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ORIGINAL ARTICLE ***Reproductive endocrinology***

High-intensity training elicits greater improvements in cardio-metabolic and reproductive outcomes than moderate-intensity training in women with polycystic ovary syndrome: a randomized clinical trial

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STUDY QUESTION: Does 12 weeks of high-intensity interval training (HIIT) result in greater improvements in cardio-metabolic and reproductive outcomes compared to standard moderate-intensity continuous training (MICT) in women with polycystic ovary syndrome (PCOS)?

SUMMARY ANSWER: HIIT offers greater improvements in aerobic capacity, insulin sensitivity and menstrual cyclicity, and larger reductions in hyperandrogenism compared to MICT.

WHAT IS KNOWN ALREADY: Exercise training is recognized to improve clinical outcomes in women with PCOS, but little is known about whether HIIT results in greater health outcomes compared to standard MICT.

STUDY DESIGN, SIZE, DURATION: This was a two-armed randomized clinical trial enrolling a total of 29 overweight women with PCOS between May 2016 and November 2019.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Women with PCOS aged 18–45 years were randomly assigned to 12 weeks of either MICT (60–75% peak heart rate, N = 14) or HIIT (>90% peak heart rate, N = 15), each completed three times per week. The primary clinical outcomes were aerobic capacity (VO_{2peak}) and insulin sensitivity (euglycaemic–hyperinsulinaemic clamp). Secondary outcomes included hormonal profiles, menstrual cyclicity and body composition.

MAIN RESULTS AND THE ROLE OF CHANCE: Both HIIT and MICT improved VO_{2peak} (HIIT; Δ 5.8 ± 2.6 ml/kg/min, $P < 0.001$ and MICT; Δ 3.2 ± 2 ml/kg/min, $P < 0.001$), however, the HIIT group had a greater improvement in aerobic capacity compared to MICT ($\beta = 2.73$ ml/kg/min, $P = 0.015$). HIIT increased the insulin sensitivity index compared to baseline (Δ 2.3 ± 4.4 AU, $P = 0.007$) and MICT ($\beta = 0.36$ AU, $P = 0.030$), and caused higher increases in sex hormone-binding globulin compared to MICT ($\beta = 0.25$ nmol/l, $P = 0.002$). HIIT participants were 7.8 times more likely to report improved menstrual cyclicity than those in the MICT group (odds ratio 7.8, $P = 0.04$).

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LIMITATIONS, REASONS FOR CAUTION: This study has a small sample size and the findings of the effect of the exercise interventions are limited to overweight reproductive-aged women, who do not have any co-existing co-morbidities that require medication.

WIDER IMPLICATIONS OF THE FINDINGS: Exercise, regardless of intensity, has clear health benefits for women with PCOS. HIIT appears to be a more beneficial strategy and should be considered for promoting health and reducing cardio-metabolic risk in overweight women with PCOS.

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Key words: exercise / high-intensity interval training / moderate-intensity continuous training / PCOS / insulin resistance / menstrual cycle

Introduction

Polycystic ovary syndrome (PCOS) is a common and complex condition, affecting 8–13% of women of reproductive age worldwide (Teede *et al.*, 2018). PCOS has diverse clinical manifestations with varying impacts on metabolic, reproductive and psychological health (Teede *et al.*, 2010; Azziz *et al.*, 2016). PCOS is characterized by androgen excess, ovulatory dysfunction and polycystic ovaries, with a combination of at least two of the three required for diagnosis (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). Arguably, the two defining characteristics of PCOS are hyperandrogenism and insulin resistance. Insulin resistance is present in up to 75% of women as determined by euglycaemic–hyperinsulinaemic clamp (Tosi *et al.*, 2017), is independent of obesity (Cassar *et al.*, 2016) and is present across all phenotypes (Tosi *et al.*, 2021). Women with PCOS are at a 4.4-fold increased risk of developing type 2 diabetes mellitus (T2DM) (Moran *et al.*, 2010), accounting for 19–28% of all T2DM cases in pre-menopausal women (Rodgers *et al.*, 2019). The synergistic relationship of insulin resistance with hyperandrogenism is also considered a key factor in the aetiology of PCOS. The main source of hyperandrogenism occurs within the ovaries where the hypothalamic–pituitary–gonadal axis stimulates hypersecretion of androgens. This is also compounded by hyperinsulinaemia, resulting from insulin resistance, directly stimulating androgen production in the ovarian theca cells and indirectly by decreasing the production of sex hormone-binding globulin (SHBG) leading to an increase in circulating free androgens (Diamanti-Kandarakis and Dunaif, 2012).

Exercise is well established as a therapy for improving health and is recommended by the international evidence-based guidelines for all women with PCOS, particularly for those who are overweight or obese (Teede *et al.*, 2018; Stepto *et al.*, 2019). A minimum of 150 min per week of moderate-intensity exercise or 75 min per week of vigorous-intensity exercise is currently recommended (Teede *et al.*, 2018). It is unclear whether these two exercise prescriptions provide equal improvements in the health of women with PCOS. High-intensity interval training (HIIT) comprises of repeated, short bouts of high-intensity exercise ($\geq$90% HR_{max} (Norton *et al.*, 2010)) interposed with periods of rest or low-intensity exercise (Kessler *et al.*, 2012). HIIT appears to be safe if performed under the supervision of an exercise physiologist and following robust clinical screening (Levinger *et al.*, 2015), and has the potential to address the time limitation barrier to exercise participation (Weston *et al.*, 2014). In apparently healthy population groups and in populations with T2DM, HIIT has been reported to induce greater increases in aerobic capacity (VO_{2peak}) when compared with moderate-intensity exercise (Hollekim-Strand *et al.*, 2014; Weston *et al.*, 2014; Milanović *et al.*, 2015). However, there is very little research conducted in women and more specifically in women with PCOS (De Nardi *et al.*, 2018) with a majority of these studies conducted predominantly in male or older populations (Batacan *et al.*, 2017). A recent meta-analysis by our group suggested that in women with PCOS, HIIT may provide some additional benefits for improving insulin sensitivity and cardiorespiratory fitness in comparison to moderate-intensity interventions (Patten *et al.*, 2020).

