Effect of Exercise Interventions on Metabolic, Reproductive and Mental Health in Overweight Women with Polycystic Ovary Syndrome

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Abstract

Polycystic ovary syndrome (PCOS) is a common and complex endocrinopathy with significant metabolic and reproductive manifestations, carrying a major health and economic burden. Exercise has consistently been found to improve clinical outcomes, but shortfalls with exercise prescription are evident, with little known about the impact of exercise intensity for improving health outcomes. Research suggests that high-intensity interval training (HIIT) is feasible, well tolerated and enjoyable for people with, or at risk of, chronic disease and can address many of the shortfalls and barriers to exercise participation. Despite this, there is limited research exploring the efficacy of HIIT in comparison to standard moderate-intensity exercise recommendations for improving the health of women with PCOS. Therefore, the optimal exercise intensity for improving health outcomes remains unknown. The overall aim of this thesis was to determine the effectiveness of exercise, and more specifically exercise intensity, on the metabolic, reproductive and mental health and health-related quality of life of women with PCOS.

The first two studies of my thesis were systematic reviews (Chapters 2 and 3), one that included a meta-analysis, to summarise and evaluate the current exercise intervention literature on key clinical metabolic, reproductive and anthropometric outcomes and on mental health and health-related quality of life (HRQoL) in women with PCOS. Briefly, results from these reviews suggested that exercise is beneficial for improving both the physical and mental health of women with PCOS. Furthermore, exercise of vigorous intensities provided additional benefits for increasing cardiorespiratory fitness, body composition and insulin resistance. There was insufficient evidence to be able to analyse the impact of exercise intensity on mental health and HRQoL, however, the available evidence indicated that exercise is effective for improving health-related quality of life and PCOS symptom distress. Exercise also shows some efficacy for improving symptoms and/or prevalence of depression and anxiety in women with PCOS.

Following this, a protocol for a clinical trial was designed (Chapter 4), and conducted to investigate whether HIIT could confer greater benefits than standard moderate intensity continuous training (MICT) on cardio-metabolic outcomes (Chapter 5), reproductive outcomes (Chapter 6) and mental health and HRQoL (Chapter 7) in overweight women with PCOS. To do this, we employed a two-arm, randomised clinical trial where twenty-four overweight women, aged 18-45 years with diagnosed PCOS were randomised to either MICT (60-75% peak HR [HR_{peak}]) or HIIT (>90% HR_{peak}). We had 13 women complete the HIIT intervention and 11 women complete the MICT intervention.

In regards to cardio-metabolic outcomes, both HIIT and MICT improved VO_{2peak} (HIIT; 23.4 ± 10.1%, P <0.001 and MICT; 14.0 ± 9.3%, P <0.001), however, the HIIT group had a significantly greater improvement compared to MICT (P = 0.004). HIIT increased the insulin sensitivity index both

compared to baseline (49.1 ± 38.2%; P = 0.014) and to MICT (P = 0.046). Overall, the improvement in VO_{2peak} was associated with the improvement in insulin sensitivity (P = 0.003, R^2 = 0.38).

In terms of reproductive health outcomes, HIIT resulted in improvements in free androgen index (FAI) (P = 0.041), percent of free testosterone (0.016) and sex hormone binding globulin (SHBG) (0.026), with no significant changes as a result of MICT. HIIT also resulted in significantly greater improvements in SHBG (P = 0.005) and percent of free testosterone (P = 0.002) compared to MICT. A significant association between Δ insulin sensitivity and Δ free testosterone was detected in HIIT group (P = 0.029, adjusted R^2 = 0.43), but not in the MICT group. In regards to menstrual cyclicity, although not significant, 69% of participants (9 of 13) and 18.2% (2 of 11) reported improvements in menstrual cyclicity following HIIT and MICT, respectively.

When examining psychosocial outcomes, reductions in depression (P <0.001) and stress (P = 0.005) scores were observed in the HIIT group. Anxiety scores were reduced in both groups (HIIT; P = <0.001, and MICT; P = 0.007), however, there was a significantly greater reduction following HIIT compared to MICT (P = 0.017). The HIIT group also significantly improved emotions, weight and menstrual problem domains of the polycystic ovary syndrome questionnaire (PCOSQ) and physical functioning, role emotional, energy and general health domains of the SF-36.

In conclusion, supervised HIIT is effective and offers greater improvements in aerobic capacity, insulin sensitivity, hormonal profiles and anxiety scores in comparison to MICT in overweight women with PCOS. We also demonstrated that HIIT is efficacious for improving menstrual cyclicity, depression and HRQoL, indicating that HIIT may be superior to MICT for improving a wide range of health outcomes in women with PCOS. Therefore, HIIT should be considered as an effective strategy to promote health, reduce the cardio-metabolic risk and improve key clinical outcomes in this population.

Student Declaration

I, Rhiannon Patten, declare that the PhD thesis entitled **Effect of Exercise Interventions on Metabolic, Reproductive and Mental Health in Overweight Women with Polycystic Ovary Syndrome** is no more than 80,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, references and footnotes. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work.

I have conducted my research in alignment with the Australian Code for the Responsible Conduct of Research and Victoria University's Higher Degree by Research Policy and Procedures.

Signature: 

Date: 30/04/2021

In memory of Professor Nigel Keith Stepto

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Details of publications included within this thesis

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The effectiveness of high intensity intermittent training on metabolic, reproductive and mental health in women with polycystic ovary syndrome: study protocol for the iHIT- randomised controlled trial	Published	#**Patten, R.**, #Hiam, D.**,** *et al.* The effectiveness of high intensity intermittent training on metabolic, reproductive and mental health in women with polycystic ovary syndrome: study protocol for the iHIT- randomised controlled trial. *Trials* **20**(221). doi:10.1186/s13063-019-3313-8 (2019).	4
High-intensity compared to moderate-intensity training results in greater cardio-metabolic and reproductive outcomes in women with PCOS	In preparation	**Patten, R.**, McIlvenna, L., Levinger, I., Garnham, A., McAinch, A., Rodgers, A., Shorakae, S., Parker, A., #Hiam, D., #Moreno-Asso, A., & #Stepto, N. High-intensity compared to moderate-intensity training results in greater cardio-metabolic and reproductive outcomes in women with PCOS	5 & 6
Efficacy of high-intensity interval training compared to moderate-intensity continuous training for improving mental health and health-related quality of life in women with polycystic ovary syndrome.	In preparation	**Patten, R.**, Moreno-Asso, A., McIlvenna, L., Rosenbaum, S., Hiam, D., Stepto, N., & Parker A. Efficacy of high-intensity interval training compared to moderate-intensity continuous training for improving mental health and health-related quality of life in women with polycystic ovary syndrome.	7

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2018 – Centre for Research Excellence in Polycystic Ovary Syndrome, poster presentation

2017 – Higher degree by research conference, Institute for Health and Sport, Victoria University, poster presentation

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List of abbreviations

AE-PCOS: androgen excess and polycystic ovary syndrome

AMH: anti-müllerian hormone

BMI: body mass index

CVD: cardiovascular disease

DASS-21: depression anxiety stress scales - 21-items

DHT: dihydrotestosterone

DXA: dual x-ray absorptiometry

FAI: free androgen index

FSH: follicle stimulating hormone

GnHR: gonadotropin releasing hormone

HIIT: high-intensity interval training

HOMA-IR: homeostatic model assessment of insulin resistance

HR_{max}: maximal heart rate

HR_{peak}: peak heart rate

HRQoL: health-related quality of life

IGT: impaired glucose tolerance

IR: insulin resistance

LH: luteinising hormone

mFG: modified Ferriman-Gallwey

MICT: moderate-intensity continuous training

NIH: national institute of health

OGTT: oral glucose tolerance test

PCOM: polycystic ovarian morphology

PCOS: polycystic ovary syndrome

PCOSQ: polycystic ovary syndrome questionnaire

RCT: randomised controlled trial

SF-36: 36-item short-form survey

SHBG: sex hormone binding globulin

T2DM: type 2 diabetes mellitus

VO_{2peak}: peak oxygen uptake

WC: waist circumference

Chapter 1.0 Literature Review

1.1 What is Polycystic Ovary Syndrome?

Polycystic ovary syndrome (PCOS) is the most common endocrine condition in women of reproductive-aged[1]. In PCOS, there is an underlying hormonal imbalance between androgens and insulin which leads to reproductive (hyperandrogenism, menstrual irregularities and sub-fertility)[2], metabolic (insulin resistance [IR], obesity and increased risk of developing type 2 diabetes mellitus [T2DM] and cardiovascular disease [CVD])[3,4] and psychological (increased symptoms of depression and anxiety, and decreased health-related quality of life [HRQoL]) impacts[5,6]. Women with PCOS also have a propensity to gain weight which worsens the clinical features of the condition[7]. Despite the adverse health implications and the high prevalence, the aetiology and optimal treatment of PCOS remain unclear and it is under-recognised by health professionals[8].

1.2 Diagnostic criteria

PCOS was first described by Stein and Leventhal in medical literature in 1935 where it was recognised as a reproductive disorder with symptoms including polycystic ovaries and amenorrhoea[9]. More recently, hyperandrogenism has been acknowledged as part of the condition which has subsequently become known as PCOS[10]. The PCOS diagnostic criteria is continuously evolving, with ongoing controversy surrounding the optimal diagnostic criteria. The three most recognised diagnostic criteria include the National Institute of Health (NIH) criteria[11], Androgen Excess and PCOS (AE-PCOS) critera[12] and the Rotterdam criteria[13] (Table 1). Currently, the Rotterdam criteria is the most internationally recognised and accepted criteria for the diagnosis of PCOS and includes any two of the following:

- Oligo- or anovulation/irregular cycles (<21 days or >35 days or less than 8 cycles per year)
- Clinical or biochemical hyperandrogenism
- Polycystic ovarian morphology (PCOM) on ultrasound

Related disorders causing hyperandrogenism (e.g. hyperprolactinaemia, hyperthyroidism and non-classic congenital adrenal hyperplasia) must be excluded[13].

Table 1. Phenotypes of PCOS

Clinical Features	Phenotype			
	A	B	C	D
Hyperandrogenism	+	+	+	
Oligo- or anovulation	+	+		+
Polycystic ovarian morphology	+		+	+
Diagnostic criteria				
NIH	✓	✓		
AE-PCOS	✓	✓	✓	
Rotterdam	✓	✓	✓	✓

NIH, National Institute of Health; AE-PCOS, Androgen Excess and Polycystic Ovary Syndrome.

The NIH criteria was the first described diagnostic criteria, developed in 1990[11]. According to this criteria, the diagnosis of PCOS required the presence of oligo- or anovulation and hyperandrogenism, regardless of the presence of polycystic ovaries, and is considered to diagnose the most severe phenotypes of PCOS (A and B; Table 1)[11]. The newer Rotterdam criteria, created in 2003, was expanded to include two additional phenotypes (C & D; Table 1). The various phenotypes of PCOS are based on the presence or absence of the above criteria with the most severe phenotypes considered to include both hyperandrogenism and ovulatory dysfunction[14], with women meeting this criteria being at greater risk for obesity, insulin resistance, and risk factors associated with T2DM and CVD[15].

Due to the evolving criteria and the heterogeneous nature of the condition, diagnosis is difficult and this means that PCOS can often go undiagnosed[16]. The clinical heterogeneity of PCOS, the ongoing development of diagnostic protocol and advancements in testing equipment lead to inconsistency in regards to the diagnosis of PCOS in clinical practice. This often results in a delayed diagnosis and unmet need for information, causing women to be dissatisfied with care[17,18]. A poor or delayed diagnosis experience may limit crucial screening for metabolic complications or lead to disengagement with health professionals resulting in a lack of information, affecting their ability to improve their lifestyle[19]. While PCOS is diagnosed based on the presence of reproductive features, it is well known that PCOS has additional features including insulin resistance, obesity and mental health issues that should be

considered[20,21]. If these go unmonitored and undiagnosed, it can lead to an increased risk of developing co-morbidities and a worsening of health-related quality of life.

1.3 Prevalence and burden of PCOS

PCOS is the most common endocrine disorder in reproductive-age women. It affects 8-13% of women worldwide depending on the diagnostic criteria used and populations studied[1,21]. In Australia, the prevalence estimates are slightly higher, with 12-21% of Australian women of reproductive-age being affected, and is more common in overweight women and those of Indigenous background[8,22]. An Australian study examining the prevalence of PCOS reported that 72% of women with PCOS had phenotype A or B, while 13% of women had phenotype C and 15% had phenotype D. Of these women, 68% did not have a pre-existing diagnosis[8]. The various phenotypes of PCOS appear to demonstrate varying degrees of metabolic disturbances, with phenotypes A and B reported to have a higher prevalence of metabolic syndrome of 29.6% and 34.5% of women, respectively, in comparison to phenotype C and D with 10% and 8.3%, respectively[23].

PCOS is a complex and chronic condition with manifestations across the lifespan, presenting a significant health and economic burden[20]. PCOS places women on track for a plethora of diverse, often chronic conditions ranging from T2DM, CVD, infertility and mental health issues[3,20,24]. The costs associated with PCOS and its co-morbidities that occur in women of reproductive-age have been estimated to exceed $4 billion in the United States of America[25]. In Australia, PCOS and the associated co-morbidities is estimated to cost the Australian health care system over $400 million each year[2]. These estimates are said to be conservative and would be significantly higher if the care of co-morbidities was extended to include women of post-reproductive age[25]. Approximately 40% of the economic burden is due to the increased risk and prevalence of T2DM in these women[25]. Only 2-3% of the cost is associated with diagnostic evaluations[25].

1.4 Pathophysiology of PCOS

Despite decades of research, the aetiology of PCOS remains largely unclear due to the highly complex and multifactorial nature of the syndrome[20]. The complex relationship between multiple biological systems may partially explain the limited understanding of the aetiology. PCOS is caused by a combination of genetic and lifestyle factors that lead to an underlying hormonal imbalance of androgens and insulin, and is further exacerbated by obesity (Figure 1)[2,20]. Hyperandrogenism is well established contributor to the aetiology and is detected in the vast majority of women with PCOS[26-29], whilst IR is present in 50-95% of women with PCOS, and is highly prevalent in both overweight and lean women[20,27,30]. PCOS is a heterogeneous condition, not only in terms of its pathophysiology but also in regards to the severity of health consequences[31]. Combined, these health consequences lead to psychological concerns including an increased prevalence of symptoms of anxiety and depression,

decreased HRQoL, poor body image and low self-esteem[2]. The diagnostic features and their health implications will be discussed in more detail throughout this literature review.

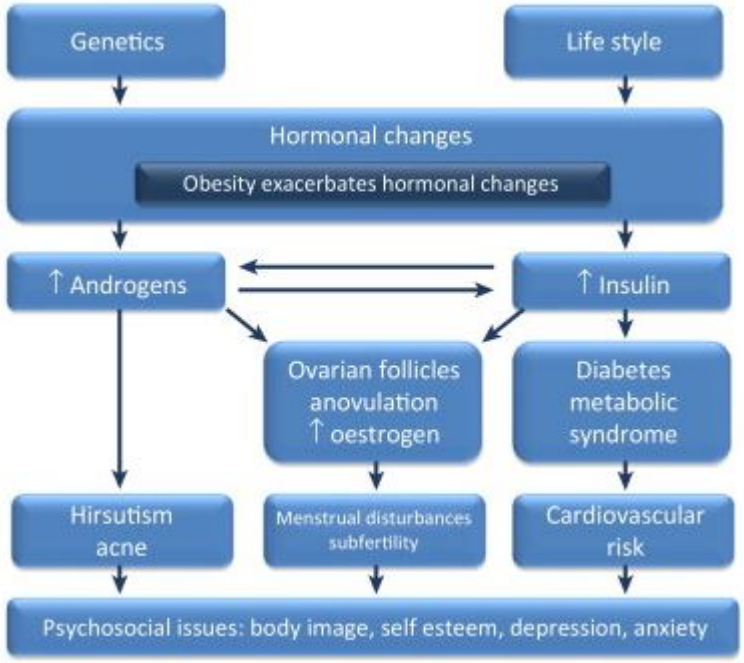

Figure 1. The aetiological and clinical features of PCOS.
Reproduced with permission from the author[2].

1.5 Reproductive features

1.5.1 Hyperandrogenism

Hyperandrogenism is a well-established contributor to the aetiology of PCOS and is considered the main clinical hallmark[10] occurring in approximately 75-85% of women with PCOS[26,29]. Hyperandrogenism can be determined by either clinical or biochemical androgen excess. Biochemical hyperandrogenism is determined by elevated serum levels of androgens including; total testosterone, free testosterone or calculated free testosterone or by the FAI[32]. FAI is calculated as the ratio of total testosterone to SHBG levels (total testosterone/SHBG) x 100[33]. Total testosterone refers to the sum of the circulating concentrations of both protein-bound and unbound testosterone[34]. Free testosterone is considered the circulating testosterone that is unbound to any plasma protein, predominantly to SHBG or albumin[34]. Free testosterone can be measured directly or calculated from total testosterone, SHBG and albumin concentrations[35]. SHBG is secreted by the liver and is the main transport protein for testosterone[36]. Measurements of hyperandrogenism are limited by poor accuracy, sensitivity and specificity, largely due to low levels of circulating concentrations of androgens in women[32,37].

The mechanisms behind androgen excess are relatively unclear with multiple mechanisms involved. Androgen excess is suggested to result from the disruption of normal ovarian or adrenal function,

resulting in excessive production of androgens. It has also been suggested to be in part caused by neuroendocrine abnormalities. In a normal menstrual cycle, the gonadotrophin-releasing hormone (GnRH) is secreted in a pulsatile manner by the hypothalamus that stimulates the release of gonadotrophins such as luteinising hormone (LH) and follicle stimulating hormone (FSH) from the pituitary gland[38,39] (Figure 2). Women with PCOS have persistently rapid GnRH pulse frequency, favouring the secretion of LH over FSH, resulting in an elevated LH:FSH ratio[39]. The elevated LH promotes the theca cells to synthesise androgens, and the deficiency of FSH contributes to the failure of the follicles to mature, resulting in PCOM[38].

IR and compensatory hyperinsulinaemia have also been considered to contribute to hyperandrogenism (Figure 1). Hyperinsulinemia is thought to enhance the GnRH pulsatile release from the hypothalamus, increasing LH pulsatility[40] (Figure 2). Insulin acts synergistically with LH to stimulate ovarian androgen production and suppress hepatic production of SHBG[41]. Low levels of SHBG contribute to high levels of free testosterone by reducing testosterone binding[42]. Insulin has also been found to bind to the insulin receptor in ovarian theca cells and granulosa cells, activating the release of LH and stimulating excessive androgen production[40,43] (Figure 2). Obesity, particularly abdominal obesity, is also known to increase circulating androgen and insulin levels, and reduce SHBG, increasing hyperandrogenism, hirsutism, sub fertility[20] and ovarian dysfunction[24,44,45]. Obesity also affects androgens not bound to SHBG, affecting production rates and metabolic clearance of dehydroepiandrosterone and androstenedione, and is significantly impacted by body fat distribution[45].

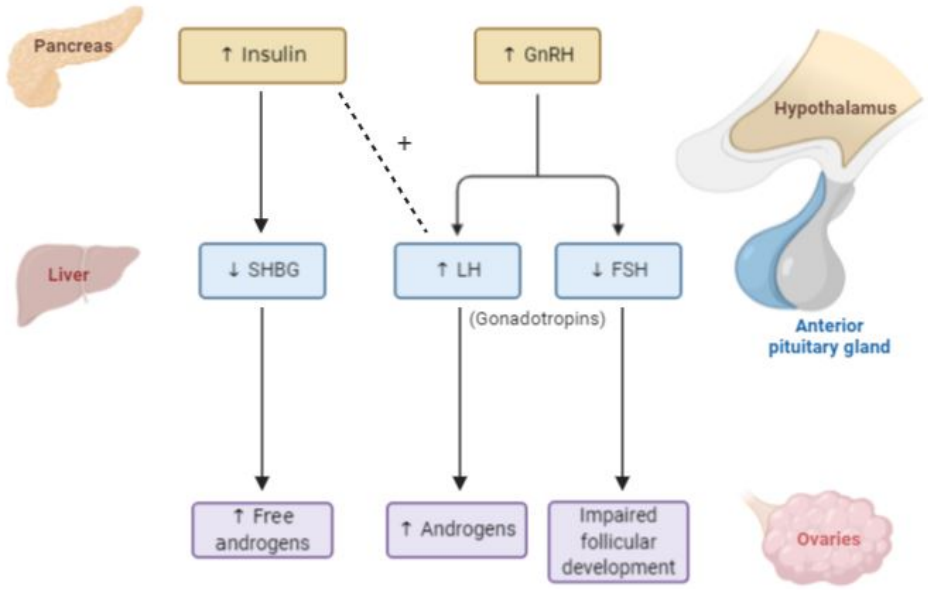

Figure 2. Disruptions in neuroendocrine function and insulin action causing reproductive features of PCOS.
GnRH – gonadotropin releasing hormone, LH – luteinising hormone, FSH – follicle stimulating hormone.
Created with BioRender.com

Clinically, hyperandrogenism can manifest as hirsutism, acne and androgenic alopecia[31], however, there is large variance depending on ethnicity, age and body mass index[21,31]. Hirsutism is defined as the

development of male pattern terminal hair growth in women[46] and is considered to be the most reliable clinical marker of androgen excess and is quantified according to the modified Ferriman-Gallwey (mFG) score[31,46]. This tool is used to evaluate hair growth of nine areas of the body, each scored from 0 to 4, where 0 indicates no terminal hair growth and 4 indicating full male pattern terminal hair growth[46]. In most populations, an mFG score of ≥ 8 is indicative of hirsutism[47]. In populations of Asian women, the overall density of facial and body hair growth is lower and therefore hirsutism is confirmed when the score is ≥ 3[47].

1.5.2 Ovulatory dysfunction

Ovarian dysfunction usually manifests as oligomenorrhoea or amenorrhoea resulting from chronic oligo-ovulation or anovulation[48] and is very common amongst women with PCOS[20]. Oligomenorrhoea is defined as less than eight cycles per year, or cycles that are less than 21 days or greater than 35 days in length. Amenorrhoea is the absence of menstruation lasting for more than three months without pregnancy[49]. However, regular cycles do not exclude ovulatory dysfunction as it does not denote whether ovulation occurred. Progesterone concentrations during the luteal phase of the menstrual cycle is required to determine ovulatory dysfunction[49]. PCOS is the most common cause of anovulatory dysfunction, accounting for 90-95% of women attending infertility clinics with anovulation[20]. Obesity independently exacerbates infertility and induces a greater risk of miscarriage[48]. A cross-sectional study of Australian women reported that, independent of BMI, infertility was 15-fold higher in women with PCOS compared to women without, with infertility reported in 72% of women with PCOS and only 16% of women without PCOS[50].

Ovulatory dysfunction in PCOS can be attributed to the impaired follicular development due to the decreased levels of FSH[51]. There is also a growing body of literature to suggest that Anti-Müllerian hormone (AMH) may be elevated in women with PCOS[52-55]. AMH is produced predominantly in the ovarian granulosa cells of pre-antral and antral follicles and has been proposed as a marker of ovarian dysfunction by diminishing follicular sensitivity to FSH and inhibiting follicle recruitment and growth[56]. AMH has also been proposed as a potential alternative diagnostic marker of polycystic ovaries[55], however, the recent international evidence-based guidelines state that AMH levels should not yet be used as an alternative for the detection of polycystic ovaries[32].

1.5.3 Polycystic ovary morphology

The definition for PCOM has been controversial due to the advancements in technology, including high-frequency and image enhancing software which have significantly enhanced the measurement capabilities. Currently, the international evidence-based guidelines for the assessment and management of PCOS have stated that, using an endovaginal transducer with a frequency bandwidth of 8MHz, the threshold for PCOM on either ovary, a follicle number per ovary of ≥20 and/or an ovarian volume of ≥10 mL[32]. This number is increased from the initial Rotterdam criteria which requires ≥12 follicles per

ovary[13] due to advancements in technology. They also state that ultrasounds should not be used in individuals who are <8 years post menarche due to the high incidence of multi-follicular ovaries at that stage of life. As stated earlier, there is emerging evidence that AMH may be used to detect PCOM, however, at this stage AMH levels are not considered and should not be used due to lack of reliable assays and lack of standardisation of cut-off levels[32].

1.6 Metabolic features

1.6.1 Insulin resistance

IR is typically defined as a decreased sensitivity or responsiveness to metabolic actions of insulin, such as insulin-mediated glucose disposal and inhibition of hepatic glucose production[57]. IR, as with many other features of the condition, can manifest differently amongst women with PCOS[58]. Despite the heterogeneity, the majority of women with PCOS have been reported to be IR[59]. IR and compensatory hyperinsulinemia are present in a large proportion of both overweight and lean women with PCOS and is now considered to have a central aetiological role[60], contributing to both the metabolic and reproductive features of the condition. Hyperinsulinemia acts on ovarian tissue, disturbing ovarian hormone regulation and menstrual cycles, resulting in fertility issues[61,62] and contribute directly to hyperandrogenism through augmenting androgen production and increasing free androgens by reducing SHBG[59,63-66]. Interestingly, it is postulated that women with PCOS have an intrinsic IR which is mechanistically distinct from obesity-associated IR[60]. Obesity-related or extrinsic IR further exacerbates the underlying intrinsic IR and thereby increases the risk of metabolic disorders[3,67].

Insulin sensitivity can be assessed using a range of methods, however, the gold standard is the euglycaemic-hyperinsulinaemic clamp. This method directly measures whole body glucose disposal at a given level of insulinaemia under steady-state conditions[57]. However, this method is not typically used in clinical practice as it is time and labour intensive and more expensive than methods such as the oral glucose tolerance test (OGTT) and other methods that are based on fasting insulin and glucose levels such as homeostatic model assessment of insulin resistance (HOMA-IR). These methods however are imprecise and unable to accurately classify all cases of insulin resistance[4]. A retrospective analysis from 375 women with PCOS found that when using the M-clamp value derived from the euglycaemic-hyperinsulinaemic clamp method, insulin resistance was detected in 74.9% of women[4]. The M-clamp value is calculated as the glucose infusion rate (mg/min) expressed per body surface area, per body mass or per fat-free mass. Due to muscle being responsible for most insulin-induced glucose metabolism, glucose infusion rate is most commonly expressed per fat-free mass (mg/FFMkg/min)[68]. They also highlighted the imprecision of surrogate indices when used to classify women with PCOS as insulin resistant. When using HOMA-IR, which is perhaps the most commonly used index in PCOS research, insulin resistance was only detected in 41.1% of women in comparison to the M-clamp[4]. A much higher prevalence of IR has also been reported in overweight and obese women, with IR being detected in 59.3% of normal-weight women, 77.5% in overweight women and 93.9% in obese women[4].

1.6.2 Obesity

Obesity is closely linked to the development of PCOS and its clinical features[65,69,70]. Excess body weight worsens the underlying hormonal disturbances, increasing insulin and androgen levels and worsening the clinical features of PCOS[64]. Women with PCOS have an increased prevalence of obesity with the majority of literature reporting estimates of between 40-60% of women with PCOS being overweight or obese depending on the population group studied[71-73]. Obesity is associated with an increased likelihood of metabolic dysfunctions with the effect of obesity having an independent yet additive effect on the symptoms of PCOS[74], substantially worsening IR and increasing the risk of developing impaired glucose tolerance (IGT), T2DM and cardiovascular risk factors[75,76]. Furthermore, PCOS often manifests following weight gain, suggesting that the current obesity epidemic may cause an increased prevalence of PCOS in the future[73,77]. Additionally, as obesity exacerbates PCOS, the condition is becoming more prevalent and severe as the population gains weight[78].

1.6.3 Metabolic health consequences

PCOS carries a major health burden with substantial metabolic health consequences. Reflecting their high metabolic risk, women with PCOS have higher rates of IGT, metabolic syndrome and T2DM[75,76,79], and a more rapid progression from normal glucose function to IGT and T2DM compared with age- and weight-matched women without PCOS[80,81]. PCOS has been identified as a significant non-modifiable risk factor associated with T2DM by the International Diabetes Federation[82]. A meta-analysis of 13 studies, reported that women with PCOS are four times more likely to develop T2DM than women without PCOS[3]. A large, prospective cross-sectional analysis of reproductive-aged Australian women reported prevalence rates of T2DM of 7.5% among women with PCOS compared to only 1.5% in age- and weight-matched women without PCOS[83]. This is an 8.8-fold increased risk of T2DM among Australian women. In addition, they reported the prevalence of gestational diabetes to be 11.2% in women with PCOS versus 3.8% in women without PCOS; a ~3-fold increased risk of gestational diabetes in women with PCOS[83].

1.7 Mental health and health-related quality of life

Depression and anxiety are the second leading causes of global disease burden[84], with women being almost twice as likely as men to meet the criteria for major depressive disorder[85]. The increased prevalence of depression correlates with hormonal changes in women, including during puberty, prior to menstruation, after child birth and at perimenopause, suggesting that hormonal fluctuations may be a trigger for depression[86]. However, the underlying mechanisms remain unclear, limiting the ability to make treatment specific to women. Adding to this, women with PCOS have an additional increased prevalence of both anxiety and depression, with the effects observed across the lifespan[87-89].

Many chronic illnesses are associated with a reduced quality of life and mental health impacts[89]. In women with PCOS, fears regarding infertility, poor body image, low self-esteem and coping with the

condition may all contribute to poorer mental health and a decreased health-related quality of life[89,90]. Compared to matched control women, and those with other chronic conditions, women with PCOS report increased symptoms of depression and anxiety[20,87,89-91]. The International evidence-based guidelines for the assessment and management of PCOS recommend that all women with PCOS are routinely screened for depression and anxiety and other aspects of emotional wellbeing by a suitably qualified health professional[32]. The prevalence of depression rates among women with PCOS vary widely according to the population group studied and also vary according to mild, moderate and severe forms of depression[5,92].

A comprehensive meta-analysis on mental health outcomes in women with PCOS, reported that women with PCOS are four times more likely to suffer from moderate to severe symptoms of depression and reported a median pooled prevalence of depression from 18 studies to be 36.6% in women with PCOS and 14.2% in the control group[5]. The same meta-analysis also reported symptoms of anxiety, finding that women with PCOS are six times more likely to suffer from severe symptoms of anxiety compared to women without PCOS and reported a median pooled prevalence of 41.9% in women with PCOS compared to 8.5% in the control group[5]. A large population-based retrospective study of the Australian women reported that women with PCOS had more hospitalisations for issues relating to mental health than women without PCOS[93]. They reported 14% of women with PCOS were hospitalised for anxiety in comparison to 5.9% for control women. In regards to depression, 9.8% of women with PCOS were hospitalised compared to 4.3% of control women[93]. In the general population, particularly in women, obesity has been associated with an increased risk of depression[94,95]. A meta-analysis exploring the effects of BMI on anxiety and depression among women with PCOS reported that BMI had only a small effect, suggesting that there may be some impact of weight on these symptoms, although it is more likely to be due to PCOS status[96].

An individual's physical, psychological, and social wellbeing is amalgamated into the concept of HRQoL, and is influenced by an individuals' perception of their health[97,98]. HRQoL is measured by both generic questionnaires (e.g. SF-36) and measures that are specific to certain medical conditions. In 1998, the first PCOS specific HRQoL questionnaire was developed[99]. This tool was developed to assess the impact of PCOS on emotional and physical health and included five domains; emotions, body hair, weight, infertility and menstrual problems. Measuring health-related quality of life can have an important role in chronic disease[100], evaluating health care or interventions from a patient's perspective[101]. This is particularly important in clinical trials where health status tools can assist in clinical decision making regarding treatment choice[102].

Previous research has suggested that PCOS and its associated symptoms vastly contribute to an overall diminished HRQoL[102]. A study utilising results from the Oxford Health and Lifestyle Survey reported that PCOS had a greater impact on psychological wellbeing compared to other chronic conditions

including asthma, epilepsy, diabetes, back pain, arthritis and coronary heart disease[103]. In regards to physical health, the impact of PCOS was similar or milder than these conditions. This is not surprising as the symptoms of PCOS are often related to a deterioration in self-esteem and self-image, resulting in a diminished psychological wellbeing[24,104]. In comparison to women without PCOS, women with PCOS consistently report poorer HRQoL[102,103,105]. Despite the evidence for an increased prevalence of poorer mental health and wellbeing in women with PCOS, the reason for this association is unclear, and information regarding prevention and treatment strategies, and the success of such interventions, are limited.

1.8 Exercise

Considering the known health consequences of PCOS, it is important to consider the factors that may aid in preventing or managing the symptoms. Lifestyle interventions which include exercise are considered the first-line therapy for treatment of symptoms and for reducing the risk of developing co-morbidities[32]. Exercise is defined as planned, structured and repetitive physical activity with the aim to maintain or improve components of physical fitness (e.g. being active with a purposeful intention including going to the gym/for a run and playing a sport)[106]. This differs from physical activity which is defined as any bodily movement produced by skeletal muscles that requires energy expenditure and includes informal, unstructured activities such as walking to the train, going shopping, walking a dog, and climbing stairs, as well as planned exercise[106]. This thesis will focus on the specific effects of prescribed exercise interventions, however, it is important to note that both physical activity and exercise provide substantial health benefits, with physical inactivity having significant implications for poor physical and mental health. The World Health Organization reports that physical inactivity is the fourth leading global risk for mortality in the world and increases the risk of developing cancer, T2DM and cardiovascular diseases[107]. Being physically active and engaging in regular exercise is a potent tool to reduce the risks for developing metabolic and cardiovascular diseases[108].

In addition to the benefits of exercise and physical activity from a public health perspective, exercise is also used as an effective treatment strategy for a wide range of medical conditions. Diverse evidence from all areas of health affirm the concept of regular exercise as medicine[109]. Exercise is well-established as a therapy for preventing and managing risk factors associated with chronic disease[109,110]. Among other established benefits, exercise enhances cardiorespiratory fitness: a well-established marker of all-cause mortality[111,112]. Both men and woman who report meeting the physical activity guidelines, have large reductions in relative risk of death (approximately 20-35%)[113]. Therefore, increasing participation in regular exercise and physical activity is vital from a public health perspective.

1.8.1 Exercise intensity

Exercise interventions and prescription are commonly varied in regards to the dose, type and intensity. In this thesis, we focus on aerobic exercise and will therefore be the type of exercise discussed

throughout. The two most common types of aerobic interventions utilised are traditional moderate-intensity training and HIIT. Moderate-intensity exercise is typically undertaken at a 55-70% of maximum heart rate (HR_{max}) or 40-60% of VO_{2max} and performed continuously for 30-60 minutes[114]. HIIT is broadly referred to as repeated, short bouts of vigorous exercise interspersed with periods of rest or low-intensity exercise as active recovery[115]. In order to be classified as high-intensity exercise, an intensity of ≥90% HR_{max} or ≥VO_{2max} needs to be reached during the exercise bout[114]. The target heart rate for HIIT is dependant in the duration of the interval prescribed. Two commonly used HIIT protocols are 4 minute intervals and 1 minute intervals (Figure 3). The longer 4 minute protocol typically has a target heart rate of 90-95% of HR_{max} and consists of longer rest or active recovery periods, usually 2 minutes[115]. The shorter 1 minute intervals usually have a target heart rate of ~100%HR_{max} and are interspersed with 1 minute of rest or active recovery. When designing or using HIIT protocols, it is important to consider the fidelity of interventions in order to determine the validity of such protocols. Evaluations of fidelity in exercise interventions should address both session attendance and compliance as these factors combine equal the dose of the intervention and impact the response to exercise[116].

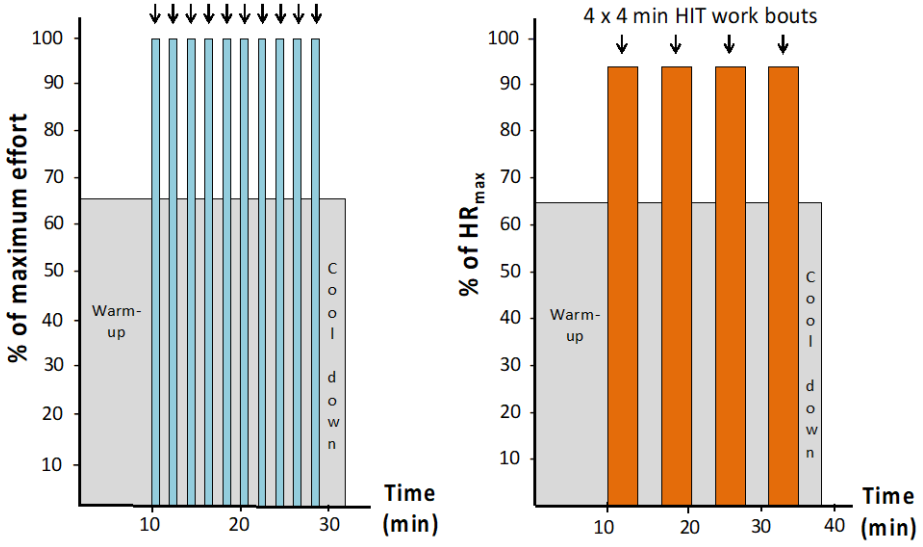

Figure 3. Low-volume HIIT consisting of 10 x 1 min work bouts at maximal intensity (~100%HRmax) separated by 1 min of rest or low-intensity exercise (left) and high-volume HIIT consisting of 4 x 4 min work bouts reaching 90-95% HRmax, separated by 2 min of rest or low-intensity exercise (right).

