Supervised aerobic exercise training and increased lifestyle physical activity to reduce cardiovascular disease risk for women with polycystic ovary syndrome: a randomized controlled feasibility trial

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Title: Supervised aerobic exercise training and increased lifestyle physical activity to reduce cardiovascular disease risk for women with polycystic ovary syndrome: a randomized controlled feasibility trial

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Abstract

Background

Polycystic ovary syndrome (PCOS) is a complex, heterogeneous endocrinopathy. Women with PCOS often present with CVD risk factors.

Physical activity (PA) interventions reduce CVD risk factors in women with PCOS. However, sedentary behaviours have a distinct deleterious effect on cardiometabolic health. Increasing PA and reducing sedentary behaviours may be a worthwhile therapeutic target to improve cardiovascular health in this population.

This study investigated the feasibility of two PA interventions to decrease CVD risk in women with PCOS.

Methods

This was a feasibility RCT of two PA interventions in thirty-six women with PCOS. Participants were randomised to a supervised exercise intervention (n=12), a lifestyle physical activity intervention (LPAG) aimed at reducing sedentary behaviours (n=12), or a control group (n=12), for 12 weeks. Primary outcomes included the feasibility and acceptability of the interventions and procedures.

Results

Recruitment rate was 56%. Adherence rate was 53% and 100% to the exercise intervention and LPAG, respectively. Secondary outcome data indicates a reduction in oxidised LDL concentrations in the exercise group, and weight loss in both intervention groups.

Conclusions

The procedures for recruitment, allocation, and outcome measurements were acceptable. However, before progression to a full-scale trial, adherence to the exercise programme should be addressed.

1 Introduction

Polycystic ovary syndrome is a complex, heterogenous endocrinopathy affecting reproductive, cardiovascular, and metabolic health in up to 20% of women of reproductive age [1]. According to the Rotterdam criteria, there are three key clinical signs/symptoms: i) clinical/biochemical hyperandrogenism, ii) chronic anovulation/oligomenorrhea, and iii) polycystic ovaries [2]. Women must present with two out of the three symptoms to receive a diagnosis. PCOS affects fertility and is characterised by various cardiovascular disease (CVD) risk factors including dyslipidemia, insulin resistance, abdominal obesity, and chronic low-grade inflammation [3][4]. In addition, PCOS is associated with psychological distress and increased rates of mental health conditions [1].

Meta-analyses have indicated that exercise interventions of 12-24 weeks in duration are effective in mitigating CVD risk factors [5][6]. However, research has also highlighted the distinct deleterious effects of sedentary behaviour on cardiometabolic health [7][8][9]. This illuminates the importance of moving more often throughout the day, even when one is engaged in regular structured exercise.. However, to the authors knowledge, no studies have been conducted to investigate the effect of increasing lifestyle physical activity and reducing sedentary behaviours in women with PCOS. This may present a novel therapeutic target to improve CVD risk in women with PCOS because it presents an alternate approach to the conventional supervised exercise intervention. This may be effective for those with less time or accessibility to interventions and reduces burden on participants.

In addition, oxidised low-density lipoprotein (oxLDL) plays a key role in the development of atherosclerosis and is an independent risk factor for CVD [10][11]. OxLDL is associated with abdominal obesity and a high total cholesterol: high-density lipoprotein (TC:HDL) ratio. These CVD risk factors are often present women with PCOS [3][4]. Despite this, to our knowledge, only three studies have examined the role of oxLDL in CVD in women with PCOS, and none included an exercise intervention.[12][13][14]

Before an adequately powered randomised-controlled trial (RCT) measuring the efficacy of exercise and/or increased lifestyle physical activity on such indicators of cardiovascular health can be designed

and implemented, 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. Indeed, intervention studies can be undermined by unexpected, but ultimately preventable, issues in study design, conduct, and analysis [15]. Thus, the Medical Research Council recommend that interventions are developed systematically, utilising a phased approach that incorporates feasibility testing [16].

