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BMJ Open Improving reproductive function in women with polycystic ovary syndrome with high-intensity interval training (IMPROV-IT): study protocol for a two-centre, three-armed randomised controlled trial

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ABSTRACT

Introduction Polycystic ovary syndrome (PCOS) is a common endocrine disorder in women of reproductive age and the leading cause of anovulatory infertility. Women with PCOS have a 15-fold higher prevalence of infertility, compared with women without PCOS, independent of body mass index (BMI). A healthy lifestyle is recommended to improve overall health and fertility in PCOS but there is limited evidence on the isolated effects of exercise, especially for reproductive outcomes. Previous findings indicate superior metabolic health benefits after vigorous compared with moderate-intensity exercise. Our primary aim is to determine the effect of high-intensity interval training (HIT) on menstrual frequency, as a proxy for reproductive function, in women with PCOS.

Methods and analysis The study is a two-centre, randomised, controlled trial with three parallel groups. Women (n=64) from Trondheim (Norway) and Melbourne (Australia) with PCOS according to the Rotterdam criteria will be randomly allocated (1:1:1) to high-volume HIT, low-volume HIT or a control group with no exercise after stratifying for BMI < or ≥ 27 kg/m² and study centre. Measurements for study end points will be undertaken at baseline, after a 16 week exercise intervention and at 12 months following baseline assessments. The primary outcome measure is menstruation frequency, measured as the number of self-reported menstrual bleedings divided by the number of expected menstrual bleedings during a 12-month period. Secondary outcome measurements include markers of cardiovascular, metabolic and reproductive health, as well as quality of life and adherence to and enjoyment of exercise.

Ethics and dissemination The Regional Committee Medical Research Ethics, Norway, and The Australian Catholic University Human Research Ethics Committee, Australia, have approved the trial protocol. This trial will provide new insight regarding the impact of exercise on fertility in PCOS. We expect this trial to contribute to new

Strengths and limitations of this study

- This will be the first randomised controlled trial to determine the isolated effects of two different high-intensity interval training protocols on reproductive and health-related outcomes in women with polycystic ovary syndrome, with a follow-up time of 12 months.
- The exercise intervention is controlled and monitored through partly supervised sessions for 16 weeks.
- We will include women within all body mass index categories.
- Investigators will not be blinded for all assessments due to the difficulty blinding investigators and participants to a behavioural intervention.
- Menstrual frequency is only a proxy for reproductive function, and we will not undertake ovulation monitoring.

therapeutic exercise strategies as part of clinical care for women with PCOS.

Trial registration number Clinical trial gov NCT02419482.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a complex endocrine disorder^{1 2} affecting 8%–13% of reproductive-aged women.³ The ovulatory disturbance is a central diagnostic feature of PCOS,⁴ and the syndrome is recognised as the leading cause of anovulatory infertility and menstrual disorders.⁵ A large community-based cohort study reported a 72% prevalence of infertility among women with PCOS, compared with 16% among women without PCOS.⁶



According to the most recent guidelines for the assessment and management of PCOS, lifestyle intervention is regarded as first-line therapy to manage reproductive and metabolic outcomes.⁴ In addition to reproductive dysfunctions, PCOS is associated with a number of adverse cardiovascular, metabolic and psychological outcomes across all categories of body mass index (BMI).^{2 4 7-9} Obesity seems to exacerbate the clinical features in PCOS, but also weight-independent insulin resistance is strongly implicated in the aetiology of PCOS, contributing to the reproductive and metabolic complications.¹⁰⁻¹³ Approximately 40% of women with PCOS have a normal BMI (<25 kg/m²) and the prevalence of insulin resistance among lean women with PCOS is estimated to be around 75%.¹⁴ Adipose tissue dysfunction, such as hypertrophic adipocytes and impairments in lipolysis and insulin action, plays a central role in the metabolic abnormalities observed in PCOS.¹⁵ Hypertrophic adipocytes are more susceptible to inflammation and chronic low-grade inflammation in PCOS.¹⁶ There is limited research on the effect of exercise training on adipose tissue function and low-grade inflammation in PCOS.