Although both moderate- and vigorous-intensity exercise is recommended for women with PCOS, there have been no studies comparing the effects of the two standard exercise recommendations on health outcomes in women with PCOS. There is a need for robust exercise intervention studies examining the impact of exercise intensity in women with PCOS. The primary aim of this study was to test the hypothesis that HIIT is more effective than moderate-intensity continuous training (MICT) in improving aerobic capacity and insulin sensitivity in overweight women with PCOS. In addition, we investigated whether there are differences in improvement of weight, lean and fat mass, hormonal profiles and menstrual cyclicity between the two interventions.

Materials and methods

Study design

This study was a two-arm randomized clinical trial, conducted at Victoria University in Melbourne, Australia from May 2016 to November 2019. The study was approved by the Victoria University Human Research Ethics Committee and is registered with the Australian New Zealand Clinical Trials Registry (ACTRN12615000242527). All women provided written informed consent prior to participation. There were some deviations from the original study design (ACTRN12615000242527). We did not include a

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non-exercise control group, there are multiple robust studies that have consistently shown that exercise improves the clinical symptoms of PCOS compared to no exercise (Jedel et al., 2011; Almenning et al., 2015; Orio et al., 2016; Lionett et al., 2020). Therefore, participants were randomized into either MICT or HIIT to answer our primary aim and determine whether HIIT is more effective than MICT for improving aerobic capacity and insulin sensitivity in overweight women with PCOS. Secondary outcomes included body composition, hormonal profiles and menstrual cyclicity. There were no changes to primary or secondary outcomes after the commencement of the trial.

Participants

Women were recruited through community and social media advertisements and enrolled by research team members. Inclusion criteria were Caucasian women aged 18–45 (pre-menopausal), with a BMI greater than 25 kg/m^2, insufficiently active (do not meet the minimum physical activity recommendations of 150 min of moderate to vigorous activity per week), and with diagnosed PCOS. PCOS was diagnosed according to the Rotterdam Criteria (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004), and confirmed by an endocrinologist. Rotterdam criteria require two of the following: (i) oligo- or anovulation; (ii) clinical (hirsutism and acne) and/or biochemical hyperandrogenism with other causes excluded; and (iii) polycystic ovaries on ultrasound and the exclusion of other causes of hyperandrogenism. Exclusion criteria included diabetes, pregnancy, smoking, illness or injury that prevented or limited exercise performance and existing participation in regular physical activity. Those taking anti-hypertensive, insulin sensitizers, dietary supplements, weight loss medication or hormonal contraceptive medications in the 3 months prior to enrolment were excluded.

Study protocol

After the completion of baseline testing, participants were randomized to a 12-week HIIT (N = 15) or MICT (N = 14) intervention. Randomization was completed by an independent biostatistician by a simple randomization procedure using computerized sequence generation at an allocation ratio of 1:1. To ensure equal proportions of BMI in each arm, randomization was stratified according to BMI brackets (25–35 or >35 kg/m^2). Following familiarization of the graded exercise tests, participants underwent a graded exercise test to assess VO_{2peak}, a body composition scan (dual-energy X-ray absorptiometry (DXA)), blood sampling for lipids, metabolic and reproductive hormones, and a euglycaemic–hyperinsulinaemic clamp, to assess insulin sensitivity (DeFronzo et al., 1979) at both baseline and post-intervention. Participants were instructed to maintain their usual diet and exercise habits throughout the duration of the intervention. An accelerometer (ActiGraph GT3X+, ActiGraph LLC, Pensacola, FL, USA) was worn for 7 days and a 3-day food diary was collected preceding baseline testing and following post-intervention testing. Nutritional analysis of the food diaries was determined using Xyris FoodWorks (Xyris Pty Ltd., Highgate Hill, QLD, Australia).

Euglycaemic–hyperinsulinaemic clamp

Insulin sensitivity was assessed using the euglycaemic-hyperinsulinaemic clamp described by DeFronzo et al. (1979). Clamp timing was standardized to a minimum of 48–72 h after the graded exercise test. Participants consumed a standardized high-carbohydrate diet the day prior, followed by an overnight fast to minimize the confounding effect of glycogen depletion. For participants with regular menstrual cycles (21–35 days (Teede et al., 2018)), this session was completed during the early follicular phase of the menstrual cycle (Days 1–7) as determined by self-report using menstrual diaries. Fasting venous blood samples were collected and stored prior to commencement. A bolus of insulin (Actrapid, Novo Nordisk, Bagsværd, Denmark) of 9 mU/kg was initially infused, followed by a constant insulin infusion rate of 40 mU/min/m^2 with glucose (20%) infused at a variable rate to meet the target blood glucose of 5 mmol/l. The euglycaemic–hyperinsulinaemic clamp was run for at least 120 min or until steady state of 5 mmol/l was achieved. Blood glucose was assessed every 5 min using a glucose analyser (YSI 2300 STAT Plus, YSI Inc., OH, USA). Glucose infusion rates (GIRs) were calculated during steady state, defined as the last 30 min of the insulin clamp. GIR is expressed relative to lean body mass as skeletal muscle is responsible for the majority of insulin-induced glucose uptake (Ferrannini et al., 1988). Plasma insulin levels were determined via Radioimmunoassay kit (Human Insulin-Specific RIA, HI-14K, Millipore, MA, USA). Insulin sensitivity index (ISI) was calculated using the following formula: (GIR (mg/lean body mass [kg]/min)/steady state insulin) × 100.

