THE UNIVERSITY OF HULL

The effects of a structured 8-week aerobic exercise intervention on anti-Mullerian hormone levels and oxidised LDL-cholesterol in the polycystic ovarian syndrome.

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Amie Woodward, BSc

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Abstract

AIM: PCOS is the most common endocrine disorder of reproductive age women, affecting 4-12%. The condition is associated with reproductive and cardiometabolic complications including obesity, insulin resistance, hyperandrogenism and infertility. Women with PCOS have a two to four-fold higher prevalence of metabolic syndrome (and higher oxidised LDL) and higher concentrations of Anti-Mullerian hormone (AMH) compared to BMI-matched controls. The present study aims to determine whether physical activity reduces AMH and OxLDL levels and therefore improves fertility and cardiometabolic outcomes. **METHODS:** Women with PCOS (n=14) and healthy controls (n=11) completed an 8-week exercise programme consisting of 3 x 1-hour treadmill sessions per week at 60% VO2max. Blood samples were analysed for AMH and oxidised LDL concentrations using the ELISA method. Pre collected anthropometric and cardiorespiratory fitness markers were also analysed to determine any significant differences in means before and after the intervention. **RESULTS**: Within the longitudinal PCOS study, mixed two-way ANOVAs found no significant differences between AMH and oxidised LDL levels pre and post intervention in PCOS or controls, despite significant improvements in body mass, BMI, WC and VO2max evident in the PCOS group.

CONCLUSION: Results from the PCOS intervention demonstrated no significant improvement of AMH or oxidised LDL levels in women with PCOS or controls following short-term structure moderate intensity exercise training. It did have a significant impact on anthropometric and cardiorespiratory fitness markers. Future research should place importance upon identifying and conducting research on the phenotypes of PCOS separately, particularly in relation to those phenotoypes with hyperandrogenism, as well as conducting exercise interventions over a longer duration.

1. Introduction

1.1 Cardiovascular Disease

Cardiovascular diseases (CVD) are the leading cause of death worldwide, as reported by the World Health Organisation (WHO), with around 30% of deaths occurring in 2005 being attributable specifically to coronary heart disease (Zhang, Xu, Li, 2014). Around one-third of cardiovascular disease cases are either caused or associated with one or more of four risk factors: smoking, high blood pressure, high cholesterol and obesity (Ray, 2012). The INTERHEART study conducted in 2004 established a standardised case-control study of acute myocardial infarction in 52 countries (Yusef et al., 2004). The study examined 15,152 cases and 14,820 controls to identify the relationship between myocardial infarction and various risk factors. The study revealed that smoking, abnormal lipids (apolipoprotein B and apolipoprotein A1 ratio), history of hypertension, diabetes, abdominal obesity, psychosocial factors, consumption of fruits and vegetables, alcohol consumption and physical activity levels were all significantly related to acute myocardial infarction, accounting for 90% of the population attributable risk in men and 94% in women (Yusef et al., 2004).

Dyslipidemia is a prominent risk factor in itself for CVD, exacerbated by the worldwide epidemic of type 2 diabetes and obesity, potentially stemming from a sedentary society (Miller, 2009; Ray, 2012). Dyslipidemia is characterised by high levels of low-density lipoprotein cholesterol (LDL-C), high levels of triglycerides and low levels of high-density lipoprotein cholesterol (HDL-C) (Miller, 2009). Various large case-control studies have found the total cholesterol to high density lipoprotein cholesterol ratio (TC-HDL-ratio) to be an accurate surrogate marker of CVD risk and a predictor of CHD (Koenig et al., 2011; Wu, Willet, Rifai, Shai, Manson & Rimm, 2006). As well as obesity and physical inactivity, insulin resistance is a large contributor to an abnormal lipid profile (and insulin resistance is, in turn, exacerbated by obesity and physical inactivity) (Miller, 2009).

CVD poses a large risk to women, particularly those over 60 years; coronary heart disease (CHD) is the specific leading cause of death for women aged 60 and older (Welty, 2001). Women are 4 to 8 times more likely to die of CVD than of any other disease (Anderson, Kochanek & Murphy, 1997). Further to this, women with diabetes

have a CHD-related mortality rate that is 3 to 7 times higher than women without diabetes (Welty, 2001). The reason for a higher risk after a certain age may be, in part, related to rising levels of LDL-C in women (by about 2mg/dL per year between the ages of 40 and 60 years), which is considered to be related to declining levels of oestrogen (Welty, 2001) whereby declining oestrogen concentrations results in the down-regulation of LDL receptors on the liver (Welty, 1996; Welty, 2001) and consequently higher plasma LDL levels. Research has demonstrated increased total cholesterol and LDL-C, and decreased HDL-C in postmenopausal women, with subsequent increase in CVD risk (Halverstadt, Phares, Wilund, Goldberg and Hagberg, 2007).

Therefore, given that women may be at a greater risk of CVD due to established causes of dyslipidemia affecting the population as a whole (obesity, physical inactivity and insulin resistance) and the decline of oestrogen in later life, particular attention must be given to the treatment of dyslipdemia and other metabolic diseases in women to reduce the mortality rates of CVD. In particular, consideration should be given to oxidised LDL concentrations which have been shown to be a measure of atherogenic potential (Assman, 1982) and an accurate predictor of CHD and myocardial infarction (Koenig et al., 2011; Weinbrenner et al., 2006).

1.2 Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder of reproductive age women, affecting 4-12% (Hutchison et al., 2011). It was first described and reported in medical literature by Stein and Leventhal (1935) who described seven women who suffered from amenorrhea, hirsutism and enlarged ovaries with multiple cysts (Stein & Leventhal, 1935). Now, the typical diagnostic tool for PCOS is the Rotterdam criteria which was derived from an ESHRE (European Society of Human Reproduction and Embryology) and ASRM (American Society for Reproductive Medicine) scientific consensus 2003. The Rotterdam criteria states that women must present with 2 out of the following 3 signs/symptoms: hyperandrogenism, chronic anovulation or oligomenorrhea, and polycystic ovaries, in the absence of other diseases that promote these symptoms (Ladson et al., 2011). However, 30% of women with PCOS will have regular menses (Sirmans & Pate, 2014). PCOS is often, though not included in the diagnostic criteria, characterised by higher amounts of visceral fat, obesity and insulin resistance (Hutchison et al., 2011). This is therefore associated with

reproductive and cardiometabolic complications, such as impaired glucose tolerance and metabolic syndrome (Harrison, Lombard, Moran & Teede, 2010). However, while anovulatory women displaying hyperandrogenism and polycystic ovaries consistent with PCOS are often insulin resistant, ovulatory women with PCOS usually are not (Robinson et al., 1993).

1.3 PCOS, Steroid Hormone Imbalance and Anti-Mullerian Hormone

In addition to hyperandrogenism, inappropriate gonadotropin secretion is a common hormonal feature in women with PCOS that is also not included in current diagnostic criteria (Chun, 2014). These abnormal patterns include increased serum luteinizing hormone (LH) concentrations, low to normal follicle stimulating hormone (FSH) levels and an increased LH:FSH ratio that have been well recognised as common characteristics of PCOS (Chun, 2014).

Anti-Mullerian hormone (AMH) is produced by recruited antral follicles in the ovary until they become sensitive to FSH (Saikumar, Selvi, Prabhu, Venkatesh & Krishna, 2013). AMH is therefore a key regulator in the recruitment process which prevents the depletion of all primordial follicles at once (Saikumar et al., 2013). While AMH impedes the transition from primordial follicles into growing, primary follicles, it limits the sensitivity of small, antral follicles to FSH (Durlinger et al., 2001). When the follicles in the growing cohort reach 6mm, they stop producing AMH; it is absent in the dominant follicle, increasing this follicle's sensitivity to FSH compared with the nondominant ones (Hehenkamp et al., 2006).

AMH has been recognised as a potential predictor of ovarian reserve; increased AMH serum levels have been found in PCOS patients who have a two to threefold higher number of pre antral and small antral follicles compared to those with normal ovaries (Laven et al., 2004; Saikumar et al., 2013). Its gradual decrease over the reproductive years is believed to be the best representation of the decline in reproductive capacity in fertile women (Hehenkamp et al., 2006).

It is also accepted that AMH is an excellent predictor of ovarian responsiveness to gonadotropin treatment for ovulation induction and *in-vitro* fertilisation (IVF); a study by Amer et al. (2013) found AMH levels to be negatively correlated with response to

human menopausal gonadotropin (hMG). It is therefore suggested that AMH levels may reflect the severity of PCOS, where severe PCOS is associated with an increased number of small antral follicles (Amer et al., 2013).

Though AMH levels are known to decrease with age in women with normo-ovulatory cycles, normal levels typically range between 5-43 pmol/L (Nybacka, Carlstrom, Fabri, Hellstrom & Hirschberg, 2013). Women with PCOS are known to have elevated circulating levels of Anti-Mullerian Hormone (AMH), often two to threefold the amount of non-PCOS women (Amer et al., 2013; Nybacka et al., 2013). Increased production of AMH may therefore have an inhibitory role in the ovary, preventing follicle selection and resulting in follicle arrest at the small antral phase (Woo, Kim, Rhee, Park & Lee, 2012). Thus, this leads to the failure of a dominant follicle from the recruited pool (Amer et al., 2013; Saikumar et al., 2013). As a result of this, 40% of women with PCOS are affected by infertility; PCOS is the most common cause of anovulatory infertility, with 90-95% of anovulatory women seeking treatment at infertility clinics having PCOS (Sirmans & Pate, 2014).

The mechanisms for this are not clear, though it has been suggested that PCOS women have an excess of small follicles (2-5mm) as compared to larger follicles; AMH is produced from the granulosa cells of antral follicles (Saikumar et al., 2013; Woo et al., 2012). Therefore, this suggests higher AMH levels are the result of a higher antral follicle count. Other research suggests that increased AMH production is due to an increase in production of AMH by each follicle (Nybacka et al., 2013; Woo et al., 2012).

It's been suggested that women with PCOS have increased intrinsic insulin resistance (Hutchison et al., 2011); insulin resistance and the resulting compensatory hyperinsulinaemia have been consistently documented in both lean and obese PCOS subjects compared to weight-matched controls (Cassar et al., 2014; Norman et al., 2004). Furthermore, insulin has been shown to serve as a co-gonadotrophin to stimulate ovarian androgen production; severe hyperinsulinemic states in women have been associated with marked hyperandrogenemia, such as leprauchaunism (Legro, 2012). The proposed mechanism behind this is that insulin is capable of reducing levels of SHBG (sex-hormone binding globulin), thereby resulting in higher levels of bioavailable testosterone (Norman et al., 2004).

Therefore, high levels of testosterone could be responsible for high levels of AMH by stimulating the development of antral follicles (Woo et al., 2012). Cassar et al. (2014) recently conducted a study on both lean and overweight women with PCOS and found that AMH levels were more likely to be linked with increased testosterone levels than insulin resistance, adiposity and gonadotropin secretion (Cassar et al., 2014). Furthermore, AMH is known to inhibit aromatase and thus the production of estradiol (E2), therefore causing dysregulation in folliculogenesis (Woo et al., 2013).

1.4 Obesity and Insulin Resistance/Dyslipidemia in PCOS

It should be noted that not all PCOS women are obese, and neither is obesity the cause of PCOS (Legro, 2013). Certainly, obesity may increase the risk of, or exacerbate PCOS, but that effect has been shown by some to be modest (Sirmans & Pate, 2014). Indeed, although a third to 50% of PCOS women are overweight or obese (Gambineri, Pelusi, Vicennati, Pagotto & Pasquali, 2002), it has been proposed that the prevalence of obesity in PCOS related studies may be a form of selection bias whereby women with PCOS are more likely to seek out medical treatment if they display symptoms such as hirsutism, menstrual disturbances and obesity (Legro, 2013; Sirmans & Pate, 2014).