1.8.2 High-intensity interval training

Recently, the recognition that exercise intensity is important for rapid health benefits has emerged[117-123], offering exciting potential to optimise treatment for and prevention of a range of health concerns. HIIT it is a popular, enjoyable, and time-efficient approach to fitness[117-121,123-126] and has been proven safe and feasible amongst clinical populations including PCOS[118,121,124,126,127]. Superior reductions in IR, cardiovascular risk factors and all-cause mortality have been observed with increasing exercise intensity

in populations with lifestyle induced chronic diseases[128-130]. These improvements are particularly important given that poor cardiorespiratory fitness is associated with all-cause mortality[111] and an increased risk of developing metabolic syndrome and T2DM[131].

HIIT has been found to confer greater health benefits compared to MICT in both healthy[118,132] and clinical populations, such as improved cardiorespiratory fitness and insulin sensitivity[118,119,121,133,134]. This is important given that time limitation is often the most cited barrier to exercise participation[135-137]. Utilising HIIT may be an effective strategy to reduce the time required while simultaneously gaining greater health benefits. In addition to the time limitation barrier, lack of enjoyment is also listed as a common barrier to exercise and is often cited as a reason to cease exercise participation[135]. Despite greater exertion, HIIT has been found to be equally or more enjoyable and may be relevant for improving adherence to exercise in healthy populations[138,139] and among other chronic conditions[134,140]. Despite the many benefits of HIIT, it is unknown whether exercise interventions of various intensities result in greater health benefits in women with PCOS with current research limited to low quality, under powered studies. Due to the limited research exploring the effects of HIIT in women with PCOS, it is unclear whether standard moderate intensity continuous exercise or vigorous- to high-intensity exercise training is more beneficial for improving the clinical features of PCOS. Furthermore, there have been no studies that have assessed the impact of HIIT on mental health and HRQoL.

1.9 Exercise in PCOS

These benefits of exercise also exist for women PCOS, with the beneficial effects being noted in a number of reviews[141-143]. In 2011, the first international evidence-based guidelines for the assessment and management of PCOS was published[2]. These guidelines acknowledge exercise as a first-line therapy, recommending 150 min/week of moderate intensity exercise or 75 minutes of vigorous intensity exercise to improve clinical outcomes. These exercise guidelines are generic, and identical to the guidelines for the general population, rather than being specific for women with PCOS[144]. Furthermore, the guidelines provided are based on one high quality systematic review which appraised 6 randomised controlled trials that primarily investigated the effectiveness of walking or cycling prescribed 3-5 times per week for 12-24 weeks[2,145]. It has been acknowledged that this evidence base is largely preliminary and that more high-quality research is needed. Despite the limitations, the interventions have elicited a number of clinically relevant adaptations, including increased insulin sensitivity, cardiorespiratory fitness, menstrual cyclicity and ovulation, body composition, hormonal profiles and improved mental health[77,145-147] (Figure 4). According to these guidelines, vigorous and moderate exercise are treated as interchangeable options, however, data supporting the equivalence of these options for promoting health benefits are lacking[117]. These guidelines were updated in 2018, however, the exercise recommendations remain the same, the limitations still exist, and the importance of exercise intensity is yet be addressed[32].

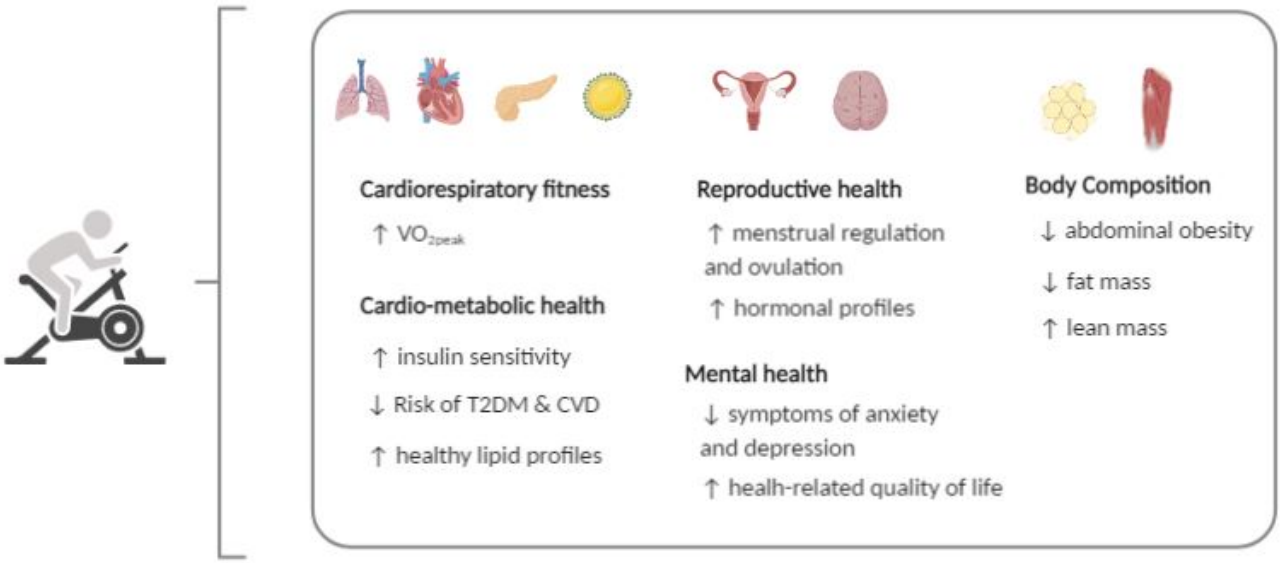

Figure 4. Summary of the benefits of exercise on the clinical outcomes for women with PCOS.

VO_{2peak} – peak oxygen consumption, T2DM – type 2 diabetes mellitus, CVD – cardiovascular disease. Created with BioRender.com.

1.9.1 Exercise and metabolic health

Considering the beneficial effects of exercise on other insulin resistant populations[148], it is not surprising that incorporating exercise as a treatment for the metabolic manifestations of PCOS is effective[149]. Previous non-PCOS studies in obesity and T2DM have demonstrated improved IR with greater insulin stimulated glucose uptake and reduced insulin secretion after aerobic exercise[150]. Moderate physical activity, at least three to five times per week, has consistently been shown to reduce the development of T2DM in high-risk groups[151]. In line with this, significant improvements in insulin sensitivity are commonly observed as a result of exercise interventions in women with PCOS[149,152]. A previous systematic review exploring the effect of exercise in women with PCOS reported a significant improvement in insulin sensitivity in 5 out of the 8 included studies with improvements ranging from 9-30%[149]. The large variation in the observed improvements is likely due to the use of surrogate indices to measure insulin sensitivity (e.g. HOMA-IR, fasting glucose, fasting insulin). Studies that have utilised the gold standard euglycaemic-hyperinsulinaemic clamp often report larger increases in insulin sensitivity[79,153,154], however, this method is costly and difficult to undertake in a clinical setting. Studies using alternative methods (e.g. HOMA-IR) have also found significant improvements in insulin sensitivity following an exercise intervention[155-158].

Current reviews and meta-analyses have also been unable to analyse the specific effects of exercise characteristics (intensity, dose, type) on insulin sensitivity due to the large variety and poor reporting of the exercise interventions used[142,143]. We address these issues in a meta-analysis conducted by our group (Chapter 2). For a summary of all relevant exercise interventions that explore the effects on cardio-metabolic health, cardiorespiratory fitness and body composition see Table 3 in Chapter 2.

1.9.2 Exercise and reproductive health

Surprisingly, given the vast amount of research exploring the effects of exercise on metabolic health in women with PCOS, there are limited studies investigating the effects of exercise on reproductive outcomes. Improvements in hormonal profiles and insulin sensitivity play important roles for improved reproduction function[149]. Regular, moderate intensity aerobic exercise has been found to improve reproductive outcomes including menstrual cycle regulation and ovulation in young, overweight women with PCOS[149]. Overall, exercise studies have shown improvements in menstrual cyclicity and/or ovulation rates in ~50% of women with PCOS[149]. In some studies, exercise has been found to improve FAI, total and free testosterone, and/or SHBG[155,159,160], however, no changes have been reported in other studies[156,161,162]. Measures such as testosterone, FSH and LH vary with the stage of the menstrual cycle, therefore requiring caution when interpreting results[163]. A previous systematic review and meta-analysis found that lifestyle (diet and exercise) intervention and exercise-alone improved hormonal outcomes in women with PCOS with limited additional benefit from diet[163]. Their analysis suggested that lifestyle intervention improves the levels of FSH, SHBG, total testosterone, androstenedione and FAI, and mFG score in women with PCOS, while exercise alone improved all of the outcomes other than LH and FAI.

Menstrual cyclicity has also been reported as a result of aerobic exercise interventions[155], even without changes in hormonal parameters[164,165]. Recent reviews exploring the effect of exercise on ovulation, menstrual cyclicity and pregnancy outcomes in women with PCOS, have been unable to meta-analyse the effects due to limited and poor quality data. A recent semi-quantitative analysis that included dietary and exercise intervention studies, reported that exercise is likely to result in improved menstrual regulation, ovulation rates and pregnancy rates in women with PCOS[142]. Several studies included in this review included a dietary intervention. Additionally, heterogeneous results have been reported for the effect of weight loss on reproductive function despite similar decreases in weight[166]. Excess body mass has been found to correlate with an increased rate of cycle disturbances[167,168], delayed time to conception and adversely affects the response to fertility treatments[169,170]. Studies have shown that a weight loss of as little as 5% is associated with improved spontaneous ovulation rates in overweight, infertile women[171-173]. Lifestyle modifications including caloric restriction and regular exercise is currently considered as the first-line treatment for overweight women with PCOS who are looking to conceive[174].

Although within the existing research, there is a wide array of improvements, there are no consistent findings, making it difficult to conclusively determine the effectiveness of exercise and exercise

characteristics that aid in promoting benefits in reproductive health. Evaluation of exercise interventions is even more challenging due to the use of different diagnostic criteria (and phenotypes) included within research, but also due to the poor reporting of the characteristics of exercise interventions (e.g. intensity and dose). Furthermore, at this stage it is unknown whether a particular type or intensity of exercise is more effective for inducing improvements in hyperandrogenism and reproductive function. Future high-quality research is required to tease out the effects of exercise, and to determine the optimal exercise characteristics in order to promote improvements in reproductive health. For a summary of all relevant exercise interventions that explore the effects of exercise on reproductive outcomes see Table 3 in Chapter 2.

1.9.3 Exercise and mental health

In a healthy population, physical activity is an effective means of managing and improving mental health[175]. This is also the case for populations with chronic conditions[176] and in overweight women[177]. It is therefore reasonable to hypothesise that physical activity is an effective method of improving the mental health of women with PCOS. It has been shown that women with PCOS that are more physically active report fewer symptoms of depression than inactive women with PCOS[178], although active women with PCOS still report higher scores on depression scales than active women without PCOS[89]. There is preliminary evidence to suggest that exercise results in positive mental health outcomes in women with PCOS[147]. However, there is little evidence available to demonstrate the effect of exercise alone on the mental health and psychological wellbeing of women with PCOS[77]. Additionally, despite the rationale for exercise improving mental health in PCOS, there is limited evidence documenting the specific effects on mental health and health-related quality of life[147].

The specific interaction between physical activity and mental health has not been explored in depth in PCOS but existing research clearly indicates that women with PCOS report higher levels of depression and anxiety and lower levels of HRQoL[147]. The optimisation of physical activity is especially relevant in the treatment of PCOS as it is vital to enhancing wellbeing and promote engagement and long-term adherence[89]. In a systematic review, we summarise the current literature, providing some support of the benefits of exercise and ultimate state that future high-quality trials with well-reported interventions and outcomes are required to adequately assess the impact of exercise on mental health and wellbeing (Chapter 3).

1.9.4 Limitations with current exercise recommendations

Although there is evidence in support of the benefits of exercise for improving a wide range of health concerns among women with PCOS, the current lifestyle strategies tend to fall short. They have high attrition rates due to general (time limitations, low enjoyment) and PCOS-specific barriers (low confidence, physical limitations), and fail to normalise IR and hyperandrogenism[89,147]. Existing research states the need for better designed studies, to compare different intensities and other exercise

characteristics in women with PCOS in order to improve mental and physical health and to ultimately inform clinical recommendations regarding exercise prescription for women with PCOS[147]. For a truly effective treatment, future interventions must therefore better address parallel needs (e.g. low time investment, confidence, motivation and enjoyment) to increase efficacy, improve participation and sustainability, and reduce attrition in this high-risk group. Future research should also endeavour to establish the necessary exercise dose, type, intensity and frequency required for treating and managing PCOS and its associated comorbidities[77].

1.10 Aims

The overarching aim of this thesis is to examine the effects of exercise training, and more specifically exercise intensity, on a comprehensive range of cardio-metabolic, reproductive and mental health outcomes in women with PCOS.

Aim 1: To conduct a systematic review and meta-analysis to synthesise and analyse the current evidence in regards to the effect of exercise interventions on cardio-metabolic and reproductive health outcomes in PCOS (Chapter 2).

Aim 2: To conduct a systematic review to summarise and evaluate the current evidence in regards to the effect of exercise interventions on health-related quality of life and mental health outcomes in women with PCOS (Chapter 3).

Aim 3: To determine whether high-intensity interval training is more effective than standard continuous moderate-intensity exercise training in overweight women with PCOS on:

i) Cardio-metabolic health outcomes (Chapter 5)

ii) Reproductive health outcomes (Chapter 6)

iii) Mental health and health-related quality of life (Chapter 7)

Chapter 2.0 Exercise interventions in polycystic ovary syndrome: a systematic review and meta-analysis

This paper has been published in Frontiers in Physiology (Appendix A), and is attached at the end of the document. An updated literature search was conducted and is reported below (page 40).

2.1 Abstract

Background: Polycystic ovary syndrome (PCOS) is a common and complex endocrinopathy with reproductive and metabolic manifestations. Exercise training has consistently been found to result in improved clinical outcomes in women with PCOS, but shortfalls with exercise prescription are evident. The aim of this systematic review and meta-analysis was to identify exercise intervention characteristics that provide favourable outcomes in women with PCOS.

Methods: A systematic review of published literature was conducted using EBSCOhost and Ovid Medline up to May 2019. The review adheres to the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines as per our PROSPERO protocol (CRD42018088367). Randomised controlled trials, non-randomised controlled trials and uncontrolled trials that evaluated an exercise intervention of at least moderate intensity in women with PCOS were included. Meta-analyses were performed using general linear mixed modelling and Bayesian inferences about effect magnitudes**.**

Results: Thirty-three articles were identified for systematic review of which 19 were meta-analysed. The meta-analysis found that improvements in health outcomes are more dependent on exercise intensity rather than dose. Fixed effects analysis reported a moderate increase in VO_{2peak} (24.2%; 90%CL, 18.5 to 30.1), and small reductions in HOMA-IR (-36.2%; 90%CL, -55.3 to -9.0) and waist circumference (-4.2%; 90%CL -6.0 to -2.3) as a result of vigorous intensity exercise. These results are confirmed in the predicted analysis which reported the greatest improvements in VO_{2peak}, BMI, and waist circumference after vigorous intensity exercise alone or when combined with diet, particularly for women with clinically adverse baseline values.

Conclusions: Exercise training in the management of PCOS is becoming more common. Results from our analysis support the use of exercise and suggest that vigorous intensity exercise has the greatest impact on cardiorespiratory fitness, body composition and insulin resistance. Our results indicate that, a minimum of 120 minutes of vigorous intensity per week is needed to provide favourable health outcomes for women with PCOS.

2.2 Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine conditions, affecting 8-13% of reproductive aged women[32]. PCOS is complex with diverse features including reproductive, metabolic and mental health complications. PCOS is diagnosed via the internationally endorsed Rotterdam criteria, which require the presence of two or more features including clinical or biochemical signs of hyperandrogenism, oligo- or anovulation, and polycystic ovaries on ultrasound, with the exclusion of other aetiologies[13]. Although not currently recognised in the diagnostic criteria, insulin resistance is a key aetiological factor contributing to the severity of reproductive and metabolic features[179,180], with obesity known to exacerbate the severity of clinical symptoms[181]. Consequently, women with PCOS are at a two to eight times greater risk of developing impaired glucose tolerance and type 2 diabetes mellitus compared to women without PCOS[3].

Exercise is well established as a therapy for preventing and managing chronic diseases in the general population[182,183], and in women with PCOS[32,141,184]. The beneficial effects of exercise in women with PCOS have been summarised in several recent systematic reviews and meta-analyses[142,143]. In addition, the international evidence-based guidelines for the management of PCOS recommend lifestyle intervention, including exercise training and diet, as the first line of therapy to improve general health, hormonal outcomes and quality of life[32]. However, the studies utilised in the development of the guidelines were limited to a small number of randomised controlled trials (RCTs), resulting in a general consensus recommendation of exercise rather than a clear exercise prescription for the management of PCOS[32,141]. In particular, there is uncertainty about suitable intensity, duration and modality of exercise and the interaction between exercise and diet. We have addressed this uncertainty by meta-analysing the effects of exercise characteristics on key clinical markers in women with PCOS, with the aim of assisting clinicians with exercise prescription and guiding future research in women with PCOS.

2.3 Methods

2.3.1 Protocol and registration

This systematic review and meta-analysis was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) and was registered on the International prospective register for systematic reviews (PROSPERO) CRD42018088367.

2.3.2 Search strategy, study selection & data extraction

We performed a systematic search of the literature in May of 2018 using EBSCOhost (MEDLINE, SPORTDiscus, PsycINFO, CINAHL) and Ovid Medline. The search was limited to peer reviewed, published, English language articles from 1980-current. The search terms were modified when required for each database and are reported in Supplementary Table 1. The reference lists of other review articles were searched to identify other potential eligible studies. After removal of duplicates, two reviewers (RB and RP) independently screened articles by title and abstract. Subsequently, the same reviewers

independently completed full-text screening. Any discrepancies were resolved by consensus or by consultation with a third reviewer (NS). After full-text screening, data extraction of eligible studies was performed independently by RB and RP using a pre-determined extraction form.

Where required, authors were contacted via email using an institutional email address in order to obtain additional or raw data. Following a second email, if no response was received within 14 days, the article was excluded from the meta-analysis. Where multiple publications resulted from the same trial, results were combined and only one result (largest participant number) for each outcome was used in the analysis.

2.3.3 Eligibility criteria

The Participant, Intervention, Comparison, Outcomes and Studies (PICOS) framework was used for this systematic review (Table 2). Briefly, included studies involved women aged 18-45 (pre-menopausal) and with a diagnosis of PCOS via any established diagnostic criteria. The interventions included RCTs, non-randomised controlled trials and uncontrolled trials that had a pre-post design and reported outcomes of an exercise training intervention greater than two weeks in duration, and of moderate intensity or greater. Exercise intensity was categorised according to Norton et al.[114], classified as moderate (55 to <70% HR_{max} or 40 to < 60% VO_{2max}), vigorous (70 to <90% HR_{max} or 60 to <85% VO_{2max}) or high (≥90% HR_{max} or ≥85% VO_{2max}) intensity. Two weeks was used as a minimum intervention duration in order to capture the effects of exercise training. We accepted exercise interventions that included aerobic exercise, resistance training or a combination. Exercise interventions that were combined with a drug therapy that may affect the outcomes measures were excluded. Exercise interventions that were combined with a dietary intervention or dietary advice were included. Comparison groups consisted of a no exercise control group or a diet only group. The primary outcomes specified for the meta-analysis were peak oxygen consumption (VO_{2peak}) used to measure cardiorespiratory fitness, homeostatic model assessment of insulin resistance (HOMA-IR) to measure insulin resistance, free androgen index (FAI) to measure androgens and body mass index (BMI) and waist circumference to assess weight related outcomes. Secondary outcomes were those that were included in the systematic review only and consist of additional reproductive, cardio-metabolic or anthropometric outcomes (Table 2).

Table 2. Eligibility criteria for study inclusion

Inclusion Criteria					
Participant	**Intervention**	**Comparator**	**Outcome**	**Study design**	**Limits**
Diagnosed with PCOS using any established definition Premenopausal women aged 18-45. Any weight category	Any exercise intervention that is: Supervised or unsupervised Greater than 2 weeks in duration Moderate intensity (>55% HR_{max} or >40% VO_{2peak}) or above	No exercise control group Diet only group	Cardiorespiratory Fitness – VO_{2peak} Metabolic – measures of insulin sensitivity, lipids Body composition – weight, BMI, W/H ratio, waist circumference Reproductive – menstrual regularities, hormonal markers	RCT Clinical trial Non-RCT Pilot Feasibility Parallel	English language Human trials Peer reviewed

HR_{max} – Maximal Heart Rate, VO_{2peak} – Peak Oxygen Consumption, BMI – Body Mass Index, W/H Ratio – Waist to Hip Ratio, RCT – Randomised Controlled Trial

2.3.4 Assessment of risk of bias in included studies

The Downs and Black Checklist for the Assessment of Methodological Quality[185] was used to evaluate the included randomised and non-randomised studies. Two reviewers (RB and RP) independently assessed the methodological quality and disagreements were resolved by consensus. Questions regarding blinding of participants were removed from the checklist (Supplementary Table 2). As per our previous meta-analysis[186] publication bias was assessed by examining a scatter plot of t-statistic associated with each study estimate value contributing to the study-estimate random effect versus log of the standard error of the effect. No outliers or publication bias was identified using this approach.

2.3.5 Data analysis

The meta-analyses were performed with the general linear mixed-model procedure in the Statistical Analysis System (Version 9.4, SAS Institute, Cary, NC, USA). The fixed effects in the model were used to estimate the main effects of exercise and its modifiers. A nominal variable represented type of exercise (moderate, vigorous, resistance, control) and a linear numeric variable represented total dose of any exercise (in hours). A nominal variable with two levels (exercise, control) was interacted with the baseline value of the dependant variable and with a dummy variable representing a dietary co-intervention (described as either a structured dietary plan, dietary advice or guidance) to estimate respectively the modifying effects of baseline and of diet in exercise and control groups. The linear numeric effect of baseline HOMA-IR produced unrealistic predictions at low values of this dependant variable, so the numeric variable was replaced by a nominal variable with three levels defined by the following: low, <2.1; moderate, 2.1-3.4; high, >3.4.

A random effect representing the identity of each study estimate was included to allow for differences in the means of study estimates not accounted for by the fixed effects, and an additional random effect representing study identity accounted for study clusters of estimates (control and/or one or more experimental estimates). In the mixed-model, each study estimate was weighted by the inverse of the square of its standard error (SE), and the random effects were estimated by setting the residual variance to unity[187]. The standard error of each estimate was either derived as the standard deviation (SD) of change scores divided by the square root of the sample size or was computed with a spreadsheet from either the exact p value or compatibility intervals for the mean change. For estimates where the standard error could not be derived from the data, it was imputed from the error of measurement averaged over other similar estimates (Supplementary Table 3). The number of imputed standard errors represented 0.1-3% of the total number, depending on the meta-analysed measure.

All study-estimates, standard errors and baseline between-subject SDs were converted to factor effects by dividing by the group mean and then log-transformed for the meta-analysis. The qualitative magnitudes of meta-analysed mean effects were evaluated via standardisation using magnitude thresholds provided by an appropriate baseline between-subject SD[188]. This SD was derived by combining (via variances) the mean of the study SDs with the between-study SD of the study baseline means, to represent the SD of women drawn randomly from a population of sub-populations. The threshold for the smallest important effect (0.2 SD) was rounded down towards the mean of the study SD to allow for differences in study means to be due partly to differences in assay technique; the thresholds for small, moderate, large, very large and extremely large were 1, 3, 6, 10 and 20 times the smallest important threshold, respectively (corresponding to 0.2, 0.6, 1.2, 2.0 and 4.0 times the SD, Supplementary Table 4)[186]. These thresholds were also used to evaluate the magnitude of the numeric linear modifying effects of baseline and training duration, by multiplying the beta-coefficients (slopes) in the model by two between-subject SD and between-study SD, respectively[188]. The thresholds were halved for evaluation of the magnitude of the random-effect SDs[189]. The meta-analysis also provided predicted population and individual-setting effects of various combinations of exercise, diet and baseline values for the dependent variable. The predicted effects for individual settings had the same value as the predicted population mean effects, but their compatibility intervals were wider, owing to the contribution of the between- and within-study random effects.

Uncertainty in the estimates of effects is presented as 90% compatibility limits. Probabilistic decisions about true (large-sample) magnitudes accounting for the uncertainty were based on one-sided hypothesis tests of substantial magnitudes[190]. The p value for rejecting a hypothesis of a given magnitude was the area of the sampling t distribution of the effect statistic with values of that magnitude. For effect modifiers, random-effect SDs, and predicted population mean effects, hypotheses of substantial decrease and increase were rejected if their respective p values were less than 0.05. For predicted effects in individual settings, hypotheses of harm and benefit were rejected if the respective

p-values were less than 0.005 and 0.25. If one hypothesis was rejected, the p value for the other hypothesis was interpreted as evidence *for* that hypothesis, since the p value corresponds to the posterior probability of the magnitude of the true effect in a reference Bayesian analysis with a minimally informative prior[191,192]. The p value is reported qualitatively using the following scale: 0.25-0.75, possibly; 0.75-0.95, likely; 0.95-0.995, very likely; >0.995, most likely[188]. This scale was also used to interpret the posterior probability of a true trivial effect, which is given by the area of the sampling distribution in trivial values. If neither hypothesis was rejected, the magnitude of the effect was considered to be unclear, and the magnitude of the effect is shown without a probabilistic qualifier. To reduce inflation of error arising from the large number of effects investigated, effects were considered decisive with more conservative p-value thresholds ($p<0.01$ for a substantial decrease or increase; $p<0.001$ for harm; $p<0.05$ for benefit) and are formatted bold in tables and figures.

2.4 Results

The combined searches identified 1476 papers for review. Of these, 692 were excluded due to duplication, 457 were removed by title screening and 270 were removed after abstract screening. Fifty-seven papers were reviewed for full-text. Twenty-four of these were excluded due to medication use, lack of data on outcomes of interest and type of intervention (Figure 5). Thirty-three publications were deemed suitable for inclusion in the systematic review however, multiple publications were found for six exercise intervention studies. In that case, articles were combined and only the outcome measure with the largest N was used for meta-analysis. The remaining 20 articles were included in the systematic review with only one being excluded from the meta-analysis due to using a non-parametric analysis[193]. A summary of study and participant characteristics, exercise intervention and study outcomes and results of the methodological quality are presented in Table 3 (for full results see supplementary Table 5).

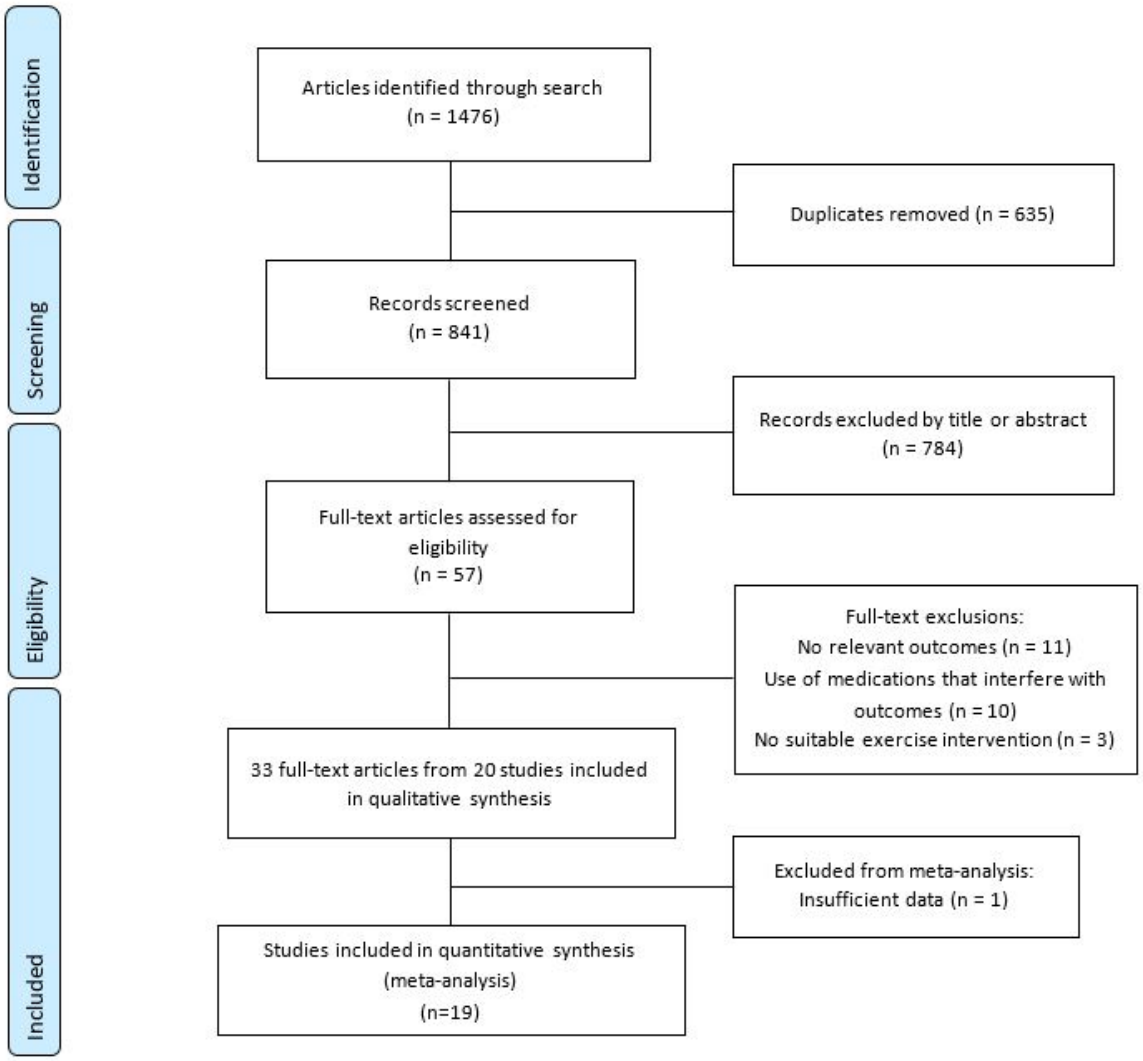

Figure 5. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) study selection flow diagram.

2.4.1 Summary of Articles

Of the 20 trials included in the systematic review, 10 were RCTs[155,156,160,161,164,165,193-196], five non-randomised uncontrolled trials[158,159,197-199], one randomised parallel trial[200] and one non-randomised parallel trial[201], one randomised cross-over trial[202], one single arm trial[203] and one non-randomised controlled trial[162]. The mean age of participants ranged from 22 to 32 years. Baseline BMI ranged from 26.1 to 38.3kg/m^2. Participants from 16 studies were diagnosed according to the Rotterdam criteria[155,157-160,162,164,165,195-197,199-203] and the remaining four studies used the NIH diagnostic criteria[156,161,193,198]. Sample sizes ranged from 5 to 62. Of the 19 reported interventions, 13 had full intervention supervision[156,158,159,162,164,165,193,195-198,201,203], two had partial supervision (at least one supervised session per week)[157,161], three had no supervision[160,199,200] and two did not report on supervision[155,202]. Practitioners that supervised the exercise intervention included exercise physiologists, physiotherapists and physical activity educators.

