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<https://orcid.org/0000-0002-9579-4012>, Broom, David, Dalton, Caroline, Metwally, Mostafa and Klonizakis, Markos (2022) 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. *Journal of Physical Activity and Health*, 19 (6). pp. 436-445.

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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

Running Head: Exercise physical activity in women with PCOS

Keywords: oxidized LDL, mHealth, sedentary behaviour

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Abstract word count: 200

Manuscript word count: 6190

Date of manuscript submission: 18/02/2022

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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.

## 27 1 Introduction

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

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

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

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

53 and implemented, the feasibility and acceptability of the interventions and procedures for recruitment,  
54 allocation, and outcome measurements must be assessed. In addition, the interventions must be  
55 refined, and a sample size must be calculated. Indeed, intervention studies can be undermined by  
56 unexpected, but ultimately preventable, issues in study design, conduct, and analysis [15]. Thus, the  
57 Medical Research Council recommend that interventions are developed systematically, utilising a  
58 phased approach that incorporates feasibility testing [16].

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

61 The objectives of this study were to: i) assess rates of recruitment and retention; ii) measure rates of  
62 attendance and compliance with the interventions; iii) obtain a standard deviation for oxidised LDL so  
63 that a sample size for a future, larger-scale RCT can be calculated.

## 64 2 Materials and Methods

### 65 2.1 Study Design

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

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

75 **2.2 Recruitment and Sampling**

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

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

84 **2.3 Eligibility Criteria**

85 The inclusion and exclusion criteria are set out below:

86 *Inclusion Criteria*

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

91 *Exclusion Criteria*

- 92 i) Post-menopausal status.
- 93 ii) Smokers.
- 94 iii) Undertaking regular structured exercise defined as >150min/week.
- 95 iv) Taking metformin for fewer than 3 months.
- 96 v) Taking the oral contraceptive pill (OCP) or have taken in the last month.
- 97 vi) Have any medical condition that may be responsible for the symptoms of PCOS.
- 98 vii) Have current, clinically defined CVD or a history of cardiac events.

99 Both short-term usage of metformin and OCP use were excluded as they may affect results [18][19].  
100 On the advice of the consultant clinician (MM), it was deemed acceptable for participants to have  
101 been taking metformin for at least three months, because changes to glucose metabolism are more  
102 stable after this period. Participants were required to inform the researcher as soon as possible after  
103 beginning such medication. They were advised that commencement of any of the above-mentioned  
104 medications during the trial is a contraindication and they would be withdrawn from the trial.

### 105 2.4 Baseline and Post-Intervention Measurements

106 Participants were asked to abstain from alcohol and vigorous exercise for 24 hours before attending  
107 their assessments. In addition, participants were asked to abstain from eating for at least two hours  
108 prior.

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

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

### 113 2.5 Randomisation

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

### 118 2.6 Withdrawals

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

124 **2.7 Adverse Events**

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

128 **2.8 Supervised Exercise Programme Group**

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

136 **2.9 Lifestyle Physical Activity Group**

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

143 **2.10 Control Group**

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

147 **2.11 Blood Sampling and Storage**

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



150 hand. Blood samples were collected into BD Vacutainer plastic serum tubes, inverted 5-6 times, and  
151 left to clot upright at room temperature for thirty minutes. Samples were then centrifuged in a Heraeus  
152 Labofuge 400 at 1300 x g (Relative Centrifugal Force) for ten minutes at 18-25°C. Serum was  
153 aliquoted and stored at -80°C until analysis. Assays were performed at the Biomolecular Research  
154 Centre, Sheffield Hallam University.

## 155 2.12 Outcome Measures

### 156 2.12.1 Feasibility Outcomes

157 Similar to other feasibility trials [26][27], the primary outcomes for this study were: acceptability and  
158 feasibility of procedures for recruitment, allocation, measurement, and retention for the intervention  
159 procedures. Recruitment rate was calculated by dividing the number of women eligible and  
160 consenting by the recruitment period. Attrition rates were established as discontinuation of the  
161 intervention and loss to follow-up measurement for both conditions. Compliance was monitored by  
162 session attendance and monitoring the data from recorded daily physical activity (that is, if  
163 participants continued to send through weekly data), with examination of reasons for drop-out or non-  
164 compliance. Reasons for drop-out were also used to assess the suitability of allocation procedures.  
165 Suitability of measurement procedures were evaluated by completion rates and reasons for missing  
166 data. Safety of the exercise intervention was assessed by exploring reasons for dropout, and the  
167 number and type of adverse events that occur in each group.