Therefore, the aim of the present study was to assess the feasibility of conducting a RCT of exercise training and increased physical activity (PA) in women with PCOS.

The objectives of this study were to: i) assess 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.

2 Materials and Methods

2.1 Study Design

A full description of the trial methods is available in our published protocol paper [17]. In summary, the study was a three-arm, randomised controlled feasibility trial conducted at one site in the UK (Sheffield, England). Health Research Authority (HRA) approval for the study was obtained and Research Ethics Committee (REC) favourable opinion granted by the North West – Greater Manchester East REC (18/NW/0454). The trial was prospectively registered at ClinicalTrials.gov (Identifier: NCT03678714).

The study took place from September 2018 and ceased in March 2020 due to the imposed government lockdown due to COVID-19. It was conducted at the Centre for Sport and Exercise Science (CSES), Sheffield Hallam University (SHU), Sheffield, UK.

2.2 Recruitment and Sampling

As this was a feasibility study, no formal sample size calculation was required. The aim was a sample size of 51 participants which is suitable for a feasibility trial; our protocol provides an in-depth explanation of how the sample size was determined [17].

Participants were mainly recruited from Sheffield Teaching Hospitals (STH), Sheffield, UK, upon a routine visit to either the fertility or gynaecology clinic led by MM. In addition, participants were recruited through social media platforms. The procedure for those responding to the announcement involved sending through an information pack by email and asked to contact the researcher to participate.

2.3 Eligibility Criteria

The inclusion and exclusion criteria are set out below:

Inclusion Criteria

i) Women clinically diagnosed with PCOS.
ii) Have experienced menarche and be at least 18 years of age.
iii) Were English speaking.
iv) Were physically able to perform exercise.

Exclusion Criteria

i) Post-menopausal status.
ii) Smokers.
iii) Undertaking regular structured exercise defined as >150min/week.
iv) Taking metformin for fewer than 3 months.
v) 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.
vii) Have current, clinically defined CVD or a history of cardiac events.

Both short-term usage of metformin and OCP use were excluded as they may affect results [18][19]. On the advice of the consultant clinician (MM), 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 required to inform the researcher 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.

2.4 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.

During visit 1, the following baseline tests and measurements were undertaken: age, anthropometric measures (stature, body mass, hip and waist measurements), capillary and venous blood sampling, aerobic fitness assessed by the Astrand-Rhyming [20] single stage test.

After completion of the 12-week intervention, all tests and measurements were repeated.

2.5 Randomisation

Participants were randomised, using block randomisation for equal numbers [21], using a computerised randomisation programme (QuickCalcs, GraphPad Software, USA). Allocation was concealed and placed in sequentially labelled opaque envelopes and offered to the participants, in sequence, by the researcher, on completion of their baseline assessments.

2.6 Withdrawals

Participants were informed that they could withdraw themselves and the data at any time without providing a reason. To preserve randomisation and produce unbiased results, intention-to-treat analysis was utilised [22]. Missing outcome data were dealt with by using the last observation carried forward (LOCF) method [23][24]. This approach minimises the number of participants excluded from the analysis [24].

2.7 Adverse Events

Adverse events were collected, reported, and assessed by the research team to determine classification and whether they were likely to be due to the trial. This may include unexpected musculoskeletal injuries arising from the trial.

2.8 Supervised Exercise Programme Group

Participants assigned to the exercise group were invited to undertake 2 sessions of supervised exercise training each week for 8 consecutive weeks and 3 sessions of supervised exercise training each week for the final 4 consecutive weeks at CSES fitness suite at SHU. Each session lasted approximately 60 minutes and involved 40 minutes of an individualised aerobic exercise protocol performed either on a cycle ergometer, elliptical trainer, rowing ergometer, or a motorised treadmill, preceded by a 10-minute warm-up and followed by a 10-minute cool down. The protocol paper describes the design and justification of the exercise protocol at length [17].

