil as the principal source of MUFA (monounsaturated fatty acid)
- fish (weekly)
- egg (0-4 per week)
- cheese, yoghurt
- moderate amounts of
    - poultry
    - wine (1-2 per day) or clear spirits
- STAY AWAY FROM
    - red meat
    - carbonated SODA drinks, carbonated water is OK
    - processed or refined foods
    - the oil/dairy products
        - butter
        - vegetable polyunsaturated & trans fatty oils and margarines

The diet should be

- ~ 55 % carbohydrates
- < 30 % fats
    - < 8-10 % saturated fat
    - NO trans fats
- ~ 15 % proteins

When on the Mediterranean Diet

- try consume **MORE**
    - fibre
    - antioxidants
    - a low glycemic load

LOW GL

GL = glycemic load or glycemic index

GL is the effect a total meal has on your blood sugar (insulin) and is not only related to the original form of the carbohydrate.

The secret to Low GL is fibre, the more the fibre the slower the absorption.

Low GL Foods	**High GL Foods**
Vegetables	Flour or flour products
Fruits	Refined grains (white rice)
Beans	Sugar in any form
Nuts	Processed foods
Seeds	Junk food
Olive oil	Large starchy potatoes
Whole grains	
Teas	
Herbs and Spices	

FEM DIET

Week 3

- olive oil as the principal source of MUFA (monounsaturated fatty acid)
- when heating foods
    - sauté is best, cooking veggies or meat in a non-stick fry pan with water or stock to prevent sticking to the pan. You can always add more water if needed.
    - use coconut oil when needed as it can withstand the higher temps
- free range
    - poultry
    - eggs
- drinks teas cold or hot
    - Green Tea is GREAT!!!
- spreads
    - avocado is a good healthy fat
    - applesauce, mashed banana, prune puree or mashed pumpkin
    - honey
- watch out for potatoes, sweet potatoes are better than white
- sourdough bread is a good alternative if the whole grains are getting boring
- pastas should not be the basic white flour pasta but the wholemeal or brown pastas
- raw is better than roasted or cooked
- be aware of the salad dressing, make your own if need be
- alcohol
    - you can consume clear spirits
        - vodka, real lime and soda water
- <u>STAY AWAY FROM</u>
    - red meat
    - carbonated SODA drinks
    - processed or refined foods
    - mayonnaise
    - high-fat dairy products
        - saturated fats and butter
        - vegetable polyunsaturated & trans fatty oils and margarines
            - WATCH OUT for the omega-3 margarines and or olive oil margarine

FEM DIET

Week 6

- Weaknesses within Med. Diet
    - Bored with diet
    - More options
        - 7th Heaven Wholefoods
            - 122 Belmore Road in Randwick
        - The Organic Meat & Poultry Shop
            - Shop 15 in the Randwick Plaza
              130 Belmore Road in Randwick
        - Go Vita Randwick
            - Shop 52 in the Royal Randwick Shopping Centre
              Belmore road in Randwick
        - Norton Street Grocer
            - They are around the city, closest one to UNSW is in the Bondi Junction Westfield
        - Asian Markets
            - Usually have great prices on healthy foods.
    - Remember, it's not a low-calorie diet!
    - Green Tea is GREAT!!!
    - Storage of oils
        - Store your oils, olive or coconut in the refridge
            - They my go solid so you will nee to scoop them out.
    - Sleep
        - Try to get enough sleep
    - Try to eat slowly and take longer to get through your meal which should help reduce stress
        - Try to make sure you sit down and relax while eating.
            - Try it for one meal a day or a week
    - IRON
        - See back sheet

- STAY AWAY FROM
    - red meat
    - carbonated soda drinks
    - processed or refined foods
    - mayonnaise
    - high-fat dairy products
        - saturated fats and butter
        - vegetable polyunsaturated & trans fatty oils and margarines
            - WATCH OUT for the omega-3 margarines and or olive oil margarine

FEM DIET

Week 9

- You already know so much about the diet but remember
  LOTS OF FRUIT AND VEG!!!
- Weaknesses within Med. Diet
  - Glycaemic load
- Will I keep up this diet after FEM??
- You made it this far, keep it up for the last 3 weeks, YOU CAN DO IT!!!!!!
  - PLEASE keep up the diet & fish oil through the post testing

- STAY AWAY FROM
  - red meat
  - skin on meat
  - carbonated soda drinks
  - processed or refined foods
  - mayonnaise
  - high-fat dairy products
    - saturated fats and **butter**
    - vegetable polyunsaturated & trans fatty oils and **margarines**
      - WATCH OUT for the omega-3 margarines and or olive oil margarine