### 168 2.12.2 Secondary Outcomes

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

173 Thiobarbituric acid reactive substances (TBARS) was also measured as a secondary outcome. Lipid  
174 peroxides created by oxidising agents that alter lipid structure result in the formation of  
175 malondialdehyde (MDA), which is thought to reflect the extent of lipid peroxidation [28]. MDA, in

176 the present of heat and acid, reacts with thiobarbituric acid to produce a coloured end product that be  
177 quantified using a plate reader. TBARS was measured by commercially available assay (R&D  
178 Systems, USA).

179 The optical density for each assay was measured using an electronic plate reader. the calibration curve  
180 was plotted using Prism (GraphPad Software, USA). Following manufacturers recommendation, an  
181 appropriate regression model was chosen based on the linearity of the data and used to interpolate  
182 unknown concentrations.  $R^2$  values were checked to ensure the good fit of the model. All  $R^2$  values  
183 were above 0.99 (considered to be a very good fit) other than the two SHBG assays. For each assay  
184 where quality controls were provided, the concentration obtained was observed to be within the  
185 acceptable detection range as specified by the manufacturer.

186 All intra-assay coefficients of variability (CV) were calculated to be below 10% other than the second  
187 SHBG plate (mean CV=18%) and the first neopterin plate (mean CV=21%). This indicates some  
188 inconsistency in results between replicates. The inter-assay CV for each plate was determined to be  
189 below 15% apart from neopterin, which had an inter-assay CV of 23%. As before, this may indicate  
190 some inconsistency in results for neopterin concentrations between plates.

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

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

195 In order to measure the amount of lifestyle physical activity (and subsequent sedentary behaviour), the  
196 long-form International Physical Activity Questionnaire (IPAQ) was administered at baseline and  
197 post-intervention to all participants [29]. A score of total MET-minutes per week was calculated, as  
198 well as total sitting time per week. METs (metabolic equivalents) are multiples of the resting  
199 metabolic rate (indicating energy output required to complete a task), and a MET-minute is thus  
200 computed by multiplying the MET score of an activity by the duration (in minutes) that it was  
201 performed [29].

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

### 203 2.13 Data Analysis and Handling

204 All quantitative measurements are presented as mean  $\pm$  standard deviation (SD) unless otherwise  
205 stated.

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

210 An exploratory analysis of oxLDL by two-factor mixed ANOVA was performed. There were no  
211 outliers in the data, as assessed by inspection of a boxplot and by examination of studentized residuals  
212 for values greater than  $\pm 3$ . Concentration was normally distributed, as assessed by Shapiro-Wilk's test  
213 ( $p > .05$ ) and visual assessment of a Q-Q Plot. Results should be interpreted with caution as statistical  
214 power has not been determined.

### 215 2.14 Criteria for Success

216 The feasibility trial was assessed against acceptability criteria as follows:

- 217 i) Sufficient oxidised LDL data is obtained to allow for a formal sample size calculation  
218 based on standard deviation of this specific dependant variable.
- 219 ii) Adherence to the exercise intervention defined as at least 74% of scheduled sessions  
220 taking place and the participant engaging i.e., undertaking all exercises. This figure has  
221 been chosen because it reflects a mean adherence level for supervised exercise  
222 interventions for people with chronic conditions, including CVD and diabetes [30].
- 223 iii) Loss to follow-up at 12-weeks is  $< 20\%$ .
- 224 iv) There are no serious adverse events (SAE) resulting from the trial procedures.
- 225 v) There are no significant difficulties for the researcher in administering the measurement  
226 procedures or the intervention, measured by missing outcome data.

227

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

## 231 3 Results

### 232 3.1 Summary

233 Figure 1 shows the flow of participants through the trial. Recruitment took place from October 2018  
234 to January 2020. Follow-up data collection was completed by March 2020; however, due to COVID-  
235 19 restrictions and the obligatory early trial termination, two participants were unable to attend the  
236 laboratory to complete the follow-up assessments.