2.9 Lifestyle Physical Activity Group

Participants randomised to the lifestyle physical activity group (LPAG) were offered advice and information on how to increase physical activity, provided using British Heart Foundation guidelines: 'Understanding Physical Activity' [25]. They were asked to monitor and track their daily physical activity using a smartphone fitness application (i.e., Google Fit or Apple Health). Participants sent their data each week, by email. Data included daily energy expenditure, step count, and distance travelled by foot in km.

2.10 Control Group

Participants in the control group did not undertake any intervention but still received standard care from their medical and health professionals. This is dependent on the clinical decisions made by the participant and their health professional.

2.11 Blood Sampling and Storage

Blood was drawn from participants on their initial and post-intervention visit to the CSES. Blood was drawn from the median cubital vein, median cephalic vein, or from the dorsal superficial veins of the

hand. 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.

2.12 Outcome Measures

2.12.1 Feasibility Outcomes

Similar to other feasibility trials [26][27], the primary outcomes for this study were: 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 physical activity (that is, if participants continued to send through weekly data), 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 was assessed by exploring reasons for dropout, and the number and type of adverse events that occur in each group.

2.12.2 Secondary Outcomes

Serum oxidised LDL was analysed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Mercodia, Sweden). ELISA was also used for quantitative analysis of neopterin (IBL International, Germany), fasting insulin (Invitrogen, USA) and sex hormone binding globulin (SHBG) (Abcam, UK). Neopterin was assayed using the principle of a competitive ELISA.

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 [28]. MDA, in

the present of heat and acid, reacts with thiobarbituric acid to produce a coloured end product that 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. 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. All R^2 values were above 0.99 (considered to be a very good fit) other than the two SHBG assays. 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.

All intra-assay coefficients of variability (CV) were calculated to be below 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.

Aerobic fitness was assessed using the Astrand-Rhyming test [20] to determine VO_2 max. This is a submaximal single-stage test performed on a cycle ergometer, lasting between 6-7 minutes.

For lipid profile, including LDL-cholesterol, HDL-cholesterol, and total cholesterol (TC), and fasting glucose, a CardioChek PA Blood Analyser (PTS Diagnostics, USA) was used.

In order to measure the amount of lifestyle physical activity (and subsequent sedentary behaviour), the long-form International Physical Activity Questionnaire (IPAQ) was administered at baseline and post-intervention to all participants [29]. 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 [29].

Waist circumference (WC) and hip circumference (HC) were measured as per our protocol [17].

2.13 Data Analysis and Handling

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

For biochemical analysis, each sample was measured in duplicate and the mean of the two wells was used as the final value. Intra-assay CVs were calculated based on concentrations of each pair of duplicates, and inter-assay CV was calculated using the known controls for each plate to check consistency between assays of the same antigen.

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. Results should be interpreted with caution as statistical power has not been determined.

2.14 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 this specific dependant variable.

ii) Adherence to the exercise intervention defined as at least 74% of scheduled sessions taking place and the participant engaging i.e., undertaking all exercises. 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 [30].

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, the criteria for success determined what modifications, if any, should be made to the procedures and intervention before proceeding.

3 Results

3.1 Summary

Figure 1 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.

3.2 Screening, Eligibility, and Recruitment

Table 1 presents a summary of feasibility and acceptability data. Of the 78 people who volunteered to take part, 64 met the eligibility criteria after screening, 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 1.

3.3 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 not related to the exercise, 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.

3.4 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%), despite flexible scheduling being offered. This indicates that the exercise intervention must be refined to increase adherence.

Two nonserious AEs were observed during the study, both from participants in the exercise group. These were incident 1) back and incident 2) ankle pain, determined after investigation to be unrelated to the exercise sessions as part of the trial. No exercise sessions were postponed or affected by the AEs. No SAEs were reported.