Those women with PCOS that are lean may not necessarily have insulin resistance per se, but rather an enhanced ovarian sensitivity to insulin, or display insulin hypersecretion despite normal insulin sensitivity (Vrbikova, Cibula & Dvorakova, 2004). Furthermore, the presence of central adiposity is highly associated with insulin resistance in subjects without PCOS, and may also hold true for both lean and obese women with PCOS, who may both exhibit central adiposity (Norman, Noakes, Wu, Davies, Moran & Wang, 2004). Either way, it is generally accepted that abnormal insulin metabolism is a major underlying feature of PCOS (Sattar, 2011), with the prevalence of insulin resistance in PCOS ranging from 50%-70% (Carmina & Lobo, 2004).

Other risk parameters for CHD displayed in women with PCOS are impaired endothelial function, including coronary artery calcification, two to four fold higher prevalence of metabolic syndrome compared to BMI matched controls and consequently around a 50% increased risk of CHD events (Sattar, 2006).

Dyslipidemia has been shown to be prevalent in up to 70% of women with PCOS (Kim & Choi, 2013). The lipoprotein profile in women with PCOS is characterised by elevated plasma triglycerides (TG) and reduced high density lipoprotein (HDL)-cholesterol concentrations, mirroring that seen in patients with type 2 diabetes (T2D) (Sattar, 2006). Furthermore, TC-HDL-ratio has been shown to be a better predictor of dyslipidemia and subsequent CVD risk than TG, HDL and LDL concentrations alone (Koenig et al., 2011; Wu et al., 2006).

Insulin resistance can lead to elevations in TG through increased hepatic synthesis of very low density lipoprotein (VLDL) particles (Sattar, 2011). As part of their dyslipidemia profile, Women with PCOS have a higher proportion of small, dense LDL (LDL-III) - which are considered to be more atherogenic than larger, less dense LDL species (LDL-I and LDL-II) and are therefore associated with a higher incidence of coronary heart disease and T2D (Sattar, 2011).

Previous literature has suggested that hyperandrogenism can increase the level of small, dense LDL levels by way of up-regulating hepatic lipase (Herbst, Amory, Brunzell, Chansky & Bremner, 2003). However, a study on fifty-two PCOS women found that PCOS women do indeed have increased hepatic lipase activity (and subsequently increased percentage of small, dense LDL), but this appears to be more closely related to adiposity and insulin metabolism rather than circulating androgen levels (Pirwany, Fleming, Greer, Packard & Sattar, 2001).

1.5 Treatment of PCOS

The correct diagnosis and subsequent treatment of PCOS is important for various reasons. The variety of symptoms of PCOS are seemingly unrelated but are often severe enough to cause concern; therefore, a correct diagnosis can not only offer an explanation but also help to rule out other medical conditions or the more sinister implications that the symptoms are indicative of, such as androgen secreting tumors (Ajayi & Ogunmokun, 2007). Furthermore, a definitive diagnosis will help to form the basis of treatment, especially when PCOS symptoms such as acne and excessive body hair may affect body image.

There are various modes of treatment used for both the symptoms and the proposed causes of PCOS (although it is hard to isolate a single influence in a feedback endocrine system, since all participants in the loop can be affected (Legro, 2012)). These range from lifestyle intervention and modification, which is said to be the cornerstone of therapy for PCOS (Ladson et al., 2011), to drug treatment, to alternative therapy such as acupuncture (Johansson & Stener-Victorin, 2013) and even surgery.

1.5.1 Drug Treatment

Various drugs have been studied and used in the treatment of PCOS in order to increase insulin sensitivity and subsequently treat infertility (Ayup et al., 2007). There were several insulin sensitising drugs commonly used in the treatment of PCOS, including metformin, and several glitazone derivatives including troglitazone, rosiglitazone and pioglitazone. However, troglitazone was removed from the market due to liver toxicity and troglitazone and rosiglitazone have been shown to induce teratogenesis in animals and is no longer recommended for the treatment of infertility (Ayup et al., 2007). Perhaps the most frequently studied and widespread drug used in the treatment of PCOS is metformin (Ayup et al., 2007; Fleming & Sattar, 2007).

Metformin lowers blood glucose levels without increasing insulin secretion or causing hypoglycaemia; its proposed mechanism is by decreasing hepatic glucose production and increasing insulin sensitivity at the post-receptor level, while decreasing intestinal glucose absorption (Ayup et al., 2007).

Another treatment for reproductive dysfunction is the oral contraceptive pill which regulates the menstrual cycle and improves hirsutism and acne. This method remains controversial for long-term and high dose use, however, due to potential adverse metabolic and cardiovascular effects including worsening of insulin resistance and arterial stiffness (Meyer, McGrath & Teede, 2007).

1.5.2 Lifestyle Interventions

Since the criteria for pharmacologic treatment in PCOS is still debatable and there remains to be no particular strategy on drug selection or use, lifestyle modification is still the first line of management for weight loss (in obese patients), reducing cardiovascular risk and improving reproductive dysfunction (Moran, Pasquali, Teede,

Hoeger & Norman, 2008). Various studies have examined the effect of lifestyle intervention (caloric restriction and exercise) in PCOS. Published studies typically include a lifestyle intervention that examines diet and exercise together (Hagg et al., 2014), or examine the effects of an exercise intervention alone (Hutchison et al., 2011). While dietary intervention, too, has been examined independently, particularly for overweight women with PCOS, these studies often have higher dropout rates, modest weight loss and difficulty in weight maintenance after the study (Moran et al., 2009).

1.6 Aims and Objectives

The study aims to examine, and add to the current body of research, whether an 8-week aerobic exercise intervention can positively impact AMH and OxLDL levels in women with PCOS, subsequently improving both fertility and cardiometabolic outcomes.

The study's objectives are therefore to collect measurable data on these variables, as well as to collate measures of body composition and markers of cardiorespiratory fitness, before and after an 8-week aerobic exercise intervention. A primary objective is to analyse previously stored blood samples, from controls and women with PCOS participating in the 8-week aerobic exercise intervention, to collect data on AMH and OxLDL levels for each participant. These data can then be analysed for significant differences in means both pre and post intervention, and between women with PCOS and controls. Since the study uses some data that are already collected (anthropometric and cardiorespiratory markers), another objective of the study is to use statistical analysis to determine if there are any significant differences in the available data between means in the variables both before and after the intervention, and between controls and women with PCOS.

The Null Hypothesis (H0) states that the 8-week aerobic exercise intervention has no significant impact on AMH and OxLDL levels in women with PCOS. The Alternative Hypothesis (H1) states that the 8-week aerobic exercise intervention will have a significant effect on AMH and OxLDL levels in women with PCOS.

2. Review of Existing Literature

2.1 Physical Activity and CVD in Women

Research has demonstrated the effect of physical activity in women on CVD risk. The INTERHEART study (Yusef et al., 2004) found moderate to strenuous physical exercise to be one of nine lifestyle modifications protective against myocardial infarction; this effect seemed to be greater in women than in men. That is, among the females, the population attributable risk (PAR) of myocardial infarction due to not participating in regular moderate to strenuous exercise was 37.3%, compared to 22.9% in men (Yusef et al., 2004), after adjusting for age.

Various studies including large female samples have reported positive effects of exercise training on CVD risk in females (Halverstadt, Phares, Wilund, Goldberg and Hagberg 2007; Katzmarzyk et al., 2003). Katzmarzyk et al. (2003) studied a sample of 333 females, and of those presenting with metabolic syndrome, 28% were no longer classified as having the syndrome after a 20-week aerobic exercise-training programme. Furthermore, Halverstadt et al. (2007) examined the effects of a 24-week aerobic exercise-training programme on lipid profile in 100 sedentary, menopausal women. Results revealed an significant increases in HDL-C and decrease in TC and LDL-C.

2.2 Oxidised LDL and its Role in Atherosclerosis

Atherosclerosis is a combination of changes in the intima of the artery, involving focal accumulation of lipids with blood and its constituents, leading to fibrous tissue formation and calcification of the arteries (Assmann, 1982). Upon occurrence in the coronary and cerebral arteries, this may lead to CHD, stroke, myocardial infarction and death. LDL-C is one of the main determining factors in the development of atherosclerosis, causing its progression through an increase in transport of plasma LDL-C into the arterial wall (Assmann, 1982). LDL particles may then become modified through lipid peroxidation, and subsequently scavenged and degraded by macrophages (Parthasarathy, Raghavamenon, Garelnabi & Santanam, 2012), leading to 'foam cell' formation (Parthasarathy, Quinn, Schwenke, Carew & Steinberg, 1989).

Without sufficient HDL, the buildup of cholesterol esters in the arterial wall can lead to fibrosis, atherosclerotic plaques and calcification of the artery wall (Assmann, 1982). Fig. 2 below demonstrates lipoprotein metabolism and defective LDL catabolism that results in foam cell formation.

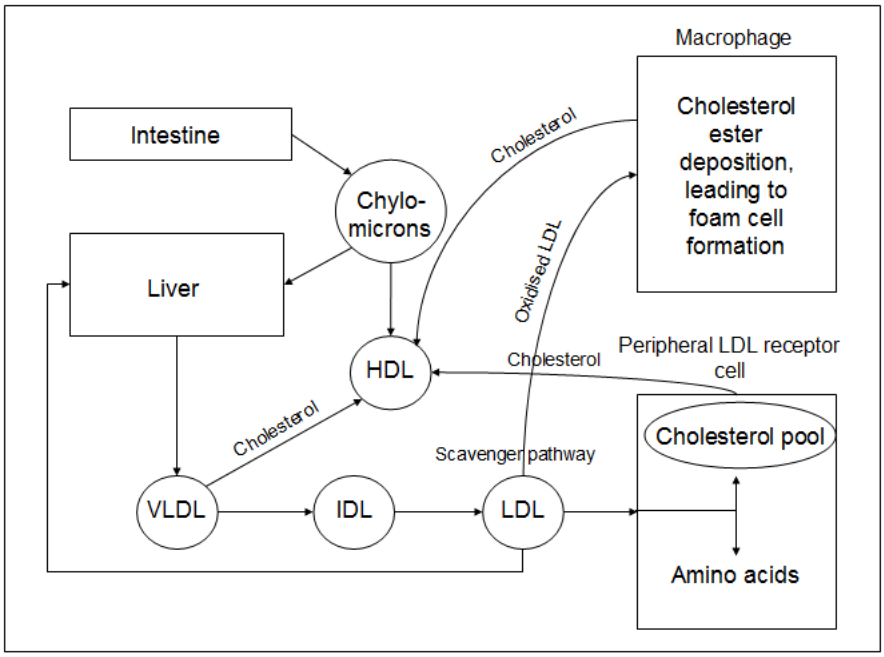

Figure 1: Lipoprotein metabolism and LDL catabolism resulting in foam cell formation. Adapted from Assman (1982).

Over 400 articles per year have been published on the topic of oxidized LDL's involvement in the development of atherosclerosis during the last decade (Parthasarathy et al., 2012).

Increased plasma oxLDL concentrations have been associated with an increased risk of CHD incidents (Holvoet, 2004; Koenig et al., 2011). Holvoet et al. (2001) analysed the predictive value of oxLDL in identifying patients with CAD. Of the 130 women in the study (77 controls and 53 CAD patients), 100% of women with high circulating levels of oxLDL (above the 90th percentile of oxLDL distribution across controls) had CAD.

Furthermore, a cross sectional study of 308 men and 279 women by Weinbrenner et al. (2006) showed a significant association between high oxLDL concentrations and higher waist circumferences (>88cm), an indirect measure of abdominal fat, in women, independent of other markers of cardiovascular risk.