### 237 3.2 Screening, Eligibility, and Recruitment

238 Table 1 presents a summary of feasibility and acceptability data. Of the 78 people who volunteered to  
239 take part, 64 met the eligibility criteria after screening, and 36 were recruited. This gives eligibility  
240 and recruitment rates of 82% and 56%, respectively. The recruitment rate over time is 2.25  
241 participants per month. Reasons for non-consent and exclusion are shown in Figure 1.

### 242 3.3 Retention

243 The retention rate was 89%, which was above the acceptable criterion of 80%. Five of the 36  
244 participants formally left the study; three from the exercise group (two for ill-health not related to the  
245 exercise, and one with no reason given), one from the LPAG (no reason given), and one from the  
246 control group (no reason given). Twenty-nine participants completed all baseline and follow-up  
247 sessions and measurements. However, due to COVID-19 restrictions in March 2020, two participants  
248 who were due to return for their follow-up visit (one from the LPAG, one from the control) were  
249 unable to visit due to the obligatory lab closure.

### 250 3.4 Exercise Attendance and Safety Data

251

252 Overall attendance to the exercise sessions was below the acceptable limit of 74%. A total of 152/285  
253 sessions were completed (53%), despite flexible scheduling being offered. This indicates that the  
254 exercise intervention must be refined to increase adherence.

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

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

### 261 3.5 Lifestyle Physical Activity Group Engagement

262 Although lifestyle data were collected for this group, these are not presented here. The primary reason  
263 for this is because as a feasibility trial, the purpose is not to test the efficacy of the interventions but to  
264 assess if the procedures could be followed and the intervention(s) were acceptable to participants.

265 Reassuringly there were no missing lifestyle data for participants in the LPAG (100% submission).

266 This indicates an extremely high engagement with the protocol and is a promising basis for a future  
267 trial. However, it should be noted that this refers to adherence to the protocol instructions to record  
268 and report weekly data, and not that participants had 100% adherence to the physical activity  
269 guidelines provided.

### 270 3.6 Outcome Measurements

271 Assessment of anthropometry, glucose, and cholesterol by capillary sample, VO<sub>2</sub> max, and  
272 administration of the IPAQ was completed without any issues for all participants at baseline and all  
273 participants who attended follow-up. Nevertheless, in some cases a blood sample could not be  
274 retrieved within two attempts.

275 3.6.1 Baseline Characteristics

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

279 3.6.2 Anthropometry, Lipids, Glucose, Physical Activity and Aerobic Fitness

280 Table 3 shows pre- and post-values for anthropometry, capillary sample, and physical fitness  
281 measurements. Data indicate body mass loss (kg) in both the exercise group and the LPAG, reflected  
282 in both WC and HC. The data also indicate improvements in VO<sub>2</sub> max in the exercise group and to a  
283 lesser extent, the control group.

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

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

290 3.6.3 Biochemical Results

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

293 The data indicates that the largest improvements in oxLDL were seen in the exercise group with a  
294 14% reduction at follow-up compared to baseline. However, this was also observed, to a lesser extent,  
295 in the control group where data indicates a 10% reduction at follow-up compared to baseline, with the  
296 smallest reduction seen in the LPAG.

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

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

## 301 4 Discussion

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

### 308 4.1 Feasibility

309 The first criterion for success stipulates that sufficient oxidised LDL data is obtained to allow for a  
310 formal sample size calculation using the SD of the variable. In the present study, 31 observations of  
311 oxidised LDL were obtained across groups at baseline. Indeed, it has been suggested that samples of  
312 between 24 and 50 are sufficient to calculate a standard deviation of an outcome that can then be  
313 entered into a formal power calculation for a full-scale RCT [31][32]. In addition, sample sizes of at  
314 least 30 are considered to provide an SD that is a sufficiently accurate estimate of a population-SD  
315 [33]. As such, this criterion is fulfilled.