M	Chicken (in any form)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
E	Beef (in any form)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A	Lamb (in any form)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
T	Pork (in any form)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Ham (in any form)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Turkey (in any form)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Sausages	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Liver (in any form)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Kidney (in any form)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Meat Pies/Pastries	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Ready Meat Meals	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Meat Free/Vegetarian Meals (soy or tofu)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Other	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
C	Cornflakes	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
E	Rice Krispies	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
R	Weetabix	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
E	Muesli	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A	Porridge	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
L	All Bran	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Shreddies	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Special K	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Fruit n Fibre	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Other	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Other	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
D	Cheese	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A	Cream	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
I	Milk	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
R	Yoghurt	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Y	Ice Cream	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Other	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
V	Leafy Greens (brocolli, spinach)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
E	Salad Vegetables (lettuce)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
G	Potatoes (boiled, mashed, baked or fried)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Peas	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Carrots	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Cabbage	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Capsicium (green, red, yellow)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Tomato, tomato juice or sauce	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Mushroom	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Marinated veggies	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Onion	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Baked Beans	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Broad Beans	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Chick Peas	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Lentils (green/brown)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Kidney Beans	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Soya Beans	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Coconut milk	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Other	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Apples	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Oranges	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
F	Bananas	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
R	Pears	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
U	Grapefruit	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20

I	Peaches	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
T	Strawberries	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Apricots	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Mangoes	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Dried Fruit (apricots, sultanas, currants)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Fruit Juice	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Avocado	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Blueberries	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Pineapple	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Grapes	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Other	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
G	White Bread	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
R	Wholemeal	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A	Sourdough	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
I	Brown Bread	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
N	Multigrain Bread	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
S	Crisps	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Rice Crackers	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Naan/pita Bread	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Flour Tortillas	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Corn Tortillas	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Pasta/noodles	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Rice	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Rice noodle	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Other	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
O	Tea	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
T	Honey	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
H	Chocolate (>70% cocoa)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
E	Lollies	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
R	Vegimite	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Nuts	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Stock (chicken, beef, seafood, etc…)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Water	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Olive oil	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Fish/Seafood	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Eggs	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Pizza	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Curry	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Chili	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Soda Drinks	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Beer	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	White wine	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Red wine	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Hard Alcohol	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20

Appendix H
(FEM fish oil intake form)

Fish Oil Intake Form

Study ID Date capsules started Date capsules ended

Please initial across cap 1 or cap 2 or 3 after ingestion

If no cap is ingested explain reason in comments box below

MON	TUES	WED	THURS	FRI	SAT	SUN
cap 1	cap 1	cap 1	cap 1	cap 1	cap 1	cap 1
cap 2	cap 2	cap 2	cap 2	cap 2	cap 2	cap 2
cap 3	cap 3	cap 3	cap 3	cap 3	cap 3	cap 3
cap 1	cap 1	cap 1	cap 1	cap 1	cap 1	cap 1
cap 2	cap 2	cap 2	cap 2	cap 2	cap 2	cap 2
cap 3	cap 3	cap 3	cap 3	cap 3	cap 3	cap 3
cap 1	cap 1	cap 1	cap 1	cap 1	cap 1	cap 1
cap 2	cap 2	cap 2	cap 2	cap 2	cap 2	cap 2
cap 3	cap 3	cap 3	cap 3	cap 3	cap 3	cap 3
cap 1	cap 1	cap 1	cap 1	cap 1	cap 1	cap 1
cap 2	cap 2	cap 2	cap 2	cap 2	cap 2	cap 2
cap 3	cap 3	cap 3	cap 3	cap 3	cap 3	cap 3
cap 1	cap 1	cap 1	cap 1	cap 1	cap 1	cap 1
cap 2	cap 2	cap 2	cap 2	cap 2	cap 2	cap 2
cap 3	cap 3	cap 3	cap 3	cap 3	cap 3	cap 3
cap 1	cap 1	cap 1	cap 1	cap 1	cap 1	cap 1
cap 2	cap 2	cap 2	cap 2	cap 2	cap 2	cap 2
cap 3	cap 3	cap 3	cap 3	cap 3	cap 3	cap 3
cap 1	cap 1	cap 1	cap 1	cap 1	cap 1	cap 1
cap 2	cap 2	cap 2	cap 2	cap 2	cap 2	cap 2
cap 3	cap 3	cap 3	cap 3	cap 3	cap 3	cap 3
cap 1	cap 1	cap 1	cap 1	cap 1	cap 1	cap 1
cap 2	cap 2	cap 2	cap 2	cap 2	cap 2	cap 2
cap 3	cap 3	cap 3	cap 3	cap 3	cap 3	cap 3
cap 1	cap 1	cap 1	cap 1	cap 1	cap 1	cap 1
cap 2	cap 2	cap 2	cap 2	cap 2	cap 2	cap 2
cap 3	cap 3	cap 3	cap 3	cap 3	cap 3	cap 3
cap 1	cap 1	cap 1	cap 1	cap 1	cap 1	cap 1
cap 2	cap 2	cap 2	cap 2	cap 2	cap 2	cap 2
cap 3	cap 3	cap 3	cap 3	cap 3	cap 3	cap 3
cap 1	cap 1	cap 1	cap 1	cap 1	cap 1	cap 1
cap 2	cap 2	cap 2	cap 2	cap 2	cap 2	cap 2
cap 3	cap 3	cap 3	cap 3	cap 3	cap 3	cap 3
cap 1	cap 1	cap 1	cap 1	cap 1	cap 1	cap 1
cap 2	cap 2	cap 2	cap 2	cap 2	cap 2	cap 2
cap 3	cap 3	cap 3	cap 3	cap 3	cap 3	cap 3
Comments						