Exercise training positively affects metabolic, cardiovascular and psychological outcomes in women with PCOS, but the effect on fertility is unclear.^{4 12 17-19} Some trials²⁰⁻²⁴ and systematic reviews^{12 25} report improved ovulation or menstruation frequency after a period of exercise training or after a combined exercise-diet intervention.²⁶ Two of these studies indicate a weight-independent effect of exercise on fertility, suggesting insulin sensitivity to be a key factor.^{21 23} Only one study to date has investigated the effect of exercise on menstruation frequency in women with PCOS having normal or low BMI (<25 kg/m²).²² In that study, they found a significant improvement in menstruation frequency (from 48 to 27 days) after 8 weeks (three sessions per week) of aerobic and resistance training. However, 8 weeks follow-up may be too short to determine any effect of exercise training on menstrual frequency. Furthermore, inconsistent reporting of fertility-related outcomes, small sample sizes and short intervention periods, make it difficult to interpret the effect of exercise on fertility.^{2 4 12 25}

Despite growing evidence on health benefits of exercise training in PCOS,^{4 12 27} there is a lack of well-designed randomised controlled trials of different exercise protocols including long intervention and follow-up periods to determine the isolated effect of exercise on fertility outcomes

. Most studies to date have only included overweight/obese participants and less is known about the potential benefits in women with PCOS who have BMI ≤25 kg/m². Although moderate-intensity exercise provides health benefits, only vigorous, and not moderate-intensity, physical activity was associated with reduced odds of insulin resistance and metabolic syndrome in a cross-sectional study of women with PCOS.¹⁸ Indeed, there

is an increased focus on high-intensity interval training (HIT) as a means of improving insulin sensitivity²⁸ and cardiorespiratory fitness²⁹⁻³¹ in clinical populations, including PCOS.³² HIT involves brief, repeated work bouts of relatively intense exercise separated by periods of rest or low-intensity exercise. Several different HIT protocols exist, and they can broadly be divided into 'high-volume' (HV) and 'low-volume' (LV) HIT. One of the most common HV-HIT protocols is the Norwegian 4×4 min HIT protocol, which induces superior improvements in cardiorespiratory fitness compared to work-matched moderate-intensity training in both healthy individuals³³ and in various patient groups.^{29 34} LV-HIT typically consists of ≤10 min of intense exercise within an exercise session lasting ≤30 min in total, such that the total weekly training time commitment is markedly lower than the current public health guidelines.³⁵ LV-HIT could therefore have the potential to overcome the most common barriers for women in fertile age, such as time commitment.³⁶ LV-HIT can improve glycaemic control in people with type 2 diabetes.³⁷ We have previously reported improved insulin sensitivity (measured with homeostatic assessment of insulin resistance, HOMA-IR), endothelial function and body composition after 10 weeks of HIT in women with PCOS, without any changes in body mass.³² However, in that pilot study, the participants undertook both LV-HIT and HV-HIT and we did not determine the effects of exercise training on any reproductive outcomes. Based on the positive results from our pilot study with a combined HV-HIT and LV-HIT protocol, we will now compare the two different HIT protocols to investigate potential differences in health outcomes.

Here we describe the design, methodology and potential clinical significance of the 'Improving Reproductive function in women with Polycystic Ovary syndrome by high-intensity Interval Training (IMPROV-IT) trial'.

AIMS

The primary aim of the IMPROV-IT trial is to test the hypothesis that 16 weeks of semi-supervised HIT, followed by home-based HIT for 36 weeks, will increase menstruation frequency, as a proxy measure of ovulation, during 1 year of follow-up in women with PCOS, compared with a non-exercising control group.

Secondary aims are to determine if there are improvements in: ovarian morphology, insulin sensitivity, endothelial function, intima-media thickness, body composition, cardiorespiratory fitness, oxidative capacity, circulation markers of reproductive and metabolic health, low-grade systematic inflammation (in blood and adipose tissue), adipose tissue morphology and function, and quality of life, after 16 weeks of semi-supervised HIT, as well as after the following 36 weeks of home-based HIT.