Table 3. Summary of studies identified for systematic review detailing participants, intervention characteristics and main outcomes measures

Included in meta-analysis										
Study	**Study Design**	**QA score**	**Exercise N (total N)**	**Participant Characteristics**	**PCOS Diagnostic Criteria**	**Exercise Intervention Characteristics**	**CRF Outcomes**	**Cardiometabolic Outcomes**	**Hormonal & Reproductive Outcomes**	**Body Composition Outcomes**
Almenning et al.[157]	RCT	17	HIIT = 8 RT = 8 (25)	Age = 27.2 ± 5.5 RT: BMI = 27.4 ± 6.9 HIIT: BMI = 26.1 ± 6.5	Rotterdam	Type: Aerobic Intervals or RT Frequency: 3/week Intensity: Vigorous-High (70-95% HR_{max}) Duration: 10 weeks Supervision: Partial	HIIT: ↑ VO_{2peak} RT: No change	HIIT: ↓ HOMA-IR, ↓ Fasting Insulin, ↑ HDL RT: No change	HIIT: No change RT: ↓ AMH ↑ SHBG, ↓ FAI	HIIT: ↓ Fat mass, ↓ BF% RT: ↓ BF%, ↑ Fat free mass
Bruner et al.[195]	RCT	10	7 (12)	Age = 32.3 ± 1 BMI = 36.2 ± 2	Rotterdam	Type: Aerobic and RT Frequency: 3/week Intensity: Vigorous (70-85% HR_{max}) Duration: 12 weeks Supervision: Full	↑ VO_{2peak}	↓ Fasting Insulin	No change	↓ WC
Costa et al.[196]	RCT	17	14 (27)	Age = 27.6 ± 4.5 BMI = 25-39.9	Rotterdam	Type: Aerobic Frequency: 3/week Intensity: Moderate-Vigorous (60-85% HR_{max}) Duration: 16 weeks Supervision: Full	↑ VO_{2peak}	No change	Not measured	↓ BMI, ↓ WC
Curi et al.[200]	Randomised parallel trial	11	12 (27)	Age = 26.3 ± 1.4 BMI = 31.8 ± 1.6	Rotterdam	Type: Aerobic Frequency: N/R Intensity: Moderate Duration: 26 weeks Supervision: None	Not Measured	No change	No change	↓ BMI, ↓ WC
Giallauria et al.[158]	Uncontrolled trial	17	62 (124)	Age = 22.8 ± 3.7 BMI = 29.2 ± 2.9	Rotterdam	Type: Aerobic Frequency: 3/week Intensity: Vigorous (60-70% VO_{2peak})	↑ VO_{2peak}	↓ Fasting Insulin, ↓ $AUCi_{ns}$, ↑ AUC_{glu}/AUC_{ins}	↑ SHBG	↓ BMI, ↓ W/H

						Duration: 12 weeks Supervision: Full				
Hutchinson et al.[153] Moran et al.[52] Hutchinson et al.[198] Harrison et al.[79]	Uncontrolled trial	15 13 13 14	13 (21)	Age = 29.75 ± 1.4 BMI = 35.6 ± 5.8	NIH	Type: Aerobic Intervals Frequency: 3/week Intensity: Vigorous (75-100% HR_{max}) Duration: 12 weeks Supervision: Full	↑ VO_{2peak}	↑ GIR, ↓ Fasting Insulin, ↓ HOMA-IR.	↓ AMH No other changes	↓ BMI
Ladson et al.[161]	RCT	17	59 (114)	Age = 28.8 ± 4.6 BMI = 38.3 ± 8	NIH	Type: Aerobic Frequency: ≥2/week Intensity: Moderate Duration: 26 weeks Supervision: Partial	No Change	↑ AUC_{glu}, ↑ HDL	No change	↓ WC
Miranda-Furtado et al.[159] Kogure et al.[204] Kogure et al.[205]	Uncontrolled trial	13 15 14	45 (97)	Age = 28.1 ± 5.4 BMI = 28.4 ± 6	Rotterdam	Type: RT Frequency: 3/week Intensity: % of 1RM Duration: 16 weeks Supervision: Full	Not Measured	No Change	↓ T, ↓ FAI, ↑ SHBG	↓ WC
Moro et al.[206] Redman et al.[207] Covington et al.[154] Covington et al.[197]	Uncontrolled trial	10 10 9 9	8 (16)	Age = 25.6 ± 3.1 BMI = 32.1 ± 5.2	Rotterdam	Type: Aerobic Frequency: 5/week Intensity: Moderate (55% VO_{2peak}) Duration: 16 weeks Supervision: Full	↑ VO_{2peak}	↑ GDR, ↑ HDL	No change	No change
Nybacka et al.[165] Nybacka et al.[208]	RCT	10 12	Exercise = 17, Diet and exercise = 12 (43)	Age = 31.8 ± 4.9 BMI = 34.9 ± 5.3	Rotterdam	Type: Aerobic Frequency: 2-3/week Intensity: Moderate Duration: 16 weeks Supervision: Full	Not Measured	No change	↑ Menstrual Cyclicity No Change in hormonal markers	↓ BMI
Orio et al.[156]	RCT	18	39 (150)	Age = 25.9 ± 2.7 BMI = 26.7 ± 2.8	NIH	Type: Aerobic Frequency: 3/week	↑ $VO2_{2peak}$	↓ Fasting Insulin, ↓ HOMA-IR,	No change	↓ BMI, ↓ W/H

						Intensity: Vigorous (60-70% VO_{2peak}) Duration: 24 weeks Supervision: Full		↑ GIR,↓ AUC_{ins}, ↓ Total Cholesterol, ↑ HDL, ↓ LDL		
Orio et al.[201]	Non-randomised parallel study	14	32 (64)	Age = 18-40 BMI = 28.9 ± 3	Rotterdam	Type: Aerobic Frequency: 3/week Intensity: Vigorous (60-70% VO_{2peak}) Duration: 24 weeks Supervision: Full	↑ VO_{2peak}	↓ Fasting Insulin, ↓ AUCins, ↑AUCglu/ins, ↑ HDL, ↓ LDL	No change	↓ BMI ↓ WC, ↓ W/H
Randeva et al.[199]	Uncontrolled trail	12	12 (21)	Age = 29.7 ± 6.8 BMI = 33.9 ± 4.5	Rotterdam	Type: Aerobic Frequency: 3/week Intensity: Moderate Duration: 26 weeks Supervision: None	↑ VO_{2peak}	No Change	Not measured	↓ W/H
Roessler et al.[202]	Randomised crossover trial	15	8 (17)	Age = 31 (SEM–3) BMI = 34.8 (SEM-2.5)	Rotterdam	Type: Aerobic and aerobic intervals Frequency: 3/week Intensity: Vigorous-High (70-75 & 80-100% HR_{max}) Duration: 8 weeks Supervision: N/R	↑ VO_{2peak}	Not measured	Not measured	↓Weight, ↓ BMI, ↓ WC
Sprung et al.[203]	Single-arm trial	15	6 (12)	Age = 28 (25 - 31) BMI = 31 (28 - 34)	Rotterdam	Type: Aerobic Frequency: 3-5/week Intensity: Moderate (30-60%HRR) Duration: 16 weeks Supervision: Full	↑ VO_{2peak}	No Change	No Change	No Change
Sprung et al.[162]	Non-RCT	14	10 (17)	Age = 29 ± 7 BMI = 34 ± 6	Rotterdam	Type: Aerobic Frequency: 3-5/week Intensity: Moderate (30-60%HRR) Duration: 16 weeks	↑ VO_{2peak}	↓ Total Cholesterol, ↓LDL No change in insulin sensitivity	No Change	No Change

						Supervision: Full				
Stener-Victorin et al.[209] Jedel et al.[160] Stener-Victorin et al.[210]	RCT	13 16 16	30 (74)	Age = 30.2 ± 4.7 BMI = 27.7 ± 6.4	Rotterdam	Type: Aerobic Frequency: 3/week Intensity: Moderate Duration: 16 weeks Supervision: None	↑ VO_{2peak}	No change	↑ SHBG, ↓ Free T, ↓ Estradiol	↓ Weight, ↓ BMI
Thomson et al.[155] Thomson et al.[211] Thomson et al.[212]	RCT	11 13 10	Diet & aerobic exercise = 18, Diet and combined exercise = 20 (52)	Age = 29.3 ± 6.8 BMI = 36.1 ± 4.8	Rotterdam	Type: Aerobic, RT or combined aerobic and RT Frequency: 5/week Intensity: Moderate-Vigorous (60-80% HR_{max} and 50-75% of 1RM) Duration: 20 weeks Supervision: N/R	↑ VO_{2peak}	↓ HOMA-IR, ↓ Fasting Glucose, ↓ Fasting Insulin, ↓ Lipids	↓ T, ↓ FAI, ↑ SHBG, ↑ Menstrual Cyclicity	↓ Weight, ↓ WC, ↓ Fat mass, ↓ BF%
Vigorito et al.[164]	RCT	15	45 (90)	Age = 21.7 ± 2.3 BMI = 29.3 ± 2.9	Rotterdam	Type: Aerobic Frequency: 3/week Intensity: Vigorous (60-70% VO_{2peak}) Duration: 12 weeks Supervision: Full	↑ VO_{2peak}	↓ Fasting Insulin, ↓ AUCins, ↑ AUCglu/AUCins	↑ Menstrual Cyclicity. No Change in hormonal markers.	↓ WC, ↓ BMI, ↓ W/H
Included for systematic review only										
Brown et al.[193]	RCT	17	8 (20)	Age = 36.5 (5) BMI = 37.9 (9.4)	NIH	Type: Aerobic Frequency: 3-5/week Intensity: Moderate (40-60% VO_{2peak}) Duration: 20-24 weeks Supervision: Full	↑ VO_{2peak}	No change	Not measured	No change

QA – Quality Assessment, CRF- Cardiorespiratory Fitness, RCT – Randomised Controlled Trial, NIH – National Institute of Health, HIIT – High Intensity Interval Training, RT – Resistance Training, BMI – Body Mass Index, HR_{max} - Maximal Heart Rate, VO_{2peak} – Peak Oxygen Uptake, HRR – Heart Rate Reserve, N/R = Not Reported, ↑ Increase, ↓ Decrease, HOMA-IR – Homeostatic Model Assessment of Insulin Resistance, AUC – Area Under the Curve, GDR – Glucose Disposal Rate, GIR – Glucose Infusion Rate, HDL – High Density Lipoproteins, LDL – Low Density Lipoproteins, AMH – Anti-Müllerian Hormone, SHBG – Sex Hormone Binding Globulin, FAI – Free Androgen Index, T – Testosterone, Free T – Free Testosterone, BF% - Body Fat Percentage, WC – Waist Circumference, W/H – Waist to Hip Ratio.

Fourteen studies involved only a continuous aerobic intervention[156,158,160-162,164,165,193,196,197,199-201,203], three had a high intensity aerobic interval training group[157,198,202], three had a resistance training group[155,157,159] and two had a combined resistance training and aerobic group[155,195]. Of the interventions that included an aerobic exercise component, three included high intensity aerobic intervals[157,198,202], five included vigorous intensity exercise[156,158,164,195,201], two included moderate to vigorous intensity exercise[155,196] and nine included moderate intensity exercise[160-162,165,193,197,199,200,203]. The duration and frequency of exercise interventions ranged from 8 to 26 weeks and 2 to 5 sessions per week, respectively. The length of individual session duration varied, ranging from 30 to 90 minutes.

Seventeen studies measured VO_{2peak}[155-158,160-162,164,193,195-199,201-203]. Of these studies, 16 reported significant improvements following an exercise intervention[155-158,160,162,164,193,195-199,201-203]. Nineteen studies measured metabolic outcomes[155-162,164,165,193,195-201,203], 11 of which reported significant changes in at least one marker of metabolic health[155-158,161,162,164,195,197,198,201]. Four studies reported significant decreases in HOMA-IR[155-157,198], eight studies reported decreases in fasting insulin levels[155-158,164,195,198,201], three reported significant improvements in glucose infusion or glucose disposal rates[156,197,198], five studies reported positive increases in HDL[156,157,161,197,201] and eight studies reported no changes in markers of metabolic health following an exercise intervention[159,160,165,193,196,199,200,203]. Sixteen studies measured changes in hormonal markers and reproductive health[155-162,164,165,195,197-201,203]. Five studies reported a significant increase in SHBG levels[155,157-160], three studies reported significant decreases in FAI[155,157,159], two studies reported significant decreases in AMH levels[157,198], three studies reported improvements in menstrual cyclicity[155,164,165] and eight studies reported no changes in reproductive outcomes post exercise intervention[156,161,162,195,197,200,201,203]. All 20 studies measured changes in body composition following exercise intervention. Sixteen reported significant changes in at least one measure of body composition[155-161,164,165,195,196,198-202]. Ten studies reported significant decreases in BMI[156,158,160,164,165,196,198,200-202], nine reported decreases in waist circumference[155,156,159,161,164,195,196,200,202], five reported decreases in waist to hip ratio[156,158,164,199,201], three report decreased weight[155,160,202]. The remaining four studies reported no significant changes in any measure of body composition[162,193,197,203].

2.4.2 Meta-analysis

The results from the meta-analysis of the effect of exercise characteristics on cardiorespiratory fitness measured by VO_{2peak}, body composition (BMI & WC), insulin resistance (HOMA-IR) and hyperandrogenism as measured by FAI are presented as the population mean effects and modifying effects of exercise characteristics (Tables 4 & 5) and predicted effects of exercise across various durations and baseline values (Figures 6 & 7).

Table 4. Meta-analysed effects on peak oxygen uptake (VO2peak), body mass index (BMI) and waist circumference expressed as mean effects in control and exercise groups, and as modifying effects of exercise duration, baseline and dietary co-intervention.

	VO_{2peak}[b]			BMI[c]			Waist circumference[d]		
	Mean (%)	**90%CL (%)**	**Magnitude**	**Mean (%)**	**90%CL (%)**	**Magnitude**	**Mean (%)**	**90%CL (%)**	**Magnitude**
Population mean effects[a]									
Control group	1.0	-2.3, 4.4	**Trivial^{ooo}**	0.7	-0.2, 1.7	**Trivial^{0000}**	0.8	-1.2, 2.8	Trivial^{00}
Moderate exercise	18.4	11.2, 26.1	**Moderate↑******	-0.9	-2.0, 0.3	**Trivial^{0000}**	-1.6	-3.7, 0.5	**Trivial^{00}**
Vigorous exercise	24.2	18.5, 30.1	**Moderate↑******	-2.6	-3.6, -1.7	**Trivial^{000}**	-3.4	-5.3, -1.5	**Small↓****
Moderate - control group	17.2	9.7, 25.3	**Moderate↑*****	-1.6	-2.9, -0.2	**Trivial^{0000}**	-2.4	-4.1, -0.6	**Small↓***
Vigorous - control group	22.9	16.9, 29.2	**Moderate↑******	-3.3	-4.5, -2.2	**Trivial^{00}**	-4.2	-6.0, -2.3	**Small↓****
Modifying effects									
Baseline in control group	4.9	-1.4, 11.6	Trivial^{0}	1.3	-1.2, 3.9	**Trivial^{000}**	0.8	-2.9, 4.7	Trivial
Baseline in exercise group	-10.3	-17.6, -2.4	Small↓**	0.8	-1.3, 3.0	**Trivial^{000}**	-2.2	-5.9, 1.8	Small↓*
30 h of exercise duration	-0.8	-7.8, 6.8	Trivial	-1.3	-2.8, 0.2	**Trivial^{0000}**	-3.6	-6.0, -1.2	**Small↓****
Diet in control group	-1.9	-11.1, 8.2	Trivial	-5.6	-8.4, -2.7	**Small↓****	-7.5	-10.7, -4.3	**Moderate↓*****
Diet in exercise group	-1.4	-10.0, 8.0	Trivial	-2.9	-4.6, -1.2	**Trivial^{00}**	-1.0	-4.3, 2.5	Trivial^{00}

[a]Evaluated at mean baseline (VO_{2peak} = 24 mL.kg.min^{-1}, BMI = 31 kg.m^2, waist circumference = 97 cm), training time = 30 h, and no dietary co-intervention.
[b]Modifying effect of baseline is evaluated per 70% difference in baseline value
[c]Modifying effect of baseline is evaluated per 40% difference in baseline value
[d]Modifying effect of baseline is evaluated per 25% difference in baseline value
90%CL 90% compatibility limits, ↑ increase, ↓ decrease.
Effects are shown with their observed magnitudes, determined by magnitudetion. Clear effects are show with the probability of either a true substantial change (*possibly, **likely, ***very likely, ****most likely) and/or a true trivial change (^{0}possibly, ^{00}likely, ^{000}very likely, ^{0000}most likely). Magnitudes in bold are clear with 99% compatibility intervals.

2.4.2.1 Effect of exercise on VO_{2peak}

Meta-analysis from 16 studies with a total population of 600 women with PCOS, revealed moderate improvements in VO_{2peak} after moderate and vigorous intensity aerobic exercise, with the largest increase seen after vigorous intensity exercise (Table 4). Across all conditions, the modifying effects of intervention duration and dietary co-intervention on VO_{2peak} were trivial.

The predicted effects analysis showed that irrespective of training dose, vigorous intensity aerobic exercise alone had the most substantial increase in VO_{2peak} (Figure 6). Moreover, it is clear that baseline value plays a major role in the magnitude of improvements, with lower baseline VO_{2peak} values resulting in the largest improvements.

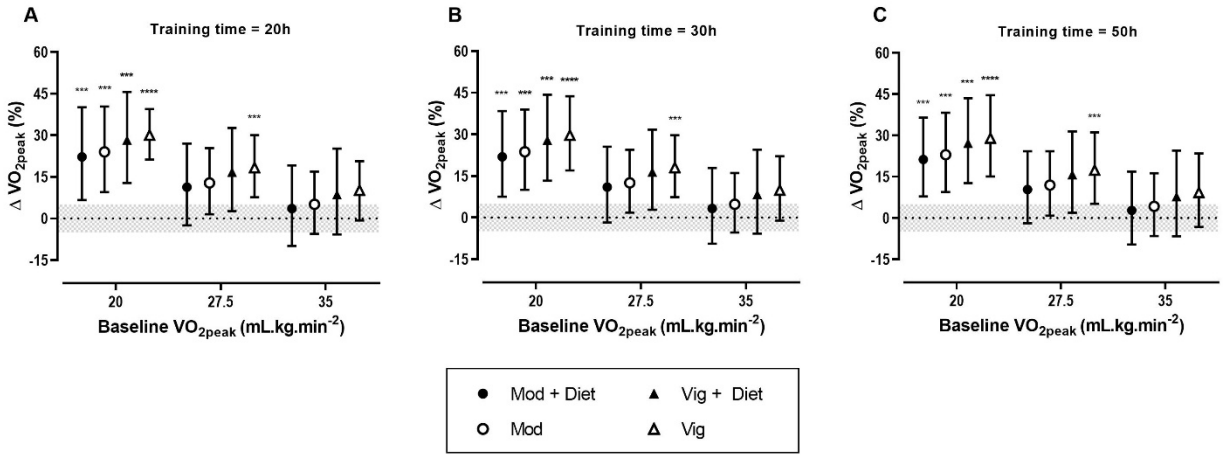

*Figure 6. Predicted effects of exercise alone or exercise plus diet versus a control group on peak oxygen uptake (VO2peak) after 20 hours (A), 30 hours (B) and 50 hours (C) of moderate (Mod) or vigorous (Vig) intensity exercise in an individual study setting. Clear effects are shown with the probability of a true substantial change (*possibly, **likely, ***very likely, ****most likely). Magnitudes in bold are clear with 99% compatibility intervals.*

2.4.2.2 Effect of exercise on BMI

Meta-analysis of 17 studies which included a total of 759 women with PCOS were included to determine the effect of exercise on BMI. The predicted mean results of each intervention were trivial (Table 4). The largest reductions in BMI were reported for women undertaking vigorous intensity exercise compared to a control group. The modifying effects of baseline BMI, duration and diet were also trivial with the exception of the effect of diet in a control group which resulted in a small decrease in BMI.

In the predicted effects analysis, training dose appears to have a limited effect on BMI outcome. The addition of diet intervention to exercise resulted in clear reductions in BMI. Notably, vigorous intensity exercise combined with a dietary intervention potentiated BMI changes, with small to moderate reductions of BMI across all baseline BMIs and training durations (Figure 7).

2.4.2.3 Effect of exercise on waist circumference (WC)

Thirteen studies which included 463 women overall were used in this analysis of the fixed effects of exercise on WC. Vigorous intensity exercise when compared to a control group resulted in the greatest reductions in WC. The modifying effect of diet in a control group resulted in a moderate decrease in WC. In contrast, there was a trivial effect of diet in the exercise group (Table 4).

The predicted effects analysis found the greatest improvement in WC with a combined vigorous intensity aerobic exercise and diet across the range of baseline WCs (Figure 7). Greater improvements were seen in women with a higher baseline WC. It was also apparent that training dose had a clear moderating effect on WC with greater decreases being reported after 50 hours of exercise in comparison to 20 hours of exercise (Figure 7).

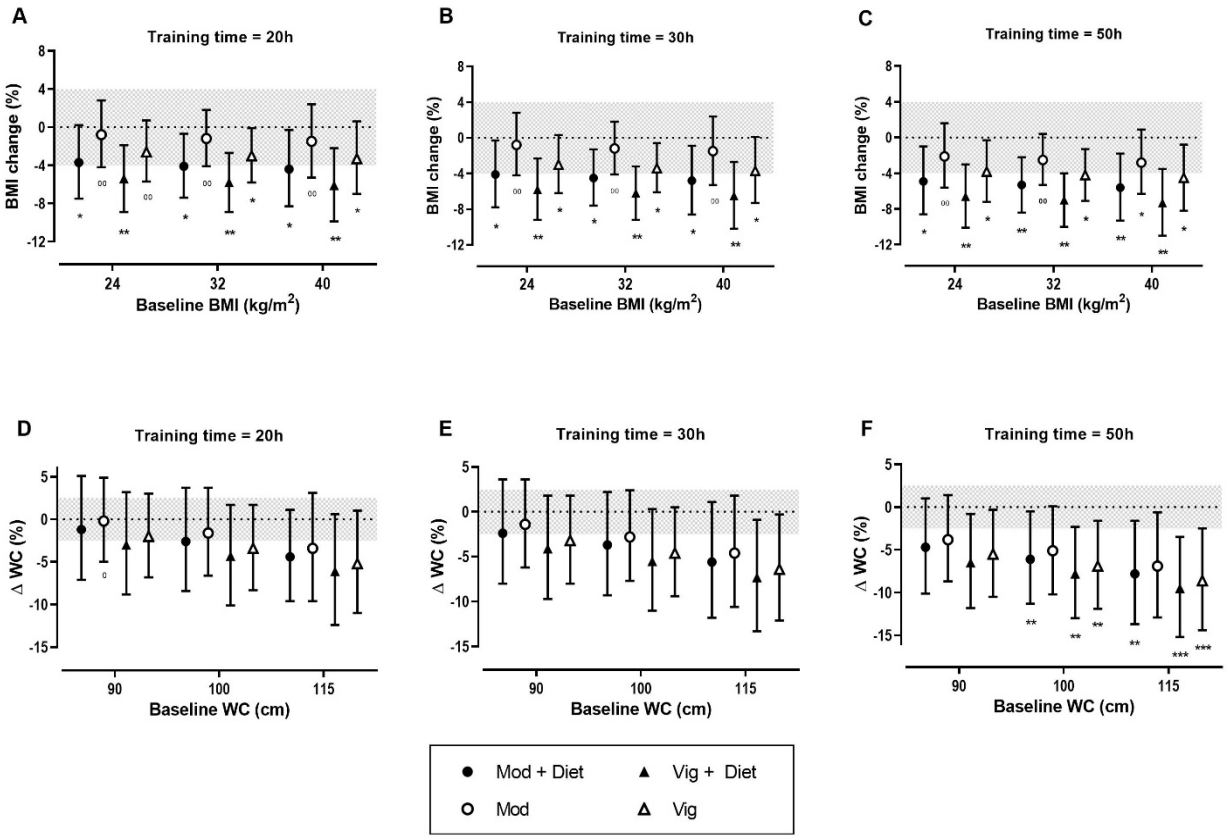

*Figure 7. Predicted effects of exercise alone or exercise plus diet versus a control group on body mass index (BMI) and waist circumference (WC) after 20 hours (A), 30 hours (B) and 50 hours (C) of moderate (Mod) or vigorous (Vig) intensity exercise in an individual study setting. Clear effects are shown with the probability of a true substantial change (*possibly, **likely, ***very likely, ****most likely). Magnitudes in bold are clear with 99% compatibility intervals.*

2.4.2.4 Effect of exercise on free androgen index (FAI)

Sixteen studies were included in the meta-analysis of exercise-induced changes in hyperandrogenism as measured by FAI and included a total of 667 women with PCOS. Of the 16 studies, 3 included a resistance training intervention. Our analysis showed that the greatest improvements in FAI occurred after resistance training (Table 5). Both moderate and vigorous aerobic exercise resulted in only trivial changes. The effect of diet resulted in a small decrease in FAI in both the exercise and control groups (Table 5).

The predicted effects analysis also reported trivial changes in FAI after aerobic exercise. Resistance training when combined with diet had the largest effect on FAI, resulting in small to moderate reductions of FAI across all baseline values and training doses, however the results were mostly unclear (Figure 8). It is apparent from the analysis that training duration plays a role in the extent of improvements in FAI, with the largest effects being seen after 50 hours of exercise.

Table 5. Meta-analysed effects on homeostatic model assessment of insulin resistance (HOMA-IR) and free androgen index (FAI) expressed as population mean effects in control and exercise groups, and as modifying effects of exercise duration, baseline and dietary co-intervention.

	HOMA-IR[b]			FAI[c]		
	Mean (%)	**90%CL (%)**	**Magnitude**	**Mean (%)**	**90%CL (%)**	**Magnitude**
Population mean effects[a]						
Control group	32.4	1.3, 72.9	Small↑**	-2.9	-11.1, 6.1	**Trivial^{000}**
Moderate exercise	10.1	-6.7, 30.0	Small↑*	2.2	-6.7, 12.0	Trivial^{000}
Vigorous exercise	-15.6	-33.2, 6.7	Small↓*	2.4	-7.4, 13.2	Trvial^{00}
Resistance exercise	-1.0	-14.4, 14.5	Trivial	-15.3	-28.4, 0.2	**Small↓***
Moderate - control group	-16.8	-38.8, 13.1	Small↓*	5.2	-6.2, 18.0	Trivial^{00}
Vigorous - control group	-36.2	-55.3, -9.0	Moderate↓**	5.4	-6.1, 18.4	Trivial^{00}
Resistance - control group	-25.2	-44.4, 0.6	Moderate↓**	-12.3	-27.3, 4.6	Small↓*
Modifying effects						
Baseline in control group	43.9	11.7, 85.4	**Moderate↑****	7.5	-11.5, 30.6	Small↑*
Baseline in exercise group	13.1	-25.1, 70.9	Trivial	1.1	-15.1, 20.3	Trivial
30 h of exercise duration	-5.4	-35.6, 38.9	Trivial	-8.1	-20.3, 6.0	Small↓*
Diet in control group	-43.1	-58.9, -21.3	**Small↓*****	-21.9	-32.8, -9.2	**Small↓****
Diet in exercise group	-19.5	-44.5, 16.6	Trivial	-11.1	-20.6, -0.4	**Small↓***

[a]Evaluated at mean baseline (HOMA-IR=moderate, FAI = 8.4%), training time = 30 h, and no dietary co-intervention.

[b]Modifying effect of baseline is evaluated for high versus low baseline

[c]Modifying effect of baseline is evaluated for a 3.0-fold difference in baseline

90%CL 90% compatibility limits, ↑ increase, ↓ decrease.

Effects are shown with their observed magnitudes, determined by standardisation. Clear effects are show with the probability of either a true substantial change (*possibly, **likely, ***very likely) and/or a true trivial change (^{0}possibly, ^{00}likely, ^{000}very likely). Magnitudes in bold are clear with 99% compatibility intervals.

2.4.2.5 Effects of exercise on insulin resistance (HOMA-IR)

Eleven studies (307 women with PCOS) were included in the meta-analysis for the effect of exercise on HOMA-IR. Vigorous intensity aerobic exercise and resistance training both resulted in moderate reductions in HOMA-IR when compared to a control group (Table 5). The modifying effect of diet on HOMA-IR resulted in a moderate reduction in a no-exercise control group, and a small reduction in an exercise group. The modifying effect of baseline in a control group resulted in moderate increases in HOMA-IR but only trivial effects in an exercise group (Table 5).

The predicted effects analysis on the effects of exercise on HOMA-IR show that clear improvements in HOMA-IR were only seen after vigorous intensity exercise both alone and vigorous exercise when combined with a dietary intervention, resulting in moderate reductions, irrespective of training dose (Figure 8).

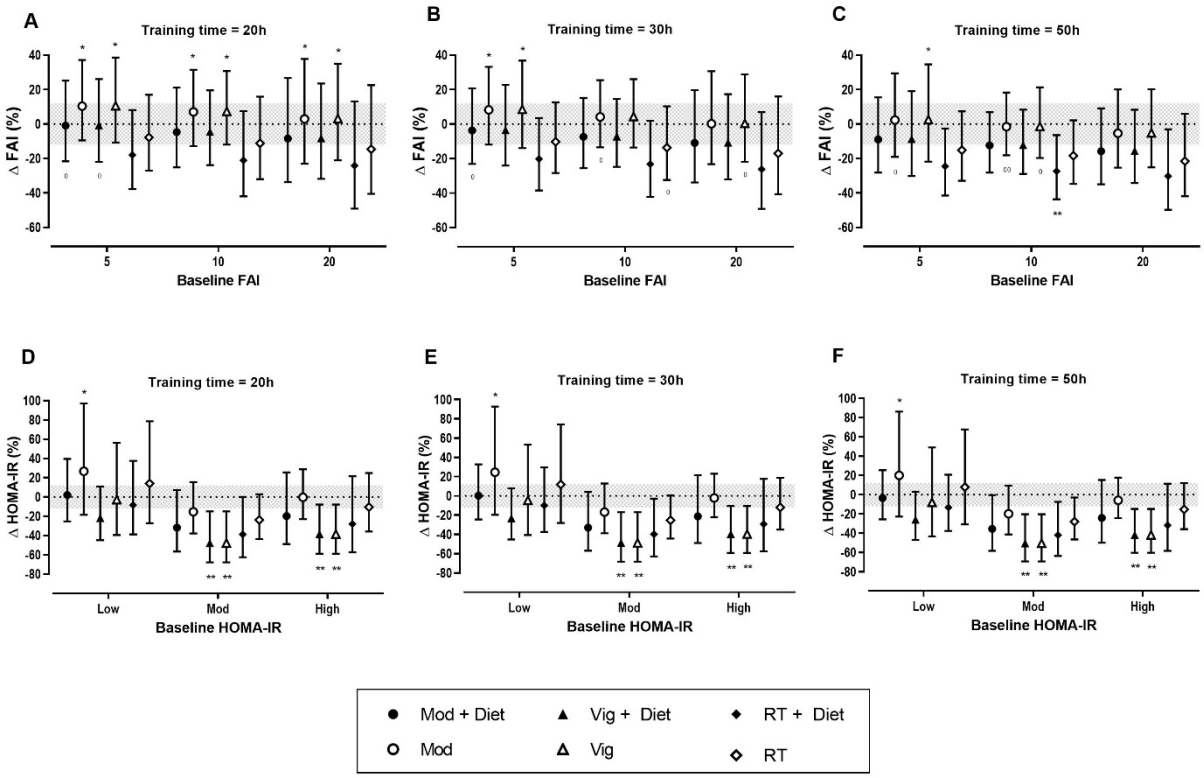

*Figure 8. Predicted effects of exercise alone or exercise plus diet versus a control group on free androgen index (FAI) and homeostatic model assessment of insulin resistance (HOMA-IR) after 20 hours (A & D), 30 hours (**B** & **E**) and 50 hours (**C** & **F**) of moderate (Mod), vigorous (Vig) intensity exercise or resistance training (RT) in an individual study setting. Clear effects are shown with the probability of either a true substantial change (*possibly, **likely) and/or a true trivial change (^{0}possibly, ^{00}likely). Baseline HOMA-IR: Low, <2.1%; Moderate (Mod), 2.1-3.4%; High, >3.4%. Magnitudes in bold are clear with 99% compatibility intervals.*

2.5 Discussion

This is the first systematic review and meta-analysis to evaluate the effectiveness of varying exercise intensities and the moderating effects of dietary co-intervention, training dose and baseline values on cardiorespiratory, metabolic and reproductive health outcomes in women with PCOS. Results from this systematic review demonstrate clear improvements in several of these outcomes following an exercise intervention in women with PCOS. The most consistent improvements were seen with cardiorespiratory fitness (VO_{2peak}), BMI, WC and various markers of metabolic health, including fasting insulin and HOMA-IR. These results are supported by our meta-analysis, which revealed improvements in VO_{2peak}, body composition and insulin sensitivity following an exercise intervention, particularly when compared to a no-exercise control group. Vigorous intensity exercise, both alone and when combined with a dietary intervention, resulted in the greatest improvements in health parameters in both the fixed effects and predicted effects analyses. Moderate intensity exercise resulted in clear improvements in

VO_{2peak}, WC, and BMI when combined with diet as seen in the predicted analysis. Interestingly, resistance training showed promising improvements in FAI and HOMA-IR in both fixed effect and predicted analyses, however further research is required to confirm these improvements.

This systematic evaluation of exercise interventions align with identified knowledge gaps in current international evidence-based guidelines[32,141] and meta-analyses[142,143] recently undertaken in this population of women. There is substantial evidence that supports the effectiveness of aerobic exercise training for improving some health outcomes in women with PCOS. In particular, aerobic exercise of various intensities has consistently been found to result in improvements in VO_{2peak} in women with PCOS[141,143,213]. VO_{2peak} is a measure of cardiorespiratory fitness and is an important indicator of health and mortality[214]. Individuals with a lower VO_{2peak} are at an increased risk of all-cause mortality and morbidity with the risk of death being more dependent on cardiorespiratory fitness than BMI[111]. To illustrate this point, with each 3.5mL/kg/min increase in VO_{2peak}, there is an associated 13% risk reduction from all-cause mortality[215]. Based on observed improvements from our meta-analysis, women with PCOS and relatively low VO_{2peak} of 24mL/kg/min are likely to experience a ~30% risk reduction in all-cause mortality after 30 hours of vigorous intensity exercise over 10-12 weeks, irrespective of any dietary co-intervention. An increase in exercise intensity becomes of paramount importance for improving VO_{2peak} in women with high baseline values. These results expand on existing studies that have reported improvements in VO_{2peak} after vigorous or high intensity exercise interventions[79,157,196,202] and highlights the importance of exercise intensity when prescribing exercise training in clinical practice or as part of a clinical trial.

A large proportion of women with PCOS are overweight or obese, with a recent meta-analysis reporting a pooled prevalence of 61%[181]. It is therefore not surprising that many exercise and dietary interventions have an ultimate aim of reducing body weight/BMI. Modest weight loss of 5-10% in overweight women with PCOS is encouraged to yield clinical improvements[32]. However, it is important to note that health benefits can occur without significant weight loss[153,162,197]. The lack of improvement in BMI following an exercise only intervention observed from our analysis is not surprising. However, when exercise is complimented with a dietary intervention, small, but clear, decreases in BMI can be achieved. In addition, our results support the inclusion of diet in order to promote improvements in WC. Research conducted by Thomson et al.[155] reported reductions in body weight and waist circumference of ~10% across three different treatments groups (diet alone, diet + aerobic exercise or diet + combined aerobic and resistance exercise) after 20 weeks. A study conducted by Bruner et al.[195] reported no significant differences in body weight or BMI following an intervention of either nutritional counselling or a combined resistance training, aerobic exercise and nutritional counselling intervention. They did, however, report significant decreases in waist circumference of 5% in both groups following the intervention period. BMI as a measure of obesity is considered to have its limitations, with changes in BMI not necessarily reflecting changes in body fat[216]. Body composition assessment using direct

methods such as dual-energy X-ray absorptiometry (DXA) may provide valuable information on changes in body composition. When deprived of DXA information, measures of WC may provide a better measure of obesity-related health risk than BMI[217]. It is possible that exercise training alone may have a limited impact on BMI but positively improves waist circumference or other markers of body composition, including increased lean mass and decreased fat mass, which can occur without changes in total body weight.

Insulin resistance is a key aetiological feature in PCOS and underpins the metabolic dysfunction present in women with PCOS[67,218]. Although not currently included in the diagnostic criteria, insulin resistance determined from insulin clamps is prevalent in 56-95% of women with PCOS[4,30,219]. It is therefore important to understand the impact of exercise type and intensity and its interaction with diet to explore effective exercise interventions to alleviate insulin resistance in women with PCOS before major complications occur. Resistance training is an effective treatment for improving insulin sensitivity in individuals with diabetes[220-222], however, there is limited evidence to support the benefits of such training in PCOS[155]. We identified moderate decreases in HOMA-IR after resistance training interventions when compared to a control group. Resistance training is yet to be widely implemented in the treatment of PCOS, with current knowledge limited to few studies with small numbers of participants. However, there is evidence to support the effects of resistance training for improving insulin sensitivity in diabetic populations and therefore this may be applicable to women with PCOS.

Our meta-analysis showed that vigorous intensity aerobic training also resulted in moderate decreases in HOMA-IR in women with PCOS. This is in line with findings from a number of other clinical populations[121,122,133,223]. Results from a study conducted by Greenwood et al.[117] support the superior health benefits of vigorous exercise compared to moderate exercise in women with PCOS. They reported that 60 minutes of vigorous intensity exercise per week was associated with a 22% reduced odds of metabolic syndrome. In addition, Harrison et al.[79] reported a 16% improvement in insulin sensitivity in women with PCOS following a 12-week vigorous intensity exercise intervention, as determined by the gold-standard euglycaemic-hyperinsulinaemic clamp method[68]. The use of the clamp method in clinical practice is impractical, however one must be cognisant that using HOMA-IR as a surrogate marker for IR has significant limitations which includes a low sensitivity in identifying IR[4]. Despite the pitfalls of using HOMA-IR to measure insulin resistance, most clinical research in PCOS continues to use this method due to its cost-effectiveness and ease of translation into clinical practice.

Elevated FAI is the most consistently observed androgenic abnormality in PCOS[2]. Current research that measures FAI prior to and following an exercise intervention show contradictory results[155,158,210]. This may relate to the complex relationship between FAI and insulin resistance, as the latter has profound effects on SHBG. Results from our meta-analysis could not provide any conclusive evidence in support of any type of exercise training or exercise intensity influencing FAI and is consistent with another

recent meta-analysis[143]. Our results suggest that resistance training may be the most likely to induce positive changes in FAI, however, due to the limited number of studies utilising resistance training, more research is required to validate this outcome. One study of 16 week study of progressive resistance training (n=45) reported decreases in FAI values of 0.82%[159]. In addition, a study comparing a 10-week intervention of either resistance training (n=8) or high intensity interval training (n=8) to a control group (n=9), reported the largest decrease in FAI in the strength training group, with a decrease of -0.7% from baseline values[157]. Although resistance training shows promising results, reductions in FAI have also been reported after aerobic exercise[154,158,199]. Further research is required to determine the effective modality, dose and intensity of exercise for improvements in hyperandrogenism. There is also a need to identify more valid measures of androgen levels in women with PCOS to monitor impacts all interventions (e.g. exercise and/or diet, pharmacotherapies).

2.5.1 Strengths and limitations

An important strength of our analysis is the inclusion of a variety of study designs with well-characterised participants. This allowed us to go beyond existing systematic reviews and meta-analyses to generate a large dataset that included a no-intervention control group. We were also able to explore the modifying effects of diet, exercise intensity, training dose and baseline values of the outcome measures, according to a particular current health and fitness level, enabling more individualised exercise prescription for women with PCOS. However, the inclusion of studies other than RCTs may be viewed as a limitation due to the possible increase in the risk of bias. However, all studies were assessed for bias and deemed of acceptable quality. It could also be argued that including a no-exercise control group in a study design could be considered of no additional use[224,225] and it is established that in many clinical conditions, most outcomes impacted by exercise remain unchanged or worsen over the course of an intervention in no-exercise controls[122]. A limitation of this analysis is the large heterogeneity among the included studies with interventions varying greatly in frequency, intensity and the extent of exercise supervision. Some studies had sparse description of the exercise interventions, further limiting our analysis. The inclusion of unsupervised exercise interventions may have under-estimated the benefits of exercise and future research should aim to document level of supervision to better gauge its effect on clinical outcomes.

2.5.2 Conclusions

This work considerably expands on previous evidence and advances the knowledge of benefits of exercise prescription in women with PCOS. Our analysis demonstrates that exercise training in women with PCOS improves cardio-metabolic outcomes, both in the presence and independent of anthropometric changes, supporting the role of exercise therapy, as the first-line approach for improving health outcomes in women with PCOS. Specifically, for greater health improvements, exercise interventions and/or exercise prescription should aim to achieve and sustain a minimum of 20 hours of vigorous intensity exercise over 10-12 weeks, equating to 120 minutes per week across this timeframe.

Once achieving this goal, women should sustain this level of exercise for continued health maintenance. Resistance training also appears to have some health benefits and could be considered for women with PCOS. Adequate reporting of exercise intervention characteristics (i.e. exercise session supervision, exercise intensity, adherence & compliance), use of gold-standard clinical outcome measures and consideration of long-term intervention sustainability is required through the application of high-quality, large clinical studies with long-term follow-up to provide definitive exercise prescription recommendations in women with PCOS.

