Physical Activity and Cardiovascular Disease Risk in Women with Polycystic Ovary Syndrome

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Collaborating organisations: Sheffield Teaching Hospitals NHS Trust

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Candidate Declaration

I hereby declare that:

1. I have not been enrolled for another award of the University, or other academic or professional organisation, whilst undertaking my research degree.

2. None of the material contained in the thesis has been used in any other submission for an academic award.

3. I am aware of and understand the University's policy on plagiarism and certify that this thesis is my own work. The use of all published or other sources of material consulted have been properly and fully acknowledged.

4. The work undertaken towards the thesis has been conducted in accordance with the SHU Principles of Integrity in Research and the SHU Research Ethics Policy.

5. The word count of the thesis is 63952

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Abstract

Background

Polycystic ovary syndrome (PCOS) is a complex, heterogeneous endocrinopathy affecting metabolic, reproductive, and cardiovascular health in women. Evidence indicates that women with PCOS present with a cluster of cardiovascular disease (CVD) risk factors.

Physical activity (PA) interventions have been shown to reduce various CVD risk factors in women with PCOS. Research also suggests that sedentary behaviours have a distinct deleterious effect on cardiometabolic health. Thus, increasing PA and reducing sedentary behaviour may be a worthwhile therapeutic target to improve cardiovascular health in women with PCOS.

The programme of research presented in this thesis investigates the feasibility and acceptability of two PA interventions to improve markers of CVD risk in women with PCOS using both quantitative and qualitive methods.

Methods

A systematic review and meta-analysis of the effects of exercise interventions on CVD risk factors in women with PCOS provided an evidence base on which to design a supervised exercise intervention.

A feasibility randomised controlled trial (RCT) of two physical activity interventions for women with PCOS was conducted. Participants were randomised to either a supervised exercise intervention, a lifestyle physical activity intervention (LPAG) aimed at reducing sedentary behaviours, or a control group, for 12 weeks.

Semi-structured interviews were conducted with a purposive sample of participants from each group on completion of the RCT to explore the acceptability of the interventions, and barriers and facilitators to PA.

Results

The systematic review and meta-analysis demonstrated that moderate intensity aerobic exercise interventions of ≥three months in duration, with a frequency of three sessions/week, had favourable effects on CVD risk factors. These results informed the design of the RCT.

Thirty-six women with PCOS were enrolled onto the feasibility RCT (12 per group). The recruitment rate was 56% and adherence rate to the exercise intervention was considered moderate at 53%. The retention rate was high at 89%, with only five participants lost to follow-up. Adherence to the LPAG was 100%. Two non-serious adverse events were reported in the exercise group, unrelated to trial procedures. For the secondary outcomes, trend data indicates a 14% reduction in oxidised LDL concentrations in the exercise group. In addition, the data indicates weight loss (kg) of 3.4% and 3.6% in the exercise group and the LPAG, respectively.

Qualitative data from the interviews (n=11) indicated that the interventions were well received, but acceptability could be improved by providing social connectivity and implementing measures that encourage the adoption of long-term health-promoting behaviours.

Conclusions

The findings suggest that the procedures for recruitment, allocation, and outcome measurements were acceptable. However, adherence to the supervised exercise intervention was below an acceptable rate. The qualitative component provides valuable contextual data that will be crucial to addressing adherence for both the progression to a full-scale RCT, and community interventions for women with PCOS.

Statement of originality

I hereby declare that all the work contained in this thesis is original and was undertaken by the author unless otherwise stated below. Where reference is made to the work of others, citations are included with the authors' name and year of publication.

The systematic review and meta-analysis were designed by Amie Woodward (AW), also referred to as the author, guided by Markos Klonizakis (MK), David Broom (DB), and Deborah Harrop (DH), an Information Scientist within the College of Health Wellbeing and Life Sciences at Sheffield Hallam University. Double screening and data verification was undertaken by MK and DB. Analysis was conducted by AW.

The feasibility randomised controlled trial was designed by AW and guided by MK, DB, Mostafa Metwally (MM), and Caroline Dalton (CD). All trial documents were designed by AW guided by MK and DB. Ethics application and approval was undertaken by AW and assisted by MK, DB, and the R&D department at Sheffield Teaching Hospitals (STH). Recruitment was conducted by AW and assisted by MM in the Jessop Wing at STH. All participant screening and enrolment was conducted by AW. Assessments, including venepuncture and intervention delivery, were undertaken by AW. Several assessments and exercise sessions were delivered with the assistance of two undergraduate students, Jo Wilson and Victoria Cullinan, supervised by DB. Analysis of samples was conducted by AW at Biomolecular Research Centre at Sheffield Hallam University, under supervision by CD.

The interview guide for the qualitative study was designed by AW and guided by MK and DB. Interviews for participants in the exercise group were conducted by Rachel Cholerton (RC), apart from one where RC was unavailable. All other interviews were conducted by AW. Coding and analysis were conducted by AW, guided by Hilary Piercy (HP).

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Publications

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Abbreviations

95% CI 95% confidence interval
ACSM American College of Sports Medicine
ADMA Asymmetric dimethylarginine
AE Adverse event
AE-PCOS Androgen Excess and Polycystic Ovary Syndrome Society
AMH Anti-Mullerian hormone
ANOVA Analysis of variance
ApoA1 Apolipoprotein A
ApoB Apolipoprotein B
ASCVD Atherosclerotic cardiovascular disease events
ASRM American Society of Reproductive Medicine
BHF British Heart Foundation
BMI Body mass index
BMRC Biomolecular research centre
CAD Coronary artery disease
CC Clomiphene citrate
CETP Cholesterol ester transfer protein
CHD Coronary heart disease
cIMT Carotid intima-media thickness
CMO Chief medical officer
COREQ Consolidated criteria for reporting qualitative research
CREPCOS The Centre for Research Excellence in Polycystic Ovary Syndrome
CRF Cardiorespiratory fitness
CRP C-reactive protein
CSES Centre for Sport and Exercise Science
CV Cardiovascular
CVD Cardiovascular disease
DBP Diastolic blood pressure
DCI D-chiro-inositol
DM Diabetes mellitus
ELISA Enzyme-linked immunosorbent assay
eNOS Endothelial nitric oxide synthase
EP Estrogen-progestin
ESHRE European Society of Human Reproduction and Embryology
FFA Free fatty acids
FFM Fat free mass
FM Fat mass
FMD Flow mediation dilation
FSH Follicle stimulating hormone
GAD Generalised anxiety disorder
GLUT Glucose transporter

GnRH Gonadotropin releasing hormone
GRADE Grading of Recommendations Assessment, Development and Evaluation
GWAS Genome Wide Association Studies
HCP Healthcare professional
HDL High density lipoprotein
HIIT High-intensity interval training
HOMA-IR Homeostatic model assessment of insulin resistance
HR Hazard ratio
HRA Health Research Authority
HRQoL Health related quality of life
IL Interleukin
IPAQ International physical activity questionnaire
IR Insulin resistance
IRS Insulin receptor substrate
LDL Low density lipoprotein
LH Luteinizing hormone
LOD Laparoscopic ovarian drilling
LPAG Lifestyle physical activity group
MDA Malondialdehyde
METS Metabolic equivalents
MI Myocardial infarction
MNC Mononuclear cell
MRC Medical Research Council
MUFA monounsaturated fatty acid
NAFLD Non-alcoholic fatty liver disease
NHS National Health Service
NICE National Institute for Health and Care Excellence
NIH National Institute of Health
NO Nitric oxide
OCP Oral contraceptive pill
OGTT Oral glucose tolerance test
OR Odds ratio
OxLDL Oxidised low-density lipoprotein
PA Physical activity
PAD Peripheral artery disease
PAR Population attributable risk
PCO Polycystic ovaries
PCOS Polycystic ovary syndrome
PICO Population, intervention, comparison, and outcome
PIS Participant information sheet
PRISMA Preferred reporting items for systematic reviews and meta-analysis
PROSPERO International Prospective Register of Systematic Reviews
PRT Progressive resistance training

QoL Quality of life
RCT Randomised controlled trial
ROS Reactive oxygen species
RPE Rate of perceived exertion
RR Risk ratio
RT Resistance training
SAE Serious adverse event
SBP Systolic blood pressure
SD Standard deviation
SE Standard error
SHBG Sex hormone-binding globulin
SHU Sheffield Hallam University
SMD Standardised mean difference
STH Sheffield Teaching Hospitals
T2D Type 2 diabetes mellitus
TBARS Thiobarbituric acid reactive substances
TC Total cholesterol
TC:HDL Total cholesterol: high density lipoprotein ratio
TG Triglycerides
TNF-α Tumour necrosis factor-α
TT Total testosterone
UK United Kingdom
US United States
VLDL Very low-density lipoproteins
WC Waist circumference
WHO World Health Organisation
WHR Waist-to-hip ratio

Reporting Conventions

Chapter 5, which covers the qualitative research undertaken as part of this thesis, is written in first person singular in line with common reporting conventions in this field. This matches the intention of giving a voice to the participants' perspectives and clarifies the role of the researcher in conveying this. In addition, pseudonyms are also used in this chapter, rather than trial ID, because this humanises the participants and emphasises the importance of their individual perspectives and experiences. The rest of the chapters are written in third person in line with conventions for quantitative research. The author is referred to as 'the principal researcher' in these chapters.

Overview of the Thesis

This thesis explores the impact of physical activity on cardiovascular disease (CVD) risk in women with polycystic ovary syndrome (PCOS). The thesis begins with an introduction to PCOS (Chapter 1), including diagnostic criteria, prevalence, pathophysiology, and approaches to treatment. Chapter 2 reviews the literature and outlines the factors that contribute to increased CVD risk in women with PCOS, including potential mechanisms. Additionally, the protective role of physical activity, and the deleterious role of sedentary behaviour, on CVD risk are reviewed and explored. The literature reporting lifestyle intervention studies in PCOS, including physical activity, is interrogated. The literature reviewed throughout the chapter is summarised, and the justification, aims, and objectives for the thesis are set forth.

Chapter 3, 4, and 5 report on the three studies conducted as part of this programme of research. Chapter 3 is a systematic review and meta-analysis of physical activity interventions in women with PCOS and provides evidence for the basis of the next study. The next study, reported in Chapter 4, is a feasibility randomised-controlled trial (RCT) that explores the acceptability of two physical activity interventions in women with PCOS. Chapter 5 reports on a qualitative evaluation of the feasibility RCT reported in Chapter 4 and provides additional evidence regarding the acceptability of the interventions, whilst also exploring barriers and facilitators to physical activity in women with PCOS.

Chapter 6 summarises and synthesises the findings from the programme of research set out in this thesis. The aims and objectives of the thesis are revisited. Practical implications arising from this research are discussed, and recommendations for future research directions are proposed. Finally, the novel contribution to knowledge is examined, as well as pertinent reflections of the researcher, and conclusions are presented.

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1 Introduction

Overview

The aim of this chapter is to contextualise the PhD thesis by introducing and exploring key concepts and phenomena related to polycystic ovary syndrome (PCOS) and cardiovascular disease risk (CVD). This chapter outlines PCOS, including diagnostic criteria, prevalence, pathophysiology and approaches to treatment. Some of the work in this chapter has been published in Woodward, Klonizakis and Broom (2020c).

1.1 Introduction to Polycystic Ovary Syndrome

1.1.1 Diagnostic Criteria

Polycystic Ovary Syndrome (PCOS) is a complex, heterogeneous endocrinopathy affecting metabolic, reproductive, and cardiovascular health in women (Teede et al., 2018; Sirmans & Pate, 2014). There are three focal clinical features: polycystic ovaries (PCO), clinical or biochemical hyperandrogenism, and chronic oligo-anovulation (Burks & Wild, 2014). PCO refers to the accumulation of antral follicles measuring between 2 and 9 mm in diameter in at least one ovary (Sirmans & Pate, 2014). As ultrasound technology has advanced, the number of follicles that can be seen in the ovary has also increased. The recommended threshold criterion was initially proposed as ≥12 follicles per ovary (Sirmans & Pate, 2014). Now, with these advancements in ultrasound technology, the most recent recommendation proposes a follicle number per ovary of ≥20 (Teede et al., 2018). Clinical or biochemical hyperandrogenism refers to an excess of androgens that can be identified symptomatically (through acne, alopecia, and/or hirsutism) or biochemically through sensitive assay. Chronic oligo-anovulation, known as ovulatory dysfunction or oligo/amenorrhoea, refers to menstrual and/or ovulatory irregularity. Menstrual irregularity is defined depending on life-stage, but in women 3 years post-menarche (the first occurrence of menstruation) and up until perimenopause, <8 cycles per year is the recommended threshold (Teede et al., 2018). Additionally, as menstrual regularity can persist even in the presence of ovulatory dysfunction, anovulation can be confirmed through serum progesterone assay (Teede et al., 2018).

Currently, three sets of diagnostic criteria are in use; 1) the 1990 National Institute of Health (NIH) criteria, 2) the 2003 American Society of Reproductive Medicine sponsored European Society of Human Reproduction and Embryology (ASRM/ESHRE) criteria, which was formulated to expand the NIH definition, and 3) the 2006 Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) Society criteria, developed to provide an evidence-based definition reflecting technological and diagnostic advancements (Burks & Wild, 2014; Wolf, Wattick, Kinkade & Olfert, 2018). The existence of three sets of criteria demonstrates the heterogenous and complex nature of PCOS, as well as the continual advancements in technology and understanding that improve the accuracy of diagnosis.

A diagnosis of PCOS according to the NIH criteria requires that chronic anovulation and clinical or biochemical hyperandrogenism are both present (Zawadski & Dunaif, 1992). The AE-PCOS criteria requires that hyperandrogenism (clinical or biochemical), and ovarian dysfunction (encompassing PCO and/or oligo-anovulation) both be present (Azziz, et al., 2009). Diagnosis according to the ASRM/ESHRE criteria (which is commonly referred to as the Rotterdam criteria) requires that any two of the three clinical features be present (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). Each set of criteria stipulates that other disorders that could be responsible for these symptoms be excluded first, such as congenital adrenal hyperplasia, Cushing's syndrome (a condition caused by excessive adrenal production of cortisol), androgen-secreting tumours, and hyperprolactinaemia (where an individual has excessive serum levels of prolactin) (Burks & Wild, 2014). Table 1 indicates the three sets of criteria.

Table 1. PCOS diagnostic criteria.

NIH (1990)	Rotterdam (2003)	AE-PCOS (2006)
Chronic oligo-anovulation *and* hyperandrogenism	At least two from PCO, chronic oligo-anovulation, and hyperandrogenism	Hyperandrogenism *and* ovarian dysfunction (which includes PCO and/or oligo-anovulation)

The Rotterdam criteria are thus the least restrictive and account for inter-personal variation in symptoms. The AE-PCOS criteria similarly allow for some variation, because either PCO or oligo-anovulation are classed as ovarian dysfunction. Thus, one could be diagnosed when presenting with hyperandrogenism and PCO, or hyperandrogenism and oligo-anovulation. The NIH criteria are the most restrictive and do not include PCO as a criterion at all.

1.1.2 Prevalence and Diagnosis

The prevalence of diagnosed women with PCOS necessarily depends on which criteria are used. Reports of prevalence range from 6.1% to 19.9% of representative samples of women (Yildiz, Bozdag, Yapici, Esinler, & Yarali, 2012). Table 2 indicates reported prevalence from studies using different diagnostic criteria in Australia (March et al., 2010), Turkey (Yildiz et al., 2012) and Iran (Mehrabian, Khani, Kelishadi & Ghanberi, 2011). The three studies applied each set of criteria to the same sample of women to create prevalence estimates. From these estimates, prevalence is over twice as high using the Rotterdam criteria than the NIH criteria.

*Table 2. Prevalence of PCOS (%) based on individual criteria**

	Diagnostic Criteria		
	NIH[a]	AES[b]	Rotterdam[c]
March et al., (2010).	8.7	12.0	17.8
Yildiz et al., (2012).	6.1	15.3	19.9
Mehrabian et al., (2011).	7.0	7.9	15.2

* adapted from Burks & Wild (2014)

[a]National Institutes of Health international conference 1990

[b] Androgen Excess Society diagnostic criteria 2006

[c]Task force sponsored by the European Society of Human Reproductive and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM), 2003

The estimation of the prevalence is more nuanced depending not only on the criteria used, but also sample sizes, geographical location, and racial or ethnic groups (Wolf et al., 2018).

Indeed, the studies outlined in Table 2 have limitations, including small sample sizes, and the authors of one study acknowledge that their sample size of 392 was likely underpowered to detect prevalence (Yildiz et al., 2010). In addition, each of the three studies was conducted at a single facility, limiting the applicability to other geographical locations.

A recent systematic review and meta-analysis investigated the prevalence figures in 21 studies where different diagnostic criteria were applied (Skiba, Islam, Bell & Davis, 2018). Each included study diagnosed PCOS using at least one of the three criteria. Their results estimated the pooled prevalence of PCOS to be 7% (95% CI: 6%, 7%) using the NIH criteria, 12% (95% CI: 10%, 15%) using the Rotterdam criteria, and 10% (95% CI: 6%, 13%) using the AE-PCOS criteria. The pooled estimates for the Rotterdam and AE-PCOS criteria were not significantly different, but there was a significant difference between the NIH and Rotterdam criteria (Skiba et al., 2018). This is thought to be due to the inclusion of ovarian morphology (PCO), which results in significantly higher prevalence estimates for PCOS. This may suggest under-reporting when the NIH criteria are used. In some cases, PCO alone may result in a misdiagnosis of PCOS. This is problematic because PCO alone should not be considered indicative of PCOS, and in some cases, menstrual irregularity and PCO can be associated with other, unrelated conditions, highlighting the need for exclusion of other conditions before a diagnosis is made (Copp et al., 2017). The differing use of the three sets of criteria globally (including lack of standardisation of diagnostic techniques and cut-offs within each set of criteria) has led to heterogeneity in prevalence estimates and women internationally reporting delayed diagnosis (Gibson-Helm, Lucas, Boyle & Teede, 2014; Gibson-Helm, Teede, Dunaif & Dokras, 2017).

Furthermore, it is likely that a high proportion of women remain undiagnosed due to variation in phenotypes and lack of clear and consistent clinical guidance (Wolf et al., 2018). Indeed, the current criteria are limited in breadth, outdated, or lack consumer involvement, resulting in a lack of consistent guidance for best clinical practice (Teede et al., 2018). There may also be sampling bias in prevalence studies where those women selected from clinics or hospitals are already actively seeking or receiving treatment for their symptoms. Those undiagnosed and not seeking treatment may be missing from such samples, skewing prevalence estimates. Indeed, a retrospective cohort study conducted by Ding et al. (2016) highlights issues in prevalence estimates, particularly with underdiagnosis. The authors conducted a search of UK primary care records for females aged 15-45 years and identified a prevalence rate of

~2%. This is lower than those prevalence rates found in epidemiology studies that provide screening to identify cases, such as those highlighted above. The difference in prevalence rate may be due to the nature of GP data collection and reporting and emphasises a need for better GP awareness of PCOS. That is, only data considered relevant at the time of a consultation are reported by clinicians. Therefore, only the initial feature of PCOS that lead to the consultation may be on the record, which may lead to underreporting of women exhibiting two or more features of PCOS (thus meeting the diagnostic criteria), even if they have been referred to a specialist. This leads to underestimation of prevalence when cases are identified through medical records. The study also found a large proportion of women with two or more features of PCOS that had not been given a diagnosis and thus were not referred for specialist treatment, increasing their risk for long-term PCOS-related complications. Again, a better public and primary care understanding of PCOS and its criteria could improve diagnostic rates and thus treatment outcomes.

Menopause further complicates the prevalence figures. Many studies describe PCOS as a condition that affects only reproductive-aged women (and enforce a cut-off age of 45 years in diagnostic studies) and thus only include pre-menopausal women in prevalence estimates. However, PCOS is likely to persist into post-menopause, particularly phenotypes associated with hyperandrogenism, and a diagnosis can still be given (Shah & Bansal, 2014; Teede et al., 2018).

Health inequality may also impact prevalence rate. For example, in the cohort study by Ding et al. (2016), the authors noted a higher incidence of PCOS in areas of greater social deprivation. Indeed, individuals with a lower socioeconomic status are more at risk for engaging in adverse health behaviours such as smoking, poor diet, and lack of physical activity (PA) (Merkin et al., 2011). This could contribute to higher rates of obesity and metabolic dysfunction in areas of greater deprivation, which may lead to exacerbation of PCOS features (Ding et al., 2016). Similarly, higher rates of co-morbidities as a result of adverse health behaviours, such as diabetes mellitus, may prompt patients to see their clinicians and thus they have a higher chance of being diagnosed with PCOS (Merkin et al., 2011).

It is therefore clear that prevalence estimates are nuanced and, in some cases, problematic. Without a global, standardised set of criteria with consistent cut-offs, it is hard to estimate

worldwide prevalence. In response to these issues, the first international evidence-based guidelines were developed in 2018 by The Centre for Research Excellence in Polycystic Ovary Syndrome (CREPCOS) in partnership with ASRM and ESHRE, which endorse the 2003 ASRM/ESHRE criteria as well as providing new recommendations regarding evaluation methods (CREPCOS, ASRM & ESHRE, 2018; Teede et al., 2018; Wolf et al., 2018).

The new recommendations highlight the potential phenotypes that are possible based on the presence of two of the three criteria needed for a diagnosis under the Rotterdam guidelines (PCO, ovulatory dysfunction, hyperandrogenism). There are four possible phenotypes, demonstrated in Table 3: A) presence of all three criteria, B) hyperandrogenism and ovulatory dysfunction, C) PCO and hyperandrogenism, and D) PCO and ovulatory dysfunction (CREPCOS, ASRM & ESHRE, 2018; Teede et al., 2018; Wolf et al., 2018). Classifying by phenotype may be useful because different pathophysiology and symptoms are likely to be associated with each, and as such, different approaches to treatment may be more appropriate or successful depending on the phenotype. Consequently, the guidelines recommend that specific phenotypes should be explicitly reported in all research (CREPCOS, ASRM & ESHRE, 2018).

Table 3. Phenotypes of PCOS possible under the Rotterdam criteria.

	Phenotype A	Phenotype B	Phenotype C	Phenotype D
PCO	X		X	X
Hyperandrogenism	X	X	X	
Ovulatory Dysfunction	X	X		X

As our understanding of PCOS evolves, as well as technological and scientific methods for detecting characteristics, indeed so should the diagnostic criteria and guidelines. The 2018 International PCOS Guidelines, as the first comprehensive single source of evidence-based recommendations, should go some way to promoting best-practice PCOS models of care and international collaboration, ultimately improving patient experiences (Teede et al., 2018). In the UK, the Rotterdam criteria are used for diagnosis and the National Institute for Health and

Care Excellence (NICE) updated their PCOS guidelines in 2018 to reflect additional recommendations from the International PCOS Guidelines (NICE, 2018).

1.1.3 Symptoms

Women with PCOS display considerable inter-personal variation in the presentation of symptoms and clinical features. As a result of hyperandrogenism, approximately 70% of women have hirsutism, and cystic acne occurs in up to 30% of adult women with the condition (Azziz et al., 2006). In addition, hyperandrogenism may promote alopecia, male-pattern baldness, and acanthosis nigricans (McCartney & Marshall, 2017; Ndefo et al., 2013).

Ovulatory dysfunction causes irregular menses and difficulty conceiving (infertility or sub-fertility). It is estimated that 40% of women with PCOS have reduced fertility, although this figure is based on those seeking treatment and is likely to be underestimated (Sirmans & Pate, 2014). Indeed, self-reports indicate that the prevalence of fertility issues may be up to 72% (Joham, Teede, Ranasinha, Zoungas, & Boyle, 2015). Of the women who present at fertility clinics, around 90% have PCOS (Sirmans & Pate, 2014).

Impaired insulin metabolism and hyperandrogenism can lead to weight gain, difficulty losing weight, and distribution of fat around the abdomen rather than hips and buttocks (Escobar-Morreale & Millan, 2007).

1.1.4 Aetiology

Aetiology refers to the cause, or set of causes, of a disease or condition. There is no single unifying theory of PCOS aetiology, and research into the aetiology of PCOS has centred on establishing the environmental and genetic contributions (Legro, 2017). There is often a family history of PCOS, but familial links to PCOS are unclear (Ndefo, Eaton & Green, 2013). Studies of PCOS in various population groups, including twin studies and ethnic group studies, seem to indicate heritability (Diamanti-Kandarakis et al., 2006), and Genome Wide Association Studies (GWAS) have identified candidate gene regions. However, these account for less than 10% of the heritability of PCOS, and the potential genes or regions do not have a clear functional relationship to the clinical symptoms (Legro, 2017). Nevertheless, those genes with altered expression that have been identified are associated with

gonadotropin production or action, steroidogenesis, or insulin action and secretion (Gur, Karadeniz, & Turan., 2015; Legro, 2017).

Evidence from controlled animal studies and observational human studies suggests that the pathophysiological basis of PCOS could originate in the intrauterine environment (Gur et al., 2015). This is known as foetal programming and suggests that PCOS is either genetically inherited or that is it caused by epigenetic alternations beginning before birth (Gur et al., 2015). There is some evidence to suggest that foetuses exposed to high androgen levels in utero develop PCOS later in life. For example, female patients with congenital adrenal hyperplasia have been shown to display features of PCOS at a higher rate than found in the normal population (Hague et al., 1990).

Lifestyle or environmental factors are thought to exacerbate PCOS and increase the prevalence, such as poor dietary choices and physical inactivity, which increase metabolic dysfunction, hyperandrogenism, and ovulatory dysfunction (Ndefo et al., 2013). Thus, PCOS is considered a polygenic trait that may result from the interaction of certain susceptible genes and environmental factors, during either the intrauterine environment or post-natal life (Diamanti-Kandarakis et al., 2006).

1.1.5 Pathophysiology

Pathophysiology refers to the disordered physiological processes that are associated with a disease or condition. The pathophysiology of PCOS is complex and circuitous, involving several body systems and pathways that have led to some speculation of whether PCOS is several disorders rather than one single disorder (Johansson & Stener-Victorin, 2013).

In normal menstrual physiology, follicular maturation and ovulation are controlled by the hypothalamic-pituitary-ovarian (HPO) axis. During the follicular phase, the hypothalamus releases gonadotropin-releasing hormone (GnRH), which stimulates the anterior pituitary to produce LH and FSH (Johansson & Stener-Victorin, 2013). The normal ratio of production between these two hormones is approximately 1:1 (Saadia, 2020). LH stimulates theca cells in the follicle, which produce androgens. FSH stimulates the production of granulosa cells in the follicle, which produce aromatase. Aromatase is an enzyme responsible for the biosynthesis of oestrogens by converting androgens to oestrogens. Thus, the production of

oestrogen is gradually increased through aromatase, and this grows the endometrial lining of the uterus (Thiyagarajan, Basit, & Jeanmonod, 2020). Once oestrogens have reached a critical level, this provides negative feedback to the anterior pituitary to lower levels of LH and FSH production. During the late follicular phase, the maturation of follicles continues to raise the oestrogen concentration, which, during the ovulation phase (14 days before menses), leads to a surge in LH that breaks the mature follicle and releases the oocyte (Thiyagarajn, et al., 2020).

The pathophysiology of PCOS is thought to involve defects in three processes. Firstly, in the hypothalamic-pituitary-ovarian (HPO) axis, secondly, in ovarian function, and thirdly, in insulin metabolism (Legro, 2017; Ndefo et al., 2013). Indeed, the three affect and exacerbate each other in an endocrine feedback loop, and it is not clear which abnormalities are a cause or consequence of PCOS (Gill & Hall, 2014; Ndefo et al., 2013). There are suggestions for which 'pathway' may be primarily disrupted (Legro, 2017). However, even if one specific pathway were the primary one, it could still eventually lead to dysfunction and disruption in the others. Figure 1 provides a summary of the pathophysiology. It demonstrates the intricate linking of the pathways and shows how disruption in one pathway can promote abnormalities in others. Below is a brief description of the disruptions in each pathway.

The neuroendocrine abnormality present in PCOS is inappropriate GnRH pulse frequency by the hypothalamus. This results in LH hyper-secretion from the anterior pituitary while FSH remains muted or unchanged (Gill & Hall, 2014; Ndefo et al., 2013). This is known as increased LH:FSH ratio and can be as high as 3:1 (Saadia, 2020). The subsequent hyper-secretion of LH results in ovarian theca cell hyperactivity and increased ovarian steroidogenesis. Because FSH remains in the lower range, granulosa cell development and aromatase production are stunted. Reduced levels of aromatase results in ovarian hyperandrogenaemia, and follicle maturation and ovulation are thus disrupted (Legro, 2017). With persistently elevated androgen levels, the negative feedback suppression of LH by ovarian steroids is diminished, thus stimulating further production of LH, perpetuating the cycle (Gill & Hall, 2014; Legro, 2017). In Figure 1, this pathway is represented in red.

The ovarian dysfunction in PCOS in characterised by excessive early recruitment of pre-antral follicles, followed by follicular arrest at the small antral stage. That is, there is a failure of the selection of a dominant follicle, preventing ovulation (Johansson & Stener-Victorin,

2013; Jonard & Dewailly, 2004). The hyper-secretion of LH and the hyperandrogenic milieu described above may be responsible for excessive early recruitment of follicles. Follicular selection may then be inhibited due to granulosa cell production of anti-Mullerian hormone (AMH), a glycoprotein that reduces follicular sensitivity to FSH in order to prevent the growth and depletion of all primordial follicles at once (Saikumar et al., 2013). However, as with the neuroendocrine abnormalities, it is not clear whether increased ovarian steroidogenesis is a consequence of LH hyper-secretion, or whether the primary defect is in ovarian and adrenal androgen production itself. Indeed, up to 30% of women with PCOS present with adrenal hyperandrogenism, suggesting that the defect in steroidogenesis is primary and affects both androgen secreting glands (Legro, 2017). In Figure 1, this pathway is represented in green.

The third pathophysiological defect is in insulin metabolism. Insulin resistance has been consistently documented in women with PCOS compared to weight-matched controls (Hutchison et al., 2011; Norman et al., 2004). Insulin resistance and the compensatory hyperinsulinemia increases circulating androgens by stimulating ovarian and adrenal androgen production (Legro et al., 2017). It also inhibits hepatic production of SHBG, thereby increasing bio-available testosterone (Normal et al., 2004). If the primary defect were in insulin metabolism, the associated hyperandrogenism could lead to both the neuroendocrine and ovarian abnormalities described. Finally, insulin resistance and hyperinsulinemia can lead to inflammation and obesity, which both, in turn, exacerbate insulin resistance (Gonzalez, 2012). This metabolic dysfunction leads to the associated increased risk of cardiometabolic disorders. Thus, there is a compelling argument for PCOS being a condition resulting from a primary defect in insulin metabolism, the mechanisms of which will be explored further in Chapter 2. In Figure 1, this pathway is represented in blue.

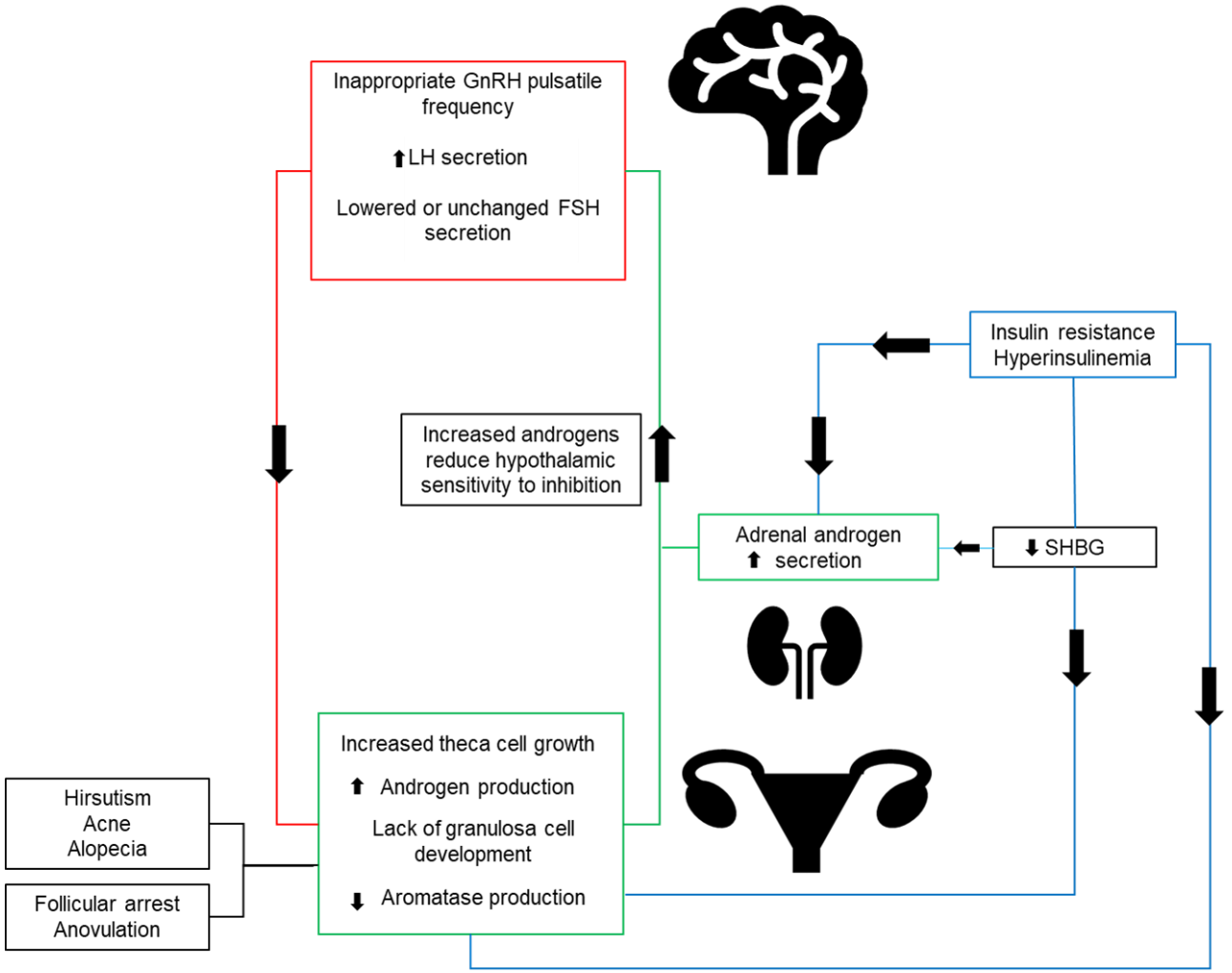

Figure 1. The three disrupted pathways in the pathophysiology of PCOS.

The pathways are interlinked, causing a negative feedback loop. GnRH = Gonadotropin releasing hormone. FSH = Follicle stimulating hormone. LH = Luteinizing Hormone. SHBG = Sex hormone binding globulin

1.1.6 Long-term Health Consequences

There are several long-term health consequences associated with PCOS. Those pertinent to and within the scope of the present programme of research are briefly outlined below.

1.1.6.1 Cardiovascular Disease Risk

PCOS is often associated with a cluster of cardiometabolic risk factors. This includes a 70% prevalence of dyslipidaemia (Kim & Choi, 2013) and up to 80% prevalence of insulin resistance (Carmina & Lobo, 2004). Other common risk factors include obesity, metabolic syndrome, impaired glucose tolerance, hypertension, and impaired endothelial and myocardial function (Dokras, 2013; Papadakis, Kandaraki, Papalou, Vryonidou, & Diamanti-Kandarakis, 2017; Sattar, 2006). Evidence suggests that sub-clinical atherosclerosis, indicated by carotid intima-media thickness (cIMT), is higher in women with PCOS compared to weight-matched controls (Dokras, 2013; Meyer, Malek, Wild, Korytkowski, & Talbott, 2012). Additionally, PCOS is associated with increased low-grade inflammation, with higher circulating concentrations of many inflammation markers that mediate CVD, such as C-reactive protein (CRP), increased white cell count, neutrophil/lymphocyte ratio, tumour-necrosis factor-alpha (TNF-a) and interleukin-6 (IL-6) (Çakıroğlu, Vural, & Vural, 2016; Calan, Kume et al., 2016; Calan, Yilmaz et al., 2016; Covington, Tam, Pasarica, & Redman, 2016; Duleba & Dokras, 2012; Orio et al., 2005).

In Chapter 2, the prevalence of CVD in PCOS is investigated, along with a review of CVD risk factors in PCOS. Additionally, potential mechanisms for increased CVD risk in PCOS, including insulin resistance and inflammation, are discussed.

1.1.6.2 Mental Health Disorders

Women with PCOS are increasingly reported to have a higher prevalence of mental health disorders. This includes increased prevalence of depression and anxiety disorders (Himelein & Thatcher, 2006), bipolar disorder (Davari-Tanha, Hosseini Rashidi, Ghajarzadeh, & Noorbala, 2014), personality disorders (Scaruffi, Gambineri, Cattaneo, Turra, Vettor, & Mioni, 2014), and binge eating disorders (Kerchner, Lester, Stuart, & Dokras, 2009). Recent literature suggests an odds ratio (OR) for women with PCOS compared to controls for depression and anxiety is 1.26 and 2.76, respectively (Blay, Agular, & Passos, 2016). In a Chinese study, the prevalence of anxiety and depression among a sample of women with

PCOS and healthy controls was reported to be 13.3% and 27.5% respectively in women with PCOS as compared to 2% and 3% in controls (Tan, Wang, Feng, Li, & Huang, 2017). Similarly, a study in India identified the prevalence of major depressive disorder and generalised anxiety disorder (GAD) to be 23.64% and 15.45% in PCOS, compared to 7.5% and 0% in controls (Hussain et al., 2015).

1.1.7 Approaches to Treatment

Treatment for PCOS typically focuses on alleviating and managing symptoms rather than attempting to treat the origin of the condition (which is not necessarily known and may not be amenable to treatment). Thus, the individual presentation of PCOS symptoms plays a role in the choice of treatment for each individual. Treatment typically falls into pharmaceutical, surgical, or lifestyle advice to address specific symptoms. In addition, complementary therapies include acupuncture, supplements, and herbal medicines (Arentz, Smith, Abbott, & Bensoussan, 2017; Lim, Ng, Cheng, Zhang, & Chen, 2019; Unfer, Facchinetti, Orru, Giordani, & Nestler, 2017). Again, these are usually geared toward a specific outcome, such as conception.

1.1.7.1 Pharmacological Treatments

Estrogen-progestin (EPs) compounds are used in the treatment of hyperandrogenaemia and its clinical manifestations in PCOS (such as hirsutism and acne) (Vrbíková & Cibula, 2005). Progestin suppresses LH which subsequently lowers ovarian androgen production, while the oestrogen component increases hepatic production of SHBG, reducing bioavailable testosterone. Further, some progestins have anti-androgenic effects by antagonising the androgen receptor and inhibiting 5*a*-reductase activity, a key catalytic enzyme involved in steroidogenesis (Vrbíková & Cibula, 2005). The combined oral contraceptive pill (OCP) is a commonly used EP. The OCP is used as a long-term management strategy for women with PCOS not attempting to conceive. Progestin suppresses ovulation via LH suppression and produces a thin endometrium which is unsuitable for implantation (The PCOS Society [India], 2018). However, combined OCPs can have a negative effect on TG and VLDL, particularly with high oestrogen dosages (de Melo, Dos Reis, Ferriani, & Vieira, 2017). There is also evidence to suggest that, in women with PCOS, long-term use or high oestrogen dosage is associated with lowering of glucose tolerance and arterial stiffness (Meyer,

McGrath & Teede, 2007). Thus, the World Health Organisation (WHO) indicates several contraindications to prescription of the combined OCP, several which are seen in PCOS, where the risk outweighs the advantages of the method. These include type 2 diabetes mellitus, dyslipidaemia, and metabolic syndrome (de Melo et al., 2017; WHO, 2015). In these cases, progestin-only contraceptives are typically considered a safer option (WHO, 2015).

Meyer et al. (2007) conducted a six-month RCT with 100 overweight (BMI >27 kg/m^2) women with PCOS. Participants were randomised into either a control group, a high oestrogen dose OCP group, a low oestrogen dose OCP group, or a metformin group. Their results indicated similar improvements in symptoms including hirsutism and menstrual cycle length across all groups. However, worsened insulin resistance and arterial stiffness were observed in the high oestrogen dose OCP group. Thus, the authors suggest that for women with CVD risk factors, a low oestrogen dose OCP may be preferable. This is in line with other reviews that indicate that OCPs may result in worsened insulin resistance (Sharma & Nestler, 2006).

Metformin is a biguanide widely prescribed for T2D and its efficacy in PCOS has been studied since 1994 (Velazquez, Mendoza, Hamer, Sosa, & Glueck, 1994). Metformin is usually prescribed in PCOS to aid in weight loss (or to improve chances of conception via weight loss) (Lashen, 2010), or to improve symptoms of hyperandrogenism. Metformin increases tissue sensitivity (including skeletal muscle, liver, adipose tissue, ovaries, and the endothelium) to insulin by increasing insulin receptors and thus cellular insulin-mediated glucose uptake, reducing serum insulin and glucose concentrations (Diamanti-Kandarakis, Christakou, Kandaraki, & Economou, 2010). It also interferes with hepatic gluconeogenesis to decrease glucose concentrations, and increases hepatic production of SHBG, reducing bioavailable testosterone (Kahn, 2008). Improvements in insulin sensitivity may thus attenuate the risk of metabolic and cardiovascular disorders (Sirmans & Pate, 2014). Additionally, the lowering of insulin levels reduces ovarian thecal and granulosa cell steroidogenesis (Diamantis-Kandarakis et al., 2010), improving ovulation rates and menstrual regularity (Pasquali, 2008).

Many trials have investigated the effects of metformin alone, or compared to lifestyle interventions, placebo or other drugs (Dashti et al., 2017). Metformin administered at a

dosage of 1.5 to 1.7 g/day for 1-6 months appears to be effective at reducing fasting blood glucose and serum insulin (Dashti et al., 2017). However, effects on BMI appear to be variable, which can be attributed to heterogeneity in studies regarding baseline BMI and whether measures of fat distribution are taken into account (Dashti et al., 2017). Thus, although it appears effective in improving metabolism-related variables, this is rarely what metformin is prescribed for, and its efficacy as a tool for weight loss is contested.

For anovulatory women with PCOS, Clomiphene Citrate (CC) is the first drug of choice for ovulation induction in those wishing to conceive (Legro, 2017). It functions as an anti-oestrogen by inhibiting the binding of oestradiol to its receptors in the hypothalamus and pituitary (Pasquali, 2018). Subsequently, it induces changes to gonadotropin releasing hormone (GnRH) pulse frequency leading to increased pituitary release of follicle stimulating hormone (FSH) (Legro, 2017; Sirmans & Pate, 2014). The increase in FSH secretion stimulates follicular growth and results in a mid-cycle LH surge and ovulation (Adashi, 1984). CC increases ovulation rate and live birth rates (Balen, 2013). However, CC is something of a short-term solution and is generally not used for more than 3-6 cycles due to risks and side effects including increased risk for some cancers, thinning of the endometrial lining, and ovarian hyperstimulation syndrome which has an increased likelihood in those with PCOS (Schram, 2016; Yilmaz, Yilmaz Sezer, Gonenc, Ilhan, & Yilmaz, (2018).

1.1.7.2 Surgery

Laparoscopic ovarian drilling (LOD) is recommended by WHO and NICE as a second-line treatment for women with PCOS who are resistant to CC (Lebbi, Temime, Fadhlaoui, & Feki, 2015). LOD works to restore ovarian function via the destruction of ovarian follicles, inducing a reduction in serum androgens and inhibin levels, which subsequently increases FSH (Api, 2009).

Reviews investigating its efficacy have produced varied results. A Cochrane review of 25 RCTs, including sub-fertile women with PCOS who were resistant to CC, concluded that there were no significant differences in rates of clinical pregnancy, live birth, or miscarriage in women undergoing LOD compared to other medical treatments (Farquhar, Brown, & Marjoribanks, 2012).

Conversely, a review investigating the effects of ovarian drilling for PCOS in 81 studies indicated different results (Fernandez et al., 2011). It concluded that ovarian drilling resulted in spontaneous restoration of fertility in 20-64% of women with CC-resistant PCOS.

Several factors may influence the likelihood of success, including LH concentrations and number of years of infertility before the procedure (Fernandez et al., 2011; Lebbi et al., 2015). Thus, LOD is considered to confer some benefits to those women with CC-resistant PCOS but is otherwise not considered to be more effective than first-line treatment with CC (Lebbi et al., 2015).

1.1.7.3 Acupuncture

Acupuncture is purported to increase fertility-related outcomes. A recent Cochrane review (Lim et al., 2019) attempted to compile the evidence of its efficacy. The review included eight RCTs with 1546 women with PCOS. It concluded that true acupuncture in comparison to placebo acupuncture may promote clinically relevant improvements in live birth rate, multiple pregnancy rate, ovulation rate, and miscarriage. However, all evidence was judged to be of low quality, with serious imprecision in confidence intervals. In addition, there was moderate quality evidence that true acupuncture may worsen adverse events, including dizziness and nausea, in comparison to placebo acupuncture.

1.1.7.4 Supplements

Although not generally prescribed for PCOS by clinicians, research has attempted to determine the efficacy of various natural supplements on PCOS-related outcomes. These include inositol, omega three fish oils, chromium, selenium, vitamin D, vitamin B and several herbal medicines. There is no high-quality evidence to support the effectiveness and safety of most nutritional supplements and herbal medicines other than inositol (Arentz et al., 2017).

Inositol is an intracellular second messenger that helps to regulate insulin signal transduction. Two stereoisomeric forms of inositol, myo-inositol and D-chiro-inositol (DCI), work to balance some of the metabolic deregulations implicated in insulin resistance. Myo-inositol enhances glucose transport into the cell through translocation of GLUT4 to the cell membrane, while downregulating the release of FFA from adipose tissues, while DCI promotes glycogen synthase to induce glucose conversion to glycogen for storage in cells (Unfer et al., 2017). Inositol has been reported to improve time to ovulation, ovulation rates,

hyperandrogenism, insulin sensitivity, WHR, cholesterol, and TG (Unfer et al., 2017; Artenz et al., 2017). However, some results suggest that inositol may take up to 24 weeks to affect changes to androgen profile (Unfer et al., 2017).

1.1.7.5 Lifestyle Advice

The options outlined above are examples of treatments that may be effective at treating or masking a specific symptom or outcome. However, each has risks, side effects, or low-quality evidence impacting on causality. In addition, they do not centre on long-term health consequences such as increased CVD risk.

Lifestyle advice is usually given to those women attempting to lose weight, or for those that are overweight and struggling to conceive (and would like to be put forward for fertility treatment) due to the general association between obesity and infertility (Dağ & Dilbaz, 2015). Lifestyle advice typically encompasses dietary and PA advice geared toward weight loss, i.e., caloric restriction, without specific recommendations for diet composition or modes of PA. However, PCOS guidelines have recently been updated to recommend lifestyle intervention for all women with PCOS to improve metabolic and reproductive outcomes, as well as reduce the risks of long-term health complications (Teede et al., 2018). Lifestyle intervention is applicable to all women with PCOS and considered to be the cornerstone treatment of PCOS (Krystock, 2014; Legro, 2017). This should be a key treatment for all women with PCOS regardless of weight or fertility status (Teede et al., 2018). Focusing on weight loss as an outcome alone, or only recommending lifestyle changes to overweight or obese women with PCOS, fails to acknowledge the wide-ranging impacts of lifestyle intervention on cardiovascular, metabolic, and reproductive health in PCOS.

Several systematic reviews have been conducted to clarify the parameters of effective lifestyle intervention to improve clinical guidelines (Harrison et al., 2011; Hutchison et al., 2011; Kite et al., 2019; Ladson et al., 2011). However, there currently remains a paucity of sufficiently homogenous randomised controlled trials (RCT) with high confidence in the evidence. In Chapter 2, the literature surrounding lifestyle intervention for women with PCOS will be discussed at length.

1.2 Conclusion

This chapter has outlined PCOS as a complex metabolic and endocrinological condition. Its clinical features, diagnostic criteria, prevalence, and pathophysiology have been presented. The issues associated with diagnosis, prevalence, and treatment, particularly regarding the four phenotypes, have been emphasised. There are various potential treatments, but they are typically not holistic and have side effects. Lifestyle advice from clinicians has the potential to be a foundation of treatment for PCOS, but tends to be generic, and further research is needed from intervention studies to provide clear clinical guidelines.

2 Literature Review

This chapter presents a review of the literature related to the factors and mechanisms that contribute to increased CVD risk in women with PCOS. It also explores the role of PA in reducing CVD risk. Finally, the literature related to lifestyle interventions in women with PCOS is reviewed. The overview of the thesis and programme of research is then set forth.

2.1 Cardiovascular Disease

This section outlines CVD and its prevalence in the general population and in women with PCOS. The CVD risk factors that are persistent in PCOS are identified, with discussion of insulin metabolism as an underpinning mechanism for their occurrence. The protective role of PA in CVD risk is then reviewed.

CVD refers to a group of disorders that affect the heart and blood vessels. These include coronary heart disease, cerebrovascular disease, peripheral arterial disease, rheumatic heart disease, congenital heart disease, and deep vein thrombosis (WHO, 2017). In 2016, approximately 17.9 million people died from CVDs, representing 31% of all global deaths. This makes CVDs the leading cause of death globally (WHO, 2017). In women specifically, CVD is responsible for approximately one third of deaths each year (American Heart Association, 2020). It is expected that 22.2 million people will die annually from CVD by 2030 (WHO, 2011). Although individual factors such as age, ethnicity, and genetics that predispose one to CVD cannot be changed, the WHO suggest that most CVDs are preventable through lifestyle change (WHO, 2017). Identifying effective strategies to attenuate risk is therefore of high importance to reduce CVD mortality.

There exists global variation in CVD rates because of temporal and regional variation in known risk factors (Gaziano, Reddy, Paccaud, Horton & Chaturvedi, 2006). For example, when countries experience economic and social growth and development, causes of mortality tend to shift from malnutrition and infectious diseases toward degenerative and human-created diseases (Olshansky & Ault, 1986). Thus, prevalence of CVD and its risk factors may depend on where countries sit in their economic development, and is not standardised globally (Gaziano et al., 2006).

The prominent INTERHEART study has been instrumental in establishing consistent information about risk factors across populations and socioeconomic levels, thus contributing

to uniform approaches to prevention that focus on behavioural and lifestyle intervention (Yusef et al., 2004; Stewart et al., 2017). The INTERHEART study, published in 2004, is a standardised international case-control study of acute myocardial infarction (MI) in 52 countries, representing every inhabited continent (Yusef et al., 2004). The study involved 15,152 cases and 14,820 controls to investigate the relationship between nine modifiable risk factors and MI. The nine modifiable factors accounted for 90% and 94% of the Population Attributable Risk (PAR) of MI in men and women respectively (Yusef et al., 2004). These risk factors were abnormal lipids, smoking, hypertension, diabetes, abdominal obesity, psychosocial factors, lack of consumption of fruits and vegetables, lack of alcohol consumption, and physical inactivity (Yusef et al., 2004).

The INTERHEART study presents odds ratios (OR) of myocardial infarction (MI) for each risk factor. Odds ratio is the odds of an event occurring given an exposure, compared to the odds of an event occurring in the absence of that exposure (Szumilas, 2010). If the OR is above 1, the odds are higher in the exposed group, whereas if the OR is below 1, the odds are lower in the exposed group. For example, smoking is the biggest risk factor for CVD with indication from European data that it doubles the 10-year CVD mortality rate (Piepoli et al., 2016). The INTERHEART study indicated that individuals smoking greater than 40 cigarettes per day had OR of 9.16 (99% CI: 6.18, 13.58) for MI compared to those who did not smoke. Similarly, if an individual avoided smoking completely the OR would be 0.35 (99% CI: 0.31, 0.39) compared to those who did smoke.

Importantly, Yusef et al. (2004) present data on the cumulative effects of risk factors, as it is unlikely that each exists in a vacuum. Indeed, it is this cumulative effect that has the greatest effect on odds; the OR of MI for each of the risk factors combined is 333.7 (99% CI: 230.2, 483.9). That is, the odds of MI are 333.7 times higher in those with all nine risk factors compared to those without them. It also quantifies the protective effects of three factors; fruits and vegetables (consumed daily), alcohol intake (consumed three or more times/week) and PA (moderate or strenuous exercise for four or more hours/week). For example, if one consumes daily fruits and vegetables and undertakes regular exercise, the OR for MI becomes 0.60 (99% CI: 0.51, 0.71) compared to those who do not. Thus, the odds of MI are lower in the group exposed to the protective factors than those not exposed.

The INTERHEART study has been influential in the development of European guidelines for prevention and rehabilitation of CVD (Piepoli et al., 2016). However, there is not an absolute

scientific consensus on the risk factors and their cut-offs. There is often conflicting research related to both risk thresholds and the predictive value of the risk factor. In addition, there are often practical considerations of a risk factor in terms of how easy and cost-effective it is to measure within a clinical environment.

2.1.1 Cardiovascular Disease Risk in PCOS

The presence of multiple CVD risk factors indicates that women with PCOS may be at increased risk of the development of CVD (Scicchitano et al., 2012). However, the risk factors in PCOS vary between phenotypes, and while it is generally acknowledged that women with PCOS have the potential for early atherosclerosis, myocardial and endothelial dysfunction, there is some debate as to how this translates into real CVD events (Papadakis et al., 2017). Based on several prospective and retrospective cohort studies, it is estimated that women with PCOS have between 20-79% increased CVD risk compared to controls (Ding, Tsai, Wang, Lin, & Sung, 2018; Glintborg, Rubin, Nybo, Abrahamson, & Anderson, 2018; Zhao et al., 2016). This wide estimation is due to various issues among studies reporting actual incidence of CVD in women with PCOS. Heterogeneity in study design (retrospective and prospective cohort, and case-control), diagnostic criteria and evaluation methods, lack of phenotype classification, and the possibility of controls having undiagnosed PCOS make it difficult to provide true estimates of risk. It may be a consequence of PCOS as a heterogenous syndrome that makes it difficult to calculate risks for PCOS *per se*. In addition, since there are phenotypes with varying CVD risk factors, an absolute relative risk for PCOS as a single group may be of limited use. Rather, risk could simply be assessed based on the presence of CVD risk factors and the dearth of research that provides relative risks and odds ratios for each.

