# EXERCISE AND SPORT: THEIR INFLUENCES ON WOMEN'S HEALTH ACROSS THE LIFESPAN

EDITED BY: Nigel Keith Stepto, Cheryce L. Harrison, Trine Moholdt and Angelica Lindén Hirschberg

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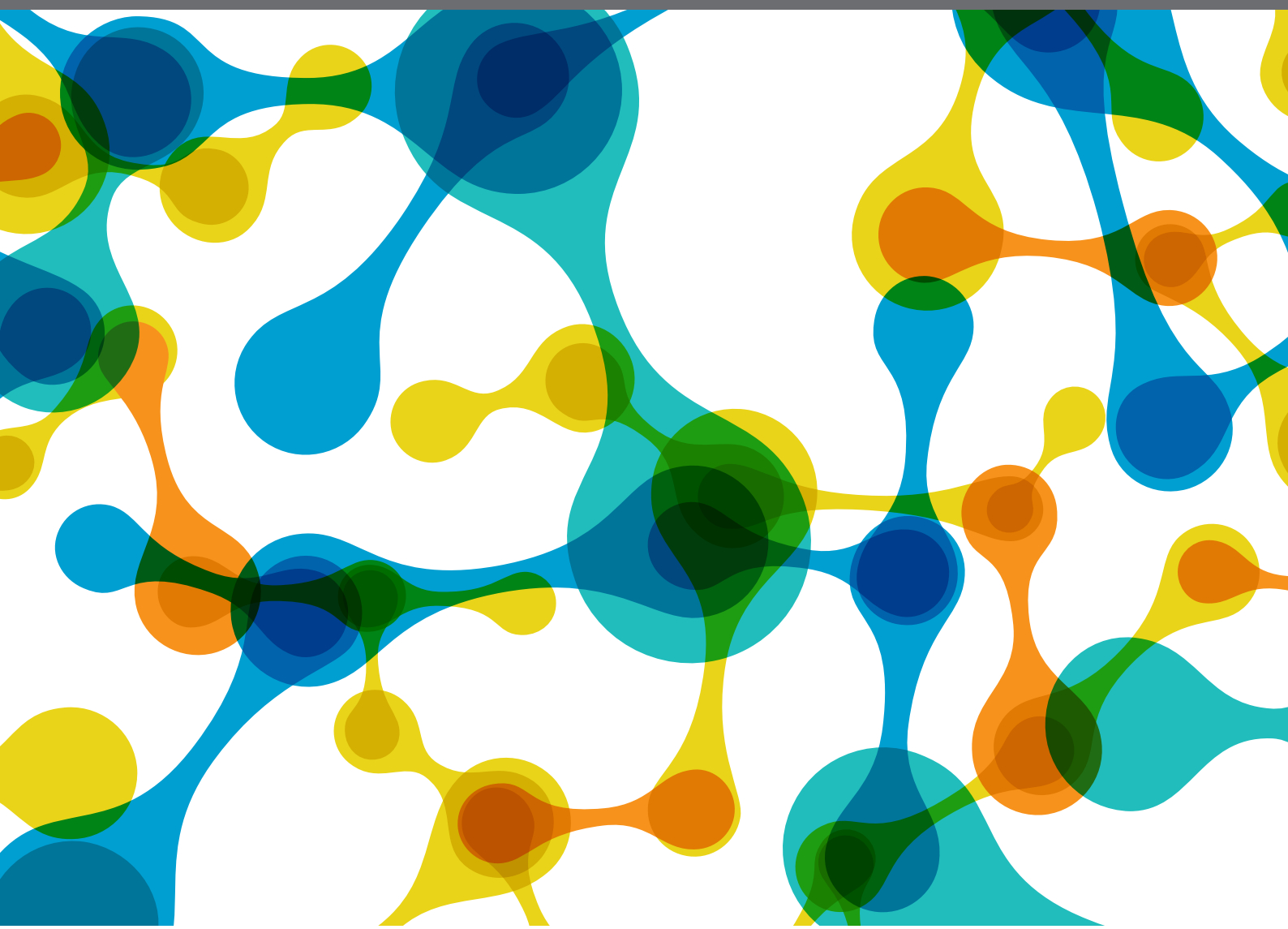

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# EXERCISE AND SPORT: THEIR INFLUENCES ON WOMEN'S HEALTH ACROSS THE LIFESPAN

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This Research Topic of Frontiers in Physiology is dedicated to the memory of Professor Nigel Stepto, the Lead Guest Editor of this collection, who sadly passed away during its formation.

Prof Stepto was a passionate and recognised world leader in the field of Exercise Physiology with outstanding contributions, particularly in the area of women's reproductive health. Nigel's research passion was in understanding the mechanistic effects of exercise for health and therapy with a special interest in insulin resistance and Polycystic Ovary Syndrome, the leading cause of anovulatory infertility in young women of reproductive age. He was the co-Deputy Director - Research Training at the Institute of Health and Sport (IHeS) at Victoria University, Melbourne, Australia and held adjunct associate professorial roles at Monash University and the University of Melbourne. He was Chair of the Exercise and Sports Science Association (ESSA) Research Committee, Project Director of the Australian Institute for Musculoskeletal Science (AIMSS) and an active member of the Australian Physiological Society (AuPS). Alongside his influential research career and leadership roles, Nigel was a strong advocate for postgraduate and early career researchers. His collaborative nature and approach to research ensured those mentored by him were considered, included and valued members across his many research projects and initiatives. Nigel's impact and influence on the careers of early researchers will continue at Victoria University with both a Nigel Stepto Travel Award and Nigel Stepto PhD Scholarship established in his honour.

Nigel was great friend and colleague to many who is very much missed. Nigel is survived by his wife, Fiona and two children Matilda (14 years) and Harriet (11 years). Vale, Professor Nigel Stepto (12 September 1971 – 4 February 2020).

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# Table of Contents

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EDITORIAL
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# Editorial: Exercise and Sport: Their Influences on Women's Health Across the Lifespan

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**Keywords: inflammation, contraceptives, adipose tissue, polycystic ovary syndrome, pregnancy, endurance training, strength training**

**Editorial on the Research Topic**

**Exercise and Sport: Their Influences on Women's Health Across the Lifespan**

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## INTRODUCTION

Physiological responses and adaptations to exercise that influence both health and sports performance is a broad and well-documented area of research with acute and prolonged effects now widely understood. Resultantly, exercise is recognized as a potent therapy for the prevention and treatment of chronic disease in adults.

Yet a significant research gender bias toward males remains with research elucidating differential reproductive and life-phase effects across clinical exercise, exercise and sports science in women, currently limited. This Research Topic was introduced to better explore physiological responses

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to exercise in women across the spectrum of health promotion to sports performance and the interplay of the reproductive lifespan on health and performance outcomes.

This Research Topic consists of nine articles, including six original research articles, two narrative review articles, and one systematic review and meta-analysis. A broad range of themes are covered across female exercise physiology, including the role of hormonal regulation and inflammation on exercise performance; the beneficial role of exercise in anovulatory conditions including polycystic ovary syndrome and perspectives of exercise during pregnancy and its role in hypertensive disorders.

## TRAINING ADAPTATIONS THROUGHOUT THE FEMALE LIFESPAN

Fluctuation in ovarian hormone levels induces physiological alterations that can produce differences in exercise performance during the menstrual cycle (Janse de Jonge, 2003). For example, progesterone has been shown to increase ventilation and body temperature at rest (Marsh and Jenkins, 2002), whereas oestradiol modulates vascular flow (Joyner et al., 2015). Mandrup et al. investigated how menopausal status and high intensity exercise training influence adipose tissue mass, glucose uptake and protein content. They demonstrate similar improvements in cardiorespiratory fitness and decreases in subcutaneous and visceral adipose tissue mass following 3 months' training in pre- and post-menopausal women. They also report of similar insulin-stimulated glucose uptake in abdominal, gluteal, and femoral adipose tissue depots in pre- vs. post-menopausal women, in contrast to earlier findings from the same trial showering skeletal muscle insulin resistance in post- compared to premenopausal women (Mandrup et al., 2018).

High-repetition, low-load resistance training in group class settings has gained popularity for weight control, especially among women. One example of this type of training is BodyPump (Les Mills International), which is claimed to result in high energy expenditure. Rustaden et al. assessed energy expenditure during BodyPump compared with heavy load resistance exercise using indirect calorimetry in women who were overweight and found that both training modalities produced similar energy expenditure during and up to 140 min after the exercise session.

## MENSTRUATION CYCLE AND ORAL CONTRACEPTIVES

Pereira et al. summarized the effects of the ovarian hormone fluctuations during the menstrual cycle on exercise-induced fatigability in a mini-review based on 46 studies comparing the follicular phase with the luteal phase of the menstrual cycle. In total, 15 studies demonstrated a statistical difference between the menstrual cycle phases studied. However, the results were inconsistent with seven studies reporting less fatigability during the luteal phase and eight studies reporting less fatigability during the follicular phase. The inconsistences could be explained by differences in exercise mode, the limb used, type of contraction and the classification of the menstrual cycle phase. The authors concluded that further studies are needed to determine the effects of a specific menstrual cycle phase on exercise-induced fatigability.

Exogenous hormones introduced through oral contraceptive use have also been found to influence exercise capacity (Lebrun et al., 2003) and change the metabolic, cardiovascular, and ventilatory responses to exercise (Charkoudian and Joyner, 2004; Isacco et al., 2012; Schaumberg et al., 2017). Schaumberg et al. observed a dampened response of central physiological adaptations to sprint interval training, demonstrated by pulmonary oxygen uptake kinetics, in women taking oral contraceptives compared to women with natural menstrual cycles. The potential underlying mechanisms for these observations include the influence of exogenous hormones on the overall endocrinological profile. Again, more knowledge is needed regarding the effect of oral contraceptives on exercise training adaptations.

In another study by Larsen et al., blood samples were collected from 53 elite female athletes prior to the Rio Olympic Games. The study showed that those who were taking oral contraceptives had higher levels of C-reactive protein (CRP) in the blood than those without hormonal contraception. CRP is a marker of inflammation and tissue damage, and it was suggested that this marker could have potential consequences for athlete performance and recovery. Other markers of stress and inflammation were comparable between groups.

## POLYCYSTIC OVARY SYNDROME

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among women of reproductive age and characterized by anovulation, hyperandrogenism, and polycystic ovarian morphology (Charkoudian and Joyner, 2004). This condition is highly associated with reproductive, metabolic and mental complications. Insulin resistance and obesity are important etiological factors contributing to the severity of the symptoms. Lifestyle intervention, including exercise, is recommended as a first-choice therapy to improve health outcomes (Charkoudian and Joyner, 2004). However, it is unclear what kind of exercise is most effective. Patten et al. performed a systematic review and meta-analysis of exercise interventions in PCOS. Based on 19 articles and 777 women, it was demonstrated that exercise training improved cardiorespiratory fitness, body composition, and insulin resistance. It was also clear that improvements were dependent on exercise intensity rather than duration. The results suggest that a minimum of 120 min of vigorous intensity per week is needed to provide beneficial health outcomes for women with PCOS.

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression post-transcriptionally and have been suggested to be of importance for the pathophysiology of insulin resistance in PCOS. Lionett et al. studied circulating and adipose tissue miRNAs in women with PCOS compared to controls and in response to randomized low-volume or high-volume

high intensity interval training. They found that women with PCOS have higher circulating levels of miRNA-27b compared to non-PCOS women but comparable adipose tissue miRNAs. miRNA-27b has been implicated in several metabolic and cellular processes, such as fatty acid metabolism, adipocyte differentiation and inflammation (Chen et al., 2012). In response to 16 weeks of low-, but not high-volume, high intensity training, levels of miRNA-27b were reduced. The clinical significance of these findings needs to be studied further.

## PREGNANCY

European and American guidelines advocate that pregnant women should be physically active at least 150 min/week to optimize gestational weight gain and prevent adverse pregnancy outcomes, such as gestational diabetes and hypertensive disorders (2015; Gynaecologists RCoO, 2015). However, <15% of pregnant women adhere to these recommendations (Gjestland et al., 2013). This Research Topic contains a Mini Review (Witvrouwen et al.) on the effects of exercise training during pregnancy on vascular health, with a focus on gestational hypertensive disorders (gestational hypertension and pre-eclampsia). Conflicting reports were found with a need for further research to fully elucidate efficacy of exercise in reducing risk of hypertensive disorders during pregnancy. The largest systematic review [22 randomized controlled trials (RCTs), $n$= 5,316] and meta-analysis to date showed that exercise during pregnancy significantly reduced the risk of gestational hypertension (OR 0.61, 95% CI 0.43–0.85), as well as pre-eclampsia (OR 0.59, 95% CI 0.37–0.94) (15 RCTs, $n$ = 3,322) (Davenport et al., 2018). However, five out of nine systematic reviews and meta-analyses showed no significant effect. The authors suggest that the discrepancies may be caused by different methodological issues. Yet given the benefits of regular physical activity on health and well-being generally, health care providers should be encouraged to discuss lifestyle behaviors with pregnant women to optimize maternal and child health outcomes. Health care provider perspectives on lifestyle advice, during pregnancy, weight gain, physical activity, and nutrition was explored by Haakstad et al. While most midwives viewed lifestyle counseling as important, nearly 40% did not give advice on gestational weight gain or gave advice discordant with the recommendations from the Institute of Medicine (Rasmussen and Yaktine, 2009), emphasizing the need for increased support and guidance for health professionals initiating healthy lifestyle conversations during pregnancy.

## PERSPECTIVES

The manuscripts included in this Research Topic contribute to much needed research into the role and influence of reproduction on physiological responses to exercise across the spectrum of female exercise physiology. Beneficial effects of exercise are reported underscoring its importance in women's health broadly. Yet importantly, as a collective, this issue emphasizes the many research gaps that remain. Exercise intervention studies are largely heterogeneous by nature, with significant variation in type, duration, frequency and intensity as well as in their overall reach, penetration and compliance. Reporting quality across studies introduces variation in the identification of particular components of exercise that contribute to positive outcomes. Taken together, this contributes to ambiguity in the field and limits translation and implementation of clinical recommendations. This issue highlights the critical need for further high quality, robustly designed research with transparent reporting in this area of exercise physiology.

## AUTHOR CONTRIBUTIONS

CH and TM drafted the manuscript, with input from ALH. All authors approved the final version.

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MINI REVIEW
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# The Effect of Exercise Training During Pregnancy to Improve Maternal Vascular Health: Focus on Gestational Hypertensive Disorders

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Hypertensive disorders of pregnancy, including gestational hypertension and pre-eclampsia, occur in up to 10% of pregnancies and are associated with increased life-long cardiovascular risk. Physical activity improves cardiovascular health in pregnancy and may lower the risk of developing hypertensive disorders of pregnancy. However, a minority of pregnant women comply with the recommended level of physical activity. Adequate knowledge on the physiological effects of exercise in healthy pregnancy could help to overcome potential barriers as pregnancy is a unique window of opportunity to improve health outcomes for both mother and child. In this mini review, we discuss structural and functional vascular adaptations during healthy and hypertensive pregnancies, we elaborate on the effects of exercise on the vasculature and review the safety and existing evidence of exercise training as preventive therapy for gestational hypertensive disorders.

**Keywords: exercise, pregnancy, vascular adaptation, pre-eclampsia, gestational hypertension**

## INTRODUCTION

Worldwide guidelines recommend aerobic training during pregnancy from 60 to 150 min/week (Savvaki et al., 2018). Little is known about the number of women practicing this, but numbers as low as 15% have been cited (Kuhrt et al., 2015). Women who exercise as recommended have 30% less risk for developing gestational hypertensive disorders (GHD), including gestational hypertension (GH), characterized by hypertension initiating after the 20th pregnancy week and pre-eclampsia (PE) defined as hypertension and proteinuria after the 20th pregnancy week (Magro-Malosso et al., 2017; Davenport et al., 2018b). Preliminary data suggest that exercise during pregnancy has a lifelong protective effect resulting in a reduced cardiovascular risk profile in the perimenopause (Clapp, 2008). Maternal physical exercise is also beneficial for the fetus, resulting in less macrosomia and consequently improved cardiovascular health of the child at a later age (Alexander et al., 2015).

Pregnancy can be considered a stress test for the cardiovascular system, imposing profound cardiovascular adaptations including increased blood volume, accompanied by a drop in vascular

resistance due to increased angiogenesis and vasodilation, generalized reduction in arterial stiffness and improved endothelial function, increased cardiac output associated with increased right and left chamber size and eccentric hypertrophy, resulting in higher stroke volume and heart rate and a fall in systemic blood pressure (Melchiorre et al., 2012; Chung and Leinwand, 2014; Osol and Bernstein, 2014; Tkachenko et al., 2014; Mannaerts et al., 2019). Regular physical exercise can boost these adaptations as has been demonstrated for angiogenesis and endothelial function (Skow et al., 2017). In women with GHD, these functional and structural vascular adaptions fail (Mannaerts et al., 2019), and may persist beyond pregnancy (Kirollos et al., 2019), explaining why these women are at a lifelong increased risk for cardiovascular disease (Lane-Cordova et al., 2019).

In this mini review, we will elucidate the vascular adaptation during normal vs. hypertensive pregnancies and we will focus on the potentially beneficial effects of exercise on the vasculature. Based on this concept, physical exercise prior to and during pregnancy may be a promising therapy to prevent GHD and GHD recurrence, however, current data to underscore this hypothesis are still limited.

## VASCULAR ADAPTATION DURING HEALTHY PREGNANCY

An optimal adaptation of the cardiovascular system is crucial for a healthy pregnancy. As early as 5 weeks amenorrhea, a significant fall in systemic vascular tone occurs, altering the set-points of the baroreceptors and the stretch receptors (Tkachenko et al., 2014). As a result, systemic vascular resistance decreases to allow sufficient placental perfusion (Clark et al., 1989). Venous tone decreases as well, resulting in expansion of the venous compartment and increased cardiac preload, ultimately leading to increased cardiac output (Melchiorre et al., 2012; Chung and Leinwand, 2014). To accommodate this blood volume expansion and increased cardiac output, the arterial bed needs to undergo structural and functional changes (Skow et al., 2017).

During pregnancy, **structural arterial remodeling** is mainly driven by placental growth factor (PlGF)-induced angiogenesis, occurring primarily at the uteroplacental unit (Osol and Bernstein, 2014). Soluble fms-like tyrosine kinase 1 (sFlt-1) is the circulating form of the VEGF receptor-1 and binds VEGF and PlGF thereby reducing their bioavailability.

The ratio of sFlt-1/PlGF is an important indicator of the angiogenic status in pregnancy and is used to predict and diagnose PE. Interestingly, this ratio appears to be indicative of future vascular dysfunction risk (Zeisler et al., 2016). The decrease in total vascular resistance is mediated by VEGF and PlGF as they induce distal angiogenesis (Hasan et al., 2002). Placental growth factor also mediates the cardiac adaptation and insufficient PlGF leads to impaired ventricular remodeling and cardiac dysfunction (Hochholzer et al., 2011).

To accommodate the increased blood volume while maintaining low blood pressure, a generalized reduction in **arterial stiffness** is of great importance. Central (aortic) pulse wave velocity (PWV), the gold standard for arterial stiffness, is known to be decreased in healthy pregnancy (Mannaerts et al., 2019).

A healthy **endothelium** controls vasomotor tone, which is essential during pregnancy. The rapidly expanding blood volume and increase in cardiac output pose an increased shear stress on endothelial cells, resulting in increased endothelial nitric oxide (NO) production (Cockell and Poston, 1997; Williams et al., 1997). Together with higher estrogen levels, this leads to a systemic vasodilation (Meah et al., 2016). In healthy pregnancy, endothelial NO synthase (eNOS) activity is significantly increased (Nelson et al., 2000) which is mirrored in improved flow-mediated dilatation (FMD), the gold standard for endothelial function measurement (Iacobaeus et al., 2017; Mannaerts et al., 2019).

## VASCULAR MALADAPTATION IN GESTATIONAL HYPERTENSIVE DISORDERS

Women who develop hypertensive disorders during pregnancy such as GH or PE appear to fail the stress test of pregnancy, in part due to insufficient cardiovascular adaptation. Therefore, the risk of developing cardiovascular disease later in life is 9.5 times higher for women with severe early PE [hazard ratio (HR) = 9.5, 95% confidence interval (CI) = 4.5–20.3] (Mongraw-Chaffin et al., 2010). Furthermore, PE has been associated with an increased risk for developing end-stage kidney disease (HR = 4.96, 95% CI = 3.9–6.3) (Khashan et al., 2019). Therefore, long-term cardiovascular monitoring and early preventive therapy are advocated (McDonald et al., 2008; Ahmed et al., 2014).

In PE, insufficient **arterial remodeling** at the spiral arteries results in placental ischemia-reperfusion damage and the production of high amounts of free radicals causing oxidative stress (**Figure 1**). Circulating free radicals activate peripheral leucocytes and platelets, resulting in an inflammatory state and disturbing proper endothelial function. The reaction of oxidative products with NO decreases its bioavailability which impairs endothelial function even more (Mannaerts et al., 2018). The abundant placental ischemia and oxidative stress in PE results in an anti-angiogenic state with a three-fold increase in antiangiogenic factors (sFlt-1) and a 90% reduction in angiogenic factors (PlGF and VEGF; Tomimatsu et al., 2017).

Women suffering from PE have increased **arterial stiffness** both during and after pregnancy, and arterial stiffness is directly correlated to the severity of the disease (**Figure 1**; Hausvater et al., 2012; Mannaerts et al., 2019). Carotid-femoral PWV is abnormal from 11 to 13 weeks in patients who develop PE later in pregnancy, which supports the concept that PE is not caused by dysfunctional placentation alone and underlying vascular disease must be present. Increased arterial stiffness may have an important influence on fetal birth weight and pregnancy outcome (Skow et al., 2017). In addition, central PWV is strongly related to an increased risk for the development of cardiovascular disease later in life, also in PE (Hausvater et al., 2012).

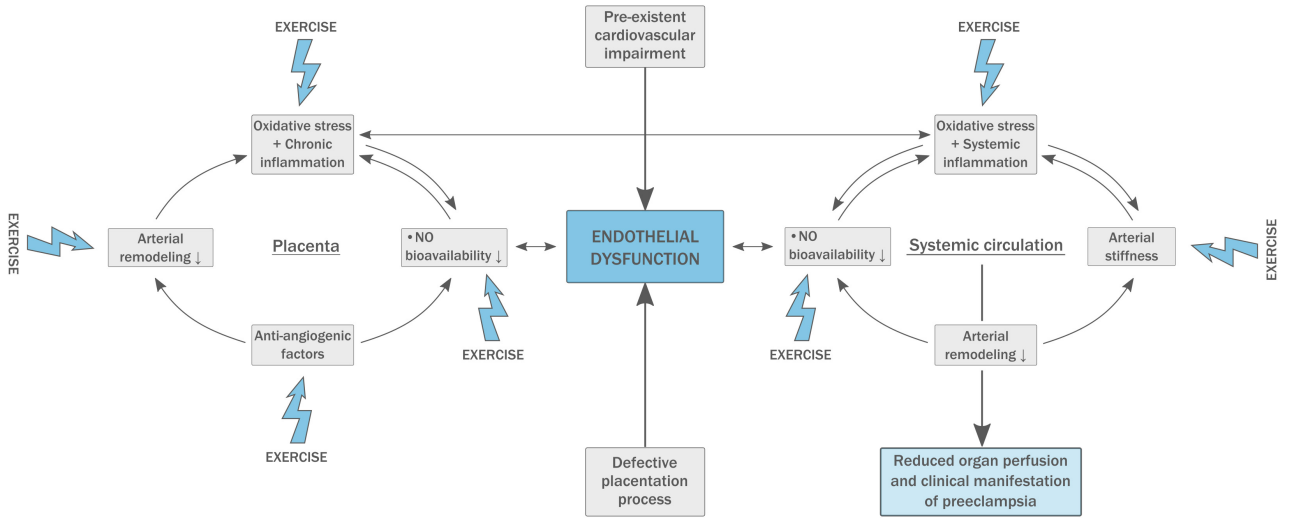


**FIGURE 1 |** Pathophysiology of pre-eclampsia (PE). A pre-existing fragile endothelial situation leads to defective placentation and high circulating levels of oxidative stress. This inadequate response to pregnancy results in arterial stiffness and exacerbates generalized endothelial dysfunction. Physical exercise has beneficial effects on multiple components of the model.

PE is characterized by dysfunction of both resting (L-FMC, low-flow mediated constriction) and recruitable (FMD) endothelial capacity (Mannaerts et al., 2019). **Endothelial dysfunction** is proven to be present prior to the development of PE, possibly serving as a predictive parameter (**Figure 1**; Weissgerber, 2014). Further, women with a history of PE appear to have reduced FMD up to 3 years postpartum (Scholten et al., 2014). Endothelial dysfunction impairs vascular smooth muscle relaxation which enhances arterial stiffness and plays an important role in the development of atherosclerosis. This suggests endothelial dysfunction to be the most plausible common link between the pathophysiology of PE and future cardiovascular disease (Mosca et al., 2011; Weissgerber, 2014).

## EFFECTS OF EXERCISE ON THE VASCULATURE

Repeated exercise bouts effectively benefit vascular function directly by exerting shear forces on the vascular wall (Hambrecht et al., 2003; Adams et al., 2005; Grimm et al., 2018) and indirectly by the release of anti-inflammatory and anabolic mediators in response to increased muscular energy demands (Goldhammer et al., 2005; Kadoglou et al., 2007; Pedersen et al., 2007; Rehm et al., 2015). This results in functional adaptation of the local and systemic vasculature to meet increased perfusion demands and to structural arterial remodeling by engagement of neuro-humoral and metabolic mechanisms (**Figure 2**; Roveda et al., 2003; Adams et al., 2005; Pedersen et al., 2007; Rehm et al., 2015).

There is clear evidence that **endothelial function** is improved by regular physical activity, both in patients with cardiovascular risk factors (Lavrenčič et al., 2000) and in patients with established cardiovascular disease (Linke et al., 2001; Van Craenenbroeck et al., 2010; Van Craenenbroeck E.M. et al., 2015). This exercise-induced benefit on endothelial function is mediated by different factors.

First, increased shear stress during exercise activates eNOS and reduces NAD(P)H oxidase activity, resulting in decreased reactive oxygen species (ROS) and increased NO bioavailability (Hambrecht et al., 2003; Adams et al., 2005). Furthermore, laminar shear stress prevents inflammation-related alterations in eNOS levels and prostacyclin/thromboxane ratio in an atherogenic environment (Grimm et al., 2018).

Second, endurance training has repeatedly been reported to lower levels of pro-inflammatory cytokines (CRP, IL-18, IL-1β, and IL-8) and increase anti-inflammatory cytokines (IL-10; Goldhammer et al., 2005; Kadoglou et al., 2007). The reduction of body fat and the anti-inflammatory and anabolic mediators released from the active skeletal muscle (referred to as "myokines"; Pedersen et al., 2007) induce systemic shifts in the innate and adaptive immunity toward a more pro-resolving and anti-inflammatory status (Rehm et al., 2015).

Third, exercise training modulates the balance between vasodilating and vasoconstricting factors, overall resulting in more vasodilation. Exercise training reduces levels of endothelin-1 and noradrenalin (Mortensen et al., 2009; Dow et al., 2017), reverses the aging-induced increase in the vasoconstrictor thromboxane (Hellsten et al., 2012) and lowers sympathetic tone (Roveda et al., 2003).

Regular physical activity and exercise interventions have been associated with the prevention of age-related increases in **arterial stiffness** (Fleenor et al., 2010). In a mouse model, the profibrotic cytokine TGF-β1 increased with aging in the carotid adventitia, where it augmented oxidative stress in fibroblasts. This resulted in increased collagen I and III deposition, and arterial stiffness (Fleenor et al., 2010). The aging-associated elevation in adventitial TGF-β1 is reduced by aerobic exercise both in mice and humans, which in turn reduces large elastic artery stiffening

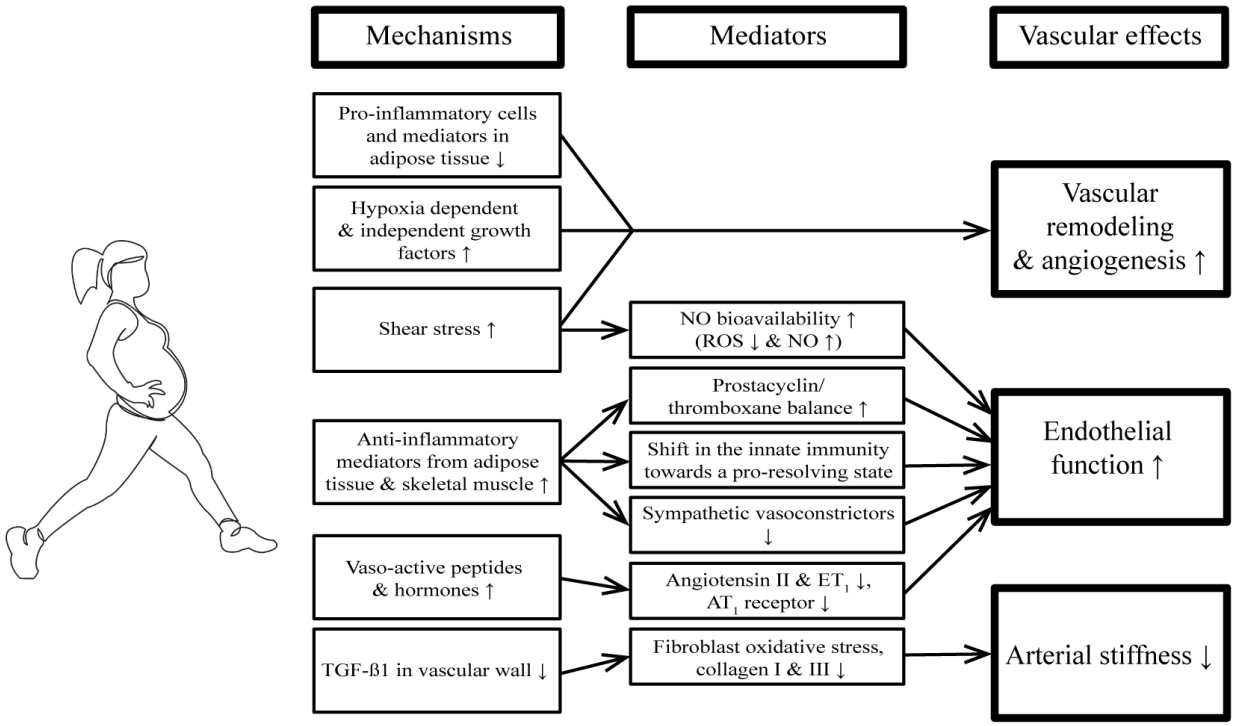


**FIGURE 2 |** Beneficial effects of repeated exercise bouts on the vasculature. AT1 receptor, Angiotensin II receptor type 1; eNOS, endothelial nitric oxide synthase; ET1, endothelin-1; NO, nitric oxide; ROS, reactive oxygen species; TGF-β, transforming growth factor beta.

(Fleenor et al., 2010). In addition, increased oxidative stress has been associated with reduced large elastic artery compliance in sedentary vs. habitually exercising postmenopausal women (Moreau et al., 2006).

Exercise has a profound impact on the process of **vascular remodeling**, which is again driven by increased blood flow and shear stress, by inflammatory cells, as well as by hypoxia-dependent and -independent growth factors (Hoier and Hellsten, 2014; Laughlin, 2016). The pro-angiogenic effect of exercise is not limited to the exercising skeletal muscle, but also induces angiogenesis in adipose tissue (Van Pelt et al., 2017) and increased coronary collateral flow in patients with coronary artery disease (Möbius-Winkler et al., 2016).

## EFFECTS OF EXERCISE IN HEALTHY PREGNANCY

Regular exercise is known to decrease cardiovascular disease in the non-pregnant population and is implemented in the treatment of heart failure and coronary artery disease patients (Karlsen et al., 2019; Witvrouwen et al., 2019a). Improved vascular health has been suggested as a major contributing factor (Myers, 2003; Van Craenenbroeck E.M. et al. 2010; 2015, Van Craenenbroeck A.H. et al., 2016).

In an uncomplicated pregnancy, current guidelines recommend moderate exercise at a frequency of two to four times a week and with an exercise duration of 30 min, throughout pregnancy (Savvaki et al., 2018). Overall, both aerobic and resistance exercises do not exert any adverse effects during pregnancy. However, evidence on resistance training is scarce and exercise with heavy loads is discommended (Savvaki et al., 2018). Most recreational exercise is safe, but sports that may cause abdominal trauma, falls or excessive joint stress and scuba diving should be avoided (Kuhrt et al., 2015; Bø et al., 2016; Savvaki et al., 2018).

Whereas the guidelines generally recommend 30 min of moderate exercise two to four times per week, 85% of pregnant women are exercising below these levels (Evenson and Wen, 2010). The most frequent barriers are fatigue, lack of time and pregnancy discomforts, but also safety concerns such as low birth weight, preterm labor and inducing fetal bradycardia could withhold pregnant women and health practitioners to prescribe the recommended amount of physical exercise (Kuhrt et al., 2015; Coll et al., 2017; Harrison et al., 2018; Witvrouwen et al., 2019b). Adequate knowledge on the physiological effects of exercise training in healthy pregnancy should help to overcome these barriers as pregnancy is a unique window of opportunity to improve health outcomes for the mother and also the future generations (Kuhrt et al., 2015).

There is no evidence for the induction of preterm delivery by regular physical activity. On the contrary, even a reduction in preterm birth of 20–50% in women performing exercise during pregnancy compared with sedentary pregnant women has been shown (Juhl et al., 2010).

The same is true for the concerns regarding exercise and low birth weight: maternal exercise was not associated with low birth weight or Apgar score at delivery (Davenport et al., 2018a). The normalization of maternal blood glucose, decrease in insulin resistance and increased placental functional capacity and nutrient delivery are suggested mechanisms to explain the beneficial effect of exercise on birth weight (Clapp, 2003; Kuhrt et al., 2015).