Clinical and biochemical measurements

Blood was collected in various anticoagulants or in serum clot activator blood tubes. Serum tubes were allowed to clot at room temperature for 60 min prior to being centrifuged. Tubes were centrifuged at 2000×*g* for 10 min at 4°C. The plasma or serum was then transferred to a clean polypropylene tube and frozen at −80°C and stored for batch analysis. Total testosterone, free testosterone, SHBG, dihydrotestosterone (DHT), oestradiol, androstenedione, total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and glycosylated haemoglobin (HbA1c) were batch analysed by an accredited pathology laboratory at Monash Health, Melbourne, Australia using standard protocols as previously described (Hiam et al., 2019). Serum steroids were measured using mass-spectrometry method using a liquid sample extraction (AB Sciex Triple Quad 5500 liquid chromatography–tandem mass spectrometry). Free testosterone was calculated by the Södergard free testosterone calculation (Södergard et al., 1982). SHBG was measured using a sequential two-step immunoenzymatic ('sandwich') assay carried out on a Unicel DXI 800 (Beckman Coulter). Free androgen index (FAI) was calculated as (total testosterone × 100)/SHBG. Anti-Müllerian hormone (AMH) and insulin were measured in-house at Victoria University. Serum AMH concentrations were determined via a commercially available ELISA kit (Ultra-Sensitive AMH/MIS ELISA, AL-105, Ansh Labs, TX, USA).

Self-reported menstrual diaries were completed by participants throughout the duration of the study and included their menstrual cycle history for a minimum of three months prior to enrolment in the study. Menstrual cycle classifications included normal cycles (cycle length >21 to <35 days), oligomenorrhoea (cycle length of ≥35 to 42 days), and amenorrhoea (absence of menstruation for 3 months or longer) (Azziz et al., 2009).

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Aerobic capacity

VO_{2peak} was assessed at baseline and following the 12-week intervention using an incremental maximal graded exercise protocol conducted on a cycle ergometer (Lode Excalibur v2.0, Groningen, The Netherlands). The initial three stages of the test consisted of three 3 min of cycling at 25, 50 and 75 Watts, respectively, followed by increases of 25 Watts each minute thereafter until volitional exhaustion (Parker *et al.*, 2017). Breath-by-breath expired respiratory gases were collected and analysed (Quark Cardio-Pulmonary Exercise Testing, Cosmed, Rome, Italy). Relative VO_2 data (ml/kg/min) was filtered to remove values that were 2 SDs above or below a seven breath mean. Smoothed data were subsequently averaged over a rolling seven breath mean and the largest value obtained was determined to be VO_{2peak}. Heart rate was recorded every minute and the peak heart rate (HR_{peak}) was also recorded (Polar H10, Polar Electro OY, Kempele, Finland).

Anthropometric measurements

Lean and fat mass were assessed using total-body DXA scans (GE Lunar iDXA, GE Healthcare, WI, USA) with participants in a fasted state and analysed using enCore Forma Software version 16. Height was taken without shoes using a calibrated stadiometer (Proscale Inductive Series I, Accurate Technology Inc., USA) and BMI was calculated (weight [kg]/height [m] squared). Waist and hip circumferences were measured as described previously (Swain and Brawner, 2014) and the waist to hip ratio (WHR) was calculated (waist circumference [cm]/hip circumference [cm]).

Exercise interventions

Participants in both groups were asked to attend three sessions per week for 12 weeks. All sessions were conducted on a stationary cycle ergometer under the supervision of an accredited exercise physiologist. Interventions were designed to match the minimum exercise recommendations for both moderate and vigorous exercise according to the international evidence-based guidelines for the assessment and management of PCOS (Teede *et al.*, 2018) and were matched for training volume (metabolic equivalent task [MET]/min/week). Both interventions progressed from 312 MET/min/week in Week 1 to 530 MET/min/week by week 4. The HIIT intervention included twice weekly sessions of 12, 1 min intervals at 90–100% peak heart rate (%HR_{peak}), separated by 1 min of active recovery at a light load and one weekly session of eight, 4 min intervals at 90–95% HR_{peak}, separated by a 2 min light load, activity recovery. The MICT consisted of three sessions per week of 45 min of continuous cycling at 60–75% HR_{peak}. Heart rate monitors (Polar H10, Polar Electro Oy, Kempele, Finland) were used in all sessions and target heart rates were achieved by altering the load on the bike according to individual fitness. For both interventions, sessions started with a 5 min warm-up at 60–65% HR_{peak} and ended with a 5 min cool down. Heart rates were recorded each minute. Adherence to exercise training was calculated as the number of sessions attended divided by the total number of scheduled sessions, reported as a percentage.

Statistical analysis

All data were analysed using R studio version 4.0.2 and conducted on an intention-to-treat basis, including all randomly assigned participants. The researchers who conducted the analyses were not blinded to group allocation of participants. Linear mixed models or generalized linear mixed models (if data was as a percentage) were used to determine the effect of exercise intensity (group) over time and to determine the interaction between time-point and group (between-group differences). All models were adjusted for age and WHR, except for body composition, which was only adjusted for age. The unique participant codes were used as a random effect to account for repeated measures. To examine the *within-group* changes, we calculated the estimated marginal means from the linear mixed models. Simple linear regression models were used to determine whether delta changes in VO_{2peak}, SHBG or FAI were associated with delta changes in ISI in response to exercise using the pooled data from all participants. Finally, a generalized linear model with a binomial distribution was performed to understand if menstrual cyclicity improvements were specific to exercise mode. Improvements in menstrual cyclicity were determined via menstrual diary monitoring. We recoded improvements in menstrual cycles, whereby 1 = 'Yes' or 0 = 'No'. Those with regular cycles at baseline and post-intervention were removed from menstrual cyclicity analysis as their cycles were already considered optimal. To meet the statistical assumptions of the regression (normally distributed residuals), ISI, fasting insulin and steady state insulin data, SHBG, calculated free testosterone, oestradiol and AMH were log transformed. Data are presented as mean ± SD and median and percentiles for boxplots. *P*-values were deemed statistically significant when <0.05. The following packages were used in our analysis; *lme4* (Bates *et al.*, 2015), *lmerTest* (Kuznetsova *et al.*, 2017), *glmmTMB* (Brooks et al., 2017) and *tidyverse* (Wickham *et al.*, 2019).