With variation in the phenotypes, it may be of more use to separate PCOS by type in such studies estimating risk. Indeed, CVD risk may be increased for those phenotypes presenting with hyperandrogenism; this phenotype has been associated with a metabolic profile that encompasses higher incidences of insulin resistance and a worse lipid profile than those women with PCOS and a normo-androgenic profile, despite comparable distributions of body weight (Dewailly, 2016).

It is hypothesised that insulin resistance underpins the worse metabolic profile in this phenotype rather than androgen excess itself. Insulin acts as a co-gonadotropin, stimulating the ovary to produce testosterone, whilst simultaneously inhibiting the production of SHBG

which leads to a higher concentration of bio-available testosterone (Norman et al., 2004). Additionally, hyperinsulinemia can lead to hepatic overproduction of very low density lipoproteins (VLDL), and elevation in triglycerides (TG) through decreased lipoprotein lipase-mediated lipolysis, leading to increased circulating chylomicrons and VLDL (Ginsberg, Zhang, & Hernandez-Ono, 2005). Skeletal muscle insulin resistance may also promote dyslipidaemia by redirecting dietary carbohydrate sources away from skeletal muscle glycogen synthesis into hepatic de novo lipogenesis, and subsequently increased circulating triglycerides assembled from glucose substrates and a reduction in high-density lipoprotein (HDL) concentrations (Petersen et al., 2007).

There is some debate about whether the increased association with CVD risk factors is due to PCOS itself, or whether it is due to commonly associated obesity. Obesity is an exacerbating factor (Legro, 2012). However, it is estimated that around half of women with PCOS have overweight or obesity, and these women display comparable hormonal and metabolic abnormalities to women without overweight or obesity with PCOS (Gambineri, Pelusi, Vicennati, Pagotto, & Pasquali, 2002; Toody, Sodi & Pappachan, 2018). Furthermore, insulin resistance is present in up to 75% of lean women with PCOS (Teede et al., 2018). Nevertheless, a common feature in both overweight and lean women with PCOS is central adiposity (Norman et al., 2004); the tendency for fat to accumulate around the abdominal area, including both visceral fat and subcutaneous fat. A woman with a BMI within the 'healthy' range could still exhibit abdominal obesity due to excessive visceral fat (Pasquali, 1994). This type of body composition may contribute to insulin resistance because visceral fat secretes IL-6, an adipokine that inhibits insulin-mediated glycogenesis and stimulates hepatic gluconeogenesis (Senn, Klover, Nowak, & Mooney, 2002). Overall, it is generally accepted that insulin resistance is a major underlying feature of PCOS (Sattar, 2011).

2.1.2 Studies of Cardiovascular Disease Prevalence in PCOS

While the increased presence of various CVD risk factors in PCOS has been well-established, the actual prevalence of CVD in women with PCOS has been less clear. Several studies have been reported, with conflicting results. One of the first studies to report this data was by Wild, Pierpoint, McKeigue, and Jacobs (2000). They conducted a retrospective cohort study of women diagnosed with PCOS in the UK before 1979. Data were collected from 319 women with PCOS and 1,060 age-matched controls, as well as analysis of cause-of-death

from 70 cohort members. They concluded that although women with PCOS had a significantly higher prevalence of risk factors, including diabetes, hypertension, hypercholesterolaemia, hypertriglyceridemia, and increased WHR, all-cause mortality in the PCOS cohort was similar to the women in the general population. They do note a higher mortality and morbidity rate from diabetes and risk of non-fatal cerebrovascular disease. However, the authors attribute this to the increased prevalence of obesity and family history of diabetes among women with PCOS. Although this study adjusted for socioeconomic status, hormone replacement therapy, and smoking rates, they did not measure testosterone or SHBG. This may have affected results, since the hyperandrogenic phenotype of PCOS is associated with greater CVD risk (Dewailly, 2016).

Similarly, Ifitkhar and colleagues (2012) undertook a retrospective cohort study in the US, comparing CV events in women with PCOS compared to those without. The cohort included 309 women with PCOS and 343 controls. Although women with PCOS had a higher BMI (29.4 kg/m^2 compared to 28.3 kg/m^2), they observed no increase in CV events, death, death due to CV disease, or stroke. Further, prevalence of T2D, hypertension, and lipoprotein profile were similar in the two groups. This study indicated that the Rotterdam criteria were used to identify women with PCOS, although they also did not report details of androgen profile, nor indices of insulin resistance.

Some studies have shown a higher incidence of CVD in PCOS compared to age-matched controls (Ding, Tsai, Wang, Lin, & Sung, 2018; Glintborg, Rubin, Nybo, Abrahamson, & Anderson, 2018). A retrospective cohort study aiming to investigate the risk of coronary artery disease (CAD) in Taiwan included 8,040 women aged 15-49 years with PCOS and 32,192 age-matched controls (Ding et al., 2018). After a mean follow up of 5.9 years, the adjusted hazard ratio (HR) of CAD was 1.44 (95% CI: 1.14, 1.81) in PCOS compared to controls. An observational, register-based Danish study of 18,112 women with PCOS and 52,769 age-matched controls found similar results (Glintborg et al., 2018). The results indicated that after a mean follow-up of 11.1 years, the HR for development of CVD in women with PCOS was 1.7 (95% CI: 1.7, 1.8) compared to controls. Both studies indicated increased risk when PCOS occurred with diabetes, obesity, and in the latter study, use of OCP.

A recent systematic review and meta-analysis was conducted to identify the association between PCOS and CVD (Zhao et al., 2016). The authors identified 10 studies, including five

case-control studies and five cohort studies, for a total of 104,392 subjects. In analysis of all included studies, PCOS was significantly associated with increased CVD risk (OR=1.30, 95% CI: 1.09, 1.56). In a sub-group analysis by study design, the results indicated that both case-control and prospective cohort study designs showed significant increased risk (OR=1.79, 95% CI: 1.16, 2.77, and OR=1.20, 95% CI: 1.06, 1.37, respectively), but retrospective cohort study designs did not.

There are several issues that lead to conflicting results. For example, there are important differences between these studies in how PCOS was initially diagnosed. In the study by Ding et al. (2018) a diagnosis of PCOS was based on blood tests for luteinising hormone (LH), follicle stimulating hormone, (FSH) and testosterone, and/or ultrasonography. LH and FSH are not included in any of the three sets of diagnostic criteria. Furthermore, there does not appear to be any consideration of menstrual irregularity in the diagnosis. It is not clear which participants also underwent ultrasonography for their diagnosis, or whether diagnosis was given on ultrasonography alone. In addition, in most studies, it is possible that age-matched controls may have undiagnosed PCOS, leading to underestimation of CVD in PCOS. Heterogeneity in study designs has thus led to conflicting results across the literature regarding actual prevalence of CVD in PCOS.

Further research is needed to determine how the increased presence of CVD risk factors translates into incidence and prevalence of CVD in PCOS. However, it is clear that PCOS is associated with several of the risk factors for CVD, namely abnormal lipids, hypertension, diabetes and abdominal obesity (Carmina & Lobo; 2004; Kim & Choi, 2013; Dokras, 2013; Papadakis et al., 2017; Sattar, 2006). Insulin resistance, a key underpinning feature of PCOS, is a potent contributor to CVD and may provide a mechanism to explain increased CVD risk factors in PCOS (Miller, 2009; Sattar, 2006).

2.1.3 The Role of Insulin Metabolism in Cardiovascular Disease

Insulin resistance is an underpinning feature of PCOS, and impaired insulin metabolism plays a key role in the development of CVD. It is therefore important to outline the mechanisms of CVD risk in PCOS as it relates to insulin metabolism. In this section, normal insulin metabolism will be outlined, before going on to discuss insulin resistance. The role of insulin resistance in the development of CVD will then be reviewed. Suggested mechanisms for impaired insulin metabolism in PCOS are also highlighted.

2.1.3.1 Normal Insulin Metabolism

Insulin is an anabolic peptide hormone produced by beta cells in the pancreatic islets of Langerhans. Its primary role is to promote the synthesis of energy storage molecules. That is, it transitions the body from a post-absorptive state, where nutrients are no longer being absorbed from meals, to an absorptive state, the postprandial period after a meal where nutrient intake is increased. During the post-absorptive state, energy stores (primarily glycogen and triglycerides) are mobilised to meet the energy needs of the cells. During the absorptive state, nutrients are plentiful and while plasma glucose serves as the primary energy source for cells, fatty acids, amino acids, and excess glucose are taken up by the liver, skeletal muscle, and adipose tissue and stored (Germann & Stanfield, 2002).

Insulin promotes the storage and synthesis of protein, triglycerides, and glycogen, whilst simultaneously inhibiting breakdown of these molecules. It works antagonistically with glucagon which is produced by alpha cells in the pancreatic islets of Langerhans. Glucagon promotes the breakdown of proteins, glycogenolysis, and lipolysis whilst inhibiting glycogenesis and lipogenesis. Thus, the two peptide hormones work contrarily to switch the body between the absorptive and post-absorptive states (Germann & Stanfield, 2002).

Both insulin and glucagon secretion are regulated by plasma glucose concentrations, amongst other factors. Glucagon promotes increases in plasma glucose concentration through glycogenolysis (the breakdown of glycogen molecules into glucose) and gluconeogenesis (the process by which the liver assembles glucose molecules from other non-carbohydrate substrates). Insulin decreases plasma glucose by suppressing gluconeogenesis and increasing transport of glucose across cell membranes. It achieves this by binding to the cell surface insulin receptor (IR), which subsequently initiates a signalling cascade initiated by tyrosine phosphorylation of insulin receptor substrates (IRS) (Abel, O'Shea, & Ramasamy, 2012). The result is increased translocation of GLUT 4, a glucose transport protein, from the cytosol to the plasma membrane. Defects in this signalling process are observed in insulin resistance, T2D, and metabolic syndrome (Abel et al., 2012; Brewer, Habtemichael, Romenskaia, Mastick, & Coster, 2014).

2.1.3.2 Insulin Resistance and CVD

Insulin resistance is characterised by an attenuated biological response from cells, typically skeletal or adipose cells, to normal or elevated insulin levels, impairing insulin-mediated glucose disposal, glycogen synthesis, and the ability to suppress lipid oxidation (Ormazabal et al., 2018; Wilcox, 2005). In response, the pancreas attempts to compensate by increasing its secretion of insulin, leading to hyperinsulinemia (Wilcox, 2005). For this reason, normal glucose tolerance can be maintained for some time in the face of insulin resistance and hyperinsulinemia (Ormazabal et al., 2018). Insulin resistance and hyperinsulinemia play key roles in the development of various abnormalities and medical conditions, including hypertension, atherosclerosis, metabolic syndrome, PCOS, non-alcoholic fatty liver disease (NAFLD), dyslipidaemia, and more (Abel et al., 2012; Wilcox, 2005). Eventually, the beta cells become unable to compensate for the prevailing insulin resistance by hyperinsulinemia, and so begins the onset of T2D (Reaven, 2004).

Various studies indicate that insulin resistance leads to and even predicts incident CV events (Bonora et al., 2007; Eddy, Schlessinger, Kahn, Peskin, & Schiebinger, 2009; Gast, Tjeerdema, Stijnen, Smit, & Dekkers, 2012). Bonora et al. (2007) conducted a prospective cohort study of 919 Italians aged 40-79 years with a 15-year follow-up. Homeostasis model assessment for insulin resistance (HOMA-IR) was measured at baseline. During follow-up, 118 participants experienced a first symptomatic CVD event. HOMA-IR was significantly higher in these participants at baseline than those free of CVD. Further, after adjustment for age, sex, smoking, PA, classic risk factors (blood glucose, LDL-C, HDL-C, BMI, triglycerides,) and novel risk factors (fibrinogen, oxidised LDL, high- sensitivity C-reactive protein, and more), the hazard ratio of symptomatic CVD relative to non-insulin resistant subjects was 2.2 (95% CI: 1.4-3.6, $P < 0.001$).

Eddy and colleagues (2009) attempted to identify the portion of coronary artery disease (CAD) incidence that could be attributed to insulin resistance in comparison to other metabolic variables and CAD risk factors. They used the Archimedes model, which is a model simulation that uses of real-person health data to chart the incidence and progression of disease. The model ensures that distribution and correlation of important variables are the same in the simulated population as those in the real population. They used the model to create a simulated population of 10,000 young adults aged 20-30 years, followed for 60 years or until they died. They were put through simulated clinical trials to assess the impact that

each variable had on CAD events over time. Their results indicated that normalising insulin sensitivity in this population could prevent 42% of myocardial infarctions. This was the single most important risk factor for CAD. Other variables and common risk factors for CAD including hypertensive SBP, obese BMI, and smoking were entered into the model. The result of normalising these variables were 36%, 21%, and 9%, respectively, reductions in myocardial infarction.

Finally, Gast et al. (2012) conducted a meta-analysis of 65 studies (n = 516,325) to compare the association between fasting glucose, fasting insulin, and HOMA-IR indices with incident CVD. Per one standard deviation increase in HOMA-IR, the relative risk of CHD was 1.46 (95% CI: 1.26-1.69), compared to glucose and insulin concentrations of 1.21 (95% CI: 1.13-1.30) and 1.04 (95% CI: 0.96-1.12), respectively.

The evidence outlined suggests that insulin resistance plays a central role in the development of CVD. Insulin resistance contributes to CVD by several mechanisms including endothelial dysfunction, dyslipidaemia, hypertension, and inflammation.

In the endothelium, Nitric Oxide (NO) mediates endothelial-dependent relaxation, and inhibits platelet aggregation, cell adhesion, and smooth muscle proliferation (Wilcox, 2005). Insulin stimulates the key catalytic enzyme that synthesises NO and endothelial nitric oxide synthase (eNOS), as well as stimulating production of its cofactor, thus enhancing NO production. In the case of insulin resistance, cofactor levels are reduced and eNOS downregulated, leading to impaired endothelial function, hypertension, and atherosclerosis (Wilcox, 2005; Abel et al., 2012). Furthermore, compensatory hyperinsulinemia increases pro-coagulant factors, contributing to increased platelet aggregation. In addition, while cells are resistant to insulin's metabolic effects, they are still sensitive to the mitogenic properties of insulin, which promote vascular smooth muscle cell proliferation, a key event in the development of atherosclerosis (Wheatcroft, Williams, Shah, & Kearney, 2003).

Insulin resistance promotes dyslipidaemia via several different processes. Insulin usually inhibits lipolysis in adipocytes, but in the presence of insulin resistance, these suppressive effects are lost, thus leading to an increase in mobilisation of free fatty acids (FFA). In addition, the influx of lipids from different sources, including de novo lipogenesis and triglyceride-rich lipoproteins, leads to increased hepatic assembly and secretion of VLDL to carry energy between the liver and the adipose tissue (Semenkovich, 2006). In the case of insulin sensitivity, these processes are usually inhibited post-prandially to prevent excessively

high plasma triglycerides. In the insulin resistant state, there is hence an excess of FFA availability and an increase in VLDL production (Ormazabel et al., 2018). Triglyceride-rich VLDL transfers triglycerides to HDL in exchange for cholesteryl esters, facilitated by cholesterol ester transfer protein (CETP). The resulting triglyceride enriched HDL then becomes a substrate for the lipolytic action of hepatic lipase, leading to a reduction in plasma HDL-C concentrations (Rashid, Watanabe, Sakaue, & Lewis, 2003). Thus, accelerated atherosclerosis in the presence of insulin resistance could be a result of increased delivery of atherogenic VLDL-derived particles into the vasculature, and a decreased availability of athero-protective HDL particles (Semenkovich, 2003).

Dyslipidaemia in the presence of insulin resistance has been linked to greater risk of CHD events. Children of the original participants of the Framingham Heart Study (n = 2,910) were followed for a mean of 14 years to determine the effect of insulin resistance on the dyslipidaemia and CHD relationship (Robins, Lyass, Zachariah, Massaro, & Vasan, 2011). Participants with diabetes or a history of CHD were excluded. In total, 128 participants experienced an incident CHD event. The cumulative incidence of a CHD event was greatest for groups with insulin resistance and either the lowest HDL-C or the highest triglycerides, and the incidence rates were significantly higher in these groups compared to those with comparable HDL-C or triglycerides but without insulin resistance ($P<0.001$). Interestingly, in the absence of insulin resistance, the risk of CHD events was not significantly increased for those with low HDL-C and high triglycerides compared to those with high HDL-C and low triglycerides. Thus, the study concludes that it is in fact insulin resistance, not obesity, associated with dyslipidaemia that best defines a high CHD-risk state (Robins et al., 2011).

Insulin resistance and inflammation are intricately linked, and it is generally accepted that systemic inflammation contributes to insulin resistance (Straub, 2011). Inflammation promotes insulin resistance through a variety of pathways. Obesity, and the accumulation of visceral adipose tissue, are major causes of increased release of pro-inflammatory chemokines, cytokines, and adipokines (Abel et al., 2012; Chen, Chen, Wang, & Liang, 2015; Rehman & Akash, 2016). Adipose tissue, particularly visceral adipose tissue, is an active endocrine organ which has the ability to produce a variety of mediators that regulate the energy metabolism and insulin sensitivity, including interleukin-1β, IL-6, and TNF-a, which lead to local inflammation in adipocytes and systemic inflammation upon entering the bloodstream (Rehman & Akash, 2016). As adiposity increases, adipocytes release chemoattractants, which initiate the migration of monocytes into the tissue. The monocytes

subsequently differentiate into macrophages, which are the primary source of cytokine production, producing large amounts of TNF-a, IL-1β, and IL-6 (Chen et al., 2015; Ormazabel et al., 2018; Rehamn & Akash, 2016). TNF-a and IL-6 increase lipolysis and reduce triglyceride synthesis and insulin-stimulated glucose uptake by interfering with insulin post-receptor signalling, resulting in an increase in circulating triglyceride levels (Ormazabel et al., 2018; Rehman & Akash, 2016).

The resulting increase in plasma FFA contributes to the hypertriglyceridaemia observed in insulin resistance (Wilcox, 2005). The excess FFA can thus result in lipotoxicity, where FFA and its metabolites, such as ceramides and diacylglycerol (DAG), are stored in non-adipose tissue not adapted for lipid storage, including skeletal muscle, liver, heart, and blood vessels (Abel et al., 2012; Orzamabel et al., 2018; Rehman & Akash, 2016). Elevated plasma FFA can also interfere with insulin signalling pathways, particularly IRS-1 serine phosphorylation (Rehman & Akash, 2016). Glucolipotoxicity, occurring from hyperglycaemia and dyslipidaemia, is responsible for the activation of various pro-inflammatory mediators that lead to specific tissue insulin resistance and impaired insulin secretion from the pancreatic beta cells (Rehman & Askash, 2016).

Hyperglycaemia and hypertriglyceridemia increase cellular oxidative stress, producing ROS (Abel et al., 2012). Pancreatic beta cells, adipocytes and peripheral tissues are vulnerable to the damaging effects of oxidative stress. Peripheral and adipose tissues thus protect themselves by inducing insulin resistance to prevent glucose and FFA entering the cells (Rehman & Akash, 2016). This occurs through activation of stress-signalling pathways that decrease insulin signalling pathways and insulin-mediated glucose uptake. This pathway is subsequently associated with endothelial dysfunction and the upregulation of TNF-a, IL-6, and CRP, contributing further to the inflammatory state (Rehman & Akash, 2016). Figure 2 provides a schematic representation of adipocytokine induced insulin resistance.

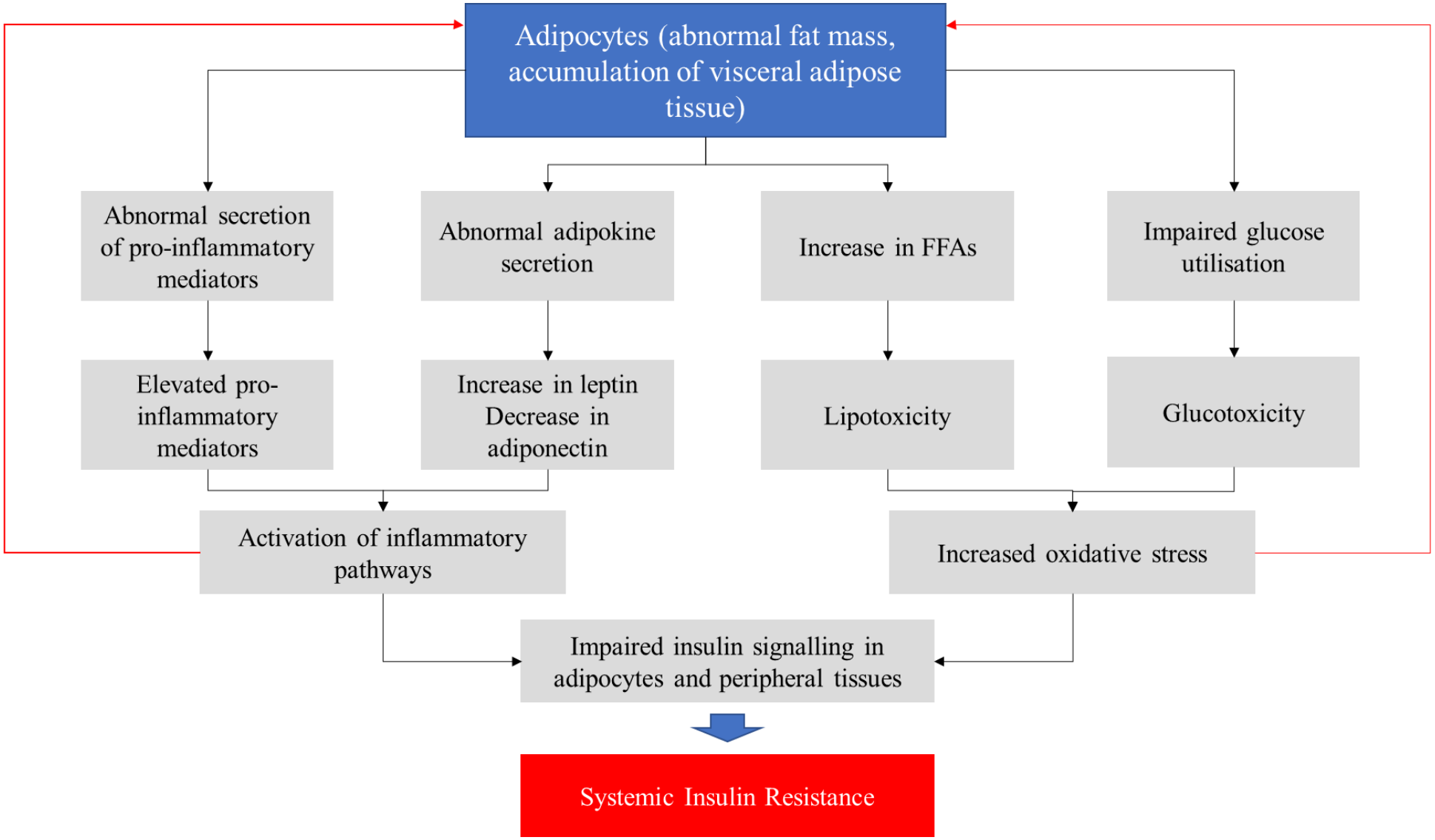

Figure 2. Schematic representation of adipocytokine induced insulin resistance. Adapted from Rehman and Akash (2016).

CRP, an acute-phase protein synthesised by the liver, is an inflammatory marker that is up-regulated significantly during inflammation (Chen et al., 2015). It is regulated by pro-inflammatory cytokines such as IL-6 and TNF-a and thus is strongly correlated to the development of insulin resistance (Chen et al., 2015; Rehman & Akash, 2016). CRP further participates in the pathogenesis of insulin resistance by inducing local and systemic inflammation (Rehman & Akash, 2016).

2.1.3.3 Impaired Insulin Metabolism in PCOS

Women with PCOS exhibit many of the abnormalities caused by insulin resistance that are associated with increased CVD risk, including dyslipidaemia, endothelial dysfunction, hypertension, central adiposity, and inflammation (Dokras, 2013; Papadakis et al., 2017). In PCOS, the cause of insulin resistance is hypothesised to be a post-receptor defect in insulin signalling, where phosphorylation of insulin-receptor substrate -1 (IRS-1) serine residues is increased while the phosphorylation of the tyrosine residues is decreased (Baptiste, Battista, Trottier, & Baillargeon, 2010). This exaggerated serine phosphorylation may cause a decrease in insulin-stimulated IRS-1 activation and subsequently a decrease in translocation of GLUT 4, leading to decreased cellular glucose uptake (Carpentier, 2008; Gonzalez, 2012).

The pro-inflammatory cytokine TNF-a is elevated in PCOS independent of obesity, and TNF-a is a known mediator of insulin resistance by inducing the exaggerated serine phosphorylation of IRS-1 (Gonzalez, 2012). TNF-a is produced by visceral adipose tissue (Carpentier, 2008), and this increased distribution of intra-abdominal fat has been shown to be more prevalent in women with PCOS compared to weight-matched controls (Carmina et al., 2007). In addition, intra-abdominal fat releases more FFA into circulation than subcutaneous fat (Baptiste et al., 2010), and the increased availability of FFA may lead to storage of lipids in non-adipose tissue such as muscle cells, leading to lipotoxicity and inflammation (Aye et al., 2018; Baptiste et al., 2010; Zhou, Wang, & Yu, 2014). The accumulation of these intra-myocellular lipid metabolites (such as diacylglycerols and ceramides) have been postulated to activate intra-cellular serine kinases which may be key to the insulin-signalling pathway defect that results in insulin resistance (Aye et al., 2018; Baptiste et al., 2010). Women with PCOS have been shown to have increased FFA availability (Aye et al., 2018).

Obesity in women with PCOS also exacerbates insulin resistance and inflammation due to hypoxia-related adipocyte death, resulting from adipose tissue expansion. This leads to

mononuclear-cell (MNC) infiltration which become macrophages, subsequently releasing TNF-a and IL-6, contributing to insulin resistance (Gonzalez, 2012; Zhou et al., 2014) However, even in the absence of obesity, MNC sensitivity to glucose is increased in PCOS, and glucose ingestion promotes an inflammatory response (Gonzalez, 2012).

2.1.4 Cardiovascular Disease Risk Factors in PCOS

This section outlines the CVD risk factors that are commonly identified in women with PCOS. For each one, this includes a review of the impact of each risk factor on the development of CVD and cardiovascular events. This is to demonstrate the importance of continued investigation to mitigate these factors in women with PCOS.

2.1.4.1 Abnormal Lipids

Abnormal lipids, characterised by high LDL-C and TG concentrations and low HDL-C concentrations, are prevalent in approximately 70% of women with PCOS (Kim & Choi, 2013). This persists in non-obese women with PCOS and is consistent with an insulin resistant state (Kim & Choi, 2013). Apolipoprotein ratio and LDL-C are risk factors for CVD, with evidence indicating that the two are similar predictors for CVD (Piepoli et al., 2016; Yusef et al., 2004). Apoproteins are the protein component of lipoproteins, where apolipoprotein B (ApoB) is the main component of LDL and apolipoprotein A1 (ApoA1) the main component of HDL (Piepoli et al., 2016). ApoA1 levels have been shown to be significantly lower in PCOS compared to controls (Valkenburg et al., 2008).

In the INTERHEART study, the authors used the ApoB/ApoA1 ratio to calculate ORs. This ratio was the second strongest risk factor after smoking. The OR for myocardial infarction for the top versus the lowest decile of ApoB/ApoA1 ratio was 4.73 (99% CI: 3.93, 5.69). This relationship was also graded, similar to smoking, with risk increasing as the ratio increased (Yusef et al., 2004). Risk decreases incrementally with reductions in LDL-C. In a meta-analysis of 22 statin versus controls trials (n=134, 537), each 1 mmol/L reduction in LDL-C corresponded to approximately 20% reduction in risk of major vascular events per each 1 mmol/L reduction (Cholesterol Treatment Trialists' Collaborators, 2012). The recommended LDL-C concentration for those with a low overall CVD-risk is ≤ 100 mg/dL, and for those with a history of CV events, lowering LDL-C to ≤ 70 mg/dL reduces the risk of recurrent CV events (Piepoli et al., 2016).

Low HDL-C is also independently associated with CVD risk, regardless of LDL-C concentrations (Cholesterol Treatment Trialists' Collaboration, 2010; Piepoli et al., 2016). HDLs confer many vasculoprotective benefits, including attenuation of oxidative stress and inflammatory responses, contribution to endothelial repair, anti-thrombotic activity, and vasodilatory activity, all of which may attenuate key events in atherosclerotic plaque formation (Chapman et al., 2011). An HDL-C level of < 40 mg/dL in men and < 45 mg/dL in women may indicate increased CVD risk (Piepoli et al., 2016).

There is evidence to suggest that measurement of either LDL-C or non-HDL-C in isolation may not provide accurate estimation of CVD risk, and that the TC:HDL ratio may be a more powerful predictor. Quispe et al. (2019) studied 14,403 participants in a prospective study to investigate the aetiology and clinical sequalae of atherosclerosis. All participants were free of atherosclerotic CVD at baseline. A proportion of participants had LDL-C and non-HDL-C levels below the mean, but their TC: HDL ratio was still at or above the mean. These participants had 24-29% greater risk for atherosclerotic CVD compared to those participants with a TC:HDL ratio less than the mean. This risk also increases incrementally as the TC:HDL ratio increases, independent of other risk factors. A prospective cohort study of 6,147 women aged 50-59 years indicated a 16% increase in the risk of MI per one unit increase in TC:HDL ratio, after adjustment for age, education, smoking, WHR, blood pressure, and socioeconomic status (Calling, Johansson, Wolff, Sundquist, & Sundquist, 2019). This suggests that TC:HDL ratio is a powerful predictor of acute myocardial infarction and should not be overlooked in the assessment of CVD risk.

2.1.4.2 Oxidised LDL

Atherosclerosis, the primary cause of CVD, is a progressive disease characterised by the accumulation of lipids and fibrous elements in the intima of the artery, leading initially to fatty streak lesion formation. The fatty streak lesions lead to advanced fibrous lesions characterised by the proliferation of smooth muscle cells and lipid-rich necrotic debris, known as atherosclerotic plaques. In the final stage, lesions become increasingly complex, undergoing calcification, ulceration, and haemorrhage (Assman, 1982; Lusis, 2000). Atherosclerotic plaques can grow sufficiently large to result in stenosis of the vascular lumen, which can progress to complete occlusion (Assman, 1982). This occlusion leads to coronary ischaemia; however, the most important clinical implication is acute occlusion caused by

rupture of the lesion, leading to blood clot and resulting in myocardial infarction or stroke (Lussis, 2000).

The term ‘oxidised LDL’ is a general term and may refer to several types of modified LDL depending on the oxidant, the modified components, and products. In atherosclerosis, the initial fatty streak lesions are caused by increased transport of lipoproteins, particularly LDL, into the arterial wall, and decreased transport of lipids out of the arterial wall (Assman, 1982). Thus, increased circulating concentrations of LDL and reduced circulating concentrations of protective HDL, which removes excess cholesterol, lead to greater accumulation (Lusis, 2000). Once LDL is trapped in the vessel wall, it undergoes modification, including oxidation, as a result of exposure to reactive oxygen species (ROS) generated as waste products of vascular cells, and by other enzymes that occur in atherosclerotic legions (Lusis, 200). The oxidative changes to LDL involve both its lipid and protein components and generate a range of products including reactive aldehydes, cholesterol oxidation products, and protein oxidation products such as protein cross-links and lipid-protein adducts, depending on the type and extent of oxidation (Parthasarathy, Raghavamenon, Garelnabi, & Santandom, 2010).

In the arterial lesion, the modification of LDL triggers an inflammatory response in the endothelial cells characterised by the recruitment of monocytes and their subsequent differentiation into macrophages, so-called ‘scavenger cells’ (Assman, 1982; Lusis 2000). Macrophages are capable of absorbing LDL through phagocytosis, where the lipoprotein deposits its cholesterol portion into the cell, thus forming foam-cells (Assman, 1982). The accumulation of cholesterol in the foam cells leads to cell death, and their lipid contents contribute to the necrotic centre of the lesion (Assman, 1982; Lusis, 2000). It is these cholesterol-filled foam cells that constitute the initial fatty streak lesions that begin the progress of atherosclerosis; thus, LDL and its oxidation is the critical element in atherogenesis (Lee, Margaritis, Channon, & Antioniades, 2012).

Since the discovery of the role of oxLDL in atherosclerosis, various studies have attempted to examine whether oxLDL is associated with, or even predictive of, the disease process (Parthasarathy et al., 2012). Variation between studies exists because of the differing types of oxLDL and their detection methods. Indeed, protocols target different epitopes of oxLDL, its receptors, its by-products, and its anti-bodies (Itabe, Obama, & Kato, 2011; Parthasarathy et al., 2012).

Meisinger et al. (2005) conducted a prospective case-control study to examine the association between plasma oxLDL and the risk of CHD. Participants included 346 men without CHD or diabetes mellitus at baseline, with a mean follow-up of 5.6 ± 2.6 years. Baseline mean plasma oxLDL concentrations were significantly higher in those participants who subsequently experienced an event compared to controls. After adjustment for smoking, hypertension, obesity, PA, education, and alcohol consumption, the HR for a future CHD event in the upper tertile of oxLDL distribution was 4.25 (95% CI: 2.09, 8.63) compared with the lower tertile. Additionally, plasma oxLDL was the strongest predictor compared with conventional lipoprotein profile and traditional CHD risk factors. These results indicate that plasma oxLDL is an independent CHD risk factor in apparently healthy men.

Amaki et al. (2004) conducted a study to compare malondialdehyde-modified LDL (MDA-LDL) concentrations in patients with severe CAD (n=43 males and 10 females, aged 65.3 ± 9.4 years) and healthy controls (n=46 males and 11 females, aged 50.4 ± 13.1 years). Malondialdehyde (MDA) is a reactive aldehyde produced from oxidative fatty acid degeneration. MDA may then further modify the protein components of LDL, resulting in MDA-LDL. Thus, the presence of MDA has long been used as a 'yard-stick' that reflects the extent of lipid peroxidation, and MDA-LDL is thought to reflect naturally occurring oxidation of LDL (Amaki et al., 2004; Parthasarathy et al., 2012). The results of the study indicated that MDA-LDL was significantly raised in patients with CAD compared to healthy controls. Furthermore, MDA-LDL was not associated with age, sex, smoking, hypertension, or hyperlipidemia, indicating that MDA-LDL is an independent risk factor for CAD.

Since then, numerous other studies have demonstrated an association between CVD and oxLDL. A recent systematic review summarised the results (Gao & Liu, 2017). Of 19 prospective cohort and case-control studies reporting the association between circulating oxLDL and atherosclerotic CVD events (ASCVD), 13 found a significant association. After adjustment for LDL-C, this association remained significant in eight studies. One of the studies that adjusted for LDL-C reported that the risk of major adverse coronary events (MACE) in 246 patients increased by 215% (95% CI: 47%, 576%) in patients with the highest quartile of oxLDL compared to those in the lowest quartile. However, six studies found no such association independent from LDL-C.

Whilst the majority of studies included in the review found a significant association, there remains some inconsistency. This may be the result of differences in laboratory detection

methods for oxLDL. While several enzyme-linked immunosorbent assays (ELISA) exist for the detection of oxLDL, they recognise different epitopes that represent varying stages and types of LDL modification. For example, one ELISA cannot detect oxLDL with fewer than 60 lysine modifications; that is, it may not detect LDL with only fatty acid modifications, or those earlier on in the stages of oxidation. The authors did not specify which detection method each study used (Gao & Liu, 2017).

Female sex has been identified as a factor associated with elevated oxLDL (Mosca et al., 1997). However, there are fewer studies incorporating female participants, and even fewer studies investigating oxLDL levels in PCOS, despite the establishment of dyslipidaemia and insulin resistance as key metabolic aberrations in PCOS. Only three studies report this, as summarised below.

Macut et al. (2006) investigated 179 women with PCOS and 56 age- and BMI-matched controls. They measured oxLDL, blood lipids and lipoproteins, insulin resistance, and testosterone and SHBG concentrations. Their results indicated that both lean and overweight women with PCOS had similarly increased oxLDL concentrations compared to controls. In addition, oxLDL concentrations were not dependent on severity of hyperandrogenism or insulin resistance. Furthermore, although both overweight women with PCOS and overweight controls displayed elevated TG and lower HDL-C concentrations, oxLDL concentrations were not elevated in controls in comparison, suggesting a primary alteration in lipid metabolism in PCOS.

Macut and colleagues (2008), in a follow up study, aimed to determine the association of key lipid and lipoprotein fractions with insulin resistance in 75 women with PCOS (mean age = 23 years) and 51 age- and BMI-matched controls (mean age = 25 years). Their results indicated again that dyslipidaemia, insulin resistance, and elevated oxLDL concentrations were significantly more pronounced in women with PCOS than controls, even after adjustment for BMI.

One study did not find evidence of elevated oxLDL in PCOS. Demiral et al. (2007) investigated the interaction between dyslipidaemia, serum leptin, and asymmetric dimethylarginine (ADMA), a NO synthetase inhibitor, in adolescent females with PCOS. The sample was constituted of 23 obese females with PCOS, 21 non-obese females with PCOS, and 31 lean, healthy controls. The study found that although markers of dyslipidaemia were higher in PCOS than in controls, there were no significant differences in oxLDL and ADMA

concentrations in any of the groups. The inconsistency may be due to age. Research suggests that the progression of atherosclerosis is not only dependent on the presence of risk factors, but also the capacity of repair mechanisms that counter endothelial injury, which subsequently become impaired with aging (Rauscher et al., 2003). Thus, the adolescent females in the latter study, despite the presence of risk factors, may have thus far attenuated the progression of atherosclerosis.

Endothelial dysfunction, characterised by reduced NO bioavailability and increased generation of ROS in the vascular wall, is an early step in the process of atherogenesis (Tousoulis et al., 2006). ROS are unavoidable by-products of various biological processes, including cellular respiration, and are used by macrophages and neutrophils to break down pathogens, among other uses. Despite their essential role, ROS are also responsible for direct damage to cellular structures. Free radicals, a particularly reactive and unstable type of ROS, initiate and propagate a chain reaction that damages molecules until two radicals meet and stabilise each other (Kimball, 2015). As such, there are usually tightly controlled pathways that contribute to both the production of ROS and their elimination via antioxidant mechanisms (Lee, Margaritis, Channon, Antoniades, 2012). Oxidative stress occurs when there is an imbalance between the production of ROS and the endogenous antioxidant mechanisms that counteract or repair the resulting damage of ROS, and increases with age (Antoniades, Antonopoulos, Bendall, & Channon, 2009).

Oxidative stress is thus a critical feature of atherosclerosis, and risk factors such as smoking, diabetes, and hypertension are linked to increased production of ROS (Lee et al., 2012). Native LDL that become trapped in the vascular wall are extremely susceptible to the oxidative damage caused by ROS. They subsequently undergo either lipid peroxidation or apolipoprotein B-100 modification to form oxLDL, which then plays a key role in many pro-atherogenic steps, including endothelial dysfunction, release of inflammatory cytokines, and the formation of foam cells (Lee et al., 2012; Parthasarathy et al., 2012).

Despite the presence of multiple risk factors in PCOS, including endothelial dysfunction, oxLDL is rarely used as a marker of CVD risk in PCOS. In a clinical setting, using oxLDL may not be practical, because it must be measured via laboratory assay. However, further research is necessary to investigate both the presence of elevated oxLDL in PCOS, and whether this indicates increased CVD risk.

2.1.4.3 Diabetes Mellitus

Women with PCOS have a higher prevalence of diabetes mellitus (DM) than the general population. In an 18-year follow-up study of 1,127 women, of which 4.7% met the NIH criteria for PCOS, PCOS was associated with an adjusted OR of 2.4 (95% CI: 1.2, 4.9) DM compared to those without PCOS (Wang et al., 2011). In another follow-up study (mean follow-up 16.9 years) of 255 Italian women meeting the NIH criteria for PCOS, the prevalence of T2D in PCOS was 39.3% at the end of follow-up. The prevalence of T2D in the Italian population of a similar age is estimated to be 5.8% (Gambineri et al., 2012). In the former study, the OR was adjusted for other risk factors including high BMI. In the latter study, the mean BMI of the participants was 29.1 ± 7 kg/m^2, thus it is unclear how much influence baseline BMI had on the incidence of DM; the authors note the increasing risk concurrent with increasing BMI. Nonetheless, both studies used the NIH criteria for PCOS diagnosis. These are the most restrictive criteria and the prevalence of DM in PCOS may be underestimated.

Diabetes mellitus (DM) (type 1 and type 2) is a significant risk factor for CVD, with the INTERHEART study indicating an OR of 2.4 (99% CI: 2.1, 2.7) for acute myocardial infarction (Yusef et al., 2004). A meta-analysis of 102 prospective studies (n = 698,782) aimed to quantify the association of DM and CVD (Emerging Risk Factors Collaboration et al., 2010). HRs for those with diabetes were approximately twofold higher for CHD and stroke compared to those without, with no appreciable difference after adjustment for lipid, inflammatory, or renal markers. This suggests that diabetes confers approximately twofold excess risk for CVD. Interestingly, HRs for CHD were significantly higher in women than in men. Women, especially young women, are generally at less CVD risk than men, but in the presence of diabetes this protective effect appears to be lost (Recarti, Sep, Stehouwer & Unger, 2015). The reasons for this are not clear. It may be due to a gender disparity in treatment and management of CVD risk. That is, women with diabetes may be less likely to receive treatment to control CVD risk factors such as lipids and blood pressure (Gouni-Berthold et al., 2008). However, it may be because women with diabetes tend to have greater endothelial dysfunction than men with diabetes, and inflammatory factors may interact with oestrogens, reducing their cardioprotective effects on insulin action and lipids (Recarti et al., 2015). Thus, the development of diabetes may confer an even greater risk of CVD events in

women with PCOS compared to women without, because endothelial dysfunction, low-grade inflammation, and impairments in insulin action are often already present.

2.1.4.4 Hypertension

There is a higher prevalence of hypertension in women with PCOS compared to women without (Amiri, Ramezani Tehrani, Behboudi-Gandevani, Bidhendi-Yarandi & Carmina, 2020; Joham, Boyle, Zoungas & Teede, 2015). In a large cross-sectional analysis of 8,612 women aged 29-33 years, where 5.8% had PCOS, prevalence of hypertension was 5.5% in PCOS compared to 2% in controls (Joham et al., 2015). In addition, although BMI was associated with hypertension in women without PCOS, there was no such association in PCOS (Joham et al., 2015). This may suggest that hypertension is independent from BMI in PCOS. Hypertension in PCOS appears to be more prevalent in pre-menopausal women than post-menopausal women (Amiri et al., 2020). This may be because androgens decrease during late reproductive age due to ovarian and adrenal aging, and the risk of hypertension in PCOS becomes congruent with the risk for older women without PCOS (Carmina, Campagna & Lobo, 2013).

Updated 2019 NICE guidelines indicate a diagnosis of hypertension should be given with a clinic-measured systolic blood pressure (SBP) >140 mmHg and diastolic blood pressure (DBP) >90 mmHg, as well as an average home-measured BP of >135/85 mmHg (NICE, 2019). Hypertension increases the risk of CAD, heart failure, cerebrovascular disease, i.e., strokes, peripheral artery disease (PAD) and atrial fibrillation (Piepoli et al., 2016). Increasing blood pressure has a linear, progressive relationship with risk beginning from as low as 115/75 mmHg (Lewington et al., 2002). A meta-analysis of 61 prospective observational studies of blood pressure and mortality in one million adults with no diagnosis of vascular disease, helped to quantify risk with increasing blood pressure. The risks were greater in old age, but the biggest proportional increases were between ages 40-69 years, where each incremental increase of 20 mmHg SBP or 10 mmHg DBP resulted in a twofold increase in deathrate from stroke, CHD, and other vascular causes (Lewington et al., 2002). Similarly, the INTERHEART study indicated that the OR for acute myocardial infarction for those with hypertension, after adjustment for all other risk factors, was 1.9 (99% CI: 1.7, 2.1) compared to those without hypertension (Yusef et al., 2004).

Despite the increase in risk from 115/75 mmHg, this may have little clinical relevance; hypertension treatment has been shown to reduce the risk of CVD events only in patients

with SBP of >140mmHg at the beginning of treatment, with no reduction for those with SBP <140mmHg (Brunstrom & Carlberg, 2016). Thus, recommendations are usually that blood pressure be lowered to <140/90 mmHg in all non-elderly hypertensive patients (Piepoli et al., 2016). For patients with blood pressure <159/99 mmHg, lifestyle interventions including salt intake reduction, weight control, and regular PA may be sufficient, and may provide added benefits for those receiving blood pressure-lowering drugs (Piepoli et al., 2016).

2.1.4.5 Abdominal Obesity

Abdominal obesity, also known as central obesity, is the accumulation of excess visceral fat (adipose tissue) stored around the organs in the peritoneal cavity. This distribution of adipose tissue is associated with higher CVD risk than subcutaneous fat stored around the thighs and buttocks (Piepoli et al., 2016; Wilklund et al., 2008). There is an increased prevalence of abdominal obesity in women with PCOS; a meta-analysis of 35 studies (n=15129) indicates a RR of 1.73 (95% CI: 1.31, 2.30) compared to women without PCOS (Lim et al., 2012). In addition, they have a greater quantity of abdominal fat than weight-matched women without PCOS (Carmina et al., 2007). Abdominal obesity is associated with insulin resistance and hyperandrogenism, independent of BMI in PCOS (Li, Lin, Pan, Yang, & Zhang, 2018).

While BMI is a risk factor for CVD, with a continuous positive relationship as BMI increases from 20 kg/m^2 (Piepoli et al., 2016), it is not a reliable measurement. Based on body mass (kg), it does not differentiate between lean muscle mass and fat mass or give any indication of visceral or subcutaneous fat distribution. Indeed, CVD risk profiles for people with overweight and obesity (classified by BMI) are often heterogenous, and this may be due to adipose distribution (Stewart et al., 2017). In the INTERHEART study, BMI showed a modest relation with acute myocardial infarction, but became non-significant when waist-to-hip ratio (WHR), a measure of abdominal obesity, was included in the multivariate model (Yusef et al., 2004). Waist circumference (WC) is another common measure of abdominal obesity. In women, risk begins to increase from WC >71cm (Flint et al., 2010). However, WC ≥88 cm represents the threshold at which weight reduction should be advised (Piepoli et al., 2016). A WC of >88cm approximately doubles the risk of CHD compared to women with a WC <71 cm (Flint et al., 2010).

In the INTERHEART study, although abdominal obesity (top vs lowest tertile) was shown to initially double the risk of acute MI, the effect was substantially diminished after adjustment for other risk factors, particularly the ApoB/ApoA1 ratio (Yusef et al., 2004). This could

indicate that body weight *per se* may not increase CVD risk, but that it is more a product of the accompanying increase in other known risk factors, particularly those associated with visceral fat deposition.

2.1.5 The Role of Physical Activity in the Reduction of Cardiovascular Disease Risk

In this section, research outlining the protective cardiovascular effects of PA and cardio-respiratory fitness is discussed. In addition, the negative impact of sedentary behaviour on CVD health is introduced and investigated. Finally, potential mechanisms for the role of sedentary behaviour in CVD risk, particularly in relation to insulin metabolism, are examined.

To assess their impact on CVD risk, it is important to clearly define several terms used in this section. 'Physical activity' refers to any bodily movement produced by skeletal muscles that produces energy expenditure. 'Exercise' is a sub-set of PA and is planned, structured, repetitive, and purposefully focused on improvement or maintenance of any component(s) of physical fitness (Caspersen, Powell, & Christenson, 1985).

'Physical inactivity', however, generally refers to the non-achievement of PA guidelines (Thivel et al., 2018). That is, in the UK, anybody not meeting the 150/minute per week of moderate, or 75 minute/week of vigorous, aerobic PA would be classed as physically inactive. 'Sedentary behaviour' is defined as any waking behaviour characterised by an energy expenditure ≤ 1.5. METS (metabolic equivalents) while in a sitting, reclining, or lying posture (Tremblay et al., 2018).

2.1.5.1 Physical Activity

The WHO recommends a PA guideline of 150 minutes/week of moderate intensity aerobic PA, or 75 minutes/week of vigorous-intensity aerobic PA, or some equivalent combination (WHO, 2020). Meeting these recommendations is associated with a lower lifetime risk of CVD in men and women, both middle-aged and elderly (Kubota, Evenson, MacLehose, Roetker, Joshu & Folsom, 2018; Lachman et al., 2018). There appears to be a graded inverse association of PA and CVD, with risk reducing as PA levels increase. (Carnethon, 2009).

PA is well-recognised for its protective benefit on CVD, both for those without current CVD and secondary prevention in those with CVD (Lollgen, Bockenhoff, & Knapp, 2009; Moore et al., 2012; Piepoli, Davos, Francis, & Coats, 2004; Sattelmair et al., 2011). There have been

attempts to establish a dose-response relationship between PA levels and CVD, morbidity, and mortality. In general, evidence appears to indicate that any activity is better than none, and more activity is better than some (Chief Medical Officer [CMO], 2019). The largest benefits are seen in those moving from being inactive (i.e., not meeting PA guidelines) to regular PA, and subsequent increases provide significant but diminishing returns (Stewart et al., 2017).

However, it is not necessarily straightforward to quantify these protective effects in relation to amount of PA. This is because studies often use terms such as 'physical inactivity' or 'sedentary behaviour' interchangeably or define 'physically active' differently. For example, the INTERHEART study indicated that for those participating in regular PA, their OR of acute myocardial infarction was 0.86 (99% CI: 0.76, 0.97) compared to those not taking part in regular PA, indicating a 14% risk reduction (Yusef et al., 2004). However, they defined regular PA as ≥4 hours/week of moderate or vigorous intensity PA. By these standards, participants meeting WHO PA guidelines of 150 min/week of moderate PA would not be included in the analysis as physically active. Thus, the OR may be higher if those people were included. There are other limitations to such studies which will be highlighted below.

Lollgen et al. (2009) conducted a meta-analysis of prospective cohort studies to establish relative risk of all-cause mortality across PA intensity categories. However, the authors acknowledge that intensity levels across included studies varied in definition. As such, their categories for the analysis included mildly active, moderately active, and highly active, with no specific definition offered. Nonetheless, for women specifically, the relative risk compared to the mildly active groups were 0.76 (95% CI: 0.66, 0.89) in the moderately active group, with a further reduction to 0.69 (95% CI: 0.54, 0.89) in the highly active group, indicating additional benefits of increased activity levels. This effect was larger in women than in men. However, the researchers indicated that PA in their study referred to leisure-time PA. PA undertaken as part of work or commuting was thus not considered, excluding participants from certain categories based on leisure time only. Additionally, PA levels were self-reported according to questionnaires (not standard across studies) and this presents another issue for PA studies. Not only may there be issues with accuracy of self-reports, but different questionnaires may vary in validity and use of definitions, calling into question the heterogeneity of the meta-analysis.

Moore et al. (2012) conducted a meta-analysis of six prospective cohort studies (n = 654,827) to establish years of life gained, after age 40 years, across various levels of PA from low to high. Compared to no leisure time PA, HRs for mortality were 0.81 (95% CI: 0.79, 0.83) for low levels of activity, 0.68 (95% CI: 0.66, 0.69) for levels at or just above the minimum level recommended by guidelines (150 min/week of moderate intensity PA), and 0.61 (95% CI: 0.59, 0.63) and 0.59 (95% CI: 0.57, 0.61) for two and three times the minimum recommended level, respectively. Thus, the results indicate a large reduction in risk when moving from low levels to the minimum recommended levels, with significant but diminishing effects for further activity above these. In addition, even low levels confer some health benefit, in line with recommendations that 'some activity is better than none'. However, PA levels in this study were also collected via self-report with non-validated questionnaires, and only leisure-time PA was recorded. This, again, gives potential for categories to have low validity, if people undertaking PA for work, commuting, or housework, but not during leisure time, are considered inactive.

Sattelmair et al. (2011) conducted an analysis of 33 prospective cohort studies to determine the relationship between dose of PA and CHD. Individuals meeting the 150 min/week basic guideline had a 14% reduced risk for CHD compared to those engaging in no PA, those meeting the advanced guideline (300 min/week of moderate activity) had a 20% reduced risk, and those engaging in PA levels five times higher than the guideline had a 25% reduced risk. This further demonstrates the diminishing returns; that is, engaging in activity 5x the recommended level does not confer 5x greater protection for CHD. Interestingly, those individuals physically active at half the recommended levels also had a 14% reduced risk compared to those undertaking no activity, further consolidating that some activity is better than none. This analysis included all types of PA, including non-leisure time PA, which may confer higher accuracy to the risk estimates. However, it is unclear how PA data was collected.

The evidence outlined above indicates that PA reduces CVD risk, and this has been well-reported in the literature. What is not necessarily clear is the dose-response relationship. This mainly stems from methodological issues or inconsistencies between studies. Studies should clearly define PA categories, including definitions of intensity, and use validated questionnaires for data collection. For example, the International Physical Activity Questionnaire (IPAQ) is a validated tool that measures both leisure and non-leisure time PA, as well as sedentary behaviours in adults (Craig et al., 2003).

PA levels may present a viable target for CVD risk reduction in women with PCOS. PA interventions have been reported to confer many benefits to the symptoms of PCOS, including lipid profile and insulin sensitivity (Harrison et al., 2011). These studies will be described in section 2.2.

2.1.5.2 Cardiorespiratory Fitness

Studies analysing PA levels and CVD risk tend to rely on self-reported data which has limitations related to objectivity, recall, and accuracy. However, evidence indicates that cardiorespiratory fitness (CRF) levels, measured by exercise-testing, are inversely associated with risk of all-cause mortality, independent of other CVD risk factors (Blair et al., 1989; Kodama et al., 2009; Mora et al., 2003). High CRF has been shown to substantially reduce, and in some cases eliminate, the higher risk of mortality associated with obesity (Lee, Blair, & Jackson, 1999; Stevens, Cai, Evenson, & Thomas, 2002).