During exercise, peripheral vasodilation in the skin and exercising muscles can lead to reduced placental blood flow. In addition to poor autoregulation of the placental circulation, this may cause reduced oxygen and nutrient delivery to the fetus. Other proposed mechanisms for possible fetal distress during maternal exercise include vagal reflex, cord compression or fetal head compression related to malposition (Artal and O'Toole, 2003). Nevertheless, a significant decrease in mean uterine artery blood flow and fetal bradycardia has only been shown in Olympic level athletes exercising at more than 90% of the maximal maternal heart rate (Salvesen et al., 2011). Moreover, it has been shown that regular exercise improves both maternal cardiovascular adaptations and placental function to maintain sufficient fetal oxygenation and growth and does not adversely affect fetal heart rate (Clapp, 2003; Kuhrt et al., 2015).

## EFFECTS OF EXERCISE FOR THE PREVENTION OF HYPERTENSIVE DISORDERS OF PREGNANCY

*Even prior to actual pregnancy, physical activity* is related to a lower occurrence of PE, with a 22–35% relative risk (RR) reduction for women with the highest vs. lowest physical activity level (Aune et al., 2014). This risk was even further reduced (RR = 0.64, 95% CI = 0.44–0.93) with combined pre- and early pregnancy physical activity. When assessing the dose-response effect of physical activity, 5–6 h of physical activity per week reduced the risk of PE with 40%, but no further reduction with increasing activity levels were reported (Aune et al., 2014). Likewise, sedentary behavior has been related to higher odds for the development of PE and GH (Aune et al., 2014; Fazzi et al., 2017; Davenport et al., 2018b).

Whether *physical activity and training during pregnancy can prevent GH and PE*, remains to be established. The largest systematic review and meta-analysis to date on GH (22 randomized controlled trials (RCTs), $n = 5{,}316$) and PE (15 RCTs, $n = 3{,}322$) showed that exercise during pregnancy significantly lowered the risk for GH (OR = 0.61, 95% CI = 0.43–0.85) and PE (OR = 0.59, 95% CI = 0.37–0.94). Moreover, 600 MET-min/week of moderate-intensity exercise (the equivalent of 140 min of brisk walking) was accompanied by a 25% reduction in the odds of developing GH, PE and gestational diabetes mellitus, with a clear dose-dependent effect (Davenport et al., 2018b).

This is in line with findings from three other large meta-analyses, where reductions in PE or GHD were observed (Aune et al., 2014; Di Mascio et al., 2016; Magro-Malosso et al., 2017). However, other systematic reviews and meta-analyses reported conflicting results depending on the type of the study-design (cohort studies vs. case-control studies vs. RCTs) and the exercise exposure that was studied (Kasawara et al., 2012; Wolf et al., 2013; Muktabhant et al., 2015; da Silva et al., 2016; Zheng et al., 2017; **Table 1**).

This controversy may be caused by methodological issues, such as heterogeneity in study designs or training programs. There is a wide variety in exercise type (strength vs. endurance vs. combined strength and endurance training, or stretching exercises), duration and frequencies of the training programs (with differences in number of sessions per week, the duration of these sessions and the total duration of the training intervention) in the current studies, and also the exercise domain (such as leisure time physical activity, occupational, domestic, or active commuting exercise) often differs. Furthermore, different evaluation of physical activity (objective measures such as accelerometry or subjective self-reported questionnaires), inadequate correction for confounding variables (some studies did not take BMI into account), or low training adherence could contribute to this discrepancy. The slightly stronger association between prepregnancy exercise and PE compared with early pregnancy physical activity, could also be due to higher achievable intensity levels before pregnancy compared with the pregnant state (Aune et al., 2014).

Conceptually, exercise in early pregnancy can reduce the risk of PE by ameliorating placentation since repetitive hypoxia bouts and reduced placental perfusion will stimulate cell proliferation and angiogenesis and lead to an improved sFlt-1/PlGF balance (Skow et al., 2017).

In elite athletes, evidence on a positive effect of vigorous exercise during pregnancy on PE or GH is lacking. A J-shaped relationship between the risk of PE and exercise, with a 40% reduction in risk with up to 5–6 h exercise per week, but no further reductions at higher activity levels has been described (Aune et al., 2014). As stated above, fetal adverse effects have only been shown in athletes exercising at more than 90% of the maximal maternal heart rate (Salvesen et al., 2011). Therefore, pregnant athletes should be referred to gynecologists for individual risk-assessment and recommendations regarding the type and intensity of exercise during pregnancy (Sma Position Statement et al., 2016; Mottola et al., 2018).

To date, only two RCTs evaluated the *effect of exercise on the recurrence of PE in a subsequent pregnancy* (Yeo et al., 2008; Kasawara et al., 2013). In the study of Kasawara et al., one training session per week in trimester 2 and 3 of pregnancy did not prevent PE recurrence. The low training intensity (heart rate 20% above resting value) and frequency demand for cautious interpretation of these results (Kasawara et al., 2013). Yeo et al. studied the effect of walking vs. stretching ($5 \times 40$ min/week) in 79 women and also did not demonstrate a reduction in the incidence, possibly affected by low adherence (Yeo et al., 2008).

In established PE pregnancies, *only one RCT assessed whether exercise* (supervised stretching vs. autogenic training) *reduced blood pressure*. In 40 PE pregnancies, both training modalities equally lowered blood pressure and proteinuria ($p < 0.05$) over time (Awad et al., 2019).

**TABLE 1 |** Summary of meta-analyses and systematic reviews on the effect of exercise before and/or during pregnancy and the occurrence of gestational hypertensive disorders.

| References | No. studies, No. participants included | Exercise exposure | Risk reduction (95% confidence interval) |
|---|---|---|---|
| (Davenport et al., 2018b) | (1) GH: 32 RCTs; *n* = 9,648 | (1) Exercise with/without cointerventions vs. no exercise during pregnancy (pooled estimate) | (1) GH: OR = 0.81, 95% CI = 0.65–1.0 |
| Meta-analysis | (2) GH: 22 RCTs; *n* = 5,316 | (2) Exercise-only interventions vs. no exercise during pregnancy (sensitivity analysis) | (2) GH: OR = 0.61, 95% CI = 0.43–0.85 |
| | (3) PE: 26 RCTs; *n* = 10,177 | (3) Exercise with/without cointerventions vs. no exercise during pregnancy (pooled estimate) | (3) PE: OR = 0.89, 95% CI = 0.73–1.08 |
| | (4) PE: 15 RCTs; *n* = 3,322 | (4) Exercise-only interventions vs. no exercise during pregnancy (sensitivity analysis) | (4) PE: OR = 0.59, 95% CI = 0.37–0.94 |
| (Aune et al., 2014) | (1) Seven cohort and four case-control studies; *n* = 168,602 | (1) High vs. low early pregnancy physical activity | (1) PE: RR = 0.79, 95% CI = 0.70–0.91 |
| Meta-analysis | (2) Two case-control and 1 cohort study; *n* = 5,194 | (2) High- vs. low-intensity activity in early pregnancy | (2) PE: RR = 0.51, 95% CI = 0.37–0.71 |
| | (3) Four cohort and one case-control study; *n* = 10,317 | (3) High vs. low prepregnancy physical activity | (3) PE: RR = 0.65, 95% CI = 0.47–0.89 |
| | (4) One case-control and one cohort study; *n* = 4,240 | (4) High- vs. low-intensity prepregnancy physical activity | (4) PE: RR = 0.55, 95% CI = 0.25–1.21 |
| | (5) One cohort and two case-control studies; *n* = 5,291 | (5) Combined physical activity before and during early pregnancy vs. no physical activity | (5) PE: RR = 0.89, 95% CI = 0.59–1.35 |
| (Di Mascio et al., 2016) Meta-analysis | Nine RCTs; *n* = 2,059 | 35–90 min of aerobic exercise for 3–4 times per weeks vs. no exercise, randomized before 23 weeks | PE and GH: RR = 0.21, 95% CI = 0.09–0.45 |
| (Magro-Malosso et al., 2017) | (1) Seven RCTs; *n* = 2,517 | 30–60 min of aerobic exercise for 2–7 times/week vs. no exercise, randomized before 23w | (1) GHD: RR = 0.70, 95% CI = 0.53–0.93 |
| Meta-analysis | (2) Sixteen RCTs; *n* = 4,641 | | (2) GH: RR = 0.54, 95% CI = 0.40–0.74 |
| | (3) Six RCTs; *n* = 2,230 | | (3) PE: RR = 0.79, 95% CI = 0.45–1.38 |
| (Kasawara et al., 2012) | (1) Six case-control studies; *n* = 9,929 | (1) LTPA, occupational activities and planned physical exercise vs. no physical activity | (1) PE: OR = 0.77, 95% CI = 0.64–0.91 |
| Systematic review | (2) Ten cohort studies; *n* = 184,243 | (2) LTPA, occupational activities and planned physical exercise vs. no physical activity | (1) PE: OR = 0.99, 95% CI = 0.93–1.05 |
| | (3) One RCT; *n* = 79 (Yeo et al., 2008) | (3) Stretching vs. walking exercise 5 times per week from week 18 until the end of pregnancy | (3) PE: OR = 6.34, 95% CI = 0.72–55.37 |
| (Wolf et al.,2013) Systematic review | Four case-control (*n* = 4,867) and 7 cohort studies (*n* = 166,822) | LTPA before and/or during pregnancy | (1) Light- or moderate-intensity LTPA: no association with PE.<br>(2) Vigorous-intensity LTPA before and/or during pregnancy may reduce the risk of PE.<br>(3) Reduced risk among women who participated in LTPA at least 25 times/month or > 4h per week<br>(4) Elevated risk of severe PE with high amounts of LTPA, defined as ≥ 4.5 h per week |

*(Continued)*

**TABLE 1** | Continued

| Reference | No. studies, No. participants included | Exercise exposure | Risk reduction (95% confidence interval) |
|---|---|---|---|
| (da Silva et al., 2016) Meta-analysis | (1) Three RCTs; *n* = 1,417 | LTPA in pregnancy vs. no physical activity | (1) PE: RR = 0.93, 95% CI = 0.55–1.57 |
| | (2) Eight cohort studies; *n* = 155,414 | | (2) Similar findings; no evidence of an association between LTPA in pregnancy and PE |
| (Zheng et al., 2017) Meta-analysis | Five RCTs; *n* = 1,872 | Exercise during pregnancy vs. usual daily activities | PE (secondary outcome); OR = 1.05, 95% CI = 0.53–2.07 |
| (Muktabhant et al., 2015) Systematic review | (1) Eight RCTs; *n* = 3,139 | (1) Diet and exercise vs. standard care | (1) PE: RR = 0.99 95% CI = 0.74–1.31 |
| | (2) Three RCTs; *n* = 1,024 | (2) Supervised exercise vs. standard care | (2) PE: RR = 0.91, 95% CI = 0.52–1.60 |
| | (3) Two RCTs; *n* = 229 | (3) Unsupervised exercise vs. standard care | (3) PE: RR = 1.60, 96% CI = 0.38–6.73 |

*GH, gestational hypertension; GHD, gestational hypertensive disorders; LTPA, leisure time physical activity; OR, odds ratio; PE, preeclampsia; RCT, randomized controlled trial; RR, relative risk.*

## CURRENT RESEARCH GAPS AND FUTURE DIRECTIONS

A large body of evidence demonstrates that exercise improves systemic endothelial function and arterial stiffness in a wide range of subjects, from children to elderly, as well as in several diseases. Surprisingly, effects of exercise on the vasculature in healthy pregnancies is understudied and data in PE pregnancies are virtually non-existent. To our knowledge, only one study examined the effect of exercise training during a healthy pregnancy on endothelial function (Ramírez-Vélez et al., 2011). In that study, FMD improved by 30% by exercise training starting between 16 and 20 weeks, at moderate intensity. Concerning arterial stiffness, a discretely improved PWV in early post-partum period was observed with prenatal exercise, but has not been studied during pregnancy (Kawabata et al., 2012). In women with a history of PE, improved FMD and venous compliance with exercise training have been shown in small patient groups (Krabbendam et al., 2009; Scholten et al., 2014, 2015), and requires confirmation in larger trials.

Whether exercise training can prevent subsequent GHD in high risk patients, is a justified research question that deserves a well-designed clinical trial. Future research should focus on strategies to improve adherence to exercise training during pregnancy (supervised vs. unsupervised training, providing information on training characteristics and safety of exercise, etc.). Also, clear definitions of exercise should be used, using the FITT acronym (frequency, intensity, type, and time). These training characteristics should be compared and their effects on vascular health and the recurrence of GHD should be assessed. The role of gestational weight gain and the socioeconomic state of the women should be explored. Furthermore, confounding variables (age, BMI, parity, and smoking) and pre-pregnancy physical activity levels should be taken into account. Physical activity should be assessed using preferably objective measures. Also, more research on the timing of initiation of exercise (first, second, or third trimester of pregnancy) and more exercise-only interventions in overweight or obese women should be performed. In addition, whether post-partum exercise in women with history of PE can reduce their increased cardiovascular risk, deserves attention.

In the meantime, physical activity in pregnant women should be stimulated, with structured advice from the treating physician. Offering eg. a smartphone-based program while considering the socioeconomic and psychological needs should ultimately lead to fitter pregnant women, with clear benefits for mother and child.

## CONCLUSION

In GHD, structural and functional adaptations of the vascular wall fail by a large amount, leading to measurable effects on blood pressure in the acute phase and increased cardiovascular risk of both mother and child in the long term. Regular physical activity has profound effects on several parts of the vascular wall by improving endothelial function, reducing arterial stiffness and inducing angiogenesis. Nevertheless, whether these beneficial

vascular effects of exercise are related to the lower risk on GHD following training remains to be confirmed. However, moderate physical exercise during pregnancy is safe and will benefit both short- and long-term outcome of mother and baby. Therefore, physical activity should be encouraged in every healthy woman considering only a few contra-indications and addressing potential barriers for exercise during pregnancy.

## AUTHOR CONTRIBUTIONS

YJ wrote the introduction. DM wrote the part on vascular adaptation in healthy pregnancy and in hypertensive disorders of pregnancy. EV elaborated on the effects of exercise on the vasculature and edited the manuscript. AV described the effects of exercise in healthy pregnancy. IW discussed the effects of exercise for the prevention of hypertensive disorders of pregnancy and edited the manuscript. DM, IW, and EV wrote the current research gaps and future directions. All authors revised and accepted the final version of the manuscript to be published.

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# Inflammation and Oral Contraceptive Use in Female Athletes Before the Rio Olympic Games

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This study investigated the association between synthetic ovarian hormone use [i.e., the oral contraceptive (OC) pill] and basal C-reactive protein (CRP), peripheral blood immune cell subsets, and circulating pro- and anti-inflammatory cytokine concentrations in elite female athletes. Elite female athletes ($n$ = 53) selected in Rio Summer Olympic squads participated in this study; 25 were taking an OC (AthletesOC) and 28 were naturally hormonally cycling (AthletesNC). Venous blood samples were collected at rest for the determination of sex hormones, cortisol, CRP, peripheral blood mononuclear memory and naïve CD4+ T-cells, CD8+ T-cells and natural killer cells, as well as pro- and anti-inflammatory cytokine concentrations. C-reactive protein concentrations were elevated ($p < 0.001$) in AthletesOC (median = 2.02, IQR = 3.15) compared to AthletesNC (median = 0.57, IQR = 1.07). No differences were reported for cortisol, cytokines, or PBMC immune cell subsets, although there was a trend ($p$ = 0.062) for higher IL-6 concentrations in AthletesNC. Female Olympians had substantially higher CRP concentrations, a marker of inflammation and tissue damage, before the Rio Olympic Games if they used an OC. Future research should examine the potential consequences for athlete performance/recovery so that, if necessary, practitioners can implement prevention programs.

**Keywords: C-reactive protein, cytokines, contraception, athletes, sex hormones**

## INTRODUCTION

Athletes are more susceptible to illness and infection during periods of intense training and competition (Walsh and Whitham, 2006; Gleeson, 2007), which may be compounded in an Olympic environment. Data from the London 2012 Olympic Games found that female athletes were 60% more likely to fall ill than male athletes, and one in five illnesses was expected to result in absence from training/competition (Engebretsen et al., 2013). This absence is associated with decreased performance outcomes (Drew et al., 2017a). Thus, factors that contribute to an increased risk of infection/illness in elite female athletes must be further examined so practitioners can implement prevention programs.

Female sex hormones play an integral role in many physiological pathways, including regulation of the immune system (Schuurs and Verheul, 1990). There is evidence to suggest that the majority of female athletes use some form of oral contraceptive (OC) (Rechichi and Dawson, 2009), which reduces the concentration and cyclical variability of endogenous estrogen and progesterone (Fleischman et al., 2010). While the OC pill may be beneficial for sportswomen by reducing menstrual cycle variability, it is possible that the administration of synthetic sex steroids interferes with normal immune homeostasis.

C-reactive protein (CRP) is an acute phase reactant used as a systemic marker of inflammation and tissue damage (Pepys and Hirschfield, 2003). Elevated CRP concentrations are also associated with an increased risk of developing cardiovascular disease (CVD) (Ridker et al., 2000) and diabetes mellitus (Freeman et al., 2002). When assessing CVD risk in healthy women, CRP thresholds of $<0.5$ mg.L$^{-1}$ (protective), 0.5–1.0 mg.L$^{-1}$ (no risk), 1.0–3.0 mg.L$^{-1}$ (intermediate risk), 3.0–10.0 mg.L$^{-1}$ (high risk), and $>10.0$ mg.L$^{-1}$ (very high risk) have been utilized (Cook et al., 2006; Cauci et al., 2017). Of interest, OC use has been associated with increased CRP concentrations in healthy female adolescents (Pirkola et al., 2010) and women (van Rooijen et al., 2006; Cauci et al., 2008; Piltonen et al., 2012; Sørensen et al., 2014; Cauci et al., 2017). However, only one of these studies (Cauci et al., 2017) utilized an athletic population, of which only 14.6% had competed at a national/international level ($n$ = 30). Regular OC use has also been associated with a higher CD8+ T-cell number and lower natural killer cell number (Auerbach et al., 2002), suggesting that it may modulate basal immune status.

Regular physical activity promotes an anti-inflammatory immune profile (Petersen and Pedersen, 2005; Gleeson, 2007), and so it is possible that the effects of OC use on inflammation differ in elite sportswomen when compared to the general community. While evidence suggests that OC use is similarly associated with elevated CRP concentrations in athletic women (Cauci et al., 2017), further studies need to be performed utilizing elite-level female athletes to confirm and extend upon these findings. Therefore, this study measured basal CRP and peripheral blood mononuclear cells (PBMCs), as well as other markers of stress and inflammation (e.g., cortisol, pro- and anti-inflammatory cytokines), to provide a snapshot of the athletes' inflammatory status prior to the 2016 Summer Olympic Games in Rio.

## MATERIALS AND METHODS

Elite female athletes ($n$ = 53) preparing for the Rio 2016 Olympics participated in this study [representing a subset of the Stay Healthy project; Phase 1 of the project is described elsewhere (Drew et al., 2017a,b)]. Athlete demographic information is presented in **Table 1**. Twenty-five athletes were taking OC (AthletesOC) and 28 were naturally cycling (AthletesNC); the type(s) of OC used by AthletesOC were not specified. AthletesOC has been taking OC for 6.5 ± 3.9 y, although it should be noted that only 12 of the 25 OC users provided information regarding length of OC use. Nonsteroidal anti-inflammatory use was reported by only three athletes (AthletesNC = 1, AthletesOC = 2). Participants completed the valid and reliable Low Energy Availability in Females Questionnaire (LEAF-Q) (Melin et al., 2014) and an illness questionnaire (to confirm they were healthy), and provided a single blood sample. Ethical approval was granted by the Australian Institute of Sport and the Griffith University Human Ethics Committee. All athletes provided written informed consent.

**TABLE 1 |** Demographic information for AthletesOC and AthletesNC.

| | AthletesOC | AthletesNC |
|---|---|---|
| Age (y) | 24.7 ± 3.5 | 24.4 ± 4.0 |
| Height (cm) | 170.88 ± 7.64 | 171.78 ± 6.45 |
| Weight (kg) | 66.77 ± 7.31 | 69.13 ± 8.71 |
| BMI | 19.51 ± 1.60 | 20.08 ± 2.11 |
| Sport | | |
| Hockey | 9 | 2 |
| Rowing | 1 | 3 |
| Soccer | 5 | 12 |
| Water polo | 5 | 4 |
| Rugby 7's | 5 | 5 |
| Triathlon | 0 | 2 |

Blood samples were collected from the antecubital vein using standard venipuncture techniques. Sample times ranged from 8:00 a.m. to 3:00 p.m. but were consistent across each sport (i.e., all water polo players had samples taken at ∼11:00 a.m.), resulting in a similar spread of sample times across AthletesOC and AthletesNC. Some athletes did not train prior to sample collection, while others performed light training or a regular training session. However, the number of athletes in each category was similar across groups; 50, 29, and 21% of AthletesNC performed no training, light training, and regular training, respectively, compared to 56, 24, and 20% of AthletesOC. On average, AthletesNC had their blood taken 2.5 h post-training, which was very similar to AthletesOC (2.0 h). Blood samples were taken randomly with respect to menstrual cycle, however, hormone concentration data (progesterone and estradiol) were used to stratify AthletesNC according to menstrual cycle phase using established reference values (Stricker et al., 2006; data were converted to relevant units for comparison where necessary).

Serum concentrations of estradiol, progesterone, and free testosterone were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Abcam, Cambridge, United Kingdom) according to the manufacturer's instructions. Serum cortisol concentrations were also determined using a commercially available ELISA kit (Abnova, Taipei City, Taiwan) according to manufacturer's instructions. Inter-assay coefficients of variation were 9.7% for estradiol, 6.0% for progesterone, 8.1% for free testosterone, and 5.1% for cortisol. All samples were analyzed in duplicate.

Serum CRP concentrations were determined via an immunoturbidimetric assay using commercially available

reagents and a COBAS Integra 400 system (Roche Diagnostics, Mannheim, Germany). Serum cytokine concentrations were assessed using commercially available 27-plex suspension array kits (Bio-Rad Laboratories Pty Ltd.; Hercules, CA, United States). This panel includes key pro- (IL1β, IL6, IL-8 TNF-α) and anti-inflammatory cytokines (IL-1ra, IL-10) as well as relevant growth factors (VEGF, PDGF) and regulatory cytokines (IFN-γ, IL-17). Assays were completed using a Bioplex 200 Suspension Array Reader (Bio-Rad Laboratories Pty Ltd.) according to the manufacturer's instructions. Standard curves of cytokine concentration vs. fluorescence intensity were automatically generated by the Bioplex Manager Software (Bio-Rad Laboratories Pty Ltd.), and sample concentrations for each cytokine were extrapolated from respective standard curves. All samples were analyzed in duplicate and allocation of samples from AthletesOC and AthletesNC groups were counterbalanced between plates. The mean inter-assay coefficient of variation for analytes included in statistical analysis was 12.7% (range: 9.9–18.1%). Those analytes where a high proportion of samples with concentrations below limits of detection, or where calculated concentrations for a given analyte were based largely on extrapolation beyond the range of the standard curve, were not included in statistical analysis.

PBMCs were isolated as previously published (West et al., 2016). Briefly, PBMCs were isolated by Ficoll (GE Healthcare, United Kingdom) density gradient separation, washed twice with PBS at 4°C, suspended at a concentration of 1–2 × 106 cells/mL in medium containing 10% DMSO, and cooled to −80°C at a rate of −1°C per min before transfer to liquid nitrogen for storage until assay. Cryopreserved PBMCs were thawed and washed in with RPMI1640 (Thermo Fisher Scientific, Waltham, MA, United States) with 10% heat-inactivated fetal bovine serum. PBMCs were stained for mass cytometry analyses as described (Newell et al., 2012) with the antibodies listed in **Table 2**. Data were acquired on a CyTOF 2 Helios upgraded instrument (Fluidigm, Toronto, Canada) at the Ramaciotti Facility for Human Systems Biology, Sydney, Australia. Flow Cytometry Standard (FCS) files were analyzed with FlowJo X 10.0.7r2 (FlowJo, LLC, Ashland, OR, United States).

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS V.24.0, Champaign, IL, United States) and Prism v7.00 (GraphPad Software Inc., San Diego, CA, United States). The distribution of the data was evaluated using Shapiro-Wilk tests. All hormone and cytokine markers (aside from RANTES) were not normally distributed, thus these data were analyzed using Mann Whitney U tests. These variables were reported as median values (interquartile range; IQR), where IQR equals the difference between the 75th and 25th quartiles. Mass cytometry data was normally distributed and, along with participant demographics (i.e., age, height, weight, BMI) and RANTES, were assessed using independent samples *t*-tests; these data were reported as mean ± standard deviation. Significance was set at $P < 0.05$.

**TABLE 2 |** Mass cytometry antibody panel.

| Isotope | Antibody | Antibody source | Receptor |
|---|---|---|---|
| 89Y | CD45 | Fluidigm | Cell marker |
| 143Nd | CD45RA | Fluidigm | $CD4^+$ and $CD8^+$ memory/naïve maker |
| 145Nd | CD4 | Fluidigm | $CD4^+$ T-cell marker |
| 148Nd | CD16 | Fluidigm | NK-cell marker |
| 149Sm | CD56 | Fluidigm | NK-cell marker |
| 170Er | CD3 | Fluidigm | T-cell marker |
| 168Er | CD8 | Fluidigm | $CD8^+$ T-cell marker |

## RESULTS

There was no difference ($p > 0.05$) in age, height, weight, or BMI between AthletesNC and AthletesOC (**Table 1**). AthletesNC had higher concentrations of estradiol ($p < 0.001$), testosterone ($p = 0.004$), and progesterone ($p = 0.001$), but not cortisol ($p = 0.41$), when compared to AthletesOC (**Table 3**).

Estradiol and progesterone concentrations showed that in the AthletesNC group, 14 athletes had hormone concentrations commensurate with the follicular phase [estradiol: 155.00 (240.00) pmol/L, progesterone: 1.52 (4.66) nmol/L], 12 athletes had hormone concentrations commensurate with the luteal phase [estradiol: 225.00 (225.00) pmol/L, progesterone: 74.05 (58.33) nmol/L], and two athletes had hormone concentrations commensurate with ovulation [estradiol: 1120.00 (460.00) pmol/L, progesterone: 3.07 (1.46) nmol/L]. There were no differences between AthletesNC in the follicular and luteal phases of the menstrual cycle for cortisol ($p = 0.471$), CRP ($p = 0.643$), or any other cytokine ($p > 0.05$), with the exception of IL-6 ($p = 0.009$) and RANTES ($p = 0.022$). IL-6 was higher in the luteal phase [4.90 (2.17) pg/mL] when compared to the follicular phase [1.47 (2.57) pg/mL], whereas RANTES was elevated in the follicular phase (10216.30 ± 2383.60 pg/mL) when compared to the luteal phase (8156.43 ± 1786.71 pg/mL).

There were no differences ($p > 0.05$) between AthletesOC and AthletesNC for any of the cytokines detected by the assay, although there was a trend ($p = 0.062$) for higher IL-6 concentrations in AthletesNC (**Table 4**). Certain cytokines (e.g., IL-2, IL-4, IL-5, IL-7, IL-9, IL-12, IL-13, IL-15) and growth factors (e.g., GM-CSF, MCP-1, VEGF) were below the limits of detection for the majority of samples and were therefore not included in the analysis.

**Figure 1** shows the difference ($p < 0.001$) in CRP concentrations between AthletesOC and AthletesNC. The number of athletes in each CRP risk stratification category is also presented (**Table 5**).

**TABLE 3 |** Hormone concentrations for AthletesOC and AthletesNC.

| | AthletesOC | AthletesNC |
|---|---|---|
| Estradiol (pg/mL) | 6.12 (23.92) | 35.01 (40.57) |
| Free testosterone (pg/mL) | 0.41 (0.40) | 0.80 (0.88) |
| Progesterone (ng/mL) | 0.28 (0.46) | 1.14 (12.07) |
| Cortisol (μg/dL) | 12.23 (6.85) | 10.40 (6.94) |

*Data are presented as median (IQR).*

**TABLE 4 |** Comparison of cytokine concentrations between AthletesOC and AthletesNC.

| | AthletesOC | AthletesNC | *p*-value |
|---|---|---|---|
| IL-1β (pg/mL) | 0.91 (0.67) | 1.02 (1.79) | 0.229 |
| IL-1ra (pg/mL) | 30.90 (39.65) | 29.06 (22.15) | 0.310 |
| IL-6 (pg/mL) | 1.22 (1.60) | 3.88 (3.61) | 0.062 |
| IL-8 (pg/mL) | 7.11 (9.83) | 10.43 (9.69) | 0.662 |
| IL-10 (pg/mL) | 5.60 (3.35) | 6.12 (4.20) | 0.687 |
| IL-17 (pg/mL) | 154.03 (133.32) | 146.59 (63.98) | 0.894 |
| TNFα (pg/mL) | 26.63 (31.31) | 28.40 (87.42) | 0.247 |
| IFNγ (pg/mL) | 29.82 (16.84) | 27.17 (16.12) | 0.940 |
| PDGF (pg/mL) | 2143.33 (1464.11) | 2111.94 (832.51) | 0.460 |
| RANTES (pg/mL) | 9460 ± 2511.97 | 9259.31 ± 2261.03 | 0.760 |

*IL-1ra, interleukin-1 receptor agonist; TNFα, tumor necrosis factor alpha; IFNγ, interferon gamma; PDGF, platelet-derived growth factor; RANTES (CCL5), regulated on activation, normal T cell expressed and secreted. Data presented as median (IQR), except for RANTES which is presented as mean ± standard deviation.*

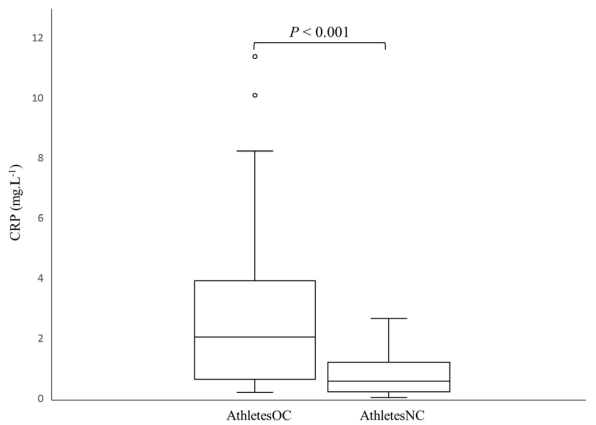


**FIGURE 1 |** Box and whisker plot of C-reactive protein (CRP) concentrations in AthletesNC and AthletesOC. The *boxes* span the interquartile range (IQR), the *line* depicts the median value, the *whiskers* represent the highest and lowest values within a 1.5 IQR of the nearest quartile, and the *circles* depict the outliers.

**TABLE 5 |** The proportion of AthletesOC and AthletesNC in each CRP risk stratification category.

| Risk stratification | AthletesOC *n* (%) | AthletesNC *n* (%) |
|---|---|---|
| <0.5 mg.L$^{-1}$ (protective) | 4 (16) | 11 (39) |
| 0.5–1.0 mg.L$^{-1}$ (no risk) | 4 (16) | 8 (29) |
| 1.0–3.0 mg.L$^{-1}$ (intermediate risk) | 8 (32) | 9 (32) |
| 3.0–10.0 mg.L$^{-1}$ (high risk) | 7 (28) | 0 (0) |
| >10.0 mg.L$^{-1}$ (very high risk) | 2 (8) | 0 (0) |
| Total | 25 (100) | 28 (100) |

Immunophenotyping of PBMCs did not reveal differences between AthletesOC and AthletesNC. **Figure 2** displays the frequency of CD4+ T-cells, memory (CD3+, CD4+, CD45RO+) and naïve CD4+ (CD3+, CD4+, CD45RO−) CD4+ T-cells, CD8+ (CD3+, CD8+) T-cells and memory (CD3+, CD8+, CD45RO+) and naïve (CD3+, CD8+, CD45RO−) CD8+ T-cells, and NK-cells (CD3-, CD20-, CD56+, HLADR−) for AthletesOC compared to AthletesNC.

## DISCUSSION

This study is the first to investigate the effect of OC use on acute phase hormonal and systemic inflammatory parameters in elite female athletes prior to the Olympic Games. C-reactive protein concentrations were significantly elevated in AthletesOC when compared to AthletesNC. Conversely, there was no difference between the two groups for cortisol or any other marker of immune function, although there was a trend for higher IL-6 concentrations in AthletesNC.

C-reactive protein concentrations were threefold higher in AthletesOC compared to AthletesNC, which supports an earlier study (Cauci et al., 2017) that reported a fourfold increase in CRP concentrations in athletic women using an OC when compared to non-users. Interestingly, none of the athletes in the prior study (Cauci et al., 2017) recorded CRP concentrations of >10.0 mg.L$^{-1}$ ("very high" CVD risk) (Cook et al., 2006; Cauci et al., 2017), whereas two OC users (8%) fell into this category in the current cohort. Only 16% of AthletesOC had "protective" CRP concentrations compared to 39% of AthletesNC, and 32% of AthletesOC fell into the combined "protective" and "no risk" categories (CRP < 1 mg.L$^{-1}$) compared to 68% of AthletesNC. It should be noted, however, that CRP is only one risk factor for CVD (Ridker et al., 2005), and elite athletes would likely otherwise be considered low risk for CVD given the protective effect of regular physical activity (Li and Siegrist, 2012), low rates of excess body mass (Wd et al., 1996), and generally good nutritional practices (Lun et al., 2009; Heaney et al., 2011). Nevertheless, 36% of AthletesOC had CRP values of >3 mg.L$^{-1}$ compared to 0% of women in the AthletesNC group, which is indicative of systemic inflammation in this cohort.