316 The next criterion is that acceptable adherence to the exercise intervention is defined as at least 74%  
317 of scheduled sessions taking place. In this study, 53% of scheduled sessions took place. Adherence to  
318 an exercise intervention is an important variable that can help to determine the validity of the  
319 findings; low adherence may not accurately reflect the potential efficacy of the intervention [34]. To  
320 boost this in our study, flexibility was offered to participants when scheduling sessions, but time  
321 constraints may still have been an issue, as this population is typically working age and may have  
322 dependents. Furthermore, using one intervention delivery location may have affected attendance.  
323 However, although multiple venues may assuage this issue to some degree, this presents further  
324 challenges in terms of resources and qualified personnel to deliver the protocol [26]. Nevertheless,

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

327 Investigation of factors that can improve adherence to exercise interventions is a heavily discussed  
328 topic [34][35]. Successful adherence-enhancing components incorporate various behaviour change  
329 techniques such as self-monitoring, reinforcement, goal setting, and feedback [34][35]. In this study,  
330 although the exercise intervention ramped in intensity every four weeks, there were no specific fitness  
331 goals or achievements to work toward. To incorporate adherence-enhancing components, a future trial  
332 could consider a pre-intervention goal-setting session in which the trainer and the participant outline  
333 some specific fitness goals for the participant, and a realistic plan for how to achieve them. This  
334 would allow regular feedback and monitoring of progress against the goal and may provide  
335 motivation and a sense of satisfaction upon achievement, or when interim goals are achieved before  
336 achievement of the end goal [35].

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

341 The criteria for success stipulated that loss to follow-up at 12 weeks should be <20%. In this study,  
342 retention rate was 89% (attrition n=5). Three withdrew from the exercise group compared to one in  
343 each of the other two groups. Two of three in the exercise group cited ill-health. This indicates that  
344 illness is a barrier to participation not experienced in the LPAG, because participants may have felt  
345 well enough to attend follow-up or take part in gentle PA, but not able to participate in structured  
346 exercise.

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



351 Analysis of reasons for missing data and completion of outcome measurements indicates that there  
352 were no significant problems with the delivery of measurement procedures.

353 The eligibility criteria were not considered to be too restrictive, as 82% of screened volunteers were  
354 eligible. Furthermore, 56% of eligible volunteers accepted the invitation to enrol into the study. This  
355 indicates that there are no significant problems with the inclusion and exclusion criteria, and  
356 recruitment rate was on par with prospective participant numbers.

357 Ten of the 28 eligible volunteers who declined to take part cited that they were unable to commit to  
358 the study. The study as designed does require a considerable time commitment for those randomised  
359 to the exercise group (2 x assessment visits, 28 exercise sessions). In order to provide added  
360 incentive, rewards or reinforcement could be offered based on the number of exercise sessions  
361 completed (and completion of follow-up). This may have the added effect of increasing adherence but  
362 also incentivising volunteers to take part. However, incentives for clinical trials are considered by  
363 some to be coercive and to encourage enrolment into trials for the wrong reasons [36]. This is  
364 particularly true of large incentives, or where the risks of the research are particularly high, or where  
365 the research is degrading [36]. ‘Tokens’, gift vouchers, and non-monetary gifts are considered less  
366 controversial [37]. Thus, it may be possible to provide small incentives/rewards to provide, for  
367 example, equal access to the study for all participants. Incentives should be based on the barriers to  
368 participation [38]. Therefore, providing travel expenses where travel costs are prohibitive may be an  
369 effective incentive, with an additional incentive to encourage the desired behaviour, such as entry into  
370 a prize lottery [38].

#### 371 4.2 Participant Characteristics

372 The purpose of this study was not to assess the efficacy of the intervention(s), and as such the study  
373 was not adequately powered to detect differences between means in participant characteristics.

374 However, the descriptive statistics provide useful information about the population in terms of CVD  
375 risk, as well as an overview of the characteristics in each of the study arms.

376 4.2.1 Anthropometry

377 The mean WC and waist-to-hip ratio (WHR) in each group at baseline were >88cm and between 0.83-  
378 0.85, respectively, at baseline. This indicates that the women in the present study had characteristics  
379 in line with previous studies that report an increased prevalence of abdominal obesity in PCOS [39].  
380 Based on proposed cut-offs, this suggests that the women in the study were at increased CVD risk  
381 [40][41][42].

