The impact of intensified exercise training on insulin resistance and fitness in overweight and obese women with and without polycystic ovary syndrome

The impact of intensified exercise training on insulin resistance and fitness in overweight and obese women with and without polycystic ovary syndrome.

Short Title: Impact of intensified exercise on IR in PCOS

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Objective: To evaluate mechanisms of insulin resistance (IR) in overweight and obese women with and without PCOS and explore relationships between IR, fitness and body mass index (BMI) at baseline and following exercise intervention. **Design**: Prospective controlled intensified exercise intervention study. **Patients**: 20 overweight (BMI >25 kg/m^2) and obese (>30kg/m^2), reproductive aged PCOS women and 13 non-PCOS overweight, healthy controls of comparable BMI and age were studied at baseline. Measures were repeated in 13 PCOS and 8 control women following 3, 1 hour exercise sessions per week over 12 weeks. **Measurements**: IR was measured by glucose infusion rate on euglycaemic-hyperinsulinaemic clamp and fitness was assessed by VO_{2max}. **Results:** At baseline, PCOS women were 46% more insulin resistant than controls (175.6 vs. 257.2mg.m^{-2}.min^{-1}, p<0.05) with IR independently associated with VO_{2max} and BMI in the PCOS group only (p<0.01). Post-exercise IR improved across both groups (p<0.01). In PCOS women, IR improved by 16% (p<0.05) but was not restored to the same level as controls (p<0.05). Improvement in IR and in VO_{2max} were related in the PCOS group (r^2 = 0.85, p<0.05), yet change in IR and in fitness were not related. No associations were found in controls. **Conclusions**: While intensified exercise improves insulin resistance in PCOS women, a higher IR persisted following exercise in PCOS women and a clear relationship between improved IR and improved fitness was not found. Therefore, other mechanisms of, and therapies for, IR must be explored in PCOS as IR remains higher than observed in non-PCOS controls.

Polycystic Ovary Syndrome (PCOS) is a common endocrinopathy affecting between 9-18% of reproductive aged women [1]. PCOS is complex, involving reproductive manifestations (hirsutism and infertility) and metabolic complications (dyslipidemia, diabetes and increased cardiovascular risk factors) [2, 3]. Insulin resistance (IR) is a key aetiological feature in PCOS, present both intrinsically and extrinsically, contributing significantly to the reproductive and metabolic complications of the disorder[4-6]. Independent of weight, women with PCOS have underlying IR and have higher rates of impaired glucose tolerance (IGT), metabolic syndrome and type 2 diabetes mellitus in comparison to weight-matched control women [7]. Extrinsic or obesity related IR, further exacerbates underlying or intrinsic IR in PCOS [4], increasing IGT and type 2 diabetes mellitus (T2DM) risk.

Mechanisms of intrinsic related IR in PCOS are yet to be fully elucidated; however previous studies have demonstrated impaired insulin signalling [5] and mitochondrial dysfunction [8] within the skeletal muscle of PCOS women. Skeletal muscle is the primary site of glucose uptake occurring predominantly via insulin-dependent activation of the insulin signalling pathways [9]. Defects within the skeletal muscle insulin signalling pathways are thought to contribute to PCOS intrinsic IR with post-receptor abnormalities contributing to overall reduced skeletal muscle responsiveness to glucose [5, 10]. Previous non-PCOS studies in other insulin resistant conditions including obesity and T2DM have demonstrated improved IR with greater insulin-stimulated glucose uptake and reduced insulin secretion after ongoing aerobic exercise [11]. Despite this, there is limited comprehensive research to date on the underlying mechanisms of IR and IR improvement following exercise in PCOS.

As IR underpins the metabolic and reproductive disturbances in PCOS, lifestyle modification, including exercise remain first line for PCOS treatment. Previous limited studies assessing exercise therapy in PCOS report improved IR following exercise using indirect measures of IR including fasting insulin, Homeostatic Model Assessment (HOMA-IR) and Quantitative Insulin-Sensitivity Check Index (QUICKI) [12]. Our group recently completed a systematic review on exercise in PCOS and clear gaps in knowledge remain, including the effects of high intensity (>80% $VO_{2\ max}$) exercise training and comprehensive gold-standard assessment of IR following exercise [12].

Therefore, we aimed to evaluate mechanisms of IR in overweight and obese women with and without PCOS and explore the effects of intensified exercise training on IR and its relationship to other cardiometabolic risk factors using the comprehensive gold standard clamp technique. Overweight and obese women were studied to control for extrinsic weight-related IR, allowing potential intrinsic mechanisms underpinning IR to be explored in PCOS.