A statistical power analysis was performed *a priori* for sample size estimation, based on VO_{2max} (ml/kg/min) data from a previous, non-PCOS intervention study comparing HIIT to MICT (Tjønna *et al.*, 2008). The resulting sample size was 12 per group with 80% power and an alpha of 0.05 (G*Power 3.1.9.4) (Faul *et al.*, 2007).

Results

Twenty-nine women completed the baseline assessments, of whom 15 were randomized into the HIIT group and 14 into the MICT group. Of these participants, 24 completed the 12-week intervention (HIIT = 13, MICT = 11). Two participants dropped out during the MICT intervention due to changes to their work schedule and one withdrew due to relocating to reside out of state. One participant withdrew from the HIIT intervention due to an injury sustained at work and another became pregnant (Fig. 1). Exercise intervention adherence was similar across the two groups (HIIT = 94 ± 3.0%, MICT = 92 ± 4.8%, $P > 0.05$). No adverse events occurred throughout the study period. There were no significant differences in dietary intake or physical activity levels from baseline to post-intervention in either group (Supplementary Table SI).

Participant characteristics

Baseline characteristics of participants in the HIIT and MICT groups are shown in Supplementary Table SII. Aside from significant differences in WHR ($P = 0.02$), they were otherwise similar. In the HIIT group, 40% (6 of 15) of participants met the criteria for Phenotype A (hyperandrogenism, oligo/anovulation and polycystic ovary morphology [PCOM]),

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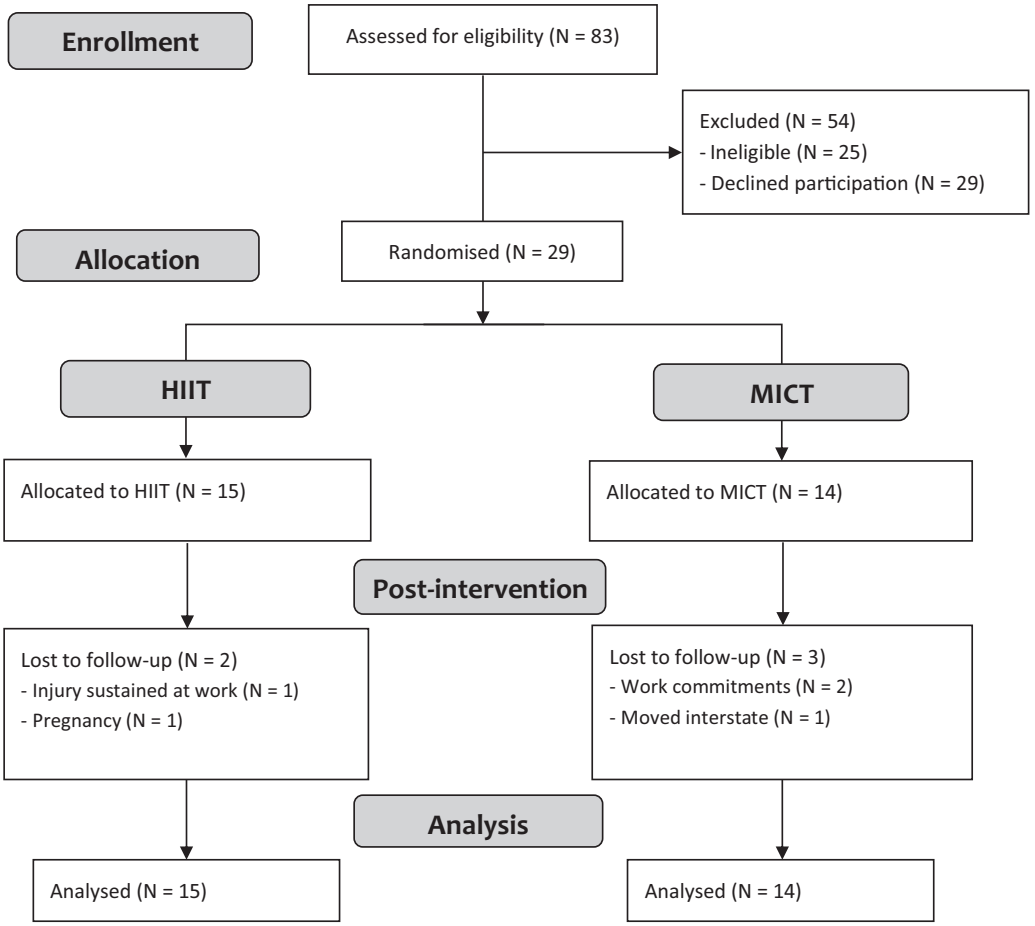

Figure 1. CONSORT trial flow diagram.

20% (3 of 15) for Phenotype B (hyperandrogenism and oligo/anovulation) and 40% (6 of 15) for Phenotype D (oligo/anovulation and PCOM). In the MICT group, 43% (6 of 14) met the criteria for Phenotype A, 7% (1 of 14) for Phenotype B, 14% (2 of 14) for Phenotype C (hyperandrogenism and PCOM) and 36% (5 of 14) for Phenotype D.

Aerobic capacity

VO_{2peak} increased after both HIIT (Δ 5.8 ± 2.6 ml/kg/min, $P < 0.001$) and MICT (Δ 3.2 ± 2 ml/kg/min, $P < 0.001$), compared to baseline (Table I). There was also a significant interaction between the exercise intervention groups, whereby the HIIT group displayed greater improvements in VO_{2peak} post-intervention compared with the MICT group ($\beta = 2.73$ ml/kg/min, $P = 0.015$, Fig. 2A). Maximal power output ($Watts_{peak}$) increased similarly in both HIIT and MICT groups from baseline ($P < 0.001$, Table I).