Additionally, we will record the adherence to exercise training and enjoyment of two different HIT protocols

CONSORT 2010 Flow Diagram

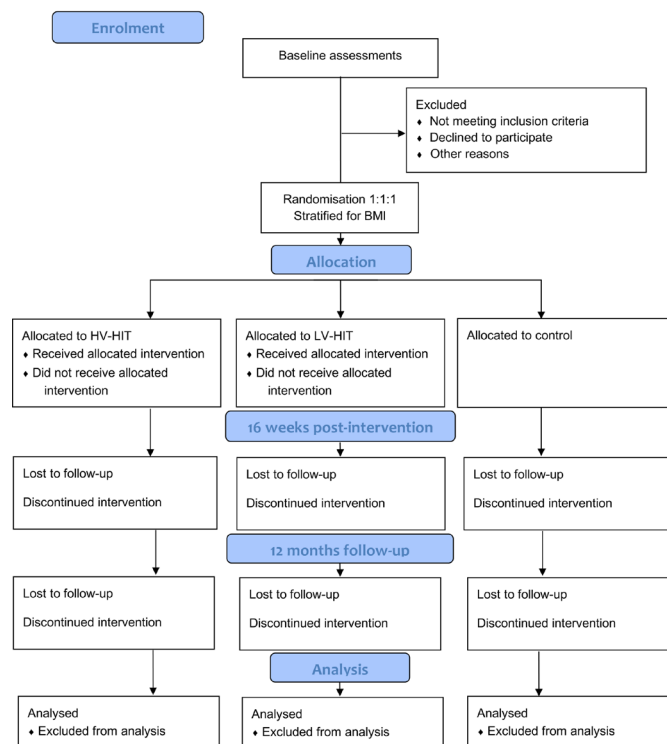


Figure 1 Consort flow diagram. BMI, body mass index; HV-HIT, high-volume high-intensity interval training; LV-HIT, low-volume high-intensity interval training.

and assess if the two protocols induce different effects on the aforementioned measures.

METHODS AND ANALYSIS

Study setting and recruitment

This is a two-centre, randomised controlled trial with three parallel groups; two training intervention groups and one control group. The two study centres are the Norwegian University of Science and Technology (NTNU) in Trondheim, Norway and the Australian Catholic University (ACU) in Melbourne, Australia. Testing and training will take place in the university research laboratories in both Norway and Australia. Participants will be recruited from the public announcement at hospitals and university homepages, at local stores, public places and social media. All participants will sign a written, informed consent. All participants will have a project-specific code that will be used during all analyses of the data. The procedures for data entry, coding and storage have been approved by the Regional Committee Medical Research Ethics in Mid-Norway.

Participants

To be eligible for inclusion in the study, women will have to meet the following criteria:

- ▶ Aged 18–45 years old.
 - ▶ PCOS diagnosis according to the Rotterdam criteria,³⁸ as confirmed by a gynaecologist, endocrinologist or general practitioner. Thus, a minimum of two of the following criteria have to be present: (1) polycystic ovary morphology (12 or more 2–9mm follicles or >10mL in volume, in at least one ovary), (2) hyperandrogenism (either clinical signs as hirsutism or acne, or biochemical), and (3) oligo/amenorrhoea. Oligomenorrhoea is defined as an intermenstrual interval >35 days and/or ≤9 menstrual bleeding in the past year. Amenorrhoea is defined as no vaginal bleeding the last 3 months for women with a previously regular menstrual cycle; no vaginal bleeding the last 12 months for women with an irregular menstrual cycle.^{39 40} Hirsutism will be defined as modified Ferriman Gallwey score⁴¹ ≥8.⁴
 - ▶ At the Norwegian centre, all participants will be screened at baseline for polycystic ovaries, hyperandrogenism and menstruation frequency to confirm the PCOS diagnosis. At the Australian centre, participants will show an ultrasound scan no older than 8 years performed by their general practitioner, gynaecologist or endocrinologist confirming polycystic ovaries. Ferriman Gallwey score for hirsutism/hyperandrogenism and information about their menstrual cycle will be obtained before entering the study to confirm PCOS diagnosis.
 - ▶ Undertaking less than 2 weekly endurance exercise training sessions with an intensity that induce heavy breathing.
- Women will be excluded from the study if they meet any of the following criteria:
- ▶ Current treatments with hormonal contraceptives including Mirena intrauterine device.
 - ▶ Insulin sensitisers or drugs known to affect gonadotropin or ovulation (with a washout period of 3 months prior to inclusion).
 - ▶ On-going pregnancy or breastfeeding within 24 weeks.
 - ▶ Cardiovascular disease or other endocrine disorders (eg, congenital adrenal hyperplasia, Cushing syndrome or androgen-secreting tumours).