Appendix I

(Correlations Between Fitness and Body Composition, Blood and Lipid Variables for Study I)

Table I1 Correlations Between Fitness and Body Composition, Blood Variables, and Lipids	
Mass (kg)	- .28*
BMI (kg/m^2)	- .44**
Waist circumference (cm)	- .15
Hip circumference (cm)	.16
Waist-height-ratio	- .36*
Trunk Body Fat (%)	- .44**
Fat free mass (kg)	.14
Body fat (%)	- .46**
Fat mass (kg)	- .38**
Insulin (µIU/ml)	- .21
HOMA-IR	- .22
Glucose (mmol/L)	- .02
C-reactive protein (ng/ml)	- .20
Triglycerol (mmol/L)	- .24
Very low density lipoproteins (mmol/L)	- .23

* Significant difference ($p < .05$)
** Significant difference ($p < .001$)
Note: Body mass index (BMI), Homeostasis model assessment of insulin resistance (HOMA-IR)

Appendix J
(Correlations on Baseline Variables for Study II)

Table J1 Blood Variable Correlations to Body Composition

	Mass (kg)	BMI (kg/m^2)	WC (cm)	HC (cm)	%BF ISAK	%BF
Fasting insulin (µIU/ml)	0.29	0.33	0.24	0.21	0.24	0.22
Fasting glucose (mmol/L)	0.42	0.27	0.18	0.25	0.36	0.35
HOMA-IR	0.30	0.32	0.23	0.21	0.25	0.23
Fasting cortisol (µg/ml)	0.00	-0.23	-0.18	-0.02	- 0.29	- 0.01
Fasting lactate (mmol/L)	- 0.49 *	- 0.52 *	- 0.52 *	- 0.49 *	- 0.38	- 0.44
Fasting adrenocorticotropic hormone (pg/ml)	0.57 *	0.43	0.37	0.52	0.32	0.47
Fasting growth hormone (ng/ml)	- 0.43	- 0.51 *	- 0.42	- 0.32	- 0.39	- 0.36
Fasting glycerol (µmol/L)	- 0.07	0.11	0.27	0.04	- 0.05	0.07
Fasting leptin (ng/ml)	0.81 **	0.89 **	0.80 **	0.84 **	0.88 **	0.83 **

**Significant difference ($p < 0.01$)
*Significant difference ($p < 0.05$)
Note: Homeostasis model assessment of insulin resistance (HOMA-IR)

Table J2 Associations Between Blood Variables Measured and Insulin, HOMA-IR, and Glucose

	Fasting Insulin (µIU/ml)	HOMA-IR	Fasting Glucose (mmol/L)
Fasting cortisol (µg/ml)	0.12	0.13	0.02
Fasting lactate (mmol/L)	0.17	0.20	0.35
Fasting adrenocorticotropic hormone (pg/ml)	0.47	0.47 *	0.24
Fasting growth hormone (ng/ml)	- 0.56 *	- 0.55 *	- 0.40
Fasting glycerol (µmol/L)	- 0.30	- 0.33	- 0.58 *
Fasting leptin (ng/ml)	0.37	0.37	0.40

* Significant difference ($p < .05$)