Participants in the control group were offered the chance to undertake the supervised exercise intervention on completion of their follow-up visit. Only 2/12 participants accepted this offer.

3.5 Lifestyle Physical Activity Group Engagement

Although lifestyle data were collected for this group, these 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 but to assess if the procedures could be followed and the intervention(s) were acceptable to participants.

Reassuringly there were no missing lifestyle data for participants in the LPAG (100% submission). This indicates an extremely high engagement with the protocol and is a promising basis for a future trial. However, it should be noted that this refers to adherence to the protocol instructions to record and report weekly data, and not that participants had 100% adherence to the physical activity guidelines provided.

3.6 Outcome Measurements

Assessment of anthropometry, glucose, and cholesterol by capillary sample, VO_2 max, and administration of the IPAQ was completed without any issues for all participants at baseline and all participants who attended follow-up. Nevertheless, in some cases a blood sample could not be retrieved within two attempts.

3.6.1 Baseline Characteristics

Table 2 summarises the baseline characteristics. Across groups, randomisation yielded comparable baseline characteristics, although participants in the exercise group were more physically active at baseline than the control group

3.6.2 Anthropometry, Lipids, Glucose, Physical Activity and Aerobic Fitness

Table 3 shows pre- and post-values for anthropometry, capillary sample, and physical fitness measurements. Data indicate body mass loss (kg) in both the exercise group and the LPAG, reflected in both WC and HC. The data also indicate improvements in VO_2 max in the exercise group and to a lesser extent, the control group.

In the exercise group, the data indicate improvements in HDL, fasting glucose, and TC/HDL ratio. In the control group, improvements in TC and TC/HDL ratio were noted.

IPAQ data indicates that in the exercise group, total MET-mins/week were increased post-intervention and sitting min/week were reduced. However, in the control group, total MET-min/week also increased, although sitting min/week remained at similar levels. Additionally, total MET-mins/week appeared to decrease for the LPAG, although sitting min/week remained largely unchanged.

3.6.3 Biochemical Results

Table 4 indicates pre- and post- values for biochemical variables. Across groups, randomisation yielded comparable baseline characteristics for all variables.

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, with the smallest reduction seen in the LPAG.

There was no statistically significant interaction between the intervention and time, nor were there any statistically significant main effects of time or group on oxLDL concentration.

However, the η^2 values for the main effects of time (partial η^2 = .147) and group (partial η^2 = .093) indicate a medium-to-large effect size.

4 Discussion

This study explored the feasibility and acceptability of two physical activity interventions for women with PCOS, encompassing both a supervised exercise intervention and a lifestyle physical activity intervention aimed at reducing sedentary behaviours. Based on the criteria for success, the main finding is that study procedures were feasible and acceptable, but the adherence to the supervised exercise intervention was not as high as intended. As such, this discussion sets out recommendations and refinements that should be made before progression to a large-scale RCT is possible.

4.1 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 between 24 and 50 are sufficient to calculate a standard deviation of an outcome that can then be entered into a formal power calculation for a full-scale RCT [31][32]. In addition, sample sizes of at least 30 are considered to provide an SD that is a sufficiently accurate estimate of a population-SD [33]. As such, this criterion is fulfilled.

The next criterion is that acceptable adherence to the exercise intervention is 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; low adherence may not accurately reflect the potential efficacy of the intervention [34]. To boost this in our study, flexibility was offered to participants when scheduling sessions, but time constraints may still have been an issue, as this population is typically working age and may have dependents. Furthermore, using one intervention delivery location may have affected attendance. However, 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 [26]. Nevertheless,

this could be resolved through the integration of the proposed intervention in a centralised "exercise referral" scheme, with special training of the exercise facilitators.

Investigation of factors that can improve adherence to exercise interventions is a heavily discussed topic [34][35]. Successful adherence-enhancing components incorporate various behaviour change techniques such as self-monitoring, reinforcement, goal setting, and feedback [34][35]. 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 [35].