CRF is an objective indicator of habitual PA, and the mechanisms that link higher CRF to reduced mortality appear to be reflective of this. That is, higher CRF is associated with improved insulin sensitivity, blood lipid and lipoprotein profile, body composition, inflammation, blood pressure, and the autonomic nervous system (Lee et al., 2010). These improvements are all seen in those undertaking regular PA (Richter & Hargreaves, 2013; Soares-Miranda, Siscovick, Psaty, Longstreth, & Mozaffarian, 2016; Stanford & Goodyear, 2014; Zhou et al., 2014) Thus, it seems that it is not CRF itself that reduces CVD risk, but that high levels of PA, resulting in greater CRF, mitigates CVD risk. CRF could therefore be utilised in a clinical setting to stratify risk and make important recommendations for disease prevention (Lee et al., 2010). In addition, it could be used as an objective indicator of PA levels in population-risk studies to attenuate inaccuracy from self-reported data.

2.1.5.3 Sedentary Behaviour

While the benefits of PA and CRF are well elucidated, research is increasingly highlighting the impact of sedentary behaviour on metabolic health. It is important to highlight, as per the definitions at the beginning of this section, that physical inactivity and sedentary behaviour are not the same thing. It is therefore entirely possible for an individual to be classed as physically active (because they meet the PA guidelines) but to spend most of their time sedentary. Indeed, in order to meet the UK PA guidelines, only 2% of one's time needs to be

spent engaging in at least moderate intensity PA; the other 98% can be spent either sedentary or engaging in very light intensity activity (van der Ploeg & Hillson, 2017). Hence, the emergence of the 'active coach potato' phenomenon (Owen, Healy, Matthews & Dunstan, 2010).

The negative impacts of excessive sedentary behaviour, even for those who exercise regularly, have begun to emerge. Sedentary behaviour and exercise, rather than being on a continuum, appear to be distinct behaviours with distinct physiologies; the cellular and molecular responses associated with each are qualitatively different (Hamilton, Hamilton & Zderic, 2007). That is to say that although one can boost health with regular exercise, one can still be affected by the distinct cellular processes associated with too much sitting. These effects appear to be characterised by metabolic alterations commonly seen in diabetes and atherosclerosis (Hamilton, Hamilton & Zderic, 2007). Indeed, television viewing time has been positively associated with abnormal glucose metabolism and the metabolic syndrome (Owen et al., 2010). Importantly, these effects persist even after adjustment for sustained moderate-to-vigorous exercise, highlighting the deleterious effects of prolonged sedentary behaviour independent of the protective effects of regular exercise (Owen et al., 2010).

Crucially, this is not to say that PA levels (including structured exercise) are not useful measurements for CVD risk. Studies have long shown that risk of CVD and all-cause mortality are reduced in proportion to PA levels, with the greatest benefits seen from moving from physical inactivity to regular PA in line with PA guidelines (Piepoli et al., 2016; Stewart et al., 2017). After this, further benefits continue to be obtained when individuals move from meeting the PA guidelines to exceeding them by two, or even three times, albeit with diminishing returns (Lollgen et al., 2009; Moore et al., 2012).

Importantly, given these diminishing returns from continually increasing PA levels, the solution may not be that physically active persons should necessarily engage in more structured exercise. Rather, the shift in focus to reducing sedentary behaviours focuses on what can be done in non-exercise time (most of one's waking time) to target CVD risk. Focusing here may provide a different or additional option for improving cardiometabolic health: light-intensity activity. Interrupting sitting with short breaks of low-intensity PA, such as walking slowly for several minutes per each 20-30 minutes of sitting, has been shown to improve glucose and insulin metabolism throughout the day (Dempsey et al., 2017; Dunstan et al., 2012). Critically, this is despite such light-intensity activity not being counted toward

the 150 min/week moderate intensity PA guideline. Thus, this is potentially a valuable public health target; in the UK, it is estimated that 27% of adults engage in less than 30 minutes of moderate or vigorous activity per week (NHS Digital, 2019). Those that do not meet the PA guidelines and spend at least eight hours/day sitting are most at risk of CVD (Stamatakis et al., 2019). Thus, for people who are less motivated, or physically unable, to engage in moderate/vigorous structured exercise, messaging that focuses on the importance of light-intensity PA (to break up sedentary behaviours) may be valuable for reducing CVD risk. Indeed, while CVD risk over time in those who do not exercise cannot logically increase further due to exercise deficiency, it still has the potential to increase due to the distinct mechanisms of sedentary behaviours (Hamilton et al., 2007). Guidelines should therefore strive to make clear that in terms of PA, any non-exercise or light-intensity PA is better than none (Piepoli et al., 2016). UK guidelines have been recently updated to emphasise that light-intensity PA should also be undertaken to break-up sedentary periods (although there are no specific guidelines on how much or how often) (CMO, 2019), and this may go some way to stating the importance of light-intensity PA. In addition, for active individuals, including this type of PA may provide further CVD protection via distinct mechanisms in addition to those conferred by moderate/vigorous exercise.

Since women with PCOS already display increased CVD risk factors and alterations in metabolic profile, sedentary behaviour may be a further exacerbating factor. This presents an additional target for reducing CVD risk in women with PCOS; as well as partaking in regular PA, sedentary behaviours should also be reduced. At the present time, no studies have examined the impact of sedentary behaviour (and minimising this) on the metabolic and cardiovascular profile of PCOS.

2.1.5.4 Physical Activity, Sedentary Behaviour, and Insulin Metabolism

PA improves glucose and insulin metabolism by restoring glucose homeostasis through increased skeletal muscle glucose disposal (Richter & Hargreaves, 2013). This is achieved via increases in: i) skeletal muscle capillarisation, ii) expression of glucose transporter proteins, and iii) mitochondrial function (Stanford & Goodyear, 2014). Indeed, exercise-mediated glucose disposal does not rely on insulin receptor or IRS-1 phosphorylation as in normal insulin signalling but does so through distinct proximal signalling mechanisms (Richter & Hargreaves, 2013). Chronic exercise increases mitochondrial content and activity, and this is associated with improved skeletal muscle insulin sensitivity and whole-body

metabolic health (Stanford & Goodyear, 2014). A possible mechanism for this is the increased mitochondrial lipid oxidation of intra-myocellular lipid metabolites, which interfere with insulin signalling (Aye et al., 2018). The subsequent improvement in insulin sensitivity may therefore reduce inflammation and the release of cytokines that promote insulin resistance.

The resulting improvement in insulin metabolism may lead to improved lipid profile through decreased mobilisation of FFA through lipolysis, and the increased uptake and storage of glucose and triglycerides (Zhou et al., 2014). Blood pressure may also be reduced by improving insulin sensitivity. In the insulin-resistant state, compensatory hyperinsulinemia results in vasoconstriction and increased sodium reabsorption which lead to hypertension (Carroll & Dudfield, 2004; Zhou et al., 2014).

However, prolonged sedentary behaviour (i.e., sitting) has distinct and independent effects on metabolic health, even after accounting for leisure-time PA (Dunstan et al., 2012; Rynders, Blanc, DeJong, Bessesen, & Bergouignan, 2018). Studies examining the impact of prolonged sedentary behaviours, such as bedrest in humans, indicate that a lack of muscular activity can reduce insulin-mediated glucose uptake in both sedentary and trained individuals (Smorawinksi et al., 2000; Stephens, Granados, Zderic, Hamilton, & Braun, 2011). A lack of ambulation leads to reduced energy expenditure, and unless dietary intake is also reduced, results in an energy surplus (Stephens et al., 2011). Thus, without PA, postprandial spikes in insulin, glucose, and lipids can promote oxidative stress, triggering inflammatory pathways and endothelial dysfunction (Dunstan et al., 2012). When repeated multiple times throughout the day, postprandial hyperglycaemia and hyperlipidaemia reduces insulin action and creates conditions that promote the development of atherosclerosis and CVD (O'Keefe & Bell, 2007). However, even just one day of prolonged sitting can considerably reduce insulin action, even when energy intake is adjusted accordingly (Stephens et al., 2011).

Studies have investigated the effects of interruptions in periods of sitting on postprandial glucose and insulin action (Dunstan et al., 2012; Rynders et al., 2018). Dunstan and colleagues (2012) compared the effects of uninterrupted sitting for five hours, with interrupted sitting (two-minute bouts of activity every 20 minutes for five hours) on plasma and insulin incremental area under curve (iAUC) in 19 men and women. The study was a crossover trial with three conditions: uninterrupted sitting, sitting with light intensity activity breaks (treadmill walking at 3.2 km/h), and sitting with moderate intensity activity breaks

(treadmill walking at 5.8-6.4 km/h). Participants had a mean age and BMI of 53.8 years and 31.2 kg/m^2, respectively. Participants consumed a high fat and carbohydrate test drink after two hours of sitting. Results indicated that compared to uninterrupted sitting, glucose and insulin iAUC was significantly reduced after the activity break conditions. This remained significant after adjustment for age, sex, weight, and pre-drink levels of glucose and insulin. Importantly, no significant differences were observed between the light and moderate intensity activity breaks. This study supports the notion that breaks in sedentary behaviour, even at a light intensity, can reduce the deleterious effects of prolonged sedentary behaviour.

In a similar crossover study of adults with T2D, Dempsey et al. (2017) investigated the effects of seven hours of uninterrupted sitting, sitting with light intensity walking breaks (three-minute bouts of treadmill walking at 3.2k/m every 30 minutes), and sitting with simple resistance activity breaks (three minute-bouts of simple resistance activities every 30 minutes) on 22-hour glucose homeostasis. Compared with uninterrupted sitting, both light walking and simple resistance activity breaks significantly reduced 22-hour hyperglycaemia which persisted until waking the following morning. This suggests that there are metabolic benefits of short periods of activity even if one is classed as physically inactive because they are not undertaking moderate-intensity PA.

Interestingly, despite the popularity of standing desks, bouts of standing may not be sufficient to reduce postprandial glycaemia. Bailey & Lock (2015) conducted a crossover trial to examine the effects of uninterrupted sitting, interrupted sitting with two minutes of standing every 20 minutes, and interrupted sitting with two-minute bouts of light intensity walking every 20 minutes, in ten non-obese adults. Participants were given a high fat and carbohydrate test drink and plasma glucose and blood pressure were assessed hourly to calculate AUC. Glucose AUC was significantly lower in the activity-break condition compared to both uninterrupted sitting and the standing-break conditions, with no differences observed between the latter groups. This is in line with the position that PA restores glucose homeostasis via enhanced skeletal muscle glucose disposal and suggests that muscle contraction is a key mechanistic factor (Rynders et al., 2018).

2.2 Lifestyle Interventions in women with PCOS

In this section, lifestyle interventions (including dietary and PA) in women with PCOS are discussed. Evidence is provided for the role of PA interventions in the reduction of CVD risk in PCOS. Areas in need of further interrogation are highlighted.

Lifestyle intervention studies for PCOS are usually aimed at improving body composition (fat mass, lean mass), hormonal profile (androgens, LH/FSH) cardiometabolic profile (lipids, insulin sensitivity), reproductive function (menses regularity, ovulation) or a combination. This is in comparison to clinical treatment, which tends to focus on one specific symptom or outcome (such as hirsutism or conception). Lifestyle intervention studies usually investigate other outcomes (that may or may not be clinical in nature) that relate back to the pathophysiology of PCOS. The research outlined in this section is distinct from clinical management, where healthcare professionals may give generic lifestyle advice to women with PCOS.

2.2.1 Dietary Interventions

Dietary interventions typically focus on weight loss as a primary outcome, with secondary outcomes including cardiometabolic profile and reproductive function. Few studies have been conducted to examine the effects of a dietary intervention alone compared to usual care in PCOS. Since weight loss was the main goal, earlier research focused on energy restriction (hypocaloric diets) rather than the investigation of dietary composition (Marsh & Brand-Miller, 2005). Thomson et al. (2008) conducted a 20-week randomised parallel study with 94 overweight and obese women with PCOS (mean age 29.3 ± 9.7 years, mean BMI 36.1 ± 0.5 kg/m^2). They compared the effects of a hypocaloric diet only (DO), diet and aerobic exercise (DA), and diet and combined aerobic-resistance exercise (DC). However, the exercise programme in DC was not matched by energy expenditure to DA, thus there may have been a difference in total calories expended during exercise between groups. In addition, there is currently no guidance for effective resistance training for women with PCOS. The diet consumed by all groups was high protein (30% of total energy intake) and consisted of 1,195-1,434 kcal/day. In all groups, weight loss was observed with no significant difference between treatments. Additionally, reductions in blood pressure, TG, TC, LDL-C, glucose, fasting insulin, testosterone, FAI (free androgen index), SHBG, and reproductive function were observed in each group, with no significant differences between treatments.

This is likely due to weight loss and is in line with previous studies that suggest weight loss from as little as 5% of total body mass can improve cardiometabolic profile and reproductive function in PCOS (Joham, Palomba, & Hart, 2016). Some studies have indicated that exercise alone is enough to induce weight loss, without any significant extra benefit with the inclusion of a dietary component (Douketis, Macie, Thabane & Williamson, 2005; Shaw, Gennat, O'Rourke, & Del Mar, 2006). However, the results from the study by Thomson et al. (2008) indicated that in the diet and exercise groups, fat mass and FFM were both significantly improved in comparison to DO. This suggests that the addition of an exercise component can confer extra benefits to body composition not obtained from caloric restriction/weight loss alone. A limitation of this study is the lack of inclusion of psychological outcomes such, as quality of life. Caloric restriction (as low as 1,195 kcal/day) and an exercise programme five days/week may lead to fatigue, feelings of hunger, and low mood (Manore et al., 2015).

A 2013 systematic review investigated different dietary compositions to improve body composition, metabolic, reproductive, and psychological outcomes for women with PCOS (Moran et al., 2013). They included five studies examining various diets, such as low-carbohydrate diets, monounsaturated fatty acid (MUFA) enriched diets, low-glycaemic index (GI) diets, high-protein diets, and fat- or carbohydrate-restricted diets, either alone or in comparison between groups. For most outcomes, including body composition, metabolic, and reproductive outcomes, there were no significant differences between diets; instead, results appeared to be associated with weight loss regardless of diet composition. Only one study observed greater weight loss with a eucaloric low-carbohydrate diet compared with a MUFA enriched diet. However, results from this study will have been confounded by lack of randomisation and an unclear cross-over protocol between diets. In addition, high attrition rates (up to 49%) were noted across the included studies. Thus, the authors conclude that improving engagement and sustainability with lifestyle change to produce weight loss (and subsequent maintenance) is of higher importance than diet composition itself (Moran et al., 2013). Further research is also required to ascertain the benefits of different diet composition in lean women with PCOS to identify whether improvements in symptoms can be obtained without weight loss.

2.2.2 Physical Activity and Exercise Interventions

PA and exercise interventions for periods from as little as 10 weeks have been shown to improve a variety of health-related outcomes, including both cardiometabolic risk factors and reproductive capacity markers (Harrison et al., 2011; Patton et al., 2020). Indeed, there have been a wide range of lifestyle interventions, including both dietary and pharmacological components in conjunction with exercise. However, there are fewer interventions that assess exercise in isolation, making it difficult to elucidate parameters for an effective intervention. Furthermore, variation in population, study design, and intervention design have led to varying and sometimes conflicting results.

In 2011, Harrison et al. conducted the first systematic review to identify the independent effects of exercise on cardiovascular and reproductive outcomes in women with PCOS. They found a lack of RCT designs, and thus captured cohort studies additionally. Even then, only eight studies where exercise could be assessed independently were identified. Sample sizes varied considerably, with enrolled participants ranging from 12 to 124. All studies included participants with overweight or obesity, with a mean BMI range of 26.8 to 37.9 kg/m^2, and all participants were pre-menopausal. Six of the eight studies were aerobic exercise only, while the other two incorporated resistance training either alone or in combination with aerobic training. Furthermore, duration and frequency varied considerably, from 12 weeks up 20 weeks, with participants exercising between 3-7 sessions/week. Although not a meta-analysis, the synthesis indicated that sustainable, less intensive studies, or those of a shorter duration, were likely to result in significant clinical benefits. These included improvements in ovulation and menstrual cycle regulation, weight loss (4.5 – 10% of body mass) and 9-30% reduction of insulin resistance, in young women with PCOS.

Since then, various other reviews and meta-analyses have been conducted to further elucidate the efficacy of PA and exercise on menstrual, cardiometabolic, and mental health outcomes in PCOS (Benham, Yamamoto, Friedenreich, Rabi, & Sigal, 2018; Domecq et al., 2013; Haqq, McFarlane, Dieberg, & Smart, 2015; Kite et al., 2019). However, several of these reviews reported results where exercise, dietary intervention, and pharmacological intervention were combined (or did not indicate if exercise was in isolation), or did not exclude participants already taking metabolism-altering medications such as metformin or OCP. Thus, despite the high quantity of reviews in this area, there remains a lack of high-

quality evidence to determine the specific parameters (mode, frequency, duration) of effective PA interventions, in isolation, on cardiometabolic outcomes.

2.2.2.1 Physical Activity Interventions and CVD Risk

The INTERHEART study found moderate to vigorous intensity PA to be one of nine lifestyle modifications that are protective against myocardial infarction (Yusef et al., 2004). Accordingly, there is compelling evidence that PA and exercise mitigate CVD risk factors in women with PCOS (Harrison et al., 2011; Kite et al., 2019; Patton et al., 2020), populations with dyslipidaemia (Mann, Beedie, & Jimenez, 2014), populations with metabolic syndrome (Carroll & Dudfield, 2004; Katzmarzyk et al., 2003), and healthy populations (Durstine et al., 2001). However, in relation to PCOS, some studies have produced inconsistent results with respect to the effectiveness of exercise only - without any additional dietary or pharmacological interventions - in improving biomarkers of CVD risk. This is particularly true regarding lipid profile (Hutchison et al., 2011), and inflammation (Beavers, Brinkley, & Nicklas, 2010).

Longer exercise interventions (e.g. >20 weeks) are associated with improved lipid profile, and the reversal of metabolic syndrome in healthy populations (Carroll & Dudfield, 2004; Halverstadt, Phares, Wilund, Goldberg, & Hagberg, 2007; Katyzmarzyk et al., 2003). This might account for some of the discrepancy in PCOS research, with exercise interventions typically ranging from eight to 24 weeks in duration. PCOS studies with longer intervention durations have found improvements in VLDL and HDL (Brown et al., 2009), whereas shorter intervention durations have found no change in LDL and HDL, despite improvements in TG and cardiorespiratory fitness (Hutchison et al., 2011).

Studies investigating the effects of PA or exercise on oxLDL concentrations in women with PCOS are currently lacking. This is despite the accepted role of oxLDL in the initiation and progression of atherosclerosis, its predictive value in CVD, and the presence of increased oxLDL concentrations in PCOS. Exercise and PA interventions have been successful in reducing oxLDL concentrations in populations at risk for CVD, including older women with obesity, and patients with CAD and/or T2D (Tiainen et al., 2018; Park, Park, Lim & Park, 2015). However, studies vary considerably, with interventions ranging from 12 weeks to two years and including a variety of different exercises. Further research is needed to ascertain whether PA plays a role in the reduction of oxLDL levels in PCOS.

Exercise interventions for 12 weeks, three sessions/week, can promote weight loss and reductions in BMI in women with PCOS (Hutchison et al., 2011). These changes are typically associated with a reduction in WHR or WC, indicating a decrease in abdominal obesity. WC and WHR may be a better indicator of health than BMI alone because of their association with other CVD risk factors, such as impaired glucose metabolism (Teede, Hutchison, Zoungas, & Meyer, 2006; Yusef et al., 2004). While reductions to BMI and WC seem to be more effectively achieved through combined exercise and dietary interventions in comparison to dietary intervention alone, weight loss is still achievable in shorter exercise-only interventions (Harrison et al., 2011). However, the amount of weight lost seems to be proportionately related to duration of the intervention (Hutchison et al., 2011). Longer duration (20 weeks+) may be the key to promote greater weight loss, irrespective of type and frequency of exercise (Thomson et al., 2008).

Hypertension is one of the key characteristics of metabolic syndrome, and there is an inverse relationship between blood pressure and insulin sensitivity (Carroll & Dudfield, 2004). Evidence supports the role of exercise as treatment for hypertension, with exercise training decreasing blood pressure in around 75% of hypertensive adults, with a more pronounced effect in women (Hagberg, Park, & Brown, 2000).

In women with PCOS, the results are less clear; some studies find no statistically significant improvements in SBP or DBP as a result of exercise interventions from 12 to 24 weeks (Brown et al., 2009; Giallauria et al., 2008), while others have found small, but clinically meaningful, improvements in SBP with exercise alone or exercise in combination with dietary intervention (Thomson et al., 2008; Vigorito et al., 2007). These conflicting results may be due to the wide range of phenotypes possible under the PCOS diagnostic criteria; indeed, prevalence of hypertension in PCOS is reported to be between 5.5-12% (Ben Salem Hachmi, et al. 2006; Joham, Boyle, Zoungas, & Teede, 2015) and as such many PCOS study participants may be normo-tensive.

There is much evidence to support the role of exercise in improving insulin metabolism. PCOS research supports the role of exercise in improving insulin sensitivity immediately after an acute bout of exercise (Aye et al., 2018), and also in the long-term with exercise interventions from three months (Giallauria et al., 2008; Vigorito et al., 2007) to 20+ weeks (Palomba et al., 2008; Thompson et al., 2009). This tentatively includes resistance training as well as aerobic exercise (Patton et al., 2020). As described, insulin resistance has been linked

to abdominal obesity, hypertension, the development of T2D (Teede et al., 2006), dyslipidaemia, and inflammation (Zhou et al., 2015), meaning it is a key indicator of CVD risk in women with PCOS, where the prevalence of insulin resistance is up to 80% (Hutchison et al., 2011), independent of weight.

Previous inconsistency in results could be due to intervention intensity. A recent review (Patton et al., 2020) of 20 studies investigated the impact of exercise interventions on PCOS and excluded any concurrent drug therapies. The authors concluded that exercise interventions from 10 weeks, involving 120 min/week of vigorous intensity exercise (60-85% VO_2 max), were likely to give greater improvements to cardiometabolic outcomes including homeostatic model assessment of insulin resistance (HOMA-IR), free androgen index (FAI), WC, and BMI, compared to moderate intensity exercise (40-60% VO_2 max). Typically, previous studies have focused on moderate intensity exercise. Thus, while there is a need to introduce deconditioned individuals to exercise carefully, gradually ramping up intensity above 60% VO_2 max may maximise results.

A limitation to exercise intervention studies in PCOS is uncontrolled or unmeasured sedentary behaviours. The 2018 PCOS guidelines (Teede et al., 2018) recommend that as well as encouraging PA, clinicians should emphasise minimised sedentary time for greater health benefits. PA and exercise have been shown to improve insulin metabolism while sedentary behaviours are distinctly antagonistic. Thus, it is possible that even those complying with an exercise intervention are spending much of their non-exercise time sedentary. The physiological impact caused by this could well affect results, with effects of exercise interventions potentially being dampened.

Measurement of sedentary behaviours can be done via self-report using diaries, logs, questionnaires (such as IPAQ), or recall interviews. They can also be measured using worn devices such as accelerometers, inclinometers, pedometers, and heart-rate monitors. The latter tend to be more accurate but may be cost prohibitive, whereas self-reported measures are cheaper and easier to apply but tend to underestimate true sedentary behaviour (Prince et al., 2020). A recent systematic review indicated that multi-item questionnaires that ask more than one question about sedentary behaviours (such as the IPAQ long-form, which asks questions about sitting time, work time, and motorised travel time) provide data comparable to that collected by devices (Prince et al., 2020) and may be an affordable option.

There have also been no trials investigating the effects of (minimising) sedentary behaviour on CVD risk in women with PCOS, either in comparison to structured exercise or otherwise. Studies utilising devices and/or fitness app-based measures of sedentary behaviour and PA in an intervention may shed light on the impact of sedentary behaviours on CVD risk in PCOS. Indeed, the 2018 PCOS guidelines recommend the use of fitness tracking devices and technology to self-monitor sedentary behaviours and PA via step count and other metrics (Wolf et al., 2018). This can be done by most via mobile-phone, where relevant freely available apps, referred to as 'mHealth interventions', have been shown to be effective in reducing sedentary behaviours (Buckingham et al., 2019; Direito et al., 2017).

Although aerobic exercise has been the focus of most PA interventions in PCOS, the study of resistance training interventions is starting to become more prolific in PCOS research. Indeed, resistance training is shown to be effective in the management of metabolic diseases, including T2D and obesity (Tresierras & Balady, 2009). In PCOS, studies have provided inconclusive results due to differences in participants, training loads and regimens, and whether resistance training was studied in isolation or additive to dietary components and/or aerobic exercise (Pericleous & Stephanides, 2018). As such, the updated PCOS guidelines do not make specific reference to resistance exercise but defer to current population recommendations of 150/minutes per week of moderate activity (or 75 minutes of vigorous activity) and muscle strengthening activities on two non-consecutive days/week (Teede et al., 2018).

Progressive resistance training (PRT) may improve body composition and metabolic health in PCOS. Kogure et al. (2018) conducted a case-control study of a 16-week progressive PRT programme, performed three/week for one hour. In total, 97 women completed the protocol (45 in the PCOS group and 52 in the non-PCOS group). The outcomes included biochemical measurements of testosterone and its intermediates, body composition and hypertrophy indicators, and muscular strength. The results indicated that PRT significantly improved hyperandrogenism and glycaemia, increased muscular strength and lean body mass, and reduced body fat percentage in both the PCOS and non-PCOS groups compared to baseline. Improvements in muscular strength were observed from eight weeks, but changes in body composition were not significant before the 16-week measurement point. However, adherence to the protocol was 46% in the PCOS group and 54% in the control group, and participants only needed to complete a minimum of 20% of the sessions to remain in the study. Thus, low adherence may have attenuated results.

Of note, the authors used measures of fat-free mass rather than body mass measurements to demonstrate changes in body composition. This is useful because muscle hypertrophy and increases in lean body mass may increase BMI, but the composition of such weight gain is more informative. For example, an increase in skeletal muscle size and quality are associated with increased insulin sensitivity and glucose tolerance, as well as increased metabolic rate and the mobilisation of visceral adipose tissue in the abdominal region (Tresierras & Balady, 2009). Indeed, a recent systematic review and meta-analysis concluded that resistance training interventions among women with PCOS lead to increases in BMI, but reductions in WC, potentially indicating an increase in fat-free mass and a reduction in visceral adipose tissue (Kite et al., 2019).

There are several RCTs investigating the effects of a resistance training intervention in comparison to other exercise modalities or control. Almenning et al. (2015) conducted a three-arm parallel RCT to identify the effects of resistance training (RT) and high-intensity interval training (HIIT), primarily on insulin resistance. Thirty-one women with PCOS were randomised to either the resistance training group, the HIIT group, or a control group (where women were advised to meet the national guidelines of 150 min/week of moderate intensity activity). The sample size was small but powered to detect significant changes in HOMA-IR; thus, other variables may have been underpowered. The participants engaged in 10 weeks of training, three/week. Compliance was high, with participants attending 87% and 90% of the RT and HIIT sessions, respectively. The results indicated that HOMA-IR was significantly improved in the HIIT group only, compared to baseline and the control group. However, in the RT group, percentage of body fat significantly decreased (-1.6% [95% CI: -2.5, -0.7]) and fat-free mass significantly increased (1.2 kg [95% CI: 0.4, 2.1]) compared to baseline, despite no changes in BMI or WC. This consolidates previous research that suggests RT can be effective at modifying body composition without absolute weight loss. Further, the women in the study were not necessarily overweight, with the mean BMI of the RT group being 27.1 kg/m^2. This suggests that lean/minimally overweight women with PCOS can still benefit from the use of RT to alter body composition without weight loss *per se*.

Vizza, Smith, Swaraj, Agho, & Cheema (2016) conducted an RCT to determine the feasibility of an intervention involving a PRT group or a usual-care (control) group. The PRT involved two supervised sessions/week of machine-based and functional exercises, and two home-based (unsupervised) sessions of low intensity calisthenics. The study had a very small number of participants, further compounded by a high withdrawal rate. Subsequently, only

six women completed in the PRT group and four in the control group. Nonetheless, in the PRT group, body weight, BMI, lean mass, and fat-free mass were significantly increased compared to the control group, accompanied by significant decreases in WC. These results may further indicate the efficacy of resistance training in modifying body composition, but the limitation of the study as an unpowered feasibility trial (that is, its primary aim is to ascertain if a full-scale intervention is possible) must be considered when interpreting these results.

PCOS guidelines currently make no specific recommendation regarding PRT, only deferring to general PA guidelines that refer to 'muscle strengthening activities two sessions/week' (Teede et al., 2018). However, the role of PRT in managing PCOS, particularly regarding body composition, appears promising. More specific guidelines related to exercises (isolated versus compound exercises) and training load (% of one repetition maximum, and repetitions/sets) based on evidence from similar populations may improve study design and provide further evidence in this area.

2.2.2.2 Physical Activity Interventions and Reproductive Health

Several studies have explored the impact of PA and exercise interventions on reproductive function, with results indicating improvements in menstrual and/or ovulation frequency following exercise in comparison to diet only or control groups (Palomba et al., 2008; Thomson et al., 2008; Vigorito et al., 2007). These improvements included a change from non-ovulatory to ovulatory cycles, restoration of cycle regularity and improvement in inter-cycle variation (Harrison et al., 2011), indicating that exercise may be more beneficial to reproductive function than caloric restriction alone. Evidence suggests that the pregnancy rate among women with PCOS trying to conceive and undertaking an exercise intervention is 35% (Palomba et al., 2008), with pregnancy being a common reason for drop-out amongst participants with PCOS in exercise trials (Pericleous & Stephanides, 2018). It has been noted that lifestyle modification for overweight or obese infertile women with PCOS is a cost-effective solution for those women wishing to conceive, either as a primary intervention or in conjunction with fertility treatment (Mahoney, 2014). This suggests that women with PCOS presenting with reduced fertility could benefit from specific advice regarding exercise and PA programmes. The effects of resistance training only on reproductive function are unclear. There are insufficient studies that examine the impact of PRT on women with PCOS (Pericleous & Stephanides, 2018), further highlighting the need for clear guidelines.

Enhanced insulin sensitivity underpins the mechanisms of how exercise can restore reproductive function (Harrison et al., 2011). Reducing hyperinsulinemia decreases ovarian steroidogenesis and increases SHBG, and the resulting return to a normo-androgenic environment may restore sensitivity of the GnRH pulse activator to steroid inhibition of LH. Subsequently, decreased levels of LH and androgens may halt the excessive recruitment of antral follicles, allowing a dominant follicle to mature, eventually leading to ovulation.

2.2.2.3 Physical Activity Interventions and Mental Health

The benefits of PA and exercise on psychological wellbeing, including improved mood, reduced depressive symptoms, and improved body-image and quality of life are documented in both overweight women (Fontaine et al., 1999; Kaukua, Pekkarinen, Sane, & Mustajoki, 2003) and adults in general (DiLorenzo et al., 1999; Byrne & Byrne, 1993). However, this is less well-documented in women with PCOS. While various studies have assessed the impact of an exercise intervention on health-related quality of life (HRQoL) in PCOS, these have mostly been combined 'lifestyle' interventions with exercise undertaken as an adjunct to different diets (Galletly et al., 2007; Thomson et al., 2010) or drug therapy (Ladson et al., 2011). Other studies have compared exercise with acupuncture or yoga (Conte, Banting, Teede, & Stepto, 2015). They indicate that exercise improves HRQoL, anxiety, and/or self-esteem (Galletly et al., 2007; Thomson et al., 2010; Stener-Victorin et al., 2013; Nidhi et al., 2012),

Moreover, cross-sectional and observational studies lend support to the idea that physically active women with PCOS are likely to have less severe depression, or any depression, compared to inactive women with PCOS (Banting, Gibson-Helm, Polman, Teede, & Stepto, 2014; Lamb et al., 2011). The psychological benefits of exercise are not necessarily related to weight loss, since an observational study of women with PCOS found that those completing a self-directed brisk walking programme improved their body image significantly in comparison to those women who did not complete the walking intervention, despite no changes to BMI (Liao, Nesic, Chadwick, Brooke-Wavell, & Prelevic, 2008).

PA may improve psychological outcomes via various physiological factors. One such factor may be the cycle of inflammation and impaired insulin metabolism present in PCOS that has been described previously. Clinical and experimental evidence links activation of the brain cytokine system to depression (Dantzer, 2001), and may be a factor in the increased prevalence of depression in women with PCOS. Subsequently, interventions that reduce

obesity-related inflammation or normalise insulin metabolism to the effect of reducing pro-inflammatory cytokines, may reduce rates of depression. In addition, severity of hyperandrogenism experienced by the individual may be related to higher levels of mental stress because of the clinical presentation. That is, cystic acne, hirsutism and thinning scalp hair, which may lead to negative self-image and poor self-esteem (Himelein & Thatcher, 2006; Sadeeqa, Mustafa, & Latif, 2018). Exercise can restore insulin sensitivity and thus reduce hyperinsulinemia (Harrison et al., 2011), which causes ovarian steroidogenesis and reduces hepatic output of SHBG, leading to hyperandrogenaemia. The subsequent reduction in androgens may therefore improve the related clinical symptoms and improve body-image.

2.2.3 Measurement tools used in PCOS and exercise studies

Studies examining the effect of exercise on PCOS frequently use measures of VO_2max or VO_2peak to quantify improvements in physical fitness (Patten et al., 2020; Stepto et al., 2019). As outlined previously, CRF is an objective indicator of habitual PA, and is correlated with lower CVD risk. This is measured using graded, maximal exercise tests to volitional exhaustion, or submaximal exercise tests that estimate VO_2max based on heart rate, such as the Astrand-Rhyming test (Astrand & Rhyming, 1954). These are typically conducted at baseline and post-intervention, but some studies also measure this at several time points throughout the intervention. The latter is more common in exercise studies because, although they are less accurate, they are simple to administer, require less technical equipment, and carry less risk of injury to those not accustomated to habitual exercise (Jackson & Ross, 1996).

In addition, interventions encompassing structured, supervised exercise interventions frequently use heart rate monitors to measure compliance to prescribed exercise regimes, including aerobic exercise and high-intensity interval training (Stepto et al., 2019). This is an objective measure of physical exertion. Studies that record and measure PA outside of a supervised setting rely on self-reported measures such as surveys (Stepto et al., 2019). While some studies use validated questionnaires such as the CARDIA physical activity questionnaire (Lin et al., 2021), other researchers designed their own questionnaires that inquire about PA behaviour (Arentz, Smith, Abbott & Bensoussan, 2021). Subsequently, while self-reported measures reduce burden on participants (by not having to use objective measures, such as pedometers), this introduces heterogeneity between studies due to varying methods of data collection and differing definitions of PA and exercise (Prince et al., 2020).

2.3 Conclusion

The evidence reviewed in this chapter has set out the prevalence and key risk factors of CVD in women with PCOS. Systemic inflammation and impaired insulin metabolism are at the root of many CVD risk factors. Indeed, women with PCOS show many of the risk factors related to impaired insulin signalling and its subsequent effects on the vascular system. As such, they are at increased risk of CVD and acute CVD events. Furthermore, research indicates that oxidised LDL may be a promising therapeutic target to reduce CVD progression. However, little research exists that elucidates whether women with PCOS can benefit from therapies aimed at reducing oxidised LDL.

Overall, results from both aerobic and resistance training studies appear to provide beneficial effects on a wide variety of PCOS symptoms. That PA is a useful tool in the management of the condition is thus undisputed (Teede et al., 2018). However, with a lack of clear reporting, major differences in study design (and a dearth of RCTs), and variations in population, more research is needed to provide conclusive results to define effective types of exercise, as well as optimal duration and frequency. The addition of this research could lead to recommendations geared toward different symptoms or phenotypes and provide clearer guidance around the additional benefits of certain exercise programmes over others. In addition, the reduction of sedentary behaviours clearly attenuates CVD risk, and should be incorporated into PCOS lifestyle interventions to ascertain what benefits may be obtained in this population. Suggestions for measurement of sedentary behaviours are provided.

Thus, this literature review highlights gaps in the literature that will be addressed by the programme of research presented in this PhD thesis.

2.4 Programme of Research and Thesis Overview

The evidence reviewed in this chapter indicates that women with PCOS have multiple risk factors for CVD, and that PA interventions in this population can be successful in attenuating many of the cardiometabolic mechanisms that contribute to this risk. However, there are several areas where further research is warranted:

i) While the threat of growing sedentary behaviours on cardiometabolic health has been outlined, the effects of maximising lifestyle PA on the pathological cardiometabolic profile of PCOS have yet to be elucidated.

ii) Oxidised LDL is implicated in the development of atherosclerosis, the primary cause of CVD. But whether PA can be effective in disrupting this has not been analysed in PA and PCOS studies.

The implementation of a full-scale trial to address these questions will likely require considerable resources and participation. Thus, before a full-scale trial can be implemented, a programme of development, feasibility testing, and evaluation must first be conducted. Developing, testing, and evaluating processes to produce a refined intervention sets up future studies for success by minimising issues, and improving study design, with minimal use of resources. Thus, a full-scale RCT, if indicated to be warranted, may be conducted subsequently with confidence in the procedures and the ability of the study to produce measurable results.

2.4.1 The Role of Feasibility Trials in Medical Research

Intervention studies can often be undermined by unexpected problems with acceptability, compliance, intervention delivery, recruitment, and retention. Indeed, weaknesses in study design, conduct, and analysis, which are ultimately preventable, can produce misleading results and waste resources (Ioannidis et al., 2014). As such, The Medical Research Council (MRC) guidance for developing and evaluating complex interventions recommends that interventions are developed systematically, using a carefully phased approach that begins with identifying relevant evidence and theory, before moving on to a feasibility or piloting approach where procedures can be tested (see Figure 3) (Craig et al., 2008).

Modelling an intervention before a full-scale evaluation can identify weaknesses, lead to refinement, or even indicate that a full-scale trial is unwarranted (Craig et al., 2008). This initial feasibility stage can also provide estimates of recruitment and retention, and aid in the accurate determination of sample sizes to ultimately produce reliable, replicable results (Ioannidis et al., 2014). The development of an intervention need not be linear, and previous stages of development can be revisited before full-scale implementation. A feasibility trial or study is thus undertaken to address the question: can the planned evaluation be done? (O'Cathain et al., 2015).

Evaluation of such feasibility studies provides valuable insight into whether an intervention is effective and how it can be optimised, or, similarly, if it is unsuccessful, it can highlight the contextual factors that influence this. Evaluation methods that also utilise qualitative analysis

can be particularly useful in this area, since contextual issues that threaten the implementation of a trial are often subtle, idiosyncratic, and complex (Wells, William, Treweek, Coyle & Taylor, 2012). The principles of this research programme are therefore shaped by the guidance for developing and evaluating complex interventions.

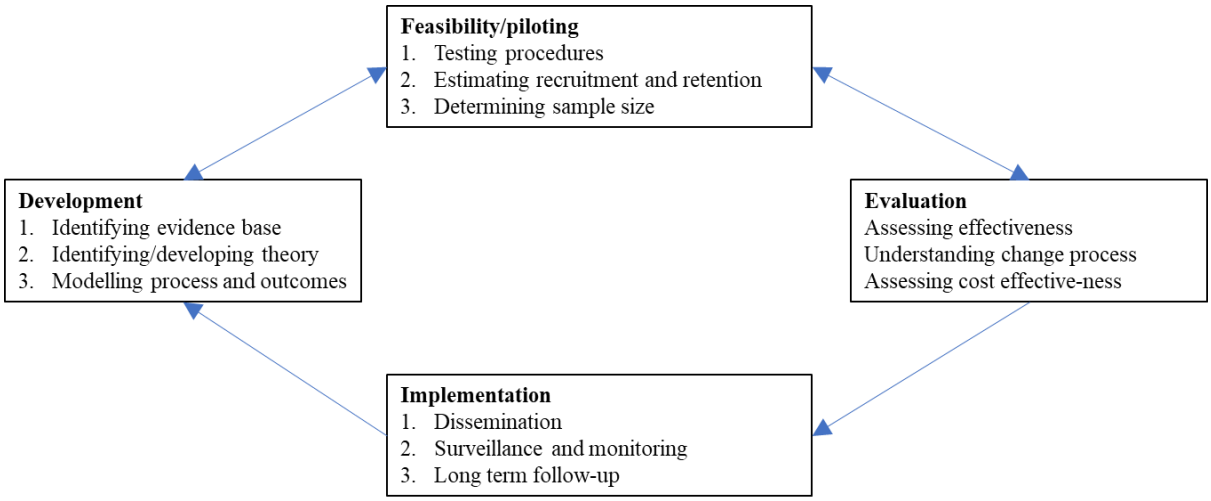

Figure 3. Key elements of the development and evaluation process (adapted from Craig et al., 2008).

2.4.2 Objectives

This body of work proposes to answer the following research question: can a lifestyle intervention comprising supervised exercise and increased lifestyle PA improve CVD risk and oxidised LDL concentrations in women with PCOS?

The objectives are to:

1. Identify and evaluate the existing evidence to determine effective types of exercise intervention for women with PCOS.
2. Evaluate the feasibility of a PA intervention comprising a supervised exercise arm and a lifestyle PA arm in women with PCOS.
3. Obtain oxidised LDL concentration data which will allow for a sample size calculation for a full-scale RCT.
4. Evaluate the acceptability and efficacy of the interventions using semi-structured interviews.
5. Identify barriers and facilitators to PA in women with PCOS and develop recommendations for the refinement of a full-scale RCT.

2.4.3 Thesis Overview

The body of work in this thesis sets out to meet the objectives using a multi-methods approach. In healthcare, this approach involves the collection and analysis of both quantitative and qualitative data to provide contextual information on the mechanisms underpinning the efficacy of interventions (Craig et al., 2008). Indeed, to evaluate interventions using solely quantitative methods may fail to capture the complexities, causalities, and nuances in public health (Craig et al., 2013). Drawing evidence from multiple robust methodologies allows for the analysis to be enhanced beyond whether the intervention is efficacious and cost effective; it asks why, when, how, and for whom the intervention is effective (Pawson, 2006). This data may be key to understanding processes that affect study outcomes, and as such, the collection and synthesis of such evidence aids in the comprehensive evaluation of complex interventions.

Thus, the objectives are met by the following: a systematic review and meta-analysis of exercise interventions in women with PCOS (Chapter 3), a feasibility RCT of a lifestyle intervention comprising supervised exercise and lifestyle PA for women with PCOS (Chapter 4), and a qualitative analysis of post-intervention semi-structured interview data (Chapter 5). Finally, the general discussion, practical implications, recommendations, and conclusions are presented in Chapter 6.

2.4.4 Philosophical Position for the Thesis

The programme of work in this thesis includes an integration of different research strategies (qualitative and quantitative research) that are typically based on different epistemological and ontological perspectives (or paradigms). That is, the quantitative meta-analysis and the RCT may potentially align with a positivist philosophical position. Positivism is concerned with identifying and elucidating an objective truth or reality that exists outside of oneself (Ryan, 2018). Conversely, the qualitative evaluative component may be more closely aligned with interpretivism. This philosophical position posits that there is no ‘true’ reality, and that the truth is subjective. That is, it is interpreted and shaped within the context of the individual’s frame of reference (Ryan, 2018). As such, the latter approach is useful for defining how an individual’s experience shapes their perception, while the former is useful for providing consistency in results and applicability to other contexts (Moon & Blackman, 2014).

However, while positivism and interpretivism are two paradigms at extreme ends of a continuum, this thesis takes an approach that aligns with pragmatism. Pragmatism is based on the epistemology that there are multiple realities, and thus multiple ways of understanding. Therefore, it has a practical focus on ‘what works’, allowing for a changing and flexible approach to the underpinning research philosophy, and posits that this stand-point should be guided by the research question itself. In particular, the integration of multiple research strategies (qualitative and quantitative) may be useful to give the researcher an understanding gained from both the lived experiences of the participants, and from the scientific analysis of objective data (Moon & Blackman, 2014; Evans et al., 2011).

3 Exercise Interventions in Women with PCOS: Systematic Review and Meta-Analysis

Overview

The purpose of this systematic review is to address gaps in the literature identified in the previous chapter, and to provide a basis for the design of a clinical trial that utilises a PA intervention for women with PCOS. The work in this chapter has been published (Woodward et al., 2019a; Woodward et al., 2019b).

3.1 Introduction

3.1.1 Aims and Objectives

The aim of the current systematic review and meta-analysis is to:

1. Provide data-backed recommendations on type, frequency and duration of exercise interventions specifically aiming to improve cardiometabolic profile in women with PCOS.

Therefore, the objectives of this study were to:

i) Conduct an updated systematic review that will include primary studies published up to April 2018.

ii) Undertake an in-depth analysis of cardiometabolic outcomes solely, without sharing the focus with fertility-related outcomes.

iii) Dependent on sufficient androgen data, aim to partition results based on androgen profile, such that any difference between normo-androgenic and hyper-androgenic phenotypes will be highlighted.

iv) Include only control groups containing women with PCOS undertaking no intervention or standard care, so that the effects of exercise can be isolated.

3.2 Methods

The review is reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidance (Appendix 1). The protocol has been summarised according to Preferred Reporting Items for Systematic review and Meta-Analysis

Protocols (PRISMA-P) and has been registered in the International Prospective Register of Systematic Reviews (PROSPERO) CRD42018086117. The full protocol has been published in a peer-reviewed journal (Woodward et al., 2019)

Table 4. Inclusion and Exclusion Criteria.

PICO	Inclusion Criteria	Exclusion Criteria
Population	Diagnosed with PCOS according to Rotterdam Criteria 2003, NIH 1990 criteria or AE-PCOS Society 2006 criteria. Be post-menarche and pre-menopausal. Be inactive (<150min/week of moderate-to-high intensity physical activity).	Receiving fertility treatment, taking metformin or OCP, or have a diagnosis of any pathology that may be promoting PCOS symptoms such as Cushing's syndrome, congenital hyperplasia or androgen-secreting tumour.
Intervention	Any sample sizes. Aerobic or anaerobic exercise training, resistance training, or combinations. At least two weeks of structured, supervised sessions. Interventions including multiple arms (such as aerobic and anaerobic exercise training, or a medication arm) will be included if it is possible to isolate the effects of the exercise intervention through a control group or placebo.	Crossover trials and interventions that are combined (such as lifestyle intervention including both exercise and diet management – where diet management refers to participants actively changing their caloric intake or the macronutrient composition of their diet in response to given targets).
Comparison	A control group of women with PCOS undertaking no interventions (not taking part in any structured exercise training), and not receiving fertility treatment, metformin, OCP or statins.	Healthy control group.
Outcome	Primary outcomes will be LDL-C, HDL-C, total cholesterol (TC), TC:HDL ratio, TG, oxidised LDL, cIMT, fasting glucose, HbA1c, blood pressure, WC, WHR, abdominal adiposity and inflammation markers. Secondary outcomes will be total testosterone, free testosterone, SHBG, fasting insulin, and HOMA-IR.	Outcome measures that have not been recorded at baseline and post intervention.

3.2.1 Eligibility Criteria

RCT, quasi-RCT and clinical trials were screened according to population, intervention, comparison, and outcome (PICO) criteria as highlighted in Table 4.

3.2.2 Searches

The electronic databases as follows were searched from inception to present: CINAHL Complete (EBSCO), Cochrane Central Register of Controlled Trials (CENTRAL) (Wiley), MEDLINE (EBSCO), Scopus (Elsevier), SPORTDiscus (EBSCO), PEDro (The University of Sydney), PubMed (US National Library of Medicine). Clinical trials were sought via searches of ClinicalTrials.gov and UK Clinical Trials Gateway. Only English language publications were sought, and no publication date limitations were applied.

The search terms were PCOS or polycystic ovary syndrome and terms relating to exercise or PA interventions. These were adapted for use in other databases.

The PubMed search strategy can be found Appendix 2.

3.2.3 Data Collection and Analysis

3.2.3.1 Study Selection

Results from the database searches were imported into RefWorks and de-duplicated once the searches are complete. The title and abstract for each paper were then exported to Microsoft Excel. Screening was undertaken in Microsoft Excel (version 16.0), with one reviewer (AW) screening each result. A second reviewer (MK and DB) screened each result in duplicate.

The full text was then retrieved for each of the initially included studies, such that they could be examined in more detail to determine their relevancy. This was undertaken by AW with screening undertaken in duplicate by a second reviewer (MK or DB). A reason was provided for any studies excluded at this stage. Throughout this stage, disagreement between two reviewers resulted in discussion and input from a third reviewer until a consensus was reached. Where the full text was not available, a request was made to the British Library.

3.2.3.2 Data Extraction

An a priori data extraction form was created in Microsoft Excel (version 16.0) which was piloted using two studies. One reviewer extracted data using the form, and all data was double-checked for consistency by a second reviewer (MK or DB).

Extracted data included bibliographic information (such as title, journal, primary author, publication date) study characteristics (such as study design, count and type of study arms, and sample sizes), participant characteristics (such as age and BMI, and PCOS diagnostic criteria), intervention and comparison data (such as type of control group, type, duration and frequency of exercise intervention, randomisation and withdrawal), and outcome data including any relevant parameters named in the primary and secondary outcomes - taken at baseline before intervention - and post-intervention, for each arm.

In the case of any missing or unclear data, two attempts were made to contact the corresponding author by email. If no response was received, the missing data was not included in the meta-analysis. Data was extracted as mean and standard deviation (SD) for baseline and post-intervention values. P-values and confidence intervals were also extracted if available.

3.2.4 Risk of Bias in Individual Studies & Heterogeneity

The Cochrane Risk Assessment tool was used to assess quality at the study level. The tool evaluates studies based on six criteria: 1) randomisation generation, 2) allocation concealment, 3) blinding of outcome assessors, 4) incomplete outcome data (that is, lost to follow-up), 5) selective outcome reporting, and 6) other risks of bias. It is not possible to blind participants to their intervention allocation due to the demands of studies requiring engagement with exercise programmes. Thus, criterion six was not included in the risk assessment.

Heterogeneity of results was assessed using the I^2 statistic. This statistic measures the consistency of results across studies; that is, whether the variation in outcomes across studies is due to chance (homogeneity), or whether there are genuine, underlying differences between the studies (heterogeneity) (Higgins, Thompson, Deeks & Altman, 2003). This statistic was chosen for its simplicity and applicability to meta-analyses regardless of the number of studies involved (Higgins et al., 2003). It describes the variability, presented as a percentage, in effect estimates that is due to heterogeneity rather than sampling error and its interpreted as follows: 0-40%: might not be important, 30-60%: may represent moderate heterogeneity, 50-

90%: may represent substantial heterogeneity, and 75-100%: considerable heterogeneity. A result of over 50% was considered significant heterogeneity (Higgins, Deeks & Altman, 2011). This then informed whether a random effect or fixed effect model would be most appropriate for meta-analysis. Sensitivity analyses were performed as appropriate by removing studies with small sample sizes (<30) or those with a high risk of selection bias.

3.2.5 Data Synthesis

Outcomes measured and presented pre- and post- intervention were quantitatively synthesised and analysed. Outcomes were recorded in tables outlining means and standard deviation, with effect size expressed as mean difference (difference between means) with 95% confidence intervals and study weighting. The mean difference was calculated as the difference between final (post-intervention) values rather than change scores. This is because baseline and final values may be reported for different numbers of participants due to missed visits or study withdrawals, leading to inaccurate change scores (Higgins & Deeks, 2011). In addition, change scores are often not presented with standard deviations and imputing them may be inappropriate because of differences between studies (Higgins & Deeks, 2011). However, in cases where there were significant differences at baseline, change scores were used if it was appropriate to impute SD. In cases where only the change score was available, efforts were made to contact authors to obtain final value scores. If this was not possible, change scores were included if presented with an SD. If there was no SD, it was imputed where appropriate. Pooled mean difference, 95% CI, P-values and I^2 statistic were also recorded for each outcome. Forest plots were generated and a P-value of <0.05 was considered statistically significant.

Statistical analysis was undertaken using RevMan 5 (The Cochrane Collaboration, 2014).

3.2.6 Subgroup Analysis

It was intended that if enough androgen data were available, data would be partitioned into normo-androgenic or hyper-androgenic profiles, based on a free testosterone measure, where >2 nmol/L indicates hyper-androgenism (Karakas, 2017). This was to highlight differences in cardiometabolic profile between these phenotypes.

To perform the subgroup analysis, free testosterone data was needed and a sufficient level of homogeneity between such studies to parse the results by androgen profile. However, this was not possible because free testosterone data was unavailable.

3.2.7 Confidence in the Findings

The Grading of Recommendations Assessment, Development and Evaluation (GRADE) was used to grade the quality of the evidence and the strength of a finding (Atkins et al., 2004). GRADE provides a systematic and explicit approach to making judgements about clinical and healthcare guidelines and recommendations, based on the quality of the evidence behind them. GRADE uses a scoring system (very low, low, moderate, high) to grade each finding in several areas including limitations, consistency, directness, and publication bias. The use of a consistent and transparent approach to evaluating recommendations increases the facilitation of critical appraisal and improves communication of these judgments (Atkins et al., 2004).

3.3 Results

The initial search of databases identified a combined total of 2,334 records. Once duplicates were removed, 2,163 records remained for title and abstract screening. Records were excluded (n = 2,136) because the title and abstract screening revealed that the articles did not meet the inclusion criteria. Twenty-seven articles were selected for full-text eligibility screening. Twenty-four were excluded for the reasons identified in Figure 4.