Randomisation and allocation

Participants will be allocated 1:1:1 to HV-HIT, LV-HIT, or control after stratifying for BMI <or ≥ 27 kg/m² and study centre (figure 1). A computer random number generator developed and administered at the Faculty of Medicine, Department of Public Health and General Practice, NTNU, Trondheim, Norway, will be used at both study centres. The investigators will be informed about the allocation results by e-mail after the registration of new participants.

Interventions

The first 16 weeks of the exercise training will be semi-supervised, with the remaining 36 weeks of the intervention organised as home-based exercise training without

any supervision. Supervised exercise interventions are effective, but do not resemble a real-life setting, and the long-term adherence often falls once the supervised exercise programme ends. Unsupervised exercise programmes are more easily implemented and flexible, but adherence is often low. During the first 16 weeks, the participants will perform at least 1 weekly supervised exercise session at the study centres. They will be given the opportunity to attend up to 3 weekly supervised exercise sessions but can also choose to do one or two of the weekly sessions as home-based training. The participants will be encouraged to continue to complete at least 2 weekly sessions (home-based and unsupervised) for the remaining 36 weeks of the study period. Participants receive a heart rate monitor that they will wear on all sessions and will be required to register all their exercise sessions during the whole study period through an online exercise-training diary (www.polar.flow.com). The researchers will have access to all sessions via the same online tool and will supervise exercise adherence at the home-based sessions; however, no motivational support or instructions will be provided during the follow-up period. We will be advising women in the control group to continue their habitual physical activity and inform them about the current recommendations of a minimum of 150 min of weekly moderate-intensity physical activity.

HIT protocols

The LV-HIT protocol consists of a 10 min warm-up at light to moderate intensity at 60%–70% of HR_{max} followed by ten 1 min work bouts at the maximal intensity the participants can complete for 1 min. In the initial training session, the intensity will be set corresponding to 100% of the workload the participant reached at the baseline VO_2max test. Participants will be instructed to try reaching 90% of maximal heart rate (HR_{max}) during the third or fourth work bout, based on previous findings from Little *et al.*³⁷ The work bouts are separated by 1 min of passive or low-intensity recovery, trying to reach 60%–70% of HR_{max} . The training session is terminated after a 3 min cool-down at 60%–70% of HR_{max} . The total exercise time is 32 min (figure 2A).

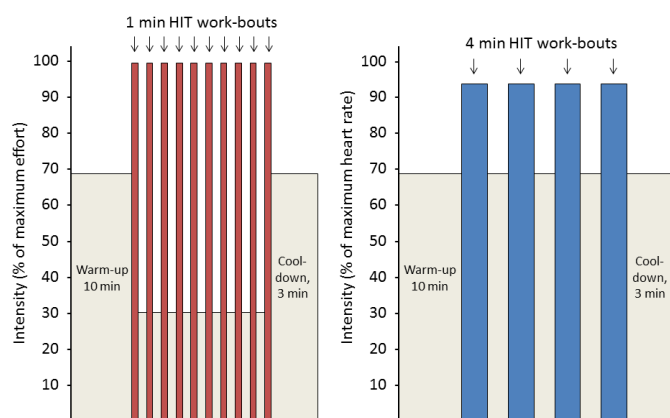


Figure 2 HIT protocol. Panel A: low-volume hit; Panel B: high-volume hit. HIT, high-intensity interval training.

The HV-HIT protocol consists of a 10 min warm-up at 60%–70% of HR_{max} , followed by four 4 min work bouts reaching 90%–95% of HR_{max} , separated by a 3 min active recovery of running/walking at 60%–70% of HR_{max} . The training session is terminated after a 3 min cool-down at 60%–70% of HR_{max} . The total exercise time is 38 min (figure 2B).

The LV-HIT and HV-HIT are not matched for time, mean workload and energy expenditure. HIT will be performed as treadmill walking or running. Heart rate monitors (Polar M400) will be used on all sessions, including on the home-based sessions, and the exercise intensity will be estimated based on their HR_{max} during the VO_2max test at baseline.⁴² Where required (due to injury, pain or discomfort), the participants will be able to exercise on other ergometers (bikes or elliptical machines). Participants can also choose to perform some of the sessions as outdoor running/uphill walking. We will adjust the absolute workload of both LV-HIT and HV-HIT throughout the intervention period to account for improvements in fitness.