2.6 Updated systematic literature search

I performed an updated literature search for the systematic review presented in Chapter 2. Using the same criteria, a further 3 exercise interventions were identified, two of which resulted in dual publications. A summary of the participant characteristics, exercise intervention characteristics and study outcomes is reported in Table 6.

Of the three recently identified clinical trials, two utilised either vigorous or high-intensity interval training. One study compared continuous aerobic training (CAT), aerobic interval training (AIT) and a no exercise control group[226]. Interestingly, they did not measure changes in cardiorespiratory fitness or insulin sensitivity, making it difficult to determine the success of the interventions. They did however report a significant decrease in total testosterone as a result of both the CAT and AIT groups, and a significant decrease in FAI in the aerobic interval group only. They also reported a significant decrease in waist circumference in the CAT group and a significant decrease in hip circumference and waist to hip ratio in the AIT group. They reported no significant differences between the two groups. This may in part be due to the similarity in intensities used for both interventions. There was only a 5-10% difference in peak heart rate prescribed throughout the intervention. In addition, they did not use a typically prescribed interval training protocol, choosing instead to utilise a 2 minute interval with a 3 minute recovery period. Lastly, the two intervention protocols were matched for session duration with the aerobic interval group working up to a 50 minute session. HIIT is typically matched for workload or energy expenditure, and utilised for its time efficiency and due to its amplified exercise stimulus, resulting in enhanced health outcomes[121]. The success of this type of interval training procedure is not commonly used or documented in both healthy and diseased populations[121,227].

The second trial that utilised interval training compared a low-volume HIIT, high-volume HIIT and a no exercise control group[228]. They reported no significant differences between groups with the exception of a significant increase in VO_{2peak} in the high-volume HIIT group compared to the control group. They did not measure changes in metabolic or reproductive outcomes and did not statistically analyse changes within the groups. They also report that they did not match their HIIT protocols for mean workload or energy expenditure. Lastly, the intervention was only partially supervised which may have contributed to their null findings.

The final study was a non-randomised uncontrolled trial which compared the effects of 8 weeks of continuous moderate-to-vigorous intensity exercise in women with PCOS compared to women without[229]. They reported significant improvements in VO_{2peak}, HOMA-IR, glucose disposal rate, waist circumference and BMI. Each exercise session was supervised and intensity was monitored to ensure the participants were reaching the desired intensity which may have contributed to the success of their intervention.

The additional studies reported here provide further support for the benefits of exercise, however, there is still little evidence which adequately compares and matches exercise intensities in women with PCOS in order to determine the most effective intervention to promote the largest improvements across all domains of health.

Table 6: Update of Table 3 with a summary of studies identified from May 2018 to January 2021 detailing participants, intervention characteristics and main outcomes

Study	**Study Design**	**Exercise N (total N)**	**Participant Characteristics**	**PCOS Diagnostic Criteria**	**Exercise Intervention Characteristics**	**CRF Outcomes**	**Cardio-metabolic Outcomes**	**Hormonal & Reproductive Outcomes**	**Body Composition Outcomes**
Aye et al.[229] & Kirk et al.[230]	Non-RCT	11	Age = 28.0 ± 6.7 BMI = 31.2 ± 6.3	Rotterdam	Type: Continuous Aerobic Frequency: 3/week Intensity: Moderate-Vigorous (~60% VO_{2peak}) Duration: 8 weeks Supervision: Full	↑ VO_{2peak}	↓ HOMA-IR, ↑ Glucose Disposal Rate	Not Measured	↓ Waist Circumference, ↓ BMI
Lionett et al.[228]	RCT	LV-HIT = 13 HV-HIT = 14 (42)	LV-HIT: Age = 31 ± 5 BMI = 28.9 ± 6.6 HV-HIT: Age = 30 ± 5 BMI = 33.1 ± 7.4	Rotterdam	Type: Aerobic Intervals Frequency: 3/week Intensity: Vigorous-High (90-100% HR_{max}) Duration: 16 weeks Supervision: Partial	LV-HIT: No Change HV-HIT: ↑ VO_{2peak}	Not Measured	Not Measured	LV-HIT: No Change HV-HIT: No Change
Ribeiro et al.[226] & Kogure et al.[231]	RCT	CAT: 28, IAT: 29 (87)	CAT: Age = 29.1 ± 5.3 BMI = 28.4 ± 5.6 AIT: Age = 29.0 ± 4.3 BMI = 28.7 ± 4.8	Rotterdam	Type: Continuous Aerobic & Aerobic Intervals Frequency: 3/week Intensity: CAT: Moderate-Vigorous (65-80% HR_{max}) IAT: Vigorous (70-90% HR_{max}) Duration: 16 weeks Supervision: Full	Not Measured	Not Measured	CAT: ↓ Testosterone IAT: ↓ Testosterone, ↓ FAI	CAT: ↓ Waist Circumference, ↓ Hip Circumference, IAT: ↓ WHR

CRF – Cardiorespiratory Fitness, RCT – Randomised Controlled Trial, LV-HIT – Low-Volume High-intensity Interval Training, HV-HIT – High-Volume High-intensity Interval Training, BMI – Body Mass Index, VO_{2peak} – Peak oxygen uptake, HOMA-IR – Homeostatic Model Assessment of Insulin Resistance, CAT – Continuous Aerobic Training, AIT – Aerobic Interval Training, FAI – Free Androgen Index, WHR – Waist to Hip Ratio.

Chapter 3.0 Effectiveness of exercise interventions on mental health and health-related quality of life in women with polycystic ovary syndrome: a systematic review

This paper was submitted in November in 2020 and is complete and currently under review at BMC Public Health. An updated systematic search was completed in January 2021, however, this did not yield any additional studies that met the inclusion criteria.

3.1 Abstract

Background: Polycystic ovary syndrome (PCOS) is a complex condition, impacting cardio-metabolic and reproductive health, mental health and health related quality of life. The physical health benefits of exercise for women with PCOS are well-established and exercise is increasingly being recognised as efficacious for improving psychological wellbeing. The aim of this review was to summarise the evidence regarding the effectiveness of exercise interventions on mental health and health-related quality of life outcomes in women with PCOS.

Methods: A systematic search of published literature was conducted in March of 2020. The review adheres to the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines as per our PROSPERO protocol (CRD42019118657). Randomised controlled trials, non-randomised controlled trials and uncontrolled trials that evaluated the effect of an exercise intervention of at least moderate intensity in reproductive aged women (aged 18 to 45 years) with diagnosed PCOS were included. Methodological quality was assessed using the modified Downs and Black checklist. Primary outcomes included symptoms of depression and anxiety, and health-related quality of life and were summarised as statistically significant within group changes or clinically meaningful.

Results: Fifteen articles from 11 trials were identified and deemed eligible for inclusion. Exercise demonstrated positive improvements in health-related quality of life in all of the included studies. Half of included studies also reported significant improvements in depression and anxiety symptoms. There was large variation in methodological quality of included studies and in the interventions utilised.

Conclusions: The available evidence indicates that exercise is effective for improving health-related quality of life and PCOS symptom distress. Exercise also shows some efficacy for improving symptoms and/or prevalence of depression and anxiety in women with PCOS. However, due to large heterogeneity of included studies, conclusions could not be made regarding the impact of exercise intervention characteristics. High-quality trials with well reported exercise intervention characteristics and outcomes are required in order to determine effective exercise protocols for women with PCOS and facilitate translation into practice.

3.2 Background

Polycystic Ovary Syndrome (PCOS) is a complex and common condition, affecting 8-13% of reproductive aged women[32] and carries a major disease burden across cardio-metabolic and reproductive health. PCOS is characterised by hyperandrogenism, ovulatory dysfunction and polycystic ovary morphology, and although not recognised in the diagnostic criteria, insulin resistance is considered a key aetiological feature, contributing to the severity of PCOS features. PCOS is the leading cause of anovulatory infertility among reproductive-aged women[232] and has significant metabolic features including insulin resistance, obesity, and an increased risk of developing type 2 diabetes[3,30,186,233]. PCOS is also known to be related to diminished mental health, including increased symptoms of depression, anxiety and lower health-related quality of life, with these comorbidities occurring and having impact across the lifespan[89].

Many chronic illnesses have an impact on mental health and are associated with a reduction in quality of life and an increase in a range of psychological symptoms[89,234,235]. Given the clinical features of PCOS, it is perhaps not surprising that women with PCOS experience mental health problems and mood dysfunction to a greater degree than women without PCOS[236]. Compared to age and weight matched control women[87,88], and those with other chronic conditions including diabetes and coronary heart disease[103], women with PCOS have poorer mental health and health-related quality of life with many reporting increased symptoms of anxiety and depression and PCOS symptom distress. Fears regarding infertility, body image concerns, low self-esteem and coping with the condition may all contribute to poorer mental health among these women[89].

In a healthy population, exercise is an effective means of promoting, improving and managing mental health[175]. This is also the case for populations with chronic conditions[176] and in overweight women[177]. The specific interaction between physical activity and mental health in PCOS has not been explored in depth, but the limited existing research indicates a positive effect of exercise for improving mental health and health-related quality of life in women with PCOS[147,237-239]. Women with PCOS who are more physically active report fewer symptoms of depression than sedentary women with PCOS[89], although active women with PCOS report higher symptoms of depression than active women without PCOS[89].

The current international evidence-based guidelines recommend 150 minutes per week of moderate intensity exercise or 75 minutes per week of vigorous intensity exercise in all women with PCOS, in order to improve general health and quality of life[32]. Despite the positive effects of exercise, low compliance with these guidelines because of general barriers (time limitations, low enjoyment experienced with exercise) and PCOS-specific barriers (low confidence, physical limitations) to exercise[89,147], means that many women with PCOS remaining inactive or insufficiently active[117].

Enhancing engagement in exercise is vital to increase adherence to exercise recommendations and increase the potential health and mental health benefits of exercise[89]. This systematic review will synthesise the existing literature and aim to determine the effectiveness of exercise for improving symptoms of mental health and health-related quality of life in women with PCOS.

3.3 Methods

Protocol and registration: This systematic review was conducted and reported in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA)[240] and was registered on the International Prospective Register of Systematic Reviews (CRD42019118657).

Information sources & search: An electronic database search was conducted in December 2018 and updated in March 2020 using EBSCOhost (PsycINFO, MEDLINE, SPORTDiscus, CINAHL) and Ovid Medline with no time restrictions. The search strategy included Medical Subject Heading (MeSH) terms and keywords for mental health, health-related quality of life, physical activity or exercise interventions and PCOS. An example of a search strategy is reported in Supplementary Table 1 and was adapted for each database. The search terms were broad in order to capture publications that may have included mental health or health related quality of life as secondary outcomes. Reference lists of relevant review articles were searched to identify additional eligible studies.

Eligibility criteria: The Participant, Intervention, Comparison, Outcomes and Studies (PICOS) framework was used for this systematic review (Table 7). Briefly, included studies recruited women aged 18-45 (pre-menopausal) and with a diagnosis of PCOS using any established diagnostic criteria (e.g., Rotterdam criteria, National Institute for Health [NIH]). Randomised controlled trials (RCT), non-randomised controlled trials and uncontrolled trials where participants, were exposed to an exercise intervention of at least moderate intensity and of two weeks or greater in duration, were included in this review. Exercise intensity was categorised according to Norton et al.[114], classified as moderate (55 to <70% HR_{max} or 40 to < 60% VO_{2max}), vigorous (70 to <90% HR_{max} or 60 to <85% VO_{2max}) or high (≥90% HR_{max} or ≥85% VO_{2max}) intensity. Two weeks was used as a minimum intervention duration to capture the chronic effects of exercise. Only published, peer-reviewed, English language trials were considered. The primary outcome measures were health-related quality of life as assessed by either the polycystic ovary syndrome questionnaire (PCOSQ) or the short form 36 (SF-36) questionnaire and symptoms of depression and anxiety assessed by any validated questionnaire (Table 7). Trials that did not report at least one of these outcome measures were not included in this review.

Table 7. Eligibility criteria for study inclusion

Inclusion Criteria					
Participants	**Intervention**	**Comparison**	**Outcome**	**Study Design**	**Limits**
Diagnosed with PCOS using any established definition Reproductive years, aged 18–45	Any intervention that included exercise of: Any type or intensity Duration >2 weeks	No exercise Alternative therapies (e.g. acupuncture, cognitive behavioural therapy) Medications	Depression symptoms Anxiety symptoms HRQoL (SF-36) PCOS symptom distress (PCOSQ)	RCT Non-RCT Cohort Case Control Parallel Clinical trial	Peer reviewed English language

PCOS – Polycystic Ovary Syndrome HRQoL – Health Related Quality of Life, SF-36 – Short Form 36, PCOSQ – Polycystic Ovary Syndrome Questionnaire, RCT – Randomised Controlled Trial

Study selection and data extraction: After duplicates were removed, two reviewers (R.P. and R.B.) independently screened each article by title and abstract. Reference lists of systematic reviews and meta-analyses that were considered relevant were manually searched to identify additional references. Following removal of irrelevant studies, full-text versions of the remaining publications were assessed for inclusion eligibility. Data relating to study, participant and intervention characteristics and outcome measures were extracted independently by reviewers using a pre-determined data extraction form. At each stage of the screening process, discrepancies were resolved by consensus or by a third reviewer (N.S.). Where required, authors were contacted using an institutional email address in order to obtain additional or raw data. Due to poor reporting of intervention characteristics and outcome measures and large heterogeneity in the interventions, a quantitative synthesis was not feasible. Study results were therefore summarised as statistically significant within group changes ($p<0.05$) or clinically meaningful changes.

Risk of bias: The Downs and Black checklist for the assessment of methodological quality was used to evaluate randomised and non-randomised studies[185] (Supplementary Table 2). Questions regarding blinding of participants were removed as blinding is not possible in exercise intervention trials; however, blinding of outcome assessors was included. This checklist included 21 items with each item receiving a 0 or a 1 response and assesses reporting, internal and external validity or bias, and power. Higher scores indicated better methodological quality. Inter-reviewer discrepancies concerning the methodological quality of included studies were resolved by consensus.

3.4 Results

The combined searches identified 1114 references. Six articles were removed due to duplication and 1033 articles were deemed irrelevant after title and abstract screening. Of the 76 papers that were deemed eligible for full-text screening, 61 were excluded due to having no relevant outcome measures (Figure 9). The remaining 15 publications were deemed eligible for inclusion and were assessed for methodological quality, with results reported in Supplementary Table 6. These 15 publications were the result of 11 trials. In cases where multiple publications arose from one trial, data were grouped together. The characteristics of the included trials are presented in Table 8 and summarised below.

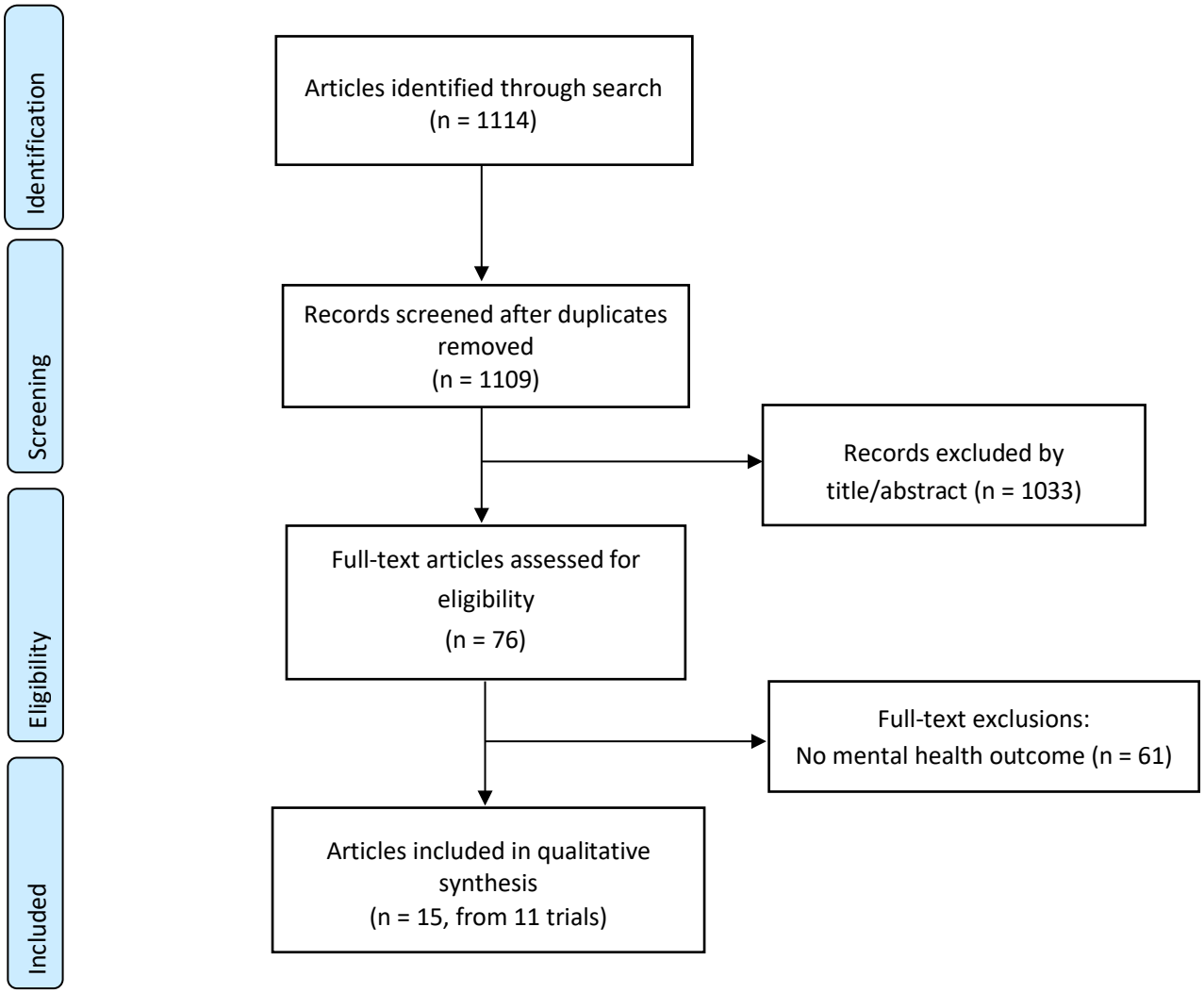

Figure 9. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) study selection flow diagram.

3.4.1 Study design and participants characteristics

Of the 11 included trials, nine were RCTs[161,196,226,237-239,241-243], one was a single arm study[244] and one was a case-control study[245]. Sample sizes ranged from 13 to 149 participants. The mean age of participants ranged from 26 to 33 years of age and the mean BMI ranged from 27.9 to 41.3kg/m^2.

Participants from nine trials were diagnosed with PCOS according to the Rotterdam criteria[196,226,237-239,241,243-245] and three trials according to the NIH criteria[161,242]. Of the included trials, nine recruited only overweight and obese women with PCOS and all studies excluded women with chronic co-morbidities, such as diabetes and cardiovascular disease. One study included only women with a positive screen for depression symptoms[242]. Three studies excluded women who were taking medication treatment for depression[196,237,241], five studies did not exclude women who were taking anti-depressant medication[226,238,239,243,245] and two studies did not report on use of medication of mental health status[161,244].

3.4.2 Assessment of study quality

Scores from the modified Downs and Black checklist varied greatly with scores ranging from 8 to 18 on a 21-point scale. The most common issues were poor reporting of aspects of the intervention or the characteristics of participants lost to follow-up. The full assessment of methodological quality is presented in supplementary Table 6.

3.4.3 Intervention characteristics

Aerobic exercise alone was delivered in eight out of the 11 trials[161,196,226,239,241-244]. One study included an aerobic exercise group, a resistance training group and a combined aerobic and a resistance training group[237] and the final two studies delivered resistance training only[238,245]. Of the included studies, only four of the interventions had full supervision by an exercise specialist[196,226,238,245], three had partial supervision[161,237,241] and four had no supervision[239,242-244]. The duration of the interventions varied from 12 to 26 weeks, with the number of session ranging from two to five per week. Exercise intensity was only adequately reported in five of the 11 included studies. Of those that reported intensity, four used moderate-vigorous intensity exercise. Adherence, classified as the percent of sessions attended in regards to the expected attendance, was reported in only three of the included studies. Two of these reported an average adherence of 81%[196] and 95%[238] for supervised sessions, with one trial reporting an average of 51% for unsupervised session adherence[238]. The third study reported that on average, only 38% of participants in the lifestyle only group reported meeting their weekly exercise goal[242]. No adverse events were reported as a result of any of the exercise interventions.

3.4.4 Outcomes

Six of the included trials had a primary outcome of mental health and/or health-related quality of life. The remaining five trials included mental health and health related quality of life as a secondary outcome.

3.4.5 PCOS symptom distress

Of the studies included, eight used the PCOSQ to assess PCOS related distress, seven of which reported statistically significant improvements in a minimum of two domains. Six studies reported statistically significant improvements in emotions[161,237,238,241,243,244] weight[161,237-239,241,244] and infertility domains[237-239,241,243,244], three reported improvements in the menstrual problems domain[237,239,241] and one reported improvements in the body hair domain[244]. The eighth study did not report a statistically significant impact of exercise on PCOS related distress, but did report minimally important clinically significant improvements (≥0.5 point increase), in all domains for the cognitive behavioural therapy plus lifestyle group, and clinically significant increases in the weight and infertility domains for the lifestyle only group[242]. In total, the weight domain had the most clinically significant improvements with six studies reporting improvements ranging from 0.7 to 1.75 point increase in scores. Clinically significant improvements were also commonly reported in the emotions domain, with four studies reporting increases in scores of between 0.5 and 0.8 points. A large proportion of interventions that utilised a range of exercise intensities, doses, types and durations, resulted in significant improvements in multiple domains of the PCOSQ, therefore suggesting that regardless of these factors, exercise can improve health-related quality of life in regards to PCOS symptom distress.

3.4.6 Health-related quality of life

Six studies used SF-36 to determine the health-related quality of life of participants. Four studies reported statistically significant increases in the physical functioning domain[196,226,238,246]. Three studies reported significant increases in general health[196,226,247], vitality [226,238,247] and mental health[196,226,238]. Two studies reported statistically significant improvements in role physical [226,243], social functioning[226,238] and role emotional domains[226,238]. No studies reported statistical improvements in the bodily pain domain. Clinically meaningful improvements were most commonly reported for the role physical domain, with 4 studies showing clinically meaningful changes after an exercise intervention[196,226,243,246], reporting increases in scores of 6.3 to 39.3. In regards to the SF-36, a change of score of 5 points or greater is considered clinically meaningful[248]. Improvements were also commonly reported for emotional and mental health domains with increases in scores of between 5.0 to 40.6, and 11.7 to 15.4 respectively. There were three studies that observed large improvements in multiple domains of the SF-36. Two of these studies used an aerobic exercise intervention of moderate to vigorous intensity, delivered three times per week for 16 weeks[196,226], while the third study conducted a 12 week progressive resistance training program delivered twice per week[238]. In summary, many studies reported improvements in domains of the SF-36 as a result of various exercise interventions, however it appears that there were no common denominators in regards to exercise characteristics.

3.4.7 Depression

Eight studies measured the effect of an exercise intervention on depression symptoms, with four reporting reductions in depression symptoms. Of the studies that reported improvements, two used the Centre for Depression Scale (CES-D) questionnaire[237,242], and two used the Hospital Anxiety Depression Scale (HADS) questionnaire[231,245]. Two studies that reported improvements delivered an aerobic exercise intervention[231,242], one delivered resistance training[245] and the final study compared three interventions (diet only, diet and aerobic exercise and diet and combined exercise)[237], all of which resulted in improved depression scores. There were no obvious common denominators in regards to exercise characteristics between studies that did, and those that did not report improvements in symptoms of depression, although all studies that utilised the CES-D and the HADS found significant improvements. One study that used the Depression Anxiety and Stress Scales (DASS-21) questionnaire reported significant changes in depression symptoms at post-intervention, in comparison to a no-intervention control group, after 12 weeks of supervised resistance training[238]. In regards to clinical significance, of the two studies that used the DASS-21 to assess depression symptoms, one study reported the exercise reduced average depression scores from 7.76 to 3.5[241], which indicates a decrease from moderate symptoms to no elevation in depression symptoms. The second study also had clinically meaning change in depression symptoms, as participants, on average, shifted from moderate to mild depression symptom severity from baseline to post-intervention[238]. One of the studies that assessed depression symptoms following a combined diet and aerobic intervention, reported decreases in depression symptoms, as scores on the CES-D moved from 18.6 at baseline and 14.0[237]. As the CES-D uses a cut-off score of ≥16 to suggest possible depression and ≥23 for probable depression[249], these results indicate that at least some women moved from depressed to non-depressed status post-intervention. The second study that used the CES-D included only women classified as possibly depressed (score of ≥16) at baseline, and reported large decreases in symptoms with scores decreasing from 24 to 18[242]. Lastly, both studies that used the HADS questionnaire found that participants had borderline abnormal scores (8-10 points) at baseline, indicating mild depression, and normal scores (0-7 points) post-intervention, indicating no depression[226,245].

3.4.8 Anxiety

Six studies also examined symptoms of anxiety, three of which reported statistically significant within group reductions in symptoms after an exercise intervention[231,245,247]. A third study reported significant reductions in anxiety symptoms compared to a control group, after exercise[238]. Results from this study are also considered clinically meaningful, as average anxiety scores (DASS-21) reduced from 10.3 at baseline to 7.4 post-intervention, indicating that symptoms decreased from extremely severe to moderate. Two studies reported the prevalence of anxiety at baseline and following an exercise intervention. One of these studies reported that 44.1% of participants had anxiety (according to the

measures used) at baseline, which decreased to 23.2% after a 16 week resistance training intervention[245]. The second study reported a drop from 15.9 to 4.7% of women considered to have clinical anxiety after a 16 week aerobic exercise intervention[247]. In summary, exercise interventions reduced symptoms of depression and anxiety in half of the reviewed studies. Due to the large variety of training interventions, conclusions cannot be made regarding the impact of a specific type or intensity of an exercise intervention, compared to another.

Table 8. Summary of studies identified for systematic review detailing participant and intervention characteristics, measures used and psychological outcomes.

Study	QA score[a]	Study Design	Exercise Intervention N (total N)	Participant Characteristics	Exercise Intervention(s) Characteristics	Comparison(s)	Measures	Main psychological findings from the exercise group
Arentz et al. 2017[241]	18	RCT	62 (122)	Age: 28.9 ± 5.6 years BMI: 35.2 ± 6.8 kg/m^2 PCOS diagnostic criteria: Rotterdam	Type: Aerobic Frequency: N/R (90-150mins/week) Intensity: 60-90% HR_{max} Duration: 12 weeks Supervision: Partial	Herbal medicine + lifestyle intervention	PCOSQ DASS-21	[b]Significant improvements were seen for all domains of the PCOSQ and DASS-21 in the herbal medicine plus lifestyle group, significant improvements only for infertility (p=0.001), weight (p=0.01), menstrual problems (p=0.02) and emotions (p=0.04) in the lifestyle only group. No significant changes in DASS-21 scores in the lifestyle only group.
Cooney et al. 2018[242]	13	Pilot RCT	8 (15)	Age: 32 (27-34) years BMI: 35 (31-40) kg/m^2 PCOS diagnostic criteria: NIH	Type: Aerobic Frequency: N/R (50-175mins/week) Intensity: N/R Duration: 16 weeks Supervision: None	CBT (weekly 30min CBT sessions) + lifestyle modification	PCOSQ CES-D	Clinically but not statistically significant improvements in all domains of the PCOSQ (≥0.5 point increase) with the exception of menstrual problems in the overall group. Statistically significant improvement in depression scores (p=0.01) in the overall group, with no differences between groups (p=0.68).
Costa et al. 2018[196]	16	RCT	14 (27)	Age: 27.6 ± 4.5 years BMI: 32 ± 4.2 kg/m^2 PCOS diagnostic criteria: Rotterdam	Type: Aerobic Frequency: 3/week Intensity: 60-85% HR_{max} Duration: 16 weeks Supervision: Full	No intervention control group	SF-36	Significant improvements in physical functioning (p=0.004), general health (p=0.012) and mental health (p=0.042) domain scores compared to baseline.
De Frène et al. 2015[244]	7	Single arm study	23	Age: 29 (5) years BMI: 33.7 (7.8) kg/m^2 PCOS diagnostic criteria: Rotterdam	Type: Aerobic Frequency: N/R Intensity: N/R Duration: 24 weeks Supervision: None	None	PCOSQ	Significant positive effect on total PCOSQ score (p=<0.001) as well as emotions (p=<0.01), weight (p=<0.001), body hair (p=<0.05) and infertility (p=<0.001) domain scores.
Ladson et al. 201 [161]	16	RCT	16 (26)	Age: 28.8 ± 4.6 years BMI: 38.3 ± 8 kg/m^2	Type: Aerobic Frequency: ≥2/week Intensity: N/R Duration: 26 weeks Supervision: Partial	Metformin + caloric restriction & exercise	PCOSQ	Significant improvements in emotions (p=0.008) and weight (p=0.002) domain scores.

				PCOS diagnostic criteria: NIH				
Lara et al. 2015[245] & Ramos et al. 2016[246]	12 13	Case-control	43	Age: 27.9 ± 5.3 years BMI: 27.9 ± 5.5 kg/m^2 PCOS diagnostic criteria: Rotterdam	Type: RT Frequency: N/R Intensity: 60-85% of 1RM Duration: 16 weeks Supervision: Full	Non-PCOS	HADS SF-36	Significant improvements in both anxiety (p=<0.01) and depression (p=<0.01) scores over time[245]. Significant improvements in SF-36 physical functioning domain (p=0.02)[246].
Legro et al. 2015[239] & Dokras et al. 2016[247]	18 12	RCT	49 (149)	Age: 28.6 ± 3.4 years BMI: 35.1 ± 4.6 kg/m^2 PCOS diagnostic criteria: Rotterdam	Type: Aerobic Frequency: 5/week Intensity: N/R Duration: 16 weeks Supervision: None	OCP or combined OCP + lifestyle intervention	PCOSQ SF-36 PRIME-MD	Significant positive effect on weight (p=<0.0001), infertility (p=<0.0001), menstrual problems (p=0.004) PCOSQ domains [239]. Significant improvement in general health (p=<0.05) and vitality (p=<0.05) domains of the SF-36. Significant decrease in the prevalence of anxiety (15.9 to 4.7%; p=0.02). Non-significant changes in the prevalence of depression (22.7 to 15.9%; p=0.17)[247].
Ribeiro et al. 2019[226] & Kogure et al. 2020[231]	17 17	RCT	CAT = 28, IAT = 29 (87)	CAT = Age: 29.1 (5.3) years BMI: 28.4 (5.6) kg/m^2 IAT = Age: 29.0 (4.3) years BMI: 28.7 (4.8) kg/m^2 PCOS diagnostic criteria: Rotterdam	Type: Aerobic Frequency: 3/week Intensity: CAT – 65-80% HR_{max} IAT – 70-90% HR_{max} Duration: 16 weeks Supervision: Full	No intervention control group	SF-36 HADS	CAT – Significant improvements in physical functioning (p=0.022), role physical (p=<0.001), general health (p=<0.001), vitality (p=<0.001), social functioning (p=<0.001), role emotional (p=<0.001) and mental health (p=<0.001) domains of the SF-36. IAT – Significant improvements in physical functioning (p=<0.001), role physical (p=0.027), general health (p=<0.001), vitality (p=0.001), social functioning (p=<0.001), role emotional (p=0.011) and mental health (p=<0.001) domains of the SF-36[226]. Significant improvements in anxiety and depression scores (p=<0.05) in both the CAT and IAT groups[231].
Stener-Victorin et al. 2013[243]	13	RCT	29 (44)	Age: 29.9 ± 4.4 years BMI: 28.1 ± 7.4 kg/m^2	Type: Aerobic Frequency: ≥3/week Intensity: N/R Duration: 16 weeks Supervision: None	No intervention control group & acupuncture group	MADRS-S BSA-S PCOSQ SF-36	No significant improvements in anxiety or depression. Significant improvements in PCOSQ domains for infertility (p=<0.05) and emotions (p=<0.001) and the role physical (p=<0.001) domain of the SF-36.

				Diagnostic criteria: Rotterdam				
Thomson et al. 2010[237] & Thomson et al. 2016[212]	12 12	RCT	Aerobic only = 15 Aerobic + RT = 20 (49)	Age: 29.3 ± 6.8 years BMI: 36.1 ± 4.8 kg/m^2 Diagnostic criteria: Rotterdam	Type: Aerobic only or combined aerobic & RT Frequency: 5/week Intensity: Aerobic = 60-80% HR_{max}, RT = 50-75% of 1RM Duration: 20 weeks Supervision: Partial	[c]Diet only (energy restricted, high protein diet)	CES-D PCOSQ	Significant improvement in depression scores in all groups (p=≤0.001) with no effect of treatment (p=0.86). Significant improvements in PCOSQ domain scores for emotions (p=≤0.001), weight (p=≤0.001), menstrual problems (p=≤0.001), and infertility (p=≤0.001) for all groups.
Vizza et al. 2016[238]	19	Pilot RCT	7 (13)	Age: 26.7 ± 7 years BMI: 41.3 ± 12.5 kg/m^2 Diagnostic criteria: Rotterdam	Type: RT Frequency: 2/week Intensity: N/R Duration: 12 weeks Supervision: Full	No intervention control group	PCOSQ SF-36 DASS-21	Significant improvements in the RT group compared to the control group for emotions (p=0.003), weight (p=0.04) and infertility (p=0.03) PCOSQ domains. Significant improvements in the RT group compared to the control group for physical functioning (p=0.02), vitality (p=0.02), social functioning (p=0.002), role emotional (p=0.009) and mental health (p=0.009) SF-36 domains. Significant improvements in the RT group compared to the control group for depression (p=0.01) and anxiety (p=0.03).

Data presented as mean ± SD or median (IQR).

1RM – One Repetition Maximum, BMI – Body Mass Index, BSA-S – Brief Scale for Anxiety, CAT – Continuous Aerobic Training, CBT – Cognitive Behavioural Therapy, CES-D – Centre for Epidemiological Studies Depression scale, DASS-21 – Depression, Anxiety and Stress Scale 21, HADS – Hospital Anxiety and Depression Scale, HR_{max} – Maximum Heart Rate, IAT – Intermittent Aerobic Training, MADRS-S – Montgomery Åsberg Depression Rating Scale, N/R – Not Reported, OCP – Oral Contraceptive Pill, PCOSQ – Polycystic Ovary Syndrome Questionnaire, PRIME-MD - Primary Care Evaluation of Mental Disorders, QA – Quality Appraisal, RCT – Randomised Controlled Trial, RT – Resistance Training, SF-36 – Short Form 36.

[a]Methodological quality score from the Downs and Black checklist. Possible range of scores 0-21.

[b]Data provided by author.

[c]All groups received the diet intervention.

3.5 Discussion

The current systemic review aimed to determine the effectiveness of exercise on mental health and health-related quality of life outcomes in women with PCOS. Exercise interventions appear to have positive effects on health-related quality of life and associated PCOS symptom distress as assessed by validated measures. The findings for mental health outcomes were less consistent, with a combination of positive and null findings regarding improvements in symptoms of anxiety and depression, although half of the included studies reported improvements in symptoms of anxiety and depression after an exercise intervention. The most common exercise program included various types of aerobic exercise of varying intensities, ranging from moderate to high intensity. Others included some form of resistance training program or a combination of the resistance and aerobic exercise. Intervention duration and the inclusion of supervised exercise also varied among interventions. There did not appear to be any common exercise characteristics that could explain differences in symptoms of depression and anxiety, PCOS associated distress, or improvements in health-related quality of life outcomes.

This systematic review expands on an existing review of seven trials that found exercise to be beneficial for improving health-related quality of life, depression and anxiety in women with PCOS[147]. Future research is required however to provide further evidence of these benefits. Observed improvements resulted from various types of exercise, exercise intensities and concurrent therapies, making it difficult to determine the components of the intervention that contribute to improved outcomes. Studies included in this review were largely heterogeneous with varying interventions, concurrent therapies, sample sizes, study designs, comparator groups and methodological quality making the independent effect of any particular type of exercise intervention or characteristics difficult to assess. These variations prevented a meta-analysis from being conducted and limited the ability to form conclusions about the effectiveness of exercise on mental health and health-related quality of life in women with PCOS. In addition, poor reporting of exercise characteristics and the large variety of intensities, duration and frequency, limited the capacity to formulate more specific exercise recommendations for promoting mental health and health-related quality of life in women with PCOS, which limits the translation of these research findings into clinical practice.