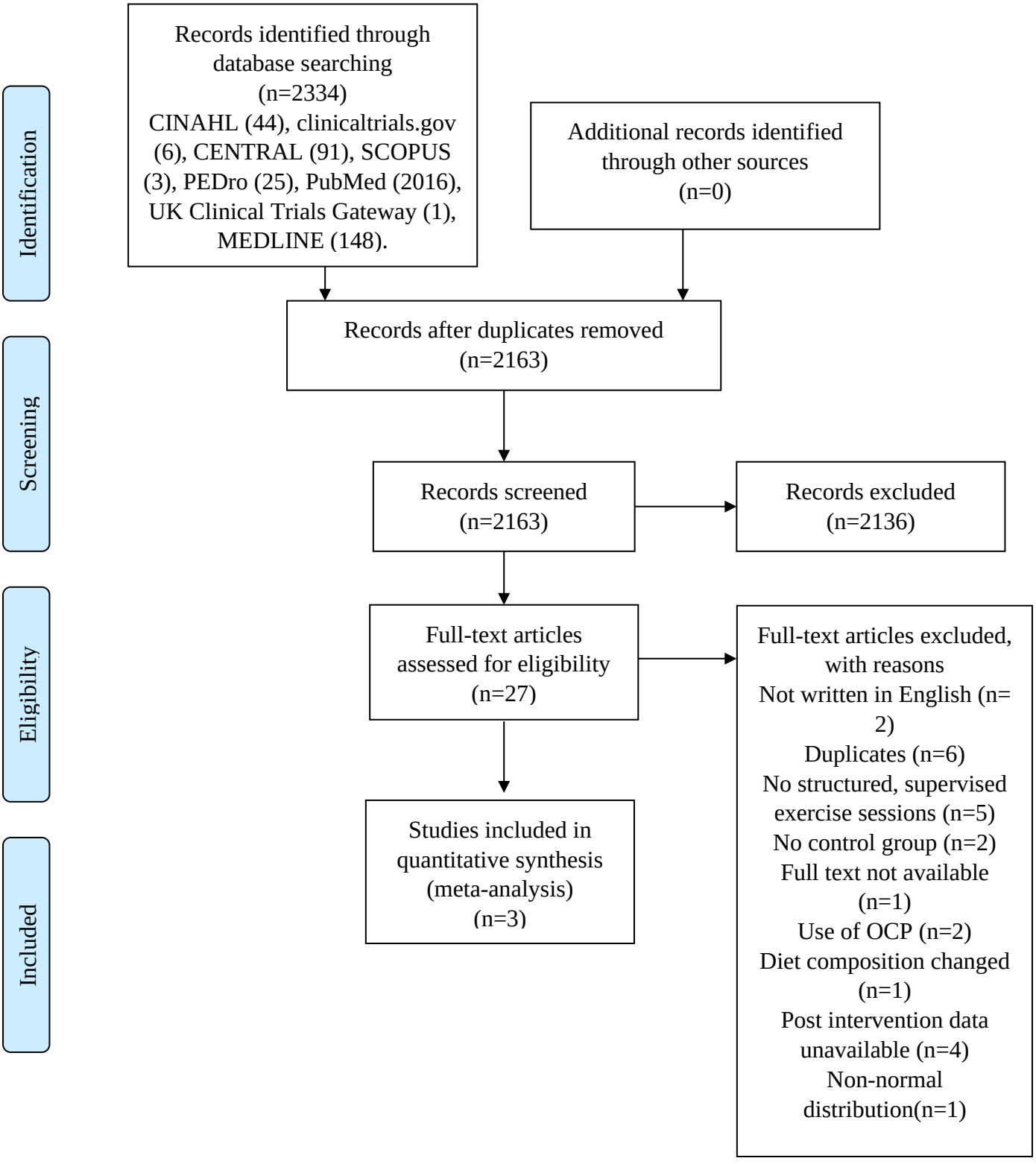

Figure 4. PRISMA flow diagram (Moher, Liberati, Tetzlaff, Altman, & the PRISMA group, 2009)

3.3.1 Study Design and Data Handling

Three studies were included in the meta-analysis. One was an exercise only RCT (Vigorito et al., 2007) and two were exercise only non-randomised clinical trials (Giallauria et al., 2008; Sprung et al., 2013). All compared an exercise intervention to a control group or standard care.

Two studies presented data as mean and SD (Giallauria et al., 2008; Vigorito et al., 2007) and one presented data as mean and 95% CI (Sprung et al., 2013). Data from the latter study were converted into mean and SD. Data were converted into the most common unit used for each variable if there were discrepancies.

Sensitivity analysis was performed either by removing studies with small sample sizes (<30 participants) from the pooled data or by removing those with a high risk of selection bias.

3.3.2 Participant Characteristics

Table 5 is a summary of characteristics of the three included studies. Across all studies, there was a total of 231 participants, with 117 receiving an exercise intervention and 114 controls. Total participants ranged from 124 (Giallauria et al., 2008) to 17 (Sprung et al., 2013). The 2003 Rotterdam criteria was used to reach a PCOS diagnosis in all three studies. The mean age of participants was 26 years, ranging from 22 (Vigorito et al., 2007) to 28 years (Sprung et al., 2013).

Table 5. Characteristics of Included Studies.

Study	Type	Diagnosis	Exercisers	Controls	Duration	Frequency	Session Length	Mode	Intensity	Outcomes Reported	Significant Improvement Between Groups[α]
Giallauria et al. 2008	CT	Rotterdam	N=62 BMI=29.2 kg/m^2	N=62 BMI=29.5 kg/m^2	3 months	3/week	30 min	Bicycle ergometer	60-70% of VO_2 max	LDL-C, HDL-C, TC, TG, Fasting Glucose, WHR, TT, SHBG, CRP, SBP, DBP	WHR* and CRP*
Sprung et al. 2013	CT	Rotterdam	N=10 BMI=31 kg/m^2	N=7 BMI=35 kg/m^2	16 weeks	3/week for 11 weeks 5/week for 5 weeks	30 min for 11 weeks, 45 min for 5 weeks	Participant preference	30% HRR for 11 weeks, 60% HRR for 5 weeks	LDL-C, HDL-C, TC, TG, Fasting Glucose, WC, TT, SHBG. HOMA-IR	TC** and LDL-C**
Vigorito et al. 2007	RCT	Rotterdam	N=45 BMI=29.3 kg/m^2	N=45 BMI=29.4 kg/m^2	3 months	3/week	30 min	Bicycle ergometer	60-70% VO_2 max	LDL-C, HDL-C, TC, TG, Fasting Glucose, WC, WHR, TT, HOMA-IR, SBP, DBP, CRP	WC, WHR

Study is lead author and year of publication. Type: CT=controlled trial, RCT=randomised controlled trial. Diagnosis refers to the specific criteria that the researchers used to confirm PCOS diagnosis: Rotterdam = European Society for Human Reproductive and Embryology/American Society for Reproductive Medicine (2003). N = number of participants randomised into each arm of the study. BMI = mean body mass index (kg/m^2) of participants in each arm at study entry. Duration, frequency, session length, mode and intensity refer to intervention characteristics. HRR = heart rate reserve, VO_2 max = maximum oxygen update, LDL-C = low-density lipoprotein cholesterol, HDL-C = high-density lipoprotein cholesterol, TC = total cholesterol, TG = triglycerides, WC = waist circumference, WHR = waist-to-hip ratio, TT = total testosterone, SHBG = sex hormone-binding globulin, HOMA-IR = homeostatic assessment of insulin resistance, SBP = systolic blood pressure, DBP = diastolic blood pressure, CRP = C-reactive protein. α = statistically significant.

3.3.3 Intervention Characteristics

The exercise intervention duration in two studies was three months (Vigorito et al., 2007; Giallauria et al., 2008) one was 16 weeks (Sprung et al., 2013). All of the studies had an exercise frequency of three times per week (Vigorito et al., 2007, Giallauria et al., 2008, Sprung et al., 2013). One study began with three sessions per week for 11 weeks and then progressed to five sessions per week for five weeks (Sprung et al., 2013). Exercise intensity was determined by a percentage of VO_2 max (Vigorito et al., 2007; Giallauria et al., 2008) or heart rate reserve (HRR) (Sprung et al., 2013). All were aerobic exercise interventions. Session length was 30 minutes in all three studies (Vigorito et al., 2007; Giallauria et al., 2008; Sprung et al., 2013) increasing to 45 minutes after 11 weeks in one (Sprung et al., 2013) .Two studies were performed on a bicycle ergometer (Vigorito et al., 2007; Giallauria et al., 2008), and one was performed on a stationary cycle, treadmill or elliptical machine according to participant preference (Sprung et al., 2013).

All three studies reported that all participants completed the study protocol (Vigorito et al., 2007; Giallauria et al., 2008; Sprung et al., 2013). All studies reported a mean adherence of ≥80%. All studies included women of reproductive age with a clinically confirmed PCOS diagnosis. All studies specifically mentioned exclusion of participants who were taking OCP, metformin, or other hormonal, anti-androgen or carbohydrate metabolism modification drugs. All studies also specifically mentioned the exclusion of other conditions that could promote hyperandrogenism, such as Cushing's Syndrome and congenital adrenal hyperplasia. All studies excluded those with thyroid dysfunction, diabetes, CVD or other renal or hepatic diseases. Only one study confirmed exclusion of smokers and the exclusion or participants who undertook regular exercise (Sprung et al., 2013). Two studies did not specify a formal sample size calculation (Vigorito et al., 2007; Giallauria et al., 2008), and another based this on an outcome of flow-mediated dilation (Sprung et al., 2013).

3.3.4 Risk of Bias in Included Studies

The author's judgements about each risk of bias category are presented as percentages across all included studies in Figure 5. A summary of the author's judgements of each risk of bias item for each included study are presented in Figure 6. Further information outlining how each judgement was reached for each category in each included study is available in Table 6.

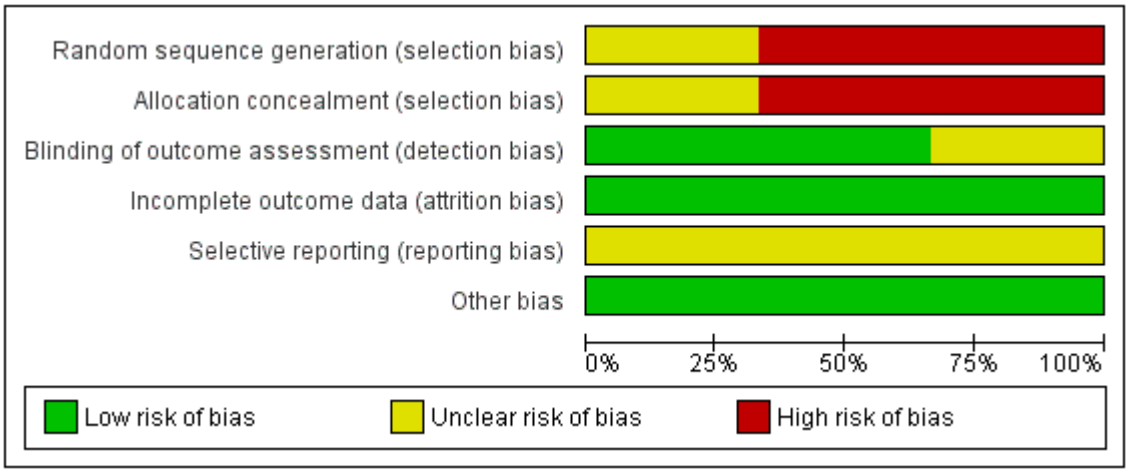

Figure 5. Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

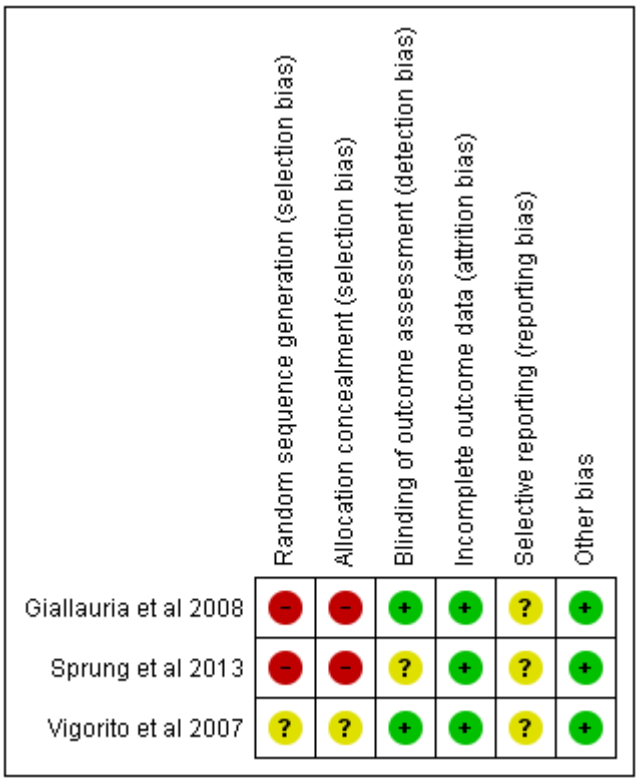

Figure 6. Risk of bias summary: author's judgements about each risk of bias item for each included study.

Table 6. How each judgement was made by the author for each category in each included study.

Trial	Bias Domain	Source of Bias	Author's judgement	Support for judgement
Vigorito et al. 2007	Selection Bias	Random sequence generation	Unclear Risk	Not reported.
		Allocation concealment	Unclear Risk	Not reported.
	Performance Bias	Blinding of participants and personnel	N/A	It was not included whether participants were blind to their allocation of intervention or to control groups, as it is often not possible (e.g., in a supervised exercise setting) to blind participants to an intervention while promoting exercise behaviour.
	Detection Bias	Blinding of outcome assessment	Low Risk	All clinical assessments were performed by the same physician who was blinded to the patient allocation.
	Attrition Bias	Incomplete outcome data	Low Risk	No attrition reported.
	Reporting Bias	Selective reporting	Unclear Risk	Unable to locate study protocol.
	Other Bias	Adherence	Low Risk	All participants completed the study protocol.
Giallauria et al 2008	Selection Bias	Random sequence generation	High Risk	Allocation by patient choice
		Allocation concealment	High Risk	Patients chose allocation.
	Performance Bias	Blinding of participants and personnel	N/A	It was not included whether participants were blind to their allocation of intervention or to control groups, as it is often not possible (e.g., in a supervised exercise setting) to blind participants to an intervention while promoting exercise behaviour.
	Detection Bias	Blinding of outcome assessment	Low Risk	A physician who was blinded to the patient allocation performed all clinical assessments.

	Attrition Bias	Incomplete outcome data	Low Risk	No attrition reported.
	Reporting Bias	Selective reporting	Unclear Risk	Unable to locate study protocol.
	Other Bias	Adherence	Low Risk	Adherence was reported as 80%.
Sprung et al 2013	Selection Bias	Random sequence generation	High Risk	Allocation by patient choice.
		Allocation concealment	High Risk	Patients chose allocation.
	Performance Bias	Blinding of participants and personnel	N/A	It was not included whether participants were blind to their allocation of intervention or to control groups, as it is often not possible (e.g., in a supervised exercise setting) to blind participants to an intervention while promoting exercise behaviour.
	Detection Bias	Blinding of outcome assessment	Unclear Risk	Not reported.
	Attrition Bias	Incomplete outcome data	Low Risk	No attrition reported.
	Reporting Bias	Selective reporting	Unclear Risk	Unable to locate study protocol.
	Other Bias	Adherence	Low Risk	91% adherence reported.

Two studies (66.6%) were judged to have a high risk of selection bias because participants were allocated to groups based on their own choice (Giallauria et al., 2008; Sprung et al., 2013) and one (33.3%) was judged to have an unclear risk of selection bias because the authors did not report a method for randomisation or allocation concealment (Vigorito et al., 2007).

Performance bias was excluded from the assessment as all the studies included supervised exercise sessions, so it is impossible to blind participants to this type of intervention while promoting exercise behaviour. Two studies (66.6%) were judged to have a low risk of detection bias because the blinding of outcome assessment was ensured, or the outcome measurement was not likely to be influenced by lack of blinding (Vigorito et al., 2007; Giallauria et al., 2008). The remaining study was judged to have an unclear risk of detection

bias because the authors did not address this outcome. All studies were judged to have a low risk of attrition bias due to zero reported attrition rate, and all were judged to have an unclear risk of reporting bias because prospective protocols could not be located. Additionally, it was assessed whether adherence (reported as <80%) may have presented a high risk of 'other sources of bias', and all were judged to be at a low risk.

3.3.5 Reporting of Outcomes

All three studies reported on outcomes relating to lipid profile (such as HDL-C, LDL-C, TC and TG) but no studies reported oxidised LDL. All studies included either WC or WHR. Two studies reported fasting blood glucose and HOMA-IR measures (Vigorito et al., 2007; Sprung et al., 2013), and one reported just fasting blood glucose (Giallauria et al., 2008). On androgen profile, all three studies reported total testosterone and two reported sex hormone binding globulin (SHBG) in addition (Giallauria et al., 2008; Sprung et al., 2013). Two studies reported systolic blood pressure (SBP) and diastolic blood pressure (DBP) (Vigorito et al., 2007; Giallauria et al., 2008). Only two studies reported inflammation markers and both of those reported C-reactive protein (CRP) (Vigorito et al., 2007; Giallauria et al., 2008).

3.3.6 Effects of Exercise Versus Control

Following the study inclusion criteria, only three studies could be included in the meta-analysis. As such, subgroup analyses of exercise intensity, type and frequency were not performed. Subgroup analysis for intervention duration may have been possible but, given that there would be two studies in one category and one in the other, it was deemed to be uninformative and potentially misleading (Deeks, Higgins & Altman, 2011). Effect estimates, 95% CI and I^2 values are listed in Table 7 for each outcome.

Table 7. Mean difference, 95% CI, P and I^2 value for each outcome analysed.

Outcome	Studies	N	MD	Lower	Upper	P	I^2 (%)
HDL-C (mg/dL)	3	231	-2.97	-6.62	0.68	0.11	0
LDL-C (mg/dL)	3	231	-4.10	-13.32	5.22	0.39	42
TC (mg/dL)	3	231	-4.78	-9.24	-0.32	0.04	14
TG (mg/dL)	2	214	1.55	-4.66	7.76	0.63	0
Fasting Glucose (mg/dL)	2	214	-1.75	-3.46	-0.04	0.04	0
WC (cm)	2	107	-1.97	-3.35	-0.59	0.005	0
WHR	2	214	-0.05	-0.08	-0.02	0.0003	0
TT (nmol/L)	3	231	-0.20	-0.38	-0.02	0.03	47
SHBG (nmol/L)	2	141	4.05	1.79	6.31	0.0004	0
CRP (mg/L)	2	214	-0.34	-0.54	-0.15	0.0006	0
SBP (rest) (mmHg)	2	214	-4.40	-7.13	-1.66	0.002	0
DBP (rest) (mmHg)	2	214	-0.80	-1.96	0.37	0.18	0

N = number of participants. MD = Mean difference. LDL-C = low-density lipoprotein cholesterol, HDL-C = high-density lipoprotein cholesterol, TC = total cholesterol, TG = triglycerides, WC = waist circumference, WHR = waist-to-hip ratio, TT = total testosterone, SHBG = sex hormone-binding globulin, HOMA-IR = homeostatic assessment of insulin resistance, SBP = systolic blood pressure, DBP = diastolic blood pressure, CRP = C-reactive protein.

Additionally, free testosterone measures were not available. Total testosterone measures indicated that the mean values for participants (231) in the studies eligible for meta-analysis were hyper-androgenemic, based on total testosterone (TT) concentrations of >2.0 nmol/L (Karakas, 2017; Mayo Clinic Laboratories, 2018; Pasquali et al., 2016), therefore subgroup-analysis of androgen profile could not be conducted.

3.3.6.1 Primary Outcomes

Blood lipids:

All three studies (231 participants) in the meta-analysis assessed changes in LDL-C, HDL-C, TC and TG (231 participants). There was no observed effect of exercise versus control on LDL-C, HDL-C or TG. There was a statistical effect of exercise on TC versus control (-4.70 mg/dl, 95% CI -9.24, -0.32, I^2 = 14%). When the study with a small sample size was removed (Sprung et al., 2013), the effect was no longer statistically significant.

Of the three studies in the analysis, one reported a significant decrease in LDL-C (-0.7 mmol/L, 95% CI -1.1 to -0.3, P=0.001) and in TC (-0.20 mmol/L, 95% CI -0.28 to -0.04, P=0.01) when compared to the control group (Sprung et al., 2013).

Fasting Blood Glucose:

Data from the three studies (231 participants) pooled in the meta-analysis showed a significant favourable effect of exercise on fasting glucose concentrations versus controls (-1.75 mg/dL, 95% CI -3.45, -0.5, I^2 =0%). When the study with a small sample size was removed, the effect remained significant (-1.75 mg/dL, 95% CI -3.46, -0.4, 214 participants, I^2 =0%).

Measures of Abdominal Obesity:

Two studies (107 participants) were pooled in the meta-analysis to assess changes to WC and WHR. A statistically favourable effect of exercise on WC (-1.97 cm, 95% CI -3.35, -0.59, I^2 =0%) and a small but statistical favourable effect of exercise on WHR (-0.05, 95% CI -0.09, -0.01, I^2 =0%) compared to the control group was observed.

One study reported a significant decrease in WC (P <0.01) and WHR (P <0.05) in the exercise group when compared to the control group (Vigorito et al., 2007). One other reported a significant decrease in WHR (P <0.05) in the exercise group compared to control (Giallauria et al., 2008).

Blood Pressure:

Two studies (214 participants) were pooled in the meta-analysis to assess changes in SBP and DBP at rest. The results indicated a statistical favourable effect of exercise on SBP in comparison to controls (-4.40 mmHg, 95% CI -7.13, -1.66, I^2 =0%) but no effect was observed for DBP.

Of the two studies reporting SBP and DBP, one did not note any statistical effect of exercise on SBP or DBP in comparison to controls (Giallauria et al., 2008). The other study (Vigorito et al., 2007) reported a significant (P < 0.01) decrease in SBP after the exercise intervention, but this was not significant in comparison to the control group.

C-Reactive Protein:

Two studies (114 participants) included in the meta-analysis recorded changes in CRP. There was a small but statistical favourable effect of exercise on CRP compared to controls (-0.34 mg/l, 95% CI -0.54, -0.14, I^2 =0%). Both studies had a sample size >30.

Of the two studies one reported significant improvement after exercise only (Vigorito et al., 2007) and the other found significant improvement after exercise and between-groups

(Giallauria et al., 2008). Both studies were ≥12 weeks in duration, with sessions of 30 minutes on a bicycle ergometer.

3.3.6.2 Secondary Outcomes

Total Testosterone and Sex Hormone Binding Globulin:

Three studies (231 participants) were pooled to assess changes in TT. There was a significant favourable effect of exercise on TT compared to controls, although moderate heterogeneity was noted (-0.20 nmol/l, 95% CI -0.38, -0.02, I^2 =47%). Removal of the study with a small sample size (Sprung et al., 2013) mitigated I^2 to 35% and increased the statistical effect estimate (-0.24 nmol/l, 95% CI -0.43, -0.05, 114 participants). The same result was also observed when removing the study with the highest risk of bias (Sprung et al., 2013).

Only two of the studies reporting TT also reported changes to SHBG (114 participants). The meta-analysis indicated a favourable effect of exercise on SHBG concentrations (4.10, 95% CI 1.79, 6.31, I^2 =0%). However, of note, both studies had a high risk of bias in two domains.

Homeostatic Model Assessment of Insulin Resistance:

Only one study eligible for meta-analysis reported HOMA-IR and as such pooled analysis could not be conducted. No studies reported any significant improvement in HOMA-IR after exercise.

Figures 7 to 18 show the comparisons for each outcome and subsequent forest plot.

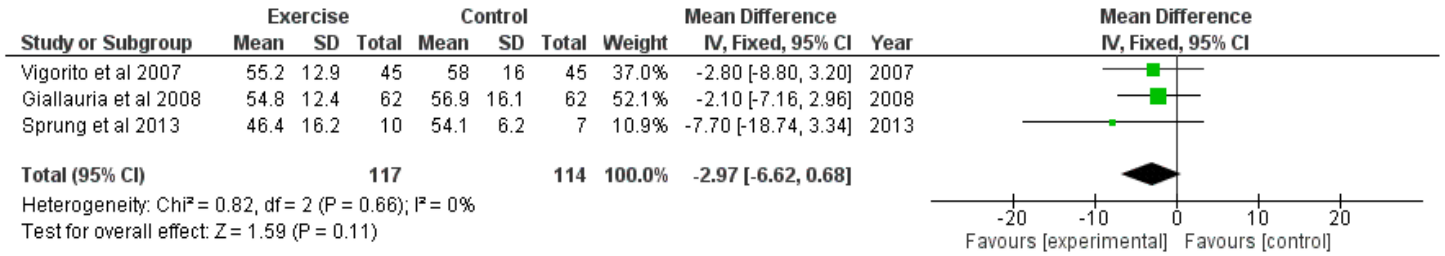

Figure 7. Forest plot of comparison: 1 – all interventions, outcome: 1.1 – HDL-C (mg/dL)

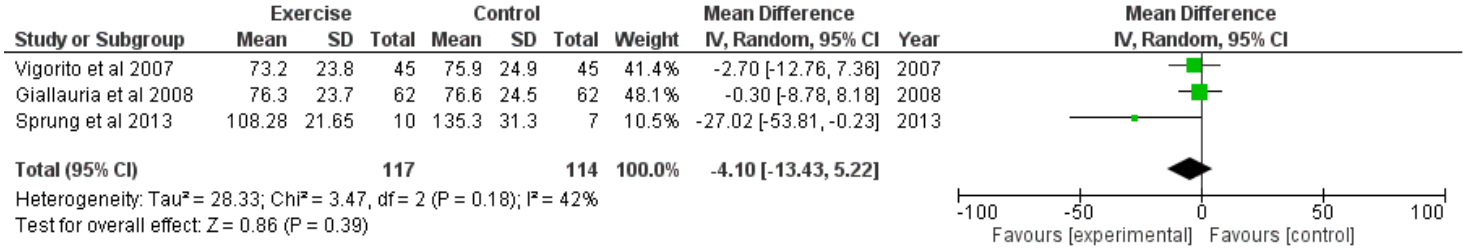

Figure 8. Forest plot of comparison: 1 – all interventions, outcome: 1.1 – LDL-C (mg/dL)

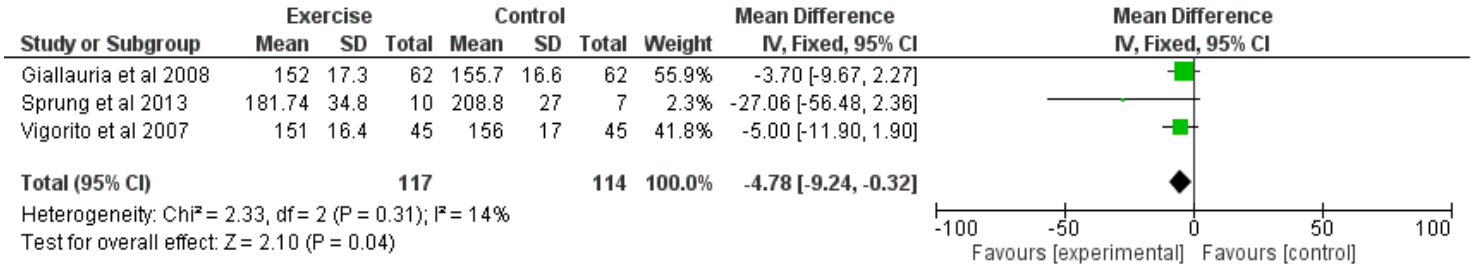

Figure 9. Forest plot of comparison: 1 – all interventions, outcome: 1.3 – TC (mg/dL)

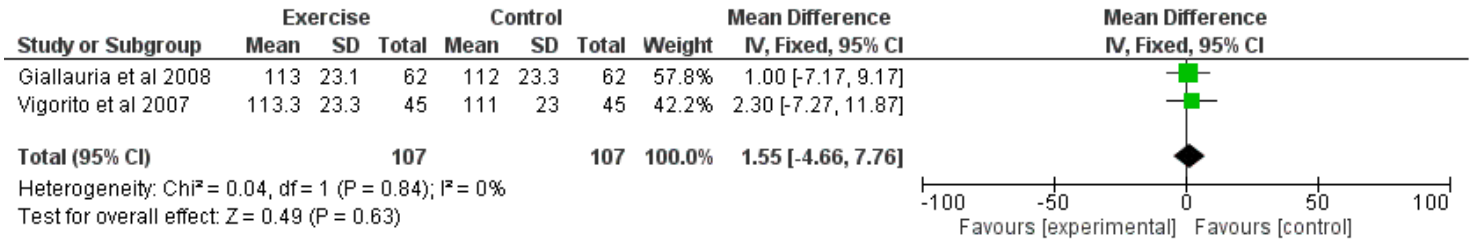

Figure 10. Forest plot of comparison: 1 – all interventions, outcome: 1.4 – TG (mg/dL)

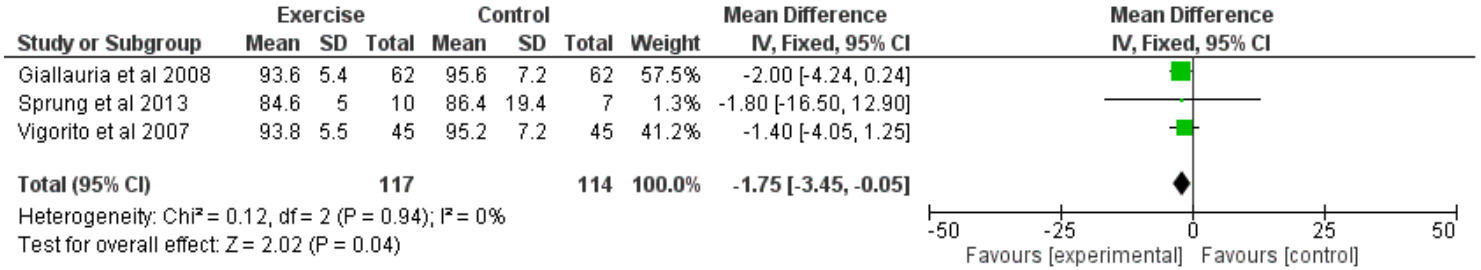

Figure 11. Forest plot of comparison: 1 – all interventions, outcome: 1.5 – Fasting blood glucose (mg/dL)

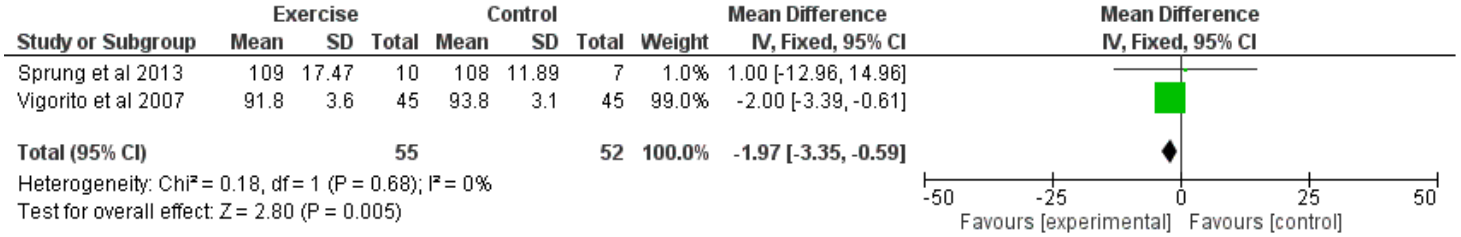

Figure 12. Forest plot of comparison: 1 – all interventions, outcome: 1.6 – Waist circumference (cm)

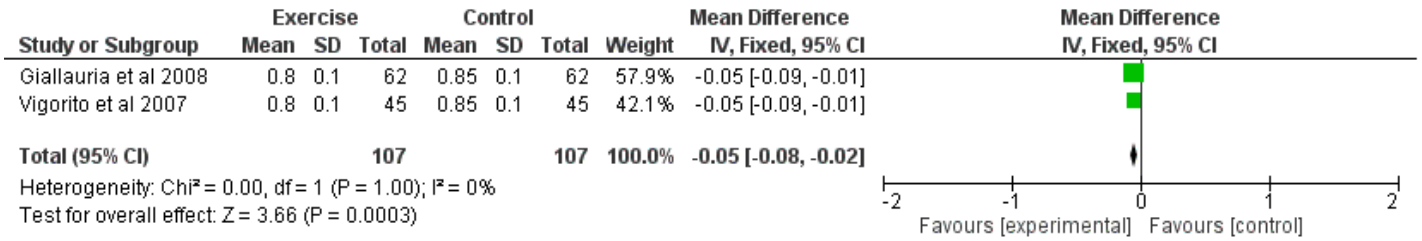

Figure 13. Forest plot of comparison: 1 – all interventions, outcome: 1.7 – Waist-to-hip ratio

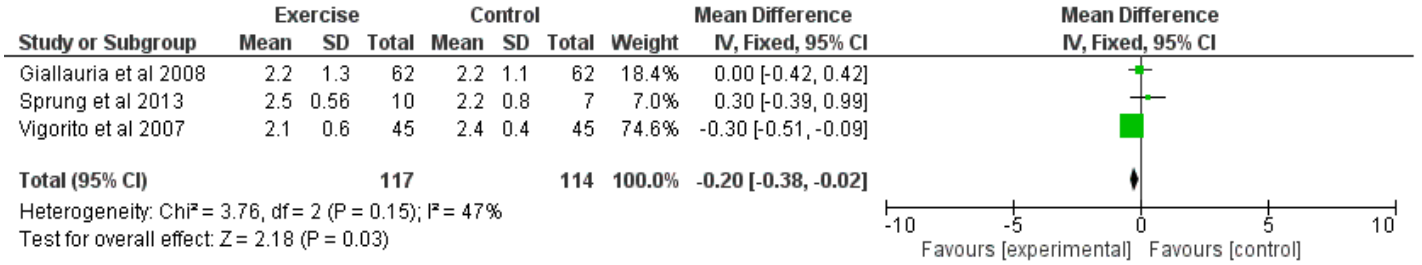

Figure 14. Forest plot of comparison: 1 – all interventions, outcome: 1.8 – Total testosterone (nmol/L)

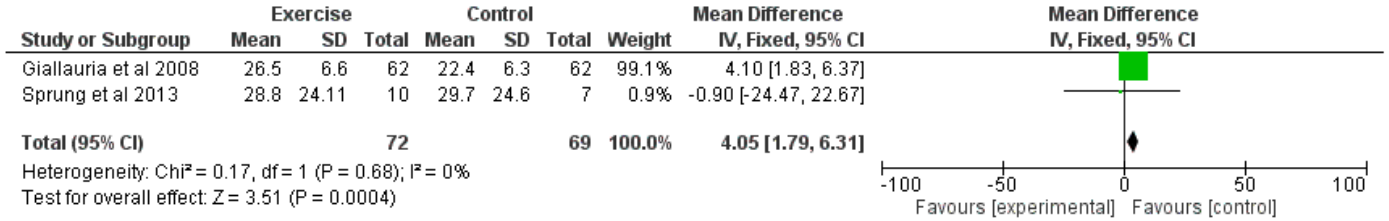

Figure 15. Forest plot of comparison: 1 – all interventions, outcome: 1.9 – Sex hormone-binding globulin (nmol/L)

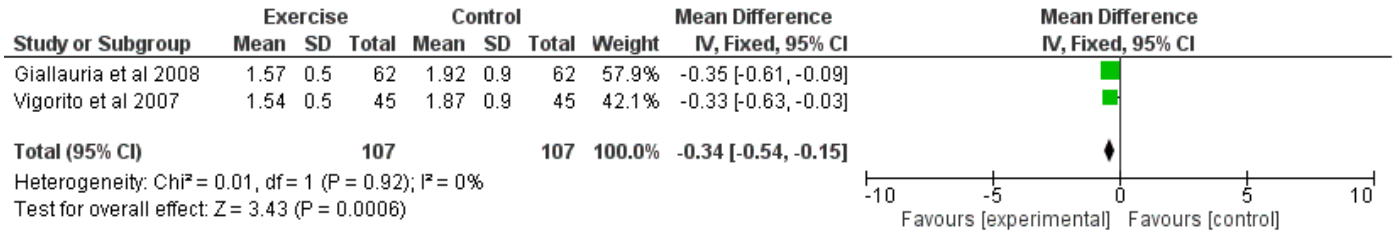

Figure 16. Forest plot of comparison: 1 – all interventions, outcome: 1.10 – C-reactive protein (mg/L)

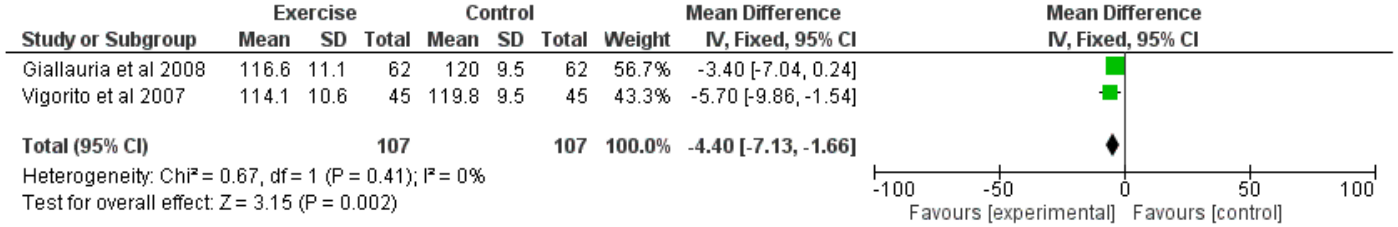

Figure 17. Forest plot of comparison: 1 – all interventions, outcome: 1.11 – Systolic blood pressure (rest) (mmHg)

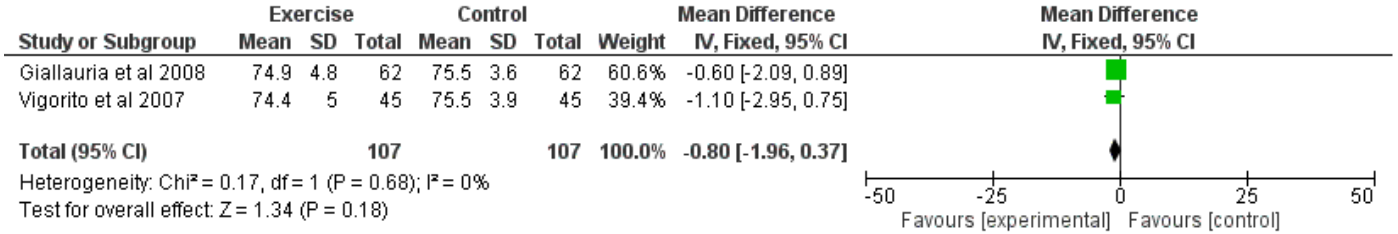

Figure 18. Forest plot of comparison: 1 – all interventions, outcome: 1.12 – Diastolic blood pressure (rest) (mmHg)

Table 8. GRADE evidence profile to assess confidence in effect estimates for each outcome.

No of Studies (No. of participants)	Quality Assessment					Summary of Findings		
	Study Limitations*	Consistency	Directness	Precision	Publication Bias	P Value	Effect (95% CI)	Quality
HDL-C								
3 (231)	Serious limitations (-1)	No important inconsistency	Direct	Imprecision (-1)[a]	Unlikely	0.11	-2.97 (-6.62, 0.68)	++, Low
LDL-C								
3 (231)	Serious limitations (-1)	No important inconsistency	Direct	Imprecision (-1)[a]	Unlikely	0.39	-4.10 (-13.43, 5.22)	++, Low
TC								
3 (231)	Serious limitations (-1)	No important inconsistency	Direct	No important imprecision	Unlikely	0.04	-4.78 (-9.24, -0.32)	+++, Moderate
WC								
2 (107)	Serious limitations (-1)	No important inconsistency	Direct	No important imprecision	Unlikely	0.005	-1.97 (-3.35, -0.59)	+++, Moderate
WHR								
2 (107)	Serious limitations (-1)	No important inconsistency	Direct	No important imprecision	Unlikely	0.0003	-0.05 (-0.08, -0.02)	+++, Moderate
Fasting Glucose								
2 (214)	Serious limitations (-1)	No important inconsistency	Direct	No important imprecision	Unlikely	0.04	-1.75 (-3.46, -0.04)	+++, Moderate
TT								
3 (231)	Serious limitations (-1)	Moderate Heterogeneity (-1)[b]	Direct	No important imprecision	Unlikely	0.03	-0.20 (-0.38, -0.02)	++, Low

SHBG								
2 (141)	Serious limitations (-1)	No important inconsistency	Direct	No important imprecision	Unlikely	0.0004	4.05 (1.79, 6.31)	+++, Moderate
CRP								
2 (214)	Serious limitations (-1)	No important inconsistency	Direct	No important imprecision	Unlikely	0.0006	-0.34 (-0.54, -0.15)	+++, Moderate
SBP								
2 (107)	Serious limitations (-1)	No important inconsistency	Direct	No important imprecision	Unlikely	0.002	-4.40 (-7.13, -1.66)	+++, Moderate
DBP								
2 (107)	Serious limitations (-1)	No important inconsistency	Direct	Imprecision (-1)[a]	Unlikely	0.18	-0.80 (-1.96, 0.37)	++, Low

*unclear randomisation and allocation, non-randomised controlled trials, small sample size (<30). [a] = confidence interval includes possible benefit in both directions. [b] = I^2 47%. LDL-C = low-density lipoprotein cholesterol, HDL-C = high-density lipoprotein cholesterol, TC = total cholesterol, TG = triglycerides, WC = waist circumference, WHR = waist-to-hip ratio, TT = total testosterone, SHBG = sex hormone-binding globulin, HOMA-IR = homeostatic assessment of insulin resistance, SBP = systolic blood pressure, DBP = diastolic blood pressure, CRP = C-reactive protein.

3.3.7 Quality of the Evidence

Table 8 provides the GRADE evidence profile. This reflects the grading of the quality of the evidence for each outcome. Evidence has been downgraded for all outcomes due to the presence of serious study design limitations, including small sample size (≤30 participants), unclear or inappropriate randomisation or allocation procedures and non-randomised controlled trials. Subsequently, all evidence could only begin at a maximum of moderate quality.

Moderate heterogeneity was observed for only one outcome. Also, there was no important inconsistency of mean post-intervention values in most of the analyses. No outcomes were downgraded for indirectness, because all studies directly compared an exercise intervention versus usual care or control, with explicit exclusions of confounding medications. Where CI were wide or indicated possible benefit in both directions, evidence was downgraded due to imprecision and uncertainty of results. Publication bias of all outcomes was considered unlikely, since the author conducted a thorough and comprehensive search of relevant databases, and no studies eligible for analysis declared any conflict of interest or funding sources that may have influenced publication.

3.4 Discussion

This systematic review and meta-analysis identified three studies, including 231 participants with PCOS, that isolated and examined the effect of structured, supervised exercise on cardiometabolic outcomes in PCOS. Various recently published reviews have examined the effects of exercise and/or lifestyle modification on facets of PCOS (Benham, Yamamoto, Friedenreich, Rabi & Sigal, 2018; Dewailly, 2016; Domecq et al., 2013; Haqq, McFarlane, Dieberg & Smart, 2015). To the author's knowledge, this is the only recent review that has aimed to isolate the effects of exercise alone in comparison with control/standard care, without the inclusion of dietary, pharmacological or behavioural modification programmes.

3.4.1 Summary of Main Findings

Analysis of pooled data indicated, in the comparison of exercise and control, statistical favourable effects of exercise on TC, fasting glucose, WC, WHR, SBP, CRP, TT and SHBG using post-intervention scores. This supports the role of exercise as a treatment in the improvement of several cardiovascular risk factors in PCOS, including abdominal adiposity, insulin sensitivity, endothelial dysfunction and androgen profile.

3.4.1.1 Primary Outcomes

A statistically significant effect of exercise was observed on TC versus control (-4.70 mg/dl, 95% CI -9.24, -0.32, I^2 = 14%), P = 0.04, but meta-analysis revealed no other significant changes to lipid profile in PCOS women. Other reviews have produced inconsistent results; a comprehensive, qualitative review (Dewailly, 2016) mostly found no significant effects of exercise only (without a dietary component) on lipid profile in PCOS, and those studies reporting significant improvements in TC involved a combined dietary and exercise component. Conversely, a recent review (Benham et al., 2018) noted a statistical effect of exercise on TC concentrations in PCOS in a pooled meta-analysis of just two studies (-0.09 mmol/L, 95% CI -0.10, -0.07), though it is not clear if this was based on exercise versus control only. Subsequently, sensitivity analysis rendered the pooled effect estimate non-significant. Additionally, since TC is the sum of LDL-C and HDL-C, the clinical relevance of this measure may be misleading, since LDL-C and HDL-C have contrasting roles within the vascular system and a change to either would affect the measure of TC (Mann, Beedie &

Jimenez, 2014). TC:HDL appears to be a better predictor of cardiovascular risk than TC or LDL-C (Carroll & Dudfield, 2004; Holvoet et al., 2001).

Despite these results, exercise has been shown to have a positive effect on HDL-C and TG in healthy populations and those presenting with metabolic syndrome (Carroll & Dudfield, 2004; Durstine et al., 2001; Katzmarzyk et al., 2003) with the latter sharing some cardiovascular risk factors with PCOS. This discrepancy may be due to the intervention characteristics shared by the three included studies (three sessions/week, 30 minute-session). It has been reported that changes to HDL-C and TG are more likely with an energy expenditure of 1,200kcal/week (Durstine et al., 2001); these interventions may be unlikely to produce this output at lower intensities. Additionally, a 2004 review (Carroll & Dudfield, 2004) indicates that interventions should be longer in duration (>20 weeks) to induce positive changes to HDL-C and TG in people with metabolic syndrome.

Pooled analysis of post-intervention values indicated a significant effect estimate of exercise versus control on fasting glucose concentrations (-1.75 mg/dl, 95% CI -3.45, -0.5, I^2 =0%), P = 0.04. This effect remained significant after sensitivity analysis. This finding is in line with a recent review that indicated a statistically significant effect estimate of lifestyle modification versus minimal intervention on fasting blood glucose in PCOS (−2.3 mg/dL, 95% CI, −4.5 to −0.1, I^2 = 72%) P = 0.04 (Domecq et al., 2013). However, statistical heterogeneity was noted, and exercise and dietary/behavioural modification were combined under 'lifestyle intervention'. Two other reviews (Domecq et al., 2013; Haqq et al., 2015) found no significant effects of lifestyle or exercise interventions on fasting blood glucose in PCOS. Despite this, various studies have demonstrated that aerobic exercise training enhances glucose disposal rate in women with PCOS (Covington, Tam, Pasarica & Redman, 2016; Redman, Elkind-Hirsch & Ravussin, 2011). The mean fasting blood glucose range for the three studies in the pooled analysis was 84.6-95.6 mg/dL, which are all considered to be in the normal range of <100 mg/dL (NICE, 2018). This is not unusual, because women with PCOS can maintain normal fasting glucose at the expense of increased insulin secretion (Karakas, 2017). Nevertheless, it is difficult to assess the clinical relevance of this outcome without comparative data on insulin sensitivity.

There was a statistically favourable effect of exercise versus control on WC (-1.97 cm, 95% CI -3.35, -0.59, I^2 =0%), P = 0.005, and WHR (-0.05, 95% CI -0.09, -0.01, I^2 =0), P = 0.003, in two studies. This is in agreement with two other reviews (Benham et al., 2018; Haqq et al.,

2015), although one combined exercise and dietary modification under lifestyle intervention (Haqq et al., 2015). WC and WHR, in some cases, have been shown to be a better indicator of health risk than BMI (Janssen, Katzmarzyk & Ross, 2004) because they measure abdominal obesity, a condition strongly associated with cardiovascular risk factors (Yusuf et al., 2004). A decrease in WC and WHR has also been associated with improvements in glucose metabolism (Thomson et al., 2008).

Exercise had a statistically significant effect on SBP in comparison to control (-4.40 mmHg, 95% CI -7.13, -1.66, I^2 =0%), P = 0.0003. This has been observed after lifestyle intervention in PCOS in another review (-5.01 mmHg, 95% CI -6.63, -3.39, P < 0.05, I^2 =0%) (Benham et al., 2018). A meta-analysis of RCTs (Fagard, 1999) has indicated that aerobic exercise training produces a small but statistical improvement in blood pressure, even in the absence of weight loss, in normotensive adults. Blood pressure values among this population have been inversely associated with insulin sensitivity (Carroll & Dudfield, 2004). The mean data from the meta-analysis indicates that participants were normotensive (≤120 mmHg). There were improvements in both WC and WHR, shown to be associated with insulin sensitivity in PCOS (Chen, Xu & Zhang, 2014). As such, improvement in insulin regulation is a plausible explanation for several of the observed effect estimates.

There was a favourable statistical effect of exercise on CRP versus control (-0.34 mg/l, 95% CI -0.54, -0.14, I^2 =0%) P < 0.001. This finding is in agreement with another review that found favourable effects of lifestyle modification versus usual care (-0.47 mmol/L, 95% CI -0.80, -0.15, P = 0.004, I^2 =0%). Indeed, PCOS has been linked to an inflammatory state characterised by increased levels of CRP (Krystock, 2014; Repaci, Gambineri & Pasquali, 2011). However, the clinical relevance of this finding may be tenuous; the mean CRP range for the studies in the pooled analysis was 1.54-1.92 mg/L, which are considered to be within the normal range (Nehring & Bhimji, 2018) and as such this may not indicate an inflammatory state in the participants. Also, the effect may not be reproduced in populations with a higher-than-normal value.

3.4.1.2 Secondary Outcomes

Pooled data analysis indicated a statistical favourable effect of exercise versus control on TT(-0.20 nmol/L, 95% CI -0.38, -0.02, I^2 =47%) P = 0.03, and SHBG (4.05, 95% CI 1.79, 6.31, I^2 =0%) P < 0.001. Both outcomes were derived from at least one study with a high risk

of bias for randomisation and allocation procedures. Nevertheless, a previous review has noted a statistical lowering of fasting insulin levels in the exercise group compared to the control group in PCOS (-0.95 μU/mL, 95% CI -1.48, -0.43, $P < 0.05$, $I^2 = 0\%$) (Benham et al., 2018). Additionally, a qualitative systematic review found evidence for improved insulin sensitivity following exercise in PCOS (Dewailly, 2016). An improvement in insulin sensitivity following exercise could therefore be an explanation for both reduced TT and increased SHBG; hyperinsulinemia causes an increase in free androgen plasmatic levels both through the stimulation of ovarian androgen synthesis, and by suppressing hepatic production of SHBG (Bellanger, Battista & Baillargeon, 2014). It was not possible to perform a meta-analysis on free testosterone; caution is advised when measuring TT alone, because women with PCOS can have TT in the normal range but have high concentrations of free and bioavailable testosterone due to lower concentrations of SHBG (Karakas, 2017). However, the data indicate that the participants in the meta-analysis had low enough SHBG concentrations (<30 nmol/L), even post-intervention, to indicate hyperandrogenism (Karakas, 2017). This provides further plausibility to the explanation that exercise may have mitigated insulin hypersecretion, thereby increasing hepatic production of SHBG and reducing ovarian androgen synthesis to the effect of reduced TT.

3.4.2 Overall Completeness and Applicability of Evidence

One study in the analysis was an RCT and two were non-RCT. This limits the overall applicability of the evidence, particularly where participants were allocated to groups based on preference. Although the studies specified no statistical differences in baseline characteristics, it is possible that the adherence and attrition rates are not truly reflective of those that would be observed in gold standard RCTs.

Only one study specified formal sample size calculations, and this study had a small sample size (17 participants). In samples of this size, variance in scores is likely to affect statistical significance and applicability to the general PCOS population is limited.

Sub-group analysis based on androgen profile was not possible, because the studies included in the meta-analysis indicated that the mean TT concentration for all participants was high enough to constitute hyperandrogenaemia. Typical cut-off values of TT for hyperandrogenaemia are generally >2.0 nmol/L (Karakas, 2017; Pasquali et al., 2016) and post-intervention values for all participants in the meta-analysis (n=231) ranged from 2.1-2.5

nmol/L. The results of the meta-analysis may therefore have limited applicability to normo-androgenic phenotypes and differences in treatment responsiveness between phenotypes have not been highlighted.

An important characteristic of the review was to only include trials where OCP was clearly excluded. This was to avoid the contamination of the data by the hormonal and metabolic changes associated with the OCP, particularly those with low or anti-androgenic properties, such as hepatic synthesis of SHBG that reduces free testosterone concentrations (de Melo, Dos Reis, Ferriani & Vieira, 2017). Additionally, in overweight or obese women with PCOS, research suggests that certain types of OCP containing desogestral or cyproterone acetate can aggravate insulin resistance and decrease glucose tolerance (Kilic et al., 2011; Meyer, McGrath & Teede, 2007; Nader, Riad-Gabrial & Saad, 1997). Because of the considerable variability in the presentation of clinical and metabolic symptoms of PCOS, including varying levels of glucose tolerance, hyperandrogenism and insulin sensitivity, as well as the variation in the types and metabolic effects of OCPs used to manage PCOS symptoms, participants taking OCP were excluded to reduce the effects of inter-person variability in the meta-analysis.

PCOS is the most common cause of infertility (Sirmans & Pate, 2013). It is estimated that 40% of women with PCOS are affected by infertility or have difficulty conceiving (Teede, Deeks & Moran, 2010). As a result, approximately up to 95% of anovulatory women seeking or receiving fertility treatment have PCOS (Durstine et al., 2001). Therefore, although OCP may be a front-line management tool in PCOS in women not aiming to conceive (Meyer et al., 2007), there exists a substantial proportion of women with PCOS that are not taking OCP, many of whom are encouraged to improve their health to increase chances of conception, indicating that the findings of this review have applicability to this subset of the population.

3.4.3 Potential Biases in the Review Process and Limitations

The eligibility criteria were restricted to articles published in the English language. Consequently, it is possible that additional information from trials that would have otherwise met the inclusion criteria may have been excluded. Also, trials were only eligible for inclusion if the full-text could be obtained; subsequently at least one eligible trial could not be included because the abstract was a conference paper, and the full-text had not been published. These factors may contribute to publication bias. Due to a lack of trials in the meta-analysis, funnel plots could not be utilised for the analysis of publication bias.

Some difficulty in study selection occurred due to a lack of trials that explicitly excluded the use of OCP and other hormonal or metabolism-altering drugs. The criteria stipulated that studies could only be included if this were specifically excluded, and as such some studies may have been excluded for not providing such a statement. Similarly, at least one gold standard RCT was excluded due to the use of non-normally distributed data and non-parametric tests. These data could have influenced findings if they could be synthesised for meta-analysis and thus had to be excluded.