Research design and methods

Subjects

Premenopausal overweight (BMI>25 kg/m^2) and obese (BMI ≥30 kg/m^2) [13] sedentary women, with (n = 20) and without (n = 14) PCOS of comparable weight and BMI were recruited through community advertisement. 21 women (13 = PCOS and 8 = control) completed the study as previously described[14]. Diagnosis of PCOS was based on the NIH diagnostic criteria as previously described[14, 15]. All non-PCOS women had regular menstrual cycles, normal testosterone and free androgen index (FAI) and no evidence of clinical hyperandrogenism. Exclusion criteria in all participants included pregnancy, smoking, T2DM, regular physical activity and recent fluctuation in weight[14]. The Southern Health

Research Advisory and Ethics Committee approved the study and all participants gave written informed consent.

Screening

At screening (3 months prior to baseline), standard diet and lifestyle advice was delivered [Heart Foundation recommendations (www.heartfoundation.org.au)] and medications affecting IR including the oral contraceptive pill (OCP) were ceased. All women were instructed to maintain a stable diet and weight during the screening and run-in process. Dietary intake was monitored periodically through food diaries during the study period to ensure diet remained stable and therefore changes to insulin sensitivity with exercise alone could be assessed. End-point data was collected in the follicular phase of the menstrual cycle at baseline and following the 12 week exercise intervention, wherever feasible, as previously reported [14].

Clinical Measures

Anthropometric assessment

Following an overnight fast, all participants completed basic anthropometric assessment including weight (Tanita TBF310, Tokyo, Japan), waist and hip circumferences and height (Stadiometer Holtain, Wales, UK**)** as previously described [14]. BMI was calculated as weight (kg) / height squared (m^2). Waist-hip ratio (WHR) was calculated as waist / hip circumference**.**

Insulin Sensitivity: Euglycaemic Hyperinsulinaemic Clamp

Insulin sensitivity was assessed using the euglycaemic-hyperinsulinaemic clamp technique [16], as previously described [14]. Briefly, an IV catheter was inserted for blood drawing in the

dorsal hand and for infusion of glucose and insulin in the contra-lateral arm. Fasting blood samples were collected and thereafter, insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) was infused at a rate of 40 mU/m2 per minute for 120 minutes. Plasma glucose levels were clamped at ~5 mmol/L, using a variable infusion rate of 25% glucose. Real time blood glucose measurement was assessed every 5 minutes using a glucose analyser (YSI 2300 STAT glucose/L-lactate analyser; Yellowsprings Instruments, USA). During the clamp period, steady state was defined as the last 30 minutes of the insulin-stimulated period. The glucose infusion rates were calculated during the last 30 min of the euglycaemic-hyperinsulinaemic clamp and expressed as glucose (mg) per body surface area (m^2) per minute.

Biochemical Measurements

Fasting glucose, glycated haemoglobin (HbA1c) and lipids (cholesterol, HDL, LDL and triglycerides) were collected under fasting conditions. LDL was calculated as previously described [14, 17].

Maximal Aerobic Capacity

VO_{2max} was assessed at baseline (approximately one week following the euglycaemic clamp) and at the completion of the intervention using the MOXUS modular VO_2 system (AEI Technologies, Pittsburgh, PA) while participants exercised on a treadmill (Biodex RTM 500 (model no. 945-295) New York, USA) until volitional fatigue as previously described [14].

Exercise Intervention

All participants completed a 12 week intensified aerobic exercise program on a motorised treadmill (Biodex 500/Life Fitness 95T). Participants attended three, one hour sessions each week which sequentially alternated between moderate intensity (walking or jogging at 70%

of $VO_{2\ max}$ or 75-85% HR_{max}) and high intensity interval training (6x5 minute intervals with 2 minutes recovery period at ~95-100% of $VO_{2\ max}$ or ~95-100% HR_{max}). Participants progressed to 8 repetitions in the high intensity training sessions by the week 4, and reduced recovery time to 1 min by week 8 of training. Target exercise intensity (percentage $VO_{2\ max}$) and heart rates for each participant were achieved by altering speed (kph) and workload (gradient; %) on the treadmill with individual increases in fitness. A second $VO_{2\ max}$ test was performed at 6 weeks to assess changes in fitness and maximal heart rate.