Previously, a higher percentage of athletes not using an OC were observed to have CRP concentrations of <0.5 mg.L$^{-1}$, and fewer had concentrations of >3 mg.L$^{-1}$, when compared to non-OC users in the general population (Cauci et al., 2008, 2017). Interestingly, this inverse relationship between CRP concentration and exercise was not observed for the athletes using an OC (Cauci et al., 2017). These findings suggest that regular exercise decreases the frequency of high-inflammatory status, while increasing the frequency of basal protective low-inflammatory status, in non-OC users but not those taking an OC (Cauci et al., 2017). Moreover, a higher percentage of AthletesOC had CRP concentrations >3 mg.L$^{-1}$ (36%) than previously observed in OC users in the general population (31%) (Cauci et al., 2008). It is not immediately clear as to why this occurred, however, periods of intensified training can have an immunosuppressive effect (Walsh and Whitham, 2006; Gleeson, 2007). Given that blood sampling occurred 3 months prior to the Olympic Games, all athletes were performing heavy training loads which may explain the results obtained. Indeed, 87% of athletic women who were not taking an OC in the recent study by Cauci et al. (2017) reported CRP concentrations of <1 mg.L$^{-1}$ compared to 68% of AthletesNC in the present study, indicating

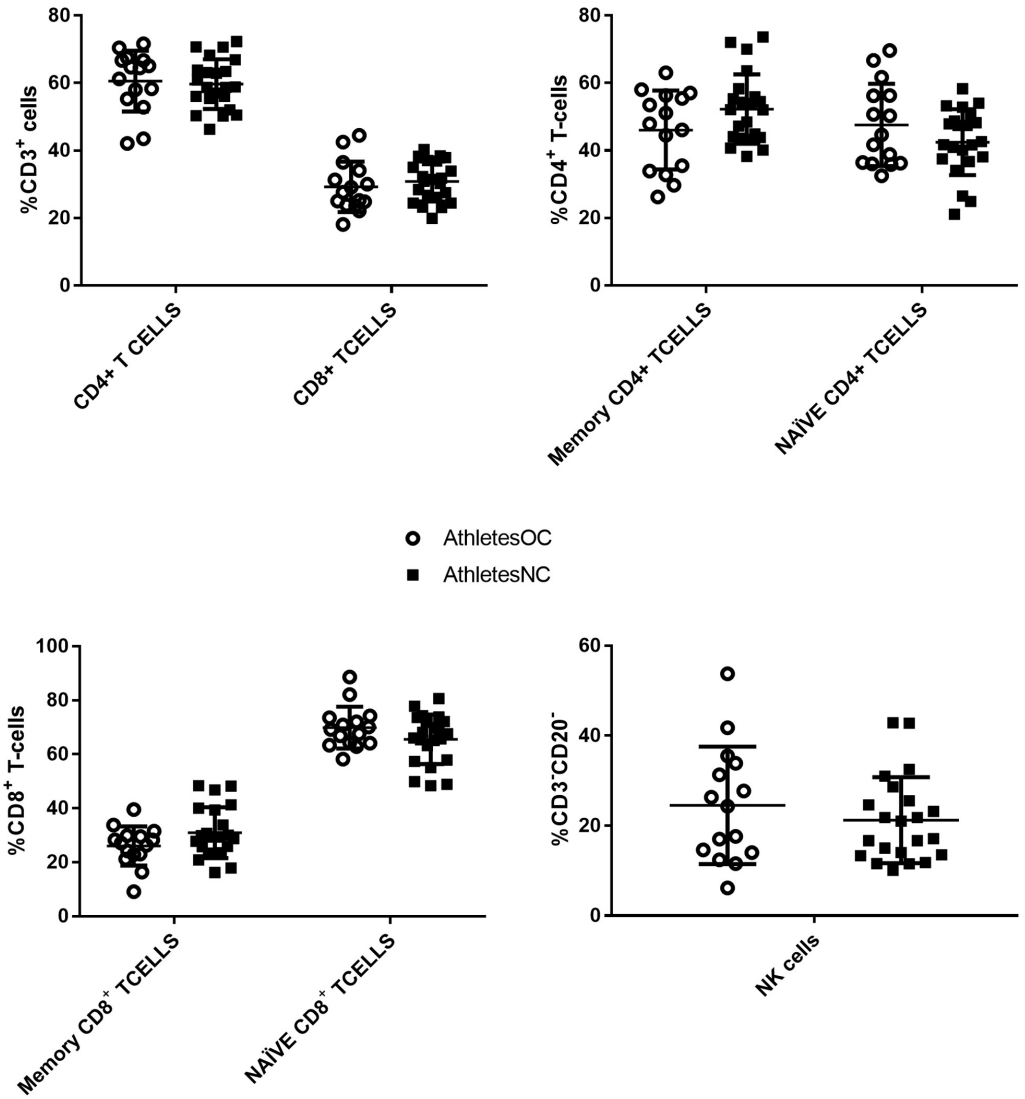


**FIGURE 2 |** PBMC subsets between AthletesOC and AthletesNC. There were no significant differences between the groups in $CD4^+$ or $CD8^+$ T-cells **(top left)**, memory ($CD3^+$, $CD4^+$, $CD45RO^+$) or naïve (CD4+, $CD45RO^-$) $CD4^+$ T-cells **(top right)**, memory ($CD3^+$, $CD8^+$, $CD45RO^+$) or naïve ($CD3^+$, $CD8^+$, $CD45RO^-$) $CD8^+$ T-cells **(bottom left)**, or NK-cells (CD20-, CD56+, HLADR−) **(bottom right)**.

an increased inflammatory status even amongst non-OC users. However, in the main, the results suggest that OC users have elevated CRP concentrations relative to athletes who do not take OC, with little difference between athletes and non-athletes in terms of the CRP response to OC use.

As expected, estradiol, progesterone, and testosterone were lower in the AthletesOC group when compared to AthletesNC (Wiegratz et al., 2003; Fleischman et al., 2010). While no previous research has compared cortisol concentrations in elite female athletes according to OC status, one study found that cortisol concentrations doubled in high school/university athletes after 10 months of OC use when compared to pre-OC levels (Rickenlund et al., 2004). Although we did not have access to pre-OC cortisol data, the comparable values reported for AthletesOC and AthletesNC do not suggest a significant relationship between OC use and basal cortisol concentrations. In non-athlete populations, no consistent effect of OC use on resting cortisol concentration has been observed, with some studies showing an elevation (Timmons et al., 2005; Boisseau et al., 2013), others showing no difference (Bonen et al., 1991; Kirschbaum et al., 1996), and one showing a decreased concentration (Reinberg et al., 1996) in OC users.

No between-group difference was observed in the frequency of PBMC immune cell subsets or for any of the cytokines measured, although IL-6 concentrations were three times higher ($p$ = 0.062) in AthletesNC. This was driven by significantly higher IL-6 concentrations in AthletesNC that were in the luteal phase at the time of blood sampling, when compared to those that fell into the follicular phase. Indeed, a separate analysis revealed no significant differences between AthletesNC

in the follicular phase and AthletesOC. There is little consensus in the literature regarding the effect of menstrual cycle phase on IL-6 concentrations, with some studies showing elevated concentrations in the follicular phase (Angstwurm et al., 1997), others in the luteal phase (Konecna et al., 2000), and others reporting no difference (O'Brien et al., 2007). Nevertheless, these findings are interesting given that IL-6 is thought to trigger CRP expression (Black et al., 2004), yet CRP was higher in the AthletesOC group though this group displayed a trend for lower IL-6 concentrations, and there was no difference in CRP between AthletesNC in the follicular and luteal phases despite differing IL-6 concentrations. Giraldo et al. (2008) found that OC use improves inflammatory status in untrained women by lowering the concentration of IL-8 and increasing the concentration of IL-13, which was not replicated in the current findings (there was no between-group difference in IL-8 and IL-13 concentrations were below the limits of detection). No differences in IL-6 were observed between OC users and non-users in this previous study (Giraldo et al., 2008). It is important to note that almost half of the athletes in the present study had trained prior to blood collection, and thus, it is possible that the samples reflect a post-exercise cytokine response. However, separate within-group analyses of the cytokine and CRP data were performed according to training status (trained before sample collection vs. no training before sample collection), and no differences in IL-6 (or CRP) were found. This suggests that it was the use of the OC, rather than a training response, that elicited the findings observed.

Timmons et al. (2005) observed an ∼80% greater IL-6 concentration immediately post-exercise in normally-menstruating women in the follicular phase when compared to women using an OC, which indicates a blunted IL-6 response in OC users. While it is speculative to suggest an influence of OC use on the exercise-induced cytokine response in elite athletes given an exercise protocol was not employed, future studies should further examine the relationship between OC use and the cytokine response to exercise in this cohort. Although IL-6 has known inflammatory properties, increased IL-6 concentrations after exercise inhibit pro-inflammatory (i.e., TNF-α) cytokines and facilitate anti-inflammatory cytokine (i.e., IL-1ra, IL-10) production (Petersen and Pedersen, 2005), and thus, a disruption to this process may have implications for recovery and performance. A recent review by Peake et al. (2017) highlights the importance of the initial pro-inflammatory response to exercise-induced muscle injury, but notes that these interactions must be tightly regulated to avoid prolonged inflammation and tissue damage.

Animal and human studies demonstrate that regular exercise alters the balance between Th-1 and Th-2 cell subsets (Yeh et al., 2009; West et al., 2016), and initial studies indicate that OC use alters circulating white cell subsets at certain times of the menstrual cycle (Auerbach et al., 2002). Our findings indicate that, in elite athletes, OC use has little effect on key immune subsets involved in a cell mediated immune response. It should be noted that Auerbach et al. (2002) reported cell differences per liter of blood, while we reported cells relative to their parent population. However, re-calculation of our data to be comparable to the previous study (Auerbach et al., 2002) made no difference to our results (data not shown). Thus, our observation suggests OC use does not modify chronic exercise-induced PBMC immune responses in elite female athletes.

This study provides unprecedented insight into the basal immune functioning of elite athletes leading into the Olympic Games. However, given the elite status of the subjects and the pivotal timing of the study, it was not possible to manipulate the athletes' schedules to control for factors such as training status prior to blood sampling (and the timing of blood sampling after training, for those that did train) and diet, which is a limitation of the study. Ideally, training status and/or sampling time would have been strictly controlled to ensure uniformity between groups. Nevertheless, AthletesNC and AthletesOC were very similar with respect to the number of athletes that trained/didn't train in each group prior to blood sampling, and the timing of blood sampling post-training (2.5 vs. 2 h, respectively). Moreover, CRP and IL-6 were the only inflammatory markers assessed that were significantly different (or approaching statistical significance, in the case of IL-6) between groups, and a separate analysis of these data according to training status (trained before sample collection vs. no training before sample collection) found no differences in either IL-6 or CRP for AthletesNC or AthletesOC. This suggests that the results obtained relate to the use of OC as opposed to reflecting a post-exercise response. An additional limitation of the study was incomplete data pertaining to the OC type used by AthletesOC, thus precluding understanding of how the different OC formulas available may differentially influence inflammation. It should also be noted that the cross-sectional study design allows limited understanding of the influence exerted by OC on inflammatory markers at different time points. Nevertheless, this study highlights a substantially increased inflammatory status in athletes taking an OC at a pivotal point in their training (in the months immediately prior to the Olympic Games). As suggested by Cauci et al. (2017), it is plausible that higher basal CRP levels may result in an exacerbated inflammatory response when exposed to physical stress or injury. Future studies should investigate whether higher basal CRP concentrations are associated with reduced performance and recovery in elite female athletes.

## CONCLUSION

Elite female athletes selected for Olympic squads had substantially greater levels of CRP if they were users of an OC. Specifically, in the months prior to the Rio Olympic Games, AthletesOC had threefold higher CRP concentrations than their teammates who were not taking an OC. There was also a trend for lower IL-6 concentrations in AthletesOC which needs to be further explored. As female sports continue to gather momentum and public interest, future research should examine the relationship between OC use, immune function, and performance/recovery in elite female populations, as this may have important implications for the athletes' training and recovery programs.

## DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Human Research Ethics Committee – Griffith University. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

BLa contributed to the data analysis and led the manuscript writing. AC contributed to the experimental design, blood collection and handling, oversaw the blood analysis, and assisted with the preparation of the manuscript. CC contributed to the experimental design, blood handling and analysis, and assisted with manuscript preparation. MD, HM, BF, DH, NV, GW, LB, and BLu contributed to the experimental design and assisted with the preparation of the manuscript. NW contributed to the experimental design, blood collection and handling, data analysis, and assisted with the preparation of the manuscript. CM contributed to the experimental design, data analysis, and preparation of the manuscript.

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# Similar Energy Expenditure During BodyPump and Heavy Load Resistance Exercise in Overweight Women

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**Purpose:** High-repetition, low-load resistance exercise in group class settings has gained popularity in recent years, with BodyPump as a prime example. For individuals using exercise for body-weight management, the energy expenditure during exercise is of interest. Therefore, we herein aimed to estimate the energy expenditure during a session of BodyPump and a time-matched session of heavy load resistance training in overweight women (BMI $\geq$ 25.0).

**Methods:** Eighteen women participated in the study (mean age 35.4 years $\pm$ 10.2, BMI 30.4 kg/m$^2$ $\pm$ 4.8), 10 exercising BodyPump (50–100 repetitions each muscle group) and eight performed a heavy load session (eight repetition maximum $\times$ three sets). The energy expenditure was assessed with indirect calorimetry during the sessions and for two intervals at rest during the recovery phase: 0–20 and 120–140 min after the sessions.

**Results:** The BodyPump group lifted significantly more loads than the heavy load group (19,485 kg $\pm$ 2258 vs 15,616 kg $\pm$ 2976, $p$ = 0.006), while energy expenditure was similar with 302 kcal $\pm$ 67 and 289 kcal $\pm$ 69 in BodyPump and heavy load group, respectively ($p$ = 0.69). With no group differences, the resting metabolic rate (RMR) was elevated with 15–22% 2 h after exercise.

**Conclusion:** Overweight women achieved an energy expenditure of approximately 300 kcal (4.7 kcal per min) during a single session of BodyPump, which was similar with the women performing a single session of heavy load resistance exercise.

**Keywords: resting metabolic rate, RMR, EPOC, group exercise, energy consumption**

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## INTRODUCTION

The worldwide prevalence of overweight (BMI $\geq$ 25.0 kg/m$^2$) and obesity (BMI $\geq$ 30.0 kg/m$^2$) have increased considerably during the last three decades (Ng et al., 2014; Di Cesare et al., 2016). According to the World Health Organization, 40% of the adult female population are classified as overweight and 15% as obese [World Health Organization (WHO), 2020]. Several lifestyle related interventions have been investigated to treat overweight and obesity and prevent weight gain, and today a combination of energy restrictions, physical activity, and behavioral change strategies are recommended (Donnelly et al., 2009; Laddu et al., 2011; Dombrowski et al., 2014;

Samdal et al., 2017). Traditionally, endurance training have been prioritized as physical activity among overweight and obese, but the American College of Sports Medicine (ACSM) also recommend adults to perform regular resistance exercise [two to three times/week with an intensity between 60 and 80% of one repetition maximum (1RM)] (Garber et al., 2011).

Increased energy expenditure is imperative if exercise is used to reduce body weight through loss of fat mass; however, traditional resistance exercise does not appreciably elevate energy expenditure relative to other exercise modalities (e.g., endurance training) (Donnelly et al., 2009; Willis et al., 2012; Swift et al., 2018). Despite this knowledge, health- and fitness clubs offer different resistance-exercise-based group classes and claim these to be effective in improving body composition and reduce body weight. A strategy used to augment the energy expenditure in many of these classes is to increase the worktime to rest ratio, i.e., apply a high duty cycle (work time divided by total exercise time). This translates into resistance exercise modes with low-loads, high number of repetitions, and short rest-intervals between sets and exercises (Stanforth et al., 2000; Rixon et al., 2006; Berthiaume et al., 2015; Harris et al., 2018).

BodyPump is the most popular resistance-exercise-based group class worldwide, available in almost 15,000 health- and fitness clubs (Les Mills International). The distributor, Les Mills International (2020), pre-choreograph the classes, all based on the same principle; a full body-workout with barbell and weights and a high duty cycle: 800–1000 repetitions per session/h, low loads (<35% of 1RM) (Rustaden et al., 2017) and short rest-intervals (<20 s). According to LesMills, this formula results in a high energy expenditure (up to 540 kcal each BodyPump session[1]).

Previously, the energy expenditure during a session of BodyPump has been assessed in young, normal weight, and trained men and women (Stanforth et al., 2000; Berthiaume et al., 2015; Harris et al., 2018). In these studies, the energy expenditure was reported to be 250–334 kcal each session. However, only Stanforth et al. (2000) measured the actual oxygen consumption during the exercise session. Berthiaume et al. (2015) and Harris et al. (2018) only estimated the energy expended based on a movement sensor (SenseWear armband) and heart rate (HR), respectively. Moreover, changes in resting metabolic rate (RMR) after the exercise session—excess post exercise oxygen consumption (EPOC)—was not assessed in any of these studies. The magnitude of EPOC seems to be positively dependent on the work done during exercise (intensity and duration) and may have relevance in body-weight management (Borsheim and Bahr, 2003).

Given that BodyPump is globally popular and available in fitness centers worldwide, it is valuable to gain knowledge of the physiological responses to this exercise mode; not the least, insight in the energy expenditure for individuals that exercise for body-weight management. Therefore, the aim of the present study was to assess the energy expenditure from BodyPump in middle-aged, overweight women. In addition, the BodyPump session was compared to a time-matched session of traditional heavy load resistance training in accordance with the ACSM recommendations (Garber et al., 2011). We hypothesized that the energy expenditure would be higher during and after BodyPump than a heavy load resistance exercise session, because BodyPump has a high duty cycle and should result in a larger total work (repetitions times load).

# MATERIALS AND METHODS

## Study Design and Participants

Participants were recruited from an on-going randomized controlled trial (RCT), where overweight and obese women were randomized to either BodyPump, heavy load resistance exercise (with or without a personal trainer), or non-exercising controls (for further details, see Rustaden et al., 2017). Inclusion criteria were BMI $\geq$ 25.0, age 18–65, and being untrained defined as "not performing regular structured exercise $\geq$ twice a week for the last 6 months."

The main aims for the RCT study were to investigate the effects of 12 weeks of these exercise interventions on muscle strength and body composition. In the present study, we wanted to investigate the energy expenditure of a single session of BodyPump and a single session of heavy load resistance exercise. All participants in the BodyPump and personal trainer group were invited to participate in the present study.

In total, 18 women volunteered to participate in the present study (mean age 36 years $\pm$ 10, weight 84 kg $\pm$ 14, height 168 cm $\pm$ 6, and BMI 30 kg/m$^2$ $\pm$ 5), 10 from the BodyPump group and eight from the heavy load resistance exercise group. The women were initially untrained but had, at the point of testing in the present study, been training either BodyPump or heavy load resistance exercise for 3–4 weeks (9–12 sessions). All participants were therefore well accustomed to the exercise routines when entering the present, acute study. In the present study, those who were part of the BodyPump group in the RCT conducted the BodyPump session, while those who were in the heavy resistance exercise group in the RCT, conducted the heavy resistance exercise session.

In the present study, we aimed to estimate the energy expenditure from a BodyPump and a heavy load resistance exercise session. In brief, the participants conducted an exercise session, BodyPump or heavy load resistance exercise, while the oxygen uptake was measured continuously. The oxygen uptake was used to estimate the energy expenditure during the exercise sessions (Compher et al., 2006). EPOC was assessed by measuring oxygen uptake at rest, i.e., RMR, conducted before and twice after the exercise sessions. The time slots for the RMR assessments were chosen based on similar, previous studies (Binzen et al., 2001; Benton et al., 2016). Total work (load $\times$ repetitions $\times$ sets) and HR were also assessed during the exercise sessions.

The participants arrived at the laboratory after 12 h fast. Caffeine and nicotine were prohibited before testing, and the participants were instructed to use car or public transportations to the laboratory. Strenuous physical activity or exercise was asked to be avoided 48 h before the test day. The test day was initiated by RMR measurements between 7:45 and 8:30 am, followed by a standard breakfast (oeatmeal) with a caloric content

[1]https://www.lesmills.com/workouts/fitness-classes/bodypump, April 2020.

equivalent to 20% of the individual's estimated RMR (between 8:30 and 9:00 am). The exercise sessions occurred between 9:00 and 10:00 am. After the exercise sessions, RMR was assessed for 20 min. The women were thereafter given a standardized lunch (same as the breakfast; at 10:30 am) and they rested in a seated position before the final RMR measurement 2 h after exercise (120–140 min). In total, the testing procedure lasted for approximately 4 h.

## Ethics Statement

The study is approved by the Regional Committee for Medical Research Ethics Norway, Oslo (REK 2012/783). All participants signed a written consent statement before entering the study, and the procedures followed the World Medical Association Declaration of Helsinki.

## Assessments

### Energy Expenditure

Energy expenditure was assessed by indirect calorimetry, applying an automatic ergospirometry system (Oxycon Pro Jaeger Instrument, Hoechberg, Germany). The participants breathed through a Hans Rudolph mask attached to a 3-m long non-rebreathing hose, allowing the participants to move freely during the exercises (an investigator manually assisted by positioning/controlling the hose). The measurements started 2 min before exercise and continued during the entire exercise sessions. $O_2$ and $CO_2$ were continuously sampled (in a mixing chamber) and averaged over 30 s periods. Prior to each test, the Oxycon Pro Jaeger Instrument was calibrated after the manufacturers' guidelines. Indirect calorimetry is a valid assessment method when estimating energy expenditure (Compher et al., 2006), and the Oxycon Pro Jaeger Instrument system applied has been found to be a highly accurate and valid system (Foss and Hallèn, 2005).

The energy expenditure (kcal) was calculated as the accumulated $O_2$ consumption during exercise multiplied by 5 kcal (McArdle et al., 2010).

### Resting Metabolic Rate

Resting metabolic rate was estimated by indirect calorimetry with a ventilated hood (Canopy-option for Oxycon Pro Instrument). The participants were in supine position on a comfortable bed, the test lab was quiet, had dimmed light, and the temperature was 22–24°C. The measurements lasted for 30 min, but the initial 10 min was discarded (Compher et al., 2006). The calorie equivalent used to estimate energy expenditure was derived from each participant's respiratory exchange ratio (RER) and ranged from 4.68 to 5.04 kcal per liter oxygen ($LO_2$) (McArdle et al., 2010). The energy expenditure was calculated as calories each minute = $VO_2$ ($Lmin^{-1}$) × kcal per $LO_2$.

### Total Workload

The work done during exercise was calculated by multiplying the load used in each exercise (kg) by the repetitions and sets for each participant. The body mass was included as load in exercises where the center of mass was moving vertically: In squats and lunges 90% of the body mass was added to the external load (e.g., 80 kg × 0.9 + 30 kg = 102 kg). In push-ups, dips, and sit-ups, 65, 50, and 40% of the body mass were used, respectively. These estimations were based on pilot testing on a force plate (AMTI, SG-9, Advanced Mechanical Technologies, Newton, MA, United States).

### Heart Rate

Heart rate was registered by using an HR monitor (Polar RS800, Kempele, Finland) during the exercise sessions. Maximal HR was estimated: 211 – 0.64 × age (Nes et al., 2013).

## Exercise Protocols

The participants conducted either a session of BodyPump (**Table 1**) or heavy load resistance exercise (**Table 2**). A personal trainer was present during all sessions to ensure proper lifting technique and assist if necessary, but did not interfere with the exercise protocol.

### BodyPump

BodyPump is a high-repetition low-to moderate group session, prechoreographed and distributed by LesMills International. Every third month LesMills releases a new BodyPump program, all based on the same model and principles (LesMills International). During the intervention period in the RCT study, BodyPump release no. 83 was present at all health- and fitness clubs worldwide, including nine music tracks (4–7 min), each exercising specific body parts. All the 1-h sessions includes approximately 800 repetitions in total, and 50–100 repetitions in each muscle group. The participants exercise with a step and free-weights (1, 2.5, or 5 kg), which they put together on a 1.25 kg bar. Between each track, there is a short rest period of approximately 1 min, used to change weights and prepare to the next exercises. Some of the tracks include short inter-session rest periods (typically 16–32 beats and 7–14 s) (LesMills International). During the assessment in the present study, participants were instructed from a LesMills video (Les Mills International, 2020), with an instructor demonstrating the whole session. As mentioned above, the participants were familiar with the exercise program, as assessments were conducted midway into the RCT study. The external loads for each exercise used were based on the instructions, and the participants experience. The video-instructor encouraged the participants to achieve muscular fatigue in each track, with proper lifting technique.

### Heavy Load Resistance Exercise

The heavy load resistance exercise group performed session 1 from week 5–8 in the RCT (Rustaden et al., 2017), including 8RM × two to four sets, and 45 and 60 s of rest between sets and the exercises, respectively (**Table 2**). The participants selected the exercise loads based on their experience and, if necessary, with assistance from the personal trainer overseeing the sessions. It was important that the loads were as heavy as possible for the eight repetitions per set.

## Statistical Analysis

Data are presented as means with standard deviation (±) for all variables. A normal distribution of the data was found

**TABLE 1 |** Description of BodyPump release no. 83, with exercises and number of repetitions.

| Music no. | Exercise | Volume (reps) |
|---|---|---|
| 1 Warming-up | Straight leg deadlift, rowing, shoulder press, squat, lunges, and biceps curl | 88 |
| 2 Leg | Squat | 95 |
| 3 Chest | Bench press | 80 |
| 4 Back | Rowing, stiff legged deadlift, clean and press, and power press | 75 |
| 5 Triceps | French press, triceps press, pullover, and overhead triceps press | 78 |
| 6 Biceps | Biceps curl | 68 |
| 7 Leg | Squat, lunges, and squat jump | 72 + 24 jumps |
| 8 Shoulders | Push-up, lateral raise, rowing, and shoulder press | 76 + 36 push up |
| 9 Abdominals | Sit-ups, sit-ups to the side, and side-plank | 51 + 30 s |

**TABLE 2 |** Description of the heavy load resistance exercise program, showed with exercises and training volume.

| Exercise | Volume (sets × reps) |
|---|---|
| Squat | 3 × 8 |
| Lunges | 4 × 8 |
| Stiff-legged deadlift | 3 × 8 |
| Forward rowing | 3 × 8 |
| Bench press | 3 × 8 |
| Dips | 2 × 8 |
| Shoulder press | 2 × 8 |
| Lateral raise | 2 × 8 |
| Clean and press | 2 × 8 |
| Triceps press overhead | 2 × 8 |
| Biceps curl | 2 × 8 |
| Sit-ups | 3 × 8 |

using a Shapiro–Wilk test, and an independent *t*-test was used to compare between-group differences in total workload and energy expenditure during the sessions. A mixed between-within subject's analysis of variance assessed the impact of the two different exercise programs on $O_2$ ml/kg, RMR (20 min), HR (beats/min), and RER at the three assessment time points. Analyses were conducted with SPSS Statistical Software version 21 (IBM Corporation, Route, Somers, NY, United States). Level of significance was $p \leq 0.05$.

# RESULTS

There were no significant differences in demographic variables between the two experimental groups (**Table 3**). The duty cycles (active time during the sessions) were 86 and 45% for BodyPump and heavy load resistance exercise, respectively.

## Energy Expenditure

The estimated total energy expenditure during exercise was not significant different between the groups ($p = 0.696$) with 302 kcal (±67) during BodyPump and 289 kcal (±69) during heavy load resistance exercise (**Table 4**). The individual range was 170–378 kcal in BodyPump and 169–347 kcal in the heavy load resistance exercise group.

**TABLE 3 |** Demographic data of all participants in the BodyPump group and the heavy load resistance exercise group (RE).

| Variable | BodyPump (*n* = 10) | RE (*n* = 8) | *p*-value |
|---|---|---|---|
| Age (year) | 36.4 ± 9.9 | 34.1 ± 11.0 | 0.651 |
| Weight (kg) | 84.7 ± 13.5 | 87.1 ± 16.4 | 0.744 |
| Height (cm) | 167.1 ± 6.6 | 168.9 ± 6.7 | 0.562 |
| BMI (kg/m$^2$) | 30.3 ± 4.7 | 30.5 ± 5.3 | 0.967 |
| Fat mass (%) | 38.1 ± 7.4 | 38.6 ± 5.2 | 0.275 |
| Muscle mass (kg) | 28.8 ± 3.2 | 30.4 ± 3.6 | 0.270 |

*Presented as mean with standard deviation (±) and differences between groups with p-value.*

**TABLE 4 |** Exercise duration (min/session), oxygen uptake ($O_2$), respiratory exchange ratio (RER), heart rate, kilocalories (kcal) each minute and total energy expenditure in the BodyPump, and heavy load resistance exercise group (RE).

| Variable | BodyPump (*n* = 10) | RE (*n* = 8) | *p*-value |
|---|---|---|---|
| Duration [min (session)] | 53.0 ± 0.0 | 57.7 ± 2.9 | 0.033* |
| $O_2$ (ml/min/kg) | 12.3 ± 2.7 | 12 ± 2.0 | 0.779 |
| RER | 0.96 ± 0.0 | 0.94 ± 0.0 | 0.373 |
| Heart rate (beats/min) | 142 ± 16 | 146 ± 13 | 0.592 |
| Kcal/min | 4.7 ± 1.2 | 4.0 ± 1.0 | 0.200 |
| Total energy expenditure (kcal) | 302 ± 67 | 289 ± 69 | 0.696 |

**Indicates a significant difference between the groups with $p < 0.05$. Presented as mean with standard deviation (±) and p-value showing group differences.*

## Resting Metabolic Rate

There were no statistically significant differences between the exercise modalities in RMR 0-20 or 120–140 min after exercise (**Table 5**). Oxygen uptake ($O_2$ ml/min), RER, RMR, and HR were assessed at supine rest for 20 min before exercise, immediately after (0–20 min) and 120–140 min after exercise. The mixed between-within subject's analysis of variance revealed no significant interaction effect between the groups. In both groups, there was a significant effect for time ($p < 0.005$), but the main effect comparing the two groups was not significant (**Table 5**). In the BodyPump group, RMR increased 29% from before exercise to immediately after exercise, and 22% from before exercise to 2 h after exercise ($p < 0.001$). For the heavy load resistance exercise group changes in RMR were 33 and 15% before to immediately after, and before to 2 h after exercise, respectively ($p < 0.001$).

**TABLE 5 |** Variables from resting metabolic rate (RMR) before exercise, after exercise acute (0–20 min), and after exercise 2 h (120–140 min) in the BodyPump and heavy load resistance exercise group (RE).

| Variable | Before exercise | | After exercise 0–20 min | | After exercise 120–140 min | | Interaction | Time | Between groups |
|---|---|---|---|---|---|---|---|---|---|
| | BodyPump | RE | BodyPump | RE | BodyPump | RE | *p*-value (pes) | *p*-value (pes) | *p*-value (pes) |
| $O_2$ ml/kg | 2.3 ± 0.4 | 2.6 ± 0.4 | 3.0 ± 0.5 | 3.4 ± 0.5 | 3.0 ± 0.4 | 3.0 ± 0.7 | 0.240 (0.17) | <0.0005* (0.75) | 0.336 (0.06) |
| RER | 0.87 ± 0.13 | 0.77 ± 0.03 | 0.78 ± 0.04 | 0.75 ± 0.04 | 0.84 ± 0.05 | 0.81 ± 0.07 | 0.473 (0.11) | <0.0005* (0.70) | 0.076 (0.21) |
| RMR kcal | 19 ± 4 | 21 ± 2 | 25 ± 4 | 29 ± 4 | 24 ± 4 | 25 ± 5 | 0.194 (0.20) | <0.0005* (0.78) | 0.181 (0.11) |
| HR (beats/min) | 65 ± 8 | 66 ± 9 | 89 ± 10 | 93 ± 8 | 72 ± 10 | 72 ± 11 | 0.474 (0.10) | <0.0005* (0.96) | 0.751 (0.01) |

**Indicates a significant difference in time within both groups with $p < 0.05$. Presented with mean values and standard deviation (±). Interaction between the groups, substantial main effect for time in the two groups, and main effect comparing the two groups are shown with p-value and partial eta squared (pes).*

## Total Workload

Including both external loads and part of the body mass, the BodyPump group lifted significantly more loads (19,485 kg ± 2258) than the heavy load resistance exercise group (15,616 kg ± 2976) ($p = 0.006$). Load lifted per minute was also significantly higher in BodyPump compared to the heavy load resistance exercise group ($p = 0.001$), with 368 kg/min (±43) and 280 kg/min (±50), respectively. Based on the participants' 1RM tests at baseline in the RCT study (Rustaden et al., 2017), the relative loads (% of 1RM) in the BodyPump group were estimated to 14% (±2.8) and 18% (±2.6) in squat and bench press, respectively. The relative loads in the heavy load resistance exercise group were 77% (±16.5) in squat and 80% (±8.0) in bench press, which were significantly higher than the BodyPump group (both $p \leq 0.001$).

# DISCUSSION

To our knowledge, this is the first study to compare the energy expenditure during BodyPump session with traditional heavy load resistance exercise in overweight women. We observed that the BodyPump participants performed more work (kg lifted) than the heavy load resistance exercise group. Nevertheless, energy expenditure during the workouts were about 300 kcal and similar between the two exercise modalities. The RMR was elevated for at least 2 h after exercise, with no differences between BodyPump and heavy load resistance exercise; i.e., the EPOC appeared similar between sessions.

The higher total workload performed in BodyPump, compared to the heavy load resistance exercise, was due to the higher number of repetitions, as well as fewer and shorter periods of rest. The BodyPump program included approximately 800 repetitions, and 10 min of rest in total. In comparison, the heavy load resistance exercise program included 248 repetitions, and approximately 28 min of rest. Thus, the heavy load resistance exercise group had a higher energy expenditure per kg lifted. It is also likely that the exercises in this group were performed with larger range of motions, compared to BodyPump, as they used 2–4 s per repetition. In BodyPump, the participants had to keep up with the choreography and music, which is approximately one lift each second. This faster lifting pace in BodyPump might have resulted in smaller range of motions, and consequently, less energy used per repetition.