Many of the outcomes were based on studies with serious limitations, including a high risk of selection bias, and small magnitude effect estimates. This limits the quality of the evidence, despite the directness and consistency of the evidence for most outcomes. As noted, the generalisability may also be limited by the high occurrence of selection bias, and particularly by study designs which allowed participant allocation based on preference rather than true randomisation.

3.4.4 Future Research Recommendations

Most studies featured moderate-intensity aerobic interventions, with less emphasis on resistance training in the literature, therefore different types of exercise intervention could not be compared. Current PA guidelines recommend that adults undertake activity to improve muscle strength on at least two days a week (CMO, 2019). As such, a greater emphasis should be placed on the inclusion of resistance exercises in exercise interventions to identify additional benefits to cardiometabolic health in PCOS. Future consideration could also be given to tools for self-reporting PA, such as the Global Physical Activity Questionnaire, as well as interventional studies.

3.5 Conclusions

The results of the pooled data analysis indicated that moderate aerobic exercise interventions ≥three months in duration, with a frequency of three sessions/week for at least 30-minute-long sessions, may have favourable effects on various cardiometabolic risk factors including TC, fasting blood glucose, WC, WHR, SBP and CRP in women with PCOS. Additionally, the data indicate that if participants have TT and SHBG concentrations outside of normal ranges, this type of intervention could improve androgen profile in comparison to usual care.

As indicated by the analysis of the quality of the evidence, various outcomes were judged to be of a moderate quality, with statistically significant, precise effect estimates. Nonetheless,

results should be interpreted with caution due to the presence of serious methodological limitations including a lack of gold standard RCTs and a high risk of selection bias.

A thorough search of nine databases was conducted from inception to present but were only able to find three eligible studies that isolated the effects of exercise alone versus usual care that explicitly excluded the use of OCP and other hormonal or metabolism-altering drugs. Only one of these was a gold standard RCT, albeit judged to have an unclear risk of selection bias due to unclear randomisation or allocation procedures. This review highlights the limitations of the available literature. More gold standard RCTs that can make direct comparisons between treatment options for PCOS, including exercise, pharmacological, behavioural and dietary interventions could provide greater precision for future recommendations of treatment options, including the efficacy of exercise in comparison to other treatments. However, this may have limited applicability to the general population; often, patients with PCOS may undertake combined interventions to get the best results, and studies designed in this manner may provide greater applicability in that regard.

4 Supervised Exercise Training and Increased Physical Activity to Reduce Cardiovascular Disease Risk in Women with PCOS: A Feasibility Randomised-Controlled Trial

Overview

This chapter reports the methods and results of the feasibility randomised-controlled clinical trial. It describes the procedures for eligibility screening, recruitment, and study procedures, before summarising the findings. Some of the work in this chapter has been published as part of the clinical trial protocol (Woodward et al., 2020).

4.1 Introduction

4.2 Aims and Objectives

Before an adequately powered RCT measuring the effects of exercise and/or increased lifestyle PA on indicators of cardiovascular health can be designed, the feasibility and acceptability of the interventions and procedures for recruitment, allocation, and outcome measurements must be assessed. In addition, the interventions must be refined, and a sample size must be calculated.

Therefore, the specific aim of the present feasibility study is as follows:

1. Assess the feasibility of conducting an RCT of exercise training and increased lifestyle PA in women with PCOS.

The objectives of this study were to:

i) Measure rates of recruitment and retention.
ii) Measure rates of attendance and compliance with the interventions.
iii) Obtain a standard deviation for oxidised LDL so that a sample size for a future, larger-scale RCT can be calculated.
iv) Identify the use of partitioning participant data by androgen profile.

4.3 Methods

4.3.1 Study Design

This feasibility study implemented an exploratory randomised controlled trial (RCT) design. The justification for a feasibility study has been described in detail in Chapter 1. Briefly, before a full-scale trial can be implemented, a programme of development, feasibility testing, and evaluation must first be conducted, to ensure that funds are not wasted and unnecessary burden to participants is avoided. Chapter 3, a systematic review and meta-analysis of PA interventions in PCOS, provided an evidence base on which to develop intervention procedures. It is then appropriate to assess the acceptability and feasibility of the intervention(s) before proceeding. As such, the overall purpose of this study is to assess the feasibility and acceptability of the intervention and procedures and identify issues which may hinder the development of a full-scale trial.

The study took place between September 2018 and March 2020. It was conducted at the Centre for Sport and Exercise Science (CSES), Sheffield Hallam University (SHU), Sheffield, UK.

4.3.2 Recruitment and Sampling

As this was a feasibility study, no formal sample size calculation was required. The sample size for a feasibility needs to strike a balance where it does not cause undue burden on participants by being too large, but not be too small that critical parameters (such as consent rate, attrition and compliance) cannot be precisely estimated and used for the calculation of a full-scale RCT. It has been posited that at least 30 participants overall should be sufficient for a feasibility trial (Lancaster, Dodd & Williamson, 2004).

Additionally, sample sizes between 24 and 50 have been recommended to calculate a standard deviation of an outcome that can then be entered into a formal power calculation for the full-scale RCT (Sim & Lewis, 2012; Whitehead, Julious, Cooper & Campbell, 2016). Measurement of oxidised LDL concentrations are a secondary outcome for the proposed study, and a sample size within this remit will provide a reliable standard deviation for oxidised LDL to be used in a power calculation for the definitive trial. Since there are three groups, a maximum sample size of 51, allowing even numbers across three groups (17 in each group), was proposed and would allow for any drop out.

Participants were mainly recruited from Sheffield Teaching Hospitals, Sheffield, UK. Upon a visit to either the fertility or gynaecology clinic, potential participants were initially screened according to inclusion criteria by a clinical member of the research team via a computerised search of their notes. If participants passed the initial screening, they were asked if they were happy to be approached by the researcher. Following agreement, a brief discussion about the trial was conducted and potential interested participants were provided with an information pack, including an invitation letter and participant information brochure. Email and contact information for the researcher was included in the packs, and potential participants were given a maximum of 14 days to respond. It was clearly stated in the invitation letter that there was no obligation or pressure to participate in the study and that if patients did not wish to participate, their future medical care would not be jeopardised. Patients who were happy to be contacted were asked to provide an email address or telephone number. Volunteers wishing to participate in the study were requested to contact the principal researcher. If no contact was made, the principal researcher contacted patients who had left contact details a maximum of two times.

Potential participants who declined to volunteer, or who did not respond to the follow-up contact attempts, were not contacted further, since it was assumed that they did not wish to participate in the study.

In addition, participants were recruited through several other social media platforms, including various Facebook groups for PCOS support locally. The public announcement(s) that were posted on social media can be found in Appendix 4. The procedure for those responding to the announcement involved sending through an information pack by email. After this, the procedure was the same; that is, potential participants were asked to contact the researcher, or were followed-up by the researcher if they had left contact details.

Volunteers who responded to the letter of invitation and satisfied triage through pre-screening were invited to the physiology laboratory at CSES, Sheffield Hallam University Collegiate Campus, for their initial session, where they provided written informed consent and had an opportunity to familiarise themselves with the protocol and ask questions. They then undertook baseline assessments.

4.3.3 Eligibility Criteria

The inclusion criteria for the sample were as follows:

i) Women diagnosed with PCOS according to the Rotterdam Criteria 2003, National Institute of Health (NIH) 1990 criteria or Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) Society 2006 criteria. In the case of participants recruited from outside of STH, a self-reported diagnosis was deemed sufficient.

ii) Have experienced menarche (their first menstrual bleeding) and be at least 18 years of age.

iii) Were English speaking.

iv) Were physically able to perform exercise.

Exclusion criteria were as follows:

i) Post-menopausal status.

ii) Smokers.

iii) Undertaking regular structured exercise defined as >150min/week.

iv) If taking metformin, have been taking it for <3 months.

v) Were taking the oral contraceptive pill (OCP) or have taken in the last month.

vi) Have any medical condition that may be responsible for the symptoms of PCOS, such as congenital hyperplasia, androgen-secreting tumour, hyperprolactinemia, or Cushing's syndrome.

vii) Have current, clinically defined CVD or a history of cardiac events.

Both metformin and the OCP are included in the exclusion criteria because both have been shown to affect androgen concentrations and glucose metabolism in PCOS, which may affect results (Diamantis-Kandarakis, 2010; Meyer et al., 2007). On the advice of the consultant clinician, it was deemed acceptable for participants to have been taking metformin for at least three months, because changes to glucose metabolism are more stable after this period. Participants were asked to let the researcher know as soon as possible after beginning such medication. They were advised that commencement of any of the above-mentioned medications during the trial is a contraindication and they would be withdrawn from the trial.

4.3.4 Baseline and Post-Intervention Measurements

Participants were asked to abstain from alcohol and vigorous exercise for 24 hours before attending their assessments. In addition, participants were asked to abstain from eating for at least two hours prior. This was to ensure accurate fasting values for several analytes.

During visit one, after written informed consent was obtained and following the confirmation of eligibility by the researcher, the following baseline tests measurements were undertaken: age, anthropometric measures (stature, body mass, hip and waist measurements), capillary and venous blood sampling (see outcome measures for a detailed description of analytes), aerobic fitness assessed by the Astrand-Rhyming (Astrand & Rhyming, 1954) single stage test (see outcome measures for detailed description).

Within two weeks after completion of the 12-week intervention, all tests and measurements were repeated.

4.3.5 Randomisation and Masking

Participants were randomised, using block randomisation for equal numbers (Suresh, 2011), between a supervised exercise programme, a lifestyle PA group and a control group. This was undertaken using a computerised randomisation programme (QuickCalcs, GraphPad Software, USA). Allocation was concealed and placed in sequentially labelled opaque envelopes by somebody outside of the research team to reduce bias (Dettori, 2010). Each envelope was offered to the participants, in sequence, by the researcher, on completion of their baseline assessments. It was not possible to mask participants or the research team to the allocated intervention.

4.3.6 Withdrawals

Participants were informed that they could withdraw at any time without providing a reason and could withdraw their data from the study. A participant was withdrawn if they requested to leave the trial, or they were lost to follow-up (defined as no response following two attempts at contact by email and/or telephone). If allocated to the exercise group, participants were considered as withdrawn if they no longer attended the supervised exercise sessions, and in the lifestyle PA group, if they no longer reported daily physical activity from fitness application. To preserve randomisation and produce unbiased estimates, intention-to-treat analysis was utilised, whereby participants' data was still included in the analysis, unless

specifically requested not to, even if they had withdrawn (McCoy, 2017). Missing outcome data were dealt with by using the last observation carried forward (LOCF) method, where the baseline measurement is retained and carried forward to be used in the analysis (Gupta, 2011; Streiner & Geddes, 2001). This approach minimises the number of participants excluded from the analysis but does introduce heterogeneity into the results (due to mixing of compliant and non-compliant participant data) (Streiner & Geddes, 2001).

4.3.7 Harms and Auditing

Adverse events were collected, reported, and assessed by the research team to determine severity and whether they were likely to be due to the trial. Serious adverse events and reactions were to be reported to the relevant ethics committees, and appropriate action taken, if any. Auditing was carried out by the sponsor, Sheffield Hallam University. In the event of harm to participants arising as a result of the management, design or conduct of the research, it was agreed that Sheffield Hallam University insurance and indemnity policies applied.

4.3.8 Supervised Exercise Programme

Participants assigned to the exercise group were invited to undertake two sessions of supervised exercise training each week for eight consecutive weeks and three sessions of supervised exercise training each week for the final four consecutive weeks at CSES fitness suite at Sheffield Hallam University Collegiate Campus. Each session lasted approximately 60 minutes and involved 40 minutes of an individualised exercise protocol performed either on a cycle ergometer, elliptical trainer, rowing ergometer, or a motorized treadmill, preceded by a 10-minute warm-up and followed by a 10-minute cool down.

The duration and intensity of the programme was selected based on evidence from exercise trials in PCOS that have identified that supervised, moderate-intensity exercise sessions between 50-70% VO_2 max, for a minimum of 12-weeks, showed improvement in cardiometabolic risk factors such as TG, inflammation and insulin resistance (Harrison et al., 2011; Hutchison et al., 2011; Giallauria et al., 2008; Vigorito et al., 2007).

Most of these studies involved three sessions per week (Hutchison et al., 2011; Giallauria et al., 2008; Vigorito et al., 2007). However, to maximise adherence and reduce inconvenience to participants, the current trial comprised two sessions per week, at a duration of one hour, increasing to three sessions per week for the final four weeks. Various research studies have

shown that two sessions per week for eight weeks can elicit improvements in microvascular endothelial function and exercise tolerance, both of which can reduce CVD risk (Klonizakis et al., 2013; Klonizakis et al., 2009), which is regarded as a key outcome in the current study. Additionally, the American College of Sports Medicine (ACSM) recommends a weekly duration of 60-150 minutes of exercise duration for inactive individuals or individuals who do not participate in any habitual PA (ACSM, 2010).

ACSM's Frequency, Intensity, Time and Type (FITT) principle recommends increasing one variable of the principle after at least one month of exercise. Extremely deconditioned to moderately deconditioned individuals are recommended to work at a moderate intensity of ~57-74% $HR_{max,}$ which correlates with previous exercise interventions in PCOS where workload is set to a VO_2 max of 50-70% (ACSM, 2010). The programme therefore increased in intensity at increments of four weeks up to 74%HR_{max}, allowing for progression while remaining moderate in effort and within the recommendations for moderately deconditioned individuals.

In order to calculate individual heart rate zones for each participant, a formula (206.9 - (0.67*age)) was used to calculate maximum heart rate (ACSM, 2010). Participants wore a Polar T31 heart rate monitor chest strap and wristwatch for the duration of each session in order to monitor their heart rate and stay in the assigned zones.

4.3.9 Lifestyle Physical Activity Group

Participants randomised to the lifestyle PA group attended CSES at Sheffield Hallam University for all the tests and measurements as in the exercise group but did not take part in the structured exercise intervention. However, advice and information on how to increase PA was provided using British Heart Foundation guidelines: 'Understanding Physical Activity' (BHF, 2018). This was discussed during the informed consent consultation. Participants were asked to monitor and track their daily PA using a smartphone fitness application. This was either Google Fit or Apple Health, since these are the default fitness apps for Android and Apple phones and are often pre-installed. Participants sent their data each week, by email. Data included daily energy expenditure, step count, and distance travelled by foot in km. Between the baseline measurements and those after 12 weeks, participants received regular email support, approximately once a week, to obtain information about their progress and provide advice as needed.

Participants in the control group did not undertake any intervention but still received standard care from their medical professional. To improve follow-up for the control group, all participants allocated to the control group were offered the opportunity to undertake supervised exercise sessions at the CSES after their successful completion of the trial.

4.3.10 Participant Timeline

The Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) diagram shown in Figure 19 outlines the study time-points.

	STUDY PERIOD													
	Enrolment	Allocation	Post-allocation											Close-out
TIMEPOINT	*-1 wk*	0	*1 wk*	*2 wk*	*3 wk*	*4 wk*	*5 wk*	*6 wk*	*7 wk*	*8 wk*	*9 wk*	*10 wk*	*11 wk*	*12 wk*	*+2 wk*
ENROLMENT:															
Eligibility screen	X														
Informed consent		X													
Allocation		X													
INTERVENTIONS:															
Exercise Group			♦	—	—	—	—	—	—	—	—	—	—	—	♦
Lifestyle Physical Activity Group			♦	—	—	—	—	—	—	—	—	—	—	—	♦
Control Group]			♦	—	—	—	—	—	—	—	—	—	—	—	♦
ASSESSMENTS:															
Aerobic fitness, oxidised LDL, blood lipids, testosterone, glucose, SHBG		X													X
Interview															X

Figure 19. Participant schedule of enrolment, interventions, and assessments.

4.3.11 Blood Sampling and Storage

Blood was drawn from participants on their initial and post-intervention visit to the CSES, by the principal researcher who was trained in venepuncture. Blood was drawn from the median

cubital vein, median cephalic vein, or from the dorsal superficial veins of the hand. If an attempt to draw blood was unsuccessful, one further attempt was conducted. If this was still unsuccessful, no sample was retrieved. Blood samples were collected into BD Vacutainer plastic serum tubes, inverted 5-6 times, and left to clot upright at room temperature for thirty minutes. Samples were then centrifuged in a Heraeus Labofuge 400 at 1300 x g (Relative Centrifugal Force) for ten minutes at 18-25ºC. Serum was aliquoted and stored at -80ºC until analysis.

Assays were performed at the Biomolecular Research Centre, Sheffield Hallam University, by the researcher.

4.3.12 Outcome Measures

4.3.12.1 Feasibility Outcomes

The primary outcomes for this study are acceptability and feasibility of procedures for recruitment, allocation, measurement, and retention for the intervention procedures. Recruitment rate was calculated by dividing the number of women eligible and consenting by the recruitment period. Attrition rates were established as discontinuation of the intervention and loss to follow-up measurement for both conditions. Compliance was monitored by session attendance and monitoring the data from recorded daily PA, with examination of reasons for drop-out or non-compliance. Reasons for drop-out were also used to assess the suitability of allocation procedures. Suitability of measurement procedures were evaluated by completion rates and reasons for missing data. Safety of the exercise intervention were assessed by exploring reasons for dropout, and the number and type of adverse events that occur in each group.

4.3.12.2 Secondary Outcomes

Serum oxidised LDL is the secondary outcome of the current study and was analysed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Mercodia, Sweden). This assay is a solid-phase assay based on the direct sandwich technique. Two monoclonal antibodies (capture and detection) are directed against distinct epitopes of the antigen. Initially the sample is incubated with the capture antibody bound to the microtitration well, followed by a washing step with phosphate-buffered saline (PBS) to remove unbound components. Then, the detection antibody, which is conjugated to an

enzyme (peroxidase), is added to the wells. After incubation and washing to remove unbound enzyme components, the enzyme substrate (TMB) is added to detect the bound conjugate. The reaction is stopped by adding sulfuric acid to give a colorimetric endpoint which is measured by spectrophotometry.

ELISA was also used for quantitative analysis of c-reactive protein (Invitrogen, USA), neopterin (IBL International, Germany), fasting insulin (Invitrogen, USA) and sex hormone binding globulin (SHBG) (Abcam, UK). Each assay other than neopterin was based on the sandwich technique described above. Neopterin, which is produced by macrophages and indicative of pro-inflammatory immune status, was assayed using the principle of a competitive ELISA. In this assay, the antigen in the sample competes for antibody binding sites with a reference antigen that is pre-coated on the microtitration plate. Depending on the amount of antigen in the sample, more or fewer free antibodies will be available to bind to the reference antigen. Thus, the colorimetric signal is produced by the labelled reference antigen, and the lower the amount of antigen in the sample, the stronger the signal will be due to higher amounts of reference antigen bound to the antibody.

Thiobarbituric acid reactive substances (TBARS) was also measured as a secondary outcome. Lipid peroxides created by oxidising agents that alter lipid structure result in the formation of malondialdehyde (MDA), which is thought to reflect the extent of lipid peroxidation (Parthasarathy et al., 2012). MDA, in the present of heat and acid, reacts with thiobarbituric acid to produce a coloured end product that can be quantified using a plate reader. TBARS was measured by commercially available assay (R&D Systems, USA).

The optical density for each assay was measured using an electronic plate reader, with the wavelength set according to manufacturers' recommendations, and was used to determine the quantity of the antigen in the serum samples using a calibration curve of known concentrations. The calibration curve was plotted using Prism (GraphPad Software, USA). Following manufacturers recommendation, an appropriate regression model was chosen based on the linearity of the data and used to interpolate unknown concentrations. R^2 values were checked to ensure the good fit of the model. For each assay where quality controls were provided, the concentration obtained was observed to be within the acceptable detection range as specified by the manufacturer.

It was proposed that free testosterone be measured using a liquid chromatography method. This method was chosen due to its ability to detect small amounts of serum testosterone,

particularly in lower concentrations in females (Gallagher, Owen & Keevil, 2007). However, due to Covid-19 restrictions in the laboratory, training for the equipment was unable to take place, and free testosterone could not be measured.

Aerobic fitness was assessed using the Astrand-Rhyming test (Astrand & Rhyming, 1954). This is a submaximal single-stage test performed on a cycle ergometer, lasting between 6-7 minutes. The goal is to obtain heart-rate values between 125 and 170 beats per minute (bpm) for a given work-rate at 50 revs/minute (50 or 75 watts for unconditioned women). Heart rate is then measured at the fifth and sixth minute if steady state (a difference $\leq$ 5 bpm is achieved), and the average of the two heart-rate measurements can be used to estimate VO_2 max according to a nomogram. This value must be adjusted for age to account for decreasing maximal oxygen uptake and maximal heart rate with age (Astrand & Rhyming, 1954).

For lipid profile (including LDL-cholesterol, HDL-cholesterol, and TC) and fasting glucose, a CardioChek PA Blood Analyser was used. A capillary blood sample was obtained and collected into a capillary tube. The sample was then inserted into the machine cassette. This produces an automated reading from the sample in which accuracy can be maintained by using control samples and calibrations.

In order to measure the amount of lifestyle PA (and subsequent sedentary activity), the long-form International Physical Activity Questionnaire (IPAQ) was administered at baseline and post-intervention to all participants. This was used to compare differences in time spent sitting, both during the week and weekend, and the amount of lifestyle PA (including transport, housework, and leisure time) undertaken. Scores were calculated using the guidelines for data processing and analysis of the IPAQ long form (IPAQ, 2005). A score of total MET-minutes per week was calculated, as well as total sitting time per week. METs (metabolic equivalents) are multiples of the resting metabolic rate (indicating energy output required to complete a task), and a MET-minute is thus computed by multiplying the MET score of an activity by the duration (in minutes) that it was performed (IPAQ, 2004).

Waist circumference was measured with the participant standing with feet together, and the tape measure placed around the narrowest part of the torso, between the umbilicus and the xyphoid process (ACSM, 2010). Hip circumference was measured with the participant standing as above, and the tape measure placed around the maximum circumference of the buttocks (ACSM, 2010).

4.3.13 Data Collection, Monitoring, Management and Storage

Data were collected by the principal researcher using anonymised data collection forms (Appendix 4) and entered into a computerised database. Range checks were carried out to ensure data quality. Data checks were completed by other members of the research team.

Participants' names were anonymised and replaced with a code using a computerised pseudonymisation programme (Open Pseudonymiser, University of Nottingham, UK). All other study data was stored securely on Sheffield Hallam University premises and/or saved on encrypted computer drives on site. Only the research team had access to the data, including the final trial dataset, and only the researcher had access to the original pseudonym data. Data will be securely archived and stored for up to seven years, when it will be destroyed. All data will be stored and managed according to Sheffield Hallam University's confidentiality and data protection policies.

Due to the low risk involved in this study, no formal data monitoring committee was formed. However, the study was monitored by the research team members being led by a senior team member (MK) who met at regular intervals throughout the study period.

4.3.14 Interview and Qualitative Methods

Interviews were conducted with participants after completion of the trial to form the qualitative evaluative component of this study. The methods and findings for this study are described in Chapter 5.

4.3.15 Data Analysis and Handling

All quantitative measurements are presented as mean ± standard deviation (SD) unless otherwise stated.

Descriptive statistics were used to characterise the groups at baseline and post-intervention. Pre- and post-intervention means were recorded for each group. An exploratory analysis of the secondary outcome, oxidised LDL, was performed using a two-factor mixed-ANOVA. The effect of two independent variables (grouping and time point) was measured on the dependent variable. The within-subjects factor is time, and the between-subjects factor is group. Data were assessed to ensure the assumptions of the mixed-ANOVA were met

including checks for normality. However, this trial is not powered to detect differences in the outcomes, and as such this was just a preliminary analysis.

For biochemical analysis, each sample was measured in duplicate and the mean of the two wells was used as the final value. Intra-assay coefficients of variability (CVs) were calculated based on concentrations of each pair of duplicates, and these reflect the consistency and performance of the assay in the hands of the user. In addition, the number of samples necessitated the use of two plates for each assay. Thus, the inter-assay CV was also calculated using the known controls for each plate to check consistency between assays of the same antigen.

All analyses were interpreted with a caveat that the study is a feasibility trial without a formal sample size calculation and as such may be inadequately powered. All statistical analysis was undertaken using the latest IBM SPSS Statistics software which is currently version 26.0.

4.3.16 Criteria for Success

The feasibility trial was assessed against acceptability criteria as follows:

i) Sufficient oxidised LDL data is obtained to allow for a formal sample size calculation based on standard deviation of the primary variable.
ii) Adherence to the exercise intervention is at least 74% (defined as at least 74% of scheduled sessions taking place). This figure has been chosen because it reflects a mean adherence level for supervised exercise interventions for people with chronic conditions, including CVD and diabetes (Bullard et al., 2019).
iii) Loss to follow-up at 12-weeks is <20%.
iv) There are no serious adverse events (SAE) resulting from the trial procedures.
v) There are no significant difficulties for the researcher in administering the measurement procedures or the intervention, measured by missing outcome data.

This criteria for success formed the basis of the interpretation of this trial and determined whether a full-scale RCT is feasible. Furthermore, they will determine what modifications, if any, should be made to the procedures and intervention before proceeding.

4.3.17 Ethical Considerations

Health Research Authority (HRA) approval for the study, including the qualitative component, was obtained and Research Ethics Committee (REC) favourable opinion granted by the North West – Greater Manchester East REC on 19th July 2018, reference 18/NW/0454. This is presented in Appendix 3.

4.4 Results

4.4.1 Summary

Figure 20 shows the flow of participants through the trial. Recruitment took place from October 2018 to January 2020. Follow-up data collection was completed by March 2020; however, due to COVID-19 restrictions and the obligatory early trial termination, two participants were unable to attend the laboratory to complete the follow-up assessments. Table 9 presents a summary of the feasibility and acceptability findings.

4.4.2 Screening, Eligibility, and Recruitment

Table 9 presents a summary of feasibility and acceptability data. Of 78 volunteers screened for participation, 64 met the eligibility criteria and 36 were recruited. This gives eligibility and recruitment rates of 82% and 56%, respectively. The recruitment rate over time is 2.25 participants per month. Reasons for non-consent and exclusion are shown in Figure 20.

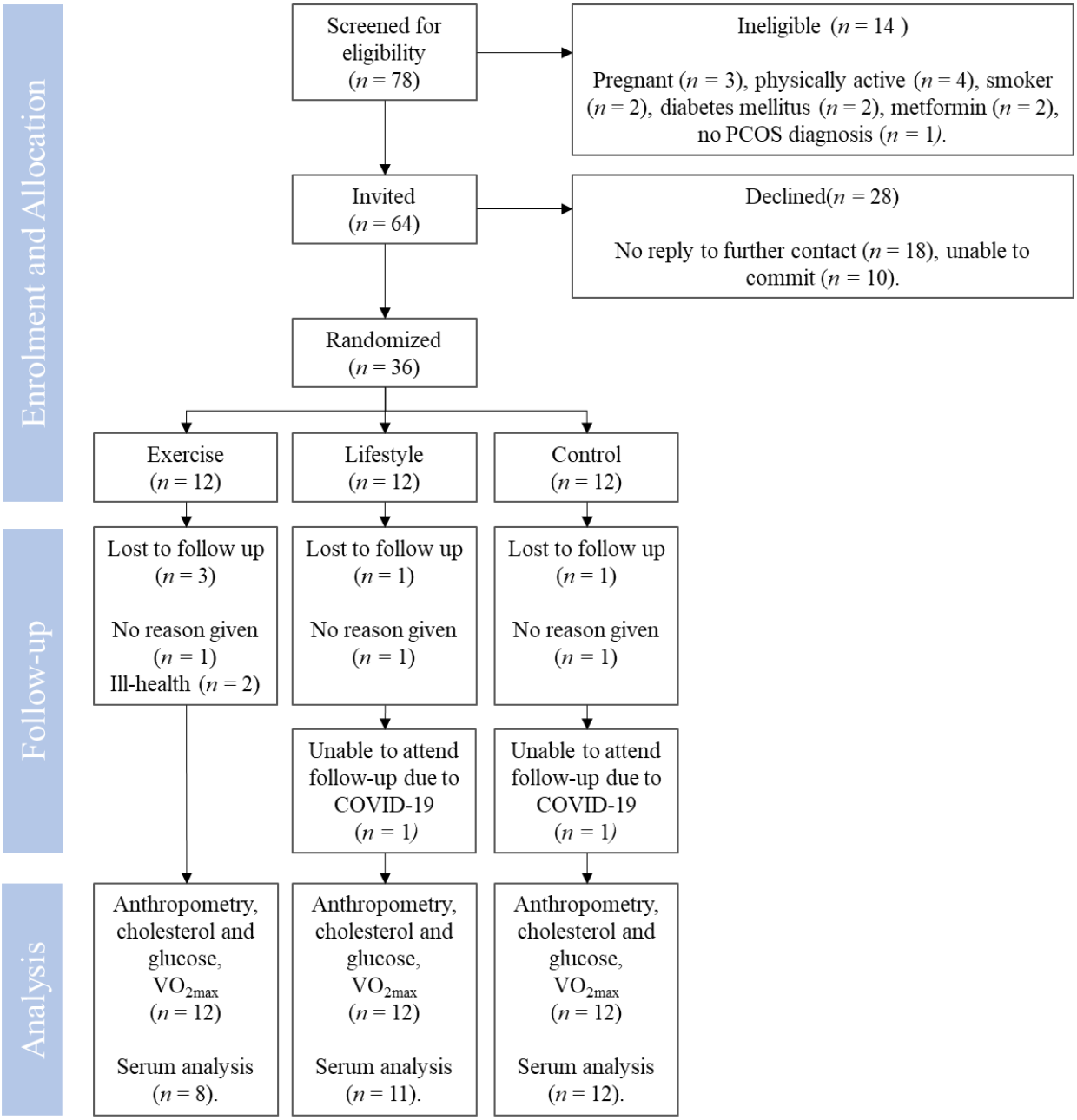

Figure 20. Flow of participants through the trial.

Table 9. Summary of Feasibility and Acceptability Findings.

Methodological Issues	**Findings**	**Evidence**
What proportion of those screened were eligible?	Over 80% of volunteers screened were eligible.	64/78 volunteers were eligible (82%).
What factors influenced eligibility?	Pregnancy, and current level of physical activity, were the most common reasons for ineligibility.	Four volunteers already met the minimum recommended levels of PA, and three volunteers were pregnant.
Was recruitment successful?	Recruitment exceeded the minimum number of participants needed for a feasibility sample.	Thirty-six participants were recruited over 16 months (2.25 participants/month).
Were eligible volunteers recruited?	Over half of the eligible volunteers were recruited into the study.	Of 64 eligible volunteers, 36 were enrolled into the study (56%).
Were participants successfully randomised and did randomisation yield equality in groups?	The randomisation process was successful in generating appropriate groups for the study.	The block randomisation procedure yielded equally sized groups. Baseline characteristics were approximately even across groups, although VO_2 max was higher in the LPAG at baseline.
Did participants adhere to the intervention(s)?	Adherence to the exercise intervention was below the acceptable limits set out in the criteria. Adherence to the lifestyle intervention was 100%.	There were no missing data for the lifestyle intervention, indicating high adherence. In the exercise group, 152/285 of the scheduled exercise sessions (53%) were completed.
What was the retention rate?	Retention rate was above the acceptable limit set out in the criteria.	Retention rate was 89%.
What influenced the attrition rate?	Most common reason for attrition was ill-health which was not attributable to the trial procedures.	Withdrawal was attributed to ill-health in 2/5 (40%) participants lost to follow-up.
Was the intervention acceptable to participants?	Quantitative data indicates that some changes may make the intervention more acceptable.	Moderate adherence rates suggest intervention could be refined to increase acceptability, although retention rate was high.

Was the intervention safe?	Safety data was favourable.	Two nonserious AEs (unrelated ankle pain and unrelated back pain) were noted during the study; no exercise sessions were affected.
Were outcome assessments completed?	Outcome completion rates were high for most variables.	Difficulty retrieving blood samples affected the outcome measurement completion. COVID-19 restrictions led to missing follow-up data for two participants, although they were not withdrawn.
Did all components of the protocol work together?	No procedural or methodological issues were identified when undertaking the protocol.	There were no difficulties identified in the procedures and the researcher's ability to implement them.
Was enough data collected on the secondary outcome to propose a sample size for a full-scale RCT?	Yes.	Thirty-one observations were obtained.

4.4.3 Retention

The retention rate was 89%, which was above the acceptable criterion of 80%. Five of the 36 participants formally left the study; three from the exercise group (two for ill-health, and one with no reason given), one from the LPAG (no reason given), and one from the control group (no reason given). Twenty-nine participants completed all baseline and follow-up sessions and measurements. However, due to COVID-19 restrictions in March 2020, two participants who were due to return for their follow-up visit (one from the LPAG, one from the control) were unable to visit due to the obligatory lab closure. Thus, their follow-up assessment could not be completed, although they were not considered to be withdrawn from the study and have not been included in the attrition statistics.

4.4.4 Exercise Attendance and Safety Data

Overall attendance to the exercise sessions was below the acceptable limit of 74%. A total of 152/285 sessions were completed (53%). This indicates that the exercise intervention must be refined to increase adherence. Sessions were commonly rearranged or cancelled due to other commitments, although flexible timing was offered. Reasons for this are explored in more depth using qualitative data in Chapter 5.

Two nonserious AEs were observed during the study, both from participants in the exercise group. These were back and ankle pain, determined after investigation to be unrelated to the exercise sessions as part of the trial. Actions taken included recommending that participants use non-weight bearing exercise equipment such as the cycle ergometer rather than the treadmill, and self-monitoring. No exercise sessions were postponed or affected by the AEs. No SAEs were reported.

Participants in the control group were offered the chance to take up the exercise intervention upon completion of their follow-up visit. Only two of 12 participants accepted this offer.

4.4.5 Lifestyle Physical Activity Group Engagement

While participants reported and sent their lifestyle data to the researcher each week, those data are not presented here. The primary reason for this is because as a feasibility trial, the purpose is not to test the efficacy of the interventions. Rather, it was to assess whether this

type of intervention was acceptable to participants and whether they would engage with the procedures. In addition, there were no specific goals or targets given to participants, they had only to attempt to increase their own baseline of PA, which differed between individuals. Thus, presenting means or ranges would have provided little descriptive value due to high heterogeneity of the data.

There were no missing lifestyle data for participants in the LPAG; that is, each participant sent their data each week. This indicates high engagement with the protocol and is a promising basis for a future trial. However, without statistical analysis of the data to track significant changes to PA levels and the outcome variables, the success of the apps in increasing lifestyle PA and improving health cannot yet be judged. This would be the focus of a fully powered RCT.

4.4.6 Outcome Measurements

Assessment of anthropometry, glucose, and cholesterol by capillary sample, VO_2 max, and administration of the IPAQ was successful for all participants at baseline and all participants who attended follow-up. This indicates no issues or difficulties with the procedures for outcome measurements. However, in several participants, at both baseline and follow-up, a blood sample could not be retrieved within two attempts. As such there are some missing biochemical variables. The researcher was trained and assessed as competent in venepuncture. However, for future studies, an alternative researcher trained in venepuncture could be made available for follow-up attempts at a blood draw.

4.4.7 Baseline Characteristics

Table 10. Summary of Baseline Characteristics.

Baseline Characteristics	Exercise (*n*=12)	LPAG (*n*=12)	Control (*n*=12)
Age (years)	29.7 ± 8.6	29.8 ± 5.8	31.5 ± 5.5
Height (cm)	164.9 ± 6.3	164.1 ± 4.8	163.2 ± 4.9
Weight (kg)	97.8 ± 25.6	94.7 ± 23.3	86.2 ± 22.5
BMI (kg/m^2)	35.8 ± 8.0	35.1 ± 8.5	32.1 ± 7.3
WC (cm)	103.3 ± 17.7	101.2 ± 18.9	96.7 ± 20.2
HC (cm)	123.8 ± 16.5	119.6 ± 15.3	117.4 ± 16.8
VO_2 max (ml/kg/min)	24.0 ± 8.8	33.4 ± 13.5	29.1 ± 13.1
IPAQ Total MET-Min/Week	3990(1654)	3188(2981)	2163(2010)
IPAQ Total Sitting Min/Week	3060(1253)	2565(1590)	2070(1530)

All data are presented as mean ± SD except IPAQ which are presented as median (IQR). HC; hip circumference, WC; waist circumference, IPAQ; International Physical Activity Questionnaire, MET; metabolic equivalents.

Table 10 summarises the baseline characteristics. Across groups, randomisation yielded comparable baseline characteristics. VO_2 max was higher in the LPAG (33.4 ± 13.6) than in the exercise group (23.9 ± 8.9). However, the wide SD indicates that this may not be a statistically significant difference. In addition, the IPAQ total MET-min/week score indicates that the exercise group were more physically active at baseline than the control group. This could introduce some bias to the results, because participants may have been more likely to participate in PA outside of the trial.

Figure 21 indicates participants' PA level at baseline according to the IPAQ scoring protocol. The categories include inactive (not meeting the criteria for the other categories), minimally active (five or more days per week of moderate PA or walking for at least 30 minutes; or three or more days of vigorous PA per week for at least 20 minutes; or five or more days per week of any combination of walking, moderate, or vigorous PA, achieving at least 600 MET-min/week), or health-enhancing PA (HEPA) (three or more days of vigorous activity per week achieving at least 1500 MET-min/week; or seven or more days per week of any combination of walking, moderate PA, or vigorous PA, achieving at least 3000 MET-min/week).

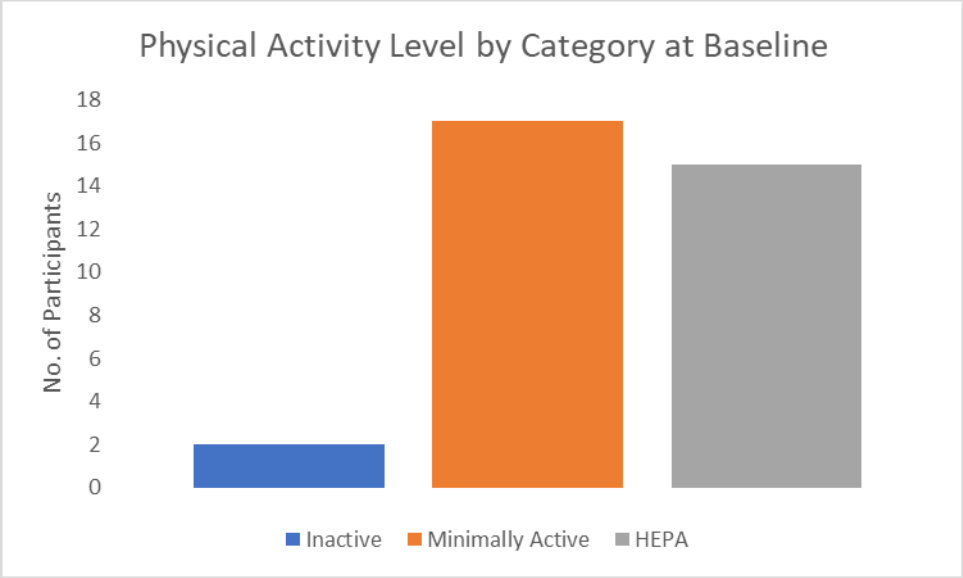

Figure 21. Column chart to show classification of participants' physical activity level at baseline.

Two participants were classified as 'inactive', 17 participants were classified as 'minimally active', and 15 participants were classified as 'HEPA'. This indicates that although the eligibility criteria required participants to be undertaking <150 min/week of structured exercise, many participants were still undertaking PA as part of their daily lives (such as for transport, work, or leisure).

4.4.8 Anthropometry, Capillary Sample, Physical Fitness

Table 11. Summary of Pre and Post Values for Anthropometry, Capillary Sample, and Physical Fitness Measurements.

Measurement	Exercise		LPAG		Control	
	Pre	Post	Pre	Post	Pre	Post
Weight (kg)	97.8 ± 25.6	94.5 ± 28.8	94.7 ± 23.3	91.3 ± 21.4	86.2 ± 22.5	89.4 ± 22.1
WC (cm)	103.3 ± 17.7	97.2 ± 17.1	101.2 ± 18.9	99.7 ± 20.3	96.7 ± 20.2	98.0 ± 19.6
HC (cm)	123.8 ± 16.5	121.1 ± 17.0	119.6 ± 15.3	116.8 ± 13.7	117.4 ± 16.8	119.6 ± 15.8
WHR	0.83 ± 0.7	0.80 ±0.7	0.84 ± 0.1	0.85 ± 0.1	0.82 ± 0.8	0.81 ± 0.8
TC (mmol/L)	4.8 ± 1.0	5.0 ± 1.0	4.8 ± 0.8	4.8 ± 0.5	4.7 ± 0.8	4.2 ± 0.8
HDL (mmol/L)	1.4 ± 0.4	1.5 ± 0.4	1.2 ± 0.4	1.0 ± 0.3	1.3 ± 0.5	1.2 ± 0.5
Glucose (mmol/L)	5.1 ± 0.8	4.9 ± 0.9	4.9 ± 0.6	5.4 ± 1.4	5.1 ± 1.1	5.5 ± 2.0
TC/HDL ratio	3.5 ± 1.1	3.4 ± 0.8	4.8 ± 2.3	5.0 ± 1.5	3.9 ± 1.3	3.7 ± 1.1
VO_2 max (ml/kg/min)	24.0 ± 8.8	33.6 ± 13.6	33.4 ± 13.5	34.8 ± 12.2	29.1 ± 13.1	32.9 ± 13.5
IPAQ Total MET-Min/Week	3990 (1654)	4460 (5459)	3188 (2981)	2760 (2743)	2163 (2010)	3138 (3019)
IPAQ Total Sitting Min/Week	3060 (1253)	2040 (660)	2565 (1590)	2520 (1365)	2070 (1530)	2100 (2205)

All data are presented as mean ± SD except IPAQ which are presented as median (IQR). HC; hip circumference, WC; waist circumference, TC; total cholesterol, HDL; high-density lipoprotein, TC/HDL; total cholesterol/high-density lipoprotein.

Table 11 shows pre- and post-values for anthropometry, capillary sample, and physical fitness measurements. Data indicate weight loss (kg) in both the exercise group and the LPAG (3.4% and 3.6% reduction in kg, respectively). This was observed in both WC and HC. This was not observed in the control group. The data also indicate that the biggest improvements in VO_2 max were observed in the exercise group (40% increase from baseline). However, a lesser improvement was also observed in the control group (13% increase from baseline).

In the exercise group, the data indicate improvements in HDL, fasting glucose, and TC/HDL, which were not observed in the LPAG. In the control group, improvements in TC and TC/HDL ratio were noted.

As expected, IPAQ data also indicates that in the exercise group, total MET-mins/week were increased post-intervention by 12% and sitting minutes per week were reduced by 33%. However, in the control group, total MET-minutes per week increased by 45%, although sitting mins/week remained at similar levels. Scores in the LPAG remained largely unchanged.

4.4.9 Biochemical Results

Table 12. Summary of baseline and follow-up values of biochemical analysis.

	Exercise		**LPAG**		**Control**	
Analyte	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
OxLDL (U/L)	95.43 ± 32.86	82.15 ± 20.38	95.78 ± 37.05	93.75 ± 17.13	106.68 ± 25.92	95.78 ± 27.03
CRP (mg/L)	1.35 ± 0.91	1.72 ± 1.79	0.70 ± 0.84	0.57 ± 0.9	0.70 ± 0.95	0.60 ± 0.45
SHBG (nmol/L)	67.74 ± 27.76	60.35 ± 33.37	51.65 ± 30.38	79.56 ± 37.79	70.40 ± 44.89	73.86 ± 51.46
TBARS (μM)	0.47 ± 0.27	0.47 ± 0.11	0.53 ± 0.20	0.87 ± 0.90	0.48 ± 0.29	0.58 ± 0.28
Neopterin (nmol/L)	11.50 ± 1.68	10.38 ± 2.54	9.97 ± 2.45	8.48 ± 3.98	9.15 ± 2.89	16.88 ± 25.19
Insulin (μIU/ml)	33.62 ± 28.28	38.85 ± 24.02	26.68 ± 9.54	51.63 ± 62.81	26.07 ± 24.76	36.24 ± 30.19

All data are presented as mean ± SD. OxLDL; oxidised LDL, CRP; c-reactive protein, SHBG; sex hormone binding globulin, TBARS; thiobarbituric reactive substances.

To calculate biochemical variables, calibration curves were plotted, and the unknown concentration of samples interpolated via application of a mathematical model, according to the linearity of the data and the recommendations of the manufacturer. OxLDL and neopterin values were determined using cubic spline regression. SHBG, insulin, and CRP values were interpolated using 4 parameter logistic regression (4PL). TBARS was interpolated using simple linear regression. R^2 values were assessed to determine the goodness of fit of the curve. All R^2 values were above 0.99 (considered to be a very good fit) other than the two SHBG assays.

All intra-assay CVs were calculated to be below the 10% other than the second SHBG plate (mean CV=18%) and the first neopterin plate (mean CV=21%). This indicates some inconsistency in results between replicates. The inter-assay CV for each plate was determined to be below 15% apart from neopterin, which had an inter-assay CV of 23%. As before, this may indicate some inconsistency in results for neopterin concentrations between plates. A technical error was made while conducting the second CRP assay that involved the addition of reagents to the plate in an incorrect order. It was not possible to re-run this analysis due to budgetary constraints. As such, there were twelve missing values and post-intervention CRP mean and SD is based on only thirteen values across groups.

Table 12 indicates pre- and post- values for biochemical variables. Across groups, randomisation yielded comparable baseline characteristics for most variables, except for CRP which is higher in the exercise group compared to the LPAG and the control group.

The data indicates that the largest improvements in oxLDL were seen in the exercise group with a 14% reduction at follow-up compared to baseline. However, this was also observed, to a lesser extent, in the control group where data indicates a 10% reduction at follow-up compared to baseline.

An exploratory analysis of oxLDL by two-factor mixed ANOVA was performed. There were no outliers in the data, as assessed by inspection of a boxplot and by examination of studentized residuals for values greater than ±3. Concentration was normally distributed, as assessed by Shapiro-Wilk's test ($p > .05$) and visual assessment of a Q-Q Plot. There was homogeneity of variances, as assessed by Levene's test of homogeneity of variance ($p > .05$). There was homogeneity of covariances, as assessed by Box's test of equality of covariance matrices ($p = .878$).

There was no statistically significant interaction between the intervention and time on oxLDL concentration, $F(2, 22) = .093$, $p = .912$, partial $\eta^2 = .008$. There was no statistically significant main effect of time on oxLDL concentration, $F(1, 22) = 3.799$, $p = .064$, partial $\eta 2 = .147$. There was no statistically significant main effect of group on oxLDL concentration, $F(2, 22) = 1.132$, $p = .340$, partial $\eta^2 = .093$.

However, the η^2 values for the main effects of group and time indicate a medium-to-large effect size. As such, this further solidifies the need for a future fully powered trial to elucidate significant differences between groups.

4.5 Discussion

This study explored the feasibility and acceptability of two PA interventions for women with PCOS, encompassing both a supervised exercise intervention and a lifestyle PA intervention.. Based on the criteria for success, the main finding is that study procedures were feasible and acceptable, other than adherence to the exercise intervention. As such, this discussion sets out recommendations and refinements that should be made before progression to a large-scale RCT is possible.

4.5.1 Feasibility

The first criterion for success stipulates that sufficient oxidised LDL data is obtained to allow for a formal sample size calculation using the SD of the variable. In the present study, 31 observations of oxidised LDL were obtained across groups at baseline. Indeed, it has been suggested that samples of 24 and 50 are sufficient calculate a standard deviation of an outcome that can then be entered into a formal power calculation for the full-scale RCT (Sim & Lewis, 2012; Whitehead, Julious, Cooper & Campbell, 2016). In addition, sample sizes of at least 30 are considered to provide an SD that is a sufficiently accurate estimate of a population-SD (Rowntree, 2018). As such, this criterion is fulfilled.

A sample size can thus be calculated using a formula where power (β), alpha level (α), SD (σ), and the desired clinically relevant difference (d) can be inserted. For RCTs with equal sized groups, the following formula can be applied to determine sample size (per each arm) (Florey, 1993; Noordzij et al., 2010):

$$n = 2(\alpha + \beta)^2 \sigma^2 / d^2$$

For this calculation, assuming, per convention, that α is set at 5% and β at 20%, the appropriate Z-scores to be inserted into the equation are 1.96 and 0.842, respectively. Additionally, σ can be inserted as 32.519, which is the SD obtained from 31 oxidised LDL observations. The SD is quite large (mean oxidised LDL across all groups at baseline = 99.27 ± 33.52), indicating that there is a large amount of variability in the sample (and thus, population). Therefore, the desired clinically relevant difference may be quite large. For this calculation, 20 U/L can be inserted as the minimum difference that the sample should be powered to detect. Inserting each value into the formula produces a sample size of 42 participants per group.

The next criterion is that adherence to the exercise intervention is recorded as at least 74%, defined as at least 74% of scheduled sessions taking place. In this study, 53% of scheduled sessions took place. Adherence to an exercise intervention is an important variable that can help to determine the validity of the findings. That is, if there are no statistically significant differences in outcomes after an intervention, but adherence statistics are very low, this may not accurately reflect the efficacy of the intervention (Room, Hannink, Dawes & Barker, 2017). Flexibility was offered to participants when scheduling sessions, but time constraints may have still been an issue; this population is typically working age and may have dependents. The setting for the exercise sessions was the university, which is located close to central Sheffield. For those living or working on the outskirts of the city, this may have presented a logistical issue based on traffic, availability of public transport, and time to travel after work. Although multiple venues may assuage this issue to some degree, this presents further challenges in terms of resources and qualified personnel to deliver the protocol (Klonizakis et al., 2018). However, this could be resolved through the integration of the proposed intervention in a centralised "exercise referral" scheme, with special training of the exercise facilitators. Exploration of factors affecting adherence is presented in Chapter 5.

Several reviews have been conducted to investigate factors that can improve adherence to exercise interventions (Aitken, Buchbinder, Jones & Winzenberg, 2015; Room et al., 2017). Successful adherence-enhancing components incorporate various behaviour change techniques such as self-monitoring, reinforcement, goal setting, and feedback (Aitken et al., 2015; Room et al., 2017). In this study, although the exercise intervention ramped in intensity every four weeks, there were no specific fitness goals or achievements to work toward. To incorporate adherence-enhancing components, a future trial could consider a pre-intervention goal-setting session in which the trainer and the participant outline some specific fitness goals

for the participant, and a realistic plan for how to achieve them. This would allow regular feedback and monitoring of progress against the goal and may provide motivation and a sense of satisfaction upon achievement, or when interim goals are achieved before achievement of the end goal (Aitken et al., 2015).

Finally, it may be useful in future trials, where participants are offered a choice of exercise equipment, to record which equipment is used most frequently. This may provide valuable information on preferences, and as such, future studies can be more economical by providing equipment that has shown to be favourable previously. This may also impact upon enjoyment and adherence.

The criteria for success stipulated that loss to follow-up at 12 weeks should be <20%. In this study, retention rate was 89%, giving a withdrawal rate of 11%. Of the five participants that withdrew, three participants gave no reasons, and two cited ill-health. Three withdrew from the exercise group compared to one in each of the other two groups. Two of three in the exercise group cited ill-health. This could indicate that illness is a barrier to participation not experienced in the LPAG, because participants may have felt well enough to attend follow-up or take part in gentle PA, but not able to participate in structured exercise.

Analysis of AEs indicates only two, unrelated, nonserious AEs, both in the exercise group, which had no impact upon attendance or performance in the exercise sessions. The intervention and procedures are hence considered to be safe, and the loss to follow-up was low. This suggests a high intention of participation from participants.

Analysis of reasons for missing data and completion of outcome measurements indicate that there were no significant problems with the delivery of measurement procedures other than obtaining venous blood samples. The researcher was trained and assessed as competent in venepuncture. However, in some participants, it was not possible to retrieve a sample within two attempts. The WHO recommend a maximum of two attempts per practitioner; if venepuncture is still unsuccessful, it is suggested to seek a second opinion (WHO, 2010). Thus, it is recommended for future trials that a second researcher trained in venepuncture is available to attempt the blood draw if the first researcher is unsuccessful. This should reduce the occurrence of missing data.

Although recruitment was slower than expected (2.25 participants enrolled per month), the eligibility criteria was not considered to be too restrictive because 82% of screened volunteers were eligible. Furthermore, 56% of eligible volunteers accepted the invitation to

enrol into the study. This indicates that there are no significant problems with the inclusion and exclusion criteria, and that recruitment rate was on par with prospective participant numbers.

Ten of the 28 eligible volunteers who declined to take part cited that they were unable to commit to the study. The study as designed does require a considerable time commitment from those randomised to the exercise group (two assessment visits, 28 exercise sessions). In order to provide added incentive, rewards or reinforcement could be offered based on the number of exercise sessions completed (and completion of follow-up). This may have the added effect of increasing adherence but also incentivising volunteers to take part. However, incentives for clinical trials are considered by some to be coercive and to encourage enrolment into trials for the wrong reasons (Vellinga et al., 2020). This is particularly true of large incentives, or where the risks of the research are particularly high, or where the research is degrading (Vellinga et al., 2020). 'Tokens', gift vouchers, and non-monetary gifts have been used effectively (Parkinson et al., 2019), and are considered to be less controversial, but also less likely to influence participation (Largent et al., 2012). Thus, it may be possible provide small incentives or rewards for the present study, since it is not considered to be high risk or degrading, that influence participation to some degree. Furthermore, it should be made clear that any compensation is given for time and inconvenience, ensuring equal access to the study for all participants, and not for compensation of risk (Largent et al., 2012). Incentives are context specific, and time should be spent designing an incentives system based on the barriers to participation (Parkinson et al., 2019). That is, providing travel expenses where travel costs are prohibitive may be an effective incentive, with an additional incentive to encourage the desired behaviour, such as entry into a prize lottery (Parkinson et al., 2019).