382 The data indicate that ~3.5% weight loss was observed in both the exercise group and the  
383 LPAG group at follow-up. This is in line with previous research that has indicated modest  
384 weight loss occurs from exercise interventions in PCOS [10]. A weight loss of approximately  
385 5% can improve CVD risk factors in women with PCOS with overweight [43].  
386 and Glucose

387 Baseline TC (mmol/L) and HDL (mmol/L) concentrations for all groups were within the healthy  
388 range (TC < 5.0 mmol/L, HDL ≥ 1.2 mmol/L) [44]. Previous research has indicated a 70% prevalence  
389 rate for dyslipidemia in PCOS. Thus, this could be normal variation within the population.

390 In addition, fasting glucose concentrations were within the healthy, non-diabetic range (4.0-5.9  
391 mmol/L) [45]. However, in PCOS this does not suggest there are no abnormalities in glucose and  
392 insulin metabolism. Since compensatory hyperinsulinemia occurs because of insulin resistance,  
393 fasting glucose can be maintained at healthy concentrations for a time before insulin resistance  
394 worsens [46].

395 4.3 Biochemical Analysis

396 Oxidised LDL is the key secondary outcome in this study. Results from the present study indicate that  
397 oxidised LDL concentrations were high across groups. Indeed, the observed values in the present  
398 study (mean for all groups =  $99.27 \pm 33.52$  U/L), are higher than those observed in other studies of  
399 oxidised LDL in PCOS using the same method. Thus, it is likely that the high concentration is due to  
400 a wide SD in a small population. Indeed, Macut et al. have reported in two studies [12][13], similarly  
401 wide SDs in women with PCOS. This indicates that there is considerable variation across the

402 population, possibly due to the lack of international reference data currently available. Thus, the  
403 relative value of the data is not undermined (that is, the difference between groups is more important  
404 than the absolute values).

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

408 Neopterin has pro-oxidative properties and is an independent marker for CVD risk [47]. The  
409 participants in the exercise group had higher neopterin concentrations than that observed in healthy  
410 controls in previous research, which are typically <10 nmol/L [48][49][50]. However, TBARS  
411 concentrations, which also indicate lipid peroxidation and inflammation, were not higher than those  
412 found in healthy controls, which is in line with other studies comparing TBARS in women with and  
413 without PCOS [48][51].

#### 414 4.4 Strengths and Limitations

415 The main strength of this feasibility study is that it is an essential step to identify methodological  
416 constraints that may impact on a full-scale trial, preventing potential waste of resources. a potential  
417 issue in adherence rates to the exercise intervention, which can be refined before the next steps.

418 Additionally, it has provided information about sources of and rates of recruitment, which are integral  
419 for planning and budgeting for a large-scale trial.

420 Another strength of the study is the use of technology in the LPAG. Fitness devices are proliferating  
421 rapidly and provide a convenient, economic way to track PA that is an alternative to a supervised  
422 exercise programme.

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

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

## 430 5 Conclusion

431 The present study has assessed the feasibility and acceptability of conducting an RCT of two PA  
432 interventions in women with PCOS. The results indicate that procedures for recruitment, allocation,  
433 and outcome measurement were acceptable. However, some changes (such as the inclusion of  
434 behavioural change support) may be required to the exercise intervention to increase adherence. In  
435 addition, sufficient oxidised LDL data has been collected for a sample size calculation for a fully  
436 powered RCT. The participant characteristics indicate that the population in the present study display  
437 some features of increased CVD risk, in line with previous research in women with PCOS.  
438 Furthermore, there appears to be potential for the PA intervention(s) to mitigate some of these factors  
439 having examined trends in the health outcomes data. Based on our findings, the next steps would  
440 involve planning for a definitive trial with an internal pilot study where any new features of the  
441 intervention can be assessed alongside the effectiveness of the intervention(s).

## 442 6 Acknowledgements

443 This work was funded by Sheffield Hallam University as part of a funded PhD programme.

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Table 1. Summary of Feasibility and Acceptability Findings.

| <b>Methodological Issues</b>  | <b>Findings</b>   | <b>Evidence</b>  |
|---|---|--|
| 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 <sub>2</sub> 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.

| <b>Baseline Characteristics</b> | <b>Exercise (n=12)</b> | <b>LPAG (n=12)</b> | <b>Control (n=12)</b> |
|---------------------------------|------------------------|--------------------|-----------------------|
| 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 <sup>2</sup> )        | 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 <sub>2</sub> 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 <sub>2</sub> 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-<br>Min/Week     | 3990 (1654)  | 4460 (5459)  | 3188 (2981)  | 2760 (2743)  | 2163 (2010)  | 3138 (3019)  |
| IPAQ Total Sitting<br>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.