In correspondence with the $VO_2$-measurements, mean HR was similar between the two exercise modalities (142 beats/min in BodyPump and 146 beats/min in the heavy load resistance exercise group). The estimated relative intensities ($HR_{max}$) were 76 and 77% in BodyPump and heavy load resistance exercise, respectively, which indicate a similar cardiovascular load. This correspond with Oliveira et al. (2009), who investigated the physiological profile during a BodyPump session, and found $HR_{max}$ to be 78 and 84%, during the tracks involving the largest muscle groups (Oliveira et al., 2009).

Total energy expenditure during BodyPump was somewhat higher in the present study, compared to previous findings. Stanforth et al. (2000) and Berthiaume et al. (2015) investigated

physiological responses during a BodyPump session in 30 and 40 trained men and women, respectively. Total energy expenditure in Stanforth et al. (2000) was 265 kcal (±60) including both genders, and for women only; 214 kcal (±26). Berthiaume et al. (2015) reported 250 kcal (±68) in both genders, and 202 kcal (±38) in the female participants (assessed with SenseWear armband, not $O_2$ uptake). Higher body mass in our participants could explain the discrepancy in energy expenditure, compared to these two studies. Since body mass makes up most of the load in exercises such as squats and lunges, our overweight participants probably used more energy per repetition as they were about 23 kg heavier than the normal weight women in Stanforth et al. (2000). Berthiaume et al. (2015) did not report the participants body weight, but mean BMI in their female participants were 22.7 kg/m$^2$ (±2.2), compared to 30.3 kg/m$^2$ (±4.7) in our participants exercising BodyPump. In addition, the assumption is further supported by differences in exercise intensity. Our women exercised with a relative intensity of 76% of HR max (mean 142 beats/min) compared to 63% of HR max (mean 124 beats/min) in the Stanforth et al. (2000) study. HR was not reported in Berthiaume et al. (2015). Different assessment methods may also explain some of the differences in exercise intensity. On the other hand, estimated energy expenditure in the present study is comparable with Harris et al. (2018), with 334 kcal in female participants with minimum 6 months of Body Pump experience. Anyhow, the findings of the present and previous studies indicate that the energy expenditure is significantly less than the energy costs (540 kcal) claimed by LesMills (LesMills International). Interestingly, Berthiaume et al. (2015) asked their participants of perceived energy expenditure after the session, and both men and women overestimated the measured energy expenditure by 50 and 84%, respectively. This emphasizes the importance of informing the public about realistic expectations to energy expenditure during exercise modes as BodyPump. In perspective, 4.7 kcal/min energy expenditure, as seen during the BodyPump session, equals a comfortable walking speed on a flat surface (3–4 km/h for an ∼84 kg person (McArdle et al., 2010).

Rixon et al. (2006) investigated energy expenditure in normal weighed women during four different group-based exercise concepts, and found that 60 min of bodycombat, step-aerobics, indoor-cycling, and aerobic pump with resistance exercises resulted in 8–10 kcal/min and moderate to high intensity (55–89% of $HR_{max}$) (Rixon et al., 2006). Compared to Rixon et al. (2006), both groups in the present study exercised with merely half of the energy expenditure per unit time (BodyPump group 4.7 kcal/min ± 1.2, and heavy load resistance exercise group 4.0 kcal/min ± 1.0). Furthermore, according to the ACSM position stand (Donnelly et al., 2009), physical activity with moderate intensity, resulting in energy costs between 1200 and 2000 kcal/week is recommended to prevent weight gain or give a moderate weight loss (Donnelly et al., 2009). Based on observations in the current study, a minimum of four weekly sessions of BodyPump or heavy load resistance exercise would contribute to achieving this recommendation. In fact, in two other studies from our study group, which the current participants were part of; body composition was unchanged after 12 weeks of BodyPump (Rustaden et al., 2017, 2018). In other words, the energy expenditure of each BodyPump session (or heavy load resistance exercise session) was too small to cause a loss in body mass.

The present results indicate elevated RMR due to EPOC after both BodyPump and heavy load resistance exercise with values in line with similar studies (Borsheim and Bahr, 2003; LaForgia et al., 2006). Based on similar changes in BodyPump and heavy load resistance exercise, we suggest that the EPOC was more related to the cardiovascular load and muscular energy turnover than the mechanical loading (i.e., differences in external loads/weights). As concluded by several authors, the magnitude and duration of EPOC after exercise seem highly correlated to cardiovascular intensity, expressed as % of $HR_{max}$ or % of $VO_{2max}$ (Borsheim and Bahr, 2003; LaForgia et al., 2006; Paoli et al., 2012). In contrast to our findings, Thornton and Potteiger (2002) found similar acute energy expenditure, but greater EPOC in a high-load resistance exercise group (85% of 8RM) than a low-load resistance exercise group (45% of 8RM). Interestingly, Thornton and Potteiger (2002) reported higher cardiovascular load and muscular energy turnover rates in the high-load group, as judged by HR and blood lactate, respectively. Thus, this could explain the higher EPOC in the high-load group. In our study, BodyPump (low load) compensated for lower loads with a higher duty cycle, and thereby eliciting similar cardiovascular and muscular stress as heavy load resistance exercise. Of note, our participants were well accustomed to the exercises before the test sessions. This contrasts with most other studies on EPOC, where the participants conducted the exercise session for the first time. Unaccustomed resistance exercise may lead to some exercise-induced muscle damage, which again may affect the EPOC values (Thornton and Potteiger, 2002; Borsheim and Bahr, 2003; LaForgia et al., 2006; Hackney et al., 2008; Paoli et al., 2012). This is a weakness in many other studies as exercise-induced muscle damage will decrease drastically after only one session—known as the repeated-bout effect (McHuge, 2003); thus, many of the EPOC values reported after resistance exercise may be an overestimate of the EPOC that can be expected after exercise sessions regularly repeated in a training period.

This study has several weaknesses. A better design to compare energy expenditure might have been a cross-over design where each individual had conducted both exercise sessions. However, since the participants were recruited from an ongoing RCT study, we could not interfere with the interventions. In addition, the study may have a small sample size, which could lead to type II error. Another limitation of the study is that EPOC was still present during our last assessment period (2 h after exercise), meaning that we did not capture the duration and total magnitude of EPOC. We can therefore not be sure there were no group differences at later time-points. Furthermore, we did not include a control day without exercise. Thus, we cannot claim that the EPOC assessed was only due to the exercise sessions. The RMR after exercise may have been affected by the two light meals and time of day (Borsheim and Bahr, 2003). However, Haugen et al. (2003) found that repeated morning and evening assessments of RMR were stable and highly correlated with only 6% variability. Thus, these design weaknesses should

not interfere with the main purpose with the present study, to compare BodyPump against heavy load resistance exercise.

## CONCLUSION

A single session of BodyPump resulted in an energy expenditure of approximately 300 kcal (or 4.1–4.7 kcal/min), which was similar as the energy expenditure from a session of heavy load resistance exercise. An energy expenditure of approximately 300 kcal during an 1 h session are considered as low to moderate, and comparable to the energy expenditure from brisk walking. Similar levels of EPOC were observed after the two exercise sessions.

## DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Regional Committee for Medical Research Ethics Norway, Oslo. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

All authors have been included in the planning of the study and the writing procedure. AR had the main responsibility of the whole study and was the main writer. CG was responsible for the assessments and been involved in the writing. KB and LH were the supervisors and involved in the writing. GP was the main supervisor and involved in the analyses and the writing.

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# Oral Contraceptive Use Influences On-Kinetic Adaptations to Sprint Interval Training in Recreationally-Active Women

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**Introduction:** Oral contraceptive (OC) use influences peak exercise responses to training, however, the influence of OC on central and peripheral adaptations to exercise training are unknown. This study investigated the influence of OC use on changes in time-to-fatigue, pulmonary oxygen uptake, cardiac output, and heart rate on-kinetics, as well as tissue saturation index to 4 weeks of sprint interval training in recreationally active women.

**Methods:** Women taking an oral contraceptive (OC; $n$ = 25) or experiencing natural menstrual cycles (MC; $n$ = 22) completed an incremental exercise test to volitional exhaustion followed by a square-wave step-transition protocol to moderate (90% of power output at ventilatory threshold) and high intensity (Δ50% of power output at ventilatory threshold) exercise on two separate occasions. Time-to-fatigue, pulmonary oxygen uptake on-kinetics, cardiac output, and heart rate on-kinetics, and tissue saturation index responses were assessed prior to, and following 12 sessions of sprint interval training (10 min × 1 min efforts at 100–120% PPO in a 1:2 work:rest ratio) completed over 4 weeks.

**Results:** Time-to-fatigue increased in both groups following training ($p < 0.001$), with no difference between groups. All cardiovascular on-kinetic parameters improved to the same extent following training in both groups. Greater improvements in pulmonary oxygen up-take kinetics were seen at both intensities in the MC group ($p < 0.05$ from pre-training) but were blunted in the OC group ($p > 0.05$ from pre-training). In contrast, changes in tissue saturation index were greater in the OC group at both intensities ($p < 0.05$); with the MC group showing no changes at either intensity.

**Discussion:** Oral contraceptive use may reduce central adaptations to sprint interval training in women without influencing improvements in exercise

performance - potentially due to greater peripheral adaptation. This may be due to the influence of exogenous oestradiol and progestogen on cardiovascular function and skeletal muscle blood flow. Further investigation into female-specific influences on training adaptation and exercise performance is warranted.

**Keywords: female, training adaptation, ovarian hormones, oral contraceptive (OC), exogenous hormones, cardiorespiratory, time-to-fatigue**

## INTRODUCTION

Exogenous hormones introduced through oral contraceptive (OC) use can reduce maximal exercise capacity (Notelovitz, 1987; Casazza et al., 2002; Lebrun et al., 2003), increase fat-mass (Berenson and Rahman, 2009) and change the metabolic (Isacco et al., 2012), thermoregulatory (Stachenfeld et al., 2000), cardiovascular (Coney et al., 2001), and ventilatory (Charkoudian and Joyner, 2004) responses to exercise. Although OC use has been shown to impede peak exercise adaptations to training (Schaumberg et al., 2017a), the physiological mechanisms and whether performance adaptations are influenced by OC use is unclear (Casazza et al., 2002; Lebrun et al., 2003; Zierke, 2010).

One potential mechanism mediating training adaptation is skeletal muscle blood flow. During incremental exercise, skeletal muscle blood flow is determined by locally induced vasodilation and sympathetically mediated vasoconstriction, both of which are influenced by ovarian hormones (Charkoudian and Johnson, 1997; Charkoudian and Joyner, 2004; Vanheest et al., 2005). Indeed, chronic estrogen exposure has known vasodilatory responses (Stathokostas et al., 2009), and there is some evidence to suggest that estrogen and progestogen supplementation and the menstrual cycle may influence blood flow during exercise (Gurd et al., 2007), the directionality of which appears to depend on multiple factors, including the phase of the menstrual cycle (i.e., the ratio of estrogen and progesterone) and the type and concentration of exogenous ovarian hormone concentrations. Altered muscle blood flow due to endogenous and exogenous ovarian hormones may influence the ability of the muscle to meet oxidative demands during exercise. However, this has not been investigated, nor has OC use been considered as a potential mediator of skeletal muscle blood flow adaptations to exercise training in women.

A further potential mechanism is the integration of pulmonary and cardiovascular systems to deliver oxygenated blood to skeletal muscle during exercise. Pulmonary oxygen uptake on-kinetics ($\tau\dot{V}O_{2p}$) provide insight into how the cardiovascular system and mitochondria integrate to increase aerobic energy production in response to exercise (Murias et al., 2011). The speed of $\tau\dot{V}O_{2p}$ is a good indicator of endurance performance (Jones and Burnley, 2009); a faster $\tau\dot{V}O_{2p}$ indicates earlier achievement of physiological steady state (indicated by the time constant, $\tau on$, or the mean response time, MRT) resulting in reduced oxygen deficit (Xu and Rhodes, 1999). Additionally, a fast $\tau\dot{V}O_{2p}$ has also been associated with reduced lactate accumulation and muscle glycogen depletion compared to a slow $\tau\dot{V}O_{2p}$ (Berger et al., 2006). There is increasing interest in optimal training methods to elicit physiological adaptations within pulmonary, cardiovascular, and muscular systems to improve exercise performance.

Sprint interval training (SIT) can elicit adaptations traditionally associated with endurance training in a shorter period (Gibala et al., 2012; Shiraev and Barclay, 2012). These adaptations appear to be independent of sex and include improved oxidative enzyme activity (Carter et al., 2001; Astorino et al., 2011) coupled with increased capillarization (Shiraev and Barclay, 2012) and more efficient blood distribution (Murias et al., 2011), which can lead to improvements in $\dot{V}O_{2peak}$. These adaptations also improve the rate at which oxygen is extracted in the lungs ($\tau\dot{V}O_{2p}$) (Shoemaker et al., 1996; Tschakovsky and Hughson, 1999; Grassi, 2001; Hughson et al., 2001; Gibala et al., 2006). Despite these known physiological adaptations, the rate of oxygen extraction in muscle (represented by change in deoxyhaemoglobin: $\Delta$[HHb]) or tissue saturation index (TSI), is not usually influenced by short training interventions (i.e., <6 weeks) (Overend et al., 1992; Berger et al., 2006; McKay et al., 2009); whether SIT can elicit adaptations at the muscular level following a shorter period of training is inconclusive.

The direct relationship between pulmonary and muscle oxygen extraction is important in understanding central (i.e., adaptations to cardiorespiratory function rather than adaptations to the peripheral vasculature and trained muscle) and peripheral (i.e., adaptations within skeletal muscle such as capillarization and/or mitochondrial biogenesis) adaptations to SIT; both parameters provide a measure of endurance capacity in recreationally active individuals (Murias et al., 2011; Spencer et al., 2013). Research in men suggests that an improvement in $\tau\dot{V}O_{2p}$ with no concurrent change in TSI following a period of exercise training indicates that increased muscle oxygen utilization is accompanied by faster muscle oxygen extraction (MacPhee et al., 2005; McKay et al., 2009; Murias et al., 2011; Spencer et al., 2013). While research in women has found similar effects, hormone status or OC use has not been previously considered (Talanian et al., 2007; Astorino et al., 2011; Murias et al., 2011). Due to the potential influence of OC use on cardiovascular function and skeletal muscle blood flow due to chronic exogenous oestradiol and progestogen exposure, as well as our previous finding that OC use dampens $\dot{V}O_{2peak}$ adaptations to SIT in women (Schaumberg et al., 2017a), the investigation of whether OC use influences pulmonary ($\tau\dot{V}O_{2p}$) and muscular oxygen extraction (TSI), as well as associated cardiovascular adaptation to SIT is warranted.

Therefore, the aim of the present study was to investigate the influence of OC use on pulmonary, cardiovascular and muscular oxygen uptake kinetics adaptation at moderate and heavy exercise intensities following 4 weeks of SIT.

# MATERIALS AND METHODS

## Overview

Physically active women with either regular menstrual cycles (MC; no current hormonal contraception) or using an OC completed two exercise tests – an incremental exercise test and a square-wave step-transition protocol (separated by a minimum of 48 h), prior to, and following a 4-week SIT program.

## Participants

Healthy, recreationally active women (regularly completing at least 150 min of self-reported moderate to vigorous physical activity per week, but not currently training for, or competing at state or national level sport competition), who were either long-term (minimum 6 months uninterrupted) monophasic combined OC users ($n$ = 25) or experiencing regular natural menstrual cycles (MC; $n$ = 22) participated in the study. All experimental procedures were approved by an ethics committee of The University of Queensland and participants provided written informed consent.

## Control Measures

The procedures relating to hormone verification and analysis and body composition assessment are described in detail by Schaumberg et al. (2017b). To summarize, all OC users completed testing in the 'active pill' phase of the oral contraceptive cycle, and all naturally menstruating women completed testing in the mid-luteal phase of the menstrual cycle with serum hormone verification conducted at each timepoint. Nutrition, hydration, and exercise control measures have also been previously described (Schaumberg et al., 2017a). In brief, prior to each trial participants completed a 24-h food diary, fasted overnight, consumed a standardized moderate carbohydrate pretrial meal 1 h before arrival at the laboratory, abstained from caffeine, alcohol, and other stimulants and depressants for 24 h, recorded any additional medications or supplements, and maintain an euhydrated state. Participants were encouraged to maintain their normal physical activity levels throughout the study; however, were asked to refrain from strenuous physical activity for 24 h before each trial and arrive at the laboratory in a rested state. A pretrial preparation checklist was completed to confirm compliance to pretesting requirements.

## Experimental Protocol

In each of the experimental trials, participants completed 3, 4-min step transitions to a moderate exercise intensity [90% of power output at ventilatory threshold ($PO_{VT}$); calculated as 90% of the power output (PO) in Watts (W) achieved at ventilatory threshold (VT)] and three, 3-min transitions to a high exercise intensity [Δ50% $PO_{VT}$; calculated as PO at VT plus 50% of the difference between PO at VT and peak power output (PPO)], as determined from the incremental exercise test (Joyce et al., 2013; Stanley et al., 2014) (**Figure 1**). The first transition was preceded by 4 min of baseline cycling (20 W) and each subsequent transition was separated by 4 min of baseline cycling (20 W). Participants were instructed to maintain a consistent cadence (70 ± 10 RPM) throughout the baseline, moderate, and high intensity cycling. Heart rate (HR) (Suunto®, United States), expired air (Parvo Medics' TrueOne® 2400 Indirect Calorimetry System, Utah, United States), cardiovascular parameters (PhysioFlow, Manatec Biomedical, France), and muscle oxygenation via near infrared spectroscopy (NIRS; Portalite, Artinis Medical Systems BV, Netherlands) were continuously monitored throughout the trial, and RPE (Borg, 1973) was recorded at the end of every step-transition.

## Measures

### Respiratory Measures

A familiarization session was completed prior to the first experimental trial. The $\dot{V}O_{2peak}$ protocol involved continuous incremental (25 $W \cdot min^{-1}$) exercise test on an electronically braked cycle ergometer (Lode Excalibur Sport, Lode BV, Groningen, Netherlands) as previously described (Schaumberg et al., 2017a). Respiratory gas exchange was continuously recorded via automated indirect calorimetry (Parvo Medics' TrueOne® 2400 Indirect Calorimetry System, Utah, United States) for calculation of ventilatory parameters. Before each test, the analyzers were calibrated in accordance with the manufacturers' recommendations. From the incremental test, data were averaged in 15-s epochs; $\dot{V}O_{2peak}$ was defined as the highest $\dot{V}O_2$ value attained during a 15-s period (Rossiter et al., 2006; Stanley et al., 2014). During the experimental trials, average $\dot{V}O_2$ was determined from 5-s interval data.

### Cardiovascular Measures

During exercise, heart rate (HR), stroke volume (SV) and cardiac output ($\dot{Q}$) were measured continuously using impedance cardiology (PhysioFlow®, Manatec Biomedical, France) (Charloux et al., 2000; Richard et al., 2001). The theoretical basis for determining cardiac output from this method and its validity during rest and exercise has been described previously (Charloux et al., 2000; Lepretre et al., 2004), and has been validated against the direct Fick method (Richard et al., 2001). Two sets of electrodes (Skintact FS-50, Leonhard Lang Gmbh, Austria) – one transmitting, one sensing – were applied above the supra-clavicular fossa at the left base of the neck, and along the xiphoid process. Another two electrodes were used to monitor a single electrocardiographic signal (ECG; CM5 position). Blood pressure was assessed (Digital blood pressure monitor, UA-767, A&D Instruments Ltd., United Kingdom) as part of standard calibration process for the PhysioFlow® prior to the exercise test. During the experimental trials (**Figure 1**), HR, SV, and $\dot{Q}$ data were sampled at 5-s intervals, with the average for each interval determined during moderate and high loads. The coefficient of variation of cardiac output measures during peak exercise using this method has been reported as 3.4–3.6% (Hsu et al., 2006).

### Near-Infrared Spectroscopy Measurements

Near-infrared spectroscopy (NIRS) (Portalite, Artinis Medical Systems BV, Netherlands) estimated the oxygenation of the right vastus lateralis muscle during the performance trial. A three-wavelength continuous wave system was used, which

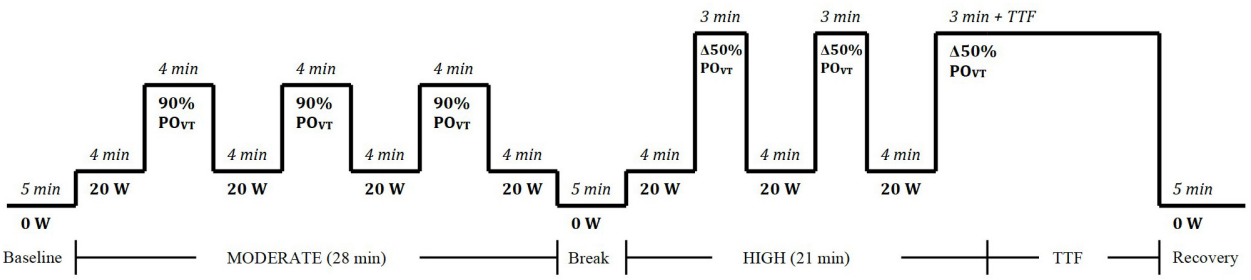


**FIGURE 1 |** Square-wave step transition exercise protocol to moderate and heavy exercise intensities. W, watts; $PO_{VT}$, power output at ventilatory threshold; min, minute; TTF, time to fatigue.

simultaneously used the modified Beer–Lambert and spatially resolved spectroscopy methods (Ihsan et al., 2013; Stanley et al., 2013). Changes in total hemoglobin ($t_{Hb}$) oxyhaemoglobin ($O_2Hb$) and deoxyhaemoglobin (HHb) were measured using the differences in absorption characteristics of light at 775, 810, and 850 mm (Stanley et al., 2013). An arbitrary value for the differential path length of 3.83 mm was used due to the uncertainty of proton path length at rest and during exercise (Stanley et al., 2013).

The NIRS device was connected via Bluetooth to a computer acquiring data at 10 Hz for later analysis. The probe was positioned one-third of the way along the vertical length of the thigh (from the quadriceps tendon on the patella). The NIRS device was wrapped in a zip-locked bag for water-proofing and covered in black material to prevent slipping and interference from ambient light and strapped to the leg securely with a bandage. Changes in $t_{Hb}$, $O_2Hb$, and HHb were reported as a change from baseline measures after first recording the tissue saturation index (TSI). The balance between oxygen consumption of the muscle tissue and oxygen supply (Equation 1) represented TSI (as a percentage).

$$TSI = [O_2Hb\ consumed]/[O_2Hb\ supplied] \times [HHb] \times 100 \quad (1)$$

Equation 1: TSI expressed as changes in $O_2Hb$ and HHb (Stanley et al., 2013). TSI, tissue saturation index; $O_2Hb$, oxygenated hemoglobin; HHb, deoxygenated hemoglobin.

## Sprint Interval Training Protocol

As previously described (Schaumberg et al., 2017a), the SIT protocol required participants to complete three supervised SIT sessions per week for 4 weeks, with a minimum of 36 h between sessions. Each session involved 1 min of work followed by 2 min of passive recovery in a 1:2 work:rest ratio (Ready et al., 1981; Gosselin et al., 2012). The work interval intensity was self-selected at the maximal sustainable effort between 100 and 120% of PPO determined in the $\dot{V}O_{2peak}$ test. Participants completed 10 1-min repetitions per session and peak heart rate, RPE, average power output and PPO were recorded. All exercise sessions were completed on an air- and magnetically braked cycle ergometer (Wattbike Ltd., Nottingham, United Kingdom).

## Data Analysis

### Assessment of Pulmonary Oxygen Uptake Kinetics

Recorded data for $\dot{V}O_2$ during each 4-min moderate (90% $PO_{VT}$) and 3-min high ($\Delta$50% $PO_{VT}$) (plus an additional 1 min prior to each interval to determine baseline $\dot{V}O_2$) loads, were interpolated into 5 s intervals; aberrant data points (caused by swallowing and coughing) were filtered out (Ozyener et al., 2001; Berger et al., 2006). To decrease the signal-to-noise ratio caused by high variability between breaths, the common practize of including multiple exercise transitions into the same protocol was employed (Whipp et al., 2005; Stanley et al., 2014). The first interval of each intensity was excluded from analysis due to the lack of a priming effect (Whipp et al., 2005; DeLorey et al., 2007; Stanley et al., 2013). Therefore, $\dot{V}O_2$ recorded during the second and third intervals for each intensity of each experimental trial was time synchronized and ensemble averaged to yield a single response for each participant for each trial. A repeated iterative technique (Sigmaplot 10, SPSS Science; Chicago, IL, United States) and a mono-exponential function (Equation 2) (Ozyener et al., 2001; Dorado et al., 2004; Whipp et al., 2005; Stanley et al., 2014) were used to model $\tau\dot{V}O_{2p}$ over moderate and high loads.

$$\dot{V}O_{2p}(t) = \dot{V}O_2 baseline + Ampl \times \left[1 - e^{-(\tau - TD/\tau_{on})}\right] \times U_1 \quad (2)$$

Equation 2: Mono-exponential function (Sigmaplot 10, SPSS Science; Chicago, IL, United States) where $u$ = if {$t$ < TD1, VO2b, VO2b + A1*[1−exp(−(t-TD1/*tau*1)]}. Initial parameters $\dot{V}O_{2b}$ = 0.5 TD1 (time delay) = −0.5; A1 (amplitude) = 1.5; Tau1 = 30. Constraints VO2 > 0; A1 > 0; Tau1 > 0; TD1 ≥ 0. $\dot{V}O_{2p}$, pulmonary oxygen kinetics; $\dot{V}O_2$, oxygen consumption; Ampl, amplitude; TD, time delay.

In Equation 2 $\dot{V}O_2$ baseline is the average $\dot{V}O_2$ during the 60 s prior to onset of the rest- (or active recovery) to-exercise transition, Ampl is the asymptotic amplitude for the exponential term and τon is the time constant of the exponential (seconds) (Stanley et al., 2014). Use of the mono-exponential function was most appropriate due to its simplicity when considering the rare occurrence of $\tau\dot{V}O_{2p}$ phase III (due to the priming effect from the first interval, the short duration of the interval and the submaximal intensity of exercise, therefore if Phase III were

to occur the amplitude would be minor) (Stanley et al., 2014). Phase I to Phase II transition occurred approximately 15 s after the onset of exercise for all participants (determined by visual examination), therefore this initial cardiodynamic component was excluded by deleting the first 20 s of data (Ozyener et al., 2001; Dorado et al., 2004; Stanley et al., 2014). Overall $\tau\dot{V}O_{2p}$, *tau*1 and mean response time (MRT = time delay + *tau*1) were calculated and very low residuals ($r^2 > 0.98$) obtained (Stanley et al., 2014), providing an overall description of on-transient oxygen uptake kinetics. While *tau*1 and MRT are closely related, MRT has been shown to be more reliable that *tau*1 in recreationally active women (Schaumberg et al., unpublished data), and therefore MRT was used as the primary measure of interest.

### NIRS Data and Assessment of De-Oxygenation Rates

Data from the NIRS device ($t_{Hb}$, $O_2Hb$, and [HHb]) were sampled down from 10 to 1 Hz and then averaged into 5 s intervals. Since tissue saturation index has been shown to provide a more accurate indication of muscle oxygenation status than Δ[HHb] (Wolf et al., 2007), TSI data was modeled. Using the Sigmaplot 10 program (SPSS Science; Chicago, IL, United States), a linear model was used to calculate the results from the average of the second and third intervals for both moderate and high intensity. Similar to $\tau\dot{V}O_{2p}$, the first interval was excluded from analysis due to the priming effect (Stanley et al., 2013). Moderate exercise prior to heavy workloads has been shown to influence Δ [HHb] (Whipp et al., 2005; Spencer et al., 2013), therefore, both workloads were analyzed using a linear model (Equation 3) (Bae et al., 2000):

$$\mathrm{TSI} = a \times t + b \quad (3)$$

Equation 3: Linear equation (Sigmaplot 10, SPSS Science; Chicago, IL, United States). TSI, tissue saturation index; *t*, time; *a*, slope; *b*, *y*-intercept.

TSI was modeled without time delay during the first 20 s of moderate intensity and 30 s of high intensity. However, the slope (*a*) was retained as an index of deoxygenation rate (Stanley et al., 2013). This particular model yielded very low residuals ($r^2 > 0.98$) and provided an overall descriptor of the muscular deoxygenation rate, and therefore muscle deoxygenation kinetic response to exercise.

### Assessment of Cardiovascular Kinetics

Heart rate and $\dot{Q}$ on-transient kinetics were modeled using the same iterative technique adopted for $\dot{V}O_2$ on-transient kinetics. HR and $\dot{Q}$ data were fitted with a mono-exponential function consistent with Equation 2 (using the same 4 or 5 min window), with the HR and $\dot{Q}$ data substituted for $\dot{V}O_2$ (Stanley et al., 2014). Unlike the $\dot{V}O_2$ on-transient kinetics analysis, the initial 20 s of data was not deleted due to lack of a cardiodynamic (Phase I to Phase II) transition. The mean response time (MRT = time delay + *tau*1) was calculated to provide an overall description of on-transient cardiovascular kinetics.

## Statistical Analysis

Data were analyzed using Microsoft Excel® 2007 and SPSS® (version 22.0, SPSS, Inc., Chicago, IL, United States). Normality of distribution was tested using the Kolmogorov–Smirnov test; when not normally distributed, data were log-transformed and re-checked for normality of distribution. Analyses included standard descriptive statistics, paired *t*-test, and two-way repeated measures analysis of variance (ANOVA) (with a main effect for training × group). To locate the source of significant differences, the Bonferroni *post hoc* test was used. Homogeneity of variance was confirmed using Mauchly's test of sphericity. When the assumption of sphericity was violated ($p < 0.05$), the *F*-statistic was adjusted using the Greenhouse–Geisser correction. Where Mauchly's test of sphericity was not found to be significant, *post hoc* analyses assumed sphericity (Vincent, 1999). Magnitude-based inferences (Hopkins et al., 2009; Batterham and Hopkins, 2015) calculated the between-trial standardized differences or effect sizes [ES, 95% confidence interval (CI)] using the pooled standard deviation (Cohen, 1988) and standard threshold values (Batterham and Hopkins, 2005). All tests were two-tailed and statistical significance was set at $p < 0.05$. Parametric results are given as the mean, standard deviation and 95% confidence interval (CI), [mean ± SD (95% CI)]; non-parametric results are given as the median and interquartile range and 95% CI, [median (IQR) (95% CI)] unless stated otherwise.

# RESULTS

## Participant Characteristics, Control Measures, and Training Protocol

Participant recruitment and retention has previously been described (Schaumberg et al., 2017a). Due to the nature of the outcome measures, we included all naturally menstruating participants within the main data set, including six of the 22 MC participants who had ovulatory, regular menstrual cycles but exhibited potential luteal phase deficiency (LPD) based on failing to meet the serum progesterone criterion of $>6$ ng.mL$^{-1}$ (Schaumberg et al., 2017b). **Supplementary Table S1** comparing the normal MC ($n = 16$) versus the potential LPD MC ($n = 8$) participants has been included; no significant differences between outcome measures pre- or post-training were found, though the MC LPD group showed dampened pulmonary oxygen uptake kinetic responses to training, discussed below. All 25 participants recruited to the OC group were taking a low-dose, monophasic combined oestradiol and progestin formulation. There were variations in androgenic ($n = 5$), antiandrogenic ($n = 5$) and non-androgenic ($n = 15$) formulations [calculated using the method of Greer et al. (2005)]; subsequent analyses confirmed androgenicity of OC type did not influence baseline characteristics or outcome measures. Physical activity, energy intake and body composition parameters were not different within or between groups at any timepoint, however, due to the inclusion of the potential LPD participants ($n = 8$) there were some differences in hormone concentrations between groups that have been previously described (Schaumberg et al., 2017b), with the MC group having higher oestradiol, progesterone and free androgen index ($p < 0.001$), and lower sex-hormone binding

**TABLE 1 |** Participant characteristics.

| | Oral contraceptive group ($n$ = 25) | | Menstrual cycle group ($n$ = 22) | |
|---|---|---|---|---|
| | **Pre-training** | **Post-training** | **Pre-training** | **Post-training** |
| **Participant demographics and control measures** | | | | |
| Age (years) | 25.5 ± 5.4 (23.1–27.8) | X | 26.4 ± 5.2 (24.0–28.8) | X |
| MC length (days) | 28.0 ± 0.0 (28.0–28.0) | X | 30.5[28.8–33.3]* (29.9–32.6) | X |
| Physical activity (min.wk$^{-1}$) | 247 ± 64 (222–272) | 235 ± 61 (211–258) | 235 ± 58 (211–260) | 222 ± 51 (201–243) |
| Energy intake (kJ.kg$^{-1}$.d$^{-1}$) | 8461 ± 3194 (6896–10026) | 8490 ± 2452 (7103–9877) | 8373 ± 2360 (7179–9567) | 8499 ± 1971 (7269–9712) |
| **Body composition** | | | | |
| Body mass (kg) | 63.6 ± 7.8 (60.3–66.8) | 63.4 ± 7.2 (60.4–66.3) | 64.6 ± 9.2 (60.5–68.8) | 64.2 ± 8.8 (60.2–68.2) |
| Body mass index (kg.m$^{-2}$) | 22.6 ± 2.1 (21.7–23.4) | 22.6 ± 2.1 (21.7–23.4) | 22.7 ± 2.3 (21.7–23.7) | 22.7 ± 2.3 (21.7–23.7) |
| Fat mass (kg) | 20.7 ± 4.8 (18.7–22.7) | 20.5 ± 4.6 (18.6–22.4) | 21.0 ± 5.2 (18.8–23.3) | 20.8 ± 5.6 (18.2–23.3) |
| Lean body mass (kg) | 40.6 ± 4.4 (38.8–42.5) | 40.6 ± 4.1 (38.9–42.3) | 41.3 ± 5.9 (38.7–43.9) | 41.2 ± 5.2 (38.8–43.5) |
| Lean body mass – legs (kg) | 13.6 ± 1.8 (12.9–14.4) | 13.8 ± 1.9 (13.0–14.5) | 13.8 ± 2.5 (12.7–14.9) | 13.8 ± 2.3 (12.7–14.8) |
| Body fat (%) | 32.3 ± 4.8 (30.3–34.3) | 32.1 ± 4.8 (30.1–34.0) | 32.3 ± 5.3 (30.0–34.7) | 32.0 ± 5.9 (29.3–34.7) |
| **Hormone measures** | | | | |
| Estradiol (pg.mL$^{-1}$) | 5.6 [5.0–10.3] (5.7–13.5) | 5.1[5.0–10.2] (11.0–63.8) | 124.4 ± 67.5$^{\Lambda}$ (92.8–156.1) | 93.2 ± 67.5$^{\#\Lambda}$ (62.4–123.9) |
| Progesterone (ng.mL$^{-1}$) | 0.6 ± 0.3 (0.4–0.7) | 0.5 ± 0.3 (0.4–0.6) | 10.0 ± 7.9$^{\Lambda}$ (6.3–13.7) | 1.0[0.7–1.4]$^{\#}$ (0.2–7.0) |
| Total testosterone (ng.mL$^{-1}$) | 0.20 ± 0.10 (0.10–0.20) | 0.13 ± 0.07 (0.10–0.16) | 0.2[0.1–0.4] (0.2–0.3) | 0.3 ± 0.2$^{\Lambda}$ (0.2–0.4) |
| SHBG (pg.mL$^{-1}$) | 209.0 ± 87.1 (170.3–247.6) | 189.6 ± 99.3 (145.6–233.7) | 62.6[40.8–90.0]$^{\Lambda}$ (50.7–86.3) | 58.5[35.6–81.9]$^{\Lambda}$ (47.5–76.9) |
| Free androgen index (%) | 7.8[4.2–8.5] (6.9–14.7) | 8.6 ± 5.6 (6.1–11.0) | 33.2[14.7–77.7]$^{\Lambda}$ (22.3–99.4) | 55.3[19.6–96.5]$^{\Lambda}$ (35.3–106.0) |

*Parametric data are presented as mean ± SD (95%CI); non-parametric data are presented as median [IQR] (95% CI).* $^{\#}p < 0.05$ *vs. pre-training;* $^{*}p < 0.001$ *vs. pre-training;* $^{\Lambda}p < 0.05$ *vs. OC group.*

globulin (SHBG; $p < 0.001$). As such, participant characteristics are re-presented in **Table 1**.