2.3 TC-HDL-Ratio and its Link with Oxidised LDL

Hypercholesterolemia has been shown to be an accurate predictor of circulating oxLDL on multivariate analysis (Holvoet et al., 2001). The TC-HDL-ratio is calculated by dividing the number of HDL-C into total cholesterol (Lopez-Jimenez, 2015). Various studies have demonstrated that it appears to be a better predictor of cardiovascular risk than total or LDL-C (Holvoet et al., 2001; Koenig et al., 2011; Wu et al., 2006).

Holvoet and colleagues (2001) examined 304 subjects, of which 178 were CAD patients and 126 were age-matched controls, to determine whether circulating oxLDL levels could predict CAD. CAD patients had significantly higher levels of oxLDL compared to controls. 100% of female subjects with high oxLDL levels (above the 90th percentile of distribution in controls) had CAD (n=53, with a mean age of 60±9.1 years). Hypercholesterolemia (defined as total cholesterol >240mg/dL or LDL-C >130mg/dL) and dyslipidemia (HDL-C <35mg/dL and triglycerides >200mg/dL) were significantly more prevalent in CAD patients compared with controls

2.4 Exercise and the TC-HDL-Ratio

Exercise has been shown to have a positive effect on HDL-C concentrations (Durstine et al., 2001). Systematic reviews by Carroll and Dudfield (2004) and Durstine et al. (2001) analysed several early controlled studies and cross-sectional literature that examined the effect of exercise training alone on various metabolic risk factors, including lipid profile. Several studies involving longer-term aerobic training (12 months), combined with dietary intervention, did show improvements in lipoprotein lipids in men and women presenting with metabolic risk factors (Dengal, 1998). Carroll and Dudfield (2004) found that, within 20 study groups examining HDL-C responses to exercise training compared to no-treatment controls, the pooled estimate of the effect size was small but statistically significant (effect size 0.245). Participants were overweight/obese adults with dyslipidemic changes characteristic of the metabolic syndrome. The review by Durstine et al. (2001) concluded that a moderate to vigorous exercise training programme, consisting of a minimum threshold of 15-20 miles per week (or 1200 to 2200 kcal/week) is associated with 2-3 mg/dL increases in HDL-C, with greater changes occurring with additional training volume. Energy expenditures

towards the higher end of 2200 kcal/week are more likely to produce improvements in HDL-C (Durstine et al. 2001).

A study by Katzmarzyk et al. (2003) examined the contribution of regular exercises to changes in CVD risk factors. The sample included 621 sedentary, but otherwise healthy participants (aged 17-65), of whom 16.9% (n=105) presented with metabolic syndrome. Participants undertook a 20-week aerobic training programme that involved 3 sessions per week on a cycle ergometer. Among the participants with metabolic syndrome, 32 no longer presented with metabolic syndrome after exercise training and 16% had improved HDL-C, 43% had decreased triglycerides and 9% had improved fasting plasma glucose, with no differences between sexes. The study did not report LDL-C, total cholesterol or TC-HDL-ratio. However, the effect on HDL-C may provide an overall improvement to TC-HDL-ratio provided there was no significant increase in LDL-C.

2.5 Research into Lifestyle Intervention on Fertility and CVD Risk in PCOS

Research concerning exercise as an intervention for PCOS has mixed results, largely due to varying designs, intensity and outcome measures (Harrison et al., 2011). A recent systematic review and meta-analysis by Hagg et al. (2014) evaluated seven RCTs for the benefits of lifestyle intervention, including exercise and dietary intervention, on the reproductive and endocrine profiles of women with PCOS. Intervention groups were defined as exercise alone or exercise plus dietary intervention. The seven studies provided data on a total of 206 pre-menopausal women with PCOS and ranged from 12 to 48 weeks in duration. Six out of seven studies involved low to moderate intensity aerobic exercise (brisk walking or cycling up to 70% VO2max). Results showed that structured exercise alone improves levels of FSH, SHBG, total testosterone, androstenedione and the Ferriman-Gallwey score (a measure of the severity of hirsutism).

Another study of structured 30-minute treadmill sessions undertaken 3 times a week, for 6 months, compared to a dietary intervention, found larger improvements in ovulation rate (65% compared to 25% respectively) and a trend towards an increased pregnancy rate (6.2% compared to 1.7% respectively) (Palomba et al., 2008). Furthermore, given that structured exercise or regular physical activity is effective at improving insulin

resistance and the metabolic syndrome, independent of weight loss, exercise alone may be an effective form of treatment for lean women with PCOS in alleviating cardiovascular risk and improving insulin sensitivity (Hamdy, Goodyear & Horton, 2001; Harrison et al., 2011; Moran et al., 2009).

The addition of dietary restriction may provide supplementary benefits in terms of weight loss for overweight and obese women with PCOS, as well as improved free androgen index (FAI), LH levels (and improved reproductive function), improved lipoprotein-lipid profiles and decreased body fat (Hamdy et al., 2001; Harrison et al., 2011). In terms of the type of diet necessary, there is currently no evidence to suggest that modifying dietary macronutrient composition (i.e. low carbohydrate/high fat diet) offers additional benefits over conventional dietary measures (energy restriction) for weight loss (Moran et al., 2009).

A recent review by Frary et al.(2014) examined the potential for a low carbohydrate dietary intervention to improve fertility, weight loss and metabolic parameters in comparison to a standard dietary intervention in women with PCOS. In the review of 15 studies, it was indeed found that a low carbohydrate diet improved weight loss by an additional 15% in comparison to a standard dietary intervention. Other than this, a low carbohydrate diet was no more effective than standard calorie restriction in improving ovulation, conception, hyperandrogenism and insulin resistance (Frary et al., 2014).

Brown et al. (2009) conducted a study on a group of PCOS women, with 8 completing a programme of structured exercise and 12 remaining as controls. The exercise program consisted of an 8-12 week 'ramp-up' period, followed by a 12 week moderate-intensity exercise programme consisting of working at 40-60% of VO2peak, for approximately 3 hours and 24 minutes per week (at the caloric equivalent of walking 12 miles per week). The results showed that despite an absence of weight loss, various lipoprotein particle parameters associated with insulin resistance and cardiovascular risk were significantly improved (Brown et al., 2009). This included significant reductions in VLDL particles and medium/small HDL particles (less anti-atherogenic than large HDL), and increased large HDL and average HDL size in the exercise group compared to the controls (Brown et al., 2009).

Dyslipidemia has been largely studied in obese or overweight women with PCOS, since the dyslipidemia associated with obesity is exacerbated by the insulin resistance associated with PCOS (Wild, 2012). However, varying dyslipidemic states have also been noted in lean women with PCOS (Kim & Choi, 2013; Conway, Agrawel, Betteridge & Jacobs, 1992). In a study of obese and lean PCOS women compared with lean, non PCOS controls, lean women with PCOS were found to have reduced HDL and HDL2, which is reported to be the most anti-atherogenic subtype of HDL (Kim & Choi, 2013) than women with normal ovaries, putting them at an increased risk of developing cardiovascular disease (Conway et al.,1992). Furthermore, Legro, Kunselman and Danaif (2001) conducted a study of 195 white women with PCOS, categorised as either obese (with a BMI above or equal to 27) or non-obese (a BMI below 27), and ethnically matched control women. Of the 42 non obese PCOS women, total cholesterol and LDL levels were increased significantly compared to non-obese controls (n=27).

Hutchison and colleagues (2011) conducted a study on 13 PCOS women and 8 non-PCOS women, of whom completed 12 weeks of supervised, aerobic exercise. Each participant took part in 3 x 1-hour session per week, with sessions alternating between moderate intensity walking/jogging (70% of VO2max) and intermittent high intensity session, equivalent to 90-100% of VO2max. At baseline, PCOS women had a more atherogenic dyslipidemic profile, with abnormal HDL (significantly lower than non-PCOS) and higher triglycerides. Though it has been suggested that exercise alone (without dietary intervention) would not be sufficient to improve lipid profile, Hutchison et al. (2011) found significantly decreased triglycerides in PCOS women after the exercise intervention, even in the absence of weight loss. Despite there being no change in LDL and HDL levels in PCOS women after the intervention, these findings are promising and indicative of the value of physical activity in PCOS women, even without weight loss. The absence of statistically significant changes in HDL and LDL levels could be due to a small sample size, or it may highlight further the need to determine a dose-response relationship between duration and intensity of exercise and lipid changes in PCOS.

2.6 Exercise and Lipid Profile in PCOS

Previous research shows that an exercise intervention of as little as 12 weeks may have a positive effect on lipid profile and lipoproteins associated with both insulin resistance

and increased cardiovascular risk (Brown et al., 2009; Hutchison et al., 2011). This effect appears to be independent of weight loss and has important implications for non-obese women with PCOS, who have also been found to have varying states of dyslipidemia characterised by increased TG and small, dense LDL particles. Brown et al. (2009), suggests that, contrary to previous research which has focused on lipoprotein particle size as a risk factor, that particle volume may actually be more important. Therefore, rather than small LDL being more atherogenic, individuals with small LDL generally have elevated numbers of LDL, thereby increasing cardiovascular risk (Brown et al., 2009).

Harrison et al. (2011) conducted a systematic review of the research to examine whether exercise therapy could have an improvement on polycystic ovary syndrome. Eight studies were examined, each involving varying levels of moderate intensity physical activity, with a programme duration of 12 or 24 weeks. They consistently found improved ovulation, a reduction in insulin resistance of 9-30% and up to a 10% decrease in weight loss. These findings were not dependent on type, or frequency, of exercise and indeed, as demonstrated by Hutchison et al. (2011), need not be dependant on weight loss. However, it was found that weight loss was maximised in the exercise studies of a longer duration. This could be important when devising interventions for both obese and overweight women with PCOS compared with lean women with PCOS. That is, a higher importance may be placed on the duration of the intervention for those that are obese, in order to produce both positive effects on fertility and cardiovascular risk and to promote weight loss.

Ladson et al. (2012) suggested that the addition of metformin to lifestyle modification would be superior to lifestyle alone in improving ovulatory frequency, hyperandrogenism and insulin sensitivity in women with PCOS. However the study found no significant benefit on ovulation and circulating androgen levels; it did, however, find improved insulin sensitivity and increased bone mineral density with the addition of metformin to lifestyle modification.

2.7 PCOS and Oxidised LDL

Oxidised LDL has also been implicated in the initiation and progression of atherosclerosis (Macut et al., 2006). Various studies have detected elevated levels of

oxidised LDL in patients with coronary artery disease (Mosca et al. 1997; Holvoet et al., 1998). Clinical predictors of elevated oxidised LDL levels were female sex, a family history of early onset cardiovascular disease, increased body mass index (BMI) and body fat percentage, and lower levels of structured exercise (Mosca et al., 1997).

Therefore, healthy women may be more at risk of elevated oxidised LDL levels if they meet the criteria above, it appears that women with PCOS are at a higher risk independent of body weight, even if they are not hyperinsulinemic.
Few research papers have directly examined the link between oxidised LDL and PCOS, despite its widely accepted role in atherogenesis and cardiovascular disease, and acknowledgement that women with PCOS are at a greater CVD risk for these pathologies.

Macut et al. (2006) conducted a cross-sectional analysis that compared 179 overweight and normal weight, middle-aged women with PCOS with 56 age and BMI matched control participants. These investigators examined levels of oxidised LDL in conjunction with dyslipidemia, insulin resistance and androgen levels in PCOS women compared to healthy women. Also, whether these differed between normal weight and overweight women with PCOS. Blood samples were collected in the follicular phase of the menstrual cycle and analysed for fasting glucose, HDL-C, LDL-C, total cholesterol, TG, oxidised LDL, ApoA1, AopB, insulin, testosterone and SHBG. It was shown that levels of oxidised LDL were significantly higher in both PCOS groups in comparison to their healthy counterparts (Macut et al., 2006), thereby indicating that PCOS increases the risk of CVD in PCOS women of a normal weight.