In addition, although the intervention(s) utilised various behaviour change techniques (such as self-monitoring and some goal setting), they were not underpinned by behaviour changes theories (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 .

The criteria for success stipulated that loss to follow-up at 12 weeks should be <20%. In this study, retention rate was 89% (attrition n=5). 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 indicates 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, 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 indicates that there were no significant problems with the delivery of measurement procedures.

The eligibility criteria were not considered to be too restrictive, as 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 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 for those randomised to the exercise group (2 x 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 [36]. This is particularly true of large incentives, or where the risks of the research are particularly high, or where the research is degrading [36]. 'Tokens', gift vouchers, and non-monetary gifts are considered less controversial [37]. Thus, it may be possible to provide small incentives/rewards to provide, for example, equal access to the study for all participants. Incentives should be based on the barriers to participation [38]. Therefore, 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 [38].

4.2 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.

4.2.1 Anthropometry

The mean WC and waist-to-hip ratio (WHR) in each group at baseline were >88cm and between 0.83-0.85, respectively, at baseline. 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 [39]. Based on proposed cut-offs, this suggests that the women in the study were at increased CVD risk [40][41][42].

The data indicate that ~3.5% weight loss was observed in both the exercise group and the LPAG group at follow-up. This is in line with previous research that has indicated modest weight loss occurs from exercise interventions in PCOS [10]. A weight loss of approximately 5% can improve CVD risk factors in women with PCOS with overweight [43].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) [44]. Previous research has indicated a 70% prevalence rate for dyslipidemia in PCOS. 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) [45]. 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 [46].

4.3 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. Thus, it is likely that the high concentration is due to a wide SD in a small population. Indeed, Macut et al. have reported in two studies [12][13], similarly wide SDs in women with PCOS. This indicates that there is considerable variation across the

population, possibly due to the lack of 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 has pro-oxidative properties and is an independent marker for CVD risk [47]. The participants in the exercise group had higher neopterin concentrations than that observed in healthy controls in previous research, which are typically <10 nmol/L [48][49][50]. 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 [48][51].

4.4 Strengths and Limitations

The main strength of this feasibility study is that it is an essential step to identify methodological constraints that may impact on a full-scale trial, preventing potential waste of resources. a potential issue in adherence rates to the exercise intervention, which can be refined before the next steps. Additionally, it has provided information about sources of and rates of recruitment, which are integral for 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 programme.

There are some limitations that need to be considered when interpreting the findings. Firstly, the use of the IPAQ to record lifestyle physical activity may have limitations because it is a self-report tool. Self-reporting has disadvantages involving both recall and/or accurate reporting [52]. Nonetheless, it is an affordable, simple, and feasible way to collect adjunct lifestyle PA data, which can be used when there is a need to reduce participant burden, as in our study. Additionally, data were not recorded on

which participants had taken metformin for >3months. Future studies should report this data so that differences arising from the effects of metformin could be investigated.

5 Conclusion

The present study has assessed the feasibility and acceptability 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 (such as the inclusion of behavioural change support) may be required to the exercise intervention to increase adherence. In addition, sufficient oxidised LDL 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 in women with PCOS. Furthermore, there appears to be potential for the PA intervention(s) to mitigate some of these factors having examined trends in the health outcomes data. Based on our findings, the next steps would involve planning for a definitive trial with an internal pilot study where any new features of the intervention can be assessed alongside the effectiveness of the intervention(s).

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Table 1. 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.	Medium 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.

Table 2. 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
Body mass (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 3. Summary of Pre and Post Values for Anthropometry, Capillary Sample, and Physical Fitness Measurements.