Insulin sensitivity

GIR increased following HIIT ($P = 0.011$) but was not altered following MICT ($P = 0.219$) (Table I). We then investigated ISI, which is a more comprehensive measure of insulin sensitivity as it also takes steady state insulin into account (Howlett *et al.*, 2008). There was a significant interaction between the exercise intervention groups, whereby the HIIT group displayed a higher ISI post-intervention compared to MICT ($\beta = 0.36$ AU, $P = 0.030$, Fig. 2B). On further analysis, improvements in ISI post-intervention were only observed in the HIIT group (Δ 2.3 ± 4.4 AU, $P = 0.007$; Table I). Following HIIT, resting fasting glucose was reduced from 5.0 ± 0.4 to 4.6 ± 0.3 mmol/l ($P = 0.016$), with no change following MICT, and no difference between the groups (Table I). Neither training intervention influenced fasting insulin levels, HbA1c levels or lipid profiles (Table I and Supplementary Table SI).

Relationship between fitness and insulin sensitivity

There was a positive relationship between changes in VO_{2peak} and ISI with exercise training, regardless of group. Changes in VO_{2peak} explained 41.4% of the changes in ISI ($P = 0.001$, adj. $R^2 = 0.414$; Fig. 2C).

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Table I Cardiorespiratory fitness, metabolic, hormonal and anthropometric outcomes at baseline and post-exercise training.

Outcome measure	High-intensity interval training			Moderate-intensity continuous training			
	Baseline N = 15	Post N = 13	*P*	Baseline N = 14	Post N = 11	*P*	*P* (time × group)
Age (years)	29.7 ± 4.8			32.5 ± 6.2			
Cardiorespiratory fitness							
VO_{2peak} (ml/kg/min)	24.8 ± 5.7	30.8 ± 6.7	**<0.001**	22.3 ± 3.2	26.1 ± 4.1	**<0.001**	**0.015**
$Watt_{peak}$	176.7 ± 30.6	219.2 ± 30.9	**<0.001**	151.8 ± 33.2	190.9 ± 25.7	**<0.001**	0.660
Body composition							
Weight (kg)	97.4 ± 19.2	97.3 ± 19.1	0.593	102.4 ± 28.9	99.8 ± 28.0	0.291	0.257
BMI (kg/m^2)	35.5 ± 6.8	35.6 ± 7.0	0.632	38.4 ± 9.3	37.3 ± 9.8	0.256	0.248
Waist circumference (cm)	99.6 ± 15.0	97.7 ± 14.6	**0.018**	109.2 ± 21.2	103.0 ± 20.8	**0.017**	0.869
Hip circumference (cm)	124.4 ± 13.2	122.6 ± 12.4	0.076	127.4 ± 21.4	124.2 ± 20.1	0.733	0.324
WHR	0.8 ± 0.1	0.8 ± 0.1	0.841	0.9 ± 0.1	0.8 ± 0.1	**0.008**	0.059
Fat mass (kg)	43.6 ± 14.3	42.5 ± 12.8	0.249	49.3 ± 20.6	50.3 ± 18.9	0.322	0.135
Lean mass (kg)	46.3 ± 4.1	47.0 ± 4.1	0.110	46.1 ± 7.2	46.8 ± 6.8	0.112	0.997
Fat mass (%)	47.8 ± 6.4	46.5 ± 7.0	0.066	51.3 ± 5.1	50.9 ± 4.7	0.400	0.055
Lean mass (%)	50.0 ± 6.1	51.4 ± 6.8	**0.022**	47.5 ± 5.0	48.1 ± 5.5	0.550	0.213
A/G ratio	1.1 ± 0.1	1.1 ± 0.2	0.418	1.1 ± 0.1	1.1 ± 0.2	0.932	0.526
Insulin sensitivity							
Fasting glucose (mmol/l)	5.0 ± 0.4	4.6 ± 0.3	**0.016**	5.0 ± 0.6	4.7 ± 0.3	0.156	0.517
Fasting insulin (μIU/ml)	17.8 ± 11.0	16.6 ± 13.4	0.859	17.7 ± 6.5	18.5 ± 6.1	0.536	0.565
GIR (mg/lean mass [kg]/min)	7.5 ± 3.2	10.0 ± 3.1	**0.011**	6.9 ± 3.3	8.5 ± 3.1	0.274	0.219
Insulin sensitivity index (AU)	8.3 ± 4.4	11.2 ± 5.3	**0.007**	7.4 ± 3.5	7.6 ± 4.1	0.501	**0.030**
Steady state insulin (μIU/ml)	108.7 ± 47.5	101.2 ± 29.2	0.226	120.1 ± 28.4	125.6 ± 31.1	0.475	0.202
Hormonal profiles							
Testosterone (nmol/l)	1.8 ± 0.8	1.4 ± 0.9	0.065	1.6 ± 0.7	1.4 ± 0.7	0.263	0.242
cFT (pmol/l)	36.2 ± 16.2	27.9 ± 16.9	0.069	34.8 ± 17.2	34.7 ± 22.4	0.538	0.890
FAI (AU)	6.9 ± 3.5	4.5 ± 2.8	**0.010**	6.2 ± 3.7	5.7 ± 3.9	0.900	0.069
SHBG (nmol/l)	32.6 ± 17.1	39.3 ± 24.5	**0.006**	31.1 ± 13.7	29.2 ± 12.0	0.166	**0.002**
AMH (pmol/l)	63.5 ± 27.2	67.4 ± 48.3	0.515	49.2 ± 21.8	42.2 ± 20.6	0.293	0.285
Androstenedione (nmol/l)	5.0 ± 1.1	4.5 ± 1.9	0.344	4.8 ± 1.8	4.2 ± 1.6	0.262	0.886
Oestradiol (pmol/l)	254.5 ± 203.8	216.4 ± 160.1	0.600	311.2 ± 260.5	269.9 ± 288.2	0.625	0.923
DHT (nmol/l)	0.3 ± 0.2	0.3 ± 0.2	0.482	0.2 ± 0.1	0.2 ± 0.1	0.591	0.382

Data are mean ± SD.
A/G ratio, android/gynoid ratio; AMH, anti-Müllerian hormone; AU, arbitrary units; BMI, body mass index; cFT, calculated free testosterone; DHT, dihydrotestosterone; FAI, free androgen index; GIR, glucose infusion rate; SHBG, sex hormone-binding globulin; VO_{2peak}, peak oxygen consumption; $Watt_{peak}$, peak watts; WHR, waist to hip ratio.
Bold indicates significance.