Statistics

All data are presented as mean ± SEM. Two-tailed statistical analysis was performed using SPSS for Windows 17.0 software (SPSS Inc, Chicago, USA) with statistical significance set at α level of $p<0.05$. At baseline, data was assessed using Independent Samples T-tests (PCOS v Non-PCOS) with univariate analysis to correct for age. The effect of exercise was assessed using repeated measures ANOVA with PCOS status as between-subject factor and exercise as within-subject factor and age as a covariate with univariate analysis for pair-wise and categorical comparative analysis. Linear regression was used to assess the impact of covariates on insulin sensitivity (glucose infusion rate) and measures of glycaemia (HbA1C, glucose) pre- and post-exercise. Relationships between variables were examined using bivariate correlation. Change in variable was defined as the percentage change between pre- and post-treatment values.

A power calculation based on a previous similar study in women with type 2 diabetes mellitus reporting a 19.8% improvement in IR measured by glucose infusion rate was used as the expected effect size and revealed a required total sample of 14 participants (7 per group) with 80% power and a significance level of 0.05 [11].

Results

At baseline, following the completion of the three month run-in, data was available for 20 PCOS (n = 2 overweight, n= 18 obese) and 14 control (n = 1 overweight, n = 13 obese) women, except for glucose infusion rate (n=29; PCOS n=17, control =12). After the exercise intervention, results are presented for 13 PCOS and 8 controls (lost to contact (n = 4 PCOS); illness (n = 1 PCOS); protocol violation (n = 1 control); discontinued intervention (n = 2 PCOS; n = 5 control)) except for glucose infusion rate (n=16; PCOS n=9, control n=7).

Baseline Characteristics

Women with PCOS were younger than control women (29 ±1.4 vs. 35±1.1 years, p=0.01). In PCOS compared to control women, weight (101.11±4.32 vs. 96.23±3.49; PCOS vs. control, p=0.39) and WHR (0.86±0.01 vs. 0.85±0.02, p=0.74) were similar between groups with no significant differences observed. There were no differences between PCOS and control groups in baseline fitness ($VO_{2\ max}$; 24.96±1.3 vs. 25.24±0.8 $ml.kg^{-1}.min^{-1}$, p=0.88) or in markers of IR, including HbA1c (5.49±0.09 vs. 5.50±0.07%, p=0.92). With direct measurement of insulin sensitivity measured by the euglycaemic hyperinsulinaemic clamp, glucose infusion rate was significantly lower (46%) in women with PCOS in comparison to controls which persisted after adjustment for age (175.6±96.3 vs. 257.2±64.3 $mg.m^{-2}.min^{-1}$, $p< 0.05$). Data on baseline characteristics have been previously reported [14].

Comparative univariate baseline analysis showed that women with PCOS in a higher BMI category (morbid obesity; ≥35.00kg/m^2) had a significantly lower glucose infusion rate in comparison to control women with similar BMI (120.57±24.79 vs. 264.48±25.48 $mg.m^{-2}.min^{-1}$, p<0.001). For those with a lower BMI (≤34.99kg/m^2) there was a non-significant difference in glucose infusion rate between PCOS and control groups (224.06±30.84 vs.

247.06±29.14 $mg.m^{-2}.min^{-1}$, p=0.25). Similarly, women with PCOS with a lower fitness at baseline (≤25.00 $ml.kg^{-1}.min^{-1}$) had a significantly lower glucose infusion rate when compared to control women with a similar fitness level (109.14±23.14 vs. 258.63±32.71 $ml.kg^{-1}.min^{-1}$, p<0.01). However, a higher fitness (≥25.01 $ml.kg^{-1}.min^{-1}$) was associated with an increased glucose infusion rate in the PCOS group, comparative to that of the controls (226.16±27.66 vs. 259.99±12.17 $ml.kg^{-1}.min^{-1}$, p=0.26). Neither BMI category nor fitness level significantly impacted on glucose infusion rate in non-PCOS control women.

Exercise Intervention Effects

Following exercise there was a significant change in whole group weight (p<0.01) and BMI (p<0.01) with no significant difference between groups. Within groups, there was a trend to reduced weight within PCOS (-1.5±0.7kg, p=0.06) and control groups (-2.4±1.2kg, p=0.09). BMI was significantly reduced in the PCOS group (-0.6±0.3 kg/m^2, p=0.03). Maximal aerobic capacity (VO_2max) was significantly improved across the whole group (p<0.01) with no significant between-group differences. IR improved across the whole group (p<0.01) no significant time by group interaction. Within groups, IR improved with exercise by 16% (p=0.03) in PCOS women with only a trend towards change in the control women (p=0.07) (Figure 1). On univariate analysis, glucose infusion rate remained significantly different between PCOS and non-PCOS control women following exercise and adjusting for age (p<0.05; Table 1). All pre- and post-exercise characteristics are depicted in Table 1 with some components reported previously [14].