While this section has outlined some effective behaviour change techniques to improve adherence and recruitment (such as goal setting and incentives), future trials should be designed with consideration of behaviour change theories (BCTs) from the outset to maximise the effectiveness of interventions. BCTs are designed to help us understand how behaviour develops and the factors that influence it over time (such as attitudes, internal motivation, and personal and societal factors) (Michie & Johnston, 2012). Designing an intervention in line with an underpinning theory will help in the selection of relevant and evidence-based behaviour change techniques, and there are several theories that have been successfully applied to PA and health promotion interventions (Michie & Johnston, 2012).

Two examples of BCTs that have been used effectively in PA and health-promoting interventions are the Theory of Planned Behaviour (TPB) (McEachan,Connor, Taylor & Lawton, 2011) and Self-determination Theory (SDT) (Ryan & Deci, 2000). TPB states that behaviour is influenced by one's intentions (the motivational component that spurs on behaviour change), and that intentions are, in turn, influenced by attitudes, subjective norms (such as others opinion on the behaviour), and perceived behavioural control (the degree to which an individual believes they have control over the behaviour) (McEachan et al., 2011). SDT posits that behaviour change is affected by intrinsic motivation (that is, not external pressure or rewards/punishment, but rather interest or enjoyment in the behaviour itself) (Ryan & Deci, 2000). In addition, the behaviour must be compatible with one's sense of self and values, and meet the individual's needs for autonomy, competency, and relatedness (Ryan & Deci, 2000). This form of self-driven motivation is thought to be stable and enduring, and as such provides a promising basis for long-term health promoting interventions (Teixeira & Mata, 2011).

When designing future interventions, researchers should adopt an approach of incorporating BCTs from the early design stages. This could involve using a framework or tool, such as the behaviour change wheel (Michie, van Stralen & West, 2011). Researchers begin with a broad approach to understanding the desired behaviours, the factors that influence it, and the target population, before selecting appropriate interventions and BCTs, and then designing and incorporating behaviour techniques (Michie et al., 2011).

4.5.2 Participant Characteristics

The purpose of this study was not to assess the efficacy of the intervention(s) and, as such the study was not adequately powered to detect differences between means in participant characteristics. However, the descriptive statistics provide useful information about the population in terms of CVD risk, as well as an overview of the characteristics in each of the study arms.

4.5.2.1 Anthropometry and PA Levels

The mean WC in each group at baseline was >88cm. As outlined in Chapter 2, WC is a measure of abdominal obesity and risk for CHD approximately doubles in women with a WC

of >88cm compared to <71cm (Flint et al., 2010). This indicates that the women in the present study had characteristics in line with previous studies that report an increased prevalence of abdominal obesity in PCOS, and a resulting increase in CVD risk.

In the present study, the mean WHR of each group was between 0.83-0.85 at baseline. WHR, a measure of the ratio of waist and hip circumference, is another measure of abdominal obesity that was found to be indicative of CVD risk in the INTERHEART study (Yusef et al., 2004). Proposed cut-off values to determine CVD from WHR vary between populations but is estimated somewhere between 0.80 and 0.87 (Al-Rubean et al., 2017; Katulanda, Jayawardena, Sheriff & Matthews, 2011; Khader et al., 2010). Taken together with the measures of WC, this suggests that the women in the study were at increased CVD risk.

The data indicate that weight loss, in terms of kg and cm around the waist, was observed in both the exercise group and the LPAG group at follow-up. Both groups lost ~3.5% of their baseline weight. This is in line with previous research that has indicated modest weight loss occurs from exercise interventions in PCOS (Harrison et al., 2011). A weight loss of approximately 5% can improve CVD risk factors in women with PCOS with overweight (Pasquali et al., 1994).

Continuous data from the IPAQ (MET-min/week) indicate that, across groups, the participants were already physically active. This is further contextualised by the categorical data. These categories classify respondents based on their lifestyle PA levels including transport, job-related PA, and leisure time PA (such as walking). The eligibility criteria required that participants not be undertaking more than 150 min/week of structured exercise. However, fifteen participants were still categorised in the HEPA category. This highlights that one can abstain from regular structured exercise, but still maintain a healthy level of PA in their daily lives (and thus not be classed as physically inactive). This may have affected results because some participants were physically active enough at baseline to gain health benefits, and as such the effect of the intervention may be dampened. In addition, this highlights the need for clearly defined terms relating to physical inactivity, PA, and exercise when selecting samples for PA interventions, to maximise effects.

The use of objective measures of PA behaviour would provide a clearer picture regarding both participants' PA levels throughout the intervention, and when assessing potential participants for eligibility. That is, rather than using structured exercise levels to screen participants, interventions could measure lifestyle PA behaviour before randomisation to

select those participants that were physically inactive or minimally active. This would prevent the selection of individuals who are very physically active, despite not participating in regular structured exercise. However, the use of objective measures of PA behaviour adds an additional burden to participants. This would require data collection and monitoring before participants are consented and randomised into the trial. The present trial was already demanding of participants' time and effort, and thus this was approach was deemed unsuitable in order to increase compliance and reduce burden.

While the LPAG used the app as an objective measure of PA, this was not replicated across groups and thus there were no objective measures of lifestyle PA for most participants. As stated previously, the addition of an objective measurement of lifestyle PA, such as an app or pedometer, would increase burden across groups by requiring significant compliance to study protocols. The IPAQ was therefore deemed a suitable measurement tool, because it has been shown to produce comparable data to devices measuring objective PA, as well as being an affordable option for a trial with budgetary constraints (Prince et al., 2020).

Despite the lack of objective measures of lifestyle PA, all participants undertook VO_2max testing at baseline and post-intervention. VO_2max, as a measure of CRF, is an objective indicator of habitual PA (Richter & Hargreaves, 2013) and thus provided valuable data on changes to physical fitness after the intervention(s). This data, as expected, indicated that those in the exercise group experienced the greatest improvements to CRF. However, the addition of an objective measure of PA across groups would provide greater detail around whether the intervention(s) were responsible for this, or whether PA outside of the trial was a factor.

An important consideration for future trials utilising apps (and thus smartphones and the internet) for PA interventions is the role of health equalities on representation of the participant group, and barriers to participation. While smartphone usage is continually increasing across all socio-economic groups (Marler, 2018), research often assumes that such usage is affordable and dependable. In fact, lower-income individuals are more likely to experience periodic disconnection, either through an inability to pay phone bills, or through being unable to replace lost, broken, or stolen devices (Marler, 2018). In addition, 'ICT poverty', whereby individuals lack competency due to a lack of computer experience, may present barriers to participation (Donner et al., 2011). To prevent a homogenous sample of educated and higher-income participants, studies should take steps to ensure that the

intervention is accessible and affordable to a diverse range of participants. This could be in the form of providing low cost devices, or pre-paid internet access for the duration of the study.

4.5.2.2 Blood Lipids and Glucose

Baseline TC (mmol/L) and HDL (mmol/L) concentrations for all groups were within the healthy range (TC < 5.0 mmol/L, HDL ≥ 1.2 mmol/L) (Heart UK, 2020). Previous research has indicated a prevalence rate for dyslipidaemia (high LDL-C and low HDL-C) in PCOS of 70%. Thus, this could be normal variation within the population.

In addition, fasting glucose concentrations were within the healthy, non-diabetic range (4.0-5.9 mmol/L) (Diabetes.co.uk, 2020). However, in PCOS this does not suggest there are no abnormalities in glucose and insulin metabolism. Since compensatory hyperinsulinemia occurs because of insulin resistance, fasting glucose can be maintained at healthy concentrations for a time before insulin resistance worsens (Veltman-Verhulst, Goverde, van Haeften & Fauser, 2013). Indeed, the fasting insulin concentrations in each group are above healthy fasting levels. However, without conducting an oral glucose tolerance test (OGTT), or a euglycemic-hyperinsulinemic clamp (which is the gold standard for measurement of insulin sensitivity) it cannot be assumed that high fasting values are due to impaired insulin metabolism and not that some participants had not sufficiently fasted before the blood draw.

In the present study, there were no noteworthy trends indicated toward blood lipids and glucose in any of the groups, at follow-up compared to baseline.

4.5.2.3 Biochemical Analysis

Oxidised LDL is the key secondary outcome in this study. Results from the present study indicate that oxidised LDL concentrations were high across groups. Indeed, the observed values in the present study (mean for all groups = 99.27 ± 33.52 U/L), are higher than those observed in other studies of oxidised LDL in PCOS using the same method. In a cross-sectional study of women with PCOS, Macut et al. (2008) observed a mean oxidised LDL of 66.9 ± 33.0 U/L. Similarly, in another study, mean oxidised LDL was reported as 65.4 ± 26.1 U/L in overweight women with PCOS (Macut et al. 2006). However, very few studies have reported oxidised LDL concentrations in women with PCOS. Other studies reporting oxidised LDL in other populations have found higher concentrations (72-84 U/L), but these are

predominantly in CHD patients (Johnston, Jernberg, Lagerqvist, Siegbahn & Wallentin, 2006; Braun et al., 2005).

Thus, it is likely that the high concentration is due to a wide SD in a small population. Indeed, Macut et al. (2006) and Macut et al. (2008) have reported similarly wide SDs. This indicates that there is considerable variation across the population, making it more susceptible to the effect of outliers. It is less likely that this is due to a technical error; the inter-assay CV was 6.2% and inter-assay CV was 7.6% and 4.6% for each assay, respectively. Additionally, the quality controls were observed to be within the acceptable range of the assay.

Furthermore, the manufacturer of the commercial ELISA (Mercodia, Sweden) indicates that the units (U/L) are an arbitrary relative unit compared against an in-house standard. As such, there is no international reference data currently available. Thus, the relative value of the data is not undermined (that is, the difference between groups is more important than the absolute values).

Across the three groups, the most noteworthy improvements in oxidised LDL occurred in the exercise group, with a lesser improvement observed in the LPAG. This may indicate potential for the intervention to improve oxidised LDL concentrations in this population.

Neopterin is produced by macrophages, which are principally responsible for the formation of fatty streak lesions that begin the process of atherosclerosis (Lee et al., 2012). Neopterin has pro-oxidative properties and is an independent marker for CVD risk (Fuchs et al., 2009). The participants in the exercise group have higher neopterin concentrations than that observed in healthy controls in previous research, which are typically <10 nmol/L (Ragab et al., 2005; Peng, Zhang, Lian, Lu & Wang, 2002; Zheng et al., 2005). In addition, CRP concentrations were higher in the exercise group in line with this. However, TBARS concentrations, which also indicate lipid peroxidation and inflammation, were not higher than those found in healthy controls, which is in line with other studies comparing TBARS in women with and without PCOS (Gonzalez, Rote, Minimum & Kirwan, 2006; Ragab et al., 2005).

Using free-testosterone data, it was proposed that participants be categorised by androgen profile as normo-androgenic or hyper-androgenic based on laboratory cut-off values (Lerchbaum, Schwetz, Rabe, Giuliani & Obermayer-Pietsch, 2014). This would have highlighted the differences between PCOS phenotypes. However, as stated, free testosterone

could not be measured due to Covid-19 restrictions that prevented necessary training. As such, this analysis could not be conducted.

4.5.3 Strengths and Limitations

This study was a feasibility study, and as such its main strength is that is an initial, informative stage that helps to identify methodological constraints that may impact on a full-scale trial. This prevents potential waste of resources when designing and implementing RCTs that may have significant methodological issues. This study has highlighted a potential issue in adherence rates to the exercise intervention, that may affect the validity of results, and as such this can be refined before the next steps. Additionally, it has provided information about sources of and rates of recruitment, which will be integral in planning and budgeting for a large-scale trial.

Another strength of the study is the use of technology in the LPAG. Fitness devices are proliferating rapidly and provide a convenient, economic way to track PA that is an alternative to a supervised exercise program. Similarly, no research has identified the impact of increased lifestyle PA on the cardiometabolic profile of women with PCOS. This study is a novel first step that could shed light on new methods of ameliorating risk whilst reducing burden on participants.

There are some limitations. Firstly, the use of the IPAQ to record lifestyle PA may have limitations because it is based on self-report. Self-reporting has disadvantages involving both recall and/or accurate reporting (van der Ploeg et al., 2010). Furthermore, the IPAQ is considered to be more effective in population-level surveillance rather than in intervention studies where individual changes in PA are more important (van der Ploeg, 2010). Nonetheless, it is an affordable, simple, and feasible way to collect adjunct lifestyle PA data.

In addition, although the intervention(s) utilised various behaviour change techniques (such as self-monitoring and some goal setting), they were not underpinned by BCTs or models at large. Thus, future interventions could be designed in line with a behaviour change framework or model in order to increase adherence using an evidence-based approach.

Finally, there are some limitations involving the experience of the researcher. Although relevant training was undertaken in laboratory techniques, relative inexperience in conducting laboratory assays can still be attributed. Thus, there may have been some errors or inconsistencies. To combat this, any technical errors have been included alongside the results,

as well as inter- and intra-assay CVs and R^2 values to allow the reader to consider the reliability of the results.

4.5.4 Conclusions

The present study has assessed the feasibility of conducting an RCT of two PA interventions in women with PCOS. The results indicate that procedures for recruitment, allocation, and outcome measurement were acceptable. However, some changes may be required to the exercise intervention to increase adherence, and this may include behavioural change support. In addition, sufficient data has been collected for a sample size calculation for a fully powered RCT. The participant characteristics indicate that the population in the present study display some features of increased CVD risk, in line with previous research. Furthermore, there appears to be potential for the PA intervention(s) to mitigate some of these factors. The next step would be the definitive trial with an internal pilot study where any new features of the intervention can be assessed alongside the effectiveness of the interventions.

5 Qualitative Evaluative Study to Assess Acceptability of the Interventions

Overview

This chapter reports the methods and results of the qualitative evaluative component of the PCOS feasibility RCT. The wider methodology relating to eligibility, recruitment, and study procedures have been described in Chapter 4. Quantitative data collection, analysis and findings have been reported in Chapter 4.

5.1 Introduction

5.1.1 Aims and Objectives

While a quantitative analysis of feasibility sheds light on objective measures of recruitment and adherence, a qualitative evaluation of the intervention helps to identify complex and nuanced factors that may affect the success of future implementation of exercise and PA interventions.

Therefore, the aims of this study were to:

1. Identify barriers and facilitators to PA in women with PCOS and develop recommendations for the refinement of a full-scale RCT.
2. Evaluate the acceptability and efficacy of the interventions to inform future research design.

The objectives of this study were threefold:

i) To examine factors that influence PA in women with PCOS.
ii) To explore the experiences of the individuals who participated in either of the intervention groups.
iii) To gather suggestions for future research design.

5.2 Methods

This was a qualitative evaluative study as part of an over-arching randomised-controlled trial (RCT) to test the feasibility of two PA interventions with women with PCOS (Woodward et al., 2020). This study is reported according to the Consolidated Criteria for Reporting

Qualitative Studies (COREQ): 32-item checklist (Tong, Sainsbury & Craig, 2007). This can be seen in Appendix 5.

5.2.1 Study Design and Theoretical Underpinning

The justification for undertaking a feasibility study at large has been described previously. Quantitative methods can be used to obtain standard deviations to calculate required sample size, and to calculate adherence, attrition, and recruitment rate to plan full-scale trials. They can also be used to evaluate and refine data collection procedures and outcome methods (Orsmond & Cohn, 2015). However, another important objective of a feasibility study is to evaluate whether the intervention is acceptable and suitable to participants (Orsmond & Cohn, 2015; Gitlin, 2013). Without careful evaluation of acceptability at the feasibility stage, a full-scale RCT may appear to be unsuccessful, for reasons which were ultimately correctable, and both participants' and researchers' time and resources may be wasted (Ioannidis et al., 2014; Gitlin, 2013). Increasingly, researchers are implementing qualitative methods before proceeding to the full trial to identify and address problems that affect the acceptability (O'Cathain, et al., 2013). Qualitative methods are particularly useful because contextual factors that threaten trial implementation are often nuanced and complex (Wells et al., 2012). In health research, qualitative methods have been employed to further understanding of complex interventions, drug and medical device trials with complex patients or trial environments, and to understand social contexts in which interventions are tested (O'Cathain et al., 2015). Indeed, the UK Medical Research Council (MRC) has developed guidelines for the development and evaluation of complex interventions using qualitative methods (Craig et al., 2008). In the current trial, based on the recommendations of O'Cathain (2013), the addition of a qualitative component was utilised to understand how actual implementation differs to planned implementation, optimise trial conduct, and to generate ideas for future research design that considered the lived experiences of the target population. The overall purpose of this was to prevent the progression to a full-scale trial that uses poor or unacceptable interventions, or that does not provide a positive experience for participants.

5.2.2 Recruitment and Sampling

Participants were recruited from all three study arms to address the first objective which was to explore factors that influence PA in order to optimise intervention procedures. Whilst this could have been addressed with recruits solely from the two intervention arms, including

those in the control arm ensured a wider range of experiences from across the sample and not just those who had participated in the interventions.

Another objective was to collect ideas for future research design from the target population; although those in the control group had not completed the intervention, they had still been recruited and undergone procedures and testing at baseline and follow-up. Thus, these participants still had valuable information about their experiences with the trial procedures and their perspectives were considered valuable.

Sample size in qualitative research is determined by several factors. Firstly, its purpose, which is to gather a deep understanding of the specific experiences of a group of participants rather than ensure statistical significance or provide statements of incidence or prevalence. Indeed, a phenomenon need only appear once to contribute to the thematic map. Secondly, a recognition that there is often a point of diminishing returns where increasing sample size no longer contributes new evidence, known as data saturation (Ritchie, Lewis, Elam, Tennant & Rahim, 2014). Finally, because qualitative research produces data that is rich in detail, sample sizes are necessarily kept small to remain manageable and enable detailed analysis (Ritchie et al., 2014).

The sampling decision needed to strike a balance between diversity, and inclusion of all three necessary groups, while remaining manageable enough to ensure quality of analysis (Ritchie et al., 2014). Adler and Adler (2012) propose a broad rule of thumb of between 12 and 60. Additionally, Guest, Bunce and Johnson (2006) contend that saturation may occur within twelve interviews. If sample sizes are very limited, purposive sampling can be used to support variation in characteristics (Ritche et al., 2014). Thus, using purposive sampling to increase diversity, I decided to interview a quarter of each group, approximately twelve in total depending on the number of participants returning for follow-up. This allowed for the analysis of a variety of experiences across the groups whilst also allowing for quality, in-depth analysis. In addition to this, I planned to conduct interviews with a proportion of participants who had dropped out of the trial in order to examine reasons for this.

Participants who successfully completed the RCT were purposively sampled and offered an interview. Purposive sampling is a technique used for the identification and selection of information-rich cases (Palinska et al., 2016). There are multiple types of purposive sampling that achieve different purposes. Some examples include: the selection of cases with maximum variation to find unique or diverse variations emerging from different conditions,

or those patterns that cut across varying conditions; selection of extreme or outlier cases to learn from unusual manifestations of the phenomena of interest; or the selection of homogenous cases to reduce variation and simplify analysis (Palinksa et al., 2016). In this study, purposive sampling for maximum variation was used to ensure that the sample was rich in terms of characteristics and constituencies that would provide maximum diversity of opinions and experiences (Ritchie et al., 2014). This is primarily because women with PCOS are a heterogenous group, encompassing women of different ages and ethnicities, and PCOS has multiple presentations. In addition, the study design had several conditions of which it was important to gather experiences from each. Increased variation may therefore increase the generalisability of results because the sample does not contain a group of women who all have the same characteristics. Thus, participants were selected based on differing trial allocation, age, adherence to the trial, and ethnicity, to provide a multitude of contextual experiences. The purposive sampling procedure began immediately after the first participants completed the trial in order to minimise the time between completion and interview.

Participants were approached at the follow-up visit and agreed to participate in the interview. A convenient time to conduct the interview, either face-to-face or by telephone, was scheduled. The telephone option was offered for participant convenience and to minimise burden.

To reduce bias, all participants who dropped out of the trial were approached for interview to examine their experiences and provide potential reasons for drop out, but none of them responded to interview invitations. All interviews were therefore conducted with participants who completed the follow-up visit. Interviews were completed within two weeks of completion while the trial was still a recent experience to maximise recall.

5.2.3 Interview Schedule

The interview schedule was devised from both the research objectives and relevant literature around factors influencing exercise participation in clinical populations. The interview schedule subsequently consisted of 13 open-ended questions. Probes were utilised where appropriate. Probes are follow-up questions that elicit further information or explanation from a participant's response, for example, 'in what way?' and 'why was that?' (Yeo et al., 2014). There were several demographic questions to provide context and transparency regarding the diversity in the sample. The full interview schedule can be seen in Appendix 6. The interview

schedule was piloted before data collection began, with one researcher and one layperson, to ensure the wording was sensitive, comprehensible, and well-paced (Ritchie et al., 2014). After piloting, minor changes were made to wording to increase coherence.

5.2.4 Data Collection

Semi-structured, in-depth interviews were chosen as the data-collection method. In-depth interviews are a powerful data acquisition tool that generate descriptions and subsequent interpretations of an individuals' social world (Yeo et al., 2014). They involve a conversation between the interviewer and interviewee, where the interviewer uses active engagement to drive the conversation about relevant issues, topics, and experiences (Mason, 2003). The style is often conversational, flexible, and iterative, being strongly influenced by the situation. One of the key advantages of the in-depth interview is that, unlike observations, it includes the participants' explicit interpretation and understanding of events (Yeo et al., 2013) Importantly, they provide the researcher with detailed knowledge or experience of a problem from a perspective other than their own (Rubin & Rubin, 2012).

In relation to structure, semi-structured interviews are characterised by a pre-determined but fluid interview schedule and open-ended questions (Given, 2008). Semi-structured interviews contrast with the more rigid and uniform style of a survey, whilst also allowing the researcher to have more control over the topics than an unstructured interview (Given, 2008; Mason, 2003). The aims of the study required significant input from participants, in their own words, about their experiences. Thus, a structured interview or a survey may have been too restrictive. Additionally, the aims and objectives included specific topics that needed to be covered, so it was important that some structure in the interview scheduled was retained.

Interviews were conducted upon successful completion of the trial; that is, if participants returned for their follow-up visit. Although data pertaining to each objective could have been collected at different time points, such as pre-trial for objective i) and at multiple time-points for objectives ii) and iii), I chose to interview participants only at follow-up for several reasons. Primarily, to minimise participant burden it was decided that the participants should only undertake a single interview. Secondly, in order to fully capture participants' experience of the whole intervention and their changing opinions throughout this, the completion of the trial was a necessary time-point. Hence, this time-point was optimal for capturing required data and reducing the impact of burden on participants.

I spent a considerable amount of time developing relationships with the exercise group as a result of delivering the 12-week supervised exercise intervention. It was expected that this relationship may, in some cases, make participants reluctant to give honest feedback. For example, participants may have wanted to be helpful in providing positive feedback rather than voicing their concerns. Having established rapport, they may have wanted to avoid causing offence with any negative opinions. As a result, a researcher external to the project (RC) was recruited to conduct most interviews with participants in the exercise group. RC is a female final-year, doctoral researcher undertaking a qualitative project and experienced in conducting semi-structured interviews. One interview with an exercise group participant was conducted by me when RC was unavailable.

To ensure consistent quality of the interviews conducted by RC, we discussed the content of the schedule beforehand, giving RC a chance to gain clarity on the objectives of each question and to ensure she understood the context. After conducting the first interview, RC and I discussed once more any issues or confusion that had arisen from the schedule. The interviews with all other participants were conducted by me, where the interactions with participants were limited to baseline and follow-up visits.

Each interview lasted up to 40 minutes. Nine were conducted via telephone, and two were conducted face-to-face. All interviews were audio-recorded using a voice recorder (Olympus, Japan). Interviews were transcribed verbatim, by me, using Audio Note Taker (Sonocent, UK) and all information relating to participant identity was removed. Each participant was given a pseudonym to maintain anonymity.

5.2.5 Data Analysis

Thematic analysis was selected as an appropriate method for interpretation of the data (Braun & Clarke, 2006). Thematic analysis, unlike a theoretically informed methodology, is a data interpretation and analysis *tool* that is independent from research paradigms and theoretical frameworks (Brown & Scaife, 2019). This allows a flexible analysis that is data-driven (or theory-driven). Because the research objectives were necessarily specific, the interview schedule was designed to shape the responses and subsequent analysis to answer the research questions. This was a deductive process because of the pre-conceived ideas that shaped a conceptual framework of expected themes. However, the use of semi-structured interviews provided an inductive element because participants were provided the opportunity to offer

their own unique insights and experiences. The subsequent analysis was therefore driven by the interpretation of the data and not by a pre-defined theoretical framework.

Braun and Clarke (2006) outline six main steps to conducting thematic analysis. Each stage and how it was applied to the present study have been described below:

1. Familiarisation

The initial step of familiarisation is vital to immerse oneself and understand the depth and breadth of the data before coding can begin. This included transcribing data verbatim and reading through the entire data set at least once, in an active way, that involved searching for meanings and patterns. It also involved forming and noting down initial ideas.

2. Generating Initial Codes

After familiarisation, the next step is to generate initial codes. These initial codes represent the most basic element of the raw data that form the basis of repeated patterns across the data set. This initial coding allowed the data to be organised into meaningful groups relating to phenomena of interest. In this study, data were coded by keeping the specific research questions in mind. Features of the data were coded and collated systematically across the whole data set. Quirkos, a qualitative data analysis software programme, was used to aid data management and consistency.

3. Searching for Themes

The next step involves sorting and grouping the long list of initial codes into fewer, broader themes based on the researcher's interpretation. This involved considering how multiple codes combine to form an overarching theme. Initial codes and excerpts were extracted into Microsoft Excel. Tables and 'thematic-piles' were created in this process to move codes around and identify how they fit into the overall thematic map, and to identify relationships between codes, sub-themes, and themes. After this step, there was a collection of potential themes and sub-themes, along with the codes that constitute these.

4. Reviewing Themes

In this step, potential themes and sub-themes are reviewed and refined. This involved the collapsing of themes into one, or the breaking down of themes into separate, more specific themes. In addition, it may become apparent that certain initial themes are not supported by the data. Thus, extracts from the data were reviewed to ensure they formed a coherent pattern. Themes were checked for fidelity against data excerpts and the entire data set using the constant comparative method and deviant case analysis method (Lewis et al., 2014). The

constant comparative method involves deriving initial hypotheses and then checking congruence with other parts of the data both within and between cases (Silverman, 2011). Deviant case analysis involves actively identifying those cases that are not congruent and adapting initial hypotheses until all cases can be incorporated therein (Seale, 2012). Any codes that did not appear to fit led to refinement of a theme or moving the erroneous codes into a new theme until they fit into the thematic map.

5. Defining and Naming Themes

In this step, once a satisfactory and cogent thematic map has been developed, themes can be further defined and named according to how they will be presented in the analysis. In this study, this included generation of additional higher order and sub-themes, and the development of clear names and definitions for each theme, ensuring they were coherent and consistent. At the end of this stage, theme names were finalised to ensure they captured the essence of each theme. Extracts were organised to form an accompanying narrative of each theme, and how it fits into the broader story.

6. Producing the Report

This step is the final opportunity for analysis, beginning once the themes are finalised, and involves telling the story presented by the data in a way that is convincing and relevant to the research objectives. In this study, vivid and compelling extracts were selected to demonstrate the essence of the themes, not only in a descriptive manner, but in a way that answered the research questions. The analysis was related back to the research objectives, describing the narrative between and within themes, and the scholarly report herein produced.

5.2.6 Quality Considerations

Several authors have developed terms that are suitable for assessing and assuring quality in qualitative research (Lewis, Ritchie, Ormston & Morrell, 2014; Lincoln & Guba, 1985). Because of the strong influence of individual and contextual factors on qualitative data, the rigid application of terms such as ‘reliability’, and ‘validity’ that are relevant in quantitative research may be inappropriate (Lincoln & Guba, 1985). Thus, one can alternatively establish credibility, transferability, and dependability. These terms refer to the robustness of the research and the degree to which findings are accurate, applicable in other contexts, consistent, and independent from researcher bias. Each of the quality considerations and how they have been established in the current study are presented below.

5.2.6.1 Credibility

Credibility, sometimes referred to as plausibility, refers to the degree to which the findings accurately reflect the experiences of the population (Lewis et al., 2014; Lincoln & Guba, 1985). Methods affecting credibility include deviant case analysis, and the provision of extracts from the data source throughout the report to demonstrate the underlying data that constitutes the findings. Purposefully searching for deviant cases ensures that themes or hypotheses can account for the majority of cases and that the data do not contradict explanations (Lincoln & Guba, 1985). I applied deviant case analysis, as described previously, throughout to ensure that developed themes incorporated divergent views. Data extracts were selected to support findings, and these have been presented in the findings section of this chapter, providing evidence that hypotheses, patterns, or explanations are supported by the data and the participants' own words.

Member-checking is also purported to improve credibility. However, it was not conducted in this study. Member-checking, or respondent validation, may involve taking a transcript back to the participant to check whether the content within is confirmed (Lewis et al., 2014). Other variations of member-checking also exist, such as taking an interpretation or analysis back to the participants for confirmation. Despite the prolific popularity of member-checking, recent literature illuminates inherent problems with the process (Smith & McGannon, 2018). The problems with member-checking begin with the ontological standpoint that there is an independent and reliable 'reality' that we can access (Lewis et al., 2014). However, there is no way to know with certainty that the account is true or that the researcher has in fact accessed this objective reality. In addition, there are numerous practical and logistical challenges. For example, how to proceed if the researcher and participant disagree over interpretations, the amount of time that has passed since the interview and transcription/analysis, and finally, the issue of having no way to check whether participants have indeed engaged in member-checking and not simply skimmed the text, agreed without reading it, or to have deferred to the researcher based on perceived authority (Smith & McGannon, 2018). In view of these considerations, including the increased burden on participants, member-checking was not conducted.

5.2.6.2 Transferability

Transferability, sometimes known as 'generalisability', refers to the extent that the findings are generalisable in other contexts including both the parent population of the sample and other settings beyond the one sampled (Lincoln & Guba, 1985; Lewis et al., 2014). Generalisability can be determined by the diversity in the sample, providing rich descriptions of participants' responses, and by providing descriptions of both the characteristics of the sample and the setting in which the research took place (Lewis et al., 2014). In this study, purposive sampling was used to provide a diverse sample. Detailed extracts in support of the findings are provided throughout. Furthermore, additional demographic data was recorded and presented to allow the reader to consider the impact of the characteristics of the sample on the transferability of the findings. Finally, clear descriptions of the research setting, both regarding the trial and the interviews, were provided to outline the specific context of the findings. This allows the reader to assess the similarity of the setting described to other settings to which the findings could be applied (Seale, 2012).

However, the nature of qualitative research, such as small sample sizes and the focus on rich, detailed analysis, inherently limits transferability. Indeed, it is not the goal of qualitative research to establish incidence or prevalence of a phenomenon (Mason, 2003). Thus, the findings are mainly applicable to the target population in other similar contexts (that is, interventions), particularly due to the narrow research objectives which shaped the responses. The element of transparency, however, allows researchers to determine for themselves if the findings are transferable to similar contexts. In addition, a diverse sample increases the scope of transferability within the target population.

5.2.6.3 Dependability

Dependability, sometimes referred to as confirmability, is similar in its meaning to the quantitative context of 'reliability'. That is, would similar findings be produced if someone else undertook the research (Lincoln & Guba, 1985)? However, due to the complexity of the phenomena being studied and the unavoidable impact of context and the researchers' personal biases and perspectives, complete replication may be an unrealistic demand (Seale, 1999). Thus, good practice in relation to dependability can be attained by taking a clear and transparent approach to the decisions made regarding processes throughout the study, so that they may be carried out by another researcher (Seale, 2012). For thematic analysis

specifically, especially given its flexible nature, Braun and Clarke (2006) suggest that rigour lies in being clear and explicit about one's approach and assumptions, and that the actual work carried out matches up with this. Indeed, in this report, I provide detailed justifications for processes and decisions undertaken throughout. Furthermore, the use of a reporting checklist (COREQ) (Tong et al., 2007) in this study contributes to the transparency and ensures key features are reported so that other researchers may follow the process. Thus, the methods and approaches in this study are transparent and open to replication. Finally, the addition of a reflexive account of how the researcher themselves had informed the research may provide additional transparency and make dependability easier to evaluate. In this chapter, I include a section on reflexivity and consider what role I played in shaping the research.

According to a recent review of qualitative research in sport and exercise psychology, an extensively used reliability technique is peer debriefing, or investigator triangulation, which involves two or more researchers independently coding data, before coming together to resolve disagreements around categorisation and interpretation (Culver, 2012; Smith & McGannon, 2018). Some researchers take this further and unitise agreement through inter-rater reliability to produce a percentage of agreement among peers; when a high level of agreement is reached, this ostensibly increases trustworthiness and replicability (Campbell, Quincy, Osserman & Pederson, 2013; Lincoln & Guba, 1985). This concept has been subject to criticism because of the underlying assumption that there is a 'correct' reality that can be captured through discussion and agreement (Smith & McGannon, 2018). On the contrary, as previously noted, themes are not regarded as existing inherently in the data but are in fact the product of the researchers' interpretation, which is essential for good qualitative research (Brown & Scaife, 2019). Additionally, the actual process of negotiation with multiple coders can also be fraught, given certain academic hierarchies, gender dynamics, and power structures that exist within teams (Smith & McGannon, 2018). However, that is not to say that reflexive discussion and subsequent feedback on the interpretation is not valuable. The idea of 'critical friends' has thus been utilised in this study (Cowan & Taylor, 2016). The difference in approach revolves around the idea that feedback given by other members of the research term is not aiming to achieve consensus or agree, but to provide a sounding board and challenge knowledge and interpretation, thus stimulating further exploration of possible explanations (Cowan & Taylor, 2016).

5.2.7 Reflexivity

In qualitative analysis, themes and concepts are not generally regarded to 'emerge' from the data, but rather the researcher is active in interpreting them (Brown & Scaife, 2019). Thus, while it is important that researchers strive to avoid conscious or systematic bias as much as possible, the researcher can never be truly 'neutral' or 'objective' because their own knowledge, ideas and beliefs shape the interpretation (Ormston, Spencer, Barnard & Snape, 2014). To this end, providing a reflexive account that transparently considers the impact of the researcher's own beliefs and behaviour on the research process can aid in highlighting potential sources of bias (Ormston et al., 2014). In this study, reflexivity was achieved by ongoing reflection on my actions, feelings and assumptions, throughout the trial and data collection process. This was facilitated by my personal involvement in each aspect of the process, including supervising participants, contact throughout the trial, and transcription of interviews. I also kept a reflective log that outlined pertinent reflections.

5.2.8 Ethical Considerations

Health Research Authority (HRA) approval for the over-arching study, including the qualitative component, has been obtained and Research Ethics Committee (REC) favourable opinion granted by the North West – Greater Manchester East REC on 19th July 2018, reference 18/NW/0454. The PIS (Appendix 4) included a separate section covering issues specific to this qualitative study, including the purposes, procedures, and data management. At the baseline visit, participants were given the opportunity to discuss the interview separately and highlight any concerns. In addition, the informed consent proforma included a specific section that dealt with consent for the interview. At the beginning of each interview, participants were reminded of the purposes of the interview and subsequent procedures, and verbal consent was confirmed before proceeding.

5.3 Results

In total, 13 participants were approached for interview. Two participants initially agreed but were subsequently lost to further contact, and an interview could not be scheduled. Subsequently, 11 interviews were conducted. Nine of the interviews were conducted by phone at the participants' preference. Five participants were in the exercise group, four

participants were in the lifestyle physical activity group (LPAG), and two participants were in the control group (no intervention).

Table 13. Demographic and Adherence Data.

Pseudonym	Age (Years)	Ethnicity	Highest Education	Income Bracket[a]	Allocation	Adherence
Carrie	19	British Pakistani	Undergraduate	£21-30k	A	75%
Corin	19	British Pakistani	Undergraduate	Under £20k	A	18%
Debbie	31	British Asian	Doctorate	£41k+	A	96%
Frances	49	White Other	Post-graduate	£31-40k	A	78%
Janet	26	White British	Undergraduate	£41k+	A	82%
Courtney	35	White British	Doctorate	£41k+	B	n/a
Kathleen	29	White British	Undergraduate	£21-30k	B	n/a
Tali	34	White British	Undergraduate	£31-40k	B	n/a
Shirley	30	White British	Undergraduate	£41k+	B	n/a
Dolores	32	White British	Post-graduate	£31-40k	C	n/a
Marissa	34	White British	Level 3	£31-40k	C	n/a

a= annual household income

Participants in the exercise group had a wide range of adherence rates to the exercise sessions, from 18% to 96%. Participants ranged in age from 19 to 49 years. In terms of self-reported ethnicity, two were British Pakistani, one was British Asian, one White Other. The rest were White British. Demographic information collected from participants can be seen in Table 13, including education level, annual household income bracket, age, and ethnicity.

In congruence with the three objectives of the study, the results have been presented in three sections. Section one reports the first two themes, Living with PCOS and Factors influencing PA behaviour, and includes data from all three groups of participants describing their experience with PA and PCOS. Section two reports on two themes specific to experiences of the intervention, including Effects of the intervention, and Challenges of the intervention, and includes data from those participants who took part in either of the two interventions. The third section is a summary of recommendations based on issues and recommendations for future study design that reports data from all three groups of participants. Below, each higher order theme is presented with a table that provides the constituting lower order themes and raw data themes. Where appropriate, results are divided by the group allocation to ensure transparency of responses.

5.3.1 Section One: General Themes

In this section, the themes Living with PCOS and Factors Influencing Physical Activity Behaviour will be reported. The section relates to objective one of the present study, which is to examine factors that influence PA in women with PCOS.

5.3.1.1 Living with PCOS

Table 14 indicates how the raw data themes were organised into the three sub themes of: Symptoms of PCOS, Management of symptoms, and Experiences of PCOS treatment.

Table 14. The thematic structure of 'Living with PCOS'.

Raw Data Theme	Lower Order Theme	Higher Order Theme
Weight	*Symptoms of PCOS*	**Living with PCOS**
Fertility		
Excess hair		
Periods		
Mood		
Exercise	*Management of PCOS*	
Alternative medicine		
Diet		
Controlling weight		
Monitoring of symptoms		
Social support		
Medication		
Lack of information given	*Experiences of PCOS Treatment*	
Told to lose weight		
Only offered help to get pregnant		
Treated dismissively		
Self-research		

Symptoms of PCOS

Participants were asked to describe the symptoms of PCOS that they feel affected them the most. PCOS has a complex profile of hormonal, metabolic, and reproductive symptoms, and a range of these symptoms were reported by participants to have a considerable effect on their lives. Weight was reported by all participants to have a significant impact. Concerns included struggling to lose weight, gaining weight easily, and weight distribution, which in PCOS is often characterised by an accumulation of abdominal fat. Ultimately, this affected participants' self-image. Self-image was further affected by hirsutism, or excess hair, which is frequently seen in PCOS with high levels of androgens. One participant said:

'I don't look at myself most of the time, erm, because what I want to see and what is there, has never matched' (Kathleen, 29).

Most participants felt that poor mental health and low mood were persistent, regardless of their other symptoms. Some participants, including Shirley, attributed this to their PCOS:

'I mean with erm... PCOS...I've always had...anxiety, almost' (Shirley, 30).

In addition, a proportion reported difficulty conceiving and those affected lamented that this was the primary concern above all others. This had a further negative impact to mental health.

Management of Symptoms

Participants reported that they had attempted many strategies to control or manage their symptoms, ranging from medication and alternative medicine, to lifestyle approaches including diet, exercise, controlling weight, and monitoring symptoms. Their overall approach seemed to be to experiment and see what worked for them. For example, in terms of diet, participants separately attempted time-restricted eating, a low-glycaemic index (GI) diet, reducing sugar intake, and a vegan diet. Sometimes these attempts produced desired results, and sometimes they did not. After a series of trial and error, some participants appeared to find something sustainable that they perceived to have a positive impact on their symptoms. For example, Marissa described how her painful periods were seemingly attenuated by beginning a vegan diet, which she has followed since:

'When I had this period, I had no abdominal pain at all, I had no symptoms… if it wasn't like physically happening, I wouldn't have even known' (Marissa, 34).

Similarly, approximately half of the participants had experimented with different exercise regimes and some had experienced beneficial effects on their symptoms of PCOS, such as an increase in menstrual regularity. Like the approach to diets, participants tried various approaches to find something that worked for them. However, a lack of guidance often meant that some felt that nothing they had tried had 'worked':

'Ah, I've done loads of different, er, programmes, err like bootcamps and all that, in the gym, and just never really worked because I didn't know what I was doing I think' (Debbie, 31).

Experiences of PCOS Treatment

When participants offered their perspective on the awareness of treatment options for PCOS, most indicated that they had not been given enough, or any, information, which led to feelings of isolation, being 'in the dark' (Debbie, 31), or feeling like there would be no help

from, or referral to, specialists. One approach had been to turn to self-research in an attempt to find out more and to find a 'community' who understood their struggles:

'I've been on reddit… just to kind of, be, not necessarily interact with but at least read up on ladies who are like me, or who've been going through the same things, so that was also helpful like, there's a nice community out there of women who are going through it' (Kathleen, 29).

Some participants were provided with limited options for treatment by healthcare professionals. For example, several participants reported simply being advised to lose weight. Additionally, participants felt that their medical practitioners had a limited view of the effects of the PCOS, and were only offered help should they decide to try to get pregnant:

'The kind of typical method there was like, if you're trying to have a baby you know, we'll, we'll sort you out and if you're not then, we won't and we're not following any of the, you know, kind of things we should be doing from a health perspective' (Frances, 49).

This approach led to some participants feeling that they were treated dismissively, with medical practitioners failing to take into account the other health effects experienced by women with PCOS. The updated NICE PCOS guidelines (2018) indicate the conditions by which women with PCOS may be at higher risk of developing diabetes or CVD and recommend that women meeting these conditions be referred for further screening. However, Shirley reports that when she asked for cholesterol and insulin testing, despite meeting the conditions set out in the guidelines, she was denied by her GP.

'[they]…always had some excuse as to why they can't do them, or they just don't proactively even… tell you that they're available' (Shirley, 30).

5.3.1.2 Factors Influencing Physical Activity Behaviour

In this section, it was reiterated to participants that the term 'physical activity' in this sense included all types of structured exercise, but also lifestyle PA such as walking from place to place, housework, shopping, and activity as part of work. Table 15 below shows the organisation of the sub-themes and raw data themes.

Table 15. Organisation of themes for 'Factors Influencing Physical Activity'.

Raw Data Theme	Lower Order Theme	Higher Order Theme
Work	*Life Factors*	**Factors Influencing Physical Activity**
Time		
Routine		
Weather		
Accountability	*Self-Regulation*	
Making excuses		
Prioritising		
Weight	*PCOS Symptoms*	
Self-esteem		
Fatigue		
Mood		
Improving Symptoms		
Understanding Exercise	*Knowledge about Exercise*	
Socialising	*Social Elements*	
Competition		
Private Environments		

Life Factors

Life factors raised by participants included work, time, routine, and weather. Work was a barrier to some due to work stress and feeling tired after long hours. However, in contrast, one participant had changed shifts so that they worked what they felt were 'regular' hours. To her, these regular hours were a facilitator to PA compared to when she worked nights, or other 'unsocial' hours.

'I feel like I've probably got more time to fit in something regularly if I wanted to, like a class or something on an evening, cause I work more regular hours' (Tali, 34).

This indicates that there is no 'ideal' working pattern that optimises PA engagement, because participants had differing perceptions on how PA could fit in around work. Time was mentioned extensively, with reasons for time as a barrier including having a busy life, looking after children, and being unable to find an activity that takes place at a time that is convenient. Although not specific to those with PCOS, these factors are important because they are a product of the characteristics of the target population; that is, primarily young to middle-aged working women, sometimes with the added responsibility of child-care.

Two participants identified the benefits of incorporating PA into their daily routine, such as walking to and from work, because this did not constitute 'extra effort' on their part, and they were more likely to engage with it. However, if it involved going back out after returning home from work, they were less likely to engage. Thus, for these participants, PA was integrated naturally into their daily lives, something that participants who did not live close to their workplace were less able to benefit from. Again, this highlights the impact of individual circumstances, including location, availability of public transport, and perceptions of time.

Self-regulation

Several factors were grouped underneath 'Self-regulation', including accountability, making excuses, and prioritising. Having somebody to hold one to account was discussed as a facilitator to PA because participants felt that they did not want to 'let somebody down'. Similarly, if participants felt that they did not 'need' to be somewhere they were less likely to be enthused. One participant said:

'I do notice that unless I have to be somewhere and… something's expected of me, then, you know, there's always that, oh I'll do it tomorrow [laughs] kind of attitude' (Frances, 49).

Whereas if something is scheduled, participants were more likely to feel increasingly motivated to keep their plans. This likely links in with participants' perceptions of time; if something is pre-arranged, schedules can be managed around this, whereas a spontaneous decision may be more likely to be postponed to 'tomorrow' in lieu of other responsibilities.

Some participants discussed their tendency to make excuses not to engage in PA despite being presented with opportunities, such as an invitation from friends:

'they're in a WhatsApp group as well, and they invite me, and it's tonight actually, but I make excuses [laughs]' (Dolores, 32).

Participants frequently cited 'time' as a barrier to a PA. However, the findings suggest that there is a more complex underlying issue of prioritisation of PA (and competing demands). Some participants highlighted that they made time, while others indicated that in a busy life, PA fell down the list of priorities. Tali's account demonstrates how the importance of PA diminished as other responsibilities increased:

'when I were younger, I loved… keeping fit and doing things like that and then it has just sort of fell by wayside cause I've got older and you've got a house and you've got things to do and you've got a job and... it just becomes sort of less of a... priority doesn't it' (Tali, 34).

PCOS Symptoms

A multitude of factors including weight, self-esteem, fatigue, mood, and improving PCOS symptoms were considered to influence PA behaviour. The onset or worsening of symptoms, particularly those related to weight gain, seemed to provide some participants with increased drive to engage in PA. This was related more to appearance and self-image than health outcomes. For example, one participant when asked what prompted them to engage in PA said:

'Normally it's just like whenever I got... very... erm... upset about my body and… you just think, oh that's it, I'm gonna do some exercise' (Carrie, 19).

Negative self-image thus served as a motivator for some but a deterrent for others:

'[Be]cause I'd been big since I was...14 to 15, a major thing that always stopped me doing anything about it was shame' (Kathleen, 29).

Similarly, although weight gain initially increased the motivation to exercise for some, the difficulty in losing weight had the opposite effect, especially when continually told by healthcare professionals to lose weight despite their efforts. This was described by one participant as 'very demotivating' (Shirley, 30). For the participants, weight as a negative reinforcer may therefore be a facilitator to PA initially but associated self-image and frustrations related to weight loss may be a barrier to long-term PA.

Mood was another factor that perpetuated a cycle of motivation and de-motivation to engage with PA. Participants acknowledged that their low mood was a barrier and that they didn't feel like exercising, whilst simultaneously knowing that PA could improve their mood if they engaged. When they did not exercise, this further worsened their low mood, developing into what was described as a 'vicious cycle' (Shirley, 30). This was further exacerbated by those who experienced fatigue as a symptom, which prevented some from exercising and subsequently fed into feelings of low mood. Thus, this perpetuates the next cycle of low mood and lack of motivation.

For some participants, despite the barriers, the knowledge that exercise could improve the chances of conception (either naturally or through treatment) was their biggest facilitator above all. Participants receiving or who wanted to receive fertility treatment were particularly motivated to use PA to give themselves the 'best chance possible' (Dolores, 32).

Knowledge About Exercise

While participants reported knowing the benefits of PA and exercise, particularly regarding their PCOS symptoms, they also discussed a lack of knowledge about exercise in general. One raw-data theme made up this sub-theme: Understanding exercise.

Participants expressed frustration at the lack of guidance around effective exercise for PCOS and described a general feeling of not knowing what they were doing when it came to exercise and PA. Most were aware of general UK PA guidelines and had also sought out information on types of exercise that could help with their symptoms. However, they felt that many fitness professionals and instructors lacked empathy and the knowledge of PCOS as a metabolic condition that comes with challenges surrounding weight loss. Indeed, this lack of knowledge lead to some participants trying out many different types of 'popular' exercise formats and classes, only to find that they did not see the benefits for which they had hoped. This in turn impacted on enjoyment and left some participants with a negative view of exercise which affected their participation:

'You don't enjoy it and you don't see the benefit so then you don't wanna do it again, so it just becomes another fad, as with diets, erm because they don't...it just doesn't work' (Debbie, 31).

This barrier may be particularly relevant to this population because of the unique challenges faced regarding PCOS. Professionals need a specific understanding of the metabolic nature of the condition and the subsequent value of losing weight, which is often prudent in PCOS due to increased risk of health conditions and difficulty conceiving. They must also understand that despite the value of weight loss for some women with PCOS, it is also difficult to achieve and there may be external pressures and time constraints that only increase this difficulty. For example, many women need to lose weight within a specific time frame to undergo fertility treatment. Thus, the women in the study indicated a specific need to access information relevant not only to effective exercise at large, but to advice and guidelines specific to PCOS that takes into considerations these pressures, demands, and challenges of PCOS.

Social Elements

Various social elements were considered to influence PA behaviour. The raw data themes under this sub-theme were socialising, private environments, and competition.

There were two largely opposing viewpoints within the themes of socialising and private environments. Many participants felt that socialising positively influenced PA participation because they felt it made it more fun, provided more of an incentive than exercising alone, and served as a distraction so that more work could be achieved with less perceived effort:

'I could always do more if I went with a friend… because if I went with a friend, I'd be sort of like chatting and you don't sort of notice' (Tali, 34).

Others felt strongly that a private environment away from the public was critically important. One participant described the thought of big group classes as 'emotionally horrifying' (Kathleen, 29). This reflects the findings from other themes, such as shame and negative self-image brought about by appearance related PCOS symptoms. These findings suggest that while some may regard social situations as a motivator, increasing enjoyment and providing an element of competition, those who were self-conscious about their image would engage more with private environments or environments limited to a few friends or trusted individuals.

5.3.2 Section Two: Intervention Specific Themes

This section reports on the themes Effects of the Intervention and Challenges of the Intervention, and relates to objective two of the present study, which is to explore the experiences of the individuals who participated in one or other of the intervention groups.

5.3.2.1 Effects of the Intervention

Participants in either the exercise group or the LPAG were asked to discuss any effects or perceived changes as a result of the intervention. This could include physical, mental, or emotional changes. Participants were asked about changes they felt to be either positive or negative in order to obtain balanced feedback. Table 16 indicates the organisation of themes.

Table 16. Organisation of themes for 'Effects of the Intervention'.

Raw Data Theme	Lower Order Theme	Higher Order Theme
Feeling fitter	*Physical Health*	**Effects of the Intervention**
Weight loss		
Adoption of health promoting Behaviours		
Increased wellbeing	*Self-Efficacy*	
Increased confidence*		
Fatigue	*Effects on PCOS*	
Low mood		
Empowerment over PCOS		
Realising exercise feels good	*Attitudes*	
Learning about the health benefits		
Developing a healthier attitude		

*= in the exercise group only.

Physical Health

In both groups, participants reported the beneficial effects of PA on their physical health including feeling fitter, weight loss, and the adoption of other healthy behaviours.

Participants in both intervention groups reported improved fitness levels, the effects of which had extended into their daily lives. These effects were felt regardless of their baseline fitness level and degree of adherence to the intervention. Among the exercise group, several participants with lower adherence felt fitter and less short of breath when walking up hills and stairs, whilst one participant who considered herself to have a good baseline fitness level reported improvement after adhering to the end of the trial. This may indicate that even small increases in PA can have beneficial effects on physical fitness, regardless of initial fitness status, for those previously classed as physically inactive.

Participants in both groups noted weight loss or change in body shape/weight distribution. This was based on their measurements at the baseline visit and the follow-up visit, which included weight (kg) and inches lost around the hips and waist:

'I think it did have a positive effect… after we'd done the intervention I'd lost weight and inches' (Shirley, 30).

Additionally, participants noted that they felt more comfortable in their clothes and that their clothing 'fit better' even if their weight had only changed minimally. Even small perceived

changes in weight appeared to produce positive effects on self-image, perhaps as a result of increased self-confidence.

Various participants from both groups indicated their intent to continue with the level of exercise or PA they were engaging in during the trial, with some having already joined gyms upon completion of the trial. One participant described how the trial gave her the motivation she needed to return to regular exercise habits:

'I've joined… a gym again now so it were kind of a push, you know, for me to get back into it' (Janet, 26).

However, this was not universal, and one participant in the LPAG reported that the trial was not enough motivation for her to change her daily habits from what she would have done regardless. This potentially links in with 'accountability' as an influential factor for PA and may suggest that for some this is needed to form new habits.

In the exercise group, participants also adopted other healthy behaviours in tandem with the exercise sessions, including eating more healthily. This bolstered the positive effects of the exercise sessions:

'When I eat poorly that makes me feel really bad, so actually why don't I eat better and exercise and I can feel better overall?' (Debbie, 31).

This suggests that making healthier choices in part of one's life does not happen in a vacuum and may encourage making healthier choices in other parts.

Self-Efficacy

Participants across both groups reported effects related to their self-efficacy in the form of increased well-being, while in the exercise group only, increased confidence was also widely discussed.

In both groups, the effects on well-being included better sleep, greater mental clarity, and feeling more positive about themselves, which subsequently had a knock-on effect that left some participants feeling more able to deal with daily challenging situations. This happened even in lieu of weight changes:

'It was nice to just kind of think, oh well at least I feel better even if I wasn't noticing any physical changes like weight wise or size wise' (Kathleen, 29).

This indicates that the positive mental effects of increased PA are not necessarily linked to weight changes. This has important implication for PCOS where weight loss is often difficult to achieve and suggests that weight loss need not be the primary objective of PA to produce positive outcomes.

In the exercise group, participants discussed an increase in self-confidence because of the intervention. This was sometimes linked in with physical changes such as weight loss, which resulted in improved self-image, but it was also linked to increased physical capabilities and a heightened perception of what they could achieve: 'It did kind of like… make me realise that, oh yeah, I could do this if I really tried' (Carrie, 19). Again, this highlights that PA can be a therapeutic tool for PCOS regardless of weight loss.