Furthermore, it appeared that these levels of increased oxidised LDL were not mediated by hyperinsulinaemia, but that there were alterations in the lipid metabolism itself, independent of insulin levels (Macut et al., 2006); though basal insulin and homeostatic model index (HOMA) were significantly higher in overweight women with PCOS in comparison to normal weight women with PCOS, these were not found to be significant predictors of oxidised LDL concentrations in either group. In normal weight patients (with PCOS), AopB to ApoA1 ratio was the only independent predictor of oxidised LDL. In overweight patients, TC-HDL-ratio was the only independent predictor of oxidised LDL.

2.8 Exercise and Diet Intervention on AMH Levels in PCOS

The fact remains that it's no longer a case of whether dietary and exercise intervention can benefit fertility, metabolic flexibility and cardiovascular risk in women with PCOS, but rather what frequencies and intensities are needed to optimise the benefits (Hamdy et al., 2001). Furthermore, it appears important to consider AMH in conjunction with this, since AMH is a contributing factor to infertility in women with PCOS. Since levels of AMH in PCOS women have been shown to indicate the success of IVF treatment (Amer at al., 2013), it must be identified whether exercise and/or dietary restriction can lower serum AMH levels. Furthermore, AMH has been proposed as a clinical predictor of improvements in reproductive function after weight loss in women with PCOS (Thomson et al., 2009). Moran et al. (2007) demonstrated that plasma levels of AMH in overweight PCOS women were an indicator of improved menstrual function after a 32-week diet intervention, consisting of 8-weeks of energy restriction and 24-weeks of maintenance including fat counting or carbohydrate counting, that resulted in weight loss. Mean daily energy intake for the energy restriction phase was 4832.1±597.0 kJ. Levels of AMH were not measured post-intervention, however.

Despite research consistently indicating that women with PCOS have increased levels of AMH regardless of body composition (Amer et al., 2013; Cassar et al., 2014; Nybacka et al., 2013) and the importance of the role of AMH and its link with high androgen levels in reproductive dysfunction (Cassar et al., 2014), very few studies have examined whether lifestyle intervention can decrease levels of AMH. Current literature is based on studies with small sample sizes and a lack of consistency in design and baseline measures of AMH, subsequently resulting in inconclusive and sometimes contrasting results (Moran et al., 2007; Nybacka et al., 2013; Thomson et al., 2009).

With regard to a dietaryintervention alone, Thomson et al. (2009) measured AMH levels and menstrual cyclicity and ovulatory function in fifty-two overweight and obese women with PCOS, before and after a 20-week weight loss programme. They found that, while lower baseline AMH levels tended to predict greater improvements in reproductive function (in line with findings by Moran et al. in 2007) and weight loss, AMH levels themselves did not change significantly after the intervention, in both those participants who lost weight or saw improved reproductive function, and those that did not (Thomson et al., 2009).

One previous study by Moran et al. (2011) has reported a decrease in AMH levels in PCOS women, but not controls, after an exercise intervention. The pilot study consisted of 7 overweight women with PCOS and 8 overweight women without PCOS of comparable age, weight and BMI. Participants undertook a 12-week endurance exercise training programme (3 x 1-hour treadmill sessions per week). The PCOS group had a significant decrease in AMH levels (-13.2±11.7 pmol/l, p=0.025).

Conversely, the only other study that examined the effects of an exercise, dietary, and combined dietary and exercise intervention on AMH levels in women with PCOS found conflicting results (Nybacka et al., 2013). Nybacka et al. (2013) used a sample of 57 overweight PCOS women, with a total of 43 completing the respective lifestyle interventions. There were three intervention groups lasting for 4 months; a dietary restriction programme, an exercise programme and a combined diet and exercise intervention. The exercise program consisted of 45-60 minutes of moderate to vigorous intensity exercise, 2 to 3 times per week, and included walking, jogging, aerobics, swimming and strength training. The intervention was aimed at increasing physical exercise to a moderate level.

Despite a decrease in BMI and improvements in reproductive function for all 3 groups, there was only a significant decrease in AMH levels for the dietary group only. Furthermore, in line with both Moran et al. (2011) and Thomson et al. (2009), lower baseline AMH levels tended to predict greater improvements in reproductive function, independent of intervention group. The significant decrease in AMH levels in the dietary intervention group was linked to a significant decrease in free testosterone in the diet group, though there was an overall, but non-significant, decrease in all groups (Nybacka et al., 2013). This is in line with previous research which has shown an association between hyperandrogenism and AMH levels in women with PCOS, with higher AMH levels compared to those who are normoandrogenic (Moran et al., 2007; Thomson et al., 2009; Woo et al., 2012).

These findings are therefore in contrast to findings from both Moran et al. (2011) who found a decrease in AMH levels after an exercise intervention, and Thomson et al. (2009) who found no change in AMH levels after a 20-week weight loss programme. A possible explanation for this variation in results, given by Nybacka et al. (2013) is the

variation in baseline AMH levels for the 3 studies. Thomson et al. (2009) reported a mean baseline AMH level of 28 pmol/L (considered to be within the normal range) and subsequently found no change in AMH levels after the dietary intervention. Conversely, Moran et al. (2011) recorded a mean baseline AMH level of 59 pmol/L, twice as high as the pretreatment level in the study by Thomson et al. (2009). This could further indicate that AMH levels reflect the severity or phenotype of PCOS and is in line with low baseline AMH levels predicting both improved reproductive function after intervention (Moran et al., 2011; Thomson et al., 2009; Woo et al., 2012), and lower baseline AMH levels predicting greater success in IVF treatment in women with PCOS (Amer et al., 2013).

However, this analysis doesn't account for the variation in results found by Nybacka et al. (2013) and Moran et al. (2011) who reported somewhat raised baseline pretreatment AMH concentrations. A further explanation for this, suggested by Nybacka et al. (2013) is that the exercise programme implemented by Moran et al. (2011) was likely more intensive than that implemented by Nybacka et al. (2013). Further to this; a decrease in free testosterone was evident in the exercise and exercise dietary group, though not significant (Nybacka et al., 2013). This could suggest that dietary intervention is more effective at decreasing androgen levels and the exercise programme dose and or/intensity was not sufficient to reduce AMH levels. However, this remains inconsistent with the the differences observed in the study by Moran et al. (2011) and Nybacka et al. (2013). Moran et al. (2011) reported no significant reduction in hyperandrogenism despite improvements in AMH levels.

2.9 Research into Metformin in PCOS Treatment

Metformin use alone has been shown to improve regular menses and spontaneous ovulation in 61 and 62%, respectively, of women with PCOS and oligo/amenorrhea (Costello & Eden, 2003). However, there is sparse data supporting an improvement in pregnancy rate (Costello & Eden, 2003; Fleming & Sattar, 2007). Data also indicates improvement in body mass index (BMI), lower LDL and higher HDL levels and improved endothelial structure and function (Fleming & Sattar, 2007; Ladson et al., 2011). Various other research, including the largest trial of metformin alone in PCOS treatment and the Diabetes Prevention Program where metformin use was compared with a placebo, demonstrated a 2 to 3kg weight loss over six months (Ladson et al.,

2011; Legro et al., 2007). Therefore, though further well-designed, randomised controlled trials with an end point of pregnancy or live-birth are necessary to assess the role of metformin in the treatment of PCOS anovulatory infertility, it would seem that longer treatment with metformin may attenuate the development of T2D and the risk of cardiovascular events (Costello & Eden, 2003; Legro et al., 2007).

2.10 Rationale for Present Study

It is clear that further research is necessary to determine whether there is a link between physical exercise interventions and improvements in AMH levels (and by extension, improve reproduction function and the severity of PCOS). Therefore, the present study intends to add to this body of research by assessing AMH levels before and after an 8-week exercise intervention in participants with PCOS. The design has similar aspects to the study by Moran et al. (2007), with 3 x 1-hour treadmill sessions per week at 60% VO2max.

As a further point of analysis, the current study will also measure levels of oxidised LDL in the PCOS women for comparison with oxidised LDL levels in healthy controls. This will help to provide more information and build on previous research on the role of oxidised LDL in the increased risk of atherogenesis in women with PCOS (Macut et al. 2006). This current research therefore aims to provide clearer answers on whether an exercise intervention alone is effective in reducing cardiovascular risk in women with PCOS. The length of the intervention (8 weeks) will also help to determine a dose-response relationship with exercise duration and lipoprotein improvements, i.e. whether as little as 8 weeks is enough to affect change, or should 12 weeks be the minimum prescription.

Since AMH has been cited as a potential indicator to the severity of PCOS in terms of ovarian responsiveness to gonadotrophins and increased number of small antral follicles (Amer et al., 2013) – where ‘severity’ is aligned with reproductive capability – it may also be useful to determine whether serum AMH levels (and intervention induced changes) are associated specifically with dyslipidemia/lipoproteins and CVD risk in PCOS.

3. Methodology

The present study utilized the stored, available blood samples obtained from a prior studies conducted within in the Department of Sports, Health and Exercise Science at the University of Hull (SHES). The previous study examined the effect of an 8-week exercise intervention on endothelial function and cardiovascular risk in women with PCOS compared to healthy controls.

Therefore, the following methodology outlines the previously conducted recruitment process, experimental design and procedures. All anthropometric data and pre, mid and post fitness measures used in the present study are taken from the prior study. The subsequent measurement and analysis of serum AMH, lipid lipoprotein sub-fractions and oxidized LDL were devised and conducted by the author.

3.1 Participant Characteristics and Recruitment

After obtaining ethical approval from Hull and East Yorkshire NHS Trust R&D and the SHES departmental ethics committee at the University of Hull, the previous author recruited healthy controls through advertisement at the university, volunteers from staff and student email services and word of mouth from local hospital staff. PCOS participants were recruited through newspaper advertisements and e-bulletins, as well as letters to patients who were seen at the Hull and East Yorkshire Diabetes, Endocrinology and Metabolism clinic with the last 5 years. Written informed consent was obtained prior to taking blood samples.

All participants were between 18-40 years of age, with pre-menopausal women with PCOS who were eligible if they met the criteria of PCOS described by the Rotterdam consensus (Ladson et al., 2011). Patients had to present with at least two out of the following: anovulation/oligomenorrhea, clinical hyperandrogenism and polycystic ovaries. Healthy controls had regular menses and no evidence of clinical hyperandrogenism. Exclusion criteria included regular exercise (≥ three times per week for the past three months), smoking, pregnancy, history of cardiovascular, renal, hepatic or thyroid disease, diabetes mellitus, family history of sudden death or any existing injury or medical condition that was a contraindication to exercise.

3.2 Experimental Design

Initially, the study included 25 female volunteers; 14 women with PCOS and 11 healthy controls. Each participant was successfully screened and commenced the exercise programme. Completion of the exercise programme was defined as the undertaking of 8 weeks of exercise sessions with at least 50% attendance each month, as well as the completion of the final, post assessment. Participants who did not complete the intervention were not included in data analysis. Other reasons for withdrawal from the study included falling pregnant, the use of any medications detailed in the exclusion criteria or if her consultant physician or general practitioner requested that it was in the best interest of the participant for her to be withdrawn.

3.3 Baseline Assessment

Within one week before the baseline assessment (pre testing), participants attended the Michael White Diabetes Centre in Hull, UK, where fasting venous blood samples were taken. They then attended the SHES department in order to carry out pre-exercise questionnaires and consent forms. They were then instructed to abstain from alcohol, caffeine and exercise 24 h prior to the testing.

Anthropometric measures were taken included stature, body mass and waist to hip measurements. After this, participants performed a ramped maximal exercise test (VO2max) to determine the target workload for subsequent exercise sessions during the exercise programme.