Comparative univariate analysis for BMI and fitness level post-exercise showed similar results to those seen at baseline. A higher BMI category (≥35.00kg/m^2) post-exercise was associated with a lower glucose infusion rate in women with PCOS in comparison to control

women (116.45±11.50 vs. 333.53±49.04mg.m^{-2}.min^{-1}, p<0.001), while a lower BMI (≤34.99kg/m^2) was associated with a similar glucose infusion rate between groups which persisted after controlling for change in glucose infusion rate (p=0.46). There was a trend for lower fitness post-exercise (≤30.00 ml.kg^{-1}.min^{-1}) to be associated with lower glucose infusion rate in PCOS when compared to control women (p=0.051); however when accounting for change in fitness post-exercise, this was not as strong (p=0.08). Interestingly, a higher aerobic capacity post-exercise was associated with increased glucose infusion rate in women with PCOS, comparative to control women within the same fitness level with no significant difference between groups, which persisted after adjusting for change in fitness (272.44±55.26 vs. 289.54±1.14 ml.kg^{-1}.min^{-1}, p=0.85).

Exercise workload as indicated by distance (km) performed on the treadmill in each moderate and high intensity exercise session significantly increased in both the PCOS (p<0.01 and p<0.05) and control groups (p<0.01 and p<0.05) over the progression of exercise (Figure 2A). Mean heart rate during each exercise session for both PCOS and control groups are presented in Figure 2B. Adherence to the exercise intervention was above 90% in both groups with no difference between groups [97% PCOS, 92% control (P=0.19)].

Correlations

At baseline, $VO_{2\ max}$ positively correlated with glucose infusion rate in PCOS (r=0.80, p<0.01, Figure 3A) but not in control women. Post-exercise, improvement in IR was associated with improvement in VO_{2max} in the PCOS group (r^2 = 0.85, p<0.05, Figure 3B), but not in the control group. At baseline and following exercise, weight inversely correlated with VO_{2max} in the whole group before and after exercise (r=-0.62, p<0.05 and r=-0.73,

241 $p<0.01$, respectively) and in the PCOS group (r=-0.64, $p<0.05$ and r=-0.77, $p<0.01$); but this
242 was not demonstrated within the control group. Following exercise $VO_{2\ max}$ inversely
243 correlated with glucose (r=-0.70, $p<0.05$), and HbA1C (r=-0.68, $p<0.05$) in PCOS but not in
244 control women.

245 When entered in to linear regression, $VO_{2\ max}$ was independently associated with glucose
246 infusion rate at baseline in PCOS ($p<0.001$) but not in the control group. Following exercise,
247 $VO_{2\ max}$ was independently associated with measures of glycaemia across the whole group
248 with including HbA1c ($p<0.05$), with a trend towards association with post-exercise glucose
249 infusion rate (p=0.07). Change in $VO_{2\ max}$ was not independently associated with change in
250 glucose infusion rate in any group despite these variables improving significantly with
251 exercise.

252 **Discussion**

253 The results of the current study, using gold standard euglycaemic hyperinsulinaemic clamps
254 affirm that women with PCOS are more insulin resistant than control women of similar
255 weight, which persisted after adjustment for age. We demonstrate the feasibility of intensified
256 exercise training in an overweight and obese group of women and show the ability of
257 exercise to alleviate IR in PCOS without change in weight or structured dietary restriction.
258 We report lower fitness and a higher BMI ($\geq$35.00kg/m^2) both independently worsen IR in
259 PCOS at baseline, a finding not observed in control women. Conversely, in PCOS women
260 with lower BMI or higher baseline fitness, insulin sensitivity was comparable to control
261 women, suggesting that both fitness and BMI independently and significantly have a greater
262 impact on IR in PCOS compared to controls. Supporting these results is a significant
263 association between improved fitness and improved insulin sensitivity in the PCOS, but not

the non-PCOS control group. In addition, although under powered to detect a difference in this setting, intensified exercise appears to impact on cardiovascular risk factors in PCOS with cases of MS and IFG resolving in the majority of cases post-exercise. Despite these results we were unable to demonstrate an independent relationship between change in $VO_{2\ max}$ and change in glucose infusion rate.