Outcome measures

Outcomes will be assessed at baseline, at 16 weeks (post intervention) and at 12 months from baseline (follow-up) in Trondheim, Norway. In Melbourne, Australia, measurements will be assessed at baseline and after 16 weeks, and only questionnaires and menstrual frequency will be assessed until 12 months from baseline. Participants will be asked not to exercise for >48 hours prior to the test visits in the laboratory and to abstain from caffeine intake for 24 hours prior to the tests. Measurements will be undertaken during the follicular phase of the menstrual cycle (1–7 days after first bleeding) in women with a regular menstrual cycle.

Menstrual frequency

The primary outcome measure is menstrual frequency. Participants will register the first day of their menstrual cycle and length (number of days) during the 12-month study period. They will send their menstruation diary to the study personnel after each menstrual cycle, and the study personnel will send out reminders to fill out the diary. In addition, participants will complete a questionnaire about their menstrual cycle at each assessment point (baseline, 16 weeks and 12 months). We will compare the number of menstrual bleedings between the groups, measured as the number of observed menstrual bleedings divided by the number of expected menstrual bleedings ($n=13$, assuming a cycle interval of 28 days) during a 12-month period. The menstrual frequency is to be measured as a proxy for ovulation. Menstrual frequency assessments will be undertaken non-blinded for the study personnel.

Ovarian morphology

Ovarian morphology will only be assessed at the site in Norway. The ultrasound assessments of the ovaries include

ovarian volume in mL (with and without a dominating follicle), dominating follicle volume in mL, number of follicles in each ovary, diameter and distribution of follicles. We will use a multifrequency transvaginal transducer on all measurements. An experienced gynaecologist (MAHR) will perform all imaging and measurements. Ovarian morphology assessments will be undertaken blinded for group allocation.

Fertility and pregnancies

Background information regarding fertility will be registered by questionnaires. These include questions about number of children, natural or assisted fertilisation, if the participants are actively seeking pregnancy or have tried to become pregnant, for how long they have tried to become pregnant, and if they have experienced miscarriages or had abortions. We will report the number of pregnancies during the intervention period, including during the 12 months of follow-up. Questionnaires will be undertaken blinded, whereas the number of pregnancies will be undertaken non-blinded for the study personnel.

Blood biochemistry and insulin sensitivity

Blood samples will be obtained after an overnight fast (no food or fluid intake except water 12 hours prior to assessments) at each time point. Analysis of fasting venous blood samples will include measurements of 17-OH progesterone (baseline only), prolactin (baseline only), haemoglobin, glycated haemoglobin, blood lipids (total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and triglycerides), blood glucose, white blood cell count (neutrophils, basophils, eosinophils, lymphocytes and monocytes) and leucocytes. Additional blood (including buffy coat) will be stored for later analyses. These later analyses will likely include, but are not limited to, androstenedione, anti-Müllerian hormone, sex hormone-binding globulin, testosterone, insulin, leptin, adiponectin and micro-RNAs. We will also obtain two Tempus RNA tubes from each participant for later assessments of gene expression.

After fasting blood samples, glycaemic control will be measured using a 2-hour oral glucose tolerance test (OGTT). The participants will consume 75 g of glucose diluted in 250 mL water. Blood will be sampled for insulin and glucose measurements at 0 (prior to the OGTT), 30, 60, 90 and 120 min from an indwelling catheter. Glycaemic control will be calculated as total area under the curve (AUC) and incremental area under the curve (iAUC; using fasting concentrations as baseline values) using the trapezoid method, for glucose and insulin concentrations, and peak concentrations during the OGTT. Insulin sensitivity will be estimated using the HOMA-IR; fasting serum insulin in $\mu\text{U}/\text{mL} \times$ fasting plasma glucose in $\text{mmol}/\text{L}/22.5$.⁴³

Biological materials collected at NTNU will be stored in the Regional Research Biobank at St Olav's Hospital (Biobank1, <https://biobank1.no/nb/>). Biological materials from collected at ACU will be stored locally until

data collection is completed in Australia, and later sent to Norway. Sample collection and handling are performed in accordance with hospital/laboratory standard procedures. Blood sampling and OGTT will be undertaken blinded in Norway and unblinded in Australia.