The test was performed on a Woodway ELG55 motorised treadmill (Woodway, Weil and Rhein, Germany) and was set at 1% gradient throughout the test. Beginning with a speed of 4.5 $km \cdot hr^{-1,}$ participants were encouraged to participate until volitional exhaustion and were therefore encouraged to provide maximal effort. Each minute, a rating of perceived exertion, heart rate and VO2/kg were manually recorded. Gas collection was taken using an Oxycon Pro Metabolic System (Jaegger, Hoechberg, Germany) and participants wore a facemask with a rotary flow sensor in the mouthpiece for gas collection. The Oxycon Pro was calibrated using a 3 L Hans Rudolph volume calibrating syringe (Hans Rudolp model 5530, Kansas, USA). Participants also wore a

Polar Heart Rate Monitor (Polar Electro, OY, Finland) to monitor heart rate throughout the exercise test.

3.4 Blood Samples

Venous blood samples were taken by a trained consultant via a standard venepuncture procedure from the antecubital vein. Venous blood samples were collected at the Michael White Diabetes Centre in Hull, UK (by the same consultant each time). Samples were taken no longer than one week prior to the baseline assessment, after four weeks of the exercise programme and for a final time no longer than a week after completing the exercise programme. The previous investigator analysed the samples immediately and was sure to never leave the samples on ice for longer than 30 minutes. The samples were then refrigerated (-80) in the specialist assay lab at Manchester Royal Infirmary until analysis.

3.5 Exercise Programme

The exercise programme consisted of 3 supervised 1 hour sessions per week for 8 weeks on a motorised treadmill. The treadmill initially used was a Woodway ELG55 (Woodway, Weil an rhein, Germany) but due to maintenance in the facility, the remainder of the programme was carried out on a HP Cosmos Pulsar Treadmill (H/P/Cosmos). Participants performed all sessions at, or as close to 60% VO2max as possible, after a 5 minute warm up at 4.5 $km \cdot hr^{-1}$. The intensity of the session was then adjusted via the speed of the treadmill in order to pertain to within ± 2.5% of the target oxygen uptake. VO2/kg was measured after the warm up for 10 minutes and then a further collection was made at 40 minutes for a period of 5 minutes. Measurements of HR and RPE were taken at 15 minute intervals throughout the session and participants were encouraged to continue if they felt fatigued by reducing the intensity for a period of time to allow them to continue. However, they were allowed to stop at any time if they felt they could not continue due to injury or fatigue. After a 5-minute cool down at 4.5 $km \cdot hr^{-1}$, participants were permitted to leave once their HR had returned to 120% of basal levels.

In order to maintain the correct exercise intensity for the sessions, a mid-point assessment was carried out that was identical in procedure to the baseline assessment.

Measurements demonstrated any changes within participants VO2max, and adjusted exercise intensity accordingly during the following sessions to ensure they exercise at their target exercise intensity (percentage of VO2/kg). A post assessment was also carried out at the end of the programme which was a repeat of the pre and mid-point assessments.

3.6 Secondary Measurement of Blood Samples

The AMH ELISA (MyBioSource) uses the competitive enzyme immunoassay technique. The microtiter plate is pre-coated with goat-anti-rabbit antibody and samples are added to the plates wells with an antibody specific for AMH and Horseradish Peroxidase (HRP) conjugated AMH. The competitive inhibition reaction is observed between HRP labeled AMH and unlabeled AMH with the antibody. After the addition of substrate, colour develops opposite to the amount of AMH in the sample. The detection range is 1 ng/ml-75 ng/ml with a sensitivity typically less than 0.5 ng/ml.

The OxLDL ELISA (OxiSelect) is an enzyme immunoassay kit contains a copper oxidized LDL standard and has a detection sensitivity limit of 150 ng/ml.

3.7 Summary of ELISA Methodology

For the AMH ELISA, 50 μL of sample is added per well, with 50 μL of HRP conjugate. 50 μL of antibody is then added. These are mixed and incubated for 60 minutes at 37 degrees Celsius. Each well is then aspirated and washed for a total of three washes. 50 μL of substrate A and B is then added to each well and mixed, incubated for 15 minutes at 37 degrees Celsius, and then 50 μL of stop solution is added. The optical density of each well is then determined within 10 minutes using a microplate reader set to 450 nm.

For OxLDL ELISA, 100 μL of sample is added to the Anti-CML Antibody Coated Plate, covered with a plate cover and incubated at room temperature for 1 hour on an orbital shaker. Microwell strips are washed 3 times with 250 μL of buffer with thorough aspiration between washes. 100 μL of diluted Biotinylated Anti-Human ApoB-100 antibody is then added to each well and incubated for 1 hour. Strip wells are washed 5 more times. 100 μL of diluted Streptaviden-Enzyme Conjugate is added to each well and incubated at room temperature for 1 hour. Strip wells are washed 5 more times.

Finally, 100 μL of room temperate Substrate is added to each well and incubated on an orbital shaker for 5-20 minutes. Read absorbance of each microwell in spectrophotometer set to 450 nm.

3.2 Statistical Analysis

Two separate two-factor mixed ANOVAs were used to determine the main effects of both time (pre and post intervention), and the participants' grouping (PCOS or control) on AMH and oxLDL levels, as well as the interaction effect of time and participants' grouping on AMH and oxLDL.

4. Results

4.1 Baseline Statistics

Blood samples were drawn from participants on two occasions pre-intervention, and two occasions post-intervention, as outlined in the methodology chapter. Values from the two visits were averaged into single baseline and post measurements. Although there were initially 25 (14 PCOS and 11 non-PCOS) participants, several participants dropped out or did not attend both blood sample sessions. These participants were removed from the analysis.

At baseline, there SBP, WHR and WC were significantly higher in the PCOS group compared to controls. VO2max was significantly higher in the control group than PCOS.

Table 1. Baseline characteristics between control and PCOS women. Body mass, SBP, DBP, VO_{2max}, BMI, WHR, and WC are compared at baseline between the two groups.

Variables	Control baseline (n = 10)	PCOS baseline (n = 11)	P value
Body mass (kg)	71.04 ± 16.42	85.45 ± 18.91	0.080
SBP (mmHg)	123.00 ± 11.44	132.36 ± 11.47	0.027*
DBP (mmHg)	77.50 ± 9.30	81.82 ± 11.21	0.290
VO_2max ($ml \cdot min \cdot kg^{-1}$)	36.26± 6.38	26.32 ± 4.63	<0.001*
BMI (kg/m^2)	25.92 ± 5.39	31.15 ± 6.30	0.056
WHR	0.79 ± 0.07	0.86 ± 0.06	0.019*
WC (cm)	83.01 ± 14.20	98.05 ± 16.35	0.033*

* Significantly different between groups ($p < 0.05$). All data represented as mean ± SD.

4.2 Effect of Exercise Intervention on Measured Variables

Some variables were missing after secondary analysis for AMH and oxLDL. This left baseline and post AMH and oxLDL measurements for 10 PCOS and 10 non-PCOS women.

Baseline and post exercise intervention statistics can be seen below in Table 2. Two-factor mixed ANOVA showed no significant main effects of grouping, $F(1, 18)= .524$, $P=.478$, time, $F(1, 18)=0.93$, $P= .195$, or grouping x time, $F(1, 18)=.093$, $P=.764$ on AMH values.

A second two-factor mixed ANOVA showed no significant effects of grouping, $F(1, 19)=.372$, $P=.549$, time, $F(1,19)=2.1$, $P=.165$, or grouping x time on oxLDL values, $F(1, 19)=.668$, P.424.

Table 2. Baseline and post 8 week aerobic exercise training AMH and oxLDL concentrations for PCOS and non-PCOS groups. Data presented as mean ± standard deviation.

	PCOS (n=10)		Non-PCOS (n=10)	
Variables	**Pre**	**Post**	**Pre**	**Post**
AMH (pmol/l)	33.11 ± 21.69	33.72 ± 21.66	26.56 ± 17.3	27.44 ± 17.65
oxLDL (U/l)	30.81 ± 7.95	31.27 ± 8.55	31.48 ± 8.34	34.11 ± 9.62

Table 3 displays changes in biomarkers from baseline, to mid intervention, to post exercise intervention. There were no changes in body mass in the control group. In the PCOS group, body mass decreased significantly from pre to mid intervention and significantly decreased from pre to post intervention. Body mass improved from 85.45kg to 84.45kg mid intervention, and then to 84.04kg upon completion of the exercise intervention. SBP significantly improved in controls from baseline (123mmHg) to post intervention (117.50mmHg). SBP also significantly improved in the PCOS group from 132.35mmHg, to 128.73mmHG mid intervention. It then further decreased to 124.91mmHg post intervention. There were no significant changes to DBP in controls. In the PCOS group, DBP significantly improved from pre (81.82mmHg) to

post intervention (76.64mmHg). Both groups saw a significant improvement in VO2max upon completion of the exercise programme. The control group increased from 36.26 $ml \cdot min \cdot kg^{-1}$ to 39.21$ml \cdot min \cdot kg^{-1}$ post intervention. Significant improvements were seen in the PCOS group from 26.32 $ml \cdot min \cdot kg^{-1}$ at baseline to 26.43 $ml \cdot min \cdot kg^{-1}$ at mid intervention, followed by a further improvement to 29.71 $ml \cdot min \cdot kg^{-1}$ post intervention. Finally, while no changes were seen in the control group for BMI or WC, there were significant improvements in the PCOS group for both variables. BMI improved from 31.1kg/m^2 to 30.79 kg/m^2 mid intervention, with a further reduction to 30.69kg/m^2 post intervention, and WC improved from 96.97 cm mid intervention to 96.12cm post intervention.

Table 3. Clinical characteristics of the control and PCOS women at baseline, mid, and post 8 weeks of aerobic exercise training. Body mass, SBP, DBP, VO2max, BMI, WHR and WC are compared between both groups.

	Non-PCOS (n = 10)			PCOS (n=11)		
Variables	**Pre**	**Mid**	**Post**	**Pre**	**Mid**	**Post**
Body mass (kg)	71.04 ± 16.42	70.66 ± 16.19	70.11 ± 15.83	85.45 ± 18.91	84.45 ± 19.02*	84.04 ± 19.54*
SBP (mmHg)	123.00 ± 11.44	120.50 ± 10.28	117.50 ± 7.65*	132.36 ± 11.47	128.73 ± 11.03*	124.91± 10.40***
DBP (mmHg)	77.50 ± 9.30	75.00 ± 6.82	73.10 ± 5.43	81.82 ± 11.21	81.36 ± 12.36	76.64 ± 8.82***
VO_2max ($ml \cdot min \cdot kg^{-1}$)	36.26 ± 6.38	37.49 ± 6.96	39.21 ± 5.82*	26.32 ± 4.63	26.43 ± 4.09	29.71 ± 5.32***
BMI (kg/m^2)	25.92 ± 5.39	25.78 ± 5.36	25.58 ± 5.18	31.15 ± 6.30	30.79 ± 6.32*	30.69 ± 6.48*
WHR	0.79 ± 0.07	0.78 ± 0.06	0.79 ± 0.07	0.86 ± 0.06	0.85 ± 0.07	0.85 ± 0.06
WC (cm)	83.01 ± 14.20	82.41 ± 14.19	82.16 ± 14.57	98.05 ± 16.35	96.97 ± 17.90	96.12 ± 14.67**

Data are represented as mean ± SD.
* Significantly different compared to pre ($p < 0.05$).** Significantly different compared to mid ($p < 0.05$).