Results reported here add to previous literature assessing IR in PCOS. Firstly, in previous studies assessing the effects of exercise with or without dietary restriction, all have used a moderate intensity (60-70% $VO_{2\ max}$) exercise protocol involving an average of 30 minute sessions ranging from three to seven sessions per week (for review see [12]). Here we demonstrate that moderate to high intensity exercise with three sessions of one hour per week is effective with training intensity achievable in both PCOS and non-PCOS overweight and obese women. Additionally, IR has not been comprehensively assessed with the insulin clamp technique in previous PCOS exercise studies [12]. Assessment of IR in PCOS is difficult with many measures utilised including fasting insulin insensitive [18] and inaccurate in this setting [19]. As IR is a central pathophysiological feature in PCOS, exploration of IR at a detailed mechanistic level using sensitive methods is important.

Previous studies using indirect measures of IR have reported a 9-30% improvement in fasting insulin following moderate exercise in PCOS [12]. In general, greater improvements in fasting insulin (23-30%) were observed in studies aiming to induce weight loss and involving and dietary component. Our results show a significant improvement in insulin sensitivity in PCOS women without the presence of weight loss or change in diet, indicating that similar or higher improvements in IR can be achieved when higher exercise intensities are used alone, without these added components. Given rigid dietary prescription may be difficult to maintain, especially long-term, these results highlight the clinical importance of exercise

prescription including vigorous components, in young PCOS women. Future randomised controlled studies assessing change in IR with high intensity exercise and diet or potentially weight loss are needed to assess whether these added components produce similar, differing or potentiating effects to intense exercise alone.

A second important difference in the current study is the use of a non-PCOS control group of comparable weight and BMI with all previous exercise studies using PCOS participants across all treatment groups [12]. Assessing the effects of exercise and change in IR in PCOS women in comparison to non-PCOS control women has offered useful insights. PCOS women exercised at the same progressive workload, demonstrating equal improvement in fitness. Following exercise IR improved across both groups demonstrating a similar effect of exercise on insulin sensitivity between groups, however we demonstrate that insulin sensitivity remained significantly lower in PCOS women in comparison to control women following exercise. Furthermore, glucose infusion rate post-exercise in PCOS women remained lower than the baseline glucose infusion rate observed in control women. Taken together, this further highlights the marked IR that is characteristic of PCOS (Figure 1).

To explore confounders of IR in PCOS we conducted a comparative sub-analysis to assess the effects of lower versus higher categories of both BMI and fitness, with results providing added insights into IR in PCOS. At baseline, both lower fitness and a higher BMI were associated with significantly worse insulin sensitivity in PCOS women and conversely higher fitness and lower BMI was associated with a comparable glucose infusion rate to control women. These associations were not observed in control women, suggesting that these modifiable and extrinsic factors more significantly impact on IR in an already inherently insulin resistant group of women with PCOS. Post-exercise, a higher BMI remained independently associated with higher IR in PCOS, however a higher fitness in PCOS resulted

in comparable glucose infusion rate to that of control women, demonstrating the potential of improved fitness and regular exercise as a therapy to reduce IR in PCOS. As IR remained worsened overall in comparison to non-PCOS control women post-exercise, these findings could indicate that BMI and fitness may impact more on extrinsic, obesity related IR with mechanisms behind intrinsic (genetic related) IR, yet to be completely explored.

Similar to insulin, exercise independently induces translocation of GLUT-4 to the plasma membrane to assist glucose uptake without activation of the insulin signalling pathways [9]. These results in PCOS may indicate that the improved, but not restored insulin sensitivity observed is due to exercise induced improvement in glucose uptake within the skeletal muscle with the intrinsic or inherent PCOS defects in the insulin signalling pathways still persistent following exercise. Previous studies have demonstrated post-receptor mitogenic and metabolic insulin signalling pathway defects in PCOS skeletal muscle, independent of obesity [5, 6], ultimately reducing glucose uptake. Future detailed analysis of insulin signalling pathways is needed in future studies to clarify mechanistic changes within the skeletal muscle of PCOS women following exercise.