## Peak Exercise Responses and Time to Fatigue

Peak exercise adaptations have previously been reported (Schaumberg et al., 2017a), with the OC group showing dampened peak exercise adaptations to SIT compared to the MC group. There was no significant difference in time-to-fatigue (TTF) between groups at any timepoint ($p > 0.05$) (**Figure 2**). Following training, TTF was increased from pre-training in both groups ($p < 0.001$). Standardized between-group differences for within-group changes (Cohen's $d$) demonstrated that there was a likely higher TTF adaptation to training in the OC-group compared to the MC-group (0.96 ± 1.04).

## Pulmonary Oxygen Uptake Kinetics

There was no significant difference between groups for $\tau\dot{V}O_{2p}$ [expressed as the mean response time (MRT) in seconds] at any time point at moderate or high intensity ($p > 0.05$). Following training, $\tau\dot{V}O_{2p}$ was improved in the MC-group at both moderate ($p = 0.021$) and high ($p = 0.015$) intensities but the OC-group showed no change from baseline at both intensities ($p > 0.05$). When the MC-group was sub-grouped for potential LPD, the LPD group showed dampened $\tau\dot{V}O_{2p}$ at both intensities ($p < 0.05$) as measured by MRT; but there was no significant group × time interaction between normal MC and LPD MC (see **Supplementary Table S1**). There was a significant group x time interaction for $\tau\dot{V}O_{2p}$ at moderate intensity ($p = 0.020$); the interaction was approaching significance at high intensity ($p = 0.086$). *Post hoc* analyses suggested that the MC-group showed greater improvement in $\tau\dot{V}O_{2p}$ at high intensity following training compared to the OC-group (OC-group −1.4 s vs. MC-group −6.1 s; $p = 0.021$); but this was not significant between groups at moderate intensity (OC-group +0.5 s vs. MC-group −4.4 s; $p = 0.097$). Data are presented in **Table 2** and the $\tau\dot{V}O_{2p}$ profile at both intensities from a representative participant is presented in **Figure 3**. Standardized between-group differences for within-group changes (Cohen's $d$) demonstrated that the OC-group had a likely lower $\tau\dot{V}O_{2p}$ adaptation to training compared to the MC-group at moderate (−0.54 ± 0.67) and high (−0.57 ± 0.49) intensities.

## Cardiac Output Kinetic Response

There were no differences between groups for cardiac output kinetic response represented as mean response time ($\dot{Q}_{MRT}$) pre-

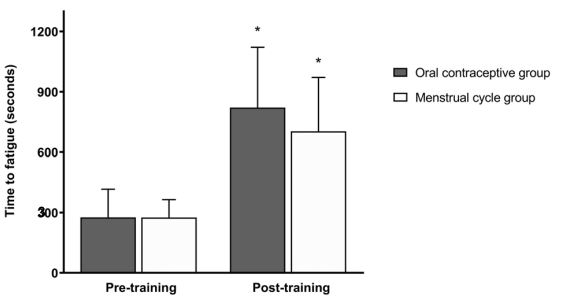


**FIGURE 2 |** Time to fatigue (seconds) at a heavy intensity ($\Delta 50\%PO_{VT}$) for the oral contraceptive group ($n$ = 25) and menstrual cycle group ($n$ = 22) pre- and post-training. Data are mean ± SD. $^{*}p < 0.001$ vs. pre-training; paired samples $t$-test.

**TABLE 2 |** Pulmonary oxygen uptake, cardiac output, and heart rate on-kinetic responses to moderate and heavy intensity exercise, pre- and post-training in oral contraceptive users ($n$ = 25) and naturally menstruating women ($n$ = 22).

| | Oral contraceptive group ($n$ = 25) | | Menstrual cycle group ($n$ = 22) | |
|---|---|---|---|---|
| | **Pre-training** | **Post-training** | **Pre-training** | **Post-training** |
| **Moderate intensity exercise (90% $PO_{VT}$)** | | | | |
| **Pulmonary oxygen uptake on-kinetic response** | | | | |
| Baseline $\dot{V}O_2$ (L.min$^{-1}$) | 0.8 ± 0.1 (0.7–0.8) | 0.7 ± 0.1 (0.6–0.7)$^{\#}$ | 0.7 ± 0.1 (0.7–0.8) | 0.7 ± 0.1 (0.6–0.7) |
| Amplitude (L.min$^{-1}$) | 0.8 ± 0.2 (0.7–0.9) | 0.8 ± 0.2 (0.7–0.9) | 1.0 ± 0.5 (0.7–1.2) | 0.7 ± 0.4 (0.6–0.9)$^{\#}$ |
| Time delay (sec) | 23.2 ± 7.3 (20.1–26.3) | 27.1 ± 8.9 (23.4–30.9) | 22.0 ± 8.0 (18.3–25.6) | 26.0 ± 9.3 (21.8–30.2) |
| Time constant (*tau*1; sec) | 32.2 ± 10.5 (27.7–36.6) | 26.9 ± 9.1 (23.0–30.7) | 36.0 ± 12.0 (30.5–41.4) | 25.9 ± 9.6 (21.5–30.3)$^{\#}$ |
| Mean response time (sec) | 55.4 ± 6.7 (52.5–58.2) | 54.0 ± 7.8 (50.7–57.3) | 58.0 ± 10.1 (53.4–62.6) | 51.9 ± 5.5 (49.3–54.4)$^{\#}$ |
| **Cardiac output on-kinetic response** | | | | |
| Baseline $\dot{Q}$ (L.min$^{-1}$) | 11.0 ± 1.3 (10.5–11.6) | 12.3 ± 2.2 (11.3–13.3)* | 11.0 ± 1.6 (10.2–11.7) | 10.6 ± 1.0 (10.2–11.1) |
| Amplitude (L.min$^{-1}$) | 6.6 ± 3.1 (5.2–8.0) | 5.2 ± 1.2 (4.7–5.8)$^{\#}$ | 4.0 ± 1.3 (3.4–4.6) | 4.6 ± 1.5 (3.9–5.3)$^{\#}$ |
| Time delay (sec) | 7.2 ± 4.0 (5.4–9.0) | 4.0 ± 1.9 (3.1–4.8) | 6.0 ± 2.5 (4.8–7.2) | 8.8 ± 6.6 (5.7–11.9)$^{\#}$ |
| Time constant (*tau*1; sec) | 45.9 ± 10.1 (41.5–50.4) | 38.4 ± 10.2 (33.9–42.9)* | 50.0 ± 10.7 (45.0–55.0) | 34.4 ± 10.5 (29.5–39.3)* |
| Mean response time (sec) | 53.1 ± 10.3 (48.6–57.7) | 38.1 ± 10.2 (33.9–42.9)* | 56.0 ± 10.0 (51.3–60.7) | 43.2 ± 5.8 (40.5–45.9)* |
| **Heart rate on-kinetic response** | | | | |
| Baseline heart rate (bpm) | 105.5 ± 10.4 (100.9–110.1) | 106.8 ± 12.1 (101.4–112.2) | 113.8 ± 8.5 (109.8–117.8) | 107.3 ± 10.0 (102.6–112.0)* |
| Amplitude (bpm) | 44.6 ± 8.1 (41.0–48.3) | 39.9 ± 9.4 (35.8–44.1)* | 32.0 ± 6.8 (28.8–35.2) | 32.4 ± 9.6 (27.9–36.9) |
| Time delay (sec) | 4.1 ± 4.3 (2.2–6.0) | 2.8 ± 1.0 (2.3–3.2) | 4.2 ± 3.4 (2.7–5.8) | 6.9 ± 6.3 (4.0–9.9) |
| Time constant (*tau*1; sec) | 55.4 ± 9.1 (51.4–59.5) | 43.2 ± 9.9 (38.8–47.6)* | 53.1 ± 15.7 (45.8–60.5) | 38.2 ± 10.4 (33.4–43.1)* |
| Mean response time (sec) | 59.5 ± 10.4 (54.9–64.1) | 46.0 ± 10.2 (41.5–50.5)* | 57.3 ± 14.0 (50.8–63.9) | 45.1 ± 6.1 (42.3–48.0)* |
| **Heavy intensity exercise (Δ50% $PO_{VT}$)** | | | | |
| **Pulmonary oxygen uptake on-kinetic response** | | | | |
| Baseline $\dot{V}O_2$ (L.min$^{-1}$) | 0.9 ± 0.2 (0.8–0.9) | 0.8 ± 0.1 (0.7–0.8) | 0.8 ± 0.2 (0.8–0.9) | 0.8 ± 0.1 (0.8–0.9) |
| Amplitude (L.min$^{-1}$) | 1.3 ± 0.3 (1.2–1.4) | 1.3 ± 0.3 (1.2–1.4) | 1.3 ± 0.4 (1.1–1.5) | 1.3 ± 0.4 (1.1–1.4) |
| Time delay (sec) | 18.2 ± 7.2 (15.2–21.3) | 22.7 ± 5.9 (20.2–25.3)$^{\#}$ | 20.6 ± 5.9 (17.9–23.3) | 23.1 ± 5.4 (20.7–25.6) |
| Time constant (*tau*1; sec) | 32.5 ± 10.0 (28.3–36.7) | 28.5 ± 10.0 (24.3–32.7) | 33.5 ± 8.5 (29.7–37.4) | 26.6 ± 9.0 (22.5–30.7)$^{\#}$ |
| Mean response time (sec) | 50.7 ± 7.6 (47.5–53.9) | 51.2 ± 8.0 (47.9–54.6) | 54.1 ± 8.9 (50.1–58.2) | 49.7 ± 6.9 (46.6–52.9)$^{\#}$ |
| **Cardiac output on-kinetic response** | | | | |
| Baseline $\dot{Q}$ (L.min$^{-1}$) | 13.0 ± 1.7 (12.3–13.8) | 14.2 ± 1.7 (13.5–15.0)$^{\#}$ | 12.7 ± 1.0 (12.3–13.1) | 12.2 ± 1.0 (11.8–12.7)$^{\#}$ |
| Amplitude (L.min$^{-1}$) | 6.7 ± 2.0 (5.8–7.6) | 6.7 ± 2.1 (5.7–7.6) | 5.4 ± 1.6 (4.7–6.2) | 6.1 ± 1.5 (5.4–6.8)$^{\#}$ |
| Time delay (sec) | 5.8 ± 4.4 (3.8–7.7) | 9.7 ± 3.8 (8.0–11.4)$^{\#}$ | 8.7 ± 3.7 (7.0–10.4) | 9.9 ± 5.0 (7.6–12.2) |
| Time constant (*tau*1; sec) | 45.4 ± 9.4 (41.3–49.6) | 31.8 ± 6.6 (28.9–34.8)* | 44.3 ± 13.8 (37.9–50.8) | 30.0 ± 10.1 (25.2–34.7)* |
| Mean response time (sec) | 51.2 ± 9.9 (46.8–56.0) | 41.5 ± 6.8 (38.5–44.5)* | 53.0 ± 13.8 (46.6–59.5) | 39.9 ± 6.3 (36.9–42.8)* |
| **Heart rate** | | | | |
| Baseline heart rate (bpm) | 122.8 ± 7.8 (119.4–126.3) | 120.3 ± 10.1 (115.8–124.8) | 125.9 ± 10.5 (121.0–130.8) | 120.7 ± 11.0 (115.6–125.8)$^{\#}$ |
| Amplitude (bpm) | 56.2 ± 8.2 (52.6–59.9) | 52.9 ± 8.0 (49.4–56.5)* | 49.0 ± 10.9 (44.0–54.1) | 48.1 ± 10.5 (43.2–53.0) |
| Time delay (sec) | 7.4 ± 5.3 (5.0–9.7) | 5.1 ± 3.8 (3.4–6.8)$^{\#}$ | 10.3 ± 3.1 (8.8–11.7) | 6.8 ± 1.9 (5.9–7.7)* |
| Time constant (*tau*1; sec) | 45.2 ± 6.1 (42.5–47.9) | 43.6 ± 10.9 (38.8–48.4) | 43.4 ± 9.8 (38.8–48.0) | 41.3 ± 4.9 (39.0–43.6) |
| Mean response time (sec) | 52.6 ± 10.1 (48.1–57.0) | 48.7 ± 12.2 (43.3–54.1)* | 53.7 ± 9.9 (49.1–58.3) | 48.1 ± 5.0 (45.8–50.4)* |

*$PO_{VT}$, power output at ventilatory threshold; sec, seconds; $\dot{V}O_2$, volume of oxygen consumed. $\dot{Q}$, cardiac output; sec, seconds. Parametric data are presented as mean ± SD (95%CI); non-parametric data are presented as median [IQR] (95% CI). $^{\#}p < 0.05$ vs. pre-training; $^{*}p < 0.001$ vs. pre-training.*

or post-training at either intensity ($p > 0.05$). Following training, $\dot{Q}_{MRT}$ improved in both groups at both intensities ($p < 0.001$). There was a significant group x time interaction for $\dot{Q}_{MRT}$ at moderate ($p = 0.003$) and high ($p = 0.039$) intensity. *Post hoc* analyses suggested there was no difference between groups for pre- to post-training improvements in $\dot{Q}_{MRT}$ at moderate [OC-group −15.0 s vs. MC-group −12.8 s; $p = 0.405$], and high intensities [OC-group −9.7 s vs. MC-group −13.1 s; $p = 0.282$]. Data are presented in **Table 2** and the cardiac output profile at both intensities from a representative participant is presented in **Supplementary Figure S1**. Standardized between-group differences for within-group changes (Cohen's $d$) demonstrated that there was no clear between-group difference in $\dot{Q}_{MRT}$ adaptation to training at both moderate (0.19 ± 0.48) and heavy (0.29 ± 0.53) exercise intensities (**Table 3**).

## Heart Rate Kinetic Response

There was no difference between groups for $HR_{MRT}$ at any time point at either intensity ($p > 0.05$). Following training, $HR_{MRT}$ improved in both groups and at each

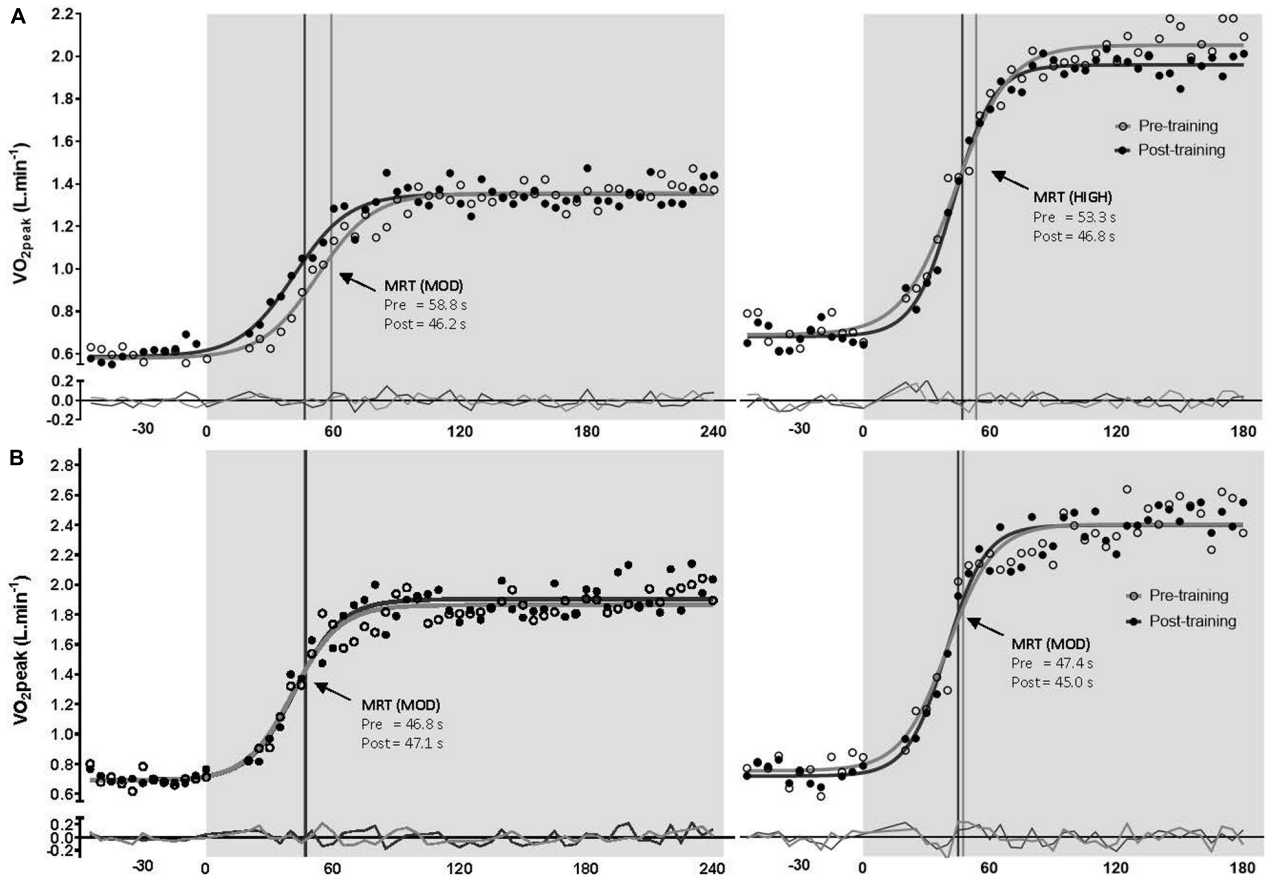


**FIGURE 3 |** Pulmonary oxygen uptake profile (with model best fit line and residuals) for a representative naturally menstruating participant **(A)** and oral contraceptive using participant **(B)** during the transition to moderate (left) and heavy (right) intensity exercise, pre-training (open circles and gray line), post-training (closed circles and black line). Mean response time (MRT) is indicated by a matched vertical line. Shading represents exercise onset.

**TABLE 3 |** Standardized between-group differences for within-group changes for the oral contraceptive group versus the menstrual cycle group following training and de-training.

| | Cohen's *d* ± SD | 95% CI | % chance for OC change to be higher/trivial/lower than MC | Descriptive of difference |
|---|---|---|---|---|
| Time to fatigue (sec) | 0.96 ± 1.04 | −0.08–2.01 | 93/6/1 | Likely higher |
| **Moderate intensity (90% $PO_{VT}$)** | | | | |
| Mean response time (sec) | 0.54 ± 0.67 | −0.13–1.21 | 85/14/2 | Likely higher |
| Tissue saturation index (slope) | −1.03 ± 0.71 | −1.74–−0.33 | 0/1/99 | V. likely lower |
| Mean response time ($\dot{Q}$) | 0.19 ± 0.48 | −0.29–0.68 | 49/46/5 | Unclear |
| Mean response time (HR) | −0.10 ± 0.46 | −0.56–0.36 | 10/57/33 | Unclear |
| **Heavy intensity (Δ50% $PO_{VT}$)** | | | | |
| Mean response time (sec) | 0.57 ± 0.49 | 0.08–1.07 | 93/6/0 | V. likely higher |
| Tissue saturation index (slope) | −0.66 ± 0.41 | −1.07–−0.25 | 0/1/99 | V. likely lower |
| Mean response time ($\dot{Q}$) | 0.29 ± 0.53 | −0.25–0.82 | 63/34/4 | Unclear |
| Mean response time (HR) | 0.17 ± 0.50 | −0.32–0.67 | 45/48/7 | Unclear |

*SD, standard deviation; CI, confidence interval; OC, oral contraceptive group; MC, naturally menstruating group; sec, seconds; Q, cardiac output; HR, heart rate; $PO_{VT}$, power output at ventilatory threshold; V. likely, very likely.*

intensity ($p < 0.05$). There was no significant group × time interaction for $HR_{MRT}$ at either moderate ($p = 0.269$) or high intensity ($p = 0.712$). Data are presented in **Table 2** and the heart rate profile at both intensities from a representative participant is presented in **Supplementary Figure S2**. Standardized between-group differences for within-group changes (Cohen's *d*) demonstrated no clear differences between groups (**Table 3**).

## Muscle Deoxygenation Rates

There was no difference between groups for TSI (presented here as an index/percentage) at baseline for either intensity (moderate: MC −0.18 ± 0.08; OC −0.16 ± 0.05; high: MC −0.24 ± 0.07; OC −0.26 ± 0.12; all $p > 0.05$). Post-training, the OC-group had improved TSI compared to the MC-group at both moderate (OC −0.24 ± 0.08; MC −0.19 ± 0.07; $p = 0.027$) and high (OC −0.34 ± 0.14; MC −0.25 ± 0.07; $p = 0.018$) intensities. Following training, TSI was improved in the OC-group at both intensities [moderate: Δ −0.08 (50%); high: Δ −0.08 (30.8%); both $p < 0.001$]; but did not change in the MC-group at either intensity [moderate: Δ −0.01 (6.0%), $p = 0.295$; high: Δ −0.01 (4.1%) $p = 0.422$]. There was a significant group × time interaction for TSI at moderate ($p = 0.001$), and high ($p < 0.001$) intensities. *Post hoc* analyses suggested the OC-group showed greater improvement in TSI at both moderate ($p = 0.004$) and high ($p = 0.002$) intensities following training compared to the MC-group. Data are presented in **Figure 4**. Standardized between-group differences for within-group changes (Cohen's *d*) demonstrated that the OC-group had a likely greater TSI adaptation to training compared to the MC-group at moderate (−1.03 ± 0.71) and heavy (−0.66 ± 0.41) intensities (**Table 3**).

# DISCUSSION

The present study investigated the influence of OC use on time to fatigue and changes in pulmonary, cardiovascular and muscle oxygen uptake kinetics to sprint interval training (SIT). Pulmonary oxygen uptake kinetics ($\tau\dot{V}O_{2p}$) as determined by MRT, improved following training in the MC-group only. However, tissue saturation index (TSI) improved in the OC-group only. Despite these differences, improvements in time to fatigue in response to SIT did not differ between groups.

Significant improvements (30%) in the mean response time for $\tau\dot{V}O_{2p}$ at both moderate and high intensity exercise were observed with training in the MC-group only; the OC-group showed no significant change from baseline. When the MC-group was sub-grouped for potential LPD, the MC LPD sub-group also demonstrated dampened $\tau\dot{V}O_{2p}$. The improvement in $\tau\dot{V}O_{2p}$ observed in the MC-group is consistent with previous research, where Murias et al. (2011) demonstrated that $\tau\dot{V}O_{2p}$ was improved in response to endurance training in young women. However, these authors did not include women using an OC and our results suggest that exogenous hormones may dampen the $\tau\dot{V}O_{2p}$ adaptations to training. It is also possible that the absence of endogenous ovarian hormones (within the probable LPD group) may also dampen $\tau\dot{V}O_{2p}$ adaptations. This is consistent with findings from our previous study (Schaumberg et al., 2017a), where $\dot{V}O_{2peak}$ improvement was lower in OC users compared to naturally menstruating women following training.

Improvement in $\tau\dot{V}O_{2p}$ can increase exercise capacity at both moderate and higher intensities of exercise (Poole and Richardson, 1997). Oxygen delivery to skeletal muscle is challenged during high intensity exercise training, thereby eliciting structural adaptations. This leads to an increased blood flow and an increase in pulmonary and/or muscle oxygen kinetic responses at the onset of constant-load exercise (Poole and Richardson, 1997; Jones and Poole, 2005). Therefore, improvements in $\tau\dot{V}O_{2p}$ may be limited by either central (e.g., cardiac output) or peripheral (e.g., skeletal muscle blood flow) adaptations (Barstow et al., 1990; Grassi, 2006). OC use has been shown to decrease collagen synthesis, and therefore capillarization, in response to exercise (Hansen et al., 2011). Therefore, reduced capillarization adaptation with exercise may dampen further central adaptations such as $\tau\dot{V}O_{2p}$. In addition, OC use increases the release of hormones (e.g., growth hormone and other glucoregulatory hormones) (62),

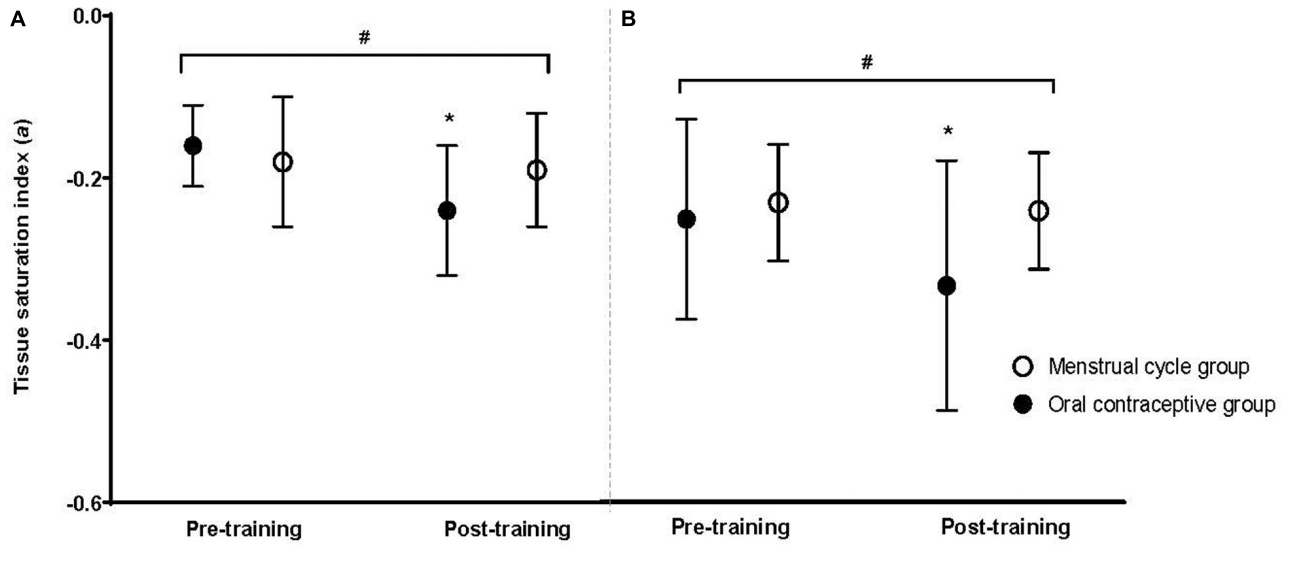


**FIGURE 4 |** Tissue saturation index at a moderate exercise intensity **(A)** and a high exercise intensity **(B)** for the oral contraceptive group ($n = 25$) and menstrual cycle group ($n = 22$) pre- and post-training. Data are mean ± SD. *$p < 0.001$ vs. pre- training; #$p < 0.05$ group × time interaction.

which can influence carbohydrate metabolism during exercise (Davidson and Holzman, 1973; Bunt, 1990; Bonen et al., 1991). As $\tau\dot{V}O_{2p}$ is controlled intracellularly via oxidative phosphorylation, glucose is required to fuel the turnover of ATP for energy production (McKay et al., 2009). If there is limited blood glucose available, this could limit the improvement of $\tau\dot{V}O_{2p}$. Luteal phase deficiency is a menstrual disturbance that may reflect early stages of low energy availability in women (De Souza et al., 2003). Therefore, the dampened $\tau\dot{V}O_{2p}$ adaptation seen within the probable LPD group further support this potential mechanism.

Cardiac output on-kinetics improved in both groups at both moderate and high intensity loads following training. It is possible that this measure was not sensitive enough to detect any between-group differences. However, it is important to note that the adaptation of the women in this study was comparable to men, where it has previously been demonstrated that the heart rate time constant to moderate intensity exercise improves with just eight sessions of HIIT, and that end heart rate is also improved (McKay et al., 2009). Recent work by Howden et al. (2015) demonstrated that women had a markedly blunted cardiovascular response to 1 year of endurance training, compared to males. Therefore, further investigation into cardiovascular on-kinetic responses is warranted to explain the lack of adaptation in the present study.

The present study investigated TSI as an overall index of the muscle deoxygenation kinetics, representative of the dynamic balance between oxygen consumption of the muscle tissue and supply. While most literature reports total oxy- or deoxy-hemoglobin, TSI arguably provides a more effective measure of the overall muscular response (because of its incorporation of both oxy- and deoxy-hemoglobin and consumption and supply of oxygen), and is closely related to [HHB] (Ihsan et al., 2013).

The first, and most obvious, conclusion for the significant TSI improvement in the OC-group and no change in the MC-group is that the OC-group showed significant peripheral adaptation with training compared to the MC-group, yet, when analyzed in conjunction with the $\tau\dot{V}O_{2p}$ adaptations, this assumption must be considered with caution, as TSI is a rate relative to both supply and utilization of oxygen at the muscle. TSI and $\tau\dot{V}O_{2p}$ are often analyzed and reported together (Gurd et al., 2007; McKay et al., 2009; Murias et al., 2011), as TSI is indicative of peripheral adaptation whilst $\tau\dot{V}O_{2p}$ is indicative of central adaptations (Murias et al., 2011). An increase in TSI (also demonstrated with the increase relative to exercise intensity) may indicate a mismatch between oxygen delivery and utilization during exercise (Spencer et al., 2013). As TSI adjusts more rapidly in response to exercise compared to $\tau\dot{V}O_{2p}$ there is insufficient oxygen delivery for the working muscles and temporal dissociation occurs, which may result in greater reliance on anaerobic energy production at the working muscles (MacPhee et al., 2005; Spencer et al., 2013), and be potentially detrimental to exercise capacity.

The concomitant changes in TSI and $\tau\dot{V}O_{2p}$ in the MC- and OC-groups can be further considered through the concept of a physiological phenomenon known as the 'transient overshoot' (Murias et al., 2011), whereby training instigates more efficient oxygen utilization as well as blood distribution, thereby causing a speeding of $\tau\dot{V}O_{2p}$ with no change in TSI. An increase in muscle oxygen extraction causes insufficient oxygen delivery, instigating a temporal dissociation period between [HHB] and $\tau\dot{V}O_{2p}$ (MacPhee et al., 2005; Spencer et al., 2013). This overshoot may be present with OC use, with greater utilization of oxygen at the muscle occurring after training due to peripheral adaptations, with concurrent blunting of oxygen supply indicated by $\tau\dot{V}O_{2p}$.

Dynamic changes in NIRS-derived muscle deoxygenation (represented by TSI) provide insights into the balance between local muscle oxygen availability and utilization during exercise (Wolf et al., 2007; Spencer et al., 2013). During exercise muscle deoxygenation adjusts to an increased workload more rapidly than $\tau\dot{V}O_{2p}$, resulting in a transient period characterized by an increased relative reliance on oxygen extraction to support a given metabolic rate. This temporary dissociation between adjustments of [HHB] and $\tau\dot{V}O_{2p}$ suggest transient oxygen delivery insufficiency (Murias et al., 2013; Spencer et al., 2013) for the rate of oxygen consumption.

Therefore, we observed an abnormal response of $\tau\dot{V}O_{2p}$ and TSI to exercise training in OC users, demonstrated by an apparent acceleration of TSI, which may suggest a mismatch of oxygen delivery and utilization at the exercising muscles. In our participant sample, oxygen delivery did not improve with training in OC users compared to naturally menstruating women, despite the apparent increase in local muscular oxygen utilization. Therefore, the next consideration based on these results is that exogenous hormones do not appear to influence peripheral adaptation to training and may indeed be beneficial. Indeed, the mechanism for the change in TSI in OC users warrants further investigation. Previous research has speculated the mismatch between oxygen utilization and delivery with OC use is due to its direct effect on the female sex hormones (estrogen and progesterone, and their exogenous forms). However, several other hormones (e.g., growth hormone, inflammatory factors, and free androgens) are also influenced by OC use and should therefore be considered. For example, growth factors, including growth hormone and free androgens are significantly different from naturally menstruating women; therefore it is necessary to further investigate this possible factor, due to their influence on capillarization and subsequent oxygen utilization at the muscle (Hansen et al., 2011; Hansen and Kjaer, 2014). Further, the influence of OC use on mitochondrial/oxidative enzymes is unknown, and further investigation into the potential influence of OC use on mitochondrial oxidative capacity is warranted.