Effects on PCOS

When participants were asked to consider the effect of the intervention(s) on their PCOS symptoms, participants across both groups indicated that they felt some of these had been improved. Resultantly, they also discussed a feeling of greater empowerment over PCOS.

Participants in each group indicated that the intervention had had an overall positive effect on their fatigue and energy levels and helped to break the vicious cycle of low mood, fatigue and PA. Participating in the intervention had made them feel more energised and subsequently much more motivated to do more the next time:

'I always felt a bit more energised the day after I'd gone and done my swimming… so I felt a bit more "let's go for it"' (Kathleen, 29).

Various participants across both groups described how they felt more in control of their PCOS symptoms and their bodies. This was due in part to seeing some to their symptoms and realising that lifestyle changes could affect these. They also mentioned how taking part in the trial was the motivating factor they needed to help themselves and regain control. However, the sense of empowerment for most participants centred around the educational aspects of the trial. This was in relation to learning about PCOS, receiving information about their own health at baseline and follow-up visits, and being directed to national treatment guidelines for PCOS. For example:

'Realising that actually the NICE guidelines had changed and that I could really kind of advocate for myself… now I feel like I've got more control over that' (Frances, 49).

This highlights the earlier issues raised regarding experiences of treatment, where many participants expressed frustration at the lack of clear guidance and reported not being told about treatment options. Learning about this information gave participants a greater sense of control over their condition.

Attitudes to PA and Exercise

Taking part in the PA intervention had served as a reminder of 'how good you do feel when you move more' (Courtney, 35). This led to a shift in attitude where participants then wanted to be more physically active just because it felt good. Similarly, upon seeing some benefits from regular PA to their health (either PCOS-related or otherwise), participants indicated an increased inclination to keep going after the trial. The ultimate effect of realising both the health benefits and that exercise feels good is that participants developed a healthier attitude toward exercise. This healthier attitude appeared to be more about enjoyment and being mindful of health rather than about weight or image. Certainly, this ties in with how participants discussed what motivated them to engage in PA; for many, it was a negative self-image or 'not wanting to be fat'. After the trial however, there appeared to be a shift from using exercise to prevent something 'negative' to using it to produce something positive:

'I think at first I were purely doing it for, like, aesthetic purposes, whereas now it is more for my health… just a bit more about… what's going on inside rather than the outside side of things' (Janet, 26).

5.3.2.2 Challenges of the Intervention

Participants were asked to outline anything they disliked or found challenging about their respective interventions, and if applicable, how they overcame those challenges. Table 17 indicates the organisation of themes.

Table 17. Organisation of Themes for 'Challenges of the Intervention'.

Raw Data Theme	Lower Order Theme	Higher Order Theme
Fitting in sessions	*Exercise Group*	**Challenges of the Intervention**
Lack of social connectivity		
Lack of weight training		
Difficulty level		
Weather	*External Factors (LPAG)*	
Practicality	*App-Related Challenges (LPAG)*	
Accuracy issues		
Phone as a distraction		
App as a motivator		

Those in the exercise group lamented that fitting in sessions around their daily schedule was sometimes difficult. This is in line with previously discussed factors influencing PA, where time and work were frequently mentioned as barriers by most participants. However, despite the difficulty, some participants overcame this challenge by elevating the priority of the exercise sessions: 'I prioritised it because it was important' (Debbie, 31). Indeed, the fact that adherence figures showed varying levels of participation despite participants citing similar time commitments supports the idea that the level of prioritisation may have been the influential factor.

Exercise sessions were one-to-one, to avoid any feelings of discomfort. Some felt that this lacked social connectivity with women who were in similar situations to themselves and suggested they would have benefited from exercising alongside others in the study. This may link in with participants describing a sense of isolation regarding their PCOS treatment, and that finding others with similar circumstances could be beneficial.

The exercise intervention was solely based on aerobic activity, beginning at a very easy intensity and ramping up in intensity (based on heart-rate zones) every four weeks until it reached a moderate intensity. This mode of activity was limiting for some who suggested they would have benefited from the inclusion of weight training. Additionally, the difficulty level of the sessions was discussed by various participants as challenging at times. Interestingly, participants tended to find the difficulty level too easy. One participant described how she felt that this was limiting, and she was not getting 'the full potential of the programme' (Carrie, 19). For some, though, as the programme ramped up, they began to find

it more physically challenging, and described how the duration of the session (forty minutes within the target heart-rate zone) made up for the moderate difficulty:

'What was brilliant about it was following this protocol that perhaps I'd train less hard but for a longer period of time… and I liked how it was… in a controlled way challenging me to like, improve my aerobic fitness' (Frances, 49).

Both challenges (weight-training and intensity) highlight the individual abilities and preferences of participants; indeed, those with PCOS are not a homogenous group, and this may suggest that flexible, more individualised sessions could produce further benefits.

Specific issues related to the LPAG intervention were: external factors including the weather, and factors related to the use of a phoned-based app. However, there was scant data relating to the LPAG because those in that group had little to say about the design of the intervention.

Using a phone-based app to record PA presented a set of specific issues. It was difficult for some to have the phone on their person at work and carrying it in a pocket rather than a bag was also risky.

'There was… one occasion, where it nearly went horribly wrong, erm, my phone fell out of my pocket while I was cycling' (Courtney, 35).

There were inherent drawbacks of using a phone-based app (rather than a wearable device) to track activity, because participants felt that constantly checking the phone for progress meant that they were getting 'distracted' and spending more time on their phone. In addition, the quality of the app itself was sometimes an issue due to a lack of accuracy or consistency in activity tracking and measurement., Finally, although some found seeing their progress throughout the day to be largely motivating, two participants asserted that this was not the case, and meeting their targets in the app was not sufficient reason to induce changing habits. This may relate again to individual prioritisation and accountability.

5.3.3 Section Three: Future Research and Scaling Up

Section three pertains to objective three of the present study, which is to gather suggestions to inform future research. This section provides a summary of issues and recommendations from all three groups of participants based on their experiences in the interventions, or their preferences for a future intervention if they were in the control group.

5.3.3.1 Recommendations for Future Research

The RCT in this thesis is a feasibility study, thus it is imperative to the objectives to obtain information on the acceptability of the procedures and interventions.

Table 18 summarises the participants' thoughts and suggestions on the intervention design and structure and the recommendations to inform design of a larger full-scale RCT which are derived from those suggestions. Issues 1-4 relate to the design of an exercise intervention. Issues 5 and 6 are concerned with more general study design considerations.

Table 18. Issues and Recommendations Raised by Participants Across Groups.

Number	Issue	Recommendation	Attribution
1	**Location**. The location must be within a reasonable distance, otherwise recruitment and adherence may be restricted.	Potential multi-site study with gyms in various locations across the city to ensure maximum reach.	Reported by all groups.
2	**Frequency**. The frequency of the sessions must strike a balance between two few (thus minimising benefits) and too many, increasing the burden on participants.	The established frequency (2/week for eight weeks and 3/week for the remaining four weeks) appeared to address these concerns and participants were satisfied.	Reported by the exercise group.
3	**Flexibility.** Work and study habits varied, and participants preferred different sessions (e.g. some evenings, some mornings). There were no suitable times for all.	Potential home-based exercise plan to be completed independently may address these concerns (and location). Technology, such as Zoom, could be used to increase accountability.	Reported by the exercise group and LPAG.
4	**Social setting.** Many felt self-conscious and would not exercise in large groups. However, many also felt isolated and desired social connectivity, particularly with other women with PCOS.	Undertake sessions in a confidential environment away from the public (as per the current protocol) but provide a choice of small groups of participants in each session or one-to-one.	Reported by all groups.
5	**Convenience.** Those in the LPAG enjoyed the convenience of engaging with the app-based intervention on their own terms and felt that had they had to commit to exercise sessions,	Consider a pragmatic approach to group allocation. If participants can choose their intervention, they may be more likely to adhere and	Reported by the LPAG.

	they would not have completed the trial.	reap benefits. This also reflects real-life circumstances, where women with PCOS have a choice in how they engage with PA, rather than engaging with an intervention under optimal circumstances	
6	**Health Monitoring.** Participants enjoyed receiving 'snapshots' of their health at baseline and follow-up visits. This impacted their behavioural choices and was connected to a feeling of empowerment over PCOS. Learning more about themselves imbued a greater sense of control.	In future designs, this component should be built in as part of the trial feedback that functions above and beyond the collection of study data and becomes a source of information for the participants. This may improve recruitment or adherence.	Reported by all groups.

5.4 Discussion

This qualitative explorative study provides detailed insight into participants' experiences with PCOS, their perceived barriers and facilitators to PA, and their experiences of participating in the present clinical trial. The themes explored in this discussion can contribute to evaluation of the clinical trial and facilitate the design of future research in this area. Importantly, it can be used to improve the experiences of participants, which subsequently may improve the success of such trials.

5.4.1 Living with PCOS

Participants from all three study groups outlined their challenges of living with PCOS. They highlighted a range of symptoms including weight gain or struggling to lose weight, difficulty conceiving, and excess body hair. In addition, many participants reported persistent low mood or poor mental health, which was sometimes the result of, or exacerbated by, their other symptoms.

Participants expressed a dissatisfaction with the lack of treatment options offered to them, a frustration with the apparent lack of knowledge medical practitioners had in relation to PCOS, and a sense of being treated dismissively or not taken seriously. Generally, in the field of women's health, women have reported feeling dismissed, talked down to, and are less

likely to be referred on for preventative screening of medical conditions including cholesterol screening (Phelan et al., 2000). The situation is similar for women in chronic pain, who have reported negative encounters with healthcare practitioners including scepticism, lack of comprehension, being belittled, being blamed for their condition, and even told their condition was psychological (Werner & Malterud, 2003).

For PCOS specifically, a worldwide internet survey of women with PCOS indicated that more than a third of women had spent over two years seeking a diagnosis to explain their symptoms, and had to see at least three separate medical providers to obtain this (Gibson-Helm, Lucas, Boyle & Teede, 2014). Furthermore, only 25% were satisfied with the information they were given concerning treatment options (Gibson-Helm et al., 2014). When asked what the respondents felt could be done to support women with PCOS, the most frequent answers were 'provide broadly available educational materials' and to support 'health professional education regarding PCOS' (Gibson-Helm et al., 2014).

The wider research is in line with the findings from the present study, where participants indicated a need for a greater understanding and empathy from healthcare professionals. In addition, the participants suggested that information regarding PCOS and how to manage symptoms should be more widely available. Women with PCOS have a great need for information in order to participate in shared decision-making with healthcare professionals and make helpful lifestyle choices (Avery & Braunack-Mayer, 2007). Participants often took a 'trial and error' approach to managing their symptoms, particularly their weight, and tried out various diets and PA regimens to find something that worked for them. Previous research has suggested that in terms of weight management, women with PCOS were more likely to engage in alternative non-lifestyle related weight management practices such as use of laxatives, diet pills, fasting, or diuretics (Moran, Brown, McNaughton, Joham & Teede 2017). Thus, providing accessible, PCOS-specific lifestyle advice may help women with PCOS find and utilise healthy, evidence-based approaches to PCOS management.

5.4.2 Factors Influencing Physical Activity Behaviour

Although trials and studies examining the physiological and psychological impact of PA and exercise interventions on PCOS are abundant, no studies have investigated how, or why, women with PCOS engage (or disengage) with PA on their own terms outside of a study

environment. The findings of the current study present a novel, in-depth look at what discourages or motivates women with PCOS to engage with PA. This information is prudent if studies and trials are to be successful, useful, and maintain adherence to interventions. In addition, information pertaining to the challenges of long-term engagement with PA could help increase participant retention and help participants forge longer-term PA habits.

In the present study, participants were asked to discuss what factors they felt influenced their PA engagement. Factors were raised that were both separate from, and inter-linked with, PCOS. For the former, participants raised several life factors (work, time, weather) and factors related to their own self-regulation (accountability, prioritising, making excuses). Indeed, several other studies have also outlined that lack of time and motivation are the most frequently reported barriers to PA, particularly for women (Arango, Patino, Quintero, & Arenas, 2011; Sharifi, Mahdavi, & Ebrahimi-Mameghani et al., 2013).

During the exercise intervention, participants were initially offered full flexibility of sessions in order to reduce time-related barriers to attendance. They could come during the day, evening, and even the weekend. Many participants appreciated this flexibility and remarked that it removed obstacles to their attendance. However, participants continued to cancel or fail to arrive at sessions despite special arrangements being made to fit in with their schedules. This may indicate that for some, lack of time is the perceived external barrier, but the actual barrier may be internal, such as the level of assigned priority to regular PA (Brinthaupt et al., 2010). Indeed, barriers to PA have been categorised by other authors as those that are objective, such as injury or inaccessible facilities, and perceived barriers, such as lack of time (Brinthaupt, Kang & Anshel, 2010). It is the latter, perceived barriers, which can present the biggest obstacles to regular PA, with 'lack of time' commonly identified as one of the most difficult barriers to overcome (Kang et al., 2007).

This is supported by the participants' responses; participants commented that exercise and PA 'fell by the wayside' in relation to other priorities such as work and home-related chores, and that they 'made excuses' despite opportunities. Furthermore, some participants in the exercise intervention commented that although it was sometimes difficult to fit in the sessions, they made this a priority because it was 'important'. In this study, these barriers represent the challenges of long-term PA engagement in women with PCOS who are commonly of childbearing and working age with multiple commitments. These factors present difficulty in committing to or prioritising PA unless there is an immediate benefit from doing so.

PCOS symptoms also present unique obstacles to lifestyle modification. In particular, research indicates a link between PCOS and increased incidence of mental health disorders including depression, anxiety (Himelein & Thatcher, 2006), bipolar disorder (Davari-Tanha et al., 2014), personality disorder (Kerchner et al., 2009), and binge eating disorder (Scaruffi et al., 2014). In this study, there was a cyclical interplay between participants' symptoms and emotions. For example, participants reported that feelings of fatigue and low mood led to low motivation and decreased PA. Subsequently, low levels of PA exacerbated low-mood, fatigue, and anxiety, thus perpetuating the cycle. Other research examining the barriers to lifestyle modification in PCOS has similarly reported tiredness, depressive and defeating thoughts, and low self-confidence as unique PCOS-related obstacles (Lim et al., 2019).

Weight and weight control were other factors that participants in the study discussed as important symptoms of PCOS that impacted their lives. Despite weight initially acting as a facilitator to PA, de-motivation from struggling to lose weight and subsequent negative self-image acted as a barrier to PA. This was related to self-consciousness when exercising around other people. Indeed, women with PCOS are more likely to have overweight compared to weight-matched controls (Lim, Davies, Norman & Moran, 2012) and to gain more weight over a period of time compared to women without PCOS (Teede et al., 2013). The subsequent negative self-image may be compounded by increased incidences of mental health disorders. Resultantly, participants stressed the importance of being able to exercise in a non-judgemental and relaxed environment in order to reduce the influence of negative self-image and low self-confidence.

Another important factor to note is the impact of shifting personal attitudes toward PA from negative reinforcement (where the behaviour is strengthened because it avoids a negative outcome, which for the participants was weight gain) to positive reinforcement (where they exercised for the inherent value of PA in making them feel good and attenuating PCOS symptoms) on PA behaviour. While participants indicated some success in using PA to prevent weight gain, many struggled to maintain this because the goal was difficult and, in some cases, unattainable. This was for a variety factors, including lack of knowledge about effective exercise and the de-motivation over time because of the perception that exercise 'doesn't work'.

Negative and positive reinforcement are principles of operant conditioning (Skinner, 1953) and both types have been shown to be effective in improving adherence to exercise

interventions (Strohacker, Galarraga & Williams, 2014) and in affecting greater lifestyle PA (Petry, Andrade, Barry & Byrne, 2013). However, the reinforcement schedule is critical to this, and interventions that provide reinforcers as quickly as possible after demonstration of the behaviour are more effective than those with delayed reinforcers (Lussier et al., 2006). Therefore, for the participants in this study, if exercise was not quickly effective in producing weight loss (the reinforcer), participants disengaged and were less likely to form long-term habits.

During the two interventions, participants became aware of new positive reinforcers that occurred more or less immediately following the demonstration of the behaviour. This included feeling good after exercising, seeing improvements in mood and fatigue, and slightly longer-term, feeling fitter and seeing improvements in PCOS symptoms such as menstrual regularity. Participants also appreciated the educational value in learning about PCOS and exercise and enjoyed seeing changes in their health-markers at baseline and follow-up visits. This led some participants to change their attitudes away from seeing PA as something only to be performed when one notices weight gain or struggles with their self-image, to something that can immediately produce myriad other positive benefits. Indeed, this was true for participants that noted either no, or inconsequential, weight change. This suggests the importance for those with PCOS of focussing on shorter-term, attainable goals where they can see tangible benefits relatively quickly to establish enduring PA habits and improved adherence rates to exercise interventions.

5.4.3 Evaluation and Feasibility of the Interventions

5.4.3.1 Intervention Effects

While the purpose of the trial was not to measure efficacy, participants' perceptions of the intervention effects have been explored. Both interventions led to reported improvements in physical health, self-efficacy, PCOS-related symptoms, and attitudes to PA. The latter, in some cases, had led to participants adopting health-promoting behaviours including joining a gym to keep up momentum, and improved diet.

In the present study, both groups of participants indicated their intent to implement health-promoting behaviours, but there was no long term (≥6 months) follow-up to assess this. A meta-analysis of PA and sedentary behaviour interventions in healthy, inactive adults

indicated that PA interventions are effective in promoting not only initial behaviour change, but also behaviour change maintenance (Howlett, Trivedi, Troop & Chater, 2019). In the meta-analysis of 16 studies, biofeedback, demonstration of the behaviour, behaviour practice/rehearsal, and graded tasks were particularly effective behaviour change techniques leading to initiation of increased PA (Howlett et al., 2019). In the present study, the two interventions included aspects of each. This included heart-rate monitors and apps that provided feedback on steps and calorie count (biofeedback), demonstration of how to use any unfamiliar equipment (demonstration of behaviour), a lengthy intervention that allowed practice of PA (behaviour practice/rehearsal) and an exercise programme that was graded in intensity, ramping up every four weeks to ensure participants could gradually increase their effort within their capabilities (graded tasks).

Participants in the study indicated improvements in self-efficacy and self-confidence, suggesting that the intervention had shown them that they could handle these activities and do more than they initially thought to control their condition. This was linked to a feeling of empowerment over their PCOS. Research indeed suggests that empowerment is an important factor that can be used as a strategy to increase self-management of chronic conditions (Tengland, 2008). In patients with T2D, studies suggest that empowerment is related to greater knowledge and ownership of their condition which leads to the development of effective self-care behaviours (Funnell et al., 2005; Hernandez-Tejada et al., 2012). Crucially, this self-management can result in improved clinical outcomes (Hernandez-Tejada, 2012). Hence, it may be a reasonable assumption that the exercise intervention initiated behaviour change, although maintenance has not been measured. To note though, some participants in the LPAG indicated that the intervention did not provide enough motivation to change long-standing habits of physical inactivity. Coupled with the presence of fewer behaviour change techniques and the suggestion that sedentary behaviour interventions are less effective in promoting behaviour change (Howlett et al., 2019), this casts some doubt on the effectiveness of the LPAG in long-term adoption of health promoting behaviours.

To counter this, particularly in lifestyle interventions where there is not a strong sense of accountability, such as in the LPAG, interventions may additionally address the psychological aspects of PCOS (Lim et al., 2019). This could be achieved by providing informative and educational material to participants to improve their sense of empowerment and control over PCOS. It could also involve signposting to psychological and/or self-help treatment or resources, or even incorporating behavioural interventions into the design.

Behavioural strategies are recommended by the International Evidence-based Guideline for the Assessment and Management of Polycystic Ovary Syndrome 2018 to optimise weight management, health, and emotional wellbeing (Teede et al., 2018). Use of these strategies may increase programme adherence and efficacy.

In the LPAG, the findings suggest that participants enjoyed the aspect of competing with oneself to out-do previous weeks' performance and push themselves to achieve more as the study progressed. This suggests that self-monitoring and goal setting played a central role in maintaining participants' motivation. In various meta-analyses of free and paid fitness-tracking apps, the most efficacious and frequently used behaviour change techniques are providing feedback on performance, self-monitoring, and goal setting (Middelweerd et al., 2014; Schoeppe et al., 2016). Indeed, the apps used by participants in the trial provided feedback (such as total time spent active, steps, and calories), self-monitoring (by providing real-time granular views on PA across each day and cumulative totals for weeks and months) and goal setting (by allowing participants to input their own PA goals).

However, these techniques were not successful for everyone, and some participants were not motivated by the app to change their behaviour. Although some apps incorporate more complex behaviour change techniques, the apps selected for use in this study were chosen due to their simplicity, ease of use, and compatibility with the majority of smart phones. Thus, more complex apps may bring about other challenges, such as being harder to use. The ideal solution would be to find apps that work for everyone based on the behaviour change techniques that work best for each individual. However, this is at variance with the principles of a clinical trial.

Thus, although the selected apps had limitations, the answer may not be different or more complex apps, but to introduce an app that can overcome some of the challenges noted by participants. This included higher phone usage, and the practicality (and even safety) of carrying around the phone to track activities. In order to increase safety, reduce distraction, and improve their practicality, interventions may consider the use of wearables instead of a phone-based app. Wearables, such as watches and wrist straps, provide all the real-time tracking features available in apps. They can then be synchronised with a smart-phone app so that all PA data can be held in the same place, but one does not need to carry the phone around continuously. As with apps, research in this area is proliferating quickly, and there is evidence to suggest that wearables have the potential to increase PA participation as part of

interventions (Brickwood, Watson, O'Brien & Williams, 2018). In addition, wearables are becoming increasingly affordable, with basic PA trackers as affordable as £10-£25, making them feasible for interventions with limited resources.

5.4.3.2 Exercise Intervention Design

When asked for their thoughts on the exercise intervention, participants frequently mentioned desiring social connectivity with other women with PCOS. This occurred when discussing factors influencing PA, was raised as a challenge in the exercise group, and was frequently suggested as a point for future research. However, participants also expressed extreme trepidation at the thought of exercising in big groups or with the public at large. Thus, organising sessions with several women with PCOS may facilitate the formation of relationships and connections, enable information and experience sharing, and subsequently may reduce feelings of isolation. This mutual support from other women may make the experience more meaningful and improve adherence and attrition rates.

This could work in much the same way as support groups. Support groups allow patients to share experiential knowledge, support one another, and share doctors' opinions (Huh & Ackerman, 2012). Additionally, social support can have a substantial impact on health, with research indicating that individuals with low social support have a higher risk of mortality compared with those with stronger social support (Cohen, Gottlieb & Underwood, 2000). Positive social networks can induce behaviour change and influence the uptake of health-promoting behaviours (Goldsmith & Albrecht, 2011). This may be especially important for patients who fear or avoid speaking out, because they suffer from health conditions that are stigmatised (Wright & Rains, 2013). Indeed, women with PCOS have described their condition as stigmatising, and have described feeling 'abnormal' and that they are not 'proper' women (Kitzinger & Willmot, 2002). Thus, the addition of social connectivity into the research design may provide broad physical and mental health benefits in addition to the PA itself and may influence the development of social support networks that could positively influence the long-term impact of the intervention.

Another challenge outlined by some participants was the difficulty level of the exercise sessions; that is, participants felt, particularly in the initial weeks, that the exercise intensity was too low. The intensity of the exercise sessions was set based on ACSM's recommendations for currently inactive individuals (ACSM, 2010). However, participants had varying fitness levels because some had never participated in regular exercise, whereas

others had in the past been regular exercisers but for various reasons were not currently meeting the guidelines. Tailoring intensity based on fitness level at baseline could subsequently improve satisfaction and have an impact on acceptability and enjoyment.

Enjoyment is a key factor in improving adherence to exercise intervention (Jekauc, 2015), and participants suggested that some personalisation in the exercise or PA would be beneficial for those with different abilities and influence enjoyment. The personalisation of an exercise programme may present some logistical challenges in the design of future interventions based on available personnel, resources, standardisation, and accessibility. However, attrition rates in lifestyle interventions for women with PCOS are often high and adherence rates low (Jiskoot et al., 2017; Moran et al., 2019; Norman et al., 2004). Thus, a balance between providing some choice to increase enjoyment whilst also maintaining a standardised programme could improve interventions.

Finally, the findings from this study indicate that participants' engagement with the intervention(s), and with PA behaviour at large, was influenced by their intrinsic motivation. That is, self-regulation, such as prioritisation of PA, as well as attitudes to PA, enjoyment, and how empowered participants felt to self-manage their condition, heavily influenced participants' engagement. These intrinsic motivators are the focus of several BCTs, notably the TPB (McEachan et al., 2011) and SDT (Ryan & Deci, 2000). Indeed, a variety of behaviour change techniques have been identified as useful in these interventions. However, designing future interventions in line with an appropriate BCT may help to develop a robust behaviour change strategy that incorporates the most successful techniques based on the factors identified here that influence the desired behaviour.

5.4.4 Strengths and Limitations

One strength of this study was the use of in-depth semi-structured interviews with open-ended questions to explore participants experiences and perspectives. This allowed participants to describe their experiences using their own frame of reference, rather than selecting pre-determined answers that would carry the researchers' own bias of pre-conceived themes. Further, the use of a qualitative component to contribute to the evaluation of a feasibility trial clarifies the social and personal contexts that may influence success in a full-scale trial. It also provides further information on the effects of the interventions that is not included in the quantitative outcomes.

Furthermore, purposive sampling allowed for more heterogeneity in the sample by selecting minority ethnicities and women of different ages. The addition of reported demographic and socioeconomic data provides transparency and allows for the evaluation of the transferability of the findings. Interviewing participants from the control group also meant that the wider experiences of living with PCOS and recommendations for future research design could be represented by participants with limited relationship to the researcher, and by those without prior significant involvement in research.

There were also several limitations. Primarily, only interviewing a small sample from each intervention group makes it harder to evaluate and transfer findings related to acceptability of the interventions, because there are likely other varying viewpoints that were missing coverage. This is particularly relevant to those participants who dropped out of the study and were lost to follow-up. However, a purposive sampling approach mitigates this effect. The recruitment approach for the interviews may also have discouraged certain participants from undertaking the interview. For example, those with only negative views or experiences of the intervention may have felt uncomfortable participating, particularly those with whom I had established rapport. In addition, social desirability bias may have affected results; RC conducted most of the interviews with the exercise group, but there may still have been an element of social desirability bias through our connection that impacted upon responses. This may also be the case for interviews that I conducted myself.

5.4.5 Reflections on the Impact of the Researcher on the Research

In this section, I will consider and acknowledge facets of my own behaviour that may have influenced this study. Firstly, it is likely that my relationship to the participants impacted the responses. I developed therapeutic bonds with participants in the exercise group, who would often discuss with me personal information related to their struggle with PCOS. I provided support and encouragement to all participants, including in the LPAG. Subsequently, many participants referred to me by name in the interviews (including those conducted by RC) as making the intervention educational, useful, and providing confidential support. Some also described me as a friend. Participants were also aware that this study was being conducted as part of my PhD. Thus, it is likely that participants were reticent to give negative feedback, or even that their experiences were biased, because of this relationship. To combat this, I maintained boundaries with participants outside of the study; I only spoke with them to arrange sessions or collect data. I also maintained, throughout the interventions and the

interview, that balanced feedback was imperative in order to improve future research in this area. I feel that most participants understood this necessity and subsequently tried to provide constructive feedback, even if it may have been perceived as negative. Further, a question in the interview schedule expressly asked participants for feedback on elements they disliked or did not enjoy.

My relationship with the participants may have also inadvertently affected who I approached for interview. I was naturally inclined to offer interviews to those participants with whom I had a rapport and where I felt that we were comfortable in conversation with one another. However, this could miss important viewpoints. To attenuate this, I purposely approached participants with whom I had less of a relationship (where possible) to minimise this impact. I strived for a balance of participants with whom I had differing relationships to provide a variety of opinions and reiterated the utility of balanced feedback at the beginning of each interview.

It is important also to consider this relationship with participants on the adherence and attrition rates of the exercise intervention. Indeed, some participants indicated that I helped to make the sessions fun or educational and this had a positive impact on their adherence. The element of support is inherent to a supervised exercise programme, and I offered this equally to all participants. However, it is also possible that some participants may have felt the opposite way and would have preferred to limit contact with me in the exercise sessions. To combat this, I made clear to all participants in the exercise sessions that I would be there to supervise and support the session, explain or demonstrate any activities, and check adherence to the protocol. However, I emphasised that participants could bring headphones to use while they were exercising if they liked and that I would be working on other things in the session. Thus, it was then up to the participants how much or how little we interacted after that.

During data analysis I tried to keep a balanced opinion on whether the interventions were acceptable or not. However, I found that I was naturally focusing on the positive aspects mentioned more than the negatives. To attenuate this, I used the comparative case method and deviant case method to ensure that negative or opposing opinions were incorporated and that the story told by the analysis made sense when all viewpoints were considered.

Finally, in order to conduct this research, I aligned my philosophical position with pragmatism. This approach most closely aligned with my view that while quantitative data is indeed valuable for capturing objective facts and figures, the addition of subjective data that

focuses on the rich perceptions and experiences of the participants allows for a more nuanced and in-depth analysis of the intervention holistically. In this way, qualitative and quantitative research strategies complement each other to answer the research question effectively.

5.5 Conclusions

This study explores the interplay between living with PCOS and PA behaviour and uses this in tandem with participants' experiences of the intervention(s) to assess the feasibility and make recommendations for future research design. In both intervention groups, there were many reported physical and mental health benefits, including the adoption of health-promoting behaviours. In the exercise group, scheduling, frequency of sessions, and the environment were found to be acceptable, with suggestions for how to improve acceptability including providing social connectivity, tailored plans for different abilities, and variation in modality. Adherence to the sessions overall was moderate, and suggestions for improving adherence are set out. In the LPAG, participants enjoyed the competitive aspect of tracking their PA data, but some were not motivated to change habits by the app. Apps that use more complex behaviour change techniques may be useful for future research. However, not all participants in the interventions were interviewed and this may impact transferability of the findings related to acceptability.

6 Discussion, Recommendations for Future Research, and Conclusions

Overview

In this chapter, the aims and objectives of the thesis are revisited and discussed. The key findings outlined in this body of work are synthesised, before considering the practical application of such findings and recommendations for future research, as well as the original contribution to knowledge. Finally, the reflections of the researcher and the conclusions are presented.

6.1 Overview of Gaps in the Literature

The literature reviewed in Chapter 2 indicated that women with PCOS have multiple risk factors for CVD, and that PA interventions can be effective in attenuating this. PA interventions of a moderate period (≥12 weeks) can be effective for improving lipid profile, ovulation, and insulin resistance independent of weight loss (Harrison, Lombard, Moran & Teede, 2011). This evidence indicates that PA interventions can have a role in improving PCOS symptoms and reducing CVD risk. However, although several systematic reviews have been conducted to clarify the parameters of effective PA intervention (Harrison et al., 2011; Hutchison et al., 2011; Kite et al., 2019; Ladson et al., 2011), there currently remains to be a paucity of sufficiently homogenous RCTs with high confidence in the evidence. Further, transparent research in this area is still needed to provide clear guidance for healthcare professionals and women with PCOS.

In addition, little research exists on the distinct mechanisms of sedentary behaviour and how this impacts the cardiometabolic features of PCOS. This has been explored in populations with similar metabolic conditions, such as T2D, with results that indicate a reduction in sedentary behaviour can have benefits for cardiometabolic health. Thus, there is justification for research examining whether this is a potential therapeutic target for women with PCOS.

The role of oxLDL in CVD risk in PCOS, and whether this is mitigated by PA, has not been explored. Numerous studies have demonstrated an association between CVD and oxLDL, with several studies indicating that oxLDL is an independent CHD risk factor (Amaki et al., 2004; Meisinger et al., 2005). Despite the presence of multiple risk factors in PCOS, including endothelial dysfunction, oxLDL is rarely used as a marker of CVD risk in PCOS.

Further research is necessary to investigate both the presence of elevated oxLDL in PCOS, and whether this indicates increased CVD risk.

Thus, there was justification for a programme of research, as part of this PhD, that includes a feasibility trial to address the gaps in this area. The MRC recommend that the development and evaluation of complex interventions begin systematically, using a carefully phased approach that begins with identifying relevant evidence and theory, before moving on to a feasibility approach where procedures can be tested (Craig et al., 2008). As such, this thesis reports on a systematic review and meta-analysis, a feasibility RCT, and a qualitative evaluative study.

6.2 Aims and Objectives of the Thesis, and Key Findings

The aim of this body of work was to investigate the acceptability and feasibility of a lifestyle intervention comprising supervised exercise and increased lifestyle PAto CVD risk in women with PCOS. This aim has been met, and new knowledge has been generated pertaining to understanding both the practical and more nuanced factors that impact the acceptability of a lifestyle intervention RCT. Below the objectives set out for the thesis are discussed, with consideration for the extent to which they have been achieved, as well as a summary of the key findings of each study.

6.2.1 Study 1: Systematic Review and Meta-Analysis

6.2.1.1 Objective One

The first objective of the thesis was to identify and evaluate the existing evidence to determine effective types of exercise intervention for women with PCOS. This objective was met as part of the research presented in Chapter 3. A thorough, comprehensive systematic review and meta-analysis were conducted to examine exercise interventions of differing modes, frequencies, durations, and intensities. The review was methodologically robust and identified areas for further investigation, as well as providing a base of critically appraised evidence from which to design a feasibility trial.

The findings of this chapter indicated that moderate aerobic exercise interventions ≥3 months in duration, with a frequency of three times per week for at least 30-minute-long sessions, has favourable effects on various cardiometabolic risk factors. However, there was a paucity of

gold standard RCT trials, and so the confidence in the evidence is limited. This finding indicates that there is a need for higher quality research, with fewer sources of bias, to provide specific recommendations to women with PCOS. In addition, few trials exist where exercise or PA were investigated in isolation; that is, without contraindications such as medications like metformin or OCP.

The findings from this chapter were in line with previous systematic reviews that have indicated that aerobic exercise interventions of ~3 months can be effective at reducing CVD risk (Harrison et al., 2011). However, in both previous research and the review conducted in Chapter 3, it was concluded that there is a lack of evidence surrounding different exercise intensities, such as anaerobic exercise, or resistance training. Thus, it was deemed suitable for the exercise intervention of the feasibility trial to contain aerobic exercise only.

The findings from the review, taken together with previous PCOS research and ACSM guidelines for exercise prescription, were used to inform the design of the next stage of development, which is the feasibility trial.

6.2.2 Study 2: Feasibility Randomised-Controlled Trial

6.2.2.1 Objectives Two and Three

The second and third objectives of the thesis were to: ii) evaluate the feasibility of a PA intervention comprising a supervised exercise arm and a lifestyle PA arm in women with PCOS, and iii) obtain oxidised LDL concentration data which will allow for a sample size calculation for a full-scale RCT.

Both objectives have been met. Objective two was met in Chapter 4, where the findings from the feasibility RCT have been presented. The trial assessed the acceptability and feasibility of two PA interventions: a supervised exercise intervention and a lifestyle PA intervention in women with PCOS. Procedures for recruitment, allocation, the intervention, and the outcome measurements were assessed against feasibility criteria to determine areas where refinements are necessary before proceeding to a full-scale RCT. Objective three was also met in this chapter; data on oxidised LDL were collected from a sufficient number of participants, and this provides an estimate of the population SD that has been used in a sample size calculation, as presented in Chapter 4.

In the trial, recruitment rate over time was slower than anticipated (2.25 enrolled per month). However, despite this, 82% of the volunteers screened were eligible. This indicates that the eligibility criteria were not too restrictive. The rate of eligible participants successfully recruited into the trial was 56%. The most frequent reason given for declining to participate was being unable to commit to the demands of the study. Indeed, the study requires a significant time commitment for those randomised to the exercise group. Thus, there is potential for small incentives (as compensation for time, only) to be considered to increase recruitment.

The retention rate was high (89%) and this was well within the criteria for acceptability. In addition, there were only two unrelated, non-serious AEs that had no impact upon attendance or performance in the exercise sessions. However, despite this, the adherence rate to the exercise intervention was lower than that seen in other supervised exercise interventions for people with chronic conditions (Bullard et al., 2019). This would present a significant issue in a future-scale trial and must be addressed. Thus, suggestions for increasing adherence using behaviour change techniques (such as self-monitoring, goal setting, reinforcement, and feedback) are set out in the findings (Aitken et al., 2015; Room et al., 2017).

Engagement with the app in the LPAG was high; that is, all participants that completed this intervention sent their data as required every week. Although this does not indicate how much participants used the app in their day-to-day life, it suggests that the procedures were acceptable to the participants. However, utilising an underpinning BCT may improve acceptability. The PA data has not been analysed here, so no judgements about the ability of the intervention to increase lifestyle PA can be made at this time. However, the engagement rate provides a promising foundation on which to build a fully powered RCT, where statistical analysis of PA data in tandem with clinical outcome data can provide information on its effectiveness.

The purpose of the trial was not to measure the efficacy of the interventions; however, baseline and post-intervention data on characteristics and biochemical analysis are presented. Trends indicate a reduction in weight, WC, and WHR in both the exercise group and the LPAG. This is in line with findings from Chapter 3, where exercise interventions of around three months have been shown to produce modest reductions in weight.

In addition, the results indicated a downward trend in oxidised LDL in the exercise group, and to a lesser extent, the LPAG, compared to baseline. However, the wide SD combined

with the relatively small sample size suggests high variability, thus this should be interpreted with caution and further research using a fully powered trial is necessary.

6.2.3 Study 3: Qualitative Evaluative Component

6.2.3.1 Objectives Four and Five

Objectives four and five were to: iiii) evaluate the acceptability and efficacy of the interventions using semi-structured interviews, and v) to identify barriers and facilitators to PA in women with PCOS, and develop recommendations for the refinement of a full-scale RCT.

Both objectives have been met in Chapter 5, which reports on the qualitative evaluative component of the feasibility RCT. In-depth, semi-structured interviews were conducted with eleven participants who were followed up after participating in the feasibility trial.

Using semi-structured interviews with participants from all three groups allowed an examination of the social and contextual factors that impact the acceptability of the intervention. The MRC recommends the evaluation of complex interventions using qualitative methods (Craig et al., 2008). In this trial, the qualitative component was utilised to increase understanding of how actual implementation of procedures differs to planned implementation. It was also employed to optimise trial conduct, and to develop ideas for future research that considers the lived experience of the target population (O'Cathain, 2013). In this vein, objective five was also met, because the interview allowed for an analysis of barriers and facilitators to PA in women with PCOS. These factors were investigated to develop recommendations that may impact the success of a full-scale RCT that employs PA interventions, particularly regarding adherence to such interventions.

The findings from this study indicated that the procedures overall were acceptable and well received. In particular, the scheduling and frequency of the exercise sessions, and the exercise environment, were acceptable. There are some suggestions for improving enjoyment, which is a key factor impacting adherence to exercise interventions (Jekauc, 2015). These included providing social connectivity for the development of social networks, plans tailored more specifically to individual fitness levels, and some variation in modality. Participants also reported various perceived effects of the intervention, including feeling fitter, less fatigued, and adopting other health-promoting behaviours.

In addition, analysis of barriers and facilitators to PA indicated that individual prioritisation of PA could be an important factor influencing PA participation, which may be applicable to PA interventions. While participants may cite 'lack of time' as a perceived external barrier, the actual internal barrier may be assigned priority of PA. Indeed, this is supported by some participants remarking that PA 'fell by the wayside' in favour of other priorities, or that they tended to 'make excuses' when presented with opportunities. Conversely, other participants indicated that they prioritised the exercise sessions because they were important, despite having other time commitments. Suggestions for encouraging prioritisation of PA included providing timely positive reinforcement in the form of biofeedback (such as weight or physical fitness changes), and setting shorter-term, attainable goals where participants can see tangible benefits relatively quickly. This may also impact self-confidence and create a feeling of empowerment, which is an important factor that increases self-management of chronic conditions (Tengland, 2008).

Indeed, this is in line with findings from Chapter 4, where adherence rates were lower than the acceptability criterion. The addition of these contextual findings aids in providing a greater understanding of the nuances affecting adherence to the intervention, beyond lack of time or logistical difficulties that may not have been illuminated without a qualitative component.

The findings from the LPAG indicated that use of the app was acceptable, with participants enjoying the aspect of competing with oneself, and self-monitoring to reach goals set for themselves. However, this was not effective for everyone, and there was not a strong sense of accountability for participants in this group. This could be attenuated by employing apps that use more complex behaviour change techniques; however, there is likely to be high individual variation in which technique works and for whom. Thus, suggestions for increasing accountability include promoting empowerment through informative and educational material on how to manage PCOS, or signposting to psychological and/or self-help treatment or resources. In addition, evidence based BCTs should be utilised in the design of the intervention to aid in selection of an app that employs effective behaviour change techniques for this population/desired behaviour. Finally, some safety and practical issues were raised regarding the use of a phone-based app, and an intervention that utilises low-cost wearables may attenuate these concerns.

6.3 Practical Implications

The findings from the studies reported in this thesis have added valuable evidence regarding PA interventions for women with PCOS. In this section, the findings from the three studies in this body of work will be considered together to set out the practical implications of the research.

6.3.1 Synthesised Findings

The evidence collected in these studies indicates that a lifestyle intervention, comprising both a supervised exercise intervention and a lifestyle PA arm, may be feasible for women with PCOS if evidence-based strategies to improve adherence are incorporated. There are several findings from this body of work that highlight the nuances and complexities of implementing PA interventions in women with PCOS.

Firstly, data from the RCT indicated a lower than acceptable adherence to the exercise intervention, which is a frequent issue in lifestyle studies. The qualitative study allowed an in-depth analysis of the complex and nuanced reasons behind this. The evidence indicated that adherence may have been impacted by individual prioritisation of PA, lack of social connectivity, and features of PCOS that lower motivation, including low mood, fatigue, or mental health comorbidities.

Secondly, data from the RCT indicates that engagement with the procedures in the LPAG was high, with no instances of missing data. However, further analysis from the qualitative study suggests that in this group, motivation to change PA behaviour was lower, potentially due to a feeling of lower accountability. This could have implications for the acceptability and the clinical benefit of the intervention.

Thirdly, the clinical outcome data, although not statistically powered, indicated that there are potentially greater benefits from the exercise intervention compared to the LPAG. However, the qualitative data suggests that participants in both groups perceived health benefits including feeling fitter and less fatigued, and signalled their intention to maintain longer-term PA habits. Furthermore, fewer participants were lost to follow-up in the LPAG compared to the exercise group. Again, this raises questions about the clinical and long-term advantages of each intervention, and what type of intervention may be of most benefit to this population.

Thus, this evidence has the potential to be translated into practical action that can be used to i) improve and refine PA interventions for women with PCOS, with particular emphasis on increasing adherence and engagement, and ii) contribute to recommendations and guidelines around initiation and maintenance of PA in women with PCOS, as part of self-management. The findings are discussed in more depth below.

6.3.2 Adherence

In addressing the issue of lower-than-acceptable adherence to the exercise intervention, the key barriers to PA in women with PCOS, as elucidated in the qualitative study, must be considered. They can be summarised as follows:

i) Self-regulation, including prioritisation of PA and accountability.
ii) Features of PCOS, including fatigue, low mood, or mental health comorbidity.

These findings can be applied to the design of PA interventions for women with PCOS to improve not only adherence, but to provide a positive experience for participants that may help to form long-term PA habits.

Overall adherence to the exercise intervention was 53%. That is, of all scheduled sessions, participants attended 53%. The qualitative interviews used purposive sampling to select participants with varying adherence levels, from 18% to 96%. In the interviews, exercise group participants indicated that a key barrier to PA was low prioritisation of exercise or a lack of accountability. This was common to all participants across adherence levels.

To improve self-regulation, there are various behaviour change techniques that can be employed. This should ideally come in the form of a planning session before participants undertake a PA intervention. Taking time to set attainable goals, including interim goals, and to provide feedback in relation to those goals, may help to improve both prioritisation of PA and accountability.

All participants that were interviewed in the exercise group indicated that attending sessions had provided benefits including improved mood, feeling more able to deal with challenges, and feeling fitter. Thus, providing reinforcement throughout, or helping participants to see these benefits of PA as a reinforcer, could help participants find PA rewarding for its inherent value, and not see it as method to improve something they do not like about themselves, such as their weight. For example, providing participants with information about the progress of

their physical fitness, or asking them to describe their mood after sessions, could help participants to recognise the widespread benefits of PA. Education sessions that outline the positive impact of PA on PCOS may also be key to affecting long-term behaviour change, which will ultimately have wider benefits to health than a short-term exercise intervention alone.

There are also several strategies that should be employed to promote enjoyment and support, which should be at the forefront of an intervention. This primarily ensures that participants have a positive experience and may also impact long-term behaviour change. For example, providing a social network of women with PCOS, in the form of small exercise groups, allows participants to engage in mutual support and information sharing. This may make the immediate intervention more enjoyable, but may also help participants to feel greater self-control over their condition and thus forge long-term PA habits. In addition, providing a variety of modes of PA is important, because it allows participants to choose a mode that suits them best. Although bespoke training plans may be difficult to implement, providing options where possible may increase enjoyment.

The features of PCOS present a unique barrier to PA that can impact on PA interventions. Fatigue, low-mood, and mental health comorbidity are some key barriers observed in this body of work that are supported by other research into lifestyle modification in PCOS (Lim et al., 2019). The implication of this is that providing behavioural support, or signposting to behavioural support and/or self-help resources, could be used to maximise not only adherence but also participant self-management, health, and emotional wellbeing (Teede et al., 2018).

These findings are also applicable to the LPAG. Although the RCT showed high engagement with the app procedures, some participants that were interviewed indicated that the app was not enough of a motivator to change their daily PA habits. That is, although they were providing data from the app, they may not have been striving to increase their daily PA. Thus, it is important that any goal setting, behavioural support, or education sessions are also applied to this group to maximise engagement and increase the clinical benefit of the intervention. In addition, BCTs should be considered in future design to incorporate evidence-based behaviour change strategies to improve acceptability and feasibility.

There are several findings that may indicate that a pragmatic design over a randomised design may work well for this population. For example, adherence to the exercise sessions was moderate. In their interviews, participants indicated that if they had been randomised to the

exercise group, they would have been unable to commit. Finally, a large proportion of eligible volunteers declined to participate because they were unable to commit to the schedule. While a randomised trial is the 'gold standard' design for pharmaceutical research, there is some criticism toward their use in highly individualised, complex interventions (Bothwell et al., 2016). Pragmatic designs can reflect real-life circumstances, where individuals with PCOS will have a choice in how they engage with PA, rather than engaging with an intervention under optimal circumstances. In addition, it may reduce participant disappointment if they are not offered the intervention according to their preference. This may create imbalanced groups and/or introduce bias that affects the validity of the results (Patsopoulos, 2011). However, this may be an acceptable trade-off if it led to increased adherence, enjoyment, and ultimately to greater benefits for participants. This may reflect real-life effectiveness of an intervention, rather than the efficacy of it under optimal, controlled conditions, a condition which is not likely to feature in the treatment of PCOS.

Finally, another potential solution to the lower-than-acceptable adherence in the exercise group is co-production. While the above implications centre on personal agency and improving motivation through individual behaviour change strategies, PA behaviour is also influenced by social and environmental barriers. That is, increasing motivation to PA in isolation may be ineffective if barriers in the social and physical environment remain (Sallis et al., 2012). Similarly, the environment alone is less likely to influence long term PA behaviour (Hunter et al., 2015). Thus, a multidisciplinary approach whereby BCTs develop desired behaviours and the physical and social environment provides opportunities for PA may be most effective (Speake et al., 2016).

Co-production is the concept of combining existing evidence with user-centred design in specialist establishments (Matheson et al., 2013); for example, co-locating community leisure facilities with NHS clinical teams, researchers, and patients. This is to promote an ethos that maintenance of good health through PA is a normal way of life and provides opportunities for individuals to choose PA as an NHS pathway (Speake et al., 2016).

Co-production may therefore have potential value in increasing adherence to (and thus effectiveness) of PA interventions for participants with health needs. Combined with an evidence-based approach to behaviour change, co-location may remove physical and social barriers to PA and help to affect change to attitudes around PA for health both personally and at a community level.

6.3.3 Exercise Intervention versus Lifestyle Physical Activity Intervention

The use of a multi-methods approach to evaluate the interventions allows for a more nuanced analysis than that which quantitative analysis alone would allow. Consideration of all the findings together indicates that there are merits and disadvantages to each intervention, and these highlight the complexity when considering which intervention is 'best'. For example, while the supervised exercise intervention provided greater accountability and one-to-one social support than an individual app-based intervention, adherence was still below acceptable limits, potentially wasting resources, and participants highlighted several issues that affected this. In addition, while the engagement with the app procedures was high, with no missing data, and resource use was low, participants lamented issues with motivation and missed out on key social support.

A large, full-scale RCT with an exercise intervention is costly and resource heavy. An exercise intervention may have statistically significant results, but if it has low adherence and does not promote behaviour change, it may not provide a health benefit to the target population in terms of long-term outcomes. Thus, its clinical significance is limited. Similarly, an app-based intervention may have a smaller magnitude of effect but is cheaper and less of a burden to participants (although it is more difficult to implement behaviour change). As such, cost-effectiveness should be considered alongside effectiveness for both interventions. A health economic evaluation, including long-term follow-up, should be conducted alongside a full-scale RCT to assess the cost per unit of effectiveness, and to maximise social benefits as well as efficiency of resources (Shiell, Donaldson, Mitton & Currie, 2002).

Ultimately, the key to providing the greatest benefit to women with PCOS in terms of their health is to use the intervention(s) to increase individual prioritisation of PA, so that long-term health-promoting behaviours - that endure past a trial - will be adopted. The behaviour change techniques that have been outlined in these findings present an opportunity to improve adherence and engagement to intervention trials. However, it is also an opportunity to affect the bigger picture; that is, promoting education and awareness of the benefits of PA to women with PCOS, so that they can be empowered to take steps which improve their health outcomes in the long term.

6.3.4 Clinical Benefits

Few studies have examined the role of oxidised LDL in PCOS. Additionally, no other studies have examined the effect of a lifestyle PA intervention on concentrations of oxidised LDL. The findings from the feasibility trial indicate that in this population, oxidised LDL, as measured by commercially available ELISA, has a wide SD, indicating high variability. This has been found in other studies measuring oxidised LDL. As such, due to the small sample size, outliers in the data may have influenced the mean to be higher than that seen in other populations. The implication of this is that a sufficiently powered trial should be conducted to detect statistical differences before inferences can be made about oxidised LDL and CVD risk in PCOS. Chapter 4 sets out a calculation to determine a sample size for such a study.

This study is the first to incorporate an intervention aimed at increasing lifestyle PA in women with PCOS. The findings indicate that this intervention was acceptable to participants. Although this study was not statistically powered, the preliminary findings suggest that there may be some cardiometabolic benefit to such an intervention. In addition, the qualitative data suggested that participants felt fitter and had improved mood. Thus, the findings from this body of work, combined with confirmatory evidence from future trials, could provide a basis to examine the effectiveness of such interventions in PCOS. This evidence would be useful for healthcare professionals when providing recommendations to women with PCOS, particularly in line with decreasing the risk of diabetes or CVD.

In clinical practice, the application of the findings from this thesis could contribute to recommendations and guidelines for self-management, which is critical to long-term management of PCOS. Lifestyle intervention is regarded as the cornerstone of treatment for PCOS, but the findings from this work indicate that the advice given by healthcare professionals could be improved. Creating educational resources for both healthcare and fitness professionals around the specific challenges of PCOS and its effect on metabolic health may lead to a more empathetic approach and higher-quality advice. In addition, resources for women with PCOS, such as support groups, should be recommended to promote information sharing, increased emotional well-being, and a greater feeling of empowerment over one’s condition. Promoting a sense of empowerment could lead to greater self-management of the condition, and long-term behaviour change in relation to PA, that has important implications for health in this population.

6.4 Future Research Recommendations or Priorities

The next priority for future research in this line of work is a full-scale definitive trial with an internal pilot study. The MRC state that a pilot study should address the main uncertainties that have been identified in the development work (Craig et al., 2008). As such, the practical implications outlined in the previous section can be used to refine the design.

An internal pilot study is part of the main study, but with an interim evaluation of the intervention and procedures using a proportion of the total sample. It can also be used to re-calculate sample sizes based on outcome data gathered in the pilot (Torgerson & Torgerson, 2008). Thus, any refinements made to the intervention and procedures resulting from the feasibility trial can be applied. The internal pilot study can be used to evaluate the impact of such refinements on recruitment and adherence, whilst the data will also contribute to the final analysis. An internal pilot study has advantages over an external pilot study (which is carried out independently before the main trial) because it prevents the delay of the main study and is more likely to obtain funding (Lancaster, Dodd & Williamson, 2004). However, a limitation is that it may be more difficult to fully apply the findings to the main design.

The sample size calculation in Chapter 4 indicates that to detect statistically significant changes in oxidised LDL, the study would need 42 participants per arm. This equates to 126 participants in total. In consideration of the retention rate (89%), 142 participants would need to be enrolled to account for the 11% attrition rate. Since the rate of recruitment was relatively slow (2.25 participants per month), a multi-centre study with three recruiting sites would allow recruitment of 6.75 participants/month. Thus, the recruitment target could be met in 19 months.

During the pilot study, various refinements to the design that have been suggested previously can be assessed. For example, incentives to improve recruitment, employing low-cost wearable fitness tracking devices in lieu of a phone-based app, pre-intervention goal-setting sessions, signposting to behavioural resources, and small group-based exercise sessions with a focus on generating social connectivity. Because the pilot study is an internal pilot, the assessment of the study can inform whether the main trial is likely to reach its recruitment and adherence targets. If this seems unlikely, the trial can be stopped to save resources (Herbet, Julious & Goodacre, 2019). The sample size calculation can also be re-assessed with updated and more comprehensive primary outcome data.