5. Discussion

5.1 PCOS Phenotypes

Although a widely accepted clinical syndrome, PCOS has a range of phenotypic characteristics that present themselves, more or less, without a constant variable or 'gold standard' of diagnosis. For example, the Rotterdam criteria has been utilised as a diagnostic tool since 2003, and stipulates that 2 out of 3 symptoms need to be present. This can be any combination of the 3, leading to several different phenotypes of PCOS that each have various complications specific to the phenotype. Table 4 demonstrates the 4 possible main phenotypes (A-D) as based on the Rotterdam criteria.

Table 4. Possible phenotypes of PCOS based on combinations of diagnostic criteria. Adapted from Dewailly (2016).

Oligo- and/or anovulation	Hyperandrogenism	Polycystic ovaries	Phenotype
yes	yes	yes	A
yes	yes	no	B
no	yes	yes	C
yes	no	yes	D

An important limitation of the Rotterdam criteria is that it excludes women with asymptomatic polycystic ovaries from the diagnosis of PCOS. This is important because various studies have revealed a prevalence of asymptomatic polycystic ovaries among 20-30% of the general female population, exhibiting neither hyperandrogenism nor oligo-anovulation (Hart, Hickey & Franks, 2004).

However, the concept of the Rotterdam criteria is useful for identifying multiple phenotypes of PCOS, and allows for the identification of more 'moderate' or latent types of PCOS (such as those without oligo-anovulation or hyperandrogenism), the way that clinicians identify each symptom is not 'standardised' (Dewailly, 2016).

For example, there is no formal definition of biological hyperandrogenism. While its clinical definition outlines three main symptoms (hirsutism, acne and alopecia), some clinicians may believe the presence of acne or hirsutism alone to be a key indicator without eliminating other causes of both that don't stem from hyperandrogenism (Dewailly, 2016). Furthermore, the FAI is artificially increased in conditions of hyperinsulinemia, which may bias the recognition of hyperandrogenism, particularly in obese women (Dewailly, 2016).

The same issue exists within the diagnosis of polycystic ovaries. While the threshold set out in the 2003 Rotterdam consensus is 12 follicles per ovary, newer ultrasound technologies allow this threshold to easily be surpassed and thus may lead to the overestimation of polycystic ovaries in the general population (Dewailly et al., 2011).

Clearly, it is important for authors to include the specific diagnostic criteria for PCOS subjects when conducting research. It may also be prudent for researchers to separate PCOS subjects into phenotypes to preserve the validity of results, because each phenotype has varying clinical and biological symptoms. It might also be important to identify whether women in the control group have asymptomatic polycystic ovaries, since the prevalence of these women may be up to 30% and including them may limit the power of statistical procedures (Dewailly et al., 2011). More pressingly, women with asymptomatic polycystic ovaries are not sub-fertile, and yet they often exhibit increased AMH levels and decreased FSH levels at baseline (Catteau-Jonard et al., 2012). Inclusion of these women in the control group as women without PCOS certainly has the propensity to compromise the validity of the results and, furthermore, raises questions about whether polycystic ovaries are a normal variant or rather a 'silent' phenotoype of PCOS (Catteau-Jonard et al., 2012).

Accordingly, it has been suggested that the serum AMH assay may emerge as the approved polycystic ovary marker, since it is increasingly shown to be an accurate substitute for follicular count (Dewailly, 2016). This will not only allow an accurate identification of polycystic ovaries and predicted ovarian responsiveness to IVF treatment for women with PCOS (Amer et al., 2013), but will also help to identify those asymptomatic women in the control group. Females with asymptomatically raised AMH should be excluded to preserve the validity of findings. It is therefore important that a universally agreed threshold of AMH is defined.

The PCOS participants in the current study were selected as detailed in chapter 3. Participants were pre-menopausal, aged 18-40 years and eligible if they met the characteristics of PCOS as described by the Rotterdam criteria. Control subjects had regular menses and no evidence of clinical or biochemical hyperandrogenism. Other causes of were eliminated before the diagnoses were made (Dewailly, 2016). Furthermore, it is not clear what methods were used to identify hyperandrogenism among control groups. Additionally, it may be possible that women in the control group had asymptomatic polycystic ovaries.

5.2 The Effects of an Exercise Intervention on AMH Levels

As previously described, AMH is believed to be an accurate substitute for follicular count in the ovary (Dewailly, 2016). To recap, AMH is produced by granulosa cells of the follicle and impedes the transition from primordial follicles into growing, primary follicles (Hehenkamp et al., 2006). AMH limits the sensitivity of the small, antral follicles that start to grow (under the influence of FSH) at the luteofollicular transition of the menstrual cycle (Hehenkamp et al., 2006). Once the antral follicles in the cohort have reached a diameter of 6mm, AMH production decreases and is thus absent in the dominant follicle (Weenen et al., 2004). Therefore, the production of AMH by small, antral follicles is believed to determine measurable AMH levels and to represent the size of the FSH-sensitive cohort; AMH values in both prepubertal girls are low, and undetectable in post-menopausal women (Hehenkamp et al., 2006).

In addition, AMH levels are presumed to be independent of the menstrual cycle and research has shown no consistent fluctuations patterns, in contrast to FSH, LH and estradiol (Hehenkamp et al., 2006).

Though the dominant follicle will show reduced AMH production as it grows beyond 6-8mm, the remaining antral follicles in the cohort will not reach the stage in which production of AMH becomes halted. Furthermore, in the case of IVF where the majority of follicles are driven into dominant growth through ovarian hyperstimulation, a clear reduction in AMH can be seen, while the remaining antral follicles in the cohort continue to be correlated with AMH levels (Fanchin et al., 2003). It therefore appears

that in order to reduce AMH levels, smaller follicles must be driven into larger stages, thereby decreasing their capacity to produce AMH (Fanchin et al., 2003).

This may provide some explanation for the previously inconsistent findings with regard to the effect of exercise interventions on serum AMH levels. That is, the intervention may reduce bio-available testosterone and improve hyperinsulinemia (Nybacka et al., 2013) which acts as a co-gonadotropin to stimulate antral follicle growth, preventing the over-stimulation of follicles to produce a high antral count. However, it is possible that before the consequent decline in AMH levels can be seen, the remaining cohort of antral follicles must first reach the stage in synthesis where their capacity to produce AMH is halted. One study with a longer intervention of 12 weeks has previously shown a decrease in AMH levels after an endurance exercise training program (3 x 1 hour treadmill sessions per week) in 7 overweight women with PCOS compared to age and BMI-matched controls (Moran et al., 2011). The women in the study also saw a significant improvement in insulin sensitivity following the exercise intervention.

Indeed, the present PCOS study involving only an 8 week intervention showed no significant improvement in mean AMH levels from baseline after the intervention, which would provide support for this alternative hypothesis.

This is consistent with two separate reports of the limited number of studies that aimed to evaluate the effect of an exercise or weight loss intervention on AMH levels in women with PCOS. Nybacka et al. (2013) analysed the effect of an exercise intervention, dietary intervention, and combined exercise and dietary intervention on AMH concentrations in a sample of 43 overweight women with PCOS. The intervention included 45-60 minutes of moderate to vigorous intensity exercise, 2-3 times per week, for 4 months. There was only a significant decrease in AMH levels in the dietary intervention group, despite a decrease in BMI and improvements in reproductive function for all 3 groups. Furthermore, the decrease in AMH levels was linked to a significant decrease in free testosterone in the dietary intervention group. In contrast, there was no significant improvement in free testosterone evident in the other 2 groups including an exercise component (Nybacka et al., 2013).

Additionally, a 20-week weight loss programme for 52 overweight and obese women with PCOS, involving a hypocaloric dietary programme, showed no improvements in

AMH levels in comparison to baseline (Thomson et al., 2009). This was accompanied by significant reductions in WC, fasting insulin and testosterone surrogates (FAI and SHBG). If the issue of intervention length prevented significant improvements in AMH concentrations in the present study, it might be expected that 16-20 weeks would be sufficient in length to allow changes to the antral follicle count once testosterone levels were reduced. However, Thomson et al. (2009) and Nybacka et al. (2013) did not demonstrate this within appropriate interventions. Therefore, there may be other factors at play that determine the success of the intervention on AMH levels.

Baseline testosterone and AMH concentration levels may be influential in the response of AMH to lifestyle interventions. On review, there are 4 studies (including the present study) that have examined the effect of exercise/weight loss intervention on AMH levels. The table below demonstrates the 4 studies and summarises the key characteristics of the investigations, including the length of intervention, baseline AMH levels and hormonal/metabolic responses.

Table 5. Comparison of studies analysing the effects of exercise and/or dietary interventions on AMH levels.

Study	Intervention Length	Intervention Type	Baseline AMH	Sig. Improvement in AMH	Sig. Improvement in testosterone
Present study	8 weeks	Exercise	33 pmol/L	No	N/A
Moran et al. (2011)	12 weeks	Exercise	59 pmol/L	Yes	No
Nybacka et al. (2013)	16 weeks	Exercise, dietary and combined	66 pmol/L (exercise), 74 pmol/L (dietary), 71 pmol/L (combined)	Only in dietary group	Only in dietary group
Thomson et al. (2009)	20 weeks	Dietary	28 pmol/L	No	Yes

Indeed, intervention length alone is not enough to account for the success of an intervention, as demonstrated by the significant AMH improvement in the 12 week study (Moran et al., 2011), in comparison to non-significant AMH concentration changes in 16 and 20 week studies (Nybacka et al., 2013; Thomson et al., 2009). However, a notable point is the range in AMH levels at baseline. While 'normal' levels can range between 5-43 pmol/L (Nybacka et al., 2013), women with PCOS are known to have two to threefold the amount of PCOS women (Amer et al., 2013; Nybacka et al., 2013). In both the present study, and Thomson et al. (2009), both baseline AMH levels in PCOS women were well within the normal range. Additionally, in the present study, mean baseline AMH levels in the control group were 26.56 ± 17.3. This was not significantly lower than the PCOS group at baseline.

This low level at baseline, that is not considered to be outside of the normal range, may well help to explain the lack of success of the intervention. However, when high AMH levels have been so frequently linked with the high antral follicle count associated with PCOS (Dewailly, 2016), the question remains of why the sample size in the present study had such a 'low' baseline concentration of AMH. The small sample size may be responsible. Given that there is such a large range of normal AMH values (from 5-43 pmol/L), it is clear that there is large, individual variation in AMH levels. This is demonstrated by the mean and standard deviation of both the PCOS group and controls in the present study at baseline (33.11 ± 21.69 pmol/L versus 26.56 ± 17.3 pmol/L, respectively). The minimum and maximum recorded AMH concentration in the PCOS group at baseline were 0.64 pmol/l and 73.52 pmol/L respectively. Therefore, since the sample only had 10 participants, it is possible that the mean was lowered by the smallest values.

However, it is perhaps more likely that the reason for the 'low' baseline AMH concentrations in PCOS group comes back to the PCOS phenotypes identified in the previous chapter. The Rotterdam consensus allows for a PCOS diagnosis if 2 out of the 3 conditions are met (oligo/anovulation, hyperandrogenism, and polycystic ovaries). This means there are 4 possible phenotypes of PCOS. Those in phenotype 'D' can be given a PCOS diagnosis if they present with oligo/anovulation and polycystic ovaries, but without the presence of hyperandrogenism. While trials involving PCOS usually test for each condition, it is not clear what proportion of the sample pertains to each phenotype. Without this specific information regarding the phenotypes of the PCOS

group, it may be difficult to identify which PCOS etiology could be improved by the 8 week exercise intervention.