## CONCLUSION

Exogenous ovarian hormones found in the oral contraceptive pill may be, at least in part, responsible for the dampened physiological adaptations to training in OC users. Although both OC users and naturally menstruating women improved TTF, we observed a dampened response of central physiological

adaptation, demonstrated by pulmonary oxygen uptake kinetics in the OC group. This may have been offset by the greater improvements in muscle oxygen utilization in OC users, compared to the MC group. These results provide insight into potential mechanisms related to training adaptation in women. Based on these results, potential mechanisms may include the lack of endogenous ovarian hormones, as well as the influence of exogenous hormones on the overall endocrinological profile, including growth hormone and free androgens, may be implicated in dampening the physiological adaptations to training with OC use. These potential mechanisms warrant investigation to further elucidate the influence of OC use on adaptations to training in women.

## DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

## ETHICS STATEMENT

This study involving human participants was reviewed and approved by The University of Queensland Human Research Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

MS, JS, DJ, XJ, LE, and TS designed the protocol and methods. MS and EH collected the data. MS, JS, and EH analyzed the data. All authors contributed to the writing and drafting of the manuscript and read and approved the manuscript.

## ACKNOWLEDGMENTS

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2020.00629/full#supplementary-material

**FIGURE S1 |** Cardiac output profile (with model best fit line and residuals; time in seconds on the *x*-axis) for a representative naturally menstruating participant **(A)** and oral contraceptive using participant **(B)** during the transition to moderate (left) and heavy (right) intensity exercise, pre-training (open circles and gray line) and post-training (closed circles and black line). Mean response time (MRT) is indicated by a matched vertical line. Shading represents exercise onset.

**FIGURE S2 |** Heart rate profile (with model best fit line and residuals) for a representative naturally menstruating participant **(A)** and oral contraceptive using participant **(B)** during the transition to moderate (left) and heavy (right) intensity exercise, pre-training (open circles and gray line) and post-training (closed circles and black line). Mean response time (MRT) is indicated by a matched vertical line. Shading represents exercise onset.

**TABLE S1 |** Pulmonary oxygen uptake, cardiac output and heart rate on-kinetic responses to moderate and heavy intensity exercise, pre- and post-training in naturally-menstruating women, sub-grouped for normal luteal phase characteristics ($n$ = 16) and apparent luteal phase deficient women ($n$ = 8); as determined by serum progesterone concentrations.

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# Effects of High-Intensity Exercise Training on Adipose Tissue Mass, Glucose Uptake and Protein Content in Pre- and Post-menopausal Women

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The menopausal transition is accompanied by changes in adipose tissue storage, leading to an android body composition associated with increased risk of type 2 diabetes and cardiovascular disease in post-menopausal women. Estrogens probably affect local adipose tissue depots differently. We investigated how menopausal status and exercise training influence adipose tissue mass, adipose tissue insulin sensitivity and adipose tissue proteins associated with lipogenesis/lipolysis and mitochondrial function. Healthy, normal-weight pre- ($n = 21$) and post-menopausal ($n = 20$) women participated in high-intensity exercise training three times per week for 12 weeks. Adipose tissue distribution was determined by dual-energy x-ray absorptiometry and magnetic resonance imaging. Adipose tissue glucose uptake was assessed by positron emission tomography/computed tomography (PET/CT) by the glucose analog [18F]fluorodeoxyglucose ([18F]FDG) during continuous insulin infusion (40 $mU \cdot m^{-2} \cdot min^{-1}$). Protein content associated with insulin signaling, lipogenesis/lipolysis, and mitochondrial function were determined by western blotting in abdominal and femoral white adipose tissue biopsies. The mean age difference between the pre- and the post-menopausal women was 4.5 years. Exercise training reduced subcutaneous (~4%) and visceral (~6%) adipose tissue masses similarly in pre- and post-menopausal women. Insulin-stimulated glucose uptake, assessed by [18F]FDG-uptake during PET/CT, was similar in pre- and post-menopausal women in abdominal, gluteal, and femoral adipose tissue depots, despite skeletal muscle insulin resistance in post- compared to pre-menopausal women in the same cohort. Insulin-stimulated glucose uptake in adipose tissue depots was not changed after 3 months of high-intensity exercise training, but insulin sensitivity was higher in visceral compared to subcutaneous adipose tissue

depots (∼139%). Post-menopausal women exhibited increased hexokinase and adipose triglyceride lipase content in subcutaneous abdominal adipose tissue. Physical activity in the early post-menopausal years reduces abdominal obesity, but insulin sensitivity of adipose tissue seems unaffected by both menopausal status and physical activity.

**Keywords: white adipose tissue metabolism, menopause, insulin sensitivity, lipid metabolism, glucose metabolism, mitochondrial enzymes**

## INTRODUCTION

Adipose tissue redistributes during the menopausal transition, leading to an accumulation of abdominal subcutaneous and visceral adipose tissue. This might be a consequence of the hormonal shift with loss of estrogens during menopause (Davis et al., 2012), but how adipose tissue metabolism is affected by menopause is sparsely investigated. Systemic glucose homeostasis and skeletal muscle insulin sensitivity are positively affected by estrogens (Louet et al., 2004; Barros and Gustafsson, 2011). In support of this, we previously found a lower insulin-stimulated glucose uptake in skeletal muscle after menopause (Mandrup et al., 2018). Importantly, a similar increase in skeletal muscle glucose uptake in pre- and post-menopausal women was seen after high-intensity exercise training (Mandrup et al., 2018). We have also observed an increase in insulin-stimulated glucose uptake in skeletal muscle of overweight males after 3 months of exercise training, whereas adipose tissue glucose uptake remained unchanged or even decreased (Reichkendler et al., 2013). Whether exercise training affects insulin-stimulated glucose uptake in adipose tissue of pre- and post-menopausal women is not known.

Studies in rodents have shown that estrogens reduce the transcription of lipoprotein lipase and thereby lipid uptake and storage in adipose tissue (Homma et al., 2000; D'Eon et al., 2005). Those mechanisms also seem to apply to humans, as lipoprotein lipase activity increased after an oral glucose tolerance test in post- compared to pre-menopausal women (Santosa and Jensen, 2013). In addition, the post-menopausal women had higher storage of free fatty acids (FFA) in adipose tissue (Santosa and Jensen, 2013). Estrogens seem to decrease lipolysis through up-regulation of adipocyte α2-adrenergic receptors, thereby preventing an excess of FFA delivered to and deposited in ectopic tissues, such as liver and skeletal muscles (Kim et al., 2014). The effect of estrogens on adipose tissue lipolysis show regional differences, as estrogens blunt β-adrenergic stimulation of lipolysis in the abdominal but not in the gluteal region in pre-menopausal women (Gavin et al., 2013).

Studies of adipose tissue mitochondrial function are sparse, and changes relating to exercise training in combination with menopausal transition have not been investigated. We previously demonstrated higher mitochondrial activity in adipose tissue after 10 weeks of swim training in rodents (Stallknecht et al., 1991), but no studies of human mitochondrial function have shown positive adaptations to exercise training in adipose tissue (Camera et al., 2010; Larsen et al., 2015; Dohlmann et al., 2018).

The first aim of the present study was to investigate the impact of menopausal status on insulin-stimulated glucose uptake and proteins associated with glucose and lipid metabolism in abdominal and femoral white adipose tissue depots. Secondly, we aimed to study the effects of exercise training on white adipose tissue mass, glucose uptake and adipose tissue protein content in pre- and post-menopausal women. Moreover, we investigated differences in metabolism between the abdominal and femoral adipose tissue depots. We hypothesized that menopause would promote abdominal adipose tissue anabolism and that exercise training would reduce adipose tissue mass in both pre- and post-menopausal women.

## METHODS

### Ethical Approval

Information about the study, including risks and discomforts associated with participation, was given in writing and orally before the participants gave their written consent to participate. The study was conducted according to the Helsinki Declaration, approved by the Ethical Committee in the capital region of Denmark, protocol no. H-1-2012-150 and preregistered at clinicaltrials.gov (no. NCT02076932).

### Participants and Study Design

Forty pre-menopausal and 39 post-menopausal women were included in the Copenhagen Women Study—Menopause. The study design has been described previously (Mandrup et al., 2017). In brief, the inclusion criteria were; healthy, sedentary, normal to overweight [Body Mass Index (BMI) 18.5–30.0 kg/m$^2$], 45–57 years of age, either pre-menopausal [regular menses and plasma estradiol in the normal fertile range; follicular phase 0.05–0.51 nmol/l, mid cycle 0.32–1.83 nmol/l, luteal phase 0.16–0.78 nmol/l, and plasma follicle stimulating hormone (FSH) <20 IU/l] or post-menopausal (no menses for at least 1 year, estrogen <0.20 nmol/l and FSH 22–138 IU/l). Sedentary behavior was defined as not engaging in planned physical activities and performing less than a total of 2 h of physical activity per week during the last 2 years. A sedentary lifestyle was supported by maximal oxygen uptake <40 ml $O_2$/min/kg at

**Abbreviations:** ACC, Acetyl-CoA carboxylase; AS160, Akt substrate 160 kDa (also known as TBC1D4); ATGL, Adipose triglyceride lipase; BMI, Body mass index; CD36, Cluster of differentiation 36 [also known as FAT (fatty acid translocase)]; CS, Citrate synthase; DXA, Dual-energy X-ray absorptiometry; [$^{18}$F]FDG, [$^{18}$F]fluorodeoxyglucose; FFA, Free fatty acids; FSH, Follicle stimulating hormone; GLUT4, Glucose transporter 4; HK, Hexokinase; IDIF, Image derived input function; MRI, Magnetic resonance imaging; $MR_{glucose}$, Metabolic rate of glucose; OXPHOS, Oxidative phosphorylation; PET/CT, Positron emission tomography/Computed tomography; SHBG, Sex hormone binding globulin; VOI, Volume of interest.

inclusion. Exclusion criteria were; smoking, use of hormonal contraception or replacement treatment, excessive alcohol intake, chronic disease, daily intake of medication or abnormal blood samples (screening for liver, kidney, and bone marrow function). Fasting abdominal and femoral adipose tissue biopsies were obtained from all participants at baseline and after the training intervention. A subgroup of 21 pre-menopausal and 20 post-menopausal women underwent a hyperinsulinemic euglycemic clamp, positron emission tomography/computed tomography (PET/CT) scan, and magnetic resonance imaging (MRI) scan. A biopsy from the abdominal and femoral adipose tissue was taken during insulin stimulation from participants of this subgroup. Skeletal muscle insulin sensitivity of this subgroup has been published (Mandrup et al., 2018). All participants were tested before and after 3 months of high-intensity aerobic exercise training, performed as supervised indoor bike exercise training three times a week. The duration of the training sessions was ∼53 min and consisted of warm-up, three blocks of different intensities with multiple periods of maximal performance, and a cool down period. Training intensity was monitored by heart rate monitors (FT2, Polar, Finland).

## Experimental Methods

*Maximal oxygen* uptake was assessed by an incremental bicycle ergometer (Monark, Ergomedic 839 E, Sports & Medical, GIH, Sweden) protocol to exhaustion as previously described (Mandrup et al., 2017).

*Body composition* was assessed by dual-energy X-ray absorptiometry (DXA) scanning (Lunar iDXA, GE Healthcare, US) after an overnight fast. Additionally, abdominal and femoral adipose tissue depots were assessed by MRI on a 1.5 T Avanto (SIEMENS, Erlangen, Germany). Abdominal and femoral axial T1-weighted images were obtained using breath-hold (water suppression, slice thickness 6 mm, spacing between slides 7.2 mm, pixel size 1.1719 × 1.1719 mm). All scans were performed by the same investigator, who was blinded for menopausal status.

*Abdominal and femoral adipose tissue mass* was assessed by automated segmentation of the MRI using in-house developed software written in MATLAB (MathWorks, Natick, Massachusetts, United States). The visceral adipose tissue was delineated by the slice containing the disc between Th11 and Th12 (upper) and the most distal slice without the iliac crest (lower). The liver was manually segmented in ITK-SNAP (Yushkevich et al., 2006) and removed from the intra-abdominal segmentation. The abdominal subcutaneous adipose tissue limitation was the disc between Th12 and L1 (upper) and the last slide not including caput femoris (lower). In the femoral scan, the adipose tissue depots were delimited by the lower delimitation of the gluteal adipose tissue (upper) and the last slide without patella (lower). All images were bias-corrected (Larsen et al., 2014) and the adipose tissue depots were automatically delineated by unrolling the images and using the graph-cut method (Li et al., 2006). The inter-muscular/visceral adipose tissue depots were delineated by thresholding, with a threshold value determined by taking the median value of a 5-component k-means clustering on the muscular/abdominal compartment. Due to the T1 weighted sequence, femur was not visible at the femoral images, but the bone marrow was delineated separately from the inter-muscular fat. The adipose tissue volumes were converted to mass using a density of adipose tissue of 0.9 kg/L. Two abdominal and three femoral scans were excluded due to errors in the acquisition or movement artifacts.

*A hyperinsulinemic euglycemic clamp was* performed as previously described (DeFronzo et al., 1979; Mandrup et al., 2018). In brief, the pre-menopausal women were investigated in the late follicular phase (days 8–13) where plasma estrogens are high, but plasma progesterone low. All participants arrived at the laboratory after an overnight fast, without having engaged in physical activity or consumed caffeine for 36 h. Arterialized venous blood samples were obtained from a hand vein. Insulin were administered through an antecubital vein at a rate of 40 $mU \cdot m^{-2} \cdot min^{-1}$ together with variable rates of glucose to maintain euglycemia. Blood samples were obtained at baseline and every 30 min in pre-coated tubes (BD Vacutainer®, Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA), immediately stored on ice and centrifuged at 4,000 rpm in 10 min before the plasma was stored at −80°C for later analyses of FFA and glycerol (clinical chemistry analyzer, Pentra C 400, Horiba, Kyoto, Japan) by investigators blinded for menopausal status. Estradiol and testosterone (competitive electrochemiluminescence immunoassay (ECLIA), Cobas 8000, e602 module, F. Hoffmann-La Roche Ltd., Rotkreuz, Switzerland) and sex hormone binding globulin (SHBG) (sandwich chemiluminescent immunometric method, Siemens Immulite 2000 XPi) were measured before insulin infusion. The participants were maintained in clamped (hyperinsulinemia, euglycemia) condition during the PET/CT scan.

## PET/CT Scan: Tracer, Image Acquisition, and Processing

PET/CT scans were performed on a Biograph mCT 128-slice (Siemens, Erlangen, Germany) as described previously (Mandrup et al., 2018). Briefly, a low-dose CT scan (120 kV, 11 mA) was performed for attenuation correction and anatomic localization prior to the PET scan. A low dose of [$^{18}$F]fluorodeoxyglucose ([$^{18}$F]FDG) (200 MBq) was injected intravenously precisely at the start of acquisition. The PET/CT scan included 60 min of dynamic acquisition of the abdomen followed by 2 × 10 min acquisition of the femoral region. The abdominal data was histogrammed into 26 timeframes of varying length (12·10, 4·120, 10·300 s) and reconstructed using the ordered subset expectation maximum algorithm with a point spread function correcting method (TrueX, Siemens), 336·336 pixels, with 8 iterations and 21 subsets. The femoral images were reconstructed as a single time frame (size: 400·400·174, voxel spacing: 2·2·2 mm, 3 iterations, and 21 subsets). All PET images were corrected for attenuation and scatter, and decay corrected to scan start. All PET/CT image analysis were performed using PMOD 3.304 (PMOD Technologies, Zurich, Switzerland) by an investigator blinded to menopausal and exercise training status. The [$^{18}$F]FDG blood concentration was determined by image derived input function (IDIF) (Christensen et al., 2014) from a cylindrical volume of interest (VOI) in aorta at the level of L2/L3 The subcutaneous abdominal (anterior and posterior) and gluteal adipose tissue depots were defined at the CT scan with a

segmentation using connected threshold with Hounsfield units from −150 to −50. The visceral adipose tissue was defined in the same way, inside a spherical VOI manually inserted in the visceral adipose tissue area. A two-pixel erosion was applied to all VOIs to avoid spillover from the intestinal walls. All VOIs were transferred to the PET images to obtain the metabolic rate of glucose ($MR_{glucose}$), which was calculated as:

$$MR_{glucose} = \frac{1,000 \cdot K_i \cdot glucose\ concentration_{plasma}}{Lumped\ Constant \cdot density_{tissue}}$$

The lumped constant (LC) accounts for differences in transport and phosphorylation rates between D-glucose and 2-fluoro-2-deoxy-D-glucose and transforms the [$^{18}$F]FDG uptake rate to glucose uptake rate. The lumped constant of adipose tissue was set to 1.14 (Virtanen et al., 2001) and the density of adipose tissue was set to 0.9 g/ml. The $K_i$ is the [$^{18}$F]FDG influx rate constant (ml blood·ml tissue$^{-1}$·min$^{-1}$) and was derived from a mathematic model fit to the dynamic time activity curve in PMOD. To estimate $K_i$ from the static scan, the mean activity of the defined VOI is divided by the [$^{18}$F]FDG blood concentration. This value is extrapolated from the area under the curve of the IDIF from 0 to 60 min to the middle of the static scan (60 min + 10 min) in MATLAB.

$$estimated\ K_i = \frac{Activity_{\ mean\ activity\ of\ the\ VOI\ [kBq \cdot ml_{tissue}{}^{-1}]}}{AUC_{available\ FDG\ i\ the\ blood\ [\ \frac{kBq \cdot \min}{ml_{blood}}\ ]}}$$

*Abdominal and femoral adipose tissue biopsies* were obtained both in the overnight fasted state and during insulin stimulation 120 min after initiation of the clamp on two separate test days. After local anesthesia (Lidocaine 20 mg/ml, SAD, Copenhagen, Denmark), the biopsies were obtained *ad modum* Bergström (Bergstrom, 1975) and immediately frozen in liquid nitrogen and stored at −80°C for later analyses.

*Adipose tissue preparation and western blotting* was performed to investigate protein content as previously described (Kristensen et al., 2007). Briefly, adipose tissue homogenates were rotated at 4°C for 60 min, centrifuged 40 min at 16,000 g, and the infranatant was harvested as the adipose tissue lysate. Total protein content in adipose tissue lysates was determined by the bicinchoninic acid method (PierceBiotechnology, Inc. Rockford, IL, USA). Lysates were solubilized in Laemmli sample buffer, and an equal amount of total protein from each sample was separated by SDS-page and transferred to PVDF-membranes. Membranes were blocked for 10 min in 2–3% skimmed milk or 3% BSA in TBS containing 0.05% Tween-20 (TBST buffer) and incubated overnight at 4°C in primary antibodies against glucose transporter 4 (GLUT4) [Thermo Fisher Scientific (# PA1-1065), MA, USA, diluted 1:1,000], akt substrate 160 kDa (AS160) [Millipore (#07-741), MA, USA, diluted 1:1,000], hexokinase (HK) [Cell Signaling Technology (#2106), MA, USA, diluted 1:1,000], cluster of differentiation 36 (CD36) [R&D systems (#AF2519), MN, USA, diluted 1:1,000], acetyl-CoA carboxylase (ACC) [Streptavidin-HRP, DAKO (#P0397), Denmark, diluted 1:2,000], adipose triglyceride lipase (ATGL) [Cell Signaling (#2138), MA, USA, diluted 1:1,000], citrate synthase (CS) [Abcam (#ab96600), Cambridge, UK, diluted 1:3,000) and oxidative phosphorylation (OXPHOS) complex 1–5 [Abcam (#ab110411), Cambridge, UK, diluted 1:10,000]. Membranes were incubated with horseradish peroxidase (HRP) conjugated secondary antibodies (Jackson ImmunoResearch, West Grove, PA, USA, diluted 1:5,000) for 45 min at room temperature before visualizing protein bands with Luminata Forte Western HRP Substrate [Millipore (#WBLUF0500), MA, USA] and ChemiDoc MP imaging system (BioRad, CA, USA). Protein signals were quantified (Image Lab version 4.0) and protein content levels expressed as arbitrary units. Protein content used for statistical analysis are expressed as a ratio to total protein content in the sample as measured by stain-free protein technology.

*Statistical analysis* was performed using SAS Enterprise Guide 7.1 (©SAS Institute Inc., Cary, NC, USA) and graphic presentations was made using GraphPad Prism 7.00 (GraphPad Software, Inc., La Jolla, CA, USA). Prior to inclusion, power calculations regarding insulin-stimulated glucose uptake measured by [$^{18}$F]FDG PET/CT and adipose tissue masses measured by MRI revealed that inclusion of 20 pre- and 20 post-menopausal women would enable detection of a 21 and a 20% change, respectively, with a likelihood of 80%. Descriptive statistics for parametric data are calculated by a two-way ANOVA and given as mean [95% confidence interval (CI)]. Masses of abdominal and femoral adipose tissue depots were analyzed by a 2-way ANCOVA with the number of analyzed slices as covariate. Adipose tissue glucose uptake determined by PET/CT and protein content determined by western blotting were assessed using a mixed model with group and time as variables and participant ID as repeated measures. Interactions between time and group were further analyzed for within group effects by ls-means. Protein content during fasting (all participants) are presented in **Figures 4**, **5**, **7**. The difference in protein content after 2 h of hyper insulin stimulation (subgroup of participants) are given as delta-values and only significant results are shown (**Figures 6**, **8**). Statistical significance was set as $p \leq 0.05$.

## RESULTS

Eighteen late pre- and 20 early post-menopausal women with a difference of 4.5 years of age were included in the subgroup of participants who underwent the hyperinsulinemic euglycemic clamp, PET/CT and insulin stimulated adipose tissue biopsies (**Table 1**). One post-menopausal woman was excluded from post-testing due to an emerging neuro-muscular disease. Characteristics of all of the participants has previously been published (Mandrup et al., 2017). Time since last menstruation for the post-menopausal women was 3.2 (2.6–3.8) years at inclusion. Plasma estradiol/SHBG index and reflected the menopausal status and the index did not change after the intervention. FSH reflected menopausal status. The post-menopausal women had lower bioavailable testosterone levels

**TABLE 1 |** Participant characteristics of pre- and post-menopausal women before and after 3 months of high-intensity training.

| | Pre-menopausal | | Post-menopausal | |
|---|---|---|---|---|
| | **Baseline (*n* = 18)** | **3 months (*n* = 18)** | **Baseline (*n* = 20)** | **3 months (*n* = 19)** |
| Age | 48.5 (47.5–49.4) | | 53.0 (51.6–54.4) | |
| Body weight (kg) | 66.1 (62.4–69.8) | 66.1 (62.4–69.4) | 67.0 (62.8–71.2) | 66.5 (61.9–71.2) |
| BMI | 23.5 (22.4–24.6) | 23.4 (22.4–24.5) | 23.6 (22.5–24.8) | 23.5 (22.3–24.8) |
| Fat mass (kg)[a] | 22.6 (20.6–24.5) | 22.1 (20.1–24.1) | 23.6 (20.8–26.4) | 22.5 (19.3–25.7) |
| Fat (%)[a] | 34.1 (32.1–36.0) | 33.4 (31.5–35.2) | 34.9 (32.3–37.4) | 33.3 (30.4–36.2) |
| Android fat (%)[a] | 35.8 (31.7–39.8) | 35.2 (31.0–39.4) | 35.9 (30.7–41.1) | 34.2 (28.4–40.0) |
| Gynoid fat (%)[a] | 40.0 (38.0–41.9) | 38.8 (37.1–40.6) | 41.0 (38.9–43.1) | 38.4 (36.0–40.8) |
| Android/gynoid ratio | 0.89 (0.80–0.99) | 0.91 (0.80–1.01) | 0.86 (0.76–0.96) | 0.87 (0.76–0.99) |
| Maximal oxygen uptake (ml $O_2$/min)[a] | 2085 (1952–2218) | 2308 (2174–2442) | 2018 (1887–2148) | 2241 (2108–2374) |
| FSH (IU/L)[b] | 12.6 (7.2–18.1) | 12.8 (7.7–18.0) | 96.0 (82.8–109.3) | 90.7 (79.7–101.7) |
| Estradiol/SHBG index[b] | 6.5 (4.3–9.8) | 7.0 (4.6–10.6) | 1.4 (1.1–1.7) | 1.3 (1.1–1.6) |
| Bioavailable testosterone (ng/dL)[b] | 5.3 (4.3–6.5) | 4.9 (4.0–6.1) | 4.0 (3.0–4.9) | 3.7 (3.0–4.6) |

*Data are presented as mean (95% confidence limits). Group differences and effect of the intervention were assessed by a two-way ANOVA. [a]Change from baseline to 3 months: ($p \leq 0.05$). [b]Difference between groups ($p \leq 0.05$). BMI, Body Mass Index; FSH, Follicle stimulating hormone; SHBG, sex hormone binding globulin.*

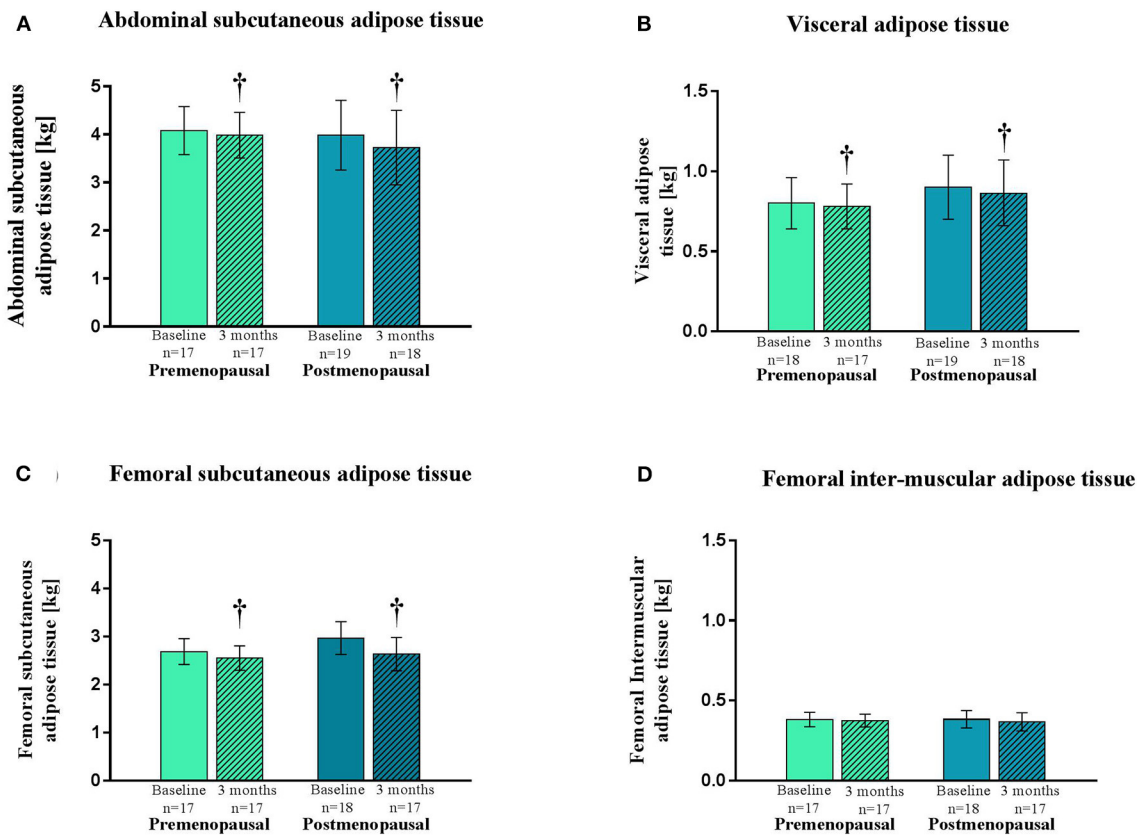


**FIGURE 1 |** Abdominal and femoral adipose tissue depots as determined by magnetic resonance imaging in pre- and post-menopausal women before and after 3 months of high-intensity training. Data are presented as mean (95% confidence limits). Changes from baseline to 3 months $^{\dagger}p \leq 0.05$. **(A)** Abdominal subcutaneous adipose tissue. **(B)** Visceral adipose tissue. **(C)** Femoral subcutaneous adipose tissue. **(D)** Femoral inter-muscular adipose tissue.

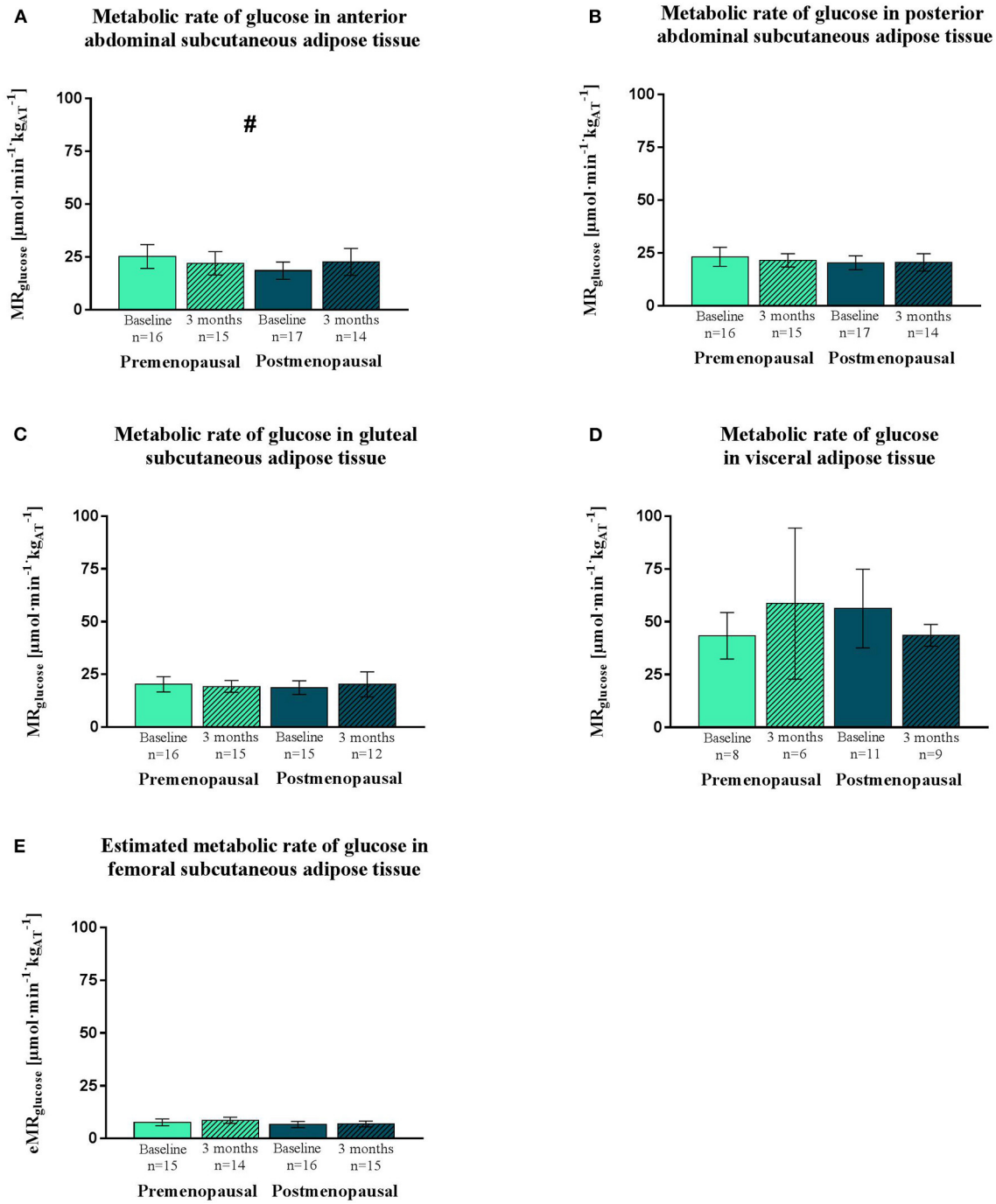


**FIGURE 2 |** Insulin-stimulated glucose uptake in adipose tissue, expressed as $MR_{glucose}$. PET/CT-derived data of $MR_{glucose}$ of abdominal subcutaneous (anterior and posterior), visceral and gluteal adipose tissue. Estimated $MR_{glucose}$ of femoral subcutaneous adipose tissue. Data was obtained during a hyperinsulinemic euglycemic clamp in pre- and post-menopausal women before and after 3 months of high-intensity training. Data are presented as mean (95% confidence limits). $MR_{glucose}$ is higher in visceral than subcutaneous adipose tissue depots. Interaction between groups and time $^{\#}p \leq 0.05$. $MR_{glucose}$, Metabolic rate of glucose. **(A)** Metabolic rate of glucose in anterior abdominal subcutaneous adipose tissue. **(B)** Metabolic rate of glucose in posterior abdominal subcutaneous adipose tissue. **(C)** Metabolic rate of glucose in gluteal subcutaneous adipose tissue. **(D)** Metabolic rate of glucose in visceral adipose tissue. **(E)** Estimated metabolic rate of glucose in femoral subcutaneous adipose tissue.