The current international evidence based guidelines for the assessment and management of PCOS states that psychological factors, including anxiety and depression, should be screened, assessed and managed[32]. It is important to ensure positive well-being to increase quality of life but also to assist in promoting engagement and adherence to lifestyle interventions. Adherence to exercise interventions has been reported to be low in clinical settings among women with PCOS[161,193,199], it is crucial that future studies report measures of adherence to determine interventions that are more effective in maintaining the interest and enjoyment of participants. Very few of the studies reviewed in the current research reported on adherence and compliance to the exercise intervention. In the studies that did report on adherence, supervised sessions had a much greater attendance rate and could ultimately contribute

to larger improvements in physical and mental health. Future studies should consider commencing with supervision of all exercise sessions, in order to address initial exercise engagement, followed by a tapering of supervision to include planned, unsupervised exercise, while concurrently promoting and encouraging self-sustainability, to promote long-term maintenance of physical activity, following the completion of the intervention.

Given that previous research shows that time limitation is reported as the biggest barrier to exercise participation both in a general population and among women with PCOS[89], the alternative of a vigorous intensity or high intensity interval training may provide a solution to this barrier. Some research also suggests that individuals experience greater enjoyment when partaking in high intensity exercise compared to continuous moderate intensity exercise[121,139,250]. Significant improvements following high intensity interval training have been reported in systematic reviews/meta-analyses for anxiety, depression[121,251] and quality of life[152,252] outcomes, however, these benefits have only been reported in patients with chronic conditions other than PCOS. In women with PCOS, there is limited evidence to suggest that high intensity exercise can result in greater health improvements compared to moderate intensity exercise[152]. However the potential effects of high intensity exercise, in particular, on mental health and health related quality of life outcomes has yet to be thoroughly investigated. Thus, more studies examining the effects of high intensity exercise on mental health outcomes in women with PCOS are needed.

Half of the studies included in this review reported significant reductions in anxiety and depression symptoms after an exercise intervention, which is a clinical meaningful outcome, especially when considering that the reviewed interventions were primarily aimed at improving the physical health, rather than the mental health, of participants. Designing future interventions with a mental health informed rationale for the exercise intervention may improve engagement and therefore result in greater mental health benefits. For example, multi-component interventions that also include additional therapies such as cognitive behavioural therapy could be considered useful and may aid to increase adherence, retention, engagement as well as the maintenance of a healthy lifestyle to improve all health outcomes in women with PCOS[32]. Although this review was focused on the effects of exercise only, one of the included studies included examined a multi-component intervention that included cognitive behavioural therapy, and reported clinically significant improvements in all domains of the PCOSQ and depression symptom scores[242]. Therefore, further examination of multi-component interventions could provide useful information for improving mental health for women with PCOS.

In addition, many of the included trials excluded women who were taking medications for the treatment of clinical anxiety and depression. Given the high prevalence of these two mental health conditions in women with PCOS, excluding these women does not adequately represent the population, and therefore, perhaps the true benefits of exercise for these women. We could benefit greatly from future research

that examines the effect of exercise in women with PCOS, who display elevated baseline levels of anxiety and depression. Such research would provide greater insight regarding the efficacy of exercise for improving mental health and health-related quality of life in women with PCOS and would increase generalisability and applicability to real-world clinical practice.

3.5.1 Strengths and limitations

This review builds on existing knowledge and provides preliminary data to support the inclusion of exercise to manage and improve mental health and health related quality of life outcomes in women with PCOS. A strength of this review is that it follows the PRISMA guidelines, including double screening of articles, data extraction and quality appraisal of each publication. This review was limited by large variations and poor reporting of exercise characteristics in the included primary studies. This hindered us from being able to conduct a quantitative synthesis of results and limited our ability to form strong conclusions about the effectiveness of exercise, and particular exercise characteristics, on mental health and health-related quality of life in women with PCOS. Future studies should endeavour to adequately report all intervention characteristics, including frequency, intensity, type, format and session duration of exercise interventions and as well as reporting both adherence and compliance to the exercise intervention inform future research.

3.5.2 Conclusions

This review found that exercise results in both clinically meaningful and statistically significant improvements in health-related quality of life in women with PCOS. Exercise also appears to have some benefit for improving symptoms of common mental health concerns with half of studies reporting significant improvements in symptoms of depression and anxiety. However, the heterogeneity of included studies, including methodological quality, and the poor reporting of the characteristics of exercise interventions delivered, limited the ability to make conclusions regarding the effectiveness of specific types of exercise. This also limited the ability to conclude the impact of specific exercise characteristics including intensity, frequency and type of exercise for improving mental health in women with PCOS. It is vital to employ strategies that can both reduce symptoms of anxiety and depression and increase adherence to interventions. Therefore, multi-component interventions that integrate psychological treatment with exercise and address the complex physical and mental health concerns of women with PCOS have the potential for improving mental health outcomes. Future studies should aim to address barriers to exercise participation and determine which intervention characteristics are associated with increased engagement and maintenance of exercise for the promotion of mental health in women with PCOS

Chapter 4.0. The effectiveness of high-intensity interval training on metabolic, reproductive and mental health in women with polycystic ovary syndrome: study protocol for the iHIT- randomised clinical trial.

This protocol paper has been published in Trials (Appendix B), however, the original publication has been amended to reflect the study that I conducted for my PhD. This chapter differs from the original protocol paper in the following ways;

- A no-intervention control group was not included due to difficulties with recruitment.
- The protocol paper included biopsies and various analyses including western blotting and DNA methylation which was not part of my PhD work and was therefore removed.
- The protocol paper stated that we would be doing intention-to-treat analysis which included all randomised participants, and that we would be using a mixed model repeated measures analysis. Instead we decided to use linear mixed models for this work. Mixed models without any ad hoc imputation has been found to provide more powerful tests than mixed model analysis with ad hoc imputations[253].
- The protocol paper states that heart rate reserve (HRR) would be used to quantify exercise intensity, however, I decided to use HR_{peak} as it is a simpler method, has good validity, is more commonly used and accepted and therefore, deemed more appropriate.
- Six and 12 month follow ups were also included in the protocol. These time points were not collected or analysed as part of my thesis due to the time schedule and difficulty contacting participants.

4.1 Abstract

Background: Polycystic ovary syndrome (PCOS) is a reproductive-metabolic condition. Insulin resistance is a hallmark of PCOS and is related to increased hyperandrogenism that drives inherent metabolic, reproductive, and psychological features of the syndrome. Insulin resistance in women with PCOS is managed by weight loss, lifestyle interventions (i.e. exercise, diet) and insulin-sensitising medications. This manuscript describes the protocol of our study evaluating the effectiveness of high-intensity interval training (HIIT) compared to moderate-intensity continuous training (MICT) for improving cardio-metabolic, reproductive and mental health in overweight women with PCOS.

Methods: We will employ a two arm, parallel-group, randomised clinical trial recruiting thirty women diagnosed with PCOS, aged between 18 to 45 years and with a body mass index (BMI) greater than 25kg/m^2. Following screening and baseline testing, women will be randomised by simple randomisation procedure using computer generated sequence allocation to undergo one of two 12-week supervised interventions: either HIIT or MICT at a 1:1 allocation ratio. The primary outcome for this trial is to measure the improvements in aerobic capacity and metabolic health; specifically changes in insulin sensitivity in response to different exercise intensities. Baseline and post intervention testing include; anthropometric measurements, cardiorespiratory fitness testing, reproductive hormone profiles (anti-müllerian hormone and steroid profiles), metabolic health, health-related quality of life, and mental health questionnaires, objective and subjective lifestyle monitoring. Reporting of the study will follow the CONSORT statement

Discussion: This trial aims to determine whether HIIT is more effective than standard MICT to advance the understanding of PCOS management and provide insight into the optimal exercise intensity for improved cardio-metabolic outcomes. Secondary outcomes will include the impact of different exercise protocols on reproductive hormone profiles, mental health and health related quality of life.

Trial registration: Trial is registered with the Australian New Zealand Clinical Trials Registry (ACTRN12615000242527).

4.2 Background

Polycystic ovary syndrome (PCOS) is a major public health concern affecting 6-10% of reproductive aged women worldwide[1]. Insulin resistance is strongly implicated in the aetiology of PCOS and is associated with the reproductive and metabolic consequences of the syndrome[30,59,79,218,254,255]. Insulin resistance in PCOS is inherent and has been demonstrated to be independent of body mass index (BMI), yet can be exacerbated by obesity[186,218], although the mechanisms remain unclear. Lifestyle interventions (diet, exercise and/or behavioural) and weight management is the first line therapy[32] for women with PCOS as they improve the clinical symptoms by increasing insulin sensitivity in these women[2,79].

Moderate intensity aerobic exercise improves metabolic and reproductive features, body composition and psychological well-being in overweight women with PCOS[79,149,153]. However even after exercise interventions a higher level of insulin resistance prevails in women with PCOS compared to women without PCOS indicating further research is warranted[79]. High-intensity interval training (HIIT) is a popular fitness program that encompasses short bouts of high intensity exercise interspersed with active recovery[256]. HIIT has been shown to be acceptable and safe and may assist in addressing barriers reported in women of reproductive age including time limitations and competing commitments and PCOS-specific barriers such as low confidence and physical limitations[89,147]. HIIT is enjoyable[79,118,121] and has been found to have a superior effect on insulin resistance, cardiovascular risk factors and all-cause mortality amongst other predominantly male and older clinical populations[128-130,196]. Despite this, there is limited comprehensive research on the efficacy of different exercise intensities and optimal exercise intensity for improved cardio-metabolic outcomes, reproductive hormone profiles, mental health and health related quality of life following exercise in PCOS.

Compared to recommended lower-intensity regimes, more positive metabolic health outcomes are now being reported for HIIT including improved glycaemic control and cardio-respiratory fitness in clinical populations and amongst women with PCOS[117,121,133,157,196]. Only one RCT has been conducted in women with PCOS to assess the benefits of HIIT to which they compared a resistance-training program and a control group[157]. After 10 weeks, they found improvements in insulin sensitivity, high-density lipoprotein (HDL) cholesterol and a decrease in fat percentage[157]. A cross-sectional study conducted by Greenwood et al. was the first to examine the effect of exercise of varying intensities amongst women with PCOS[117]. The results of their study supported superior health benefits of vigorous exercise including; a lower BMI, and HOMA-IR, higher levels of HDL, and SHBG when compared with moderate exercise in women with PCOS, independent of age, BMI and total exercise volume[117]. Specifically, it was found that 60 minutes of vigorous activity per week was associated with a 22% reduction in odds ratio of metabolic syndrome (OR 1.11; 95% CI, 1.04, 1.18)[117]. Although these studies provide positive results in favour of high intensity exercise, more objective data and further investigation is required to find the optimal intensity in promoting the greatest health benefits for

women with PCOS. Therefore, the aim of this protocol paper is to detail an exercise intervention that explores the clinical effectiveness HIIT in comparison to moderate-intensity continuous training (MICT) on: metabolic (body composition and insulin sensitivity), reproductive (anti-müllerian hormone [AMH] and steroid profiles) and mental health (depression and health related quality of life) in women with PCOS.

4.3 Methods

4.3.1 Hypothesis

Based on previous literature, we hypothesise that HIIT will result in greater health outcomes such as; greater insulin sensitivity, increased lean muscle mass, decreased androgens, increased SHBG and improved mental health, and health-related quality of life compared to MICT.

4.3.2 Design

We will employ a two-arm, parallel-group, randomised clinical trial (Figure 10) and recruit 30 women diagnosed with PCOS from the community and health clinics that are located in Melbourne Australia, via advertisement flyers in local media, information boards in clinics and from PCOS websites. They will be randomised to undergo one of two 12-week supervised interventions: either HIIT or MICT.

	Enrolment	**STUDY PERIOD**					
TIMEPOINT	$-t_1$	**Baseline (1-14 days)**	***Randomisation***	***1-4 weeks***	***5-8 weeks***	***9-12 weeks***	***Post (2-7 days)***
ENROLMENT:							
Eligibility screen	X						
Informed consent	X						
Medical History and confirmation of diagnosis	X						
Allocation			X				
INTERVENTION							
HIIT				◆—	—	—◆	
MICT				◆—	—	—◆	

ASSESSMENTS:							
GXT+ECG		X					X
DXA scan		X					X
Insulin Clamp		X					X
Questionnaires		X					X
Lifestyle Intervention		X					

Figure 10. SPIRIT flow diagram of randomised clinical trial.

GXT, graded exercise test; ECG, electrocardiogram; Insulin clamp, euglycaemic-hyperinsulinaemic clamp; DXA, dual energy x-ray absorptiometry; HIIT, high-intensity intermittent training; MICT, moderate-intensity continuous training.

4.3.3 Inclusion criteria

Women aged 18-45 years with a BMI greater than 25kg/m^2 and diagnosed with PCOS using the Rotterdam criteria[13] will be recruited for this study. Their medical practitioner will have previously diagnosed PCOS and the research endocrinologist will confirm this prior to participation in the study. Diagnosis of PCOS by the Rotterdam criteria requires confirmation of two of the following (i) oligo- or anovulation (ii) clinical (hirsutism and acne) and/or biochemical hyperandrogenism (iii) polycystic ovaries on ultrasound and exclusion of other causes of hyperandrogenism[13]. Features of PCOS will be recorded to allow phenotyping as recommended by the National Institutes of Health[257].

4.3.4 Exclusion criteria

Exclusion criteria will be other causes of menstrual disturbance and hyperandrogenism, known cardiovascular (cardiac arrhythmias), uncontrolled asthma, uncontrolled hypertension (resting systolic blood pressure >160 mmHg and/or diastolic blood pressure >105mmHg), bleeding disorders, skin or anaesthetic allergies, musculoskeletal injuries that may be aggravated by the exercise protocol, pregnancy, type 1 or 2 diabetes, or taking anti-hypertensive, insulin sensitising, anti-obesity or oral contraception medications.

4.3.5 Screening

Prospective participants will be screened to check eligibility and to assess potential risks of adverse events during exercise. If identified as having multiple risk factors (family history [first-degree] of cardio-metabolic risk factors, chronic conditions [that do not exclude from participation] but could interfere with testing or exercise, or have a BMI >40kg/m^2) exercise clearance will be required by their general practitioner. A member of the research team will obtain written informed consent from all participants prior to participation in the study.

Data Monitoring: Details of procedures for data management have been reviewed and approved by the Victoria University Human Research Ethics Committee (Reference- HRE15-298). All electronic data will be stored on encrypted hard drives or password-protected computers. Hard copies of any data will be kept in locked filing cabinets in a secure office. All questionnaire data will be de-identified.

Harms: All adverse events associated with the study, or that accrue whilst participating in the study, will be recorded. All adverse events will be reported to the Victoria University Human Research Ethics Committee.

Auditing: No formal auditing process, is proposed for the current trial. The principal investigator, Professor Nigel Stepto, is responsible for the integrity of the current trial and has extensive experience as a lead investigator in numerous human clinical trials.

4.3.6 Ethics

The study has been approved by the Victoria University Human Research Ethics Committee (Reference- HRE15-298) and is registered with the Australian New Zealand Clinical Trials Registry (ACTRN12615000242527). Any modifications or changes to the protocol will be provided in writing to the Victoria University Human Research Ethics Committee for approval. Reporting of the study will follow the CONSORT statement[258].

4.3.7 Baseline assessment

Baseline assessment will involve three sessions. Two sessions will be required to complete a symptom-limited graded exercise protocol (the first session being a familiarisation of the test). In the 7 days preceding session three, participants will wear an Actigraph^{TM} accelerometer to establish levels of habitual physical activity. The third baseline session will involve a euglycaemic-hyperinsulinaemic clamp and a dual X-ray absorptiometry scan (DXA) [GE Lunar iDXA]. Participants will also be asked to complete validated questionnaires to assess physical activity (International Physical Activity Questionnaire [IPAQ])[259], quality of life (SF-36 & PCOSQ)[102,260], depression, anxiety, and stress (Depression Anxiety Stress Scale [DASS-21])[261] and a 3-day food diary. Participants will monitor their menstrual cycles using a menstrual diary and which will be used to assess menstrual cyclicity throughout the study.

4.3.8 Randomisation and blinding

Following baseline testing, participants will be randomised to one of two 12-week interventions; HIIT, or MICT (details of the two treatment arms in Table 9). An independent biostatistician will randomise subjects by a simple randomisation procedure using computerised sequence generation with an allocation ratio of 1:1. Two tables based on BMI (<35kg/m^2 or >35 kg/m^2) will be created. As exercise interventions cannot be blinded from participants and staff (accredited exercise physiologists) implementing the intervention, only staff undertaking sample analysis and endpoint data processing will be blinded to group allocation.

4.3.9 Intervention

Every participant will receive a menstrual diary to monitor menstrual cycles. As recommended by the Australian evidence-based PCOS guidelines and International evidence-based PCOS guidelines, all women will receive evidence-based behaviour change coaching[2,32] based on social cognitive theory and the behaviour change wheel[262]. This will involve a 2-hour session including goal setting and goal striving, education regarding the importance of physical activity and healthy eating, and education on effectively using media and resources for healthy eating and diet[263].

4.3.10 Exercise interventions

Exercise will be conducted on stationary bikes in an individual setting. Exercise intensities will be prescribed and monitored using heart rates (% of peak heart rate [HR_{peak}]), as described in Table 9. Sessions will be conducted at Victoria University fitness centres/exercise clinics under the supervision of accredited exercise physiologist [79,153,155,237,264,265]. Adherence and compliance will be determined from supervised exercise session attendance and completion of prescribed exercise (duration and intensity [% HR_{peak}]) respectively. Data from participants with less than 75% adherence will be included in the intention to treat analysis only. Any musculoskeletal injuries or changes in health status will be recorded via a provided web-based diary.

Volume matching and training progression: The HIIT and MICT intervention arms will be matched for training volume (MET.min/week) and progressed weekly by manipulating session duration and intensity. Both HIIT and MICT groups will progress from 312 MET.min/week in week 1 to 530 MET.min/week in weeks 6-12, meeting exercise guidelines[266]. Exercise sessions will include warm-up and cool down protocols, and will be adjusted weekly to individual capabilities and training adaptations[79,124]. See Table 9 for details.

Table 9. Details of the two treatment arms.

Intervention	Details
MICT	Minimum physical activity recommendations (150 min per week)[266], in three supervised 50 min sessions/week of continuous moderate intensity exercise sessions of cycling at 60-70% HR_{peak} (~3.5 METs)
HIIT	Minimum vigorous physical activity recommendations (~75 min per week)[266], in three supervised sessions/week of HIIT exercise (cycling). Based on existing literature, pilot data[267] and patient consultation, we will use a practical weekly training program encompassing two successful HIIT protocols[118,121,268]: • Two sessions/week of short constant load cycling of 12 x 1 min at 90-100% HR_{peak} (>9METs; [1 min HIIT]) with 1 min active recovery. • One session/week of cycling 6–8 x 4 min at >90% HR_{peak} (>9 METS; [4 min HIIT]) with 2 min active recovery.

MICT, moderate intensity continuous training; HIIT, high-intensity interval training; min, minutes; METs, metabolic equivalent task; HR_{peak}, peak heart rate.

Preventing and managing injury: Absolute risk of cardio-vascular disease (CVD) in this young female population is low. However, participants will be screened with appropriate clinical monitoring during all exercise testing (ECG), and training sessions (heart rate and blood pressure monitoring) with individualised training prescription and progression reducing the risks of injuries and adverse events[79,153,155,237].

4.3.11 Post-intervention assessment

After 48 hours but within four days of the final exercise session (HIIT or MICT) the euglycaemic-hyperinsulinaemic clamp, questionnaires (SF-36, PCOSQ, IPAQ and DASS-21), body composition measures (DXA, weight, waist and hip circumferences) and graded exercise tests will be repeated. Women will be asked to abstain from training or physical exercise during this period. An accelerometer will be given 7 days before post-intervention testing to assess exercise in this period. They will again be asked to complete a 3-day food dairy.

4.3.12 Outcome measurements

Outcome measures will be taken pre-intervention and post-intervention (Figure 10). The primary outcome is the change in VO_{2peak} the two interventions. Secondary outcomes include reproductive health via hormone profiles (AMH and steroid profiles), mental health and health related quality of life, body composition and cardiorespiratory fitness.

4.3.13 Data collection and analysis

Anthropometric assessment

Following an overnight fast, participants will be weighed lightly clothed and without shoes (HW-PW200, associated scales services). Height will be taken without shoes using a calibrated stadiometer (Proscale Inductive Series I, Accurate Technology Inc.) and BMI calculated [weight (kilograms)/height squared (squared metres)]. Waist and hip circumference measurements will be taken[269] and the waist to hip ratio calculated. Fat mass, abdominal fat mass and fat free mass will be assessed using DXA (iDXA GE Lunar Prodigy scanner) and analysed by a qualified operator.

Fitness parameters

Cardiorespiratory fitness via peak oxygen uptake (VO_{2peak}) will be assessed using a symptom-limited graded exercise protocol on a cycle-ergometer[267]. The test will start after a 5-minute period at rest. The protocol will consist of three minutes stages at an intensity of 25 watts (W), 50W, 75W respectively, and then an increased by 25W every minute. The test will be terminated objectively when:

- Participant can no longer sustain a pedal rate greater than 60rpm;
- Respired expiratory rate (RER) reaches 1.1 or greater;
- Appearance of clinical signs or symptoms of metabolic or cardiorespiratory abnormalities;
- Patient wishes to stop.

Expired respiratory gases will be collected by the COSMED cardio pulmonary exercise test system breath-by-breath connected to automated gas analysers. The system will be calibrated before conducting each test using Hans Rudolph syringe and gases of known O_2 and CO_2 content (BOC gas). As a precautionary measure, 12-lead ECG will be monitored to minimise adverse events from undiagnosed cardiac arrhythmias during this testing.

Physical activity and diet

Dietary habits will be assessed by a consecutive 3-day food diary before baseline and post intervention testing. Food diaries will be analysed by FoodWorks® (Xyris) for total energy and macronutrients. Self-reported physical activity questionnaires (IPAQ)[259] will be used to assess physical activity behaviour pre- and post-intervention.

Mental health measures

Mental health and health-related quality of life measures will be completed pre- and post-intervention to identify any changes in mental health and wellbeing after an exercise intervention and to determine the relationship with other outcome measures (DASS, SF-36 and PCOSQ) [260,261].

Euglycaemic-hyperinsulinaemic clamp

Participants will undergo a euglycaemic-hyperinsulinaemic clamp to measure insulin sensitivity[30,198,270]. Human insulin (NovoNordisk ActRapid) will be infused at a constant rate [40mU/min/m^2] while a variable rate glucose solution is infused to meet the target of 5mmol.L^{-1} blood glucose in the last 30 minutes of the clamp. During the clamp, one hand will be warmed to improve blood flow for blood sampling. Blood samples will be taken every 5 minutes to monitor circulating blood glucose and an additional blood sample will be taken every 30 min during the clamp for insulin analysis. To reduce the risk of low potassium levels (hypokalaemia) participants will be given a single dose (600mg) of slow-release potassium before the commencement of the insulin clamp.

Pathology analysis

Glucose will be measured by using an automated analyser (YSI 2300 STAT Plus). Insulin concentrations will be determined by radioimmunoassay according to manufacturer instructions (HI-14K, EMD Millipore, UniQ PIIINP RIA #68570, UniQ PINP RIA #67034, Orion Diagnostica). Standard clinical pathology testing including; lipid profiles, haemoglobin A1c (HbA1c), AMH will be performed by a Health Pathology service. Serum steroid profiles including testosterone, dihydrotestosterone, estradiol, androstenedione and progesterone will be determined by LC-MS mass spectrometry.

4.3.14 Statistical methods and determination of sample size

The HIIT group will be compared with the MICT group using a planned contrast of change from baseline to the week 12 endpoint using linear mixed-model analyses. Stratification variables will be evaluated and retained in analyses where they are measured as significant or quasi-significant.

Transformation of scores, including categorisation, may be undertaken to meet distributional assumptions and accommodate outliers. Comparisons of changes in insulin sensitivity scores from baseline to other time points will be undertaken as secondary analyses. Data will be analysed by R studio version 4.0.2 and significance will be accepted when $p<0.05$. Where required p-values from the statistical analysis will be adjusted for multiple comparisons using the false discovery rate[271] and statistical significance will be accepted when false discovery rate (FDR) $q<0.1$.

Using the primary outcome of VO_{2peak} (as measured by the graded exercise test) with an effect size of 0.25[124], we require 12 participants per group to achieve a power of 80% ($\alpha=0.05$). With an approximate attrition rate of 30% based on previous research, we will aim to recruit 30 participants[79,153].

4.4 Discussion

PCOS is under-recognised by health professionals and leaves women on track for a plethora of chronic conditions ranging from anxiety, and depression to diabetes, subfertility, CVD and stroke[1,8,20,22]. Despite affecting ~1 million Australian women and costing over $800M nationally each year[2,25,272] there is no optimal treatments.

Overall HIIT in PCOS promises greater metabolic benefits with demonstrated acceptability and safety[117]. It has the potential to address general and PCOS specific barriers (low confidence and physical limitations) to standard exercise participation[89,147]. However, evidence from prospective studies comparing volume-matched HIIT and standard moderate-intensity exercise for efficacy and enjoyment is lacking in women generally and more importantly in women at high risk of metabolic disease (i.e. PCOS).

Here, we describe the protocol of a study evaluating the effectiveness of a practical allied health supervised 12-week HIIT or MICT program on cardio-metabolic, reproductive, and mental health in overweight women with PCOS. This trial aims to demonstrate the efficacy of HIIT in comparison to MICT to inform vital large-scale clinical trials and ultimately best clinical practice in treatment of PCOS. It will advance the understanding of PCOS management by providing insights into the best exercise intensities to improve insulin sensitivity. Further helping to address the limitations highlighted by the recently released international guidelines in PCOS lifestyle management[32]. Finally, we will explore the impact of different exercise protocols on reproductive health (anti-müllerian hormone and steroid profiles), mental health and health related quality of life. The impact of this work is likely to be significant due to the unprecedented public health challenge of PCOS in young Australian women for which we currently have no optimal treatment.

Chapter 5.0 Efficacy of high-intensity interval training compared to moderate-intensity continuous training for improving cardio-metabolic health in women with polycystic ovary syndrome

The following three chapters detail the results from the large clinical trial described in Chapter 4, assessing the efficacy of high-intensity interval training (HIIT) in comparison to moderate-intensity continuous training (MICT), with results dividing according to cardio-metabolic (Chapter 5), reproductive (Chapter 6) and mental health and health-related quality of life outcomes (Chapter 7).

5.1 Abstract

Context: Polycystic ovary syndrome (PCOS) is a common and complex endocrinopathy with metabolic manifestations, carrying a major health burden. Exercise training is recognised to improve clinical outcomes in women with PCOS, but little is known about the most effective exercise intensity for improving health outcomes.

Objective: To investigate the effectiveness of 12 weeks of high-intensity interval training (HIIT) in comparison to moderate-intensity continuous training (MICT) on aerobic capacity and insulin sensitivity in women with PCOS.

Methods: Twenty-four overweight women with PCOS aged 18-45 years were randomly assigned to 12 weeks of either MICT (60-75% HR_{peak}, n=11) or HIIT (>90% HR_{peak}, n=13). The primary outcomes were change in aerobic capacity (assessed using VO_{2peak}) and insulin sensitivity (assessed by euglycaemic-hyperinsulinaemic clamp) following exercise.

Results: Both HIIT and MICT improved VO_{2peak} (HIIT; 23.4 ± 10.1%, P <0.001 and MICT; 14.0 ± 9.3%, P < 0.001), however, the HIIT group had a significantly greater improvement compared to MICT (*P* = 0.004). HIIT increased the insulin sensitivity index both compared to baseline (49.1 ± 38.2%; P = 0.014) and to MICT (P = 0.046). Overall, the improvement in VO_{2peak} was associated with the improvement in insulin sensitivity (P = 0.003, R^2 = 0.38).

Conclusion: Supervised HIIT is effective and offers greater improvements in aerobic capacity and insulin sensitivity than MICT in women with PCOS. HIIT should be considered as an effective strategy to promote health and reduce the cardio-metabolic risk in this population.

5.2 Introduction

Polycystic ovary syndrome (PCOS) is a common and complex condition, affecting 8-13% of women of reproductive age[32]. PCOS has a diverse clinical manifestations with varying impact on metabolic, reproductive and psychological health[20,21]. PCOS is characterised by androgen excess, ovulatory dysfunction and polycystic ovaries, with a combination of at least two of the three required for diagnosis[13]. Insulin resistance also appears to be a key feature of the condition, detected in up to 75% of women as measured by euglycaemic-hyperinsulinaemic clamp[4]. This results in women with PCOS being at high-risk for developing T2DM[3]. 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[32,141]. A minimum of 150 minutes per week of moderate-intensity exercise or 75 minutes per week of vigorous intensity exercise is currently recommended[32]. It is unclear whether these two exercise prescriptions provide equal improvements in health in women with PCOS.

High-intensity interval training (HIIT) comprises repeated, short bouts of high-intensity exercise (≥90% HR_{max}[114]) interposed with periods of rest or low intensity exercise[118]. HIIT appears to be safe if performed under the supervision of an exercise physiologist and following robust clinical screening[273], and has the potential to address the time limitation barrier to exercise participation[121]. Importantly, HIIT has been reported to induce greater increases in peak oxygen uptake (VO_{2peak}) when compared to moderate-intensity exercise[274]. VO_{2peak} is an important marker of aerobic capacity and a higher VO_{2peak} is associated with a lower mortality rate, independent of age, sex, race and comorbidities[275].

HIIT has been suggested to improve cardio-metabolic health to a greater extent than standard moderate-intensity exercise recommendations in those with chronic conditions[121,276]. However, the majority of research comparing the benefits of varying exercise intensities in people with chronic cardio-metabolic conditions has been conducted in predominantly male or older populations[132]. Little research has been conducted in women and more specifically in women with PCOS[277]. A recent review 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[152]. Therefore, there is a need for robust exercise intervention studies examining the impact of exercise intensity in women with PCOS. This will aid in the identification of the optimal exercise intensity for improving health and contribute to more specific exercise guidelines for women with PCOS. As such, the primary aim of this study was to test the hypothesis that HIIT is superior to moderate-intensity continuous training (MICT) for improving aerobic capacity and insulin sensitivity in overweight women with PCOS. Secondary outcomes included changes in weight, lean and fat mass and lipid profiles.

5.3 Methods

5.3.1 Study design

This study is a two-arm randomised clinical trial and was conducted at Victoria University in Melbourne, Australia, from June 2016 to October 2019. The study was approved by the Victoria University Human Research Ethics Committee and was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12615000242527). All women provided written informed consent prior to participation.

5.3.2 Participants

Women were recruited through community and social media advertisements. Inclusion criteria were women aged 18-45 (pre-menopausal), with a BMI greater than 25 kg/m^2, sedentary, and with diagnosed PCOS. PCOS was diagnosed according to the Rotterdam Criteria[13], 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; (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 sensitisers, dietary supplements, weight loss medication or hormonal contraceptive medications in the three months prior to enrolment were excluded.

5.3.3 Study protocol

After the completion of baseline testing, participants were randomised to a 12-week HIIT or MICT intervention. Randomisation was completed by an independent biostatistician by a simple randomisation procedure using computerised sequence generation at an allocation ratio of 1:1. To ensure equal proportions of body mass index (BMI) in each arm, randomisation was stratified according to BMI brackets (<35 kg/m^2 or >35 kg/m^2). Following familiarisation, at baseline and post-intervention, participants performed a graded exercise test to assess VO_{2peak}, a dual-energy x-ray absorptiometry (DXA) to assess body composition and a euglycaemic-hyperinsulinaemic clamp, to assess insulin sensitivity[68]. Lipid profiles were also assessed at baseline and post-intervention.

5.3.4 Euglycaemic-hyperinsulinaemic clamp

Insulin sensitivity was assessed using the euglycaemic-hyperinsulinaemic clamp described by DeFronzo *et al*[68]. Clamp timing was standardised to 48 hours after exercise, and included a standardised high-carbohydrate diet before an overnight fast. For participants with regular menstrual cycles (21-35 days[32]), 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. Insulin (Actrapid, Novo Nordisk, Bagsværd, Denmark) was infused at

a constant rate of 40 mU/min/m^2 for 120 minutes, with glucose (20%) infused at a variable rate to meet the target blood glucose of 5 mmol/L. Blood glucose was assessed every 5 minutes using a glucose analyser (YSI 2300 STAT Plus, YSI *Inc., Ohio, USA)*. Glucose infusion rates (GIR) were calculated during steady state, defined as the last 30 minutes 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[278]. Plasma insulin levels were determined via Radioimmunoassay kit (Human Insulin-Specific RIA, HI-14K, Millipore, USA). Insulin sensitivity index (ISI) was calculated using the following formula: (GIR (mg/lean body mass [kg]/min)/steady state insulin) * 100.

5.3.5 Lipids and HbA1c

Total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and glycosylated haemoglobin (HbA1c) were collected under fasting conditions, stored and batch analysed in an accredited pathology laboratory at Monash Health, Melbourne, Australia using standard protocols as previously described[279].

5.3.6 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 minutes of cycling at 25, 50 and 75 watts, respectively, followed by increases of 25 watts each minute thereafter until volitional exhaustion[267]. 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 two standard deviations 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).

5.3.7 Anthropometric measurements

Lean and fat mass were assessed using total-body DXA scans (GE Lunar iDXA, GE Healthcare, Wisconsin, 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 squared[m]). Waist and hip circumferences were measured as described previously[269] and the waist to hip ratio was calculated (waist circumference [cm]/hip circumference [cm]).

5.3.8 Physical activity and diet

Participants were instructed to maintain their usual diet and exercise habits throughout the duration of the intervention. Dietary habits were assessed by a consecutive 3-day food diary prior to baseline testing and following post-intervention testing. Food diaries were analysed by FoodWorks® (Xyris) for the major food groups, total energy and macronutrients. Self-reported physical activity questionnaires (IPAQ)[259] were used to assess physical activity behaviour pre- and post-intervention.

5.3.9 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[32] and were matched for training volume (chapter 4). The HIIT intervention included twice weekly sessions of 12, 1 minute intervals at 90-100% peak heart rate (%HR_{peak}), separated by 1 minute of active recovery at a light load and one weekly session of eight, 4 minute intervals at 90-95%HR_{peak}, separated by a 2 minute light load, activity recovery. The MICT consisted of three sessions per week of 45 minutes 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 minute warm-up at 60-65%HR_{peak} and ended with a 5 minute 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.

5.3.10 Statistical analysis

Data are presented as mean ± SD and median and percentiles for boxplots. All data was analysed using R studio version 4.0.2. Data were assessed for normality using Shapiro-Wilk test and log transformed where necessary. Linear mixed models were used to determine the effect of exercise intensity (group) over time and to determine the interaction between timepoint and group (between-group differences). The unique participant codes were used as a random effect to account for repeated measures and age was included as a fixed effect. For models examining markers of insulin sensitivity, fat mass was also included as a fixed effect. A simple linear regression model adjusted for age was used to determine whether delta changes in VO_{2peak} were associated with delta changes in insulin sensitivity index as a result of exercise using the pooled data from all participants. P values of main outcomes were adjusted for multiple comparisons using the false discovery rate (FDR)[271]. P values were deemed statistically significant when <0.05. The following packages were used in our analysis; *lme4*[280], *lmerTest*[281] and *tidyverse*[282].

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[124]. The resulting sample size was 12 per group with 80% power and an alpha of 0.05 (G*Power 3.1.9.4)[283].

5.4 Results

Thirty-one women completed the baseline assessments, of whom 24 completed the 12-week intervention (HIIT = 13, MICT = 11). Two women withdrew after being assigned to the MICT group with no reason cited. Two participants dropped out during the MICT intervention due to changes to their work schedule and one withdrew due to moving interstate. One participant withdrew from the HIIT intervention due to an injury sustained at work and another became pregnant (Figure 11). No adverse events occurred during the study period. Exercise intervention adherence was similar across the two groups (HIIT = 93.9 ± 3.0%, MICT = 92.0 ± 4.8%, $P > 0.05$) and no participants fell below the required >75% adherence. There were no significant differences in body composition, fitness or insulin sensitivity markers at baseline between participants who dropped out and those that completed the study. There were no significant differences in baseline data between the two groups, except age, which was adjusted for in all analyses. There were also no significant changes in dietary or exercise behaviour ($P > 0.05$; Supplementary Table 7).