Measurement	Exercise		LPAG		Control	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
Body mass (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 4. 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
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 substance

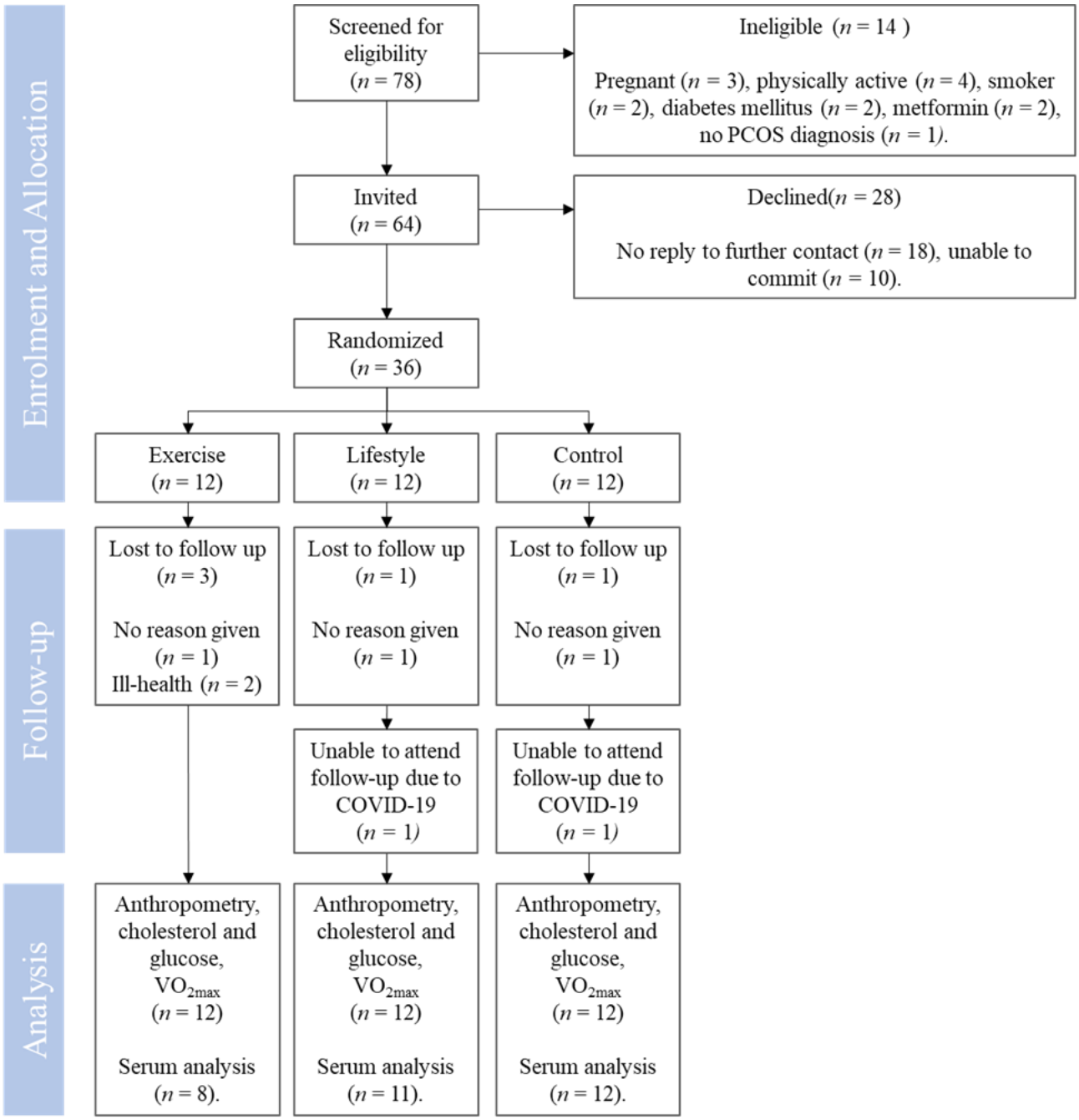

Figure 1. Flow of participants through the trial.