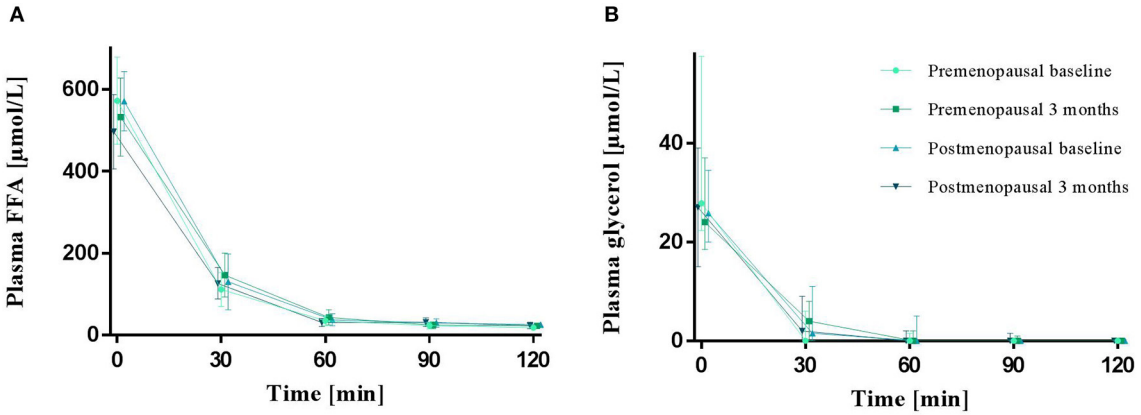


**FIGURE 3 |** Plasma FFA **(A)** and glycerol **(B)** in the fasted state (0 min) and during a hyperinsulinemic euglycemic clamp in pre- and post-menopausal women before (baseline) and after 3 months of high-intensity training. Plasma FFA are presented as mean (95% confidence limits) and glycerol as median (25–75 percentile). FFA, Free fatty acids.

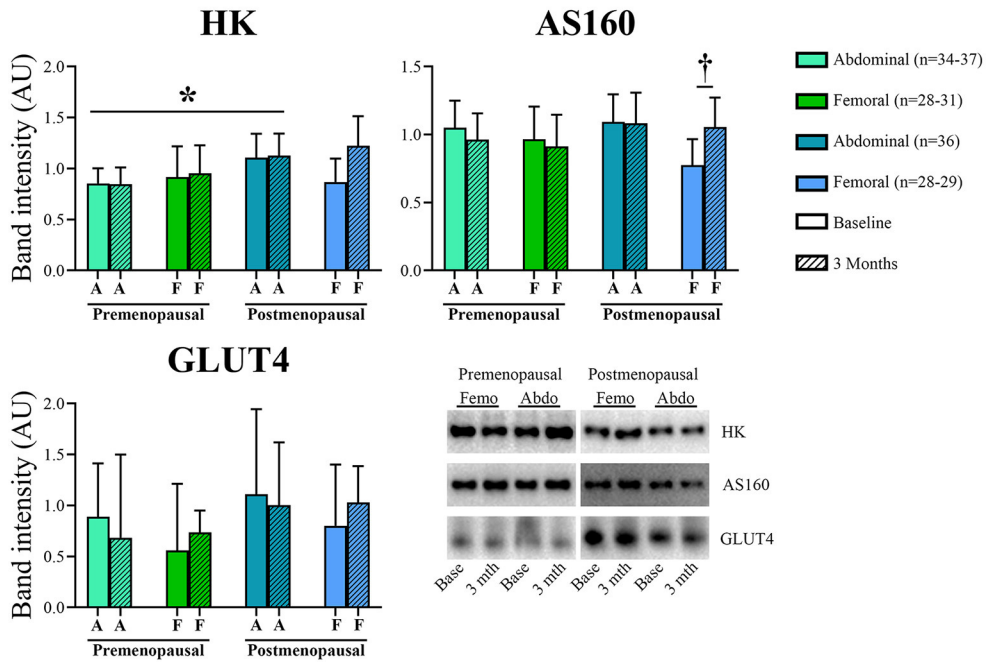


**FIGURE 4 |** Abundance of proteins associated with insulin signaling and glucose metabolism in abdominal and femoral subcutaneous adipose tissue in pre- and post-menopausal woman before and after 3 months of high-intensity training. In abdominal adipose tissue, HK content was higher in post- compared to pre-menopausal women. In femoral adipose tissue, exercise training increased AS160 content in post-menopausal women. Changes from baseline to 3 months $^{\dagger}p \leq 0.05$. Difference between groups $^{*}p \leq 0.05$. HK and AS160 protein abundances are presented as mean (95% confidence limits). GLUT4 protein abundance is presented as median (25–75 percentile). HK, Hexokinase; AS160, Akt substrate 160 kDa; GLUT4, Glucose transporter 4.

than the pre-menopausal women ($p = 0.04$), and bioavailable testosterone tended ($p = 0.06$) to decrease similarly in both groups after the intervention. Cardiorespiratory fitness increased ($p < 0.0001$) similarly in both groups after the intervention (**Table 1**).

## Adipose Tissue Mass

DXA scans: Whole body fat mass, fat percentage, and android and gynoid fat percentages did not differ between the pre- and the post-menopausal women (all: $p > 0.05$) and the masses were reduced similarly in the two groups with 3 months of

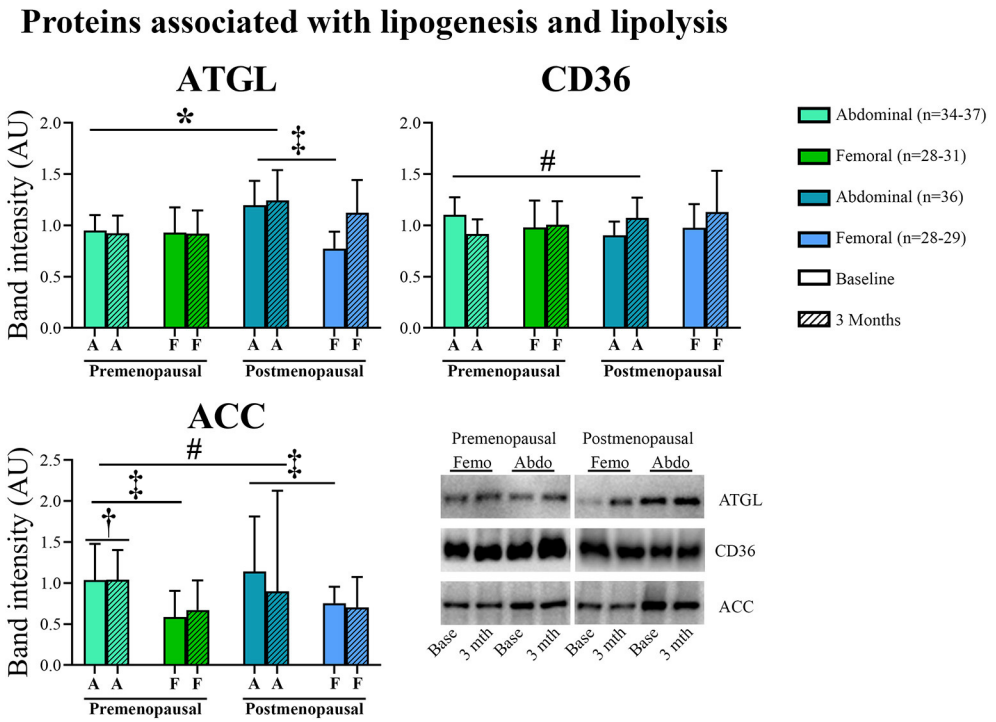


**FIGURE 5 |** Abundance of proteins associated with lipogenesis and lipolysis in abdominal and femoral subcutaneous adipose tissue in pre- and post-menopausal woman before and after 3 months of high-intensity training. Abdominal ATGL content was higher in post- compared to pre-menopausal women. ATGL content was higher in abdominal compared to femoral adipose tissue in the post-menopausal women. Abdominal CD36 content decreased after exercise training in pre- but increased in post-menopausal women. Abdominal ACC content increased after exercise training in pre- and decreased in post-menopausal women. Pre-menopausal women increased abdominal ACC content after the training intervention. ACC content was higher in abdominal compared to femoral adipose tissue. Interaction between groups and time $^{\#}p \leq 0.05$. Changes from baseline to 3 months $^{\dagger}p \leq 0.05$. Difference between abdominal and femoral depots $^{\ddagger}p \leq 0.05$. Difference between groups $^{*}p \leq 0.05$. ATGL and CD36 protein abundances are presented as mean (95% confidence limits). ACC protein abundance is presented as median (25–75 percentile). ATGL, Adipose triglyceride lipase; CD36, Cluster of differentiation 36; ACC, Acetyl-CoA carboxylase.

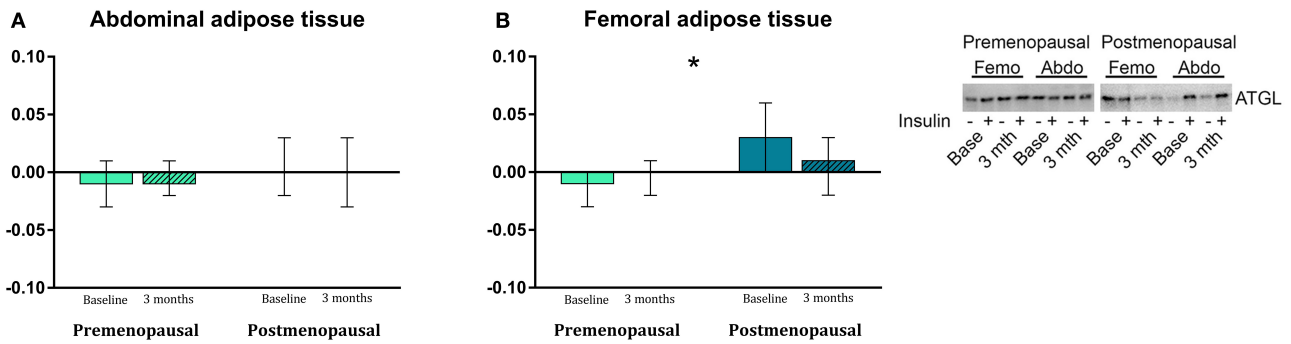


**FIGURE 6 |** The effect of 120 min of hyper insulin stimulation on protein abundance of ATGL (delta value, arbitrary units) in abdominal **(A)** and femoral **(B)** adipose tissue in pre- and post-menopausal women before and after 3 months of high-intensity training. Insulin stimulation suppressed ATGL in femoral subcutaneous adipose tissue in pre- but not in post-menopausal women. Difference between groups $^{*}p \leq 0.05$. Data are presented as mean (95% confidence limits). ATGL, Adipose triglyceride lipase.

high-intensity exercise training ($p < 0.01$, $p < 0.001$, $p < 0.01$, $p < 0.0001$, respectively) (**Table 1**). The android/gynoid ratio was similar in the two groups and did not change after the intervention.

MRI scans: Abdominal subcutaneous and visceral adipose tissue masses did not differ between the pre- and the post-menopausal women and a dipose tissue masses decreased similarly in the two groups after the intervention ($p < 0.005$ and

$p = 0.03$, respectively) (**Figure 1**). Also, femoral subcutaneous and inter-muscular adipose tissue mass did not differ between groups (**Figure 1**). The femoral subcutaneous adipose tissue was reduced similarly in the two groups after the intervention ($p = 0.01$), whereas the inter-muscular adipose tissue mass remained unchanged (**Figure 1**).

### Insulin-Stimulated Glucose Uptake in Adipose Tissue

The insulin-stimulated glucose uptake was higher in visceral compared to all subcutaneous adipose tissue depots (all: $p < 0.0001$) (**Figure 2**). The training response differed ($p = 0.02$) between the pre- and the post-menopausal women as the glucose uptake in the anterior abdominal subcutaneous adipose tissue decreased in the pre- and increased in the post-menopausal women. *Post-hoc* analysis showed no within-group difference (**Figure 2**). No significant differences in insulin-stimulated glucose uptake between or within groups were observed in the posterior abdominal subcutaneous, visceral, gluteal, or femoral subcutaneous adipose tissue depots (**Figure 2**).

### Plasma Free Fatty Acids and Glycerol

Plasma FFA and glycerol did not differ between pre- and post-menopausal women during fasting (0 min). In addition, the decline in plasma FFA and glycerol during hyperinsulinemic stimulation (30–120 min) was similar between groups. The exercise training intervention did not change fasting or insulin suppression of plasma FFA or glycerol (**Figure 3**).

### Proteins Associated With Insulin Signaling and Glucose Metabolism (Figure 4)

In abdominal adipose tissue, HK content was higher in post- compared to pre-menopausal women ($p = 0.03$), and exercise training did not affect HK content. In femoral adipose tissue, exercise training led to no change in the pre-menopausal group but increased AS160 content in the post-menopausal group (interaction: $p = 0.04$, *post-hoc* analysis: within-group effect in post-menopausal women $p = 0.02$). Neither menopausal state nor exercise training were associated with the abundance of adipose tissue GLUT4.

### Proteins Associated With Lipogenesis and Lipolysis (Figure 5)

In abdominal adipose tissue, ATGL content was higher in post- compared to pre-menopausal women ($p = 0.03$). Further, the ATGL content was higher in abdominal compared to femoral adipose tissue in the post-menopausal women ($p = 0.005$). In femoral adipose tissue, insulin stimulation suppressed ATGL content in pre- but not in post-menopausal women ($p = 0.05$, **Figure 6**). In abdominal adipose tissue, exercise training resulted in a different response in pre- vs. post-menopausal women, as CD36 content decreased in pre- but increased in post-menopausal women (interaction: $p = 0.02$). Post hoc analyses detected no within-group effect. In contrast, exercise training increased the ACC content in abdominal adipose tissue in the pre-menopausal women and decreased ACC content in the post-menopausal women (interaction: $p = 0.05$). *Post-hoc* analyses showed a within-group increase ($p = 0.05$) in pre-menopausal women after the training intervention. In both groups, ACC content was higher in abdominal compared to femoral adipose tissue (pre-menopausal: $p = 0.005$, post-menopausal: $p = 0.03$).

### Proteins associated with mitochondria (Figure 7)

In post-menopausal women, the OXPHOS complex 1 content was higher in abdominal compared to femoral adipose tissue ($p = 0.05$). Insulin stimulation suppressed OXPHOS complex 3 content after exercise training in both pre- and post-menopausal women in abdominal ($P = 0.04$) and femoral ($p = 0.05$) adipose tissue (**Figure 8**). The effect of exercise training on OXPHOS complex 5 content in abdominal adipose tissue differed between the groups as it increased in post- but not in pre-menopausal women (interaction $p = 0.04$. *Post-hoc* analysis, within-group effect in post-menopausal women $p = 0.03$).

## DISCUSSION

This study demonstrates that insulin-stimulated glucose uptake in abdominal, gluteal, and femoral adipose tissue depots are similar in pre- and post-menopausal women, despite skeletal muscle insulin resistance in post- compared to pre-menopausal women in the same cohort (Mandrup et al., 2018). Also, insulin-stimulated glucose uptake in adipose tissue depots was not altered markedly by 3 months of high-intensity exercise training in either group, whereas skeletal muscle insulin sensitivity increased after exercise training in both groups (Mandrup et al., 2018). Insulin sensitivity was higher in visceral compared to subcutaneous adipose tissue depots and the mass of visceral and subcutaneous adipose tissue depots diminished during the intervention to the same extent in pre- and post-menopausal women.

We are the first to investigate insulin-stimulated glucose uptake in adipose tissue of pre- and post-menopausal women by [$^{18}$F]FDG PET/CT and our observation of similar glucose uptake in adipose tissue depots before and after menopause contributes to the understanding of physiological changes with the menopausal transition. Skeletal muscle is responsible for ∼85% of whole-body insulin-stimulated glucose uptake (DeFronzo et al., 1979). In comparison, adipose tissue insulin-stimulated glucose uptake measured by PET/CT in non-obese and moderately obese men (BMI: 19–24 and 27–34 kg/m$^2$, respectively) has been reported to comprise 2.6 and 4.2%, respectively, of the total glucose disposal (Virtanen et al., 2002). In morbidly obese individuals (BMI > 40 kg/m$^2$), the insulin-stimulated glucose uptake in adipose tissue has been reported to contribute substantially to total glucose uptake, accounting for up to 20% of glucose disposal (Gniuli et al., 2010). Clearly, a larger adipose tissue mass yields a larger glucose uptake. In addition, hypothetically, insulin resistance in skeletal muscle could cause subsequent spillover of glucose to adipose tissue. Insulin resistant subjects had a diminished fractional uptake of glucose in skeletal muscle compared to insulin sensitive subjects, but the fractional uptake of glucose in abdominal, femoral, and intraperitoneal adipose tissue depots was comparable between insulin resistant and insulin sensitive subjects (Ferrannini et al., 2018). This is consistent with our findings, where skeletal muscle insulin sensitivity was diminished

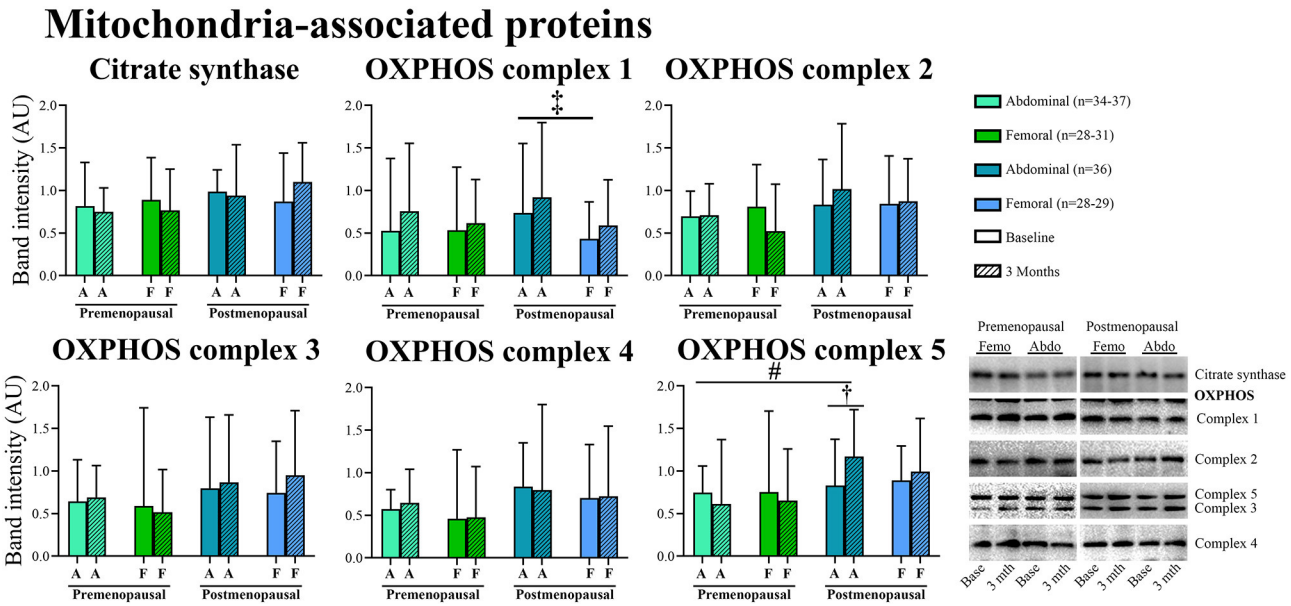


**FIGURE 7 |** Abundance of proteins associated with mitochondria in abdominal and femoral subcutaneous adipose tissue in pre- and post-menopausal woman before and after 3 months of high-intensity training. Abundance of OXPHOS complex 1 was higher in abdominal compared to femoral adipose tissue in post-menopausal women. Abdominal OXPHOS complex 5 content increased after the training intervention in post- but not in pre-menopausal women. Difference between abdominal and femoral depots $^{\ddagger}p \leq 0.05$. Interaction between groups and time $^{\#}p \leq 0.05$. Changes from baseline to 3 months $^{\dagger}p \leq 0.05$. Data are presented as median (25–75 percentile). CS, Citrate synthase.

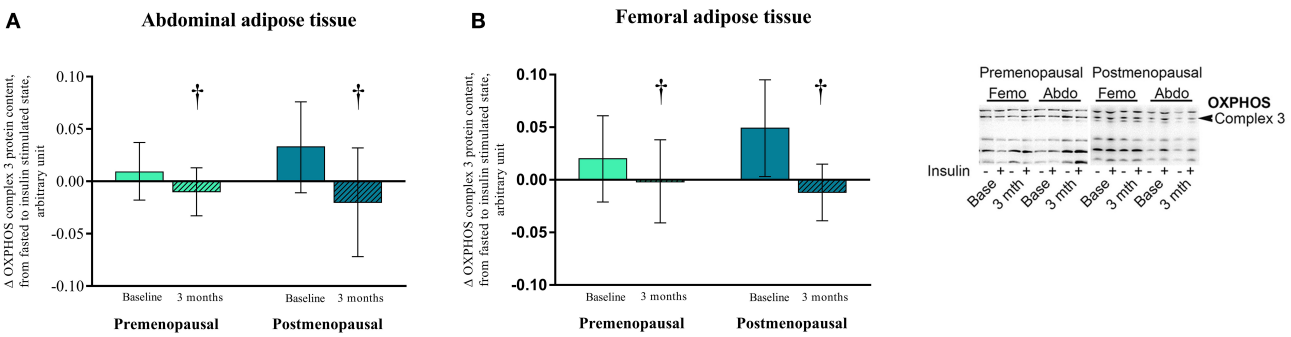


**FIGURE 8 |** The effect of 120 min of hyper insulin stimulation on OXPHOS complex 3 (delta value, arbitrary units) in abdominal and femoral adipose tissue in pre- and post-menopausal women before and after 3 months of high-intensity training. Difference from baseline to 3 months $^{\dagger}p \leq 0.05$. Data are presented as mean (95% confidence limits).

in post- compared to pre-menopausal women (Mandrup et al., 2018) with no influence of menopausal state on glucose uptake in visceral or subcutaneous abdominal or femoral adipose tissue.

We found no change in adipose tissue insulin sensitivity after exercise training in either pre- or post-menopausal women. In young overweight men, we previously found that insulin-stimulated glucose uptake in various adipose tissue depots, as determined by [$^{18}$F]FDG PET/CT, was similar or even decreased after 3 months of exercise training (Reichkendler et al., 2013). In addition, 3 months of endurance training did not influence adipose tissue glucose uptake in healthy men with or without a family history of type 2 diabetes as determined by microdialysis or *in vitro* (Dela and Stallknecht, 2010). However, in a cross-sectional study we found higher insulin sensitivity in adipose tissue of endurance-trained compared to sedentary young men (Stallknecht et al., 2000) and another cross-sectional study showed increased insulin-stimulated glucose transport in adipocytes from trained compared to sedentary humans (Rodnick et al., 1987). Also, in rodents, it has been shown, that high-capacity running rats have a higher glucose uptake in brown and visceral adipose tissue, and tend to show a higher uptake in subcutaneous adipose tissue compared to low-capacity running rats (Park et al., 2015). However, ovariectomy in both groups of rats had no effect on glucose uptake in adipose tissue. Hence, insulin-stimulated glucose uptake in adipose tissue seems to be increased in trained individuals, whereas 3 months of exercise

training or menopausal status do not influence adipose tissue insulin sensitivity.

Glucose uptake has previously been reported to be higher in visceral compared to subcutaneous adipose tissue, both during fasting (4–6 h) (Christen et al., 2010) and steady-state insulin infusion measured by [$^{18}$F]FDG PET/CT in gender mixed cohorts (Ng et al., 2012) and in obese men (Reichkendler et al., 2013). This implies a higher metabolic activity in the visceral adipose tissue, which also applies to the pre- and post-menopausal women of the present study. Visceral adiposity is associated with metabolic dysregulation, although the mechanisms are not clear (Després et al., 2008). Visceral adipose tissue mass increases with age, but the consequences of menopause *per se* on visceral adipose tissue accumulation is debated (Kuk et al., 2009). We observed no difference in visceral adipose tissue mass between non-obese pre- and post-menopausal women.

Exercise training is known to reduce abdominal obesity (Ross and Després, 2009), but the effect in post-menopausal women is not clear. A cross-sectional study reported that post-menopausal women had a higher visceral adipose tissue mass compared to pre- and perimenopausal women, although the three groups were comparable with respect to subcutaneous adipose tissue mass (Dugan et al., 2010). Within the subgroups of the pre-, peri-, and post-menopausal women, who were physically active, the post-menopausal women still had significantly increased visceral adipose tissue depots, proposing that post-menopausal women had a blunted response to exercise training in terms of reducing visceral adipose tissue mass. Although it has been reported that post-menopausal women are able to reduce visceral adipose tissue mass after a 1-year aerobic exercise intervention (Friedenreich et al., 2011), there was no dose-response effect in reducing visceral depots (Friedenreich et al., 2015). We did not observe a reduced capability of post- compared to pre-menopausal women to decrease the visceral adipose tissue mass after 3 months of high-intensity exercise training, suggesting that physical activity in the early post-menopausal years can contribute to reduced visceral adipose tissue mass.

Hexokinase is an important enzyme that phosphorylates glucose initially after cellular uptake, before storage as glycogen, or consumption via glycolysis or the pentose phosphate pathway. The protein content of HK was higher in the abdominal adipose tissue of the post- compared to the pre-menopausal women and, as expected, no increase was observed after 120 min of insulin stimulation. Previously, we demonstrated an increase in HK content in skeletal muscle in post- compared to pre-menopausal women of the same cohort (Mandrup et al., 2018), suggesting that protein content of HK is upregulated in both skeletal muscle and abdominal adipose tissue after menopause, perhaps to overcome insulin resistance. It has been shown that HK mRNA in adipose tissue is upregulated after 180 min of insulin stimulation in metabolically healthy and obese men and women, but not in type 2 diabetes patients, suggesting that acute insulin dependent upregulation of HK is impaired due to or maybe as a path in adipose tissue insulin resistance (Ducluzeau et al., 2001).

Traditionally, adipose tissue insulin sensitivity is evaluated as insulin suppression of adipose tissue lipolysis, measured by reduced release of FFA to the blood. This measure is relevant as FFA are the most metabolically important products of adipose tissue lipolysis and high plasma concentrations are linked to muscle and liver insulin resistance, hypertriglyceridemia, and impaired vascular function (Jensen, 2007). We demonstrated no difference in FFA suppression between pre- and post-menopausal women, and no change after high-intensity exercise. This is opposite to a study of mix-gender, upper body obese (BMI 28–36 kg/m$^2$) but non-diabetic subjects, where 40 weeks of combined diet and exercise intervention let to ∼50% lower plasma oleate concentration during a steady-state hyperinsulinemic clamp (Shadid and Jensen, 2006). Our participants were metabolically healthy which might explain the absence of differences between and within groups. However, a more frequent blood sampling in the initial phase of the hyperinsulinemic clamp might have given us more accurate information on insulin suppression of lipolysis (Jensen and Nielsen, 2007).

During fasting, adipocyte ATGL content is upregulated (Nielsen et al., 2011) to mobilize metabolites for energy production as an alternative to glucose. We found an increased abundance of ATGL in abdominal adipose tissue in post- compared to the pre-menopausal women suggesting a higher capacity for lipolysis in post-menopausal women. Similarly, Jensen et al. found higher palmitate flux during estrogen-deficiency than after estrogen treatment of post-menopausal women (Jensen et al., 1994). Insulin stimulates adipose tissue triacylglycerol and fatty acid synthesis (Dimitriadis et al., 2011). As expected, we observed that insulin stimulation suppressed ATGL content in femoral adipose tissue in pre-menopausal women but, surprisingly, not in post-menopausal women. Theoretically, this could contribute to mobilization of FFA and ectopic fat deposition in post-menopausal women.

Recently, Dohlmann et al. found that mitochondrial DNA in adipose tissue is not changed after 6 weeks of high-intensity training but adipose tissue mitochondrial respiratory capacity was reduced (Dohlmann et al., 2018). In the present study, content of OXPHOS complex 5 in abdominal adipose tissue in post-menopausal women increased after 3 months of high-intensity training, but we observed no other increase in mitochondrial enzymes CS or OXPHOS complexes 1–5. Apparently, exercise training does not increase mitochondrial enzymes in human adipose tissue to the same extent as we have previously found in rat adipose tissue (Stallknecht et al., 1991). However, a new intriguing finding of the present study is that insulin decreased the content of OXPHOS complex 3 in both abdominal and femoral adipose tissue of both pre- and post-menopausal women after compared to before exercise training.

We sought to evaluate the effect of menopausal status on adipose tissue mass, glucose uptake, and protein content without obesity or aging as confounding factors. We thereby compromised on generalizability, as the included post-menopausal women were leaner than the general population. Our power calculations were based on a higher change in glucose uptake and adipose tissue reduction than we found in the study. Therefore, a type II error could explain our negative finding regarding glucose uptake. However, insulin-stimulated glucose uptake was comparable within groups in different

subcutaneous depots as well as before and after the training intervention. A strength of the study is the investigation of *in vivo* insulin-stimulated glucose uptake in visceral and various subcutaneous adipose tissue depots via [18F]FDG PET/CT. Furthermore, extensive work to quantify adipose tissue protein content associated with glucose and lipid metabolism has been performed.

In conclusion, early menopause is not associated with increased insulin-stimulated glucose uptake in abdominal or femoral adipose tissue. However, post-menopausal women exhibited increased HK and ATGL content in abdominal adipose tissue suggesting increased capacity for adipose tissue glucose phosphorylation and lipolysis. Three months of high-intensity exercise training reduced subcutaneous and visceral adipose tissue masses similarly in pre- and post-menopausal women. This emphasizes that exercise training is important for mid-life women and should be encouraged.

## DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethical Committee in the capital region of Denmark, protocol nr. H-1-2012-150. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

BS, YH, AK, LE, JW, CM, JE, and MN substantially contributed to the conception or design of the work. CM, CR, CS, JE, and BU mainly contributed to the acquisition of data. ACl, ACh, and JK were the key-persons in the PET/CT data analysis (ACl and ACh) and the process of performing western blots (JK). All authors contributed significantly to the interpretation of data, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved, approved publication of the content, revised the manuscript critically, and added important intellectual content. JW and RF-S performed biochemical profiling. CR, JK, and JW performed western blotting. ACl, ACh, AK, and LE performed PET/CT. BU and ACh performed MRI. CS perfomred DXA-scans.

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REVIEW
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# Menstrual Cycle Effects on Exercise-Induced Fatigability

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Estrogen and progesterone have distinct concentrations across the menstrual cycle, each one promoting several physiological alterations other than preparing the uterus for pregnancy. Whether these physiological alterations can influence motor output during a fatiguing contraction is the goal of this review, with an emphasis on the obtained effect sizes. Studies on this topic frequently attempt to report if there is a statistically significant difference in fatigability between the follicular and luteal phases of the menstrual cycle. Although the significant difference (the *P*-value) can inform the probability of the event, it does not indicate the magnitude of it. We also investigated whether the type of task performed (e.g., isometric vs. dynamic) can further influence the magnitude by which exercise-induced fatigue changes with fluctuations in the concentration of ovarian hormones. We retrieved experimental studies in eumenorrheic women published between 1975 and 2019. The initial search yielded 921 studies, and after manual refinement, 46 experimental studies that reported metrics of motor output in both the follicular and luteal phases of the menstrual cycle were included. From these retrieved studies, 15 showed a statistical difference between the luteal and follicular phases (seven showing less fatigability during the luteal phase and eight during the follicular phase). The effect size was not consistent across studies and with a large range (-6.77; 1.61, favoring the luteal and follicular phase, respectively). The inconsistencies across studies may be a consequence of the differences in the limb used during the fatiguing contraction (upper vs. lower extremity), the type of contraction (isometric vs. dynamic), the muscle mass engaged (single limb vs. full body), and the techniques used to define the menstrual cycle phase (e.g., serum concentration vs. reported day of menses). Further studies are required to determine the effects of a regular menstrual cycle phase on the exercise-induced fatigability.

**Keywords: endurance, strength, time to task failure, progesterone and estradiol, menstrual cycle, fatigue**

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## INTRODUCTION

Regular fluctuations in ovarian hormone levels, particularly estrogen and progesterone during the normal ovulatory cycle, produce profound alterations on the body homeostasis of women between the ages of ˜13–50 years (Marsh and Jenkins, 2002; Janse de Jonge, 2003). For example, estradiol, which is a potent estrogen, is known to strongly modulate vascular flow (Tostes et al., 2003; Joyner et al., 2015) and glycogen utilization (Hackney, 1999), whereas progesterone can increase ventilation and body temperature at rest (Marsh and Jenkins, 2002). These hormone-induced

physiological alterations have the potential to produce considerable differences in performance during fatiguing exercises. Serum concentrations of estrogen and progesterone fluctuate markedly throughout the menstrual cycle, which lasts ˜23–32 days, and these fluctuations also vary among women (Stricker et al., 2006). On average ovulation occurs at day 14, and it is preceded by a follicular phase and followed by a luteal phase (on average, 12–14 days each). The hallmark of the early follicular phase (days 1–7) is the low levels of estrogen and progesterone. During the mid-follicular phase (days 7–10), estrogen slowly starts to increase and peaks in the late follicular phase (days 10–14) followed by a sharp drop just before ovulation. After ovulation, estrogen and progesterone increase during the luteal phase reaching a plateau during the mid-luteal phase (days 20–26) and later decrease again during the late luteal phase.

Fatigability is typically defined as an exercise-induced reduction in force (Enoka and Duchateau, 2008), and this construct may be influenced by the individuals' subjective perceptions during the task (Kluger et al., 2013). Fatigability is also task dependent (Enoka and Stuart, 1992; Bigland-Ritchie et al., 1995). More specifically, the demands of the task (e.g., isometric vs. dynamic contractions) will stress different physiological sites that in turn also receive strong regulatory input from the ovarian hormones. Any influence these hormones have during fatiguing contractions across a menstrual cycle is complex because of the several systems involved (cardiorespiratory, neuromuscular, etc.). Several studies attempted to address this topic by investigating the effects of the menstrual cycle on the exercise-induced reduction in force (see results). Studies evaluating the effects of menstrual cycle on the fatigability typically report if there is a statistical significant difference between cycle phases (e.g., follicular vs. luteal), and while a significant difference (*P*-value) can inform the reader about the probability of the event, it does not indicate its magnitude (Cohen, 1988). Effect sizes are a useful tool to quantify the magnitude of difference between conditions, such as the magnitude of fatigability across the menstrual cycle. Understanding the effect size of the fluctuations in the ovarian hormones together with the *P*-value has important implications. For example, one can determine if an intervention has a greater effect size than the regular fluctuations promoted by the concentrations of estrogen and progesterone, given that both have a significant effect. Additionally, the obtained effect sizes may provide valuable information for studies that need to control and test for fatigability across the cycle.