| Analyte            | Exercise      |               | LPAG          |               | Control        |               |
|--------------------|---------------|---------------|---------------|---------------|----------------|---------------|
|                    | 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



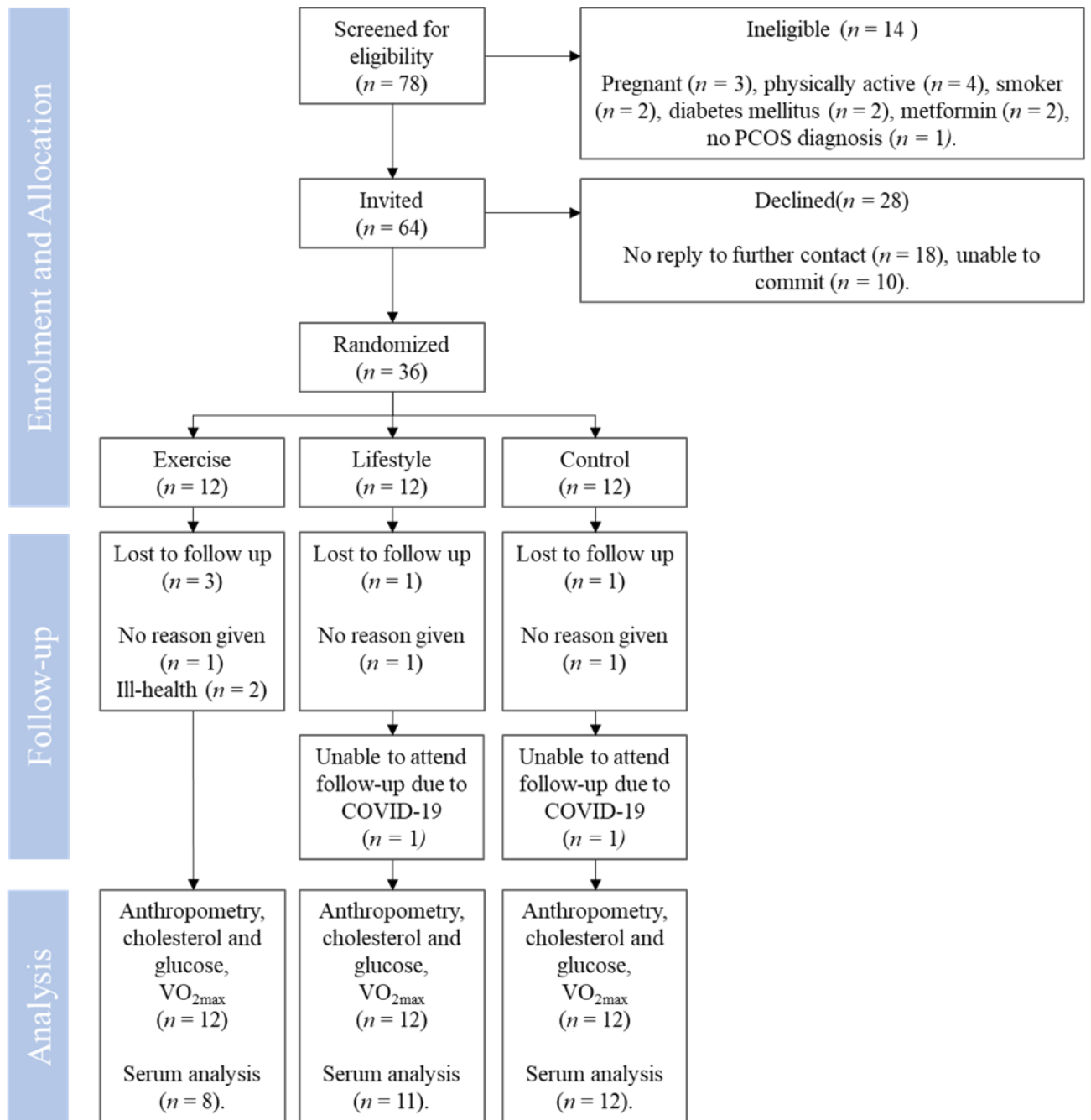


Figure 1. Flow of participants through the trial.