There are some limitations to the current study. Despite adequate power, inclusion of more control women may have shown a relationship between fitness and IR in controls. Future studies need to explore and compare other exercise modalities, including resistance exercise training, previously noted to improve IR and potentiate glucose infusion rate in women with type 2 diabetes in comparison to aerobic exercise alone [11]. Similar findings have yet to be demonstrated in PCOS with one previous study finding no added effect of resistance training with aerobic exercise [20]. Comparison of exercise across differing PCOS phenotypes, including lean women, to investigate intrinsic related IR in the absence of extrinsic, obesity related IR would be beneficial. In line with previous studies[12], our drop-out rate was 38%,

highlighting the need for lower intensity lifestyle intervention studies to improve compliance in this setting. Although this study did have a small sample size, primary outcomes were adequately powered and similar non-PCOS studies have involved similar or less participants [11]. Strengths of this study include the use of comprehensive, gold-standard techniques to measure IR, supervised exercise and a well defined non-PCOS control group of similar weight.

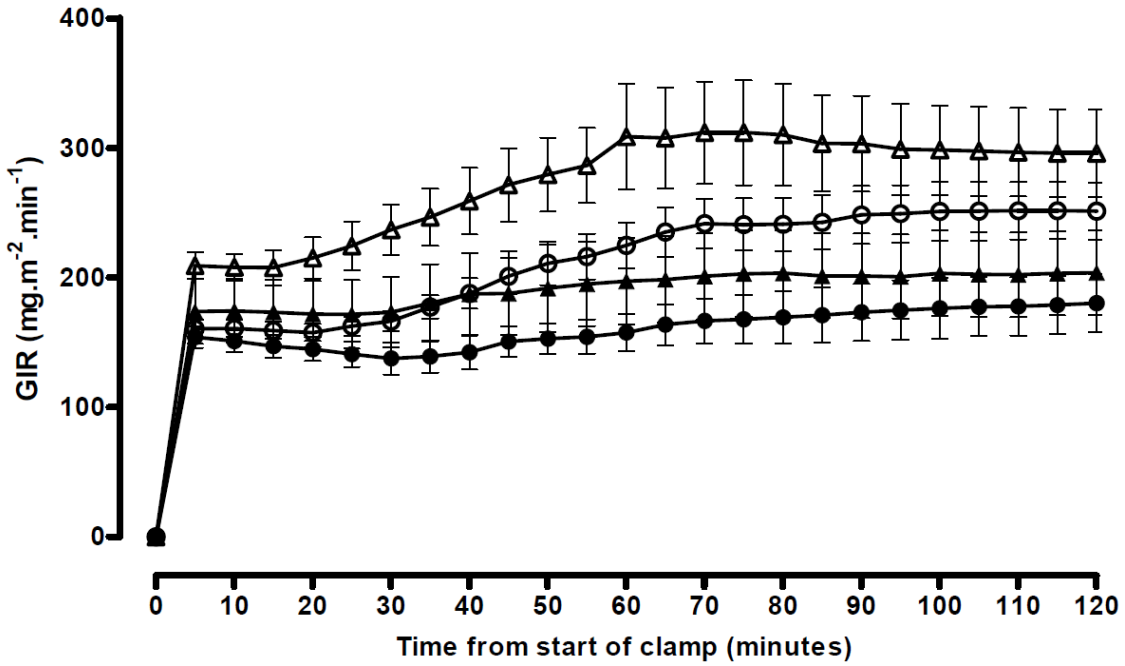
We confirm using gold standard clamp studies that PCOS women have worsened IR compared to non-PCOS control women of similar weight. In this setting we report novel data that clamp derived IR improves with intensified exercise in overweight and obese women with and without PCOS, but still remains more severe in PCOS women compared to controls post exercise. We also show for the first time that in PCOS specifically, there was a more profound impact of higher BMI and lowered fitness on IR pre and post exercise, than seen in control women. These findings suggest that modifiable factors are even more important targets for improvement of IR, in this highly insulin resistant group compared to in controls. This further emphasises the importance of regular physical activity prescription, preferably with a vigorous exercise component for women with PCOS. Randomised controlled trials on assessment of additional medical interventions to target intrinsic IR, in combination with intensified exercise, would be of future benefit to improve IR in this common clinical condition.

Table 1. Anthropometric, metabolic and fitness characteristics in PCOS and Non-PCOS women pre- and post-exercise.