Cardiorespiratory fitness and substrate utilisation

Peak oxygen uptake will be measured on a treadmill, using indirect calorimetry (Oxycon Pro, Jaeger, Germany in Norway/TrueOneRMR, Parvo Medics, USA in Australia). Participants will walk or run until voluntary exhaustion using an individualised protocol. After a 10 min walking warm-up, the test will start by walking at moderate intensity for 3 min. The speed or inclination will then be increased every 1–2 min, by 0.5–1.0 km/h or 1%–2%. The cardiorespiratory fitness test will be considered successful with a plateau in VO_2 with a further increase in workload, and a respiratory exchange ratio ≥ 1.05 . Peak oxygen uptake will be calculated as the highest consecutive 30 s measured, both absolute (mL/min) and relative ($\text{mL}/\text{min}/\text{kg}$). The maximal heart rate obtained during the exercise test at baseline will serve as the basis for calculating the intensity during the HIT sessions.

After an overnight fast (no food or fluid intake except water 12 hours prior to assessments), participants will complete a submaximal test on a treadmill. The protocol includes a 20 min warm-up, followed by 20 min steady-state expired gas sampling at 60% of peak oxygen uptake. We will ask participants to record their diet the day before the baseline test of oxidative capacity and to repeat this diet before the subsequent measures (at 16 weeks and 12 months). Fat oxidation rates (g/min) will be calculated from 5 min of stable oxygen uptake at the final stage of the test (during the last 10 min), using the following equation: $1.695 \times \text{VO}_2 - 1.701 \times \text{VCO}_2$,⁴⁴ where VO_2 is oxygen uptake and VCO_2 is the volume of expired carbon dioxide. Assessments of these outcomes will be undertaken non-blinded for the study personnel.

Adipose tissue composition, morphology and function

Abdominal and gluteal subcutaneous adipose tissue biopsies (~300–500 mg) will be collected under local anaesthesia (1% xylocaine) using a 14-G sterile needle. For a subsample of participants, ~80 mg of each biopsy will be allocated for immediate analyses of mitochondrial respiration using high-resolution respirometry (Oxygraph-2K, Oroboros, Austria). Approximately 200–300 mg will be snap-frozen in liquid nitrogen and stored at -80°C for later analyses, such as micro RNAs, gene expression and protein profiling. The remaining tissue will be immediately fixed in phosphate-buffered formalin, processed and embedded in paraffin and sectioned at $4\mu\text{m}$ for morphology and inflammation analyses. The sections will be incubated with CD45 and CD68 antibodies to detect leucocytes and macrophages. These sections will be captured with an EVOS FL Auto 2 Imaging System (ThermoFisher Scientific, USA) and analysed with the

open-source software ImageJ (Fiji).⁴⁵ Assessments of these outcomes will be undertaken blinded.

Physical activity and diet

We will monitor physical activity by questionnaires and 5-day activity monitoring (Sensewear Armband, APC Cardiovascular, UK) and record diet through a 4-day diet recall, at each measurement point. Assessments of physical activity and diet will be undertaken blinded.

Quality of life

Quality of life will be assessed using the Polycystic Ovary Syndrome Questionnaire.⁴⁶ Assessment of this outcome will be undertaken blinded.

Anthropometrics and body composition

Anthropometric measurements will be conducted when participants are fasted (no food or fluid intake except water 12 hours prior to assessments). Height will be measured standing, without shoes using a standard stadiometer. Total body mass and body composition will be measured wearing light clothing with no metal items and without shoes or socks using bioelectrical impedance analysis (InBody720 bioimpedance scale, Biospace CO, Korea) in Norway and dual-energy X-ray absorptiometry (DXA; GE Lunar iDXA Pro, encore software version 16, General Electric, Boston, Massachusetts, USA) in Australia. For the DXA scan, participants are required to lay supine on the scanning bed for the duration of the scan, which is approximately 15 min with one or two scans depending on the body shape of the participant. Waist and hip circumference will be measured to the nearest 0.5 cm horizontally at the level of the umbilicus, while standing and at normal expiration, using a metric tape. Assessments of these outcomes will be undertaken non-blinded for the study personnel.

Blood pressure and resting heart rate

Blood pressure and resting heart rate will be measured in the seated position after 15 min rest with an automatic blood pressure device three times on the left arm (diastolic and systolic, in mm Hg). The mean of the three measurements will be used to calculate blood pressure and resting heart rate. Assessments of these outcomes will be undertaken blinded in Norway and unblinded in Australia.