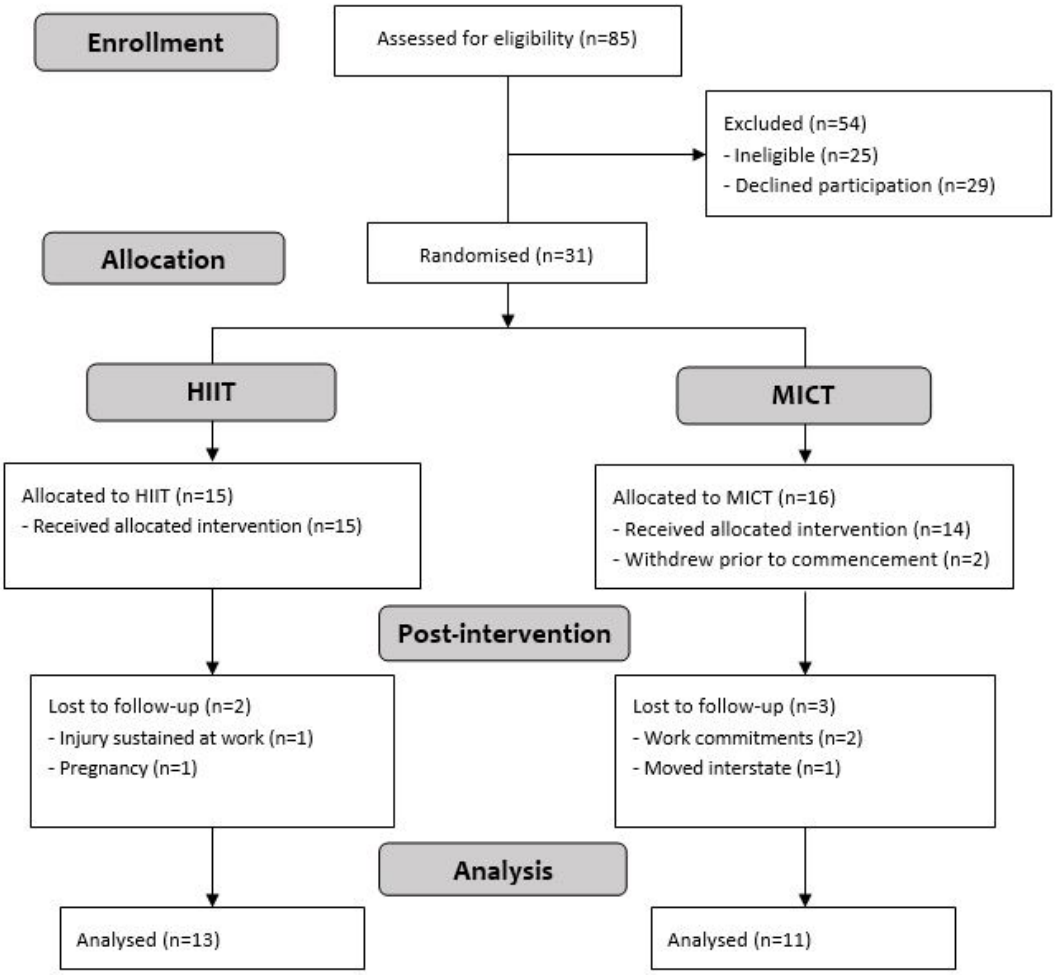

Figure 11. PRISMA trial flow diagram

5.4.1 Effect of exercise interventions

Aerobic capacity

VO_{2peak} increased in both HIIT (23.4 ± 10.1%, P < 0.001) and MICT (14.0 ± 9.3%, P < 0.001), compared to baseline (Table 10). Those in the HIIT group had greater improvements in VO_{2peak} than those in the MICT group ($P = 0.004$, Figure 12). Maximal power output (peak Watts) increased similarly following both HIIT (21.2 ± 10.9%, P < 0.001) and MICT (24.4 ± 20.9%, P < 0.001, Table 10).

Insulin sensitivity and lipid profiles

GIR increased following HIIT (47.8 ± 50.5%, $P = 0.028$), but no significant change was detected after the MICT intervention, and no difference between-groups (Table 10). The insulin sensitivity index increased following HIIT (49.1 ± 38.2%, $P = 0.014$; Table 10), with no change in the MICT group, resulting in a significant between-group difference ($P = 0.046$; Figure 12). Following HIIT, resting fasting glucose was reduced from 5.0 ± 0.4 to 4.6 ± 0.3 mmol/L in the HIIT group ($P = 0.019$), with no

significant change following MICT, and no difference between the groups (Table 10). Neither training intervention influenced fasting insulin levels, HbA1c levels, or lipid profiles (Table 10).

Body composition

There were no observed changes in weight or BMI as a result of either intervention. Both the HIIT and MICT groups significantly decreased waist circumference, by 2.2 ± 2.0 cm and 2.3 ± 2.4 cm, respectively ($P < 0.05$; Table 10). The MICT group also improved their waist to hip ratio ($P = 0.032$; Table 10) with no significant change in the HIIT group. Despite changes in waist circumference, no change in lean mass or fat mass was observed in either group. However, when assessed as a percentage of total mass, the HIIT intervention resulted in a significant increase in lean mass percentage (2.0 ± 1.8%, $P = 0.026$), likely due to a trend towards decreased fat mass (-2.0 ± 2.8%, $P = 0.069$) with no changes observed in the MICT group (Table 10).

Table 10. Cardiorespiratory fitness, body composition, insulin sensitivity, and lipid profiles at baseline and post-intervention.

	HIIT (N = 13)			**MICT (N = 11)**			
Outcome measure	**Baseline**	**Post**	**P**	**Baseline**	**Post**	**P**	**P (time × group)**
Age (years)	29.6 ± 5.3			34.6 ± 4.8			
Cardiorespiratory fitness							
VO_{2peak} (mL/kg/min)	25.1 ± 6.1	30.8 ± 6.7	**<0.001**	22.9 ± 3.2	26.1 ± 4.1	**<0.001**	**0.010**
$Watt_{peak}$	178.9 ± 26.7	215.4 ± 29.8	**<0.001**	154.6 ± 36.8	190.9 ± 25.7	**<0.001**	0.660
HR_{peak}	182.2 ± 9.7	183.2 ± 7.8	0.652	179.9 ± 10.9	182.9 ± 11.5	0.506	0.489
Body composition							
Weight (kg)	97.7 ± 19.7	97.3 ± 19.1	0.644	98.9 ± 28.4	99.8 ± 28.0	0.207	0.257
BMI (kg/m^2)	35.8 ± 7.3	35.6 ± 7.0	0.677	36.9 ± 9.8	37.3 ± 9.8	0.186	0.248
Waist circumference (cm)	99.9 ± 15.9	97.7 ± 14.6	**0.037**	105.3 ± 21.3	103.0 ± 20.8	**0.016**	0.869
Hip circumference (cm)	125.0 ± 13.8	122.6 ± 12.4	0.127	124.7 ± 21.6	124.2 ± 20.1	0.693	0.324
WHR	0.80 ± 0.07	0.80 ± 0.07	0.815	0.84 ± 0.06	0.83 ± 0.05	0.186	0.059
Fat mass (kg)	43.6 ± 14.3	42.5 ± 12.8	0.252	49.3 ± 20.6	50.3 ± 18.9	0.248	0.135
Lean mass (kg)	46.3 ± 4.1	47.0 ± 4.1	0.151	46.1 ± 7.2	46.8 ± 6.8	0.866	0.997
Fat mass (%)	47.4 ± 7.2	46.5 ± 7.0	0.069	50.5 ± 5.3	50.9 ± 4.7	0.476	0.069
Lean mass (%)	50.4 ± 6.8	51.5 ± 6.8	**0.026**	47.8 ± 5.1	48.1 ± 5.5	0.564	0.228
Insulin sensitivity							
Fasting glucose (mmol/L)	5.0 ± 0.4	4.6 ± 0.3	**0.019**	5.0 ± 0.7	4.7 ± 0.3	0.153	0.517
Fasting insulin (µIU/mL)	17.8 ± 11.0	17.6 ± 13.8	0.385	17.7 ± 6.5	18.5 ± 6.1	0.608	0.263
GIR (mg/lean mass[kg]/min)	7.6 ± 3.2	10.0 ± 3.1	**0.028**	7.9 ± 3.0	8.5 ± 3.1	0.245	0.216

Insulin sensitivity index	8.3 ± 4.4	11.2 ± 5.3	**0.014**	7.4 ± 3.5	7.7 ± 4.2	0.860	**0.037**
Steady state insulin (µIU/mL)	108.7 ± 47.5	101.2 ± 29.2	0.901	120.1 ± 28.4	125.6 ± 31.1	0.286	0.179
HOMA-IR	3.8 ± 2.4	3.5 ± 3.0	0.174	3.8 ± 1.6	3.9 ± 1.5	0.690	0.207
HbA1c (%)	5.2 ± 0.3	5.1 ± 0.3	0.537	5.3 ± 0.2	5.4 ± 0.3	0.485	0.403
Lipid profiles							
Total cholesterol (mmol/L)	4.8 ± 0.8	4.8 ± 1.0	0.500	4.8 ± 1.0	4.9 ± 0.7	0.511	0.376
Triglycerides (mmol/L)	1.0 ± 0.4	1.0 ± 0.4	0.398	1.4 ± 0.7	1.5 ± 0.6	0.737	0.489
HDL (mmol/L)	1.5 ± 0.3	1.5 ± 0.4	0.767	1.4 ± 0.3	1.4 ± 0.3	0.292	0.229
LDL (mmol/L)	2.9 ± 0.7	2.8 ± 0.9	0.417	2.8 ± 0.9	2.9 ± 0.8	0.240	0.139

VO_{2peak}, peak oxygen consumption; $Watt_{peak}$, peak Watts; HR_{peak}, peak heart rate; BMI, body mass index; WHR, waist to hip ratio; GIR, glucose infusion rate; HOMA-IR, homeostatic model assessment of insulin resistance; HbA1c, glycated haemoglobin; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol. Data are mean ± SD. All analyses are adjusted for age and individual baseline levels.

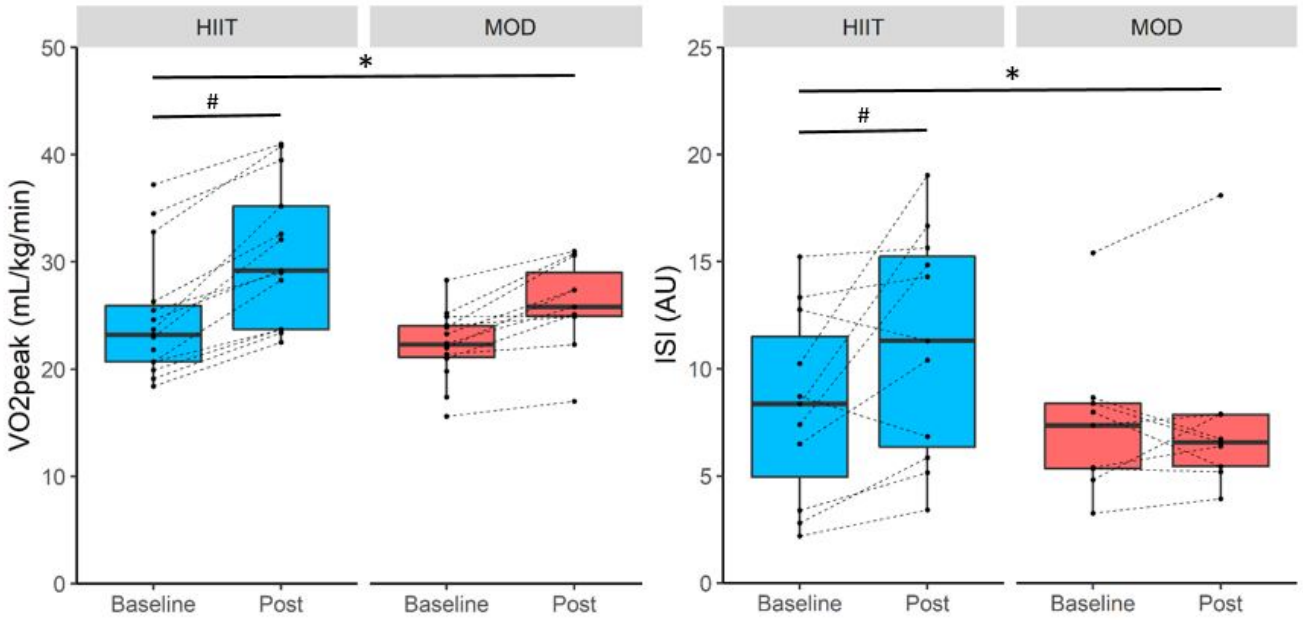

*Figure 12. VO_{2peak} (left) and insulin sensitivity index (ISI; right) at baseline and post-intervention, stratified by group and using boxplots showing the median (central line) and 25th to 75th quartiles (box). #P <0.05 indicates significant within-group differences, *P <0.05 indicates a significant interaction between HIIT and MICT, adjusted for age and baseline values.*

5.4.2 Relationship between fitness and insulin sensitivity

No significant association between changes in VO_{2peak} and changes insulin sensitivity was detected in HIIT or MICT groups alone. Once data were pooled, the improvement in VO_{2peak} was associated with the improvement in the insulin sensitivity index ($P = 0.003$, $R^2 = 0.38$; Figure 13).

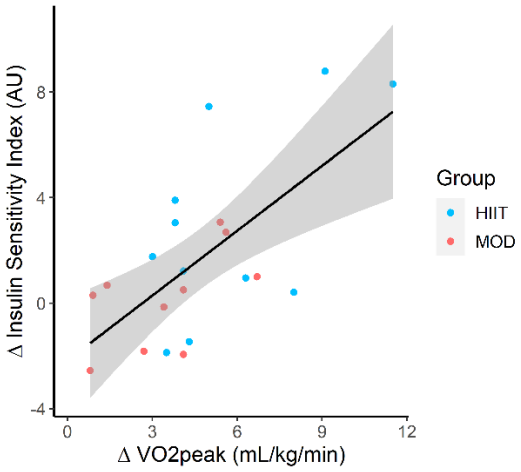

Figure 13. C: Relationship between Δ VO2peak and Δ insulin sensitivity index as a result of exercise training (linear regression P = 0.003, adjusted R2 = 0.38 for all participants regardless of group).

5.5 Discussion

We report here that HIIT increases VO_{2peak} and insulin sensitivity to a greater extent than MICT in women with PCOS, despite no reduction in body weight. To the best of our knowledge, this is the first study to match and compare the standard moderate-intensity exercise recommendations to a HIIT protocol in women with PCOS.

Given that women with PCOS are at significantly increased risk of developing insulin resistance and T2DM[3], it is important to determine the most effective exercise intervention to promote increased fitness and increased insulin sensitivity. Previously, it was reported that HIIT can improve VO_{2peak} in women with PCOS, compared to a non-exercise control group[228]. HIIT has also been found to be effective for improving VO_{2peak} and insulin sensitivity compared to age- and weight-matched control women[79]. However, it was not known whether HIIT was superior to MICT in women with PCOS. We found that while both HIIT and MICT increased VO_{2peak} in women with PCOS, there was a larger improvement following HIIT. VO_{2peak} is an important indicator of health and a predictor of premature mortality in clinical populations, with poor VO_{2peak} being a stronger predictor of mortality than BMI[111]. Poor cardiorespiratory fitness has also been associated with an increased risk of developing T2DM[284]. In line with that, we also report an association between fitness and insulin sensitivity in women with PCOS, with increases in VO_{2peak} accounting for 38% of the variation in improvements in insulin sensitivity.

We also observed a significant improvement in insulin sensitivity index after HIIT as opposed to no change following MICT. This finding is consistent with a previous study that utilised a HIIT protocol in women with PCOS and reported a 16% improvement in insulin sensitivity after training[153]. Similarly, a second study that utilised a HIIT protocol reported a significant decrease in HOMA-IR, by 17%, with no change after a resistance training intervention, also resulting in a significant between-group difference[157]. Here we suggest for the first time that HIIT has a stronger impact on the metabolic health of women with PCOS than MICT, the alternative recommended exercise intervention for women with PCOS[32]. HIIT may be favourable not only to improve fitness in these women, but also to reduce the likelihood of developing insulin resistance and early onset of T2DM, where PCOS presents a 4-fold greater risk[3].

Obesity is present in a large proportion of women with PCOS and is known to independently exacerbate both metabolic and reproductive features[7,20]. Given the high prevalence of obesity in women with PCOS, weight loss has been a major focus in regards to the treatment of PCOS[2]. Among a range of healthy and clinical cohorts, there is an on-going debate about the importance of exercise in comparison to weight loss for improving insulin sensitivity[285]. Interestingly, we report significant reductions in waist circumference in both groups, and a significant increase in lean mass percentage only with HIIT. Despite no change in body weight or fat mass, insulin sensitivity and fasting blood glucose levels were significantly improved after HIIT only. This finding is consistent with previous research where significant improvements in insulin sensitivity were reported without change in body weight in women with PCOS[152,153,206,286], suggesting that improved metabolic health can be achieved regardless of a reduction in body weight.

A potential limitation of this study is the relatively small sample size to detect significant associations between VO_{2peak} and insulin sensitivity in the HIIT group alone, but not when HIIT and MICT groups were combined. We were adequately powered to detect significant changes in VO_{2peak} and insulin sensitivity, the main outcomes of this study. Larger studies as well as follow-up studies are required to confirm these findings, and determine whether engagement in exercise and the observed improvements can be maintained and translate into reduced incidence of T2DM in this high risk population. A major strength of this study is the use of gold standard and comprehensive assessments of insulin sensitivity and body composition. In addition, all exercise sessions were supervised by accredited exercise physiologists and we report high levels of adherence to both exercise interventions.

In conclusion, HIIT appears to be safe, effective and can offer superior improvements in insulin sensitivity and VO_{2peak} compared to MICT, despite no reduction in weight, and as such it should be considered when implementing exercise programs to reduce the metabolic risk in women with PCOS.

Chapter 6.0 Efficacy of high-intensity interval training compared to moderate-intensity continuous training for improving reproductive health in women with polycystic ovary syndrome.

6.1 Abstract

Background: Polycystic ovary syndrome (PCOS) is a common and complex endocrine condition. Exercise training is recommended for women with PCOS to improve clinical outcomes, however, there is limited evidence for the isolated effects of exercise, particularly in regards to reproductive outcomes.

Objective: To investigate the effectiveness of 12 weeks of HIIT in comparison to MICT for improving hormonal profiles and menstrual cyclicity in overweight women with PCOS.

Methods: Twenty-four overweight women with PCOS aged 18-45 years were randomly assigned to perform 12 weeks of moderate intensity continuous exercise (MICT, 60-75% HR_{peak}, n=11) or HIIT (>90% HR_{peak}, n=13). Hormonal profiles, insulin sensitivity and anthropometric outcomes were assessed at baseline and post-training.

Results: HIIT resulted in improvements in FAI (P = 0.041), percent of free testosterone (P = 0.016) and SHBG (0.026), with no significant changes as a result of MICT. HIIT also resulted in significantly greater improvement in SHBG (P = 0.005) and percent of free testosterone (P = 0.002) compared to MICT. A significant association between Δ insulin sensitivity and Δ free testosterone was detected in HIIT group (P = 0.029, adjusted $R^2 = 0.43$), but not in the MICT group. In regards to menstrual cyclicity, although not significant, 69% of participants (9 of 13) and 18.2% (2 of 11) reported improvements in menstrual cyclicity following HIIT and MICT, respectively.

Conclusion: HIIT resulted in greater improvements in important hormonal outcomes in comparison to MICT. HIIT also appears to show promising results for improving menstrual cyclicity in overweight women with PCOS. HIIT should be considered as a useful therapy to improve reproductive health in women with PCOS.

6.2 Introduction

Polycystic ovary syndrome (PCOS) is a common condition, affecting 8-13% of reproductive aged women[32]. PCOS is a complex condition with diverse clinical presentations and varying severities of reproductive, metabolic and psychological impacts [20,21]. The diagnostic features of PCOS include clinical or biochemical hyperandrogenism, oligo- or anovulation and the presence of polycystic ovaries on ultrasound, with a combination of at least two features required for diagnosis[13]. Obesity is also highly prevalent among women with PCOS and is known to worsen both the reproductive and metabolic features[287]. Insulin resistance and the compensatory hyperinsulinemia are also considered to be a key factor in the aetiology of PCOS, impacting ovarian androgen production and decreasing the production of SHBG, leading to an increase in free androgens[59] and dysregulation of menstrual cyclicity, contributing to fertility issues[61,62].

Improving lifestyle behaviours by encompassing regular physical activity and healthy eating is recommended for all women with PCOS, particularly those who are overweight or obese[32]. Exercise has well-established benefits for improving the metabolic health of women with PCOS, however, the effects on reproductive health are not as clear[152]. In a recent review, only half of the included studies reported an improvement in markers of reproductive health[152]. Furthermore, only half of exercise interventions report improvements in FAI and other biochemical measures of hyperandrogenism[152]. Exercise interventions have been reported to improve menstrual regularity, ovulation rates and pregnancy in women with PCOS[142], however, interventions utilised in women with PCOS are often multi-component and involve a dietary intervention designed to induce weight loss, making it difficult to determine whether these changes occurred due to exercise or as a result of weight loss[142]. Complicating matters further, there is also large variation in the exercise prescription of current studies making it difficult to determine the characteristics of exercise interventions that may provide favourable impacts. One of such characteristics is the intensity of the exercise utilised.

There is an ongoing debate about the importance of exercise intensity and whether vigorous-intensity exercise has the potential to provide more favourable health impacts. Vigorous-intensity exercise has been found to improve cardio-metabolic health to a greater extent than moderate-intensity exercise in women with PCOS, however, the impact on reproductive outcomes is less clear[152]. The aim of this study was to compare the effects of HIIT in comparison to MICT for improving hormonal profiles and menstrual cyclicity in women with PCOS.

6.3 Methods

6.3.1 Study design, participants and study protocol

See Chapter 5 for details on study design, participant details and study protocol. Briefly, baseline and post-intervention testing consisted of a maximal graded exercise test to assess VO_{2peak}, a euglycaemic-hyperinsulinaemic clamp and a dual x-ray absorptiometry (DXA) scan. For information regarding the details of these assessments, please refer to Chapter 5.

6.3.2 Clinical and biochemical measurements

Participants arrived in a fasted state (≥12 hours). For participants with regular menstrual cycles (21-35 days[32]), this session was completed during the early follicular phase of the menstrual cycle (days 1-7) as determined by self-report using menstrual diaries. Blood was collected in various anticoagulant and 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 35000 revolutions per minute for 10 min at 4 °C. The plasma or serum was then transferred to a clean polypropylene tube and frozen at -80 °C for future analysis. Stored blood samples were batch analysed by Monash pathology for the following measures: total testosterone, free testosterone, SHBG, dihydrotestosterone (DHT), oestradiol, and androstenedione. The SHBG assay was performed using a sequential two-step immunoenzymatic ("sandwich") assay carried out on a Unicel DXI 800 (Beckman Coulter). 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[288]. Additional bloods analysis for anti-müllerian hormone (AMH) and insulin were conducted at Victoria University. Serum AMH concentrations were determined via a commercially available ELISA kit (Ultra-Sensitive AMH/MIS ELISA, AL-105, Anash Labs, Texas, USA). Plasma insulin levels were determined via radioimmunoassay kit (Human Insulin-Specific RIA, HI-14K, Millipore, Massachusetts, USA). FAI was calculated as (total testosterone x 100)/SHBG.

Menstrual diaries were completed by participants throughout the duration of the study and were used to assess menstrual regularity. 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)[12].

6.3.3 Aerobic capacity and anthropometric measurements

VO_{2peak} was assessed at baseline and following the 12 week intervention using an incremental maximal graded exercise protocol and is described in detail previously (Chapter 5). Height and weight were taken and BMI was calculated (weight[kg]/height squared[m]). For details on these outcomes, please refer to Chapter 5.

6.3.4 *Exercise interventions*

Participants in both groups were asked to attend three weekly sessions for 12 weeks as previously described[289] (Chapter 4).

6.3.5 *Statistical analysis*

Data are presented as mean ± SD and median and percentiles for boxplots. Data was analysed using R studio version 4.0.2. Data were assessed for normality using Shapiro-Wilk test and log transformed where necessary. Linear mixed models were used to determine the effect of exercise intensity (group) over time and to determine the interaction between timepoint and group (between-group differences). The unique participant codes were used as a random effect to account for repeated measures. Age was included as a fixed effect in all models. Simple linear regression models adjusted for age were used to determine whether delta changes in insulin sensitivity were associated with delta changes in SHBG as a result of exercise using the group and pooled data from all participants. Finally, a Fisher exact test was used to understand whether HIIT was more likely to improve menstrual cycles than MICT. Those with amenorrhoea at baseline and post intervention were removed from menstrual cyclicity analysis as this is considered optimal. P values of main outcomes were adjusted for multiple comparisons using the false discovery rate (FDR)[271]. P values were deemed statistically significant when <0.05. The following packages were used in our analysis; *lme4*[290], *lmerTest*[291] and *lavaan*[292], *tidyverse*[293]. No power analysis was performed *a priori* as this was a secondary analysis.

6.4 Results

Thirty one women completed the baseline assessments, of which, 24 completed the 12-week intervention (HIIT = 13, MICT = 11). Two women withdrew after being assigned to the MICT group with no reason cited. Two participants dropped out during the MICT intervention due to changes to their work schedule and one withdrew due to moving interstate. One participant withdrew from the HIIT intervention due to an injury that was sustained at work and another dropped out due to becoming pregnant (see Figure 11 in Chapter 5 for details). The PCOS phenotypic distribution for both groups is reported in Table 11. No adverse events occurred during the study period. Exercise intervention adherence was similar across the two groups (HIIT = 93.9 ± 3.0%, MICT = 92.0 ± 4.8%, $P > 0.05$). There were no differences in baseline data between HIIT and MICT with the exception of age, which was adjusted in all analyses. There were no significant differences in body composition, fitness, hormone levels or insulin sensitivity between participants who dropped out and those that completed the study. There were also no significant changes in dietary or exercise behaviour from baseline to post-intervention (P > 0.05; Supplementary Table 7).

Table 11. Breakdown of PCOS phenotypes

PCOS Phenotype	HIIT	MICT
A (HA, OA, PCOM)	5	5
B (HA, OA)	3	0
C (HA, PCOM)	0	2
D (OA, PCOM)	5	4

HA – Hyperandrogenism, OA – Oligo/Anovulation, PCOM – Polycystic Ovary Morphology.

Table 12. Clinical outcomes, hormonal profiles and insulin sensitivity at baseline and post-intervention

	HIIT (n = 13)			MICT (n = 11)			
Outcome measure	**Baseline**	**Post**	**P (within group)**	**Baseline**	**Post**	**P (within group)**	**P (time x group)**
Clinical outcomes							
Age	29.6 ± 5.3			34.6 ± 4.8			
Weight	97.7 ± 19.7	97.3 ± 19.1	0.644	98.9 ± 28.4	99.8 ± 28.0	0.207	0.257
BMI	35.8 ± 7.3	35.6 ± 7.0	0.677	36.9 ± 9.8	37.3 ± 9.8	0.186	0.248
VO_{2peak}	25.1 ± 6.1	30.8 ± 6.7	**<0.001**	22.9 ± 3.2	26.1 ± 4.1	**<0.001**	**0.004**
Hormonal profile							
Testosterone (nmol/L)	1.8 ± 0.8	1.4 ± 0.9	0.127	1.6 ± 0.7	1.4 ± 0.8	0.169	0.547
Free T (pmol/L)	34.9 ± 16.3	27.9 ± 16.9	0.114	34.8 ± 17.2	34.7 ± 22.4	0.988	0.276
Free T (%)	2.2 ± 0.8	2.0 ± 0.7	**0.016**	2.2 ± 0.6	2.2 ± 0.6	0.094	**0.002**
FAI (AU)	6.7 ± 3.3	4.5 ± 2.8	**0.041**	6.2 ± 3.7	5.7 ± 3.9	0.349	0.123
SHBG (nmol/L)	29.4 ± 17.1	39.3 ± 24.5	**0.026**	31.1 ± 13.7	29.2 ± 12.0	0.117	**0.005**
AMH	63.5 ± 27.2	67.4 ± 48.3	0.594	49.2 ± 21.8	42.2 ± 20.6	0.130	0.264
Androstenedione (nmol/L)	5.0 ± 1.2	4.5 ± 1.9	0.382	4.7 ± 1.9	4.2 ± 1.6	0.263	0.705
Oestradiol (pmol/L)	254.5 ± 213.8	216.4 ± 160.1	0.629	305.4 ± 273.8	269.9 ± 288.2	0.445	0.912
DHT (nmol/L)	0.3 ± 0.2	0.3 ± 0.2	0.557	0.2 ± 0.1	0.2 ± 0.1	0.808	0.514
Insulin sensitivity							
Insulin sensitivity index (AU)	8.3 ± 4.4	11.2 ± 5.3	**0.014**	7.4 ± 3.5	7.7 ± 4.2	0.860	**0.046**

AU - Arbitrary Units, Free T – Free Testosterone, FAI – Free Androgen Index, SHBG – Sex Hormone Binding Globulin, AMH – Anti-Müllerian Hormone, DHT – Dihydrotestosterone.
Values are mean ± SD.

6.4.1 Effect of the exercise interventions

The metabolic outcomes after both HIIT and MICT have previously been described (Chapter 5). Briefly, while both interventions improved VO_{2peak}, the HIIT group showed significantly greater improvement

when compared to MICT. The ISI increased following HIIT but there was no change in the MICT group, resulting in a significant between-group difference post-intervention. Finally, changes in VO_{2peak} were positively associated with changes in ISI.

Biochemical measures of hyperandrogenism

There were significant reductions in FAI (-32.8%, P = 0.041; Table 12) and percent of free testosterone (-9.1%, P = 0.016; Table 12) and a significant increase in SHBG (17.7%, P = 0.026; Table 12) after 12 weeks of HIIT. Interestingly, total testosterone was not altered by HIIT (P >0.05. There were no significant changes in hormonal outcomes as a result of the MICT intervention (P >0.05; Table 12). There were significant between group differences for percent of free testosterone (P = 0.002, Figure 14) and SHBG levels (P = 0.002, Figure 14) with HIIT being superior to MICT. There were no significant changes in AMH, androstenedione, oestradiol or DHT as a result of either intervention (P > 0.05).

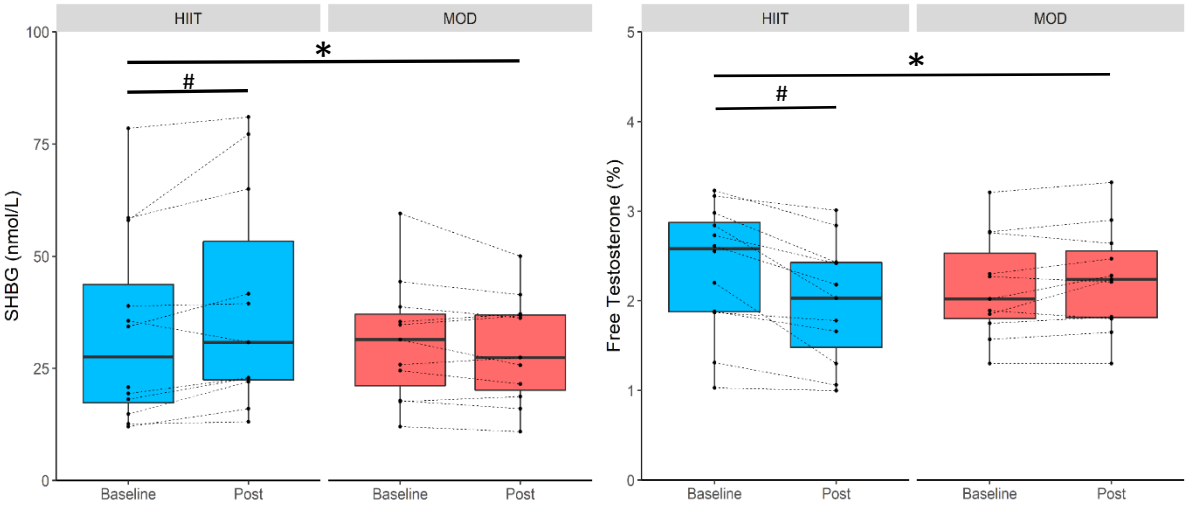

*Figure 14. Sex hormone binding globulin (SHBG; left) and free testosterone (%; right) at baseline and post-intervention, stratified by group and using boxplots showing the median (central line) and 25th to 75th quartiles (box). #P <0.01 indicates a significant within-group differences, *P <0.005 indicates a significant interaction between HIIT and MICT, adjusted for age and baseline values.*

Relationship between insulin sensitivity and free testosterone

A significant association between Δ insulin sensitivity and Δ SHBG was detected in HIIT group (P = 0.029, adjusted R^2 = 0.43), but not in the MICT group. Using the pooled data, there was a signification association between improvements in the insulin sensitivity index and SHBG (*P* = 0.009, R^2 = 0.35; Figure 15). There was no significant association between Δ insulin sensitivity and Δ percent of free testosterone (P > 0.05; data not shown).

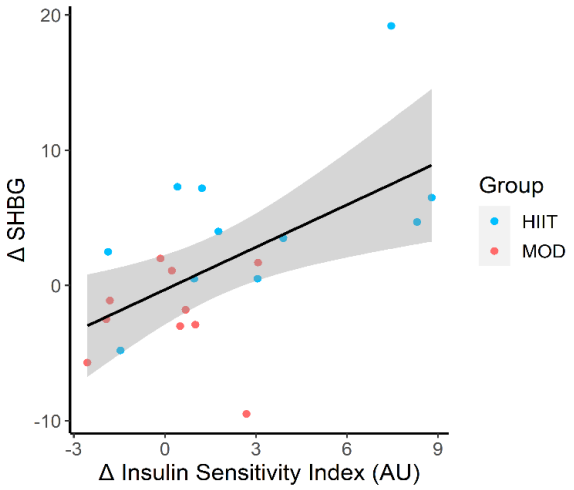

Figure 15. Relationship between Δ insulin sensitivity index and Δ SHBG as a result of exercise training (linear regression; P = 0.009, adjusted R2 = 0.35) for all participants regardless of group allocation.

Menstrual cyclicity

At baseline, all participants in the HIIT group reported menstrual irregularities, with 23% and 77% of women experiencing amenorrhoea and oligomenorrhoea, respectively. While in the MICT group, 27.3%, 54.5% and 18.2% reported amenorrhoea, oligomenorrhoea or normal cycles, respectively (Figure 16).

Post-intervention, 69% of participants (9 of 13) reported improvements in their menstrual cyclicity following HIIT (Figure 16). Three women reported improvements from amenorrhoea to oligomenorrhoea, 6 reported improvements from oligomennorrhoea to regular cycles, and 4 reported no change. In the MICT group, 18.2% (2 of 11) of women reported improvements in cyclicity post-intervention, with one women changing from amenorrhoea to oligomenorrhoea, one reporting improvements from oligomenorrhoea to regular cycles, and nine women reported no change (Figure 16).

Figure 16. Menstrual cyclicity at baseline (inner donut) and post-intervention (outer donut) in both HIIT (left) and MICT (right).

6.5 Discussion

We report that HIIT improves SHBG levels and the percent of free testosterone in comparison to MICT. These improvements occurred despite no reduction in weight or BMI. HIIT also resulted in a significant improvement in FAI, however, it was not significantly greater than MICT. Delta changes in the percent of free testosterone and SHBG that occurred with exercise were associated with delta changes in the insulin sensitivity index. Although not significant, HIIT also appeared to result in greater improvements in menstrual cyclicity than MICT.

An elevated FAI is a commonly observed androgenic abnormality in women with PCOS[2]. Some[155] but not all[156,200] studies have reported that exercise can significantly reduce FAI. A recent meta-analysis reported only small effects of exercise for reducing FAI[152]. Here we report a significant reduction in FAI following HIIT, but not MICT. This may suggest that perhaps a higher intensity of exercise is required to promote changes in FAI. It should be noted that the significant decrease in FAI reported here as a result of HIIT is predominantly due to the increased SHBG levels as there was only a trend towards a decrease in total testosterone. Low levels of SHBG are also a common feature of PCOS, with low levels of SHBG contributing to higher levels of free testosterone[42]. We report significant improvements in SHBG levels as a result of HIIT. The significant increase in SHBG reported here is comparable to that of studies using medications such as metformin to reduce insulin levels, causing a subsequent rise in SHBG levels[294], suggesting that HIIT should be considered as an effective therapy to reduce SHBG. Similar improvements in SHBG are also reported in other exercise intervention studies[155,158]. Interestingly, one study which compared 3 groups, a diet only intervention, a combined diet, and aerobic exercise (moderate-vigorous intensity), and a combined diet, resistance training and aerobic exercise (moderate-vigorous intensity) intervention reported significant increases in SHBG levels as a result of both interventions which included moderate-vigorous intensity aerobic exercise and not in the diet alone group, suggesting that exercise was the cause of the increase in SHBG levels[155]. In addition to the increase in SHBG levels, we also report a significant decrease in free testosterone percentage. Surprisingly, we saw no significant change in total testosterone[35]. Total testosterone is the sum of the both protein-bound and unbound circulating testosterone, with the majority of testosterone binding to SHBG[295]. An increase in SHBG decreases the bioavailability of testosterone and therefore decreases the levels of free testosterone which can occur despite a non-significant decrease in total testosteronee[295]. It is postulated that a decrease in circulating androgen concentrations may be related to weight loss rather than the exercise training itself[200]. Here we report improvements in hormonal profiles including an increase in SHBG and a decrease in free testosterone levels as a result of HIIT despite no reduction in weight in women with PCOS. This is in agreement with other studies that have reported improved hormonal profiles independent of weight change[165,296].