Body composition

There were no observed changes in weight or BMI as a result of either intervention. Both the HIIT and MICT groups decreased waist circumference ($\beta = -0.98$ cm, $P < 0.02$; Table I), but there was no significant difference between the exercise interventions (Table I). Interestingly, WHR decreased post-intervention in the MICT group only ($\beta = 0.5$, $P = 0.008$). Despite these changes in WHR, there were no changes in lean mass (kg), fat mass (kg) or android/gynoid ratio (Table I). We then assessed lean mass and fat mass as a percentage of total mass. The HIIT intervention increased lean mass percentage ($\beta = 0.04\%$, $P = 0.022$) with no change in MICT (Table I). There was no change in percentage of fat mass after HIIT or MICT (Table I).

Biochemical measures of hyperandrogenism

Neither total testosterone nor calculated free testosterone was altered by either MICT or HIIT ($P > 0.05$, Table I). There was a significant interaction between exercise groups for SHBG, whereby SHBG levels were 22% higher in the HIIT group compared to MICT ($\beta = 0.25$ nmol/l, $P = 0.002$, Fig. 3A). FAI significantly decreased

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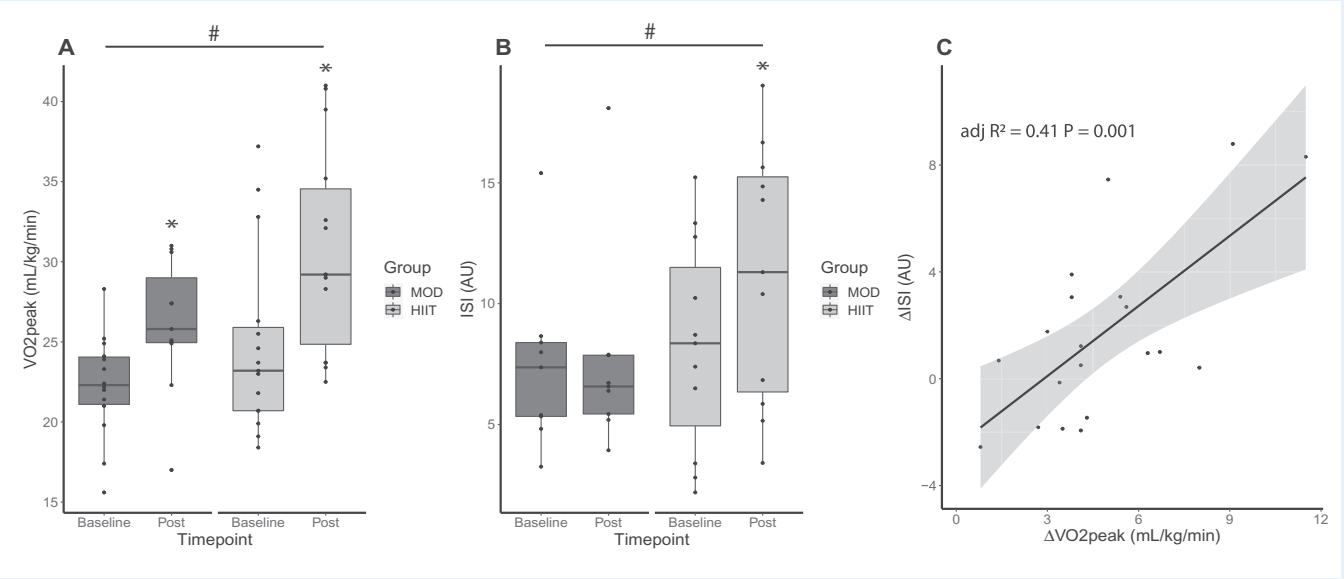

Figure 2. Effect of exercise intensity on aerobic capacity (VO_{2peak}) and insulin sensitivity index (ISI). (**A**) VO_{2peak}, (**B**) ISI. Data are shown at baseline and post-intervention, stratified by group and using boxplots showing the median (central line), 25th to 75th quartiles (box) and the minimum and maximum values (whiskers). *Indicates significant between-group differences $P < 0.005$, and #indicates a significant interaction between groups $P < 0.05$. (**C**) Relationship between Δ VO_{2peak} and Δ ISI as a result of exercise training (linear regression $P = 0.001$, adjusted $R^2 = 0.414$ for all participants who completed the intervention, regardless of group).