Future PA interventions in this area should consider whether they are accessible to those from socioeconomically disadvantaged groups. Indeed, the socioeconomic data collected from participants in the qualitative study indicated a high proportion of participants educated to at least undergraduate level, with annual household incomes of at least £31k. This could indicate that the barriers and facilitators identified are relevant only to those from similar backgrounds, and the challenges may be different in other groups. A full analysis of socioeconomic data was not undertaken in the feasibility trial. However, it is important to consider that people from socioeconomically disadvantaged groups are more likely to experience adverse health outcomes associated with inactive lifestyles, whilst also having low response rates and high attrition in PA intervention studies (Craike, Wiesner, Hilland, & Bengoechea, 2018). Thus, PA interventions may benefit those who need it the least; that is, those already willing and able to engage in PA (Bonell, Jamal, Melendez-Torres, & Cummins, 2014). As such, women with PCOS in socioeconomically disadvantaged groups may be the most likely to benefit from PA interventions, but least likely to be able to access them.

Suggestions for ensuring the intervention is accessible and appealing to those from socioeconomically disadvantaged backgrounds include active and targeted recruitment in this area. This should include partnering with community stakeholders and organisations, and ensuring study personnel are well trained and ethnically, linguistically, and culturally matched to the population of interest (Carroll et al., 2011). In addition, to improve retention, the intervention should consider cultural tailoring, demonstrate efficient tracking of participants, and demonstrate an overall caring attitude toward participants (Carroll et al., 2011).

The impact of health inequality and the use of internet-based smartphone apps in interventions should be considered as a future priority. Areas of greater deprivation have been shown to have higher incidences of PCOS (Ding et al., 2016). In addition, socioeconomically disadvantaged groups engage in lower levels of PA and have higher rates of obesity and metabolic dysfunction (Merkin et al., 2011). However, smartphone and internet access/usage may be less dependable and reliable for these groups. Thus, a group that may benefit the most from the intervention may be unable to access it due to affordability or lack of a dependable device. These barriers to participation may have an impact on the validity of the results. That is, if these issues are not addressed, a sampling bias may occur whereby the participants in the study are not from socioeconomically disadvantaged groups and the health-related

outcomes are disparate from those in the actual population. The findings may then appear promising; however, these are not likely to translate into real-world impact where additional barriers are faced by those from disadvantaged groups. This emphasises the importance of representation of the true population in study samples.

When designing a full-scale RCT, researchers should consider the costs and/or resources that participants will need to participate and minimise these where possible. While apps may be free at the point of usage, these still require a working device, typically with a mobile phone contract to access the internet. Thus, prepaid internet access could be provided alongside low-cost devices, or consideration could be given to apps that work 'offline' or use less data. Importantly, the use of PPI to engage with individuals from the target population (including those from socioeconomically disadvantaged groups) is a valuable tool that includes the end-user in the design of an intervention that works for them, rather than being prescribed to them without their input (Speake et al., 2016). This could be used to identify any barriers to the use of devices and technology in this population.

An objective of the feasibility trial was to identify the value of stratifying women with PCOS based on their PCOS phenotype. In particular, whether or not they display hyperandrogenism as a characteristic of their PCOS profile. However, restrictions placed on the laboratory due to Covid-19 prevented necessary equipment training that would have allowed for the quantification of testosterone concentrations. As such, it was not possible to assess whether this would be feasible or indeed useful to a full-scale trial. However, previous research suggests that those with a phenotype encompassing hyperandrogenism have a worse metabolic profile than other phenotypes of PCOS (Dewailly, 2016). It is therefore important to parse data on effectiveness of PA interventions based on PCOS phenotype in future studies. This would allow more specific recommendations for women with PCOS that move away from the current one-size-fits-all approach. This is especially prudent given that research continues to illuminate the myriad pathophysiological disruptions that occur in PCOS. As such, the best approach to management may differ based on the specific pathophysiological disruptions that are present.

The trial outlined in this thesis assessed the feasibility and acceptability of a lifestyle PA intervention that aimed to increase lifestyle PA behaviour in women with PCOS. This is the first such study, to the researcher's knowledge, to examine this. The findings from this study indicated such an intervention to be feasible and well received. However, more research is

required to justify this approach; in particular, cost effectiveness in comparison to a supervised exercise intervention should be analysed, and efficacy data is necessary to justify whether this could be a viable and worthwhile therapeutic target in women with PCOS. The potential advantages of such an intervention are increased agency and convenience for participants, and potentially fewer associated costs and resources than a fully supervised exercise intervention. However, the effectiveness may also be inferior, depending on adherence, and so future studies should look to analyse the factors that impact the overall worth of such an intervention, and where it might fit in the approach to management of PCOS.

Finally, although numerous studies examine the effect of PA interventions for women with PCOS, studies tend to be short and intensive (approximately three months). Few studies conduct long-term follow-up to identify whether a PA intervention has an impact on long-term behaviour change. This is an important oversight, because interventions that encourage enduring behaviour change may have significant health economic advantages. That is, if interventions can increase empowerment, encourage self-management, and promote positive attitudes toward PA, this may reduce the financial impact on health services. Indeed, the estimated annual costs of treating PCOS-related infertility in the UK range from £16-£22 million (Rajora, Goli, Savner, Singh & Bhutani, 2019). This does not include the costs of secondary conditions arising from PCOS such as diabetes. Thus, future studies should look to employ behaviour change techniques that encourage the adoption of long-term, health-promoting behaviours, and ensure adequate follow-up of participants to assess these.

As previously outlined, a way to promote the long-term uptake of health-promoting behaviour whilst maximising the effectiveness of the interventions would be to incorporate BCTs into the design of future interventions. By integrating these with the intervention design from the beginning, an appropriate BCT can be selected that will help researchers to understand the factors that influence the desired behaviour in a specific population, and how these change over time. As such, BCTs that are effective in improving PA behaviour, such as TDF (McEachan et al., 2011) and SDT (Ryan & Deci, 2000) are more likely to produce stable and enduring changes to participants' attitudes, motivations, and intentions toward PA. This may increase not only engagement with the intervention, but also promote long-term behaviour change that improves self-management of PCOS, and ultimately have an additional positive economic benefit.

While the individual limitations of each study have been set out in the corresponding chapters, there are some overarching limitations of the studies in this thesis. One such limitation is that only women with PCOS who are not taking any other medications, such as OCP or metformin, have been considered in this thesis. For example, the criteria in the systematic review stipulated that medications were a contraindication to inclusion. Further, the eligibility criteria in the feasibility trial also excluded women taking OCP or recent administration of metformin. Thus, the findings set out herein may only be applicable to a subset of women with PCOS that are not taking medications as a management tool. In real terms, this may be few women, since medications are often administered alongside lifestyle advice. However, it is important that non-pharmaceutical avenues of treatment are thoroughly explored, and the findings here contribute to evidence for such an approach. In addition, the analysis of socioeconomic data across the feasibility trial and the qualitative component may have provided valuable contextual information about the acceptability of PA interventions in subsets of women from differing backgrounds. Finally, the interventions were not designed in line with an underpinning BCT. Indeed, several behaviour change techniques were identified and suggested for use in a future trial. However, the incorporation of an appropriate BCT (selected by use of a tool or framework) will help to design interventions with evidence-based and effective behaviour change techniques based on the population and desired behaviour.

6.5 Contribution of the Thesis to Knowledge

To summarise, the studies reported in this thesis have made the following original contributions to knowledge:

1. The systematic review and meta-analysis provide clear confirmation that there are a lack of RCTs examining the effects of PA interventions in women with PCOS. In particular, it highlights inconsistencies across studies that include differing diagnostic criteria, heterogenous inclusion of medications, and a lack of supervised exercise interventions.
2. The feasibility trial has shown that an RCT of two PA interventions is a feasible intervention for women with PCOS with the potential to attenuate CVD risk factors if adherence is addressed.
3. The feasibility trial provides novel evidence that a lifestyle PA intervention, aimed at increasing lifestyle PA behaviour

4. may be feasible and acceptable to women with PCOS if motivation is addressed. It also indicates that the use of technology to monitor and engage participants with such an intervention is feasible and well-received.
5. The feasibility trial provides a clear basis for future research examining the effects of PA on oxidised LDL in women with PCOS.
6. The qualitative component contributes valuable and novel insight into the complex and nuanced contextual factors that affect acceptability, and particularly adherence, in PA interventions for women with PCOS.
7. The qualitative component has examined and elucidated barriers and facilitators to PA in women with PCOS.

6.6 Dissemination of Findings

The protocol for the systematic review and meta-analysis, as well as the full review, have been published in two peer-reviewed journals (Woodward et al., 2019a; Woodward et al., 2019b). The protocol for the feasibility trial has also been published in a peer-reviewed journal (Woodward et al., 2020). It is expected that the findings from the feasibility trial, and the qualitative evaluative study, will be published separately as full manuscripts in due course.

6.7 Reflections of the Researcher

In this section, the researcher will refer to themselves in the first person to discuss pertinent reflections, challenges, and learnings from the PhD journey.

If a PhD is a licence to practice as an independent researcher, I strongly believe that I have earnt that licence. The PhD programme has been incredibly rewarding and valuable, and I have gained a multitude of skills and experience that will contribute to my progression as a researcher. In particular, the qualitative study in this thesis is the first qualitative study I have conducted. I therefore undertook significant training in this area that was an addition to my previous skill set. This presented a steep learning curve because the application of key principles of validity and reliability that are seen in quantitative research are not necessarily relevant or transferable to qualitative research. In addition, the researcher must take a more active role in the interpretation of the data. Thus, learning how to conduct robust, transparent qualitative research has been a challenging and enjoyable feature of this programme.

Other key additions to my skillset and experience include the successful design, planning, and implementation of a clinical trial. Conducting each key aspect of a clinical trial has been demanding and illuminating, and I have learned how to pre-empt challenges where possible, and how to re-assess and adapt to challenges when they are unavoidable. As part of my training for the clinical trial, I also undertook a variety of training in practical skills. This included venepuncture training, which allowed me to be independent in conducting assessments with participants, and extensive training in laboratory techniques. This also afforded me the ability to conduct my own assays independently and has provided me with experience that can be drawn upon in my future career.

There have been several challenges throughout my three-year programme that have been successfully surmounted. One key challenge involved an incident with a -80 freezer where I had been storing my serum samples before analysis. An electrical malfunction caused a power cut, and the back-up power for the freezer was not connected. As such, the freezer reached a temperature above zero, and the samples were thawed for an unknown period. This caused uncertainty about the stability of the analytes and whether they would have been compromised. To overcome this, I immediately researched the stability of each analyte and determined how many freeze-thaw cycles were acceptable before degradation occurred. Most of the analytes appeared stable other than TBARS which is volatile. I conducted an interim analysis of the thawed samples as soon as possible and checked that the observed values were within the expected range. I also compared this to the samples collected after the incident that had not been thawed. The results indicated that the samples had not been adversely affected.

Finally, the Covid-19 pandemic presented a significant challenge to the research. In March 2020, face-to-face research was suspended indefinitely, and several of my participants were unable to return for their post-intervention assessment. Thus, there is some missing data which was unavoidable. However, participants sent in data by email if possible, including weight measurements and the IPAQ, to minimise this impact. Similarly, recruitment had to be immediately halted, and as such under different circumstances further participants could have been recruited.

In addition, restrictions prevented access to the biomolecular sciences laboratory, and I was unable to conduct the final analysis of my samples. I was unable to gain access until October 2020, and as such I applied for an extension of my end date to allow time for this. However, key training in high-pressure liquid chromatography was necessary before I could assay

testosterone in my samples. It was determined by my advisor that this training would be too logistically difficult to conduct with the current restrictions on personnel. Thus, I had to forego this training and unfortunately could not conduct this analysis.

Despite these challenges, the delay in practical work allowed valuable time to focus on writing up the thesis, and significant progress was made in this area during this time. Overall, the research was delayed by these challenges, but ultimately the quality of the work has not been affected.

6.8 Conclusions

The studies reported in this thesis are a novel contribution to knowledge that provide valuable evidence on the acceptability and feasibility of both a supervised exercise intervention and a lifestyle PA intervention for women with PCOS. The findings of the studies indicate that the interventions and procedures for recruitment, allocation, and outcome measurements are acceptable and feasible. However, the findings indicated that the adherence to the supervised exercise intervention was below an acceptable rate. Thus, this issue must be addressed as it impacts the feasibility of the intervention, and refinements made to the intervention before progression to a full-scale trial. The addition of a qualitative evaluative component has hence provided valuable contextual data that will be crucial to addressing adherence for both the progression to a full-scale RCT in this area, and to intervention studies for women with PCOS at large.

Key suggestions for improving adherence, and the positive experience of participants throughout the trial, include implementing various behaviour change techniques as part of the trial. This can include goal setting, reinforcement, feedback, and the cautious use of incentives, which may subsequently improve recruitment rates over time. In addition, providing an environment of social support, and including self-help resources or signposting to behavioural support, may improve enjoyment and increase the participants' feeling of empowerment over their condition. In addition, the design should be guided by BCTs. The result of this may potentially be both a better experience for participants during the trial, and the promotion of long-term behaviour change that includes the adoption of health-promoting behaviours. This may improve self-management for women with PCOS in the long term.

Suggestions for future research and next steps are set out, including the use of an internal pilot study to assess any refinements made to the intervention. Furthermore, future studies

should aim to clearly elucidate the therapeutic effects of an intervention to reduce sedentary behaviour on metabolic health in PCOS. The use of technology, such as wearables, makes this type of intervention easier to implement and monitor with greater reliability of results than self-reported measures. Finally, further research should place a priority on ensuring PA interventions for women with PCOS are accessible to those from socioeconomically disadvantaged backgrounds, so that those who may benefit most from such an intervention are able to access them.

7 References

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8 Appendices

Appendix 1: PRISMA Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	

Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	
DISCUSSION			

Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: **www.prisma-statement.org**.

Appendix 2: PubMed Search Strategy

The search was performed using PubMed syntax as demonstrated below, and amended for other databases:

1. “polycystic ovary syndrome”
2. PCOS
3. PCO*
4. polycystic ovar*
5. stein leventhal
6. (1 OR 2 OR 3 OR 4 OR 5)
7. Exercise[majr]
8. exercise therapy [majr]
9. Physical activity [majr]
10. Physical Fitness [majr]
11. Physical Endurance[majr]
12. exercis*[tw]
13. ((physical or motion) AND (fitness or therapy or therapies))[tw]
14. ((strength or resistance or circuit or enduran* or aerob* or physic* or fit*) AND train*)[tw]
15. (7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14)
16. (6 AND 15)

(((((("polycystic ovary syndrome"[Title/Abstract]) OR PCOS[Title/Abstract]) OR PCO*[Title/Abstract]) OR polycystic ovar*[Title/Abstract]) OR stein leventhal[Title/Abstract]))) AND (((((((Exercise[majr]) OR exercise therapy [majr]) OR Physical activity [majr]) OR Physical Endurance[majr]) OR exercis*[tw]) OR (((physical or motion) AND (fitness or therapy or therapies))[tw])) OR (((strength or resistance or circuit or enduran* or aerob* or physic* or fit*) AND train*)[tw]))

Appendix 3: Notice of HRA Approval and REC Favourable Opinion

Ms Amie Woodward
Chestnut Court, Room S002
Sheffield Hallam University
Collegiate Campus
S10 2BP

Email: hra.approval@nhs.net Research-permissions@wales.nhs.uk

19 July 2018

Dear Ms Woodward

HRA and Health and Care Research Wales (HCRW) Approval Letter

Study title:	**Exploring the feasibility of assessing the impact of exercise on oxidised LDL and cardiometabolic profile in women with polycystic ovary syndrome.**
IRAS project ID:	**244352**
Protocol number:	**N/A**
REC reference:	**18/NW/0454**
Sponsor	**Sheffield Hallam University**

I am pleased to confirm that **HRA and Health and Care Research Wales (HCRW) Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

How should I continue to work with participating NHS organisations in England and Wales? You should now provide a copy of this letter to all participating NHS organisations in England and Wales, as well as any documentation that has been updated as a result of the assessment .

Following the arranging of capacity and capability, participating NHS organisations should **formally confirm** their capacity and capability to undertake the study. How this will be confirmed is detailed in the "*summary of assessment*" section towards the end of this letter.

You should provide, if you have not already done so, detailed instructions to each organisation as to how you will notify them that research activities may commence at site following their confirmation of capacity and capability (e.g. provision by you of a 'green light' email, formal notification following a site initiation visit, activities may commence immediately following confirmation by participating organisation, etc.)It is important that you involve both the research management function (e.g. R&D office) supporting each organisation and the local research team (where there is one) in setting up your study . Contact details of the research management function for each organisation can be accessed here.

How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?

HRA and HCRW Approval does not apply to NHS/HSC organisations within the devolved administrations of Northern Ireland and Scotland.

If you indicated in your IRAS form that you do have participating organisations in either of these devolved administrations, the final document set and the study wide governance report (including this letter) has been sent to the coordinating centre of each participating nation. You should work with the relevant national coordinating functions to ensure any nation specific checks are complete, and with each site so that they are able to give management permission for the stu dy to begin.

Please see IRAS Help for information on working with NHS/HSC organisations in Northern Ireland and Scotland.

How should I work with participating non-NHS organisations?

HRA and HCRW Approval does not apply to non-NHS organisations. You should work with your non- NHS organisations to obtain local agreement in accordance with their procedures.

What are my notification responsibilities during the study?

The document "*After Ethical Review – guidance for sponsors and investigators*", issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments

- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

I am a participating NHS organisation in England or Wales. What should I do once I receive this letter?

You should work with the applicant and sponsor to complete any outstanding arrangements so you are able to confirm capacity and capability in line with the information provided in this letter.

The sponsor contact for this

application is as follows: Name: Dr

Keith Fildes
Tel: 0114 225 4530
Email: researchsupport@shu.ac.uk

Who should I contact for further information?

Please do not hesitate to contact me for assistance with this application. My contact

details are below. Your IRAS project ID is **244352**. Please quote this on all

correspondence.

Yours sincerely

Miss Lauren Allen Senior Assessor

Email: hra.approval@nhs.net

Copy to: *Dr Keith Fildes*
Ms Angela Pinder, Sheffield Teaching Hospitals NHS Foundation Trust

List of Documents

The final document set assessed and approved by HRA and HCRW Approval is listed below.

Document	*Version*	*Date*
Copies of advertisement materials for research participants [Poster]	2	18 April 2018
Copies of advertisement materials for research participants [Radio and social media announcement]	2	24 May 2018
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [TWIMC Letter 2017-18]		
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [Insurance Certificate]		01 August 2017
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [EL Certificate 2017-18]		
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [EL PL 2017-18]		
HRA Schedule of Events	1.0	25 May 2018
HRA Statement of Activities	1.0	25 May 2018
Interview schedules or topic guides for participants [Post-Trial Interview Script]	1	17 May 2018
Interview schedules or topic guides for participants [PAR-Q]	1	24 May 2018
IRAS Application Form [IRAS_Form_29052018]		29 May 2018
IRAS Checklist XML [Checklist_29052018]		29 May 2018
Letters of invitation to participant [Invitation Letter]	2	06 July 2018
Letters of invitation to participant [Eligibility Screening Questions]	1	05 July 2018
Participant consent form [Consent Form]	3	05 July 2018
Participant information sheet (PIS) [Participant information sheet]	5	05 July 2018
Research protocol or project proposal [Study Protocol]	5	17 May 2018
Response to Request for Further Information		09 July 2018
Summary CV for Chief Investigator (CI) [CI CV]		
Summary CV for supervisor (student research) [Supervisor CV]		
Summary CV for supervisor (student research) [Mostafa Metwally CV]	1	25 May 2018
Summary CV for supervisor (student research) [David Broom CV]	1	25 May 2018

Summary of assessment

The following information provides assurance to you, the sponsor and the NHS in England and Wales that the study, as assessed for HRA and HCRW Approval, is compliant with relevant standards. It also provides information and clarification, where appropriate, to participating NHS organisations in England and Wales to assist in assessing, arranging and confirming capacity and capability.

Assessment criteria

Section	Assessment Criteria	Compliant with Standards?	Comments
1.1	IRAS application completed correctly	Yes	No comments
2.1	Participant information/consent documents and consent process	Yes	Recruitment posters will be displayed at the site, not at GP practices. The Participant Information Sheet was updated to bring in line with assessment standards (e.g. samples).
3.1	Protocol assessment	Yes	No comments
4.1	Allocation of responsibilities and rights are agreed and documented	Yes	The Statement of Activities will act as the agreement between the sponsor and site.
4.2	Insurance/indemnity arrangements assessed	Yes	No comments
4.3	Financial arrangements assessed	Yes	No funding will be provided to the site.

5.1	Compliance with the Data Protection Act and data security issues assessed	Yes	The applicant has confirmed participants' data will be anonymised using a code number linked to their identifiable data rather than using patient identifiers (postcode, initials, date of birth) within the code.
5.2	CTIMPS – Arrangements for	Not Applicable	No comments

Section	Assessment Criteria	Compliant with Standards?	Comments
	compliance with the Clinical Trials Regulations assessed		
5.3	Compliance with any applicable laws or regulations	Yes	The Material Transfer Agreement appendix in the Statement of Activities will be used.
6.1	NHS Research Ethics Committee favourable opinion received for applicable studies	Yes	No comments
6.2	CTIMPS – Clinical Trials Authorisation (CTA) letter received	Not Applicable	No comments
6.3	Devices – MHRA notice of no objection received	Not Applicable	No comments
6.4	Other regulatory approvals and authorisations received	Not Applicable	No comments

Participating NHS Organisations in England and Wales

This provides detail on the types of participating NHS organisations in the study and a statement as to whether the activities at all organisations are the same or different.
There is one participating site. Potential participants will be identified by the consultant gynaecologist at the site. Members of the research team may attend the site to discuss the study with potential participants (where the patient has agreed to be approached). Some participants may be recruited outside the NHS and all study activities other than identification of participants will be conducted outside the NHS. HRA and HCRW Approval does not cover activity outside the NHS. Before undertaking activity outside the NHS the research team must follow the procedures and governance arrangements of responsible organisations. The Chief Investigator or sponsor should share relevant study documents with participating NHS organisations in England and Wales in order to put arrangements in place to deliver the study. The documents should be sent to both the local study team, where applicable, and the office provi ding the research management function at the participating organisation. Where applicable, the local LCRN contact should also be copied into this correspondence. If chief investigators, sponsors or principal investigators are asked to complete site level forms for participating NHS organisations in England and Wales which are not provided in IRAS, the HRA or HCRW websites, the chief investigator, sponsor or principal investigator should notify the HRA immediately at hra.approval@nhs.net or HCRW at Research-permissions@wales.nhs.uk. We will work with these organisations to achieve a consistent approach to information provision.

Principal Investigator Suitability

This confirms whether the sponsor position on whether a PI, LC or neither should be in place is correct for each type of participating NHS organisation in England and Wales, and the minimum expectations for education, training and experience that PIs should meet (where applicable).
A Local Collaborator should be identified at the site to facilitate access arrangements for the external research team where needed. GCP training is not a generic training expectation, in line with the HRA/HCRW/MHRA statement on training expectations.

HR Good Practice Resource Pack Expectations

This confirms the HR Good Practice Resource Pack expectations for the study and the pre-engagement

checks that should and should not be undertaken
External staff (e.g. University) who are coming on to the site to provide study information to potential participants will be expected to obtain a Letter of Access. This should confirm Disclosure and Barring Service and Occupational Health checks.

Other Information to Aid Study Set-up

This details any other information that may be helpful to sponsors and participating NHS organisations in England and Wales to aid study set-up.
The applicant has indicated that they do not intend to apply for inclusion on the NIHR CRN Portfolio.

Health Research Authority

North West - Greater Manchester East Research Ethics Committee

3rd Floor, Barlow House

4 Minshull Street
Manchester

M1 3DZ

Please note: This is the favourable opinion of the REC only and does not allow

you to start your study at NHS sites in England until you receive HRA Approval

Telephone: 0207 104 8009

19 July 2018

Ms Amie Woodward
Chestnut Court, Room S002
Sheffield Hallam University
Collegiate Campus
S10 2BP

Dear Ms Woodward

Study title:	**Exploring the feasibility of assessing the impact of exercise on oxidised LDL and cardiometabolic profile in women with polycystic ovary syndrome.**
REC reference:	**18/NW/0454**
Protocol number:	**N/A**
IRAS project ID:	**244352**

Thank you for your letter of 09 July 2018, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact hra.studyregistration@nhs.net outlining the reasons for your request.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for HRA and HCRW Approval (England and Wales)/ NHS permission for research is available in the Integrated Research Application System, at www.hra.nhs.uk or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from the HRA. Guidance on where to register is provided on the HRA website.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Non-NHS sites

The Committee has not yet completed any site-specific assessment (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. We will write to you again as soon as an SSA application(s) has been reviewed. In the meantime no study procedures should be initiated at non-NHS sites.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Copies of advertisement materials for research participants [Poster]	2	18 April 2018
Copies of advertisement materials for research participants [Radio and social media announcement]	2	24 May 2018
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [Insurance Certificate]		01 August 2017
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Summary CV for supervisor (student research) [Mostafa Metwally CV]	1	25 May 2018
Summary CV for supervisor (student research) [David Broom CV]	1	25 May 2018

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website:
http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success

of this project. Yours sincerely

Signed on behalf

of Mr Simon Jones Chair

Email: nrescommittee.northwest-gmeast@nhs.net

Enclosures: "After ethical review – guidance for researchers"

Copy to: *Dr Keith Fildes*

Ms Angela Pinder, Sheffield Teaching Hospitals NHS Foundation Trust

Appendix 4: Participant Documents and Proformas

Participant Consent Form

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

Sheffield Hallam University

CONSENT FORM FOR THE POLYCYSTIC OVARY SYNDROME STUDY

Participant Identification Number: | | | | |

This form will be kept confidential and not released to anyone outside of the research team

*Please **initial** each box*

1. I confirm that I have read and understand the information sheet [version 3 dated 15/07/2018] for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving reason, without my medical care or legal rights being affected. I also understand that if I withdraw, I can ask for all record of my contact details to be deleted and I can withdraw any other data collected on me. ☐
3. I understand that relevant sections of my medical notes and/or study data may be looked at by responsible individuals from the study team, the sponsor, the NHS Trust or from regulatory authorities where it is relevant to my taking part in the research. I give permission for these individuals to access my records. ☐
4. I agree to provide blood samples for testing purposes, and I give my consent to allow the research team to inform my GP if any abnormalities are revealed by the blood tests. ☐
5. I agree to be interviewed after the trial about my opinions and experiences. I give permission for the interview to be recorded via an audio recording device. ☐
6. I agree to this consent form and other data collected as part of this research study to be kept at Sheffield Hallam University. ☐
7. I understand that records relating to me will be kept confidential. No information will be released or printed that would identify me without my permission unless required by law. ☐
8. I agree to take part in the above study. ☐

Name of participant (*please print*)	Date	Signature of participant
Name of person taking consent	Date	Signature of person taking

consent (*please print*)

Name of witness (*please print*)	Date	Signature of witness [**only if applicable**]

1 for patient; 1 (original) for Investigator Site File; 1 for medical notes

Participant Information Sheet

Information for participants

The impact of exercise on oxidised LDL and cardiometabolic profile in women with polycystic ovary syndrome.

Introduction

We are inviting you to participate in a research study. Before you decide whether you would like to take part, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully and discuss it with us and/or friends if you wish. If you require more information or any further clarification on the information given to you we will gladly be at your disposal to answer any relative inquiry. Please take your time to decide whether or not you wish to take part.

Background and purpose of the study

Polycystic Ovary Syndrome (PCOS) is a multifaceted hormonal disorder that has been shown to affect women's fertility. It can also share many symptoms with pre-diabetes, and women with PCOS often have an increased risk for type 2 diabetes, heart attack and stroke.

Past research has shown that exercise can have beneficial effects on this increased risk, with or without weight loss. However, a key molecule, known as oxidised low-density lipoprotein, which has been to shown to contribute to the build-up of plaque in the arteries, has not been studied in this context. Therefore, the research will attempt to identify the impact that exercise has on this molecule, and other molecules contributing to increased risk, in women with PCOS.

Additionally, recent research has shown that long periods of inactivity, such as sitting for long hours at desk jobs, may have a negative effect on heart health, even if one undertakes regular exercise. As such, the research will attempt to compare the effect of a structured exercise program with the effects of decreasing periods of inactivity. This will further help to clarify the impact of physical activity on heart health.

Am I suitable participant for the study?

Women over the age of 18 and pre-menopausal, with diagnosed PCOS, who are not taking any medication for the condition will be eligible to take part in the study. The study will involve being randomly allocated into either an exercise program group, a lifestyle physical activity group or a control group. The exercise program will involve coming into the physiology

laboratory at Sheffield Hallam University and undertaking exercise several times a week for 12 weeks. The lifestyle physical activity group will attempt to increase their general physical activity unsupervised and will use a fitness tracking app to record this. The control group will not undertake an intervention. Blood samples will be taken and analysed to measure cardiovascular disease risk.

What will happen if I take part?

If you are eligible for the study and are happy to take part we will arrange for you to attend the Centre for Sport and Exercise Science at Sheffield Hallam University where the baseline measurements will take place (See table 1 below). We will provide you with instructions on how to get to the centre and where you can park for free. Unfortunately we are unable to pay other travel costs. You will be asked to sign a consent form agreeing to take part in the study, and to complete a pre-exercise questionnaire to ensure your readiness for physical activity. You will be given a copy of your signed consent form and this information sheet to keep.

The study design is a randomised controlled trial which means that you will be allocated to either the exercise group, the lifestyle physical activity group, or the control group by chance (random).

Baseline & Post Trial Assessment Visits: For all participants, we will arrange a visit prior to the group randomisation, and a second visit following completion of the trial. We will take some measurements including your height, weight, waist and hip measurements, and a finger-prick and venous blood sample (9ml in volume). All participants will be required to perform a submaximal oxygen uptake test performed on a cycle ergometer (stationary bike). The submaximal oxygen uptake test will help us assess your physical fitness, identify reasons related to your PCOS that might impair your ability to perform exercise and examine the differences before and after the exercise programme on several outcomes. This visit will take approximately 1 hour.

Before the baseline and post-trial intervention measurements you will be requested to abstain from vigorous exercise, alcohol, caffeine and tobacco for a period of 24h but also to be at least 2h fasting prior to the assessment as these parameters could affect your responses. We encourage you to wear sport clothing that will allow for a more comfortable movement during the exercise test.

The submaximal oxygen uptake test: This involves cycling on a bicycle ergometer at a moderate pace for six minutes. You will wear a heart rate monitor throughout, and your heart rate will be recorded each minute. We will increase the work rate of the bicycle until your heart rate is within 125-170 beats per minute (bpm). Then, we will take an average of your final two

heart rate readings, and use this, along with your body mass and age, to estimate your maximal oxygen update.

Exercise group: If you are randomised to the exercise group, a training period of 12 weeks will commence where you will be required to attend the gym located at the Centre for Sport and Exercise Science at Sheffield Hallam University two times per week for the first 8 weeks, and 3 times a week for the final 4 weeks, to perform a supervised exercise session.Each session will last approximately 60 minutes and will involve 40 minutes of an aerobic individualized exercise protocol performed either on a cycle ergometer, elliptical machine or a motorized treadmill accompanied by the warm-up (10 minutes) and cool down (10 minutes) period. The warm-up is an important component of the exercise session that will help to prevent injury and prepare the body for physical activity. The cool down will help the body gradually return to its pre-exercise state.

Training hours and dates will be fixed according to your eligibility and in agreement with the personal trainer. Straight after the 12-week training period you will be asked to visit our laboratories at the Centre for Sport and Exercise Science at Sheffield Hallam University to be assessed in the same tests as prior the exercise intervention (post-trial measurements-see Table 1).

Lifestyle Physical Activity Group: If you are randomised to the lifestyle physical activity group you will be asked to attend the Centre for Sport and Exercise Science at Sheffield Hallam University for all the measurements but will not take part in the exercise intervention. However, we will provide advice and information on how you can reduce your time spent inactive during the day. Participants will be asked to monitor and track their daily physical activity using a smartphone fitness app. We will then arrange for the research team to have access to this recorded activity. Between the baseline measurements and those after 12 weeks you will be receiving regular calls, approximately once a week, to obtain information about your progress and provide advice.

Control Group: If you are randomised to the control group, you will undertake the baseline and post-intervention assessments, but you will not take part in any intervention. After the trial is complete, you will be offered the chance to undertake exercise sessions at the university (not as part of any research) if you wish.

Interview visit: After the trial has concluded, participants in the exercise and lifestyle physical activity groups will be asked to attend the Centre for Sport and Exercise Science at Sheffield Hallam University for one final time, where a researcher will conduct an interview with you. The

interview will cover your opinions and experiences with the intervention, and factors that may influence or be a barrier to continuing increased physical activity in the future. The interview audio will be recorded for analysis purposes. This visit should take approximately 1 hour.

All the visits will take place at the Centre for Sport and Exercise Science at Sheffield Hallam University.

Table 1: Overview of the study

Visit number	Purpose of visit	Duration of visit
1	**Baseline measurements** Body weight, height and waist and hip measurements. Finger-prick and venous blood sample. Submaximal oxygen uptake test.	60 minutes
2-28	**Training sessions (exercise group only)** • 2 sessions per week for 8 weeks • 3 sessions per week for 4 weeks 40 minutes aerobic exercise either on cycle ergometer or motorised treadmill.	60 minutes
29 (or 2 for lifestyle physical activity group)	**Post-trial measurements** Body weight, height and waist and hip measurements. Finger-prick and venous blood sample. Submaximal oxygen uptake test.	60 minutes
30 (or 3 for lifestyle physical activity group).	**Post-Trial Interview** Covering opinions and experiences of the trial, and barriers and facilitators to increasing physical activity in the future.	60 minutes

What are the possible benefits of taking part in this study?

There are no direct benefits of taking part. This study is being undertaken for research purposes and to advance our knowledge of PCOS mechanisms and how symptoms can be alleviated. Previous research examining the effect of structured exercise on women with PCOS has shown various benefits, including increased physical fitness, weight loss, lower blood pressure and

better regulation of blood sugar. Therefore, it is possible that you may see some benefit to your health and well-being if you are randomised to the exercise group.

Research has also shown that similar benefits can be achieved by reducing periods of time spent sitting. Therefore, if you are in the lifestyle physical activity group, you may still experience some benefit to your health by increasing your time spent being physically active (including walking breaks during work and other general activities). Tracking physical activity can also help to give you a view of where you may like to make improvements, or for setting goals and targets.

Please note that we do not expect participation in the study to influence your chances of conception.

What are the risks of taking part?

All of the experimental procedures that will be used in this study have been rigorously tested to ensure that they meet health and safety standards. These tests are all routinely and regularly performed on patients and healthy volunteers alike. The researchers who perform the tests are all trained and skilled to do so. If we notice any signs regarding your health status, that may cause you harm by participating, you will be informed and withdrawn immediately from the study.

Overall the risks of the procedures included in this study are low. The potential risks associated with chance of injury from taking part in regular exercise will be minimised through the use of appropriate warm-down and cool-downs, and by ensuring exercise is performed at an individualised output based on your own fitness abilities. There is some risk of bruising from having blood drawn. These procedures will be conducted by appropriately-trained staff. Taking part in the study will require time commitment, particularly for the exercise group.

Do I have to take part?

No, you do not have to take part. You are free to withdraw from the study at any time. If you decide to withdraw, we may ask you to consider attending one final assessment, but this is entirely optional. You can choose to leave the study at any time without having any further assessments. We would like to use all of your data up to the point of withdrawal as this will help with our analysis. However, if you would prefer us not to use any of your data you may request for all of your data to be removed from the study. A decision not to carry on with the study will not affect the quality of care you receive in any way.

Will taking part in this study be kept confidential?

If you consent to take part in this study, the records obtained while you are in this study as well as related health records will remain strictly confidential at all times. The information will be held securely on paper and electronically at Sheffield Hallam University under the provisions of the 1998 Data Protection Act. Your name will not be passed to anyone else outside the research team or the sponsor, who is not involved in the trial. You will be allocated a trial number, which will be used as a code to identify you on all trial forms.

The information collected about you may also be shown to authorised people from the UK Regulatory Authority, the local NHS Trust and Independent Ethics Committee; this is to ensure that the study is carried out to the highest possible scientific standards. All the study research team will have a duty of confidentiality to you as a research participant.

If you withdraw consent from further study involvement, unless you object, your data will remain on file and will be included in the final study analysis.

In line with Good Clinical Practice guidelines, at the end of the study, your data will be securely archived for a minimum of 7 years from the end of the study (the end of the study is defined as the last visit of the last patient in the study). Arrangements for confidential destruction will then be made.

Coded results from the study may be stored indefinitely for subsequent analyses in the future. Any identifying information is kept strictly confidential, and access will be limited strictly to the original study team and database team. Researchers analysing the clinical data in the future will be unable to identify you.

If your blood tests reveal any abnormalities, we will inform your GP with your consent. Upon completion of the blood tests, your blood sample will be destroyed in accordance with the university's laboratory guidelines for safe disposal of human blood.

Who will be working on the study?

The researcher in charge is Ms. Amie Woodward (PhD student in Sport and Exercise Science), supervised by Dr. Markos Klonizakis (Senior Research Fellow in Clinical Physiology) and the leading NHS clinician collaborating to the study is Dr. Mostafa Metwally (Consultant Gynaecologist).

What will happen to the results of the study?

Once the study has been completed all data will be anonymised and stored as per current data protection laws. The results will be written up for publication in academic journals and possibly

used at academic conferences and will also contribute to a Doctor of Philosophy degree (PhD) completion. Anything with your personal details (name, DOB, contact details etc.) will be kept securely in a locked filing cabinet by the Principal Investigator. Overall study results will also be made available to you on request at the end of the study. Moreover, information provided by the participant will be stored at Sheffield Hallam Research Facilities in Sheffield for further analyses until the end of the project.

What if I have further questions or would like more information about the study?

If you would like more information about the study you are invited to contact:-

Ms. Amie Woodward, Sheffield Hallam University tel. 07488477786.

What happens if I have a complaint?

If you have any cause to complain about any aspect of the way in which you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms are available to you and are not compromised in any way because you have taken part in a research study. If you have complaints or concerns please contact the project co-ordinator Dr. Markos Klonizakis Tel 0114 225 5697. Or alternately you can contact the Patient Services Team (PST) on 0114 271 2400, PST@sth.nhs.uk. If you require an independent individual to complain about this study through Sheffield Hallam University, you may contact Dr Nikki Jordan-Mahy , Chair of Health and Wellbeing Faculty Research Ethics Committees, n.jordan-mahy@shu.ac.uk, or by telephone on 0114 225 3120, or by letter at Academy of Sport and Physical Activity, Faculty of Health & Wellbeing, Sheffield Hallam University, A225 Collegiate Hall, Collegiate Crescent, Sheffield, South Yorkshire, S10 2BP.

What if I am harmed?

In the event that something does go wrong and you are harmed during the research study, there are no special compensation arrangements. If you are harmed as a result of someone's negligence then you may have grounds for legal action for compensation, but you may have to pay your legal costs.

Who is organizing and funding the research?

This study is being funded by the Sheffield Hallam University and supported by the Sheffield Teaching Hospitals NHS Trust. The investigators of this study will not receive any payment for conducting this research.

Who has reviewed the study?

All research in the NHS is looked at by an independent group of people called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity.

Legal basis for research for studies

The University undertakes research as part of its function for the community under its legal status. Data protection allows us to use personal data for research with appropriate safeguards in place under the legal basis of public tasks that are in the public interest. A full statement of your rights can be found at https://www.shu.ac.uk/about-this-website/privacy-policy/privacy-notices/privacy-notice-for-research. However, all University research is reviewed to ensure that participants are treated appropriately, and their rights respected. This study was approved by UREC with Converis number ER 6262197. Further information can be found at: https://www.shu.ac.uk/research/ethics-integrity-and-practice.

You should contact the Data Protection Officer if:	**You should contact the Head of Research Ethics (Professor Ann Macaskill) if:**
• you have a query about how your data is used by the University • you would like to report a data security breach (e.g. if you think your personal data has been lost or disclosed inappropriately) • you would like to complain about how the University has used your personal data DPO@shu.ac.uk	• you have concerns with how the research was undertaken or how you were treated a.macaskill@shu.ac.uk

Postal address: Sheffield Hallam University, Howard Street, Sheffield S1 1WBT
Telephone: 0114 225 5555

Thank you for taking the time to read this information sheet and to consider this study.

Study Team Contact Details

Health and Wellbeing Faculty, Centre of Sport and Exercise Science,

Collegiate Hall, Collegiate Campus, Sheffield, S10 2BP.

Researcher:

Ms Amie Woodward

E-mail: amie.woodward@shu.ac.uk

Tel. 07488477786

Invitation Letter

Dear patient,

We are undertaking a research study to investigate the impact of supervised exercise training and increased physical activity in people diagnosed with Polycystic Ovary Syndrome.

You may have been identified as being a potentially suitable candidate from attending Sheffield Teaching Hospitals Jessop Wing clinic.

Please find enclosed a participant information sheet, which describes the study in detail and answers the most frequently asked questions.

If you are interested in participating in this study, or would like to obtain further information, please phone Ms Amie Woodward (07488477786) or email at amie.woodward@shu.ac.uk. It is important to note that there is no pressure to participate in this study and your standard care will not be affected in any way by your decision to take part in this study.

Yours sincerely,

Amie Woodward

Public Poster

Public poster version 2.0 12-04-2019

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

Sheffield Hallam University

Do you have polycystic ovary syndrome?

Would you be interested in participating in a research study which is exploring the effects of exercise and physical activity on people with polycystic ovary syndrome?

Sheffield Hallam University in collaboration with Sheffield Teaching Hospitals NHS Foundation Trust are undertaking a non-invasive, ethically-approved study (part of a PhD program), that is exploring the impact of exercise and physical activity on cardiovascular health in polycystic ovary syndrome.

If you are a non-smoker, not currently taking the oral contraceptive pill and are pre-menopausal, please get in touch.

If you are interested to receive more information, please contact Amie Woodward:

PCOS@shu.ac.uk

Public announcement(s) – wording to be used for radio advertisement and social media posts.

A team of researchers at Sheffield Hallam University are running a research study in collaboration with clinicians at Sheffield Teaching Hospitals NHS Foundation Trust for patients with polycystic ovary syndrome. The study (as part of a PhD research programme) is exploring the implications of exercise and physical activity on cardiovascular health in patients with polycystic ovary syndrome.
If you would be interested to know more about the study and potentially take part, please contact Ms. Amie Woodward on amie.woodward@shu.ac.uk or 0114 225 2347.

Wanted: Women with PCOS near Sheffield
Do you have polycystic ovary syndrome?
Would you be interested in participating in a research study which is exploring the effects of exercise and physical activity on people with polycystic ovary
syndrome?
Sheffield Hallam University in collaboration with Sheffield Teaching Hospitals NHS Foundation Trust are undertaking a non-invasive, ethically approved study, that is exploring the impact of exercise and physical activity on cardiovascular health in polycystic ovary syndrome.
If you are a non-smoker, not currently the combined oral contraceptive pill and are pre-menopausal, please get in touch.
If you are interested to receive more information, please email PCOS@shu.ac.uk.

Wanted: Women with PCOS near Sheffield for clinical trial.

I'm a PhD researcher at Sheffield Hallam University and I am currently running a clinical trial (in collaboration with Sheffield Teaching Hospitals NHS Foundation Trust) to examine the effects of exercise on people with polycystic ovary syndrome (PCOS).

As I'm sure you will be all too aware of, PCOS affects up to 20% of women and is the leading cause of infertility. It is also associated with various mental health disorders and an increased risk of cardiovascular disease.

If you, or anyone you know, have PCOS and might be interested in taking part in the trial, please get in touch at PCOS@shu.ac.uk and I can send you more information - there is of course no obligation to take part.

PRE-TEST MEDICAL QUESTIONNAIRE

Project Title: The impact of exercise on oxidized LDL and cardiometabolic profile in women with polycystic ovary syndrome.

Name:.....

Date of Birth:..... Age:..... Sex:.....

Please circle the appropriate response or fill in the blank.

1. How would you describe your present level of activity?

   Sedentary Moderately active Active Highly active

2. How would you describe your present level of fitness?

   Unfit Moderately fit Trained Highly trained

3. How would you consider your present body weight?

   Underweight Ideal Slightly over Very overweight

4. Smoking Habits Are you currently a smoker? Yes No
   How many do you smoke …….. per day

   Are you a previous smoker? Yes No
   How long is it since you stopped? ..... Years

   Were you an occasional smoker? Yes No
   ..... per day

   Were you a regular smoker Yes No
   ..... per day

5. Do you drink alcohol? Yes No
   If you answered Yes, do you have?

   An occasional drink A drink every day More than one drink a day

6. Have you had to consult your doctor within the last six months? Yes No
   If you answered **Yes**, please give details…….
   …….
   ……. ……...

7. Are you presently taking any form of medication? Yes No
   If you answered **Yes**, please give details……..
   ……..
   …….

8. As far as you are aware, do you suffer or have you ever suffered from:

Diabetes?	Yes	No	Asthma?	Yes	No
Epilepsy?	Yes	No	Bronchitis?	Yes	No
*Any form of heart complaint?	Yes	No	Raynaud's Disease?	Yes	No
*Marfan's Syndrome?	Yes	No	*Aneurysm/embolism?	Yes	No
Aneamia	Yes	No	Irritable bowel syndrome	Yes	No

9. Is there a history of heart disease in your family? Yes No

If **Yes**, please give details……
……
……..……...

10. Do you currently have any form of muscle or joint injury? Yes No
If **Yes**, please give details……
……
……..……...

11. Have you had to suspend normal training in the last two weeks? Yes No
If the answer is **Yes,** please give details……...
……
……

12. As far as you are aware, is there anything that might prevent you from successfully completing the tests that have been outlined to you? Yes No

If blood samples are to be taken, please answer the following questions:

a)	Are you suffering from any known serious infection?	Yes	No
b)	Have you had jaundice within the previous year?	Yes	No
c)	Have you ever had any form of hepatitis?	Yes	No
d)	Are you HIV antibody positive?	Yes	No
e)	Have you had unprotected sexual intercourse with any person from an HIV high-risk population?	Yes	No
f)	Have you ever been involved in intravenous drug use?	Yes	No
g)	Are you hemophiliac?	Yes	No

* If the answer to any of the above is **Yes** then please discuss the nature of the problem with your Sport and Exercise Scientist.

Signature:……...

Date:……/……/……

International Physical Activity Questionnaire

(October 2002)

LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation

Translation from English is encouraged to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ

International collaboration on IPAQ is on-going and an ***International Physical Activity Prevalence Study*** is in progress. For further information see the IPAQ website.

More Information

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective.* Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

   ☐ Yes

   ☐ No → ***Skip to PART 2: TRANSPORTATION***

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

   _____ **days per week**

   ☐ No vigorous job-related physical activity → ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

   _____ **hours per day**
   _____ **minutes per day**

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

   _____ **days per week**

   ☐ No moderate job-related physical activity → ***Skip to question 6***

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

_____ **hours per day**
_____ **minutes per day**

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ **days per week**

☐ No job-related walking → ***Skip to PART 2: TRANSPORTATION***

7. How much time did you usually spend on one of those days **walking** as part of your work?

_____ **hours per day**
_____ **minutes per day**

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ **days per week**

☐ No traveling in a motor vehicle → ***Skip to question 10***

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ **hours per day**
_____ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No bicycling from place to place → ***Skip to question 12***

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ **hours per day**
_____ **minutes per day**

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No walking from place to place → ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

13. How much time did you usually spend on one of those days **walking** from place to place?

_____ **hours per day**
_____ **minutes per day**

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ **days per week**

☐ No vigorous activity in garden or yard → ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

☐ No moderate activity in garden or yard → ***Skip to question 18***

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐ No moderate activity inside home → ***Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY***

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**
_____ **minutes per day**

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

☐ No walking in leisure time → ***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ **hours per day**
_____ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

☐ No vigorous activity in leisure time → ***Skip to question 24***

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ **hours per day**
_____ **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ **days per week**

☐ No moderate activity in leisure time → ***Skip to PART 5: TIME SPENT SITTING***

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?

_____ **hours per day**
_____ **minutes per day**

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ **hours per day**
_____ **minutes per day**

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

_____ **hours per day**
_____ **minutes per day**

This is the end of the questionnaire, thank you for participating.

Case report form: The impact of exercise on oxidised LDL and cardiometabolic profile in women with polycystic ovary syndrome

Do not write allocation on this form.

Date …….

Session …….

Participant Code …….

Height …… Weight …… WC …… HC …… Age ……

Astrand Results

Predicted VO_2max (mL/kg/min) corrected for age …….

Cardiochek Results

TC (mmol/L) …….. HDL (mmol/L) …….. Glucose (mmol/L) …….. TC/HDL ……..

Time of venous blood draw ……..

Time of venous blood drop-off ……..

Notes ……

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Appendix 5: Consolidated criteria for reporting qualitative research (COREQ) checklist

COREQ (COnsolidated criteria for REporting Qualitative research) Checklist

A checklist of items that should be included in reports of qualitative research. You must report the page number in your manuscript where you consider each of the items listed in this checklist. If you have not included this information, either revise your manuscript accordingly before submitting or note N/A.

Topic	Item No.	Guide Questions/Description	Reported on Page No.
Domain 1: Research team and reflexivity			
Personal characteristics			
Interviewer/facilitator	1	Which author/s conducted the interview or focus group?	
Credentials	2	What were the researcher's credentials? E.g. PhD, MD	
Occupation	3	What was their occupation at the time of the study?	
Gender	4	Was the researcher male or female?	
Experience and training	5	What experience or training did the researcher have?	
Relationship with participants			
Relationship established	6	Was a relationship established prior to study commencement?	
Participant knowledge of the interviewer	7	What did the participants know about the researcher? e.g. personal goals, reasons for doing the research	
Interviewer characteristics	8	What characteristics were reported about the inter viewer/facilitator? e.g. Bias, assumptions, reasons and interests in the research topic	
Domain 2: Study design			
Theoretical framework			
Methodological orientation and Theory	9	What methodological orientation was stated to underpin the study? e.g. grounded theory, discourse analysis, ethnography, phenomenology, content analysis	
Participant selection			
Sampling	10	How were participants selected? e.g. purposive, convenience, consecutive, snowball	
Method of approach	11	How were participants approached? e.g. face-to-face, telephone, mail, email	
Sample size	12	How many participants were in the study?	
Non-participation	13	How many people refused to participate or dropped out? Reasons?	
Setting			
Setting of data collection	14	Where was the data collected? e.g. home, clinic, workplace	
Presence of non-participants	15	Was anyone else present besides the participants and researchers?	
Description of sample	16	What are the important characteristics of the sample? e.g. demographic data, date	
Data collection			
Interview guide	17	Were questions, prompts, guides provided by the authors? Was it pilot tested?	
Repeat interviews	18	Were repeat inter views carried out? If yes, how many?	
Audio/visual recording	19	Did the research use audio or visual recording to collect the data?	
Field notes	20	Were field notes made during and/or after the inter view or focus group?	
Duration	21	What was the duration of the inter views or focus group?	
Data saturation	22	Was data saturation discussed?	
Transcripts returned	23	Were transcripts returned to participants for comment and/or	

Topic	Item No.	Guide Questions/Description	Reported on Page No.
		correction?	
Domain 3: analysis and findings			
Data analysis			
Number of data coders	24	How many data coders coded the data?	
Description of the coding tree	25	Did authors provide a description of the coding tree?	
Derivation of themes	26	Were themes identified in advance or derived from the data?	
Software	27	What software, if applicable, was used to manage the data?	
Participant checking	28	Did participants provide feedback on the findings?	
Reporting			
Quotations presented	29	Were participant quotations presented to illustrate the themes/findings? Was each quotation identified? e.g. participant number	
Data and findings consistent	30	Was there consistency between the data presented and the findings?	
Clarity of major themes	31	Were major themes clearly presented in the findings?	
Clarity of minor themes	32	Is there a description of diverse cases or discussion of minor themes?	

Developed from: Tong A, Sainsbury P, Craig J. Consolidated criteria for reporting qualitative research (COREQ): a 32-item checklist for interviews and focus groups. *International Journal for Quality in Health Care*. 2007. Volume 19, Number 6: pp. 349 – 357

Once you have completed this checklist, please save a copy and upload it as part of your submission. DO NOT include this checklist as part of the main manuscript document. It must be uploaded as a separate file.

Appendix 6: Interview Schedule

The impact of exercise on oxidised LDL and cardiometabolic profile in women with polycystic ovary syndrome: post-trial interview draft script.

Interviewer:
Audio device will be used to record conversation.

Ensure participant is briefed on what the information is going to be used for, gain verbal consent – (read the PIS – right to withdraw or not answer questions). Remind them that they can speak freely, and we are interested in honest opinions.

Turn on recording – announce time, date and participant code

Demographics – confirm age, ethnicity (self-identify), ask for highest education level and ask for annual household income – everyone in the house (if participant is comfortable answering):

- under £20k
- £21k to £30k
- £31k to £40K
- £41k+

1. Which intervention did you take part in? (closed question – exercise or lifestyle physical activity). Exercise/lifestyle/control - specific
2. Did you complete it? Did you find it difficult or easy to complete? Why?
3. What did you like (enjoy/find positive) and dislike about your intervention? How did you overcome the challenges
4. Did you notice any changes in yourself (probe physical/mental/emotional) throughout or after taking part in your intervention? How quickly? Positive/Negative changes?
5. Did any of these changes meet/not meet your expectations of what would happen during the intervention? Why?
6. Which aspect(s) of PCOS has the biggest effect on your life? (probe Appearance, weight, health issues, fertility).
7. Did you feel that the intervention had any effect on this aspect(s)?
8. What factors do you feel influenced how regularly you exercised before joining the trial?
9. Are you likely to take up regular exercise because of the intervention? Probe Attitude to exercise?
10. If not, why not?
11. If yes, what kind? How? What kind of support would be needed?
12. Would you take part in future research on PCOS and exercise?

13. If you could take part in an exercise intervention for another study, what factors would you like to be considered? Probe - Location, type of exercise, frequency

Thank you for taking part – summary report of findings – announce time