It is therefore likely that any given sample involves a proportion of women with the 'D' phenotype of PCOS. The proposed mechanism responsible for high AMH levels suggests that women with PCOS have increased intrinsic insulin resistance (Hutchison et al., 2011), or an enhanced ovarian sensitivity to insulin (Vrbikova et al., 2004). Insulin acts as a co-gonadotrophin to stimulate ovarian androgen production by reducing levels of SHBG, thereby resulting in higher levels of bioavailable testosterone (Legro, 2012; Norman et al. 2004). In turn, high levels of testosterone stimulate the development of antral follicles, thereby leading to high levels of AMH (Cassar et al., 2014; Woo et al., 2012). Some studies presented in table 5 may provide support for this theory. Nybacka et al. (2013) saw a significant improvement in testosterone levels only in the dietary group, accompanied by a significant decrease in AMH. The researchers suggested that their exercise intervention had not been of sufficient intensity to reduce testosterone levels, unlike in the study by Moran et al. (2007) (Nybacka et al. 2013). Thomson et al. (2009) saw a significant decrease in testosterone across all subjects, but no change in AMH levels; however, AMH levels at baseline were in the normal range to begin with, thus a proportion of subjects may have fallen into the 'D' phenotype category and affected the results. Testosterone levels were not measured in the present study which means this theory cannot be tested against the PCOS sample.

Thus, if women pertaining to the 'D' phenotype of PCOS, without hyperandrogenism, were included in the PCOS sample of the present study, they may not have had high AMH levels to begin with. The data on which criteria were met by each individual participant in the study is not available for this analysis.

In addition to potentially having AMH levels within the normal range, phenotype D has been shown to have a worse metabolic profile and increased cardiovascular risk factors than other phenotypes. Women presenting with hyperandrogenism, including the ovulatory phenotype, have a higher incidence of insulin resistance and worse lipid profile than women with the normo-androgenic phenotype, even despite comparable distributions of body weight (Dewailly, 2016; Hayek et al., 2016). It therefore remains to be questioned whether phenotype 'D' should be included in research that aims to

reduce AMH levels and cardiovascular risk, since phenotype 'D' is distinctly different from the other phenotypes that involve hyperandrogenism.

This raises important questions in relation to the selection and diagnosis of participants for PCOS studies.

Dewailly (2016) suggested that the diagnosis of PCOS using the Rotterdam consensus must always be a diagnosis of exclusion. That is, the clinician must systematically search for, and eliminate, any other condition that may give hyperandrogenism and/or oligo/anovluation. It is also possible that women in the control group had asymptomatic polycystic ovaries, since these are prevalent in 30% of the population (Dewailly et al., 2011). It was ensured that controls had regular menses, however, women with asymptomatic polycystic ovaries may not be subfertile, and may have regular cycles, and yet they may still have high AMH levels which can affect the statistical power of a comparison of means between the PCOS and control groups (Catteau-Jonard et al., 2012; Dewailly et al., 2011).

In summary, the present study adds to a body of research with inconsistent findings regarding the effect of exercise interventions on AMH levels. However, it provides further support for the suggestion that an intervention may need to be longer in length to allow for changes in ovarian morphology (i.e. reduced antral follicle count) that may subsequently lead to a decrease in AMH levels. Additionally, it is suggested that studies differentiate between the phenotypes of PCOS within their samples when presenting baseline and post intervention variables. This may allow for interactions and mechanisms to be seen and explained more clearly. Finally, the importance of identifying controls that do not present with asymptomatic polycystic ovaries is emphasised, in order to ensure an accurate comparison of AMH levels between controls and oligo/anovulatory women with PCOS.

A possible solution to this, as suggested by Dewailly (2016), is to use AMH levels as a polycystic ovary marker, since it is increasingly shown to be an accurate substitute for follicular count and may preserve the validity of results. Furthermore, AMH levels could also be used to define inclusion criteria of women with PCOS into future studies. That is, if a study is aiming to reduce high levels of AMH, women with serum AMH levels with the normal range of 5-43 pmol/L (Nybacka et al., 2013) should be included.

Similarly, in order to provide further information on the link between hyperinsulinemia, hyperandrogenism and high levels of AMH, measures of fasting insulin and/or insulin resistance (such as HOMA-IR), and hyperandrogenism (such as SHBG and/or free testosterone) should be included alongside AMH. The lack of availability of these measurements in the present study is a current limitation to understanding the lack of success of the intervention.

5.3 PCOS and Cardiovascular Risk

Though obesity is not a cause of PCOS, and neither is the mere presence of PCOS sufficient to cause obesity, there is a high prevalence of obesity among women with PCOS. Its prevalence varies depending on environmental factors, ethnic backgrounds and lifestyle (Hayek, Bitar, Hamdar, Mizra & Daoud, 2016), but reaches 80% in the United States and 50% outside of it (Balen et al., 1995). Certainly, there is an interaction between the two, whereby obesity exacerbates insulin resistance and features of metabolic syndrome, and women with PCOS have increased visceral and subcutaneous fat distribution brought about by increased androgen production (Hayek et al., 2016).

Additionally, women with PCOS present with an atherogenic lipid profile, consisting of elevated LDL-C and triglycerides, and decreased levels of HDL-C (Hutchison et al., 2011). This cardiovascular profile is associated with various complications including atherosclerosis, arterial stiffness and endothelial dysfunction (Hart & Norman, 2006), in both lean and overweight women with PCOS, even in comparison to BMI-matched controls (Balen et al., 1995; Sattar, 2006).

Certain phenotypes of PCOS have been shown to have a worse metabolic profile and increased cardiovascular risk factors than others. That is, those women presenting with hyperandrogenism, including the ovulatory phenotype, have a higher incidence of insulin resistance and worse lipid profile than women with the normo-androgenic phenotype, even despite comparable distributions of body weight (Dewailly, 2016; Hayek et al., 2016). A cross-sectional study of 2,288 women with PCOS, of reproductive age (18-45 years), revealed that hyperandrogenic women with PCOS (n=1210) presented with a higher incidence of CV risk factors including obesity, insulin

resistance and metabolic syndrome, in comparison with normo-androgenic women with PCOS (Daan et al., 2014).

Indeed, cardiovascular events may be more likely post-menopause in women with PCOS in comparison to women without. An analysis by Shaw and colleagues (2008) considered risk of CV events in post-menopausal with clinical features of PCOS (Shaw et al., 2008). Of 390 post-menopausal women, 104 had clinical features of PCOS including pre-menopausal irregular menses and current biochemical evidence of hyperandrogenemia. Women with PCOS were significantly more diabetic, obese, had a higher incidence of metabolic syndrome, and a higher incidence of CAD. Cumulative 5-year CV event-free survival was 78.9% among women with PCOS and 88.7% among women without PCOS. PCOS emerged as a significant predictor of diabetes, waist circumference, hypertension and CAD (Shaw et al., 2008).

The baseline characteristics of the participants in the current study reflects the literature with regard to an increased prevalence of CV risk factors in PCOS compared to controls. Women with PCOS had significantly higher waist circumference (98.05±16.35cm versus 83.01±14.20cm), waist to hip ratio (0.86±0.06 versus 0.79±0.07) and systolic blood pressure (SBP) (132.36±11.46mmHg versus 123.00±11.44mmHg). Hypertension is well recognised as a risk factor for CV (Assmann, 1982). Hypertension is defined as a SBP above 140mmHg (NHS, 2014). While the mean SBP of the PCOS group does not reach 140mmHg, they are at a higher risk to develop hypertension than controls.

The results from the present study indicate that the 8-week exercise programme improved several variables associated with cardiovascular risk in the PCOS group. Significant improvements ($P<0.05$) were seen in body mass, SBP, diastolic blood pressure (DBP), BMI, and WC in the PCOS group post-intervention compared to baseline. VO2max was significantly improved in both groups in comparison to baseline. In controls, the only other variable to significantly improve from baseline to post-intervention was SBP.

This indicates an improvement in cardiovascular risk for the PCOS group and is in line with various research that demonstrates the efficacy of an exercise programme in improving cardiovascular risk factors in PCOS. Certainly, improvements in BMI, body

mass and waist circumference after an exercise intervention have been associated with improved insulin resistance and lipid variables in women with PCOS (Bruner, Chad & Chizen, 2006; Thomson et al., 2008). Improvements in lipid variables have also been seen in women with PCOS after an exercise intervention without significant weight loss (Brown et al., 2009).

Thomson et al. (2008) studied the effects of a 20-week intervention on 94 overweight and obese women with PCOS (age 29.3 ± 0.7 years, BMI 36.1 ± 0.5). Participants were randomised to either diet only (high protein, energy restricted to 5000-6000 kJ/day, n=30), diet and aerobic exercise (walking/jogging for 5 sessions per week, progressing from 25-30 minutes at 60-65% HRmax during the first week and 45 minutes at 75-80% HRmax by study end, n=31) or diet and combined aerobic-resistance exercise (aerobic exercise programme 3 times per week, and 2 days per week performing a progressive resistance training programme at 50-60% 1RM during the first 2 weeks, and increasing to 65-75% 1RM by study end, n=33). All interventions significantly reduced weight, waist circumference, blood pressure, triglycerides, total cholesterol, LDL-C, fasting glucose, fasting insulin, testosterone, FAI and SHBG (Thomson et al., 2008). Despite a significant additional improvement in fat mass and preservation of fat free mass in both exercise-related conditions, there were no other significant differences in cardiometabolic parameters between groups, suggesting the improvements were primarily related to weight loss (Thomson et al., 2008).

In a similar study, Bruner and colleagues (2006) assessed the effects of exercise and nutritional counseling on 12 reproductive-aged, moderately obese women with PCOS. Participants were randomly assigned to endurance and resistance exercise plus nutritional counseling (n=7, mean age 31.3±1 years, BMI 36.2±2) or nutritional counseling only (n=5, mean age 28.4±2.7, BMI 37.1±3.4). The exercise protocol included 3 sessions per week or combined endurance and resistance training. This consisted of 30 minutes of treadmill walking/stationary cycling at 70-85% of maximum heart rate, followed by 3 sets of 15 repetitions of 12 exercises, increasing mass incrementally. The intervention was 12 weeks duration. In both groups, there were significant decreases in sum of 2 skinfolds and waist girth after the intervention. There were also significant improvements in insulin levels in both groups. However, no changes to lipid profile (total cholesterol, HDL-C, LDL-C, triglycerides and TC-HDL-ratio) were reported in either group (Bruner et al., 2006).

Positive lipid changes after an exercise intervention without significant weight loss in women with PCOS have also been described (Brown et al., 2009). Brown and colleagues (2009) randomised 20 women with PCOS to either a 12 week moderate intensity exercise programme (n=8, median BMI 37.9, median age 36.5) or a control group (n=12, median BMI 31.3, median age 28). The exercise programme consisted of 228 minutes per week at 40-60% VO2 peak. 8 exercisers and 12 controls completed the study. In comparison to controls, the exercise group had significantly decreased insulin resistance that was associated with significant reductions in VLDL, triglycerides and medium/small HDL, with significantly increased HDL and average HDL size (Brown et al., 2009).

Although data on insulin sensitivity and lipid profile were not available for the present sample, previous research has indicated that a moderate intensity aerobic exercise intervention can improve insulin sensitivity and lipid profile in association with reductions in body weight and waist circumference (Bruner et al., 2006; Thomson et al., 2008). In addition, the study by Brown et al. (2009) indicates that improvements in insulin resistance and lipid profile are possible after an exercise intervention even in the absence of weight loss. The exercise intervention in the present study was shorter than those given in the review above (ranging from 12 to 20 weeks), however, given that the exercise intervention in the present study had a significant impact on BMI, body mass and waist circumference, it is likely that there would have been subsequent improvements in insulin resistance and potentially lipid profile had these measurements been available.

5.4 The Effect of an Exercise Intervention on Oxidised LDL Concentrations

Very few studies have looked to examine the relationship between PCOS and oxLDL concentrations. Early findings are inconsistent, but have provided a view on the lipid profile of women with PCOS in comparison to healthy controls (Macut et al., 2006; Macut et al, 2008; Demiral et al., 2007).