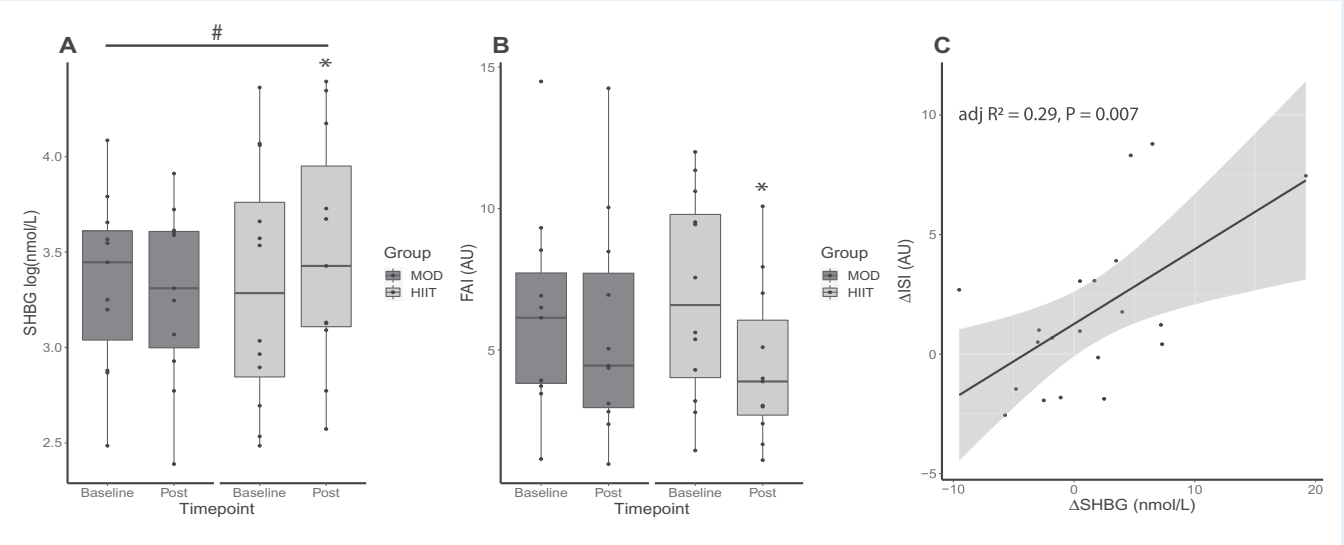

Figure 3. Effect of exercise intensity on sex hormone-binding globulin (SHBG) and free androgen index (FAI). (**A**) SHBG, (**B**) FAI. Data are shown at baseline and post-intervention stratified by group and using boxplots showing the median (central line), 25th to 75th quartiles (box), and the minimum and maximum values (whiskers). *Indicates significant between-group differences $P < 0.01$, and #indicates a significant interaction between groups $P = 0.002$. (**C**) Relationship between Δ SHBG and Δ insulin sensitivity index (ISI) as a result of exercise training (linear regression $P = 0.007$, adjusted $R^2 = 0.292$ for all participants who completed the intervention, regardless of group).

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following HIIT ($P = 0.010$; Fig. 3B) but not MICT. AMH, androstenedione, oestradiol and DHT were not altered by either intervention ($P > 0.05$; Table I).

Relationship between insulin sensitivity and SHBG

There was a positive association between changes in SHBG and ISI with exercise training, regardless of group ($P = 0.008$, adj. $R^2 = 0.292$; Fig. 3C), with changes in SHBG explaining 29.2% of the changes in ISI.

Menstrual cyclicity

At baseline, in the HIIT group, one participant reported regular cycles (7%) and the remaining participants reported menstrual irregularities, with 73% and 20% of women experiencing oligomenorrhoea and amenorrhoea, respectively. In the MICT group, 14%, 64% and 21% reported normal cycles, oligomenorrhoea or amenorrhoea, respectively (Table II). After the completion of HIIT intervention, participants were 7.8 times more likely to report improvements in their menstrual cycle than those in the MICT group (odds ratio 7.8, $P = 0.04$). Specifically, post-intervention, 69% of participants (9 of 13) reported improvements in their menstrual cyclicity following HIIT (Table III), while in the MICT group, 22% (2 of 9) of women reported improvements in cyclicity post-intervention (Table III).

Discussion

We report that HIIT increases VO_{2peak} and SHBG and improves insulin sensitivity and menstrual cyclicity to a greater extent than MICT in women with PCOS. These benefits from training were independent of changes in body weight, fat mass or fat distribution.

VO_{2peak} is an important indicator of health and a predictor of premature mortality in clinical populations, with a lower VO_{2peak} being a stronger predictor of mortality than BMI (Barry *et al.*, 2014). Poor cardiorespiratory fitness has also been associated with an increased risk of developing T2DM (Wei *et al.*, 1999) for which women with PCOS are at a higher risk of developing (Moran *et al.*, 2010). Lifestyle interventions which include exercise are considered the first-line therapy for PCOS, however, optimizing exercise prescription is important for promoting increased fitness and insulin sensitivity, reducing the risk of co-morbidities (Teede *et al.*, 2018). HIIT has previously been shown to improve VO_{2peak} and insulin sensitivity in women with PCOS, compared to a non-exercise control group (Lionett *et al.*, 2020). In addition, here we report that although both MICT and HIIT increased VO_{2peak}, the magnitude of the improvement following HIIT is greater than MICT. This was associated with improvements in insulin sensitivity, whereby VO_{2peak} explained ~41% of the variation in insulin sensitivity. Furthermore, using a gold-standard assessment of insulin sensitivity, we observed a significant improvement in ISI following HIIT but not MICT. This finding is consistent with a previous study that utilized a HIIT protocol in women with PCOS and reported a 16% improvement in insulin sensitivity after training (Hutchison *et al.*, 2011). Similarly, another study reported that HIIT reduced insulin resistance by 17% as assessed by the HOMA-IR, with no change after a resistance training intervention (Almenning *et al.*, 2015). Here, we suggest that HIIT has a stronger impact on the cardiorespiratory and metabolic health of women with PCOS than MICT, the alternative recommended exercise intervention for women with PCOS (Teede *et al.*, 2018). HIIT may be favourable not only to improve fitness in these

Table II Self-reported menstrual cyclicity data at baseline.

Exercise intervention	Menstrual cyclicity	n	Frequency (%)
High-intensity interval training	Regular	1	7
	Oligomenorrhoea	11	73
	Amenorrhoea	3	20
Moderate-intensity continuous training	Regular	2	14
	Oligomenorrhoea	8	57
	Amenorrhoea	4	29

Table III Self-reported menstrual cyclicity improvement post intervention.

Exercise intervention	Cycle improvements	n	Frequency (%)
High-intensity interval training	Yes	9	69
	No	4	31
Moderate-intensity continuous training	Yes	2	22
	No	7	78

Two participants were removed from analysis as they had regular cycles at baseline and post-intervention which is considered optimal. A further five were removed due to dropping out of the intervention and therefore data were not available for the post-intervention time-point.

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women but may also have the potential to reduce the likelihood of developing insulin resistance and early onset of T2DM. Therefore, we recommend that vigorous-intensity exercise be recommended to women with PCOS rather than moderate-intensity exercise.