Improvements in both androgen and insulin sensitivity have not been consistently reported across exercise and lifestyle intervention studies in women with PCOS. We report significant improvements

in both, the percent of free testosterone and SHBG levels together with a significant increase in insulin sensitivity as a result of HIIT, however, no significant change in these variables as a result of MICT. The relationship between androgens, SHBG and insulin is well documented with hyperinsulinaemia contributing to hyperandrogenism by augmenting ovarian androgen production and by reducing SHBG production ultimately increasing free testosterone levels[294,297]. We found a significant relationship between improvements in SHBG and improvements in insulin sensitivity. This relationship was the strongest when including the HIIT group only due to the large improvements in both variables resulting from this intervention. Altogether, this suggests that a higher intensity of exercise might be required to provoke changes in both reproductive and metabolic health outcomes in women with PCOS. In addition, these improvements were found without weight loss.

Given that exercise is considered the first-line therapy for improving both reproductive and metabolic outcomes, it is surprising how few studies evaluate changes in reproductive health outcomes are in comparison to metabolic outcomes[152]. A large community-based cohort study reported that PCOS was associated with a 15-fold increased risk of issues with fertility, independent of BMI[50]. It is estimated that 50% of women with PCOS will seek treatment related to sub/infertility[25]. Results from a recent semi-quantitative analysis revealed that exercise may improve menstrual regularity, and ovulation and pregnancy rates in women with PCOS[142]. It has also been hypothesised that insulin resistance is the driver of infertility independent of BMI[50], which could partially explain the fertility improvements observed with exercise. Although fertility was not measured in our study, improvements in menstrual cyclicity were observed as a result of HIIT, could translate to improved fertility in women with PCOS. Future studies examining the impact of HIIT on fertility would be beneficial.

Ovarian dysfunction is a common characteristic of PCOS and usually manifests as oligo- or amenorrhoea, resulting from chronic oligo-or anovulation[48] and is present in up to 95% of women with PCOS[298]. In adult women, irregular menstrual cycles clinically reflect ovulatory dysfunction, however, ovulatory dysfunction can occur with regular cycles[32]. There is increasing support suggesting that exercise improves menstrual regularity and ovulation as a result of exercise[155,164,165,296]. Similar to our findings, improvements in menstrual cyclicity were reported after 12 weeks of vigorous-intensity exercise, with 60% of women with amenorrhoea at baseline, reporting normal cycles post-intervention[164]. One study comparing a diet alone intervention to two different diet + exercise interventions reported that 49% of women improved ovulation and/or menstrual cyclicity with no difference between groups[155]. Here, we report large improvements in menstrual cycle regulation as a result of HIIT with 9 of 13 women (69%) reporting improvements in menstrual cyclicity compared to 2 out of 11 (18.2%) women in the MICT group. Although we were unable to detect significant changes between the two groups, HIIT resulted in a larger percentage of women who reported improvements in menstrual cyclicity and thereby may provide a useful strategy to improve menstrual regulation.

However, it needs to take into consideration that this study used menstrual frequency as a proxy for ovulation and other assessments are required to determine change in ovulatory status.

In conclusion, we report that HIIT is beneficial for improving menstrual cyclicity and hormone profiles in overweight women with PCOS. HIIT is also more beneficial than standard MICT for improving free testosterone and SHBG levels, key clinical hormonal markers in women with PCOS. Combined, these results suggest that exercise, particularly HIIT, is an effective treatment for improving both insulin sensitivity and hormone profiles. Future large-scale studies are required to confirm these results and provide further validation for HIIT in both lean and overweight women with PCOS.

Chapter 7.0 Efficacy of high-intensity interval training compared to moderate-intensity continuous training for improving mental health and health-related quality of life in women with polycystic ovary syndrome.

7.1 Abstract

Background: Polycystic ovary syndrome (PCOS) is a common condition characterised by reproductive, metabolic and mental health consequences. Women with PCOS have substantially greater symptoms of depression and anxiety, and a lower health-related quality (HRQoL), of life compared to women without PCOS. The aim of this secondary analysis was to determine if high-intensity interval training (HIIT) can provide greater improvements in mental health outcomes than standard moderate-intensity continuous training (MICT).

Methods: Twenty-four overweight women with PCOS aged 18-45 years were randomly assigned to perform 12 weeks of either MICT (60-75% HR_{peak}, n=11) or HIIT (>90% HR_{peak}, n=13). Outcome measures included symptoms of depression, anxiety and stress (DASS-21), general HRQoL (SF-36) and PCOS specific HRQoL (PCOSQ) collected at baseline and post-intervention.

Results: Reductions in depression (P <0.001) and stress (P = 0.005) scores were observed in the HIIT group. Anxiety scores reduced in both groups (HIIT; P = <0.001, and MICT; P = 0.007), however, there was a significantly greater reduction following HIIT compared to MICT (P = 0.017). The HIIT group also significantly improved emotions, weight and menstrual problem domains of the PCOSQ and physical functioning, role emotional, energy and general health domains of the SF-36.

Conclusion: This study demonstrated that HIIT is efficacious for improving mental health and HRQoL in overweight women with PCOS, indicating that HIIT may be superior to MICT for improving mental wellbeing.

7.2 Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine condition, affecting 8-13% of women of reproductive age[8,10]. PCOS is characterised by hyperandrogenism, menstrual irregularities and polycystic ovary morphology, with a combination of at least two of the three required for diagnosis[13]. PCOS is underpinned by reproductive and metabolic abnormalities, resulting in an increased risk of comorbidities including obesity, insulin resistance, T2DM and infertility[3,30,50]. PCOS is also recognised to be associated with diminished mental health and health-related quality of life (HRQoL)[6,96]. Women with PCOS are over three times more likely to experience moderate to severe symptoms of depression, and over five times more likely to experience severe symptoms of anxiety, compared to women without PCOS[5]. It is postulated that the symptoms and comorbidities of PCOS may contribute to poorer mental health and reduced HRQoL[20,90]. Poor mental health is associated with a decreased exercise participation and adherence to exercise[299], potentially further increasing the risk of developing co-morbidities.

Exercise is recommended in all women with PCOS to improve general health, HRQoL and to maintain or achieve a healthy weight[32]. Exercise participation has been associated with lower scores of depression in both women with and without PCOS[89]. Women with PCOS are encouraged to engage in a minimum of 150 minutes per week of moderate intensity physical activity or 75 minutes per week or vigorous intensity exercise[32]. Exercise is well established as a therapeutic tool to improve metabolic and reproductive health outcomes in women with PCOS [142,152], however, the impact on mental health and HRQoL is less clear[147]. In populations with other chronic conditions, exercise is known to be an effective tool for managing mental health[300,301]. The limited existing research of the effects of exercise on mental health and HRQoL in women with PCOS does suggest that exercise interventions may promote improvements in these outcomes[147,226,245], although, it is limited by large heterogeneity and poor reporting of current exercise interventions. Regular exercise has also been associated with improving specific mental health symptoms, namely reductions in symptoms of depression[302] and anxiety[303]. This relationship has also been observed in women with PCOS[178]. A minimum of 20 minutes of exercise per week associated with reduced odds of psychological distress[304], although, a greater volume or intensity of exercise has been associated with greater risk reduction[304]. Despite the known benefits, exercise participation in both women with and without PCOS is low, with 64% of women without PCOS and only 48% of women with PCOS reporting any engagement with exercise[89].

High-intensity interval training (HIIT) has received considerable attention over the past two decades due its time efficiency and potent training stimulus[134]. HIIT involves alternating short bouts of high-intensity exercise with periods of rest or light exercise[121]. HIIT has been found to result in greater improvements in cardio-metabolic health outcomes in comparison to moderate-intensity continuous training (MICT) among healthy populations and those with lifestyle induced chronic disease[121,134]. HIIT also appears to result in greater improvements in body composition and metabolic health outcomes

among women with PCOS[152]. Despite the growing body of evidence supporting the physiological benefits of HIIT, there is little information regarding its effect on mental health and HRQoL. Limited evidence does suggest that HIIT can improve HRQoL and symptoms of anxiety and depression among populations with chronic conditions[121,305]. It may be postulated that HIIT may provide greater improvements in mental health and HRQoL in women with PCOS due to its large impact on reducing the severity of symptoms associated with PCOS. The primary aim of this chapter was to determine the efficacy of HIIT in comparison to MICT for improving mental health and HRQoL in women with PCOS.

7.3 Methods

7.3.1 Study design, participants and study protocol

See Chapter 5 for details on study design, participant details and study protocol.

7.3.2 Aerobic capacity

Aerobic capacity was assessed at baseline and following the 12 week intervention using an incremental maximal graded exercise test, described in detail in Chapter 5.

7.3.3 Mental Health and Health Related Quality of Life Measures

The Depression, Anxiety & Stress Scale (DASS-21) is a self-report measure which is well-established, reliable and valid instrument for measuring depression, anxiety and stress[306]. The questionnaire consists of 21 questions, with 7 items per domain. The severity of symptoms is scored on a scale 4-point scale. The total score for each category is added together. Higher scores denote more severe symptoms.

Health-related quality of life was assessed using the generic SF-36 (36-item Short-Form Health Survey). This measure is well-validated, including in PCOS[307]. It consists of 8 health domains: physical functioning (10 questions), role limitations due to physical health problems (4 questions), role limitations due to emotional problems (3 questions), emotional well-being (5 questions), social functioning (2 questions), bodily pain (2 questions), energy/fatigue (4 questions), and general health perceptions (5 questions). Scoring was calculated according to the RAND procedure[308] where responses are recoded to a score between 0 and 100, with scores represent a percentage of total possible score achieved. Scores are then averaged for each of the 8 domains.

Health-related quality of life associated with PCOS symptom distress was measured using a validated self-administered questionnaire (PCOSQ)[99,309]. The PCOSQ consists of 26 questions with 5 domains each relating to a common symptom of PCOS; emotions (7 questions), menstrual problems (4 questions), infertility (5 questions), weight (5 questions) and body hair (5 questions). Questions are scored on a 7-point likert scale in which 1 represents poorest function and 7 represents optimal function.

For each domain, scores are added together and then divided by the number of questions to reach the domain score. Lower scores indicate greater PCOS symptom distress.

7.3.4 Exercise interventions

See Chapter 5 for details regarding exercise interventions. as previously

7.3.5 Statistical analysis

Data are presented as mean ± SD and median and percentiles for boxplots. All data was analysed using R studio version 4.0.2. Data were assessed for normality using Shapiro-Wilk test and log transformed where necessary. Linear mixed models were used to determine the effect of exercise intensity (group) over time and to determine the interaction between timepoint and group (between-group differences). The unique participant codes were used as a random effect to account for repeated measures and age was included as a fixed effect. Simple linear regression models adjusted for age were used to determine whether baseline, post-intervention or delta changes in VO_{2peak}, insulin sensitivity, free testosterone (%) or SHBG were associated with improvements in depression and anxiety scores using the group and pooled data from all participants. P values of main outcomes were adjusted for multiple comparisons using the false discovery rate (FDR)[271]. P values were deemed statistically significant when <0.05. The following packages were used in our analysis; *lme4*[290], *lmerTest*[291] and *tidyverse*[293].

7.4 Results

Thirty-one participants completed the baseline assessments, of whom 24 completed the 12-week intervention (HIIT = 13, MICT = 11). See Figure 11 in Chapter 5 for the trial flow diagram. There were no significant differences in study outcomes at baseline between participants who dropped out and those that completed the study. There was a significant difference in age between the two groups, which was adjusted for in all analyses. Exercise intervention adherence was similar across the two groups with an average adherence of 93.9 ± 3.0% in the HIIT group and 92.0 ± 4.8% in the MICT group (P > 0.05). No adverse events occurred during the study period.

7.4.1 Effect of the exercise interventions

The cardio-metabolic and reproductive outcomes have been previously described (Chapters 5 & 6). Briefly, while both interventions improved VO_{2peak}, the HIIT group showed significantly greater improvement when compared to MICT. HIIT also resulted in significantly greater improvements in insulin sensitivity, SHBG and the percent of free testosterone. Finally, changes in VO_{2peak} were positively associated with changes in ISI, and changes in ISI were associated with improvements in SHBG.

Mental health outcomes

The HIIT intervention significantly reduced scores for depression (P <0.001), anxiety (P <0.001) and stress (P = 0.005; Table 13). The MICT intervention also significantly reduced stress scores (P = 0.007). There was a significant time by group interaction between the two groups with the HIIT group improving anxiety scores significantly more so than the moderate group (P = 0.017, Figure 15). There were no between group differences for depression or stress scores (Table 13).

There were significant associations between depression and insulin sensitivity index at baseline (P = 0.040), and between both anxiety and insulin sensitivity index (P = 0.004) and anxiety and VO_{2peak} (0.031) using the pooled data from the combined group (HIIT and MICT). These associations were no longer significant post-intervention or when using the delta changes. There were no other significant associations between mental health outcomes and markers of hyperandrogenism at any time point.

PCOS specific HRQoL

The HIIT intervention significantly improved scores in 3 of the 5 domains of the PCOSQ; physical functioning (P = 0.014), weight (P <0.001) and menstrual problems (P = 0.002; Table 13). The MICT intervention also resulted in a significant improvement in the weight domain scores (P = 0.037). There were no significant group by time interactions between the HIIT and MICT groups for any domain (Table 13).

General HRQoL

The HIIT intervention significantly improved physical functioning (P = 0.014), role emotional (P = 0.035), energy (P = 0.031) and general health (P = 0.001) domains of the SF-36 (Table 13). The MICT group significant improved role emotional (P = 0.025) and general health (P = 0.046) domain scores. There were no significant between group differences for any domains of the SF-36 (Table 13).

Table 13. Mental health and health-related quality of life outcomes at baseline and post-intervention.

	HIIT (N = 13)			MICT (N = 11)			
Outcome measure	**Baseline**	**Post**	**P (within group)**	**Baseline**	**Post**	**P (within group)**	**P (time x group)**
Age	29.6 ± 5.3			34.6 ± 4.8			
Weight (kg)	97.7 ± 19.7	97.3 ± 19.1	0.644	98.9 ± 28.4	99.8 ± 28.0	0.207	0.257
BMI	35.8 ± 7.3	35.6 ± 7.0	0.677	36.9 ± 9.8	37.3 ± 9.8	0.186	0.248
VO_{2peak}	25.1 ± 6.1	30.8 ± 6.7	**<0.001**	22.9 ± 3.2	26.1 ± 4.1	**<0.001**	**0.010**
ISI	108.7 ± 47.5	101.2 ± 29.2	0.901	120.1 ± 28.4	125.6 ± 31.1	0.286	**0.037**
SHBG	29.4 ± 17.1	39.3 ± 24.5	**0.026**	31.1 ± 13.7	29.2 ± 12.0	0.117	**0.026**
Free testosterone (%)	2.2 ± 0.8	2.0 ± 0.7	**0.016**	2.2 ± 0.6	2.2 ± 0.6	0.094	**0.016**
DASS-21							
Depression score	5.0 ± 3.7	3.5 ± 2.9	**<0.001**	4.7 ± 5.0	3.7 ± 3.6	0.212	0.442
Anxiety score	6.5 ± 5.0	3.1 ± 4.1	**<0.001**	4.1 ± 3.6	3.0 ± 2.4	0.059	**0.017**
Stress score	8.1 ± 4.5	5.7 ± 4.2	**0.005**	7.7 ± 5.3	4.8 ± 3.9	**0.007**	0.635
PCOSQ							
Emotions	4.2 ± 1.4	4.7 ± 1.2	**0.027**	4.3 ± 1.1	4.8 ± 0.8	0.078	0.936
Body hair	3.2 ± 1.4	3.5 ± 1.7	0.190	3.9 ± 1.8	4.6 ± 1.6	0.122	0.535
Weight	2.0 ± 1.1	2.7 ± 1.2	**<0.001**	2.2 ± 1.0	2.6 ± 1.1	**0.037**	0.266
Infertility	4.5 ± 1.7	4.8 ± 2.1	0.116	4.1 ± 2.2	4.9 ± 1.9	0.122	0.363
Menstrual problems	4.0 ± 1.2	4.8 ± 1.2	**0.002**	3.7 ± 1.4	4.1 ± 1.3	0.374	0.248
SF-36							
Physical functioning	80.0 ± 18.6	88.5 ± 18.4	**0.014**	79.5 ± 20.2	85.9 ± 17.7	0.119	0.732
Role physical	86.5 ± 24.2	94.2 ± 15.0	0.224	88.6 ± 23.4	95.5 ± 10.1	0.137	0.971
Role emotional	46.2 ± 37.4	71.8 ± 32.9	**0.035**	69.7 ± 34.8	87.9 ± 22.5	**0.025**	0.582
Energy	39.6 ± 15.5	53.1 ± 17.7	**0.031**	42.3 ± 19.0	45.9 ± 19.5	0.371	0.483
Emotions	60.9 ± 12.8	67.4 ± 1478	0.108	68.0 ± 17.2	69.1 ± 20.1	0.747	0.300
Social	71.2 ± 23.6	75.0 ± 24.5	0.711	71.6 ± 33.1	78.4 ± 30.2	0.306	0.563
Pain	70.2 ± 21.0	79.2 ± 21.2	0.059	74.8 ± 18.8	79.8 ± 12.2	0.254	0.638
General health	42.3 ± 18.9	54.6 ± 19.2	**0.001**	55.9 ± 18.4	61.8 ± 19.3	**0.046**	0.165

Values are presented as mean ± SD.
HIIT: high-intensity interval training, MICT: moderate-intensity continuous training, BMI: body mass index, VO_{2peak}: peak oxygen consumption, ISI: insulin sensitivity index, DASS-21: depression, anxiety and stress scale – 21 items, PCOSQ: polycystic ovary syndrome questionnaire, SF-36, short-form survey – 36 items.

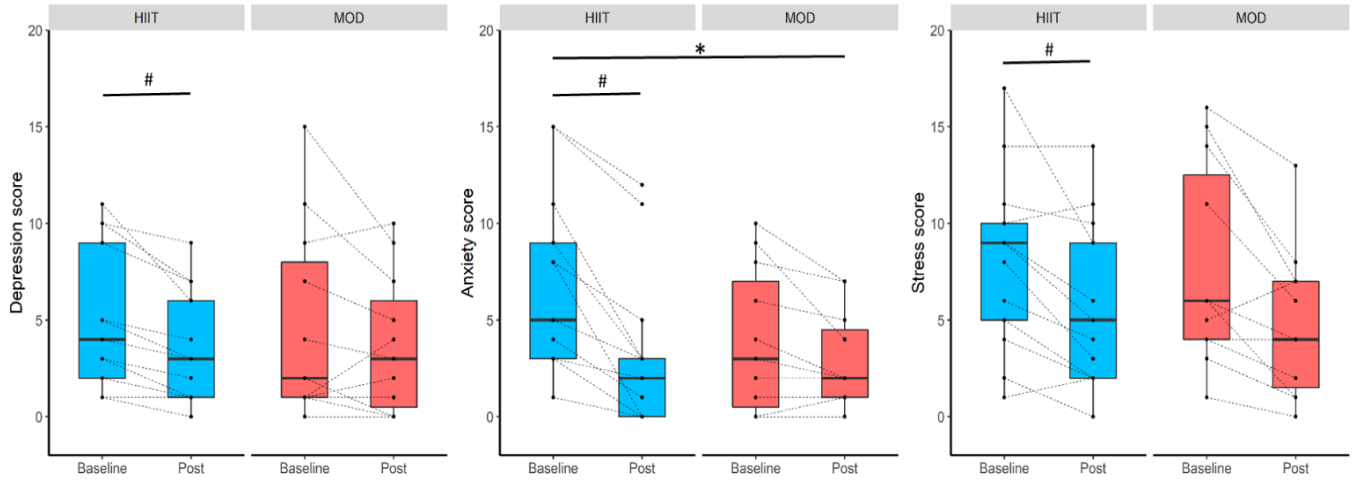

*Figure 17. Baseline and post-intervention scores for depression, anxiety and stress, stratified by group and using boxplots showing the median (central line) and 25th to 75th quartiles (box). *P <0.05 and **P <0.01 indicate significant within-group differences, # P <0.05 indicates a significant between-group differences, adjusted for age and baseline values.*

7.5 Discussion

This study demonstrated that participation in a 12-week HIIT intervention leads to a reduction in depression, anxiety and stress symptoms in overweight women with PCOS. The MICT intervention also led to a reduction in stress scores. HIIT resulted in a significantly greater reduction in anxiety symptoms compared to MICT. HIIT also resulted in improvements in multiple domains of both general and PCOS specific HRQoL. To the best of our knowledge, this is the first study to compare work-matched HIIT and MICT exercise interventions on mental health and HRQoL in women with PCOS.

It is well-established that women with PCOS display a higher prevalence of anxiety and depression disorders compared to women without PCOS[96]. Active women with PCOS report fewer depressive symptoms compared to inactive women, however, the prevalence is still greater compared to women without PCOS[89]. Although exercise is an effective means for managing mental health concerns in a general population, this relationship has been relatively understudied in women with PCOS. Existing research has a combination of positive and null findings, although approximately half of existing studies report significant improvements in symptoms of anxiety and/or depression[147]. Here we report significant improvements in depression, anxiety and stress scores as measured by the DASS-21 as a result of HIIT. Similar improvements have been reported for depression and anxiety scores using the HADS questionnaire in a recent study using a 16-week aerobic interval training intervention[231]. However, this study also found significant improvements after 16 weeks of continuous aerobic training. Unlike our study, their continuous aerobic training group was of moderate to vigorous intensity and was matched with the interval group for time rather than work. Other successful interventions for improving symptoms of depression and anxiety have used multi-component interventions which have also included either diet[237], medication[247] or cognitive behavioural therapy[242]. Resistance training

interventions have also reported improvements in depression and anxiety scores[238,245]. The large variation in the interventions implemented in prior studies makes it difficult to adequately assess the impact of exercise alone on symptoms of depression and anxiety, however, our current findings suggest that HIIT may be a promising therapy for improving mental health in women with PCOS. Further research with larger sample sizes and long-term follow-up is required to confirm these findings.

Women with PCOS also display reductions in health-related quality of life compared to women without PCOS[307]. Challenges with poor body image and dissatisfaction, low self-esteem, fertility, acne, hirsutism and long-term health concerns have been shown to compromise HRQoL in women with PCOS[2,24]. Here we report significant improvements in the emotions and menstrual problem domains of the PCOSQ as a result of HIIT and significant improvement in the weight domain as a result of both interventions. This is an unexpected result as neither group resulted in significant improvements in body weight or BMI. Similar results have been reported by previous studies that utilised a lifestyle modification intervention which also included diet[161,244], however, the majority of studies that reported change in the weight domain of the PCOSQ also reported significant weight loss[237,241,247]. Weight loss has also been assumed to be a significant contributor to improvements in distress caused by PCOS symptoms. Our findings indicate that exercise, despite a lack of weight reduction, can improve this distress.

We have previously shown that HIIT results in improvements in aerobic capacity, insulin sensitivity, menstrual regulation and hormonal profiles (Chapters 5 & 6). In this chapter, we report significant improvements in depression, anxiety and stress scores as a result of HIIT. The high adherence rate to exercise sessions likely contributed to these significant improvements, suggesting that HIIT holds promise as a time-effective and efficacious intervention for both physiological and psychological outcomes for women with PCOS when adequately adhered to. This is extremely important given the high rates of mental health concerns and distress resulting from the symptoms of PCOS, contributing to a decreased HRQoL. Here, we were only able to detect a significant association between depression and anxiety scores and insulin sensitivity index, and anxiety scores and VO_{2peak} at baseline, however, it is likely that there may be a bidirectional relationship between physiological and psychological outcomes. Future, large-scale studies are required to determine the extent of this relationship and whether improvements in physiological outcomes have a direct influence on mental health.

Given the high prevalence of mental health disorders in women with PCOS, this area is considerably under studied and additional research is required. We recommend that future studies include a follow-up period to determine whether HIIT is sustainable post-intervention and to determine whether the improvements observed here can be maintained long-term. In conclusion, supervised HIIT is safe, well-tolerated and efficacious for improving symptoms of depression, anxiety and stress, and can improve some domains of both general and PCOS specific HRQoL. The results of this chapter suggest that

further investigation into the benefits of HIIT for improving mental health and HRQoL outcomes in women with PCOS is warranted, and has the potential to inform the clinical management guidelines and ultimately decrease the prevalence of mental health concerns in women with PCOS.

Chapter 8.0 General discussion and conclusions

8.1 Thesis aims

The overall aim of this thesis was to explore the effects of exercise, and more specifically exercise intensity, on multiple domains of health in women with PCOS. Chapters 2 & 3 synthesised the current literature to advance our understanding, summarised the potential effects of exercise and aimed to determine whether certain exercise characteristics could promote greater change in key clinical outcomes in PCOS. Using the knowledge gained from Chapters 2 & 3, the aim of Chapter 4 was to develop and report the methods of our intervention, designed to compare moderate-intensity (MICT) to vigorous-intensity exercise recommendations using a HIIT protocol. Chapters 5, 6 & 7 examined the impact of HIIT compared to standard MICT recommendations on cardio-metabolic, reproductive and mental health outcomes in overweight women with PCOS.

8.2 Summary of key findings

Chapter 2

In Chapter 2, a systematic review and meta-analysis explored the impact of exercise intensity, duration, modality of exercise and the interaction between diet and exercise on key clinical outcomes in PCOS. Our analysis demonstrated that exercise training improves cardio-metabolic outcomes, both in the presence of and independent to changes in body composition. We also identified that the greatest health improvements were observed after 20 hours of vigorous-intensity exercise over a 12-week period.

Chapter 3

In Chapter 3, we conducted a systematic review to synthesise the current literature that aimed to determine the effectiveness of exercise for improving symptoms of mental health and HRQoL in women with PCOS. This review found that exercise resulted in both statistically and clinically meaningful improvements in HRQoL. Half of all included studies also reported significant improvements in symptoms of depression and anxiety. The limited number of studies that reported mental health outcomes, the heterogeneity of included studies and the poor reporting of exercise intervention characteristics limited the ability to meta-analyse and form conclusions regarding the specific effect of exercise intensity.

Chapters 5, 6 & 7

Chapters 5, 6 and 7 reported the results of a randomised clinical trial which explored the effectiveness of HIIT in comparison to MICT on cardio-metabolic, reproductive and mental health in overweight women with PCOS. Chapter 5 explored the effects of HIIT in comparison to MICT on cardio-metabolic health, finding that HIIT elicited significantly greater improvements in aerobic capacity and insulin sensitivity. There were also significant improvements in waist circumference, lean mass percentage and

fasting glucose as a result of HIIT, whilst the MICT group only significantly improved aerobic capacity and waist circumference. These results occurred despite no reduction in weight or BMI in either group.

Chapter 6 investigated the effect of HIIT in comparison to MICT for improving reproductive health outcomes. We found greater improvements in hormonal profiles in HIIT compared to MICT, with a significant increase in SHBG, and significant decreases in FAI and the percentage of free testosterone following HIIT. Although there was no significant between-group differences, it appears that HIIT may be more effective for improving menstrual cyclicity.

In Chapter 7, we examined the impact of HIIT in comparison to MICT for improving mental health and HRQoL in women with PCOS. HIIT resulted in superior improvements in anxiety scores in comparison to MICT, but also significant within-group improvements in depression and stress scores. HIIT also significant improved emotions, weight and menstrual problem domain scores of the PCOSQ, and physical functioning, role emotional, energy and general health domain scores of the SF-36. MICT significantly improved stress scores, weight domain scores of the PCOSQ, and role emotional and general health domains of the SF-36.

To the best of my knowledge, this study is the first to match and compare moderate-intensity to vigorous-intensity exercise recommendations in overweight women with PCOS. There were no adverse outcomes during the course of this study, and all participants adhered to the exercise protocols. In conclusion, HIIT is effective for improving cardio-metabolic, reproductive and mental health and HRQoL. Despite the overall superior effect of HIIT over MICT, MICT appears to be effective for improving aerobic capacity, stress scores and some domains of HRQoL. Taken together, HIIT is advantageous and appears to be more beneficial for improving key clinical features of PCOS. Therefore, we recommend HIIT to be considered as an effective method for improving health outcomes in overweight women with PCOS.

8.3 General discussion

PCOS has major health implications with a substantially increased risk of developing IGT, T2DM and sub-fertility. Consequently, women with PCOS also often report a poor quality of life and increased symptoms of depression and anxiety[5,307]. The benefits of exercise for improving health and mortality are irrefutable[111] and exercise is well-established as an effective tool for managing symptoms and decreasing the likelihood of developing co-morbidities in women with PCOS[142,143]. However, current exercise recommendations often have high attrition rates due to general barriers such as time limitation and low enjoyment, and fail to normalise androgen excess, menstrual cyclicity, IR and mental health[89,147]. Despite substantial evidence indicating that exercise is beneficial for promoting a range of health outcomes in women with PCOS, the current exercise recommendations have low uptake and poor adherence, and many women with PCOS remain inactive[89]. Conclusions from existing research consistently states the need for well-designed studies to determine the optimal exercise

recommendations to improve the physical and mental health of women with PCOS[142,147,152]. This thesis contributed to addressing these needs with the objective to inform future clinical recommendations regarding exercise prescription for women with PCOS. HIIT may provide a solution, addressing many of the known barriers to exercise participation and providing a potent form of exercise to improve critical clinical outcomes.

8.3.1 Exercise and cardio-metabolic health

PCOS is strongly linked with insulin resistance and the development of T2DM. A meta-analysis of 35 studies of women with PCOS reported increased odds for insulin resistance (OR 2.54, CI 1.44-4.47) and T2DM (OR 4.00, CI 1.97-8.10)[3]. Furthermore, 15.0%-35.6% of all incident cases of T2DM in white woman have been estimated to be attributable to PCOS[310], reflecting a need to adequately prevent or treat insulin resistance and T2DM in this population group. We report superior improvements in insulin sensitivity as a result of HIIT in women with PCOS, which likely lowers the metabolic risk in these women. We also report superior improvements in cardiorespiratory fitness as a result of HIIT. Cardiorespiratory fitness is well known to be an important indicator of health and all-cause mortality, with a VO_{2peak} being a stronger predictor of mortality than BMI[111]. Results reported here are consistent with studies in other chronic conditions[121,132,311] and further the argument that HIIT is effective for improving insulin sensitivity and aerobic capacity in women with PCOS. Recently, a systematic review and meta-analysis was conducted exploring the effects of HIIT in women with PCOS[312]. They reported that HIIT alone is an effective strategy for reducing HOMA-IR and BMI in women with PCOS[312]. Although this review is helpful, studies which also included a pharmacological treatment (metformin), or dietary interventions alongside HIIT were included in this review, therefore making it impossible to separate the impact of HIIT in comparison to other treatments. In addition, multiple included studies stated that HIIT was used but did not specify exercise intensity or protocol. The results of our clinical trial detailed in Chapter 5 provide further support for the beneficial effect of HIIT alone for improving aerobic capacity and insulin sensitivity in women with PCOS.

Although there is limited data on the effects of HIIT in women with PCOS, there is a multitude of evidence among other populations with chronic disease and obesity[118,121,133,134,313]. A meta-analysis which included studies with individuals with lifestyle-related metabolic diseases reported that increases in cardiorespiratory fitness are approximately doubled after HIIT in comparison to MICT[121]. Other reported benefits included improvements in insulin sensitivity and reduced cardiovascular risk factors with these benefits occurring despite no reduction in BMI[121]. These findings have possible implications for women with PCOS as the symptoms of insulin resistance and obesity are often that of a lifestyle-related metabolic condition. This meta-analysis along with the results from our study (Chapter 5) are supportive of the benefits of improving fitness rather than weight loss, suggesting that it may be more beneficial for both patients with lifestyle-related conditions and women with PCOS to improve physical fitness rather than focusing solely on weight loss.

Adding to this, physical activity has been found to attenuate the health risks of obesity, with active obese individuals having a lower morbidity and mortality rate than healthy weight sedentary individuals[314]. Given the high risk of women with PCOS developing IGT and T2DM, it is particularly important for women with PCOS to be active and focus on increasing fitness rather than weight loss. Furthermore, HIIT or vigorous-intensity exercise could be recommended as an effective way to reduce this risk and to engage in exercise in a time efficient manner.

In the first study to include a HIIT component within their intervention for women with PCOS, Hutchison et al. reported significant improvements in both GIR and fasting insulin[153]. A second study utilising a HIIT only intervention reported decreased HOMA-IR and decreased fasting insulin levels post-intervention[157]. Although there is limited evidence of the benefits of HIIT for improving insulin sensitivity in women with PCOS, exercise of vigorous-intensity (continuous exercise) also appears to provide greater benefits in comparison to moderate-intensity exercise. In the meta-analysis conducted by our group (Chapter 2), we reported that the greatest improvements in HOMA-IR were observed as a result of vigorous intensity exercise. Combined with the results of our clinical trial (Chapter 5), there is sufficient evidence in support of superior benefits of HIIT or vigorous-intensity exercise in comparison to MICT for improving metabolic health in women with PCOS.

It is important to note that we observed significant improvements in the HIIT intervention when using outcomes from the gold standard insulin clamp, including insulin sensitivity index and glucose infusion rates, but not when using HOMA-IR. These discrepancies are consistent with a large study in 375 women with PCOS, which determined that insulin resistance was present in 74.9% of women using the insulin clamp but this percentage was significantly lower when using surrogate indexes, with HOMA-IR identifying 41.1% of women as insulin resistant[4]. Results from our study support the indication that HOMA-IR may not be sensitive enough to detect changes in insulin sensitivity. When feasible, it is fundamental to use gold standard measurements to identify impairments in insulin action in women with PCOS due to their ~4 fold increased risk of developing T2DM[3], and the bidirectional association between insulin resistance and hyperandrogenism. Early identification of insulin resistance would also allow for early intervention and reduce the likelihood of developing T2DM.

8.3.2 Exercise and reproductive outcomes

Hyperandrogenism is a hallmark feature and is present in the vast majority of women with PCOS[315]. Testosterone is the major circulating form of androgen, with a large proportion being bound to SHBG[316]. Therefore, FAI is considered a more sensitive indicator of hyperandrogenism than total testosterone[317]. We observed no change in total testosterone, however, we saw significant improvements in both SHBG levels and FAI, suggesting that the amount of bioavailable testosterone was reduced as a result of HIIT. There has been very little research into the effects of HIIT on reproductive health in women with PCOS, however, significant increases in SHBG and FAI have also been observed as a result of other exercise

interventions in women with PCOS[155,157-160]. Insulin resistance and the resultant hyperinsulinaemia also contribute to the decreased SHBG levels and increased circulating androgens[59]. Exercise is well known to improve insulin sensitivity in women with PCOS[153,155,158,164,206], which likely contributes to lower levels of circulating androgens[318]. In our results, we report a significant association between the delta changes of both insulin sensitivity and SHBG levels, suggesting that exercise may be able to directly interrupt the vicious cycle between androgen concentrations and insulin resistance in women with PCOS.

There is also a relationship between menstrual dysfunction and the degree of insulin resistance, with women with PCOS that have a cycle length greater than 35 days displaying significantly higher HOMA-IR levels[319]. This elevated risk has been highlighted in a recent biobank study where they reported the risk of T2DM was 37% (OR 1.37, CI 1.22-1.53) higher for every 1 SD increase in bioavailable testosterone[320]. They also found evidence for a protective effect of SHBG on the risk of T2DM[320] suggesting that by increasing the levels of SHBG, we can lower the risk of women with PCOS developing T2DM. Furthermore, in a large retrospective cohort study it was reported that women with increased serum testosterone levels have an increased incidence of T2DM[321]. Improving insulin sensitivity is said to restore reproductive function via improvements in hormonal levels[149], suggesting that there is likely a link between the improvements observed in this study. Although we found no significant between-group different in menstrual cyclicity (Chapter 6), it is important to note that 69% of participants in the HIIT group reported an improvement after the intervention. The lack of between-group differences is likely due to the small sample size which was further reduced due to 2 women in the MICT group having regular cycles at baseline.