Macut et al. (2006) conducted a large, cross-sectional analysis comparing 179 PCOS women with 56 age and BMI-matched controls. It was determined that oxLDL concentrations were significantly higher in both normal and overweight women with

PCOS compared to controls. A later study by Macut et al. (2008) looked to further define the lipid and lipoprotein profile in women with PCOS. Seventy-five women with PCOS (aged 23.1±5.1 years), with a BMI of 24.9±4.7, were compared with age and BMI-matched controls. The researchers investigated fasting glucose, total, HDL and LDL cholesterol, oxLDL, triglycerides, SHBG, HOMA index and FAI. PCOS patients were shown to have significantly increased indices of insulin resistance, decreased HDL cholesterol, elevated triglycerides and increased oxidized LDL in comparison to controls.

An additional study conducted by Demirel and colleagues (2007) aimed to investigate oxLDL concentrations and its interaction with dyslipidemia in adolescent girls with PCOS. Participants included 44 girls with PCOS (23 obese and 21 non-obese) and 31 lean, healthy controls. Although results revealed significantly higher total cholesterol, triglycerides, LDL-C and VLDL-C in the obese PCOS in comparison to controls, and significantly higher LDL-C in non-obese PCOS in comparison to controls, oxLDL did not differ significantly between the three groups. Furthermore, a poorer lipid profile was associated with leptin, which was significantly higher in the obese PCOS group compared with both other groups. This may indicate that the dyslipidemia seen in the obese PCOS group may be influenced by obesity, rather than the presence of PCOS alone. More research is required to investigate the association of oxLDL with cardiovascular risk across each PCOS phenotype, so that individual mechanisms can be identified to inform treatment.

In the present study, the PCOS group had significantly higher SBP, WHR, and WC and significantly lower VO2max than controls at baseline. The mean BMI at baseline was 25.92±5.39kg/m2 for controls compared to 31.15±6.30kg/m2 for the PCOS group. According to the WHO classification, controls can therefore be considered overweight, while the PCOS group falls into the obese class 1 category (Public Health England, 2015). Mean WC in controls was 83.01±14.20cm and 98.05±16.35cm for the PCOS group.

The National Heart, Lung and Blood Institute (NHBLI) classifies overweight and obese persons by disease risk dependent on their BMI and WC (NHBLI, 2016). The control group, with a WC of less than 88cm and a mean BMI of 25.92±5.39kg/m2, are classified as at an 'increased' risk for T2D, hypertension and CVD (NHBLI, 2016). The

PCOS group, with a WC of more than 88cm and a mean BMI of 31.15±6.30, are classified as at 'very high' risk for development of T2D, hypertension and CVD.

However, despite the PCOS group falling into a higher risk category for CVD, there were no significant differences in mean baseline oxLDL between the two groups (30.11±7.28 u/l in the PCOS group versus 31.27±8.55 u/l in the control group).

Though sample sizes were small, it would be expected that the PCOS group, a group that not only meets the NHBLI criteria for a 'very high' risk of CVD in terms of BMI and WC, but is also a population at an established higher risk for CVD than a healthy population (Harrison et al., 2010; Hutchison et al., 2011; Norman et al., 2004; Sattar, 2006) would exhibit higher concentrations of oxLDL than healthy controls, when research suggests it to be an indicator of CV risk and an accurate predictor for CHD events (Holvoet et al., 2001; Holvoet, 2004; Koenig et al., 2011).

Additionally, this finding is in contrast to the findings of Weinbrenner et al. (2006), who showed a significant association between high oxLDL concentrations and higher waist circumferences (>88cm) in apparently healthy women, independent of other markers of cardiovascular risk.

The explanation for the finding of no significant differences in ox-LDL concentrations between the PCOS and control group at baseline may be due to a small sample size. However, it may also be due to a variation of PCOS phenotypes included the study sample. The previous chapters discuss the metabolic differences between each phenotype, specifically that those with hyperandrogenism have a worse metabolic profile than the normo-androgenic phenotype (Dewailly, 2016).

A large study by Daan et al. (2014) considered the cardiovascular and metabolic profiles among different PCOS phenotypes in a large cross-sectional study. Among 2,288 women with PCOS, aged 18-45 years, over half (53.3%) exhibited a hyperandrogenic form of PCOS (hyperandrogenism and oligo/anovulation, hyperandrogenism and polycystic ovaries, and hyperandrogenism, oligo/anovulation and polycystic ovaries). Women with a hyperandrogenic phenotype of PCOS were shown to have significantly higher BMI, waist circumference, SBP, DBP, fasting insulin, LDL-C and lower HDL-C than non-hyperandrogenic PCOS phenotypes.

Furthermore, multiple regression analysis that corrected for age, BMI, smoking and ethnicity did not attenuate the differences in CV risk factors between hyperandrogenic and nonhyperandrogenic women with PCOS.

Thus, the results from this study suggest that around 50% of a population of women diagnosed with PCOS (according to the Rotterdam criteria) may be normo-androgenic. In the study by Daan et al. (2014), the prevalence of CV risk factors such as metabolic syndrome and hypertension in non-hyperandrogenic women with PCOS (6.5% and 11.8% prevalence, respectively) corresponds with the prevalence of these CV risk factors in a non-PCOS population of similar age and BMI. It is therefore possible that the normo-androgenic women included in the PCOS sample of the present study had a cardiometabolic profile that closely resembles that of a non-PCOS groups. This might explain why PCOS did not exhibit higher ox-LDL concentrations than controls.

Additionally, the TC-HDL-ratio has been shown to be a significant predictor of ox-LDL level. Since normo-androgenic women with PCOS have been shown to have higher HDL-C levels than hyperandrogenic women with PCOS (Daan et al., 2014), it might therefore be probable that normo-androgenic women with PCOS subsequently have lower oxLDL levels. However, without data on total cholesterol, LDL-C and HDL-C, or which participants exhibited a hyperandrogenic form of PCOS, it is not possible to explore this within the current study sample.

In a study by Wu and colleagues (2006) 470 women in the healthy control group (aged 30-55 years), with a mean BMI of 25.1±4.6kg/m2 had a mean oxLDL concentration of 34.4±12 u/l (Wu et al., 2006). In a study by Macut et al. (2006) reported lipid metabolism between PCOS and non-PCOS women, reported that normal weight controls (mean age 23.6±4.4 years, n=36) and overweight controls (mean age 25.7±5.9, n=20) had mean oxLDL concentrations of 44.3±8.5 u/l and 44.8±7.8 u/l respectively.

Given this variation of oxLDL levels in healthy women of varying ages and BMI, the oxLDL concentrations of the PCOS women (aged 18-40 years, mean BMI 31.13±6.30kg/m2) of 30.81±7.95 u/l, in comparison to controls (mean BMI 25.92 ± 5.39kg/m2) of 31.48 ± 8.34 u/l, it is likely that this difference is a natural variation of healthy oxLDL levels.

The mean oxLDL concentrations of the PCOS group fall close to or below that of those identified in healthy female controls in other studies (Macut et al., 2006; Wu et al. 2006) providing further support that those PCOS women included in the sample may not have been at a high CV risk, likely due to the prevalence of normo-androgenic phenotypes in the sample.

There were also no significant changes to oxLDL levels post intervention, for both the control group and the PCOS group, despite a significant improvement in VO2max for both groups. As described above, the oxLDL concentrations for both PCOS and controls are close to measurements found in healthy populations (Macut et al., 2006; Wu et al., 2006). This 'low' level at baseline may indicate that oxLDL levels were not CV risk factors for either group and as such were not sensitive to the exercise intervention.

Furthermore, the study did not record overall physical activity levels outside of the structured exercise sessions. Therefore it is not possible to look at total accumulated physical activity or total sedentary time for either group. Physical activity is an important factor in the regulation of insulin and glucose metabolism, with high levels of sedentary activity shown to have a negative effect on insulin sensitivity and glucose concentrations, even in those people who take part in structured exercise sessions (Balkau et al., 2008; Dunstan et al., 2005). It is possible that the participants engaged in long periods of sedentary activity that may have mitigated the effects of the exercise intervention.

6. Conclusions

The PCOS study did not reveal any significant changes in AMH and oxLDL levels after an 8-week aerobic exercise intervention, despite significant improvements in VO2max for both groups, and significant improvements in BMI, WC and SBP in women with PCOS. This indicates that the exercise intervention reduced CVD risk, by modifying significant metabolic risk factors.

6.1 Limitations

The limitations of the PCOS study largely revolve around data collection. The blood samples used were subject to a secondary analysis to collect AMH and oxLDL concentrations. However, in order to better understand oxLDL and its mechanisms, other data is needed. As shown by the regression analysis, this includes fasting glucose concentrations and TC-HDL-ratio. It would therefore have been beneficial to obtain measures of insulin sensitivity, fasting glucose concentrations, total cholesterol and HDL and LDL particle levels in order to measure where changes were made.

A further limitation of the data set is that only data from the secondary analysis was provided for each participants. Data on all other variables, such as BMI, waist circumference and VO2max were only given as a mean and standard deviation. Therefore, it was not possible to split the data set by BMI or cardiorespiratory fitness to ascertain whether this had any effect on the results. It was therefore also not feasible to adjust for differences within the statistical model. WC, WHR, SBP and WHR were significantly different between PCOS subjects and controls at baseline. These measures of fitness and body composition could have skewed the results of the intervention since the control group had higher aerobic fitness and body composition values that were closer to a normal range than the PCOS subjects.

Additionally, without the PCOS data being sorted into phenotypes, it is not possible to see which participants were hyperandrogenic and therefore likely to have high AMH levels and a worse cardiometabolic profile. Information on asymptomatic polycystic ovaries in the control group would also have been useful in identifying those controls who may not be subfertile but have high levels of AMH.

The small sample size is a limitation with regard to weak statistical power.

6.2 Future Research

Future studies looking to evaluate the effects of exercise on oxLDL should ensure that oxLDL is measured in conjunction with TC-HDL-ratio, glucose concentrations and measures of insulin sensitivity. Research should therefore look to examine the effects of an aerobic long-term endurance program to reduce glucose, TC-HDL-ratio and subsequently and CV risk. Additionally, total accumulated physical activity and total time spent sedentary should be measured. This could be self reported or involve the use of a pedometer or an activity tracking smart-phone app. This would help to control for the mitigating effects of long periods of sedentary activity on CV risk.

Furthermore, subsequent PCOS studies should look to split their participants by phenotype to ensure distinction in the results between groups of PCOS women with different characteristics of the syndrome. This will help to identify those who are likely to have high AMH levels of be at a higher CV risk. Measurement of AMH levels could be used to exclude asymptomatic polycystic ovaries in controls and additionally used as inclusion criteria for AMH studies; that is, it can be used to check that participants exhibit an AMH concentration higher than the 'normal' range. This could also be used as an indirect measure of testosterone levels and insulin resistance, and may help to identify or confirm hyperandrogenism in those women displaying clinical symptoms such as acne and excessive hair growth.

Since the hyperandrogenic phenotype is shown to have a worse cardiometabolic profile than normo-androgenic phenotypes of PCOS, and testosterone and hyperinsulinemia are shown to be associated with high levels of AMH, future research could look to identify associations between oxLDL and AMH concentrations.

The 8-week exercise intervention did not produce any significant changes in AMH or oxLDL concentrations in PCOS women or controls. The exercise intervention should therefore be longer to account for the development of antral follicles into later stages of growth where they will cease to produce AMH. It is possible that only once the current cohort of antral follicles have progressed, can a change in AMH levels be seen in the absence of a large number of growing antral follicles. AMH levels should also be

measured in conjunction with testosterone levels and measures of insulin sensitivity in order to better understand the mechanisms that lead to a reduction in AMH concentrations.

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