Endothelial function and intima-media thickness

Endothelial function and intima-media thickness (IMT) will be assessed at the Norwegian site only. Following a 20 min supine rest period, the diameter of the brachial artery will be imaged (12 MHz Doppler probe, GE Vingmed Ultrasound AS, Horten, Norway). A cuff will be placed around the forearm (immediately distal to the olecranon) to produce the stimulus of forearm ischaemia. When an optimal image is obtained, the probe will be held stable and the ultrasound parameters will be set to optimise the longitudinal, B-mode image of the lumen-arterial wall interface. The ultrasound will

also be used to attain simultaneous continuous Doppler velocity using the lowest possible insonation angle (60°). A recording of resting diameter and velocity will be taken for 1 min, then the forearm cuff will be inflated (>200 mm Hg) for 5 min. Both diameter and velocity recordings will resume 30 s before cuff deflation and continue for 3 min after deflation.⁴⁷ This flow-mediated dilation (FMD) test is a measure of endothelial function. We will also measure carotid artery IMT in three angles: transversal, longitudinal and anterolateral using ultrasound (12 MHz Doppler probe, GE Vingmed Ultrasound AS, Horten, Norway). Measurements of the diameter will be imaged 1 cm below the bifurcation on the right side. Images will be obtained where the near wall is clearly visualised, with a double-line pattern and at the minimal diameter during the cardiac cycle as previously described elsewhere.⁴⁸ The same person (IAK) will perform all the FMD and IMT measurements. Assessments of these outcomes will be undertaken non-blinded for the study personnel.

Enjoyment

A subgroup of participants (the last 40 to be included in the trial) allocated to one of the training groups will complete a Physical Activity Enjoyment Scale (PACES)⁴⁹ as well as The Borg's scale⁵⁰ to assess enjoyment and perceived exertion at one supervised weekly training session. Assessments of these outcomes will be undertaken non-blinded for the study personnel.

Sample size and statistical analysis

We computed the sample size for a one-way analysis of variance test with three groups. In women with PCOS, a menstrual frequency of on average 4.5 menstrual bleeding during 1 year is expected.⁵¹ With a statistical power of 80%, a significance level of 0.05 and a SD of two menstrual bleeding during a 12-month period, we calculated that 48 women will be required to detect an increase in menstrual bleeding to 7.5 in the intervention groups.²⁶ Because of the non-normality of menstrual frequency, it may be necessary to use a non-parametric test. Non-parametric tests require more participants and we, therefore, added 15% to the required sample size. Additionally, to allow for expected dropouts of 10%–15%, we aim to include 64 women in the study.

We will perform all the statistical analyses blinded for group allocation. The primary analysis will be a comparison between groups for the number of menstrual bleeding during 12 months. We will adjust for the self-reported menstrual frequency at baseline. We will include all women who have reported menstrual frequency in the primary analysis, independent of adherence to the interventions, that is, intention-to-treat analysis. The number of pregnancies during the 16 weeks of intervention and during the 12-month follow-up will be compared between groups. Secondary outcome measures will be compared between groups after 16 weeks and after 12 months and adjusted for baseline values. We will do additional 'per protocol' analyses where we include women in the HIT

groups that have completed >75% of the scheduled exercise sessions during the intervention period (for comparisons after 16 weeks) and a mean minimum of one HIT session/week during the 12-month follow-up (for comparisons at the end of follow-up).

Results will be reported as means with 95% CIs and/or SD. We will report the effect of HIT as mean changes from baseline to 16 weeks of intervention and after 12 months of follow-up. We will use mixed models to test differences between groups. P values <0.05 will be considered significant for both primary and secondary outcomes. We will also perform subgroup analyses to investigate differences between BMI categories and compare adherence rates and enjoyment of the two HIT protocols.

Blinding

We are unable to blind group allocation to participants or study personnel due to the nature of the intervention (supervised exercise training). All baseline assessments will be undertaken prior to randomisation and some assessments will be undertaken blinded for group allocation (as outlined for each outcome measure).

Monitoring

The investigators are responsible for the documentation of any adverse event or serious adverse event. Participants will be told to contact the investigator if they have any unusual symptoms. We will record all medical events during the study in the Case Report Form. In addition, we record serious adverse events in a Serious Adverse Events Report Form. All serious adverse events will be reported to the sponsor within 24 hours after the site has gained knowledge of the event.

Patient and public involvement

No patients were involved in the development of the research question or design of the study. All participants will be invited to a 1-hour educational session about healthy diet and physical activity. We will also give general information about PCOS in these meetings and encourage the participants to ask questions and give us feedback about relevant topics or issues related to their disorder and to the trial implementation. Individual test results will be disseminated to each participant after testing. We will also send out a summary of the study results to all participants at the completion of the study.