Anovulation can also result from hyperinsulinaemia and insulin resistance[322], therefore, by improving both menstrual function (as seen in Chapter 6) and reducing insulin resistance (as seen in Chapter 5), it is likely that HIIT may contribute to improved fertility. Infertility is the most prevalent concern among women with PCOS[323]. PCOS accounts for approximately 80% of women with anovulatory infertility[232]. Assessing ovulation in women who have oligo- or amenorrhoea is difficult and often menstrual cyclicity is used a proxy. Fertility is negatively impacted by obesity, reducing the likelihood of naturally becoming pregnant[232]. In addition, obesity increases the risk of miscarriage, and reduces the long-term health of both the mother and child by increasing the risk of metabolic diseases and congenital abnormalities[232]. Significant benefits have been reported with a weight loss of 5-10% in overweight women with PCOS[324,325], however, lifestyle interventions may also improve infertility risk factors independent of weight loss. In women with PCOS, lifestyle interventions which include both diet and exercise, have also shown promising fertility outcomes with weight loss resulting in spontaneous ovulation, spontaneous pregnancy and the success of reproductive therapies[172,173,296,326]. Interventions which solely focus on the impact of exercise on fertility have not yet been conducted in women with PCOS, however, similar to our findings, exercise studies have reported improvements in menstrual

function[164,165]. Despite not having assessed fertility outcomes in our study, the observed improvements in menstrual cylicity (Chapter 6) may translate into improved fertility. Furthermore, HIIT shows more potential for regulating menstrual cycles. Future studies which assess the impact of HIIT on menstrual cycles, ovulation, and fertility are required to confirm the findings observed in our study.

8.3.3 Exercise and mental health

Aside from the physical consequences of the condition, many women with PCOS also experience issues relating to mental health and report a decreased quality of life in comparison to women without PCOS. Depression can further increase the burden associated with PCOS, reducing the likelihood of participating in exercise and worsening the severity of the syndrome. There is a strong relationship between exercise and depression. Individuals with depression are typically less likely to be active[327], and those who exercise regularly are far less likely to have depression[328,329]. This is also true among women with PCOS, with active women displaying fewer symptoms of depression in comparison to inactive women[89].

Exercise is known to have a positive effect on mental health, reducing the symptoms or severity of depression and assisting in improving quality of life in the general population[175], in individuals with chronic conditions[176], and in individuals with depression[330]. The limited existing research exploring the effects of exercise on mental health in women with PCOS indicates a positive effect, with half of all studies reporting either a decreased prevalence of depression or fewer depressive symptoms post-intervention. We also reported a significant decrease in depression scores as a result of HIIT, but not MICT (Chapter 7). This is in contrast with other studies examining the impact of exercise intensity among other population groups, which often report that exercise of both moderate and vigorous intensities are sufficient for improving mental health[331-333].

The mechanisms behind the anti-depressant effects of exercise are not well understood and have not been extensively studied[334], however, there are multiple proposed mechanisms in PCOS. First, there is evidence to suggest that symptoms of PCOS such as infertility, obesity, hirsutism and acne may be contribute to poorer mental health[90,335,336]. Elevated testosterone[337] and insulin resistance[91,338,339] have also been associated with depression in women with PCOS. Exercise has the potential to address all of these factors. In Chapter 6, we reported decreased bioavailable testosterone levels, increased SHBG and insulin sensitivity as a result of HIIT, which may potentially reduce the severity of depressive symptoms. In line with this, we did note a decrease in depressive symptoms as a result of HIIT, however, we were unable to determine whether the metabolic and hormonal changes, such as the decrease in bioavailable testosterone, were a contributing factor.

Two recent meta-analyses which examined the impact of HIIT on depression in individuals with mental illness reported moderate improvements in depression[340,341]. They both also reported a modest advantage of HIIT in comparison to MICT. A randomised clinical trial comparing HIIT to MICT in

healthy women reported a significant decrease in depressive symptoms post-intervention, with no between-group differences[342]. They also reported that there were no within or between group differences on anxiety scores[342]. This is in contrast with our findings where we reported significant decreases in anxiety scores in both groups, although, HIIT resulted in a significantly greater decrease in anxiety scores compared to MICT. HIIT also resulted in significant improvements in depression and stress scores, whereas MICT did not. Somewhat similar to our results, a recent study which compared continuous aerobic training, intermittent aerobic training and strength training in women with PCOS reported significant improvements in anxiety and depression scores across all three groups, however, the greatest improvements were observed in the intermittent aerobic training group, also suggesting a greater beneficial role for higher-intensity exercise training. Overall, it appears that exercise, irrespective of intensity, has a positive impact on mental health, however, HIIT may provide additional benefits compared to moderate-intensity exercise. Future large-scale studies are required to confirm the efficacy of HIIT in comparison to MICT in women with PCOS.

8.3.4 Resistance training

Although the intervention studies reviewed and tested within this thesis focused on aerobic exercise, resistance training has shown some promise for improving health outcomes in women with PCOS. Resistance training is included in the physical activity and sedentary behaviour guidelines for healthy populations[144], and in the PCOS exercise guidelines, where they recommend strengthening exercise on 2 non-consecutive days per week[32], however, resistance training is currently not widely used in PCOS research. Results from our meta-analysis (Chapter 2) suggest that resistance training may improve FAI and HOMA-IR in women with PCOS, however, results from these analyses were limited to only three studies. A multi-component intervention which included both aerobic exercise and resistance training reported significant improvements in FAI, SHBG, total testosterone and HOMA-IR, although only an aerobic intervention was used and found no significant differences between the groups[155]. A second study which compared a HIIT group to a resistance training group reported significant improvements in reproductive outcomes as a result of resistance training, with no change in these outcomes as a result of HIIT[157]. However, they only reported improvements in insulin sensitivity as a result of HIIT[157]. Therefore, the question remains whether adding a resistance training component to an intervention could provide additional benefits for some health outcomes.

8.3.5 Exercise adherence

We demonstrated high adherence rates to both of our exercise interventions, with a mean adherence of 93.9 ± 3.0% in the HIIT group and 92.0 ± 4.8% in the MICT group. The high adherence rate likely contributed to the significant improvements observed as a result of these interventions. Improving adherence to exercise is crucial given the substantial health and economic implications of physical inactivity. It is well established that longer-term maintenance of engagement in exercise is required for individuals to sustain the accompanying health benefits[343]. Worldwide, 1 in 4 adults do not meet the

global recommendations for physical activity[344]. The 2017-2018 Australian National Health Survey indicated that only 55.5% of Australian adults participated in sufficient physical activity. Furthermore, women were less likely to be physically active compared to men, with only 50% of women and 59% of men being sufficiently active[345]. A similar rate of physical inactivity has been reported in women with PCOS [89,178].

Exercise adherence is known to be affected by multiple factors including perceived enjoyment, time availability, biophysical and psychosocial factors and intrinsic motivation[346]. Engaging in exercise that has the potential to addresses these factors can promote adherence and compliance to exercise interventions. HIIT is time efficient and has been reported to be equally or more enjoyable than MICT[126,139,250]. There is also strong evidence stating that women who are motivated to exercise in order to reduce weight or for concerns with body image or body dissatisfaction are less likely to adhere to exercise in comparison to women who are intrinsically motivated by factors such as enjoyment, competence, and social interaction[347]. Moreover, there is evidence to suggest that psychosocial health, including quality of life, plays a role in the adherence to exercise[348]. Results from this study suggest that HIIT may also improve psychosocial health which may have contributed to the high adherence rates observed. Given the increased risk of developing co-morbidities, including a reduced quality of life, participation in regular exercise is critical for women with PCOS. The high adherence rates reported as a result of our intervention suggest that it is feasible for women with PCOS to undertake exercise at both moderate and high-intensities. However, all as exercise sessions were supervised, requires further exploration to determine whether or not HIIT protocols can be initiated and maintained in a real-world setting in women with PCOS.

One recent 16 week study in women with PCOS compared two semi-supervised HIIT interventions (low-volume and high-volume HIIT) to a control group[228]. They reported a much lower adherence rate with participants in both groups only attending an average of 66.6% and 73.3% in the high-volume and low-volume HIIT groups, respectively. They reported very few changes as a result of their interventions, which they attribute in part to the poor adherence[228]. Unsupervised exercise and lifestyle interventions in women with PCOS have poor adherence rates which likely contributes to the heterogeneity of findings, including a mix of positive and null findings, from such interventions[152,156,164,199]. One unsupervised study which compared a lifestyle intervention (diet and exercise) only group to a lifestyle plus cognitive behavioural therapy group reported a high adherence rate among women who also received with cognitive behavioural therapy (CBT)[242] offering a potential strategy for improving adherence. Although promising, only 59% of women in the combined group compared to 38% in the lifestyle group met their weekly exercise goals. This suggests that perhaps supervised exercise is initially required, followed by a tapering of supervision, and combined with CBT or goal setting to improve adherence.

8.4 Limitations and considerations

This thesis provides insight into the importance of exercise intensity and the potential health implications for women with PCOS, however, as with any study, the results must be considered in context with the inherent limitations. Chapter specific limitations have been discussed throughout.

Briefly, a limitation of both the meta-analysis (Chapter 2) and systematic review (Chapter 3) was the large heterogeneity among the studies included, with considerable variations in the exercise interventions utilised. In the case of the meta-analysis, this reduced the amount of studies used for each sub-group and limited the ability to form adequate conclusions regarding the effectiveness of certain exercise characteristics (e.g. type and dose) on key clinical cardio-metabolic and reproductive outcomes. In regards to the systematic review, the large heterogeneity combined with poor reporting of exercise interventions hindered our ability to meta-analyse and form conclusions regarding the effectiveness of exercise characteristics (intensity, type and dose) on mental health and HRQoL outcomes.

8.4.1 Limitations of the clinical trial

The following provides a brief discussion of limitations that relate to the study design and outcomes of the clinical trial (Chapters 5, 6 and 7), and how these might influence the key findings and conclusions resulting from this research.

Participant recruitment and sample size:

Women with PCOS were only eligible for this study if they met the stringent inclusion and exclusion criteria (outlined in Chapter 4), and any potential participants who were taking oral contraceptives or insulin sensitisers were excluded. These are the two most common medications prescribed to women with PCOS and therefore results from this study are not representative of the entire population of women with PCOS. In addition, only overweight and obese women participated in this study. Furthermore, due to the limited sample size, we were not able to determine if the various PCOS phenotypes responded differently to the training stimulus.

A limitation of this thesis is the relatively small sample size, and although we were powered to detect changes both within groups and between-groups, we were limited in terms of the regression analyses in which only observed significance was used when using pooled data rather than analysing according to group allocation (i.e., intention to treat analysis). It could also be considered that the use of the MICT group as opposed to a no-exercise intervention is a potential limitation. However, it is unlikely that changes in health status would occur following a no-intervention control and therefore would not address the aim of this thesis. In addition, according to the CONSORT statement for non-pharmacological treatments, an active treatment can also be considered the control group[349]. In the case of our study, the MICT group was equivalent to the general aerobic exercise recommendations for all

women with PCOS and enabled all participants to receive exercise training, resulting in health benefits, as opposed to including a non-intervention control group.

Participant recruitment was also particularly challenging due to the relatively small number of women with PCOS who meet the criteria, the large time commitment for the research assessments and interventions, and the invasive nature of the study, which included euglycaemic-hyperinsulinaemic clamps. We were also unable to collect blood samples from two participants within the HIIT protocol due to difficulty with cannulation, which further reduced the sample size for these outcomes.

Outcome measures:

A second limitation is the use of menstrual diaries as a proxy for ovulation. Although ovulatory data would have added strength to this thesis, given the time and cost involved in addition to the potential detrimental impact this may have had on recruitment, we decided it was not feasible to include.

Influence of habitual physical activity and dietary habits:

Although participants were asked to maintain their usual physical activity and diet, it is possible that their usual routines were altered, potentially impacting the study outcomes.

8.5 Practical implications

Exercise is widely recommended for women with PCOS, however, the importance of exercise intensity is not well understood, and it is often assumed that exercise of moderate and vigorous intensities have equivalent benefits for physical and mental health outcomes. The work included in this thesis provides new insight into the importance of exercise intensity and provides initial validation in support of HIIT in comparison to standard MICT for improving a range of health outcomes in women with PCOS. The findings observed here are of critical importance for women with PCOS given the increased risk of developing T2DM and the substantial implications of PCOS symptoms on reproductive and mental health. Improvements in insulin sensitivity, cardiorespiratory fitness, menstrual cyclicity, hormonal profiles and mental health, will likely reduce the need for ongoing health care, leading to a reduction in economic burden for the individual and reducing the cost to the healthcare system.

The HIIT intervention utilised here was a potent stimulus for health improvements and occurred despite the substantial difference in the total work time required. Considering that time limitation is the most commonly cited barrier for exercise participation in women with PCOS[89], HIIT provides an attractive alternative. Furthermore, we had an average attendance of 94% within the HIIT intervention, demonstrating that HIIT is feasible to undertake and adhere to for women with PCOS. This is important as many clinical trials in women with PCOS report poor adherence to the exercise protocols. We cannot state whether this protocol led to long-term engagement with exercise, or sustained health improvements and therefore future studies with long-term follow ups are required. Lastly, the observed

improvements occurred despite weight loss, suggesting that a focus towards improving fitness rather than reducing weight may be advantageous.

The findings observed here may also have implications for clinical practice. Although large studies exploring the efficacy and effectiveness of HIIT studies are required in both overweight and lean women with PCOS, HIIT protocols could be utilised by exercise professionals to improve the clinical outcomes of PCOS. In addition, HIIT protocols can also be adapted to suit both private practice and public health settings. However, although HIIT has been found to be safe among clinical populations, in order to maximise safety, appropriate screening and regular monitoring is required. It may also be important to consider whether HIIT could be delivered in a group setting rather than an individual settings as done in most clinical trials. Group HIIT sessions may provide an opportunity for social interaction, which may have the potential to increase adherence and make the sessions more enjoyable for those participating.

8.6 Suggestions for future directions

Overall, this thesis advances our current knowledge surrounding the importance of exercise intensity for promoting health outcomes in overweight women with PCOS, however, future research is required to confirm, and build upon these findings. Firstly, we could benefit from exercise studies, particularly HIIT interventions with long-term follow-up to determine whether these improvements are sustainable post-intervention and to determine the level of activity that is required to sustain the observed improvements. Given the high prevalence of infertility among women with PCOS, it is important to determine whether HIIT is effective for improving fertility. Secondly, larger studies that are sufficiently powered for all measures are required to confirm the observed improvements reported here. Studies with larger samples sizes would also offer the opportunity to examine the impact of exercise on the various phenotypes of PCOS and to determine whether or not the phenotypes respond differently to exercise intensities. We could also benefit from exploring HIIT among lean women with PCOS to determine if they experience the same improvements observed in overweight and obese women. Thirdly, it would be relevant for translation into clinical practice to examine the impact of varying levels of supervision of exercise sessions. For long-term compliance, studies that commence with full supervision, and progress to partial and non-supervised interventions may provide an opportunity to encourage self-motivation and self-reliance to hopefully promote longer-term adherence and compliance to exercise interventions. Examining any difference in attendance and adherence to individual or group-based delivery of HIIT would also be of relevance to both clinical and community settings. Fourthly, interventions that include both HIIT and resistance training may provide additional health benefits. Future studies incorporating these two elements are required.

8.7 Conclusion

PCOS carries a major health burden across metabolic, reproductive and mental health, and places women on a trajectory for developing a plethora of diverse, often chronic, conditions. This thesis provides evidence in the form of systematic reviews and a randomised clinical trial for the beneficial effects of HIIT in improving a range of physical and mental health outcomes in women with PCOS. HIIT was more effective in improving aerobic capacity, insulin sensitivity, menstrual cyclicity, SHBG, free testosterone, anxiety, and domains of health-related quality of life in comparison to MICT. HIIT should be considered as an effective method for improving the health of women with PCOS. Further studies are required to confirm these findings and determine the long-term benefits of this form of exercise.

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Supplementary material

Supplementary Table 1. Search terms for systematic review

PCOS	**Exercise**	**Limits**
Polycystic ovary syndrome or Polycystic ovar* or PCO* or Stein Leventhal or Leventhal	And – Exercise or Physical or Sport or Strength or Resistance or Lifestyle	Not – Insulin Resistance

Supplementary Table 2. Modified Downs and Black checklist for assessing methodological quality (Downs and Black, 1998).

Number	Question	Score		
Reporting				
1	Is the hypothesis/aim/objective of the study clearly described?	Yes = 1	No = 0	
2	Are the main outcomes to be measured clearly described in Intro or Methods section?	Yes = 1	No = 0	
3	Are the characteristics of the patients included in the study clearly described?	Yes = 1	No = 0	
4	Are the interventions of interest clearly described?	Yes = 1	No = 0	
5	Are the main findings of the study clearly described?	Yes = 1	No = 0	
6	Does the study provide estimates of the random variability in the data for the main outcomes? (distribution - SE, SD, CI)	Yes = 1	No = 0	
7	Did they report adherence to intervention?	Yes = 1	No = 0	
8	Have all important adverse events that may be a consequence of the intervention been reported?	Yes = 1	No = 0	
9	Have the characteristics of patients lost to follow-up been described?	Yes = 1	No = 0	
10	Was all exercise supervised?	Yes = 1	No = 0	Unable to determine = 0
11	Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the p<0.001?	Yes = 1	No = 0	Unable to determine = 0
Validity - bias				
12	Was an attempt made to blind those measuring the main outcomes of the intervention?	Yes = 1	No = 0	Unable to determine = 0
13	Were the statistical tests used to assess the main outcomes appropriate	Yes = 1	No = 0	Unable to determine = 0
14	Was compliance with the intervention/s reliable?	Yes = 1	No = 0	Unable to determine = 0
15	Were the main outcomes measures used accurate (valid and reliable)	Yes = 1	No = 0	Unable to determine = 0
16	Were the patients in different intervention groups and control group recruited from the same population?	Yes = 1	No = 0	Unable to determine = 0
17	Were study subjects in different intervention groups and control group recruited over the same period of time?	Yes = 1	No = 0	Unable to determine = 0
18	Were study subjects randomised to intervention groups?	Yes = 1	No = 0	Unable to determine = 0
19	Was the randomisation concealed from both patients and health care staff until baseline testing was complete?	Yes = 1	No = 0	Unable to determine = 0
20	Were losses of patients to follow-up taken into account?	Yes = 1	No = 0	Unable to determine = 0
Power				
21	Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5% (size of smallest intervention group)	Yes = 1	No = 0	Unable to determine = 0

Supplementary Table 3. Summary of results from individual studies for each meta-analysed outcome measure.

				VO_{2peak} (mL.kg.min^{-1})					BMI (kg/m^2)					Waist Circumference (cm)				
Study	**Diet**	**Group**	**N**	**Mean Baseline**	**Baseline SD**	**Delta**	**SE**	**SEM**	**Mean Baseline**	**Baseline SD**	**Delta**	**SE**	**SEM**	**Mean Baseline**	**Baseline SD**	**Delta**	**SE**	**SEM**
Almenning et al.[157]	No	RT	8	39.3	10.2	0.9	0.9	1.7	27.1	6.6	0.4	0.3	0.6	93.7	17.6	-1.4	0.7	1.4
	No	HIIT	8	37.4	4.7	3.7	0.5	0.9	23.8	4.8	0.1	0.2	0.3	86.8	12.9	0.4	2.1	4.1
	No	Control	9	36.8	7.8	-0.8	0.8	1.7	26.3	5.2	-0.2	0.3	0.7	92.6	15.5	-0.3	1.6	3.4
Bruner et al.[195]	Yes	AE	7						36.2	5.3	-0.3	0.8	1.4	98.3	13.2	-5.2	0.9	1.6
	Yes	Control	5						37.1	7.6	-1.2	0.9	1.5	99.8	11.2	-5	0.6	0.9
Costa et al.[196]	No	AE	14	27.9	3.3	5.9	0.9	2.3	32.0	4.2	-0.7	0.2	0.6	92.8	10.3	-3.7	1.3	3.3
	No	Control	13	26.8	6.2	0.2	0.9	2.3	33.6	5.1	0.7	0.4	0.9	94.1	11.6	3.8	1.3	3.2
Curi et al.[200]	Yes	AE + RT	12						31.8	1.6	-1.7	0.5	1.2	101.8	3.9	-6.7	1.5	3.7
Giallauria et al.[158]	No	AE	62	19.0	3.9	5.5	1.6	8.9	29.2	2.9	-1.3	0.1	0.7					
	No	Control	62	18.8	3.3	-0.2	0.2	0.9	29.5	3.5	-0.2	0.1	0.7					
Hutchinson et al.[153], Moran et al.[52], Hutchinson et al.[198], Harrison et al.[79]	No	AE	13	25.9	6.5	5.5	1.8	4.6	35.6	5.8	-0.6	0.3	0.8	104.0	13.3	-1.0	1.2	3.0
Ladson et al.[161]	Yes	AE	16											107.1	18.4	-4.1	1.1	3.2
Miranda-Furtado et al.[159] Kogure et al.[204] Kogure et al.[205]	No	RT	45						28.5	6.0	-0.2	0.1	0.7	81.7	12.8	-1.2	0.3	1.6
Moro et al.[206] Redman et al.[207] Covington et al.[154] Covington et al.[197]	No	AE	8	27.5	1.3	3.4	1.0	1.9	32.1	5.2	-0.3	0.6	1.2					
Nybacka et al.[165]	No	AE	17						34.9	5.3	-0.9	0.4	1.2					
Nybacka et al.[208]	Yes	AE	12						38.8	7.9	-1.9	0.9	2.1					
	Yes	Control	14						34.7	5	-1.7	0.4	1.1					
Orio et al.[156]	Yes	AE	39	19.0	2.1	6	1.8	7.8	26.7	2.8	-1.4	0.1	0.6					
	No	Control	50	19.0	2.7	-0.5	0.3	1.6	27.0	2.9	-0.1	0.1	0.6					
Orio et al.[201]	Yes	AE	32	17.5	2.2	8.1	2.2	8.9	28.9	3	-2.1	0.2	0.7	94.6	4.3	-5.1	1.4	5.6
	Yes	AE	32	17.2	2.3	5.9	1.6	6.5	28.9	2.3	-1.2	0.2	0.7	95.1	4.4	-3	0.8	3.3
Randeva et al.[199]	No	AE	12						34.0	4.5	-0.2	0.2	0.7					
	No	Control	9						37.6	9.7	0.7	0.4	0.9					
Roessler et al.[202]	No	AE	8	25.0		3.2	1.5	2.9	34.8	7.1	-0.4	0.2	0.4	112.2	14.1	-6.0	2.5	5.15.1
Sprung et al.[203]	No	AE	6	27.1	5.2	5.0	1.6	2.8	31.0	5.7	-0.3	0.5	0.8	100.0	15.7	-4.0	2.6	4.5
Sprung et al.[162]	No	AE	10	29.0	5.4	4.7	0.8	1.8	31.0	4.2	-0.2	0.3	0.7	100.0	7.7	-3.0	2.0	4.5
	No	Control	7	23.8	2.5	-0.5	1.1	2.1	35.0	4.9	0.2	0.2	0.4	109.0	15.7	-1.0	1.5	2.8

Stener-Victorin et al.[209,210] & Jedel et al.[160]	No	AE	30	33.9	8.5	4.1	1.4	5.5	27.7	6.4	0.1	0.2	0.8	91.1	13.9	-0.6	0.4	1.6
	No	Control	15	35.2	9.3	2.2	0.6	1.6	26.8	5.6	0.2	0.2	0.5	86.0	12.0	-0.1	0.3	0.9
Thomson et al.[155,211,212]	Yes	AE	18	25.5	3.7	4.4	1.0	3.1						100.2	12.2	-11.7	1.4	4.3
	Yes	AE + RT	20	24.4	2.8	2.6	0.6	1.9						103.8	12.6	-11.0	1.4	4.5
	Yes	Control	14	23.8	3.7	-0.5	0.8	2.1						103.0	12.6	-10.8	1.9	5.0
Vigorito et al.[164]	No	AE	45	17.6	2.5	6.1	1.7	8.2	29.3	2.9	-1.3	0.1	0.7	94.5	3.4	-2.7	0.8	3.6
	No	Control	45	17.7	2.2	0.2	0.2	1.1	29.4	3.5	-0.1	0.1	0.7	94.0	3.0	-0.2	1.2	5.8

Supplementary Table 3 continued…

	HOMA-IR					**FAI**				
Study	**Mean Baseline**	**Baseline SD**	**Delta**	**SE**	**SEM**	**Mean Baseline**	**Baseline SD**	**Delta**	**SE**	**SEM**
Almenning et al.[157]	3.3	1.3	-0.3	0.3	0.6	2.8	1.7	-0.7	0.3	0.6
	4.9	1.7	-0.8	0.3	0.6	1.5	1.2	0.4	0.9	
	3.6	2.1	0.7	0.5	1.0	2.6	1.4	0	0.5	1.1
Bruner et al.[195]						13.9	6.9	0	1.8	3.6
						20.3	10.3	-0.5	3.6	5.2
Costa et al.[196]	3.0	2.0	-0.6	0.7	1.9					
	1.5	1.0	-0.5	0.3	0.8					
Curi et al.[200]	3.4	0.8	-0.6	0.8	1.9					
Giallauria et al.[158]						10.1	7.9	-1.8	1.3	9.8
						9.7	8.1	0.1	1.4	11.1
Hutchinson et al.[153], Moran et al.[52], Hutchinson et al.[198], Harrison et al.[79]						10.7	5.0	-0.6	1.0	2.8
Ladson et al. [161]						12.5	8.8	-1.6	1.6	5.1
Miranda-Furtado et al.[159] Kogure et al.[204] Kogure et al.[205]	2.3	1.9	0.2	0.3	1.2	8.3	6.4	-0.8	0.3	1.8
Moro et al.[206] Redman et al.[207] Covington et al.[154] Covington et al.[197]	3.6	2.7	-0.1	0.5	1.0	18.8	14.2	-1.6	1.3	2.8
Nybacka et al.[165] Nybacka et al.[208]										

Orio et al.[156]	4.2	1.2	-1.0	0.3	1.2	13.3	6.4	-0.3	1.5	7.9
	4.0	1.1	0.2	0.3	1.3	13.0	5.9	-0.2	1.3	7.7
Orio et al.[201]						8.5	2.1	0.3	1.1	5.1
						8.5	2.4	0.1	1.1	5.1
Randeva et al.[199]						9.0	5.7	-0.9	0.9	2.3
						12.7	8.8	-2.1	1.5	3.3
Roessler et al.[202]										
Sprung et al.[203]	3.5	2.3	0.5	1.1	1.8	8.5	2.5	0.7	0.8	1.4
Sprung et al.[162]	3.4	3.2	0.4	0.3	0.7	8.7	9.8	1.1	0.6	1.4
	3.4	1.9	1.1	0.5	1.0	8.7	9.3	0.6	0.7	1.4
Stener-Victorin et al.[209,210] & Jedel et al.[160]	1.6	1.0	-0.3	0.8	1.2	7.1	4.3	1.7	1.1	1.8
	2.0	1.5	-0.4	0.3	0.6	5.8	4.0	-0.4	0.8	1.5
Thomson et al.[155,211,212]	1.9	1.0	-0.5	0.2	0.6	8.5	5.6	-2.6	1.0	3.3
	2.0	1.0	-0.6	0.2	0.6	9.1	5.7	-2.9	0.9	3.1
	2.3	1.0	-0.6	0.2	0.6	11.2	5.5	-2.8	1.1	3.3
Vigorito et al.[164]						8.5	3.4	0.2	1.3	8.2
						8.6	3.6	-0.1	1.3	8.3

VO_{2peak} – Peak Oxygen Consumption, BMI – Body Mass Index, HOMA-IR – Homeostatic Model Assessment of Insulin Resistance, FAI – Free Androgen Index, SE – Standard Error, SEM – Standard Error of Measurement, RT – Resistance Training, HIIT – High Intensity Interval Training, AE – Aerobic Exercise

Supplementary Table 4. Magnitude thresholds based on standardisation factors.

	Beneficial effects (%)				
	Small	**Mod**	**Large**	**V.Large**	**X.Large**
VO_{2peak}	5.0	16	34	63	170
BMI	-4.0	-12	-22	-33	-56
WC	-2.5	-7.3	14	-22	-40
FAI	-12	-32	-54	-72	-92
HOMA-IR	-12	-32	-54	-72	-92
	Harmful effects (%)				
VO_{2peak}	-5.0	-14	-27	-40	-64
BMI	4.0	13	27	48	120
WC	2.5	7.7	16	28	64
FAI	12	41	97	210	860
HOMA-IR	12	41	97	210	860

Mod – Moderate, V.Large – Very Large, X-Large – Extra Large, VO_{2peak} – Peak Oxygen Consumption, BMI – Body Mass Index, WC – Waist Circumference, FAI – Free Androgen Index, HOMA-IR – Homeostatic Model Assessment of Insulin Resistance.

Supplementary Table 5. Results for the modified Downs and Black methodological quality assessment (Downs and Black, 1998).

	Reporting											Validity - bias									Power	Total
Author	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**Score**
Almenning et al. (2015)	1	1	0	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	0	1	17
Bruner et al. (2006)	0	0	1	1	0	1	0	1	0	0	1	0	1	0	1	1	0	1	1	0	0	10
Costa et al. (2018)	1	1	1	1	1	1	1	1	0	0	1	1	1	0	1	1	1	1	1	0	1	17
Curi et al. (2012)	1	0	1	0	1	1	0	0	0	1	1	0	1	0	1	1	0	1	0	0	1	11
Giallauria et al. (2008)	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	0	17
Hutchison et al. (2011)	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	0	0	0	0	1	1	15
Moran et al. (2011)	1	1	1	1	1	1	1	1	0	0	1	0	1	1	1	0	0	0	0	0	1	13
Hutchison et al. (2012)	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	0	0	0	0	0	1	14
Harrison et al. (2012)	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	0	0	0	0	0	1	14
Ladson et al. (2011)	1	1	1	0	1	1	0	0	1	1	1	1	1	0	1	1	1	1	1	1	1	17
Miranda-Furtado et al. (2016)	1	0	1	1	0	1	1	1	0	1	1	0	1	1	1	0	1	0	0	0	1	13
Kogure et al. (2016)	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	0	1	0	0	0	1	15
Kogure et al. (2018)	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	0	1	0	0	0	0	14
Moro et al. (2009)	1	1	1	1	1	1	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	10
Redman et al. (2011)	0	0	1	0	1	1	1	1	0	0	1	0	1	1	1	0	0	0	0	0	1	10
Covington et al. (2015)	1	1	0	1	1	1	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	9
Covington et al. (2016)	1	0	0	1	1	1	0	1	0	0	1	0	1	0	1	0	0	0	0	0	1	9
Nybacka et al. (2011)	1	1	1	0	1	1	0	0	0	0	0	0	1	0	1	1	0	1	0	0	1	10
Nybacka et al. (2012)	1	1	1	0	1	1	0	1	0	0	0	0	1	0	1	1	1	1	0	0	1	12
Orio et al. (2016)	1	1	1	1	1	1	0	1	0	1	0	1	1	1	1	1	1	1	1	1	1	18
Orio et al. (2008)	1	0	0	1	1	1	1	1	1	0	1	1	1	1	1	1	0	0	0	0	1	14
Randeva et al. (2002	1	1	1	1	1	1	1	0	0	0	1	0	1	0	1	1	1	0	0	0	0	12
Roessler et al. (2013)	1	1	1	1	1	1	1	0	0	1	1	0	1	1	1	1	0	1	1	0	0	15
Sprung et al. (2013a)	1	1	1	1	1	1	1	1	0	0	1	0	1	1	1	1	0	0	0	0	1	14
Sprung et al. (2013b)	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	0	0	0	0	1	1	15
Stener-Victorin et al. (2009)	1	1	0	1	1	1	0	0	0	1	0	1	1	0	1	1	1	1	1	0	0	13
Jedel et al. (2011)	1	1	1	1	1	1	0	0	1	1	0	0	1	0	1	1	1	1	1	1	1	16
Stener-Victorin et al. (2012)	1	1	1	1	1	1	0	0	1	0	1	1	1	0	1	1	1	1	1	1	0	16

Thomson et al. (2008)	1	0	1	1	1	1	0	0	0	1	1	0	1	0	1	1	0	1	0	0	0	11
Thomson et al. (2012)	1	1	1	1	1	1	0	0	0	1	1	0	1	0	1	1	1	1	0	0	0	13
Thomson et al. (2016)	1	0	1	1	0	1	0	0	0	1	0	0	1	0	1	1	1	1	0	0	0	10
Vigorito et al. (2007)	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	0	15
Brown et al. (2009)	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	0	0	17

Supplementary Table 6: Results for Downs and Black checklist for assessing methodological quality of included studies.

Author	Year	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Total
Arentz et al.	2017	1	1	1	1	1	1	0	1	1	0	1	1	1	0	1	1	1	1	1	1	1	18
Cooney et al.	2018	1	1	1	0	0	1	1	0	1	0	1	0	1	0	1	1	1	1	0	1	0	13
Costa et al.	2018	1	1	1	1	1	1	1	0	0	1	1	0	1	1	1	1	1	1	0	0	1	16
De Frène et al.	2015	1	1	0	0	0	1	0	0	1	0	1	0	1	0	1	0	0	0	0	1	0	8
Ladson et al.	2011	1	1	0	0	1	1	0	1	1	0	1	1	1	0	1	1	1	1	1	1	1	16
Lara et al.	2015	1	1	0	1	1	1	0	0	0	1	1	0	1	1	1	1	1	0	0	0	0	12
Ramos et al.	2016	1	1	1	1	1	1	0	0	0	1	1	0	1	1	1	1	1	0	0	0	0	13
Legro et al.	2015	1	1	1	0	1	1	0	1	0	0	1	1	1	0	1	1	1	1	1	1	1	16
Dokras et al.	2016	1	1	1	0	1	1	0	0	0	0	1	0	1	0	1	1	1	1	0	0	0	11
Ribeiro et al.	2019	1	1	1	1	1	1	0	1	0	1	1	0	1	1	1	1	1	1	1	0	1	17
Kogure et al.	2020	1	1	1	1	1	1	0	1	0	1	1	0	1	1	1	1	1	1	1	0	1	17
Stener-Victorin et al.	2013	1	1	1	1	1	1	0	0	0	0	1	1	1	0	1	1	1	0	0	1	0	13
Thomson et al.	2010	1	1	1	1	1	1	0	0	0	0	1	0	1	1	1	1	0	1	0	1	0	12
Thomson et al.	2016	1	1	1	1	1	1	0	0	0	0	1	0	1	0	1	1	1	1	0	1	0	12
Vizza et al.	2016	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	18

Supplementary Table 7: Food diary and IPAQ results at baseline and post-intervention

	HIIT (N = 13)			MICT (N = 11)			P (time x group)
Outcome measure	**Baseline**	**Post**	**P**	**Baseline**	**Post**	**P**	
Food Diary							
Total Energy (kJ)	7694.0 ± 1019.2	7550.9 ± 913.0	0.289	7640 ± 448.4	6981.1 ± 976.0	0.084	0.141
Protein (g)	86.8 ± 32.6	91.3 ± 29.9	0.487	100.8 ± 23.2	90.1 ± 15.1	0.246	0.161
Carbohydrates (g)	173.9 ± 58.6	151.1 ± 31.1	0.247	202.9 ± 47.5	180.0 ± 46.1	0.355	0.999
Fats (g)	73.4 ± 17.7	67.3 ± 15.4	0.116	63.9 ± 1.5	57.5 ± 12.4	0.162	0.949
IPAQ							
Work	550.6 ± 1166.7	538.0 ± 999.8	0.921	156.1 ± 358.9	147.8 ± 285.8	0.346	0.721
Transport	555.9 ± 559.7	571.2 ± 558.6	0.836	667.1 ± 628.3	414.0 ± 470.5	0.191	0.157
Domestic	599.2 ± 456.8	745.0 ± 346.4	0.164	1240.9 ± 899.8	1167.3 ± 951.0	0.835	0.516
Leisure	539.4 ± 503.8	766.0 ± 627	0.247	345.1 ± 559.1	615.4 ± 570.5	0.254	0.885
Walking	1232.4 ± 1309.8	1219.7 ± 1183.3	0.928	838.5 ± 925.8	699.0 ± 750.2	0.555	0.627
Moderate-intensity	715.4 ± 568.8	908.1 ± 395.7	0.180	1300.9 ± 858.9	1285.5 ± 1069.9	0.935	0.505
Vigorous-intensity	273.8 ± 448.7	415.4 ± 396.8	0.386	283.6 ± 595.8	360.0 ± 500.6	0.735	0.808
Total	2117.8 ± 1702.3	2840.1 ± 1642.4	0.075	2524.9 ± 1591.2	2344.5 ± 1499.8	0.704	0.136
Sitting	3143.1 ± 1003.0	3013.1 ± 1003.0	0.303	2765.5 ± 962.0	2590.9 ± 850.9	03.07	0.821