Characteristic	PCOS (n=13)		Non-PCOS (n = 8)		P* (time; whole group)	P* (time & PCOS v Non PCOS)
	Pre	Post	Pre	Post		
Age (years)	29.75±1.4		35.29±1.1			0.01
Weight (kg)	96.9 ± 17.5	95.3 ± 17.8	99.4 ± 15.3	96.9 ± 12.7	<0.01	0.51
BMI (kg/m²)	35.6 ± 5.8	35.0 ± 5.8†	36.9 ± 5.9	35.9 ± 5.0	<0.01	0.50
WHR	0.87±0.0	0.88±0.1	0.84±0.0	0.83±0.1	0.89	0.22
VO_2max ($ml.kg^{-1}.min^{-1}$)	25.9±7.0	31.5±7.3†	26.1±3.2	30.7±3.3†	<0.01	0.53
RER	0.97±0.1	0.86±0.1	1.0±0.1	1.0±0.1	0.26	0.60
HR_{max} (beats/min)	184±11.5	184±6.7	181±11.2	178±7.3	<0.05	0.83
IR and Glucose Metabolism						
Fasting glucose (mmol/l)	5.0 ± 0.5	4.9 ± 0.3	4.8 ± 0.4	4.9 ± 0.4	0.93	0.56

Glucose infusion rate ($mg.m^{-2}.min^{-1}$)	171.3 ± 120.6‡	199.2 ± 105.2†‡	240.4 ± 53.0	297.5 ± 91.9	<0.01	0.28
HbA1c (%)	5.5 ± 0.4	5.6 ± 0.4	5.5 ± 0.3	5.5 ± 0.2	0.38	0.51
Lipids & BP						
Cholesterol(mmol/L)	4.5 ± 0.3	4.4 ± 0.2	4.6 ± 0.4	4.8 ± 0.4	0.74	0.23
Triglycerides(mmol/L)	1.1 ± 0.6	0.9 ± 0.4†‡	1.1 ± 0.3	1.3 ± 0.4	0.33	<0.01
HDL(mmol/L)	1.0 ± 0.3	1.0 ± 0.2	1.2 ± 0.4	1.2 ± 0.4	0.46	0.61
LDL(mmol/L)	3.0 ± 0.9	3.0 ± 0.7	2.9 ± 0.9	3.1 ± 1.0	0.68	0.59
Systolic Blood Pressure (mmHg)	108 ± 14.6	109 ± 10.4	118 ± 16.7	116 ± 16.2	0.81	0.54
Diastolic Blood Pressure (mmHg)	72 ± 10.2	69 ± 7.4	75 ± 8.8	73 ± 10.5	0.13	0.92
Metabolic Syndrome	4	1	1	2		
IFG	2	0	1	1		

WHR (waist-to-hip ratio); RER (respiratory exchange ratio); HR (heart rate); HbA1c (glycated haemoglobin); IFG (Impaired Fasting Glucose; ≥100<126mg/dL). *All results age-adjusted † Significant change within group (p<0.05). ‡ Significant difference between PCOS and non-PCOS at baseline or week 12 after age adjustment with univariate analysis. Metabolic syndrome classification using the International Diabetes Federation criteria includes central obesity (WC >88cm) plus two of raised triglycerides >150mg/dL; raised blood pressure >130/85mmHg; IFG >100 mg/dL; T2DM or reduced HDL <50mg/dL). Age, weight, $VO_{2\,max}$, glucose, glucose infusion rate and lipids have been previously reported [14].



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399 **Figure 1.** Insulin sensitivity as measured by glucose infusion rate (glucose infusion rate) before and
400 after exercise training in PCOS and Non-PCOS control women. Black circle = PCOS pre exercise;
401 Black triangle = PCOS post exercise; White circle = control pre exercise; White triangle = control
402 post exercise.

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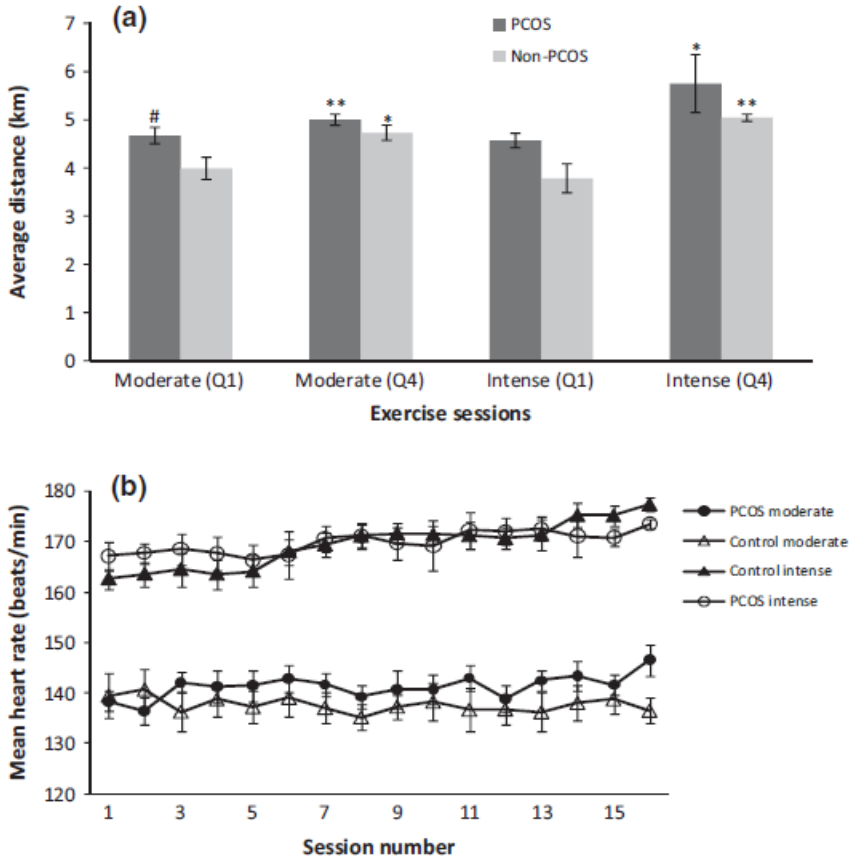