ETHICS AND DISSEMINATION

The study is approved by the Regional Committee Medical Research Ethics in Mid-Norway and The ACU Human Research Ethics Committee and has its origin in the Declaration of Helsinki and is consistent with ICH/Good Clinical Practice and applicable regulatory requirements. All protocol modification must be approved by the Regional Committee Medical Research Ethics in Mid-Norway and by The ACU Human Research Ethics Committee.

We will publish the results from the study as peer-reviewed articles in international journals. Our results will also be communicated through the Norwegian society of infertility, through social media channels as well as at national and international conferences.

DISCUSSION

Infertility and risk of lifestyle-related diseases (obesity, metabolic, cardiovascular and psychological diseases) are the main health challenges associated with PCOS, both for the individuals affected by the syndrome, the healthcare systems and society.^{2,4} To date, no treatment fully reverses or cures the symptoms of PCOS⁴⁰ and lifestyle interventions are recommended as first-line therapy according to recent guidelines.^{4, 52} Nevertheless, there is limited research available to give women with PCOS sufficient recommendations on physical activity and exercise. Some studies have demonstrated positive effects in reproductive outcomes after exercise, with concomitant improvements in insulin sensitivity and/or visceral fat.^{20–24} However, more knowledge is needed about the underlying mechanisms for improvement in fertility after exercise training, as discussed in several systematic reviews on lifestyle interventions and fertility.^{12, 25, 53, 54} HIT has shown to be time efficient, safe and well tolerated, with positive effects on metabolic and cardiovascular risk factors in several studies.^{29, 55, 56} Larger studies investigating the adherence to HIT are lacking among women in general, and among women with PCOS in particular. This is of great interest, as we need to find exercise and lifestyle programmes that are feasible over time and that can be easily implemented in everyday life. Exercise programmes that are efficient in a research setting are not necessarily effective in a real-life setting. Thus, there is a great need for studies with a longer intervention and follow-up period.

Our trial has some limitations. The use of menstrual frequency as a proxy for ovulation/reproductive function is one of these. Due to practical reasons, we are not able to undertake ovulation monitoring. The Inbody 720 and DXA are both reliable methods to measure fat-free mass (FFM), body fat percentage (BF%) and fat mass (FM) but it has been shown that the InBody 720 overestimates FFM and underestimates BF% and FM compared with DXA.⁵⁷ For each individual participant, the same measurement method will be used (InBody 720 in Norway and DXA in Australia) and our purpose is to evaluate changes in body composition (ie, delta changes). Assessing enjoyment of exercise only at the supervised sessions could imply a bias. On the supervised sessions, the participants will receive motivational support from the researchers and potentially also social support from other participants that can affect their ratings of enjoyment. The enjoyment ratings are, however, implemented to compare the perceived enjoyment of the two HIT protocols. The semi-supervised nature of the trial could imply that some participants choose to undertake all sessions supervised, whereas others choose to exercise at home for most



sessions. We chose to implement a semi-supervised exercise programme to account for the individual preferences of the participants and to be able to include women living further away from the study centres. Such protocols can both control the exercise intervention and motivate participants, and at the same time give more flexibility based on participants' preferences.

The IMPROV-IT trial will determine the effect of HIT on reproductive, cardiovascular, metabolic and psychological health in women with PCOS. The trial will contribute evidence of more specific exercise protocols in the treatment and management of PCOS for clinicians and answer some of the current limitations addressed by both the International evidence-based guidelines for the assessment and management of PCOS⁴ and several systematic reviews.^{2 12 25 58} This trial is highly relevant and important as PCOS is a major burden for the women with PCOS, the health services and society. As for now, we do not have an optimal treatment.

Trial status

We have recruited 64 women with PCOS to the IMPROV-IT study, with the last included participant in March 2019. The last follow-up will be in March 2020.

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Contributors IAK: drafted the manuscript. IAK, TM, EV, ØS and HJ: conceived and contributed to the design of the study. IAK, SL, TM and EP coordinated the study at the two sites and supervised the exercise training. IAK, SL, TM, MAHR and EP: performed measurements on test-days. EV: provided medical advice and support during the study. All authors provided feedback and approved the final manuscript.

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