Obesity is present in a large proportion of women with PCOS and is known to independently exacerbate both metabolic and reproductive features (Teede et al., 2010; Lim et al., 2013). Given the high prevalence of obesity in women with PCOS, weight loss has been a major focus in regards to the treatment of PCOS (Teede et al., 2011). Among a range of healthy and clinical cohorts, there is an on-going debate about the importance of improved fitness in comparison to weight loss for improving insulin sensitivity in exercise interventions (Bird and Hawley, 2017). In our study, we did not observe changes in body weight, fat mass or body distribution, but we did observe an increase in lean mass percentage after 12-weeks of HIIT. Although we did not see the same improvement after MICT, a longer intervention may have resulted in a similar increase. Interestingly, despite no change in body weight, fat mass or body fat distribution, we report significant improvements in insulin sensitivity after HIIT only. Based on previous research when comparing HIIT to moderate-intensity exercise, it was found that there is a higher recruitment of type II fibres and greater phosphorylation of skeletal muscle AMPK in type II fibres and was associated with a greater depletion of skeletal muscle glycogen and the recruitment of type II fibres in response to HIIT (Kristensen et al., 2015). The activation of AMPK and downstream signalling has been shown to play a role in post-exercise increases in glucose uptake, regulating skeletal muscle insulin sensitivity (Kjøbsted et al., 2017, 2019). These findings may indicate a potential mechanism for the differential response between MICT and HIIT, however, further studies are warranted. There are a number of possible mechanisms by which improvements in muscle metabolism could improve insulin sensitivity independent of weight loss, including (but not limited to) capillarization, enhanced substrate utilization capacity, increased basal insulin sensitivity and increases in the expression of GLUT4 and mitochondrial proteins (Sylow and Richter, 2019). Overall, our findings are in agreement with previous studies, whereby significant improvements in insulin sensitivity were reported without change in body weight in women with PCOS (Moro et al., 2009; Hutchison et al., 2011; Scott et al., 2017; Patten et al., 2020), suggesting that improvements in metabolic health can be achieved without a reduction in body weight.

Low levels of SHBG are a common feature of PCOS, and contribute to increased levels of free testosterone (Baptiste et al., 2010). Unlike previous studies (Hutchison et al., 2011; Almenning et al., 2015), we report that SHBG levels increase as a result of HIIT. Improvements in SHBG have similarly been reported among other studies that utilized an aerobic exercise intervention (Giallauria et al., 2008; Thomson et al., 2008). Interestingly, one study comparing a diet alone intervention to two different combined diet and exercise interventions, reported increases in SHBG levels as a result of the combined diet and exercise interventions, but not in the diet alone group, suggesting that aerobic exercise may have been the responsible for the increase in SHBG levels (Thomson et al., 2008). The observed increase in SHBG levels following HIIT in our study may have contributed to the decrease in FAI. An elevated FAI is a commonly observed androgenic abnormality in women with PCOS (Teede et al., 2011). Some (Thomson et al., 2008), but not all studies (Curi et al., 2012; Orio et al., 2016) have reported that exercise can significantly reduce FAI. A recent meta-analysis reported only small effects of exercise for reducing FAI (Patten et al., 2020). Here we report a significant reduction in FAI following HIIT, but not MICT. Altogether, the data suggest that a higher intensity of exercise might be required to provoke beneficial changes that may impact the metabolic and reproductive health of women with PCOS.

Ovarian dysfunction is a common characteristic of PCOS present in up to 95% of women with PCOS (Kumarapeli et al., 2008). It is usually manifested as oligo- or amenorrhoea, resulting from chronic oligo-or anovulation (Brassard et al., 2008). There is increasing evidence to support that exercise improves menstrual regularity and ovulation (Vigorito et al., 2007; Palomba et al., 2008; Thomson et al., 2008; Nybacka et al., 2011). Improvements in menstrual cyclicity have been previously reported after 12 weeks of vigorous-intensity exercise, with 60% of women with amenorrhoea at baseline reporting normal cycles post-intervention (Vigorito et al., 2007). Similar to these findings, we found that 69% of women in the HIIT intervention reported improvements in menstrual cyclicity. Furthermore, we found that compared to MICT, those in HIIT intervention were 7.8 times more likely to report improvements in their menstrual cycle, indicating that HIIT may be a more beneficial strategy to improve menstrual regulation in women with PCOS.

A potential limitation of this study is the relatively small sample size, however, we were adequately powered to detect significant changes in VO_{2peak} and insulin sensitivity, the main outcomes of this study. The researchers who conducted the analyses were not blinded to group allocation. Additionally, the findings of this study are limited to overweight women with PCOS of reproductive age. Larger studies and follow-up studies are required to confirm these findings and to determine whether engagement in exercise and the observed improvements can be maintained and translate into improvements in symptoms and reduced incidence of T2DM in this high-risk population.

In conclusion, our study suggests that HIIT is effective and can offer superior improvements in cardio-metabolic and reproductive health outcomes compared to MICT. As such, HIIT should be considered when implementing exercise programmes to reduce the metabolic risk and to potentially improve reproductive function in women with PCOS. Larger and longer follow-up studies are required to confirm these findings and determine whether engagement in exercise and the associated improvements can be translated into long-term beneficial health outcomes, and therefore reduce the incidence of metabolic disorders in this high-risk population. If confirmed, we recommend that the guidelines be updated to recommend vigorous-intensity exercise rather than moderate-intensity exercise.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Data availability

The data underlying this article are available in the article and in its online supplementary material. The raw data underlying this article will be shared on reasonable request to the corresponding author.

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Authors' roles

R.K.P. and A.M-A. assisted in study design, collected and analysed data, evaluated results and prepared the manuscript. D.H. assisted in study design, collected data, assisted with data analysis and evaluated the manuscript. L.C.M. collected data and evaluated the manuscript. I.L. assisted with data collection and preparation of the manuscript. A.P.G. and S.S. collected and/or interpreted clinical data and critically analysed the manuscript. A.J.M., A.G.P. and R.J.R. reviewed the manuscript providing substantial academic input. N.K.S. designed the study and assisted with data collection. All authors approved the final manuscript.

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Conflict of interest

The authors have no conflicts of interest to declare.

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