Figure 2 **(A).** Mean work performed (km) in moderate and intense training sessions in the first month (Q1) versus the last month (Q4) of training in PCOS and Non-PCOS control women. **(B).** Mean heart rate during moderate and intense interval training sessions for PCOS and Non-PCOS control women. **$p<0.01$; *$p<0.05$ time effect; # significantly different ($p<0.05$) from control at the same time point.

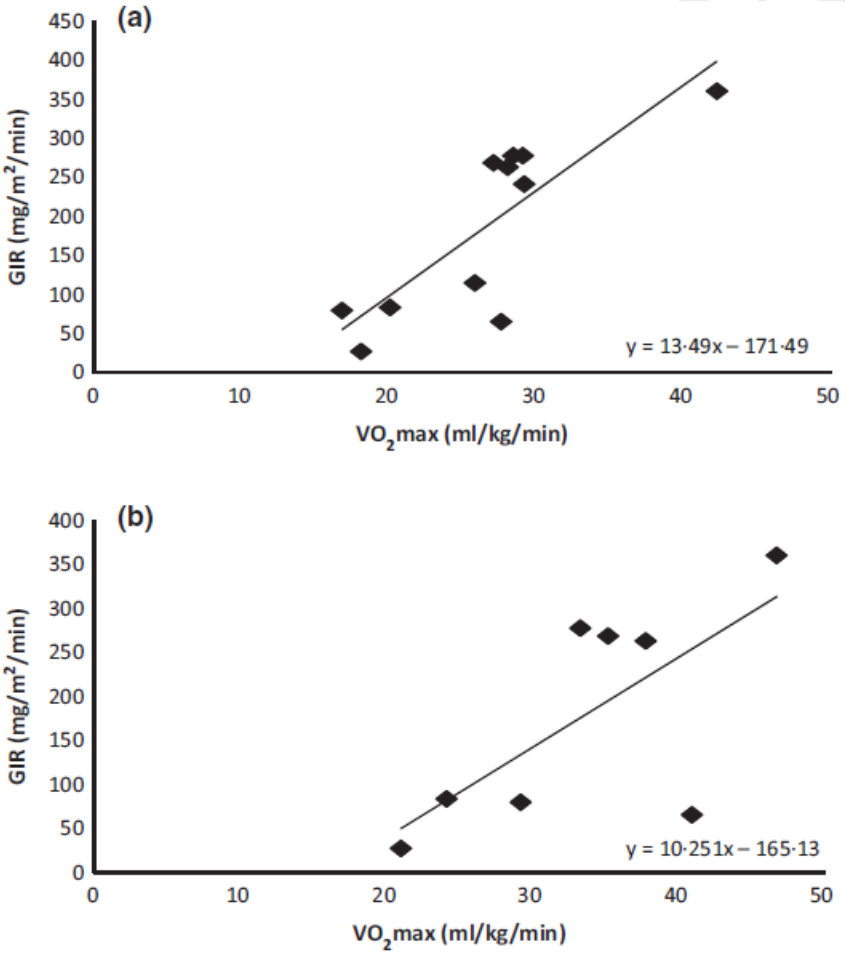

Figure 3 (A). Correlation between glucose infusion rate and $VO_{2\ max}$ pre exercise (r = 0.80, $p<0.01$) and **(B)** post exercise (r = 0.85, $p<0.05$) in PCOS women.

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