The goal of this mini-review is to summarize the effects of the ovarian hormonal fluctuations on the exercise-induced reduction in force during fatiguing contractions with emphasis on the effect size. Our hypothesis is that the menstrual cycle phase will influence the exercise-induced decline in force, but the effects will vary according to the task performed and the limb used. Data was retrieved from database searches using a combination of terms: "menstrual cycle," "menstrual phase," "menstruation," "progesterone," "estrogen," "follicular phase," "luteal phase," "fatigue," "fatigability," "time to task failure," "endurance performance." Moreover, wildcard terms such as "menstru"* and "fatig"* were also used. In this initial search, 921 studies were obtained. The retrieved manuscripts were further refined by including experimental studies in eumenorrheic women not taking oral contraceptives published in English between 1975 and 2019. We focused on studies that describe the metrics of motor output (time to failure, maximal voluntary contraction, power, work, etc.), and we included studies that reported exercise-induced reduction in force during both the luteal and follicular phases. These inclusion criteria returned 46 experimental studies used in this review.

## Data Analyses

To estimate the effects of the menstrual cycle on the exercise-induced fatigability, we calculated the Hedges's *g* effect size, as it is adequate for small sample sizes typically found in the retrieved studies (Hedges, 1981). The mean difference between the follicular phase and the luteal phase was calculated for each variable using the follicular phase as a reference (e.g., Hedge's *g* = follicular phase – luteal phase/pooled standard deviation), so a positive effect size indicates that the follicular phase had greater values on average. For each study, we carefully indicated the specific menstrual cycle phase used for the effect size calculation (e.g., early follicular vs. mid follicular; reported in each table) to account for variations across studies. It was not possible to calculate the effect size for the manuscripts that did not report exact standard deviations and/or average values (for example studies that included standard deviation or averages only in the figures). Additional interpretations throughout the text were obtained by calculating the percentage difference between the cycle phases. **Tables 1–6** also indicate if the original report found a statistical significant difference ($P < 0.05$) between the phases of the menstrual cycle.

## Fatigability During Isometric, Isotonic, and Isokinetic Tasks

### Time to Task Failure

The menstrual cycle phase has equivocal effects on the time to task failure (**Table 1**). The different results across studies may be a consequence of the type of muscle contraction (e.g., intermittent vs. sustained) or the muscle group used (upper vs. lower extremity vs. whole body). For example, for the knee extensor muscles, some reported a ∼26% greater time to task failure during the mid-luteal phase compared to the early follicular phase during an intermittent isometric contraction (effect size: −0.84; **Table 1**; Ansdell et al., 2019). During sustained isometric contractions with the knee extensors, although the follicular phase had a trend to show greater time to task failure, there were no statistical differences between the menstrual cycle phases (Tenan et al., 2016). For the upper extremity muscles, three studies showed no difference between the luteal phase and the follicular phase during a sustained isometric contraction with the hand or elbow flexor muscles (Wirth and Lohman, 1982; Hoeger Bement et al., 2009; Jarvis et al., 2011). However, two studies reported that the follicular phase had approximately a 7–60% longer time to task failure than the luteal phase during a sustained isometric contraction with the hand muscles (Petrofsky et al., 1976, 2007). The greater endurance time during the follicular

**TABLE 1 |** Time to task failure.

| References | *n* | Training status | BMI (kg/m$^2$) | Age (yr) | Muscle | Contraction type | Intensity | Cycle phase | Effect size [95%CI] | Stat diff |
|---|---|---|---|---|---|---|---|---|---|---|
| Ansdell et al. (2019) | 30 | Not reported | 24 | 25 ± 4 | KE | Isometric intermittent | 60% of MVC | *e*F vs. *m*L | −0.84 [−1.37; −0.32] | Y (↑ *m*L) |
| Birch and Reilly (1999) | 17 | Not reported | 21 | 18–32 | Whole body | Isometric sustained | 45% of MVC | *m*F vs. *m*L | 0.24 [−0.43; 0.92] | N |
| Birch and Reilly (2002) | 10 | Not reported | – | 24 ± 3 | Whole body | Isometric sustained | 45% of MVC | F vs. L | −0.22 [−1.10; 0.66] | N |
| Hoeger Bement et al. (2009) | 20 | Not reported | 23 | 21 ± 1 | Elb. Flex | Isometric sustained | 25% of MVC | *m*F vs. *m*L | 0.02 [−0.61; 0.64] | N |
| Jarvis et al. (2011) | 11 | Not endurance trained | 23 | 33 ± 10 | Handgrip | Isometric sustained | 40% of MVC | *e*F vs. *m*L | – | N |
| Petrofsky et al. (1976) | 4 | Not reported | 21 | 25 ± 5 | Handgrip | Isometric sustained | 40% of MVC | F vs. L | – | Y (↑ F) |
| Petrofsky et al. (2007) | 8 | Non-athletes | 18 | 25 ± 9 | Handgrip | Isometric sustained | 20,40, and 60% of MVC | F vs. L | – | Y (↑ F) |
| Tenan et al. (2016) | 9 | Recreationally active | – | 25 ± 5 | KE | Isometric sustained | 25% of MVC | *e*F vs. *l*F vs. *m*L vs. *l*L | – | N |
| Wirth and Lohman (1982) | 10 | Not reported | – | 18–33 | Handgrip | Isometric sustained | 50% of MVC | F vs. L | −0.33 [−1.21; 0.55] | N |

*eF, Early follicular; Elb. Flex, Elbow Flexors; F, Follicular; KE, Knee extensors; KF, Knee flexors; L, Luteal; lF, Late follicular; MVC, Maximal Voluntary Contraction; mL, Mid luteal; lL, Late luteal; N, No statistical difference between the phases; Y, yes, there was a statistical difference between the menstrual cycle phases. Upward arrow indicates which menstrual cycle phase had greater time to task failure (i.e., lower fatigability). yr, years old.*

**TABLE 2 |** Percentage decline in maximal strength after a fatiguing contraction.

| References | *n* | Training status | BMI (kg/m$^2$) | Age (yr) | Muscle | Contraction type | Intensity | Cycle phase | Effect size [95%CI] | Stat diff |
|---|---|---|---|---|---|---|---|---|---|---|
| Ansdell et al., 2019 | 30 | Not reported | 24 | 25 ± 4 | KE | Isometric intermittent | 60% of MVC | *e*F vs. *m*L | – | N |
| Dibrezzo et al., 1988 | 21 | Not reported | – | 18–36 | KE | Isokinetic 240 °/s | Maximum | *e*F vs. L | 0.10 [−0.50; 0.71] | N |
| | | | | | KF | | | | 0.03 [−0.58; 0.63] | N |
| Friden et al., 2003 | 10 | No more than two training session/week | 23 | 25 ± 4 | KE | Isokinetic 120 °/s | Maximum | F vs. L | −0.16 [−1.04; 0.72] | N |
| Janse de Jonge et al., 2001 | 15 | Not reported | – | 30 ± 8 | KF | Isokinetic 240 °/s | Maximum | *l*F vs. L | 0.19 [−0.52; 0.91] | N |
| | | | | | KE | Isokinetic 240 °/s | | | −0.01 [−0.72; 0.71] | N |
| Nicolay et al., 2008 | 11 | Not reported | | 17–30 | Hand | Isometric Intermittent | Maximum | *e*F vs. L | 0.94 [0.06; 1.82] | Y (↑ L) |
| Pallavi et al., 2017 | 100 | Not reported | 21 | 18 ± 1 | Finger Flexors | Isotonic | – | F vs. L | 1.10 [−1.39; −0.80] | Y (↑ F) |

*eF, Early follicular; F, Follicular; KE, Knee extensors; KF, Knee flexors; L, Luteal; lF, Late follicular; MVC, Maximal Voluntary Contraction; mL, Mid luteal; N, No statistical difference between the phases; Y, yes, there was a statistical difference between the menstrual cycle phases. Upward arrow indicates which menstrual cycle phase had greater decline in maximal strength (i.e., greater fatigue). yr, years old.*

phase was larger at the lower contraction intensity compared to larger ones (20 vs. 60% of maximum) (Petrofsky et al., 2007).

## Fatigue Index

Six studies reported fatigue index calculated as the percent decline in maximal voluntary contraction relative to baseline. For the lower extremity muscles, the menstrual cycle phase did not influence the fatigue index (Dibrezzo et al., 1988; Janse de Jonge et al., 2001; Friden et al., 2003; Ansdell et al., 2019). However, for the upper extremity muscles, some indicated a ∼4% greater decline in force (i.e., greater exercise-induced fatigability) during the follicular phase compared to the luteal phase (52 ± 4 vs. 56 ± 4% of baseline, respectively) (Pallavi et al., 2017), whereas others reported a ∼15% larger reduction in force during the luteal phase (follicular: 96 ± 19 vs. luteal: 81 ± 11% baseline) (Nicolay et al., 2008; **Table 2**). Differences in the task performed may also have contributed to the mixed results. For example, the fatigue index was assessed in the upper extremity muscles during isotonic and isometric intermittent tasks whereas for the lower extremity the tasks were isokinetic and isometric intermittent (**Table 2**). Isometric intermittent was performed in both the upper extremity and lower extremity muscles, and the luteal phase had greater exercise-induced fatigability (i.e., greater decline in force) for the former muscles only.

## Maximal Strength

We examined changes in maximal strength across the menstrual cycle as they can strongly influence the time to task failure (Carlson and McCraw, 1971; Hunter and Enoka, 2001). Out of the studies retrieved, only four found a statistical difference between the follicular phase and the luteal phase (**Table 3**) and better detailing of their effect sizes according to the limb involved and the task performed is described below:

**TABLE 3 |** Maximal voluntary strength.

| References | *n* | Training status | BMI (kg/m$^2$) | Age (yr) | Muscle | Contraction type | Cycle phase | Effect size [95% CI] | Stat diff |
|---|---|---|---|---|---|---|---|---|---|
| Ansdell et al. (2019) | 30 | Not reported | 24 | 25 ± 4 | KE | Isometric | *e*F vs. *m*L | −0.04 [−0.54; 0.47] | N |
| Boot et al. (1999) | 15 | Not reported | 22 | 30 ± 8 | Handgrip | Isometric | F vs. L | 0.32 [−0.40; 1.04] | Y (↑ F) |
| | | | | | KE | Isometric | | -0.04 [−0.75; 0.68] | N |
| Dibrezzo et al. (1988) | 21 | Not reported | – | 18–36 | KF | 60 °/s | *e*F vs. L | 0.23 [−0.37; 0.84] | N |
| | | | | | | 180 °/s | | 0.16 [−0.45; 0.76] | N |
| | | | | | | 240 °/s | | 0.12 [−0.48; 0.73] | N |
| | | | | | KE | 60 °/s | | 0.12 [−0.49; 0.73] | N |
| | | | | | | 180 °/s | | 0.14 [−0.47; 0.74] | N |
| | | | | | | 240 °/s | | 0.08 [−0.52; 0.69] | N |
| Friden et al. (2003) | 10 | No more than two training session/week | 23 | 25 ± 4 | KE | Isokinetic 120 °/s | F vs. L | −0.16 [−1.04; 0.72] | N |
| | | | | | Handgrip | Isometric | | −0.16 [−1.04; 0.72] | N |
| Higgs and Robertson (1981) | 12 | Not reported | – | 19–23 | Handgrip | Isometric | *m*F vs. L | – | N |
| | | | | | KE | Isometric | | – | N |
| Jarvis et al. (2011) | 11 | Not endurance trained | 23 | 33 ± 10 | Handgrip | Isometric | *e*F vs. *m*L | – | N |
| Janse de Jonge et al. (2001) | 15 | Not reported | – | 30 ± 8 | KE | Isometric | *l*F vs. L | −0.17 [−0.88; 0.55] | N |
| | | | | | | Isokinetic 60 °/s | | −0.14 [0.85; 0.58] | N |
| | | | | | | Isokinetic 240 °/s | | −0.10 [−0.82; 0.61] | N |
| | | | | | KF | Isokinetic 60 °/s | | −0.15 [−0.86; 0.57] | N |
| | | | | | | Isokinetic 240 °/s | | −0.14 [−0.86; 0.57] | N |
| | | | | | Handgrip | Isometric | | 0.02 [−0.69; 0.74] | N |
| Lebrun et al. (1995) | 16 | Trained: ($V0_2$ max: 54 ± 1 ml/kg/min) | 21 | 28 ± 4 | KE - RL | Isokinetic 30°/s | F vs. L | 0.19 [−0.50; 0.89] | N |
| | | | | | KE - LL | | | 0.002 [−0.69; 0.70] | N |
| | | | | | KE - RL | | | −0.56 [−1.27; 0.14] | N |
| | | | | | KE - LL | | | −0.21 [−0.91; 0.48] | N |
| Nicolay et al. (2008) | 11 | Not reported | 23 | 17–30 | Handgrip | Isometric | *e*F vs. L | – | N |
| Pallavi et al. (2017) | 100 | Not reported | 21 | 18 ± 1 | Handgrip | Isometric | F vs. L | 1.61 [1.29; 1.93] | Y (↑ F) |
| Petrofsky et al. (1976) | 4 | Not reported | 21 | 25 ± 5 | Handgrip | Isometric | F vs. L | – | N |
| Quadagno et al. (1991) | 12 | Weight lifters (3× /week) | – | 24.3 | Bench Press | Dynamic | *e*F vs. *l*L | −0.01 [−0.81; 0.79] | N |
| | | | | | Leg press | Dynamic | | 0.01 [−0.80; 0.80] | N |
| Sarwar et al. (1996) | 10 | Not reported | 21 | 21 ± 1 | Handgrip | Isometric | *e*F vs. *l*F vs. *m*L | – | Y (↑/F) |
| | | | | | KE | | | – | Y (↑/F) |
| Sipaviciene et al. (2013) | 18 | Physically Active | 20 | 20 ± 2 | KE | Isometric | *e*F vs. *l*F | 0.26 [−0.91; 0.40] | N |
| Tenan et al. (2016) | 9 | Recreationally active | – | 25 ± 5 | KE | Isometric | *l*F vs. *m*L | – | Y (↑/F) |
| Wirth and Lohman (1982) | 10 | Not reported | – | 18–33 | Handgrip | Isometric | F vs. L | 0.30 [−0.58; 1.18] | N |

*eF, Early follicular; F, Follicular; KE, Knee extensors; KF, Knee flexors; lL, Left Leg; RL, Right leg; L, Luteal; lF, Late follicular; mL, Mid luteal; N, No statistical difference between the phases; Y, yes, there was a statistical difference between the menstrual cycle phases. Upward arrow indicates which menstrual cycle phase had greater strength. yr, year old.*

**TABLE 4 |** Cycling endurance time.

| References | *n* | Training status | BMI ($kg/m^2$) | Age (yr) | Protocol | Intensity | Cycle phase | Effect size [95%CI] | Stat diff |
|---|---|---|---|---|---|---|---|---|---|
| Bailey et al. (2000) | 9 | Trained ($VO_2$ peak: 50 ± 4 mL/kg/min) | 22 | 27 ± 7 | Constant | 70% of $VO_2$ peak | F vs. L | – | N |
| Campbell et al. (2001) | 8 | Endurance trained: ($VO_2$ peak: 54 ± 1 mL/kg/min | 21 | 24 ± 2 | Mixed | Time trial test after cycling 2 h at 70% $VO_2$ peak | F vs. L | −1.31 [−2.39; −0.23] | Y (↑ F) |
| Jurkowski et al. (1981) | 9 | $VO_2$ peak: 50 ± 4 mL/kg/min | 21 | 22 ± 1 | Mixed | 20 min at 1/3 max power, 20 min at 2/3 of max power and later 90% of max power until failure | F vs. L | −2.67 [−3.9; −1.4] | Y (↑ L) |
| Janse et al. (2012) | 8 | Recreationally active ($VO_2$ peak: 40 ± 7 mL/kg/min) | 24 | 24 ± 4 | Mixed | 60 min at 60% of $VO_2$ max + incremental until failure | *e*F vs. *m*L | 0.23 [−0.75; 1.22] | N |
| Kraemer et al. (2006) | 8 | Active ($VO_2$ peak not reported) | 22 | 27 ± 1 | Incremental | 1 kp/2 min | F vs. L | 1.14 [0.09; 2.2] | N |
| Lara et al. (2019) | 13 | Well-trained ($VO_2$ peak: 48 ± 7 mL/kg/min) | 21 | 31 ± 6 | Incremental | 25 W/min until failure | *e*F vs. *m*L | – | N |
| McLay et al. (2007) | 9 | Moderately trained ($VO_2$ Peak: 50 ± 4 mL/kg/min) | 24 | 25 ± 7 | Time trial | Self-paced | *m*F vs. *m*L | −0.14 [−1.07; 0.78] | N |
| Nicklas et al. (1989) | 6 | Moderately trained ($VO_2$ Peak: 45 ± 2 mL/kg/min) | 22 | 26 ± 2 | Constant | 70% of $VO_2$ peak | *m*F vs. *m*L | −0.75 [−1.92; 0.42] | N |
| Oosthuyse et al. (2005) | 11 | $VO_2$ Peak: 30–45 mL/kg/min | 22 | 24 ± 3 | Time trial | Self-paced | *e*F vs. *l*F vs. *m*L | 0.03 [–0.81; 0.86] | N |
| Redman et al. (2003) | 14 | Sedentary ($VO_2$ Peak: ∼42 mL/kg/min) | 23 | 21 ± 4 | Incremental | 25 W/2 min until failure | F vs. L | 0.00 [−0.74; 0.74] | N |

*eF, Early follicular; F, Follicular; mF, Mid Follicular; mL, Mid luteal; L, Luteal; N, No statistical difference between the phases; Y, yes, there was a statistical difference between the menstrual cycle phases. Upward arrow indicates which menstrual cycle phase had lower fatigability. yr, years old.*

**TABLE 5 |** Running endurance time.

| References | *n* | Training status | BMI ($kg/m^2$) | Age (yr) | Protocol | Intensity | Cycle phase | Effect size [95%CI] | Stat diff |
|---|---|---|---|---|---|---|---|---|---|
| Bandyopadhyay and Dalui (2012) | 45 | Sedentary (estimated $VO_2$ max: ∼39 ml/kg/min | 21 | 23 ± 3 | Constant | 8–10 Km/h | *e*F vs. L | −6.77 [−7.84; −5.70] | Y (↑ L) |
| Beidleman et al. (1999) | 8 | Physically active ($VO_2$ max: ∼ 47 ml/kg/min) | 22 | 33 ± 3 | Constant | 70% of $VO_2$ maximum | F vs. L | −0.10 [−1.08; 0.88] | N |
| Bemben et al. (1995) | 5 | Moderately active ($VO_2$ max: ∼ 43 ml/kg/min) | 24 | 22 ± 4 | Incremental | Increased 1% grade/min | *e*F vs. *m*L | 0.02 [−1.22; 1.26] | N |
| Bryner et al. (1996) | 3 | $VO_2$ max: ∼ 40 ml/kg/min | | 18–30 | Constant | 80% of maximum HR | *m*F vs. *m*L | 0.17 [−1.43; 1.78] | N |
| De Souza et al. (1990) | 16 | Trained ($VO_2$ max: 53 ± 4 ml/kg/min) | 19 | 29 ± 4 | Incremental | 2% every 2 min until max | *e*F vs. *m*L | 0.32 [−0.37; 1.02] | N |
| Lebrun et al. (1995) | 16 | Trained ($VO_2$ max: 54 ± 1 ml/kg/min) | 21 | 28 ± 4 | Constant | 90% of $VO_2$ maximum | F vs. L | 0.25 [−0.94; 0.45] | N |
| | | | | | | 8 mph at 20% incline | F vs. L | 0.04 [−0.65; 0.74] | N |
| McCracken et al. (1994) | 9 | Trained, non-athletes ($VO_2$ max: 46 ± 3 ml/kg/min) | - | 18–32 | Incremental until 30 min then constant after it | 35, 60, 75% of $VO_2$ max before 30 min. Then a 90% $VO_2$ max was performed after 30 min | *m*F vs. *m*L | 0.06 [−0.86; 0.99] | N |
| Higgs and Robertson (1981) | 12 | Not reported | - | 19–23 | All out, workload greater than $VO_2$ max | Maximum | *e*F vs. *l*F | – | Y (↑/F) |

*eF, Early follicular; F, Follicular; HR, Heart Rate; mF, Mid follicular; mL, Mid luteal; L, Luteal; lF, Late follicular; N, No statistical difference between the phases; Y, yes, there was a statistical difference between the menstrual cycle phases. Upward arrow indicates which menstrual cycle phase had greater endurance time (i.e., less fatigability). yr, years old.*

### *Isometric tasks*

For the lower extremity muscles, some indicated no change across the menstrual cycle (Boot et al., 1999; Janse de Jonge et al., 2001; Bambaeichi et al., 2004; Sipaviciene et al., 2013; Ansdell et al., 2019; Sung and Kim, 2019), whereas others indicated the maximum strength was 10% greater during the follicular and ovulatory phases (Sarwar et al., 1996; Tenan et al., 2016). For the upper extremity muscles, the results are also mixed as some reported 5–20% greater strength during the follicular (i.e., low levels of estrogen and progesterone) (Bassey et al., 1995; Boot et al., 1999; Pallavi et al., 2017), and 10% greater strength during the ovulatory or luteal phases (i.e., greater estrogen concentration

**TABLE 6 |** Ratings of perceived exertion.

| References | *n* | Training status | BMI ($kg/m^2$) | Age (yr) | Task | Intensity | Cycle phase | Effect size [95% CI] | Stat diff |
|---|---|---|---|---|---|---|---|---|---|
| Ansdell et al. (2019) | 30 | Not reported | 24 | 25 ± 4 | Isometric intermittent | 60% of MVC | *e*F vs. *m*L | – | Y [↑ *m*L] |
| Bailey et al. (2000) | 9 | Trained ($VO_2$ peak: 50 ± 4 mL/kg/min) | 22 | 27 ± 7 | Cycling | 70% of $VO_2$ peak | F vs. L | – | N |
| Beidleman et al. (1999) | 8 | Physically active ($VO_2$ max: ∼47 ml/kg/min) | 22 | 33 ± 3 | Running | 70% of $VO_2$ max | F vs. L | 0.26 [−0.72; 1.25] | N |
| Birch and Reilly (1999) | 17 | Not reported | 21 | 18–32 | Isometric | 45% of MVC | *m*F vs. *m*L | – | N |
| Birch and Reilly (2002) | 10 | Not reported | – | 24 ± 3 | Isometric | 45% of MVC | F vs. L | – | Leg RPE: Y (↑ F) |
| | | | | | | | | – | Whole Body RPE: N |
| De Souza et al. (1990) | 16 | Trained ($VO_2$ max: 53 ± 4 ml/kg/min) | 20 | 29 ± 4 | Running | Incremental 2%/2 min until maximum | *e*F vs. *m*L | 0.11 [−0.58; 0.81] | N |
| Gamberale et al. (1975) | 12 | $VO_2$ max: 36.3 ml/kg/min | – | 27 | Cycling | 40 and 70% of $VO_2$ peak | *e*F vs. *l*F vs. *l*L | – | Y (↑ *e*F) |
| Hackney et al. (1991) | 6 | $VO_2$ peak: 44 ml/kg/min | 22 | 26 ± 6 | Cycling | 70% of $VO_2$ peak | *m*F vs. OV vs. *m*L | – | Leg RPE: Y (↑ OV) |
| | | | | | | | | | Whole Body RPE: N |
| Hooper et al. (2011) | 73 | Nonactive (<90 min of intense physical activity/week) | 24 | 25.5 ± 7 | Running | 65% of $VO_2$ max | *e*F vs. *L* | – | Y (↑ *e*F) |
| Janse et al. (2012) | 12 | Recreationally active ($VO_2$ max: 40 ± 7 mL/kg/min) | 25 | 24 ± 4 | Cycling | 60 min at 60% of $VO_2$ max + incremental until failure | *e*F vs. *m*L | 0.67 [−0.15; 1.5] | N |
| Kraemer et al. (2006) | 8 | Active ($VO_2$ max not reported) | 22 | 27 ± 1 | Cycling | 1 kp/2 min | F vs. L | −0.76 [−1.77; 0.26] | N |
| Lara et al. (2019) | 13 | Well trained ($VO_2$ max: 48 ± 7 mL/kg/min) | 21 | 31 ± 6 | Cycling | 25 W/min until maximum | *e*F vs. *m*L | −0.15 [−0.92; 0.62] | N |
| Sunderland and Nevill (2003) | 7 | Well trained ($VO_2$ max: 51 ± 1 mL/kg/min) | 22 | 20 ± 0 | Running: Rep. sprints | Maximum | F vs. L | −0.55 [−1.62; 0.52] | N |

*eF, Early follicular; F, Follicular; mL, Mid luteal; L, Luteal; lF, Late Follicular; lL, Late luteal; OV, Ovulatory; N, No statistical difference between the phases; Y, yes, there was a statistical difference between the menstrual cycle phases. Upward arrow indicates which menstrual cycle phase had greater Ratings of Perceived Exertion (greater perceived fatigability). yr, years old.*

compared to the follicular phase) (Phillips et al., 1996; Sarwar et al., 1996). Others showed no changes across the cycle (Janse de Jonge et al., 2001; Friden et al., 2003; Nicolay et al., 2008; Jarvis et al., 2011; Sakamaki-Sunaga et al., 2016; **Table 3**).

### *Isokinetic tasks*

No effect of the menstrual cycle phase was observed during tests performed with the knee extensors and flexors at 30, 60, 90, 120, or 240 degrees/second (Dibrezzo et al., 1988; Lebrun et al., 1995; Janse de Jonge et al., 2001; Friden et al., 2003; Bambaeichi et al., 2004; Sipaviciene et al., 2013; Wikstrom-Frisen et al., 2017).

### *Dynamic constant resistance*

No influence of the menstrual cycle phase was observed for one-repetition maximum (1-RM) during the bench press, bicep curl, half-squat, and leg press tests (Quadagno et al., 1991; Kraemer et al., 1995; Markofski and Braun, 2014; Sakamaki-Sunaga et al., 2016; Romero-Moraleda et al., 2019a,b).

## Cycling

**Table 4** summarizes the data for endurance time during a cycling task. The follicular and luteal phases produced similar time results in a 15–30 km event (time trial) (Oosthuyse et al., 2005; McLay et al., 2007), or when cycling at 60 or 70% of the $VO_2$ peak to exhaustion (Nicklas et al., 1989; Bailey et al., 2000; Janse et al., 2012). During an incremental protocol, the menstrual cycle had no effect on the endurance time (Redman et al., 2003; Kraemer et al., 2006) or the power output (Casazza et al., 2002; Redman et al., 2003; Smekal et al., 2007). Conversely, when the intensity was increased to a 90% $VO_2$ peak after the individuals had cycled at lower intensities earlier in the protocol, the time to task failure was ∼50%

longer during the luteal phase compared to the follicular phase (Jurkowski et al., 1981).

The influence of the menstrual cycle phase on exercise-induced fatigability during cycling was also evaluated during single or repeated sprints, and the cycle phase had no effect on the peak power or the drop-off in work during the sprints (Giacomoni et al., 2000; Middleton and Wenger, 2006; Shaharudin et al., 2011; Wiecek et al., 2016; Tounsi et al., 2018). However, the average work was slightly greater during the luteal phase compared to the follicular phase for the 10 × 6 s sprint (39.3 vs. 38.3 $J.kg^{-1}$, respectively) (Middleton and Wenger, 2006).

## Running

The time to exhaustion was similar between the follicular and luteal phases when running at 70 or 90% of the $VO_2$ max, 80% of the maximum heart rate, or performing an anaerobic speed test (i.e., 8 miles/hour at a 20% incline), or an incremental protocol (McCracken et al., 1994; Bemben et al., 1995; Lebrun et al., 1995; Bryner et al., 1996; Beidleman et al., 1999; **Table 5**). The menstrual cycle phase had no effect on the maximum running velocity (Burrows and Bird, 2005). However, at a supramaximal intensity (greater than $VO_2$ max), women had a greater endurance time (∼15%) during the late follicular phase compared to the early follicular phase (Higgs and Robertson, 1981). Endurance time was also longer (∼17%) during the luteal and mid follicular phases compared to the early follicular phase in a constant protocol at 8–10 $km.h^{-1}$ (Bandyopadhyay and Dalui, 2012). During repeated sprints there was no difference between the follicular and luteal phases in the distance run (Sunderland and Nevill, 2003; Julian et al., 2017), or peak power (Tsampoukos et al., 2010).

Differences in running economy across the menstrual cycle have been suggested to impact running performance (Williams and Krahenbuhl, 1997). These suggestions are based on the observation that concentrations of progesterone, that peak in the luteal phase, are positively associated with ventilation at rest (Skatrud et al., 1978) and increased inspiratory muscle endurance during a breathing test (Chen and Tang, 1989). Ventilation during exercise, however, has mixed results. While some report greater ventilation during the luteal phase (Williams and Krahenbuhl, 1997), others report no change across the cycle (De Souza et al., 1990; Lebrun et al., 1995). A confounding factor may involve the training status of the individuals. Athletes, for example, had lower changes in ventilation across the menstrual cycle compared to non-athletes during an incremental cycling test (Schoene et al., 1981). Whether any change in running economy across the menstrual cycle is translated to performance fatigability, represented by the endurance time or sprint time, is not well-understood.

## Ratings of Perceived Exertion (RPE)

The exercise-induced reduction in force or power is potentially influenced by the individual's psychological state and perception of the task performed (Mosso et al., 1903; Enoka and Duchateau, 2016). Because progesterone concentration may be associated with perceptual responses (Gonda et al., 2008; Romans et al., 2013; Reynolds et al., 2018), we also investigated the influence of menstrual cycle phase on the individual's perception during the task performed, which was typically estimated with the RPE scale. The effects of the menstrual cycle phase on the RPE during fatiguing tasks was mixed. The effect sizes were variable and in both directions without a common trend (i.e., to the follicular or luteal phases) (**Table 6**; De Souza et al., 1990; Beidleman et al., 1999; Sunderland and Nevill, 2003). For example, for running the RPE was similar between the follicular and luteal phase (1% difference between the follicular and luteal phases with no statistical difference) (De Souza et al., 1990; Beidleman et al., 1999; Sunderland and Nevill, 2003) or approximately 2 points greater during the early follicular compared to luteal phase (Hooper et al., 2011). For cycling, the majority of studies do not show a statistical difference across the menstrual cycle (Stephenson et al., 1982; Bailey et al., 2000; Kraemer et al., 2006; Janse et al., 2012; Lara et al., 2019). However, one study reported the RPE was ˜1 point greater during the early follicular phase (menstruation) compared with the late follicular and late luteal when cycling either at 40 or 70% of $VO_2$ max (Gamberale et al., 1975). Conversely, the local leg RPE during cycling was 1–2 points greater during the ovulatory phase, but there was no change in total body RPE across the cycle (Hackney et al., 1991). During a functional isometric fatiguing task using the whole body (similar to a lifting a box from the ground), there was no effect of menstrual cycle phase on the total body RPE results (Birch and Reilly, 1999, 2002), but the local leg RPE was greater during the follicular compared to the luteal phase (Birch and Reilly, 2002). During an intermittent isometric fatiguing contraction with the knee extensor muscles, the RPE was greater during the luteal phase compared to the follicular phase (Ansdell et al., 2019).

# DISCUSSION

The goal of this mini-review is to summarize the magnitude of changes in the exercise-induced reduction in force across the regular menstrual cycle. From the retrieved studies, 9 out of 32 indicated the menstrual cycle phase had an effect (i.e., statistical difference) on the exercise-induced reduction in force (i.e., lower time to task failure or greater fatigue index) during either a single limb exercise with the lower or upper extremity, or whole body exercise (constant or incremental) (**Tables 1**, **2**, **4**, **5**). The results are also equivocal for maximal strength (4 out of 16 showing statistical difference, **Table 3**) and perception of the effort (5 out of 13 showing statistical difference, **Table 6**). The calculated effect size was variable, and the exercise-induced fatigability was shown to be greater either at the luteal or the follicular phase (-6.77; 1.61, respectively) (**Tables 1**–**6**). Task dependency of fatigability (i.e., exercise mode, intensity of the task, limb involved and environment) may influence the equivocal results across studies. Confounding factors such as the serum concentration of ovarian hormones, presence of ovulatory vs. anovulatory cycles, training and nutritional status should be considered in future studies. There is ample opportunity for investigations on the effects of regular hormonal fluctuations accounting for the task performed, environment and the limb involved. Below is a discussion about

the potential mechanisms driving the influence of the above-mentioned factors on the performance fatigability across the menstrual cycle.

## Metabolism

Metabolic responses are largely influenced by the concentration of estrogen with potential implications for motor performance (Lebrun, 1993; Wismann and Willoughby, 2006; Oosthuyse and Bosch, 2010). For example, an animal study indicated that supplementation of estrogen in ovariectomized rats increased endurance time of the animals and this finding was associated with the muscle glycogen-sparing effect (Kendrick et al., 1987). The greater concentration of estrogen during the luteal phase has potential to reduce fatigability in humans. During a cycling task performed at 65 or 70% of $VO_2$ max, women had less glycogen utilization (Hackney, 1999; Devries et al., 2006) and lower leg RPE values (Hackney et al., 1991) when the level of estrogen was high (i.e., mid-luteal phase) compared to the mid-follicular phase (low levels of estrogen). However, the upper and lower extremity muscles have different metabolic responses to ovarian hormones that may influence the exercise-induced fatigability. More specifically, the arm muscle exercise was shown to require greater reliance on glycogen compared to leg muscles (Ahlborg and Jensen-Urstad, 1991). Because the glycogen sparing is somewhat greater during the luteal phase (Wismann and Willoughby, 2006), perhaps the lesser reliance on glycogen in the legs is enhanced during the luteal phase allowing less fatigability compared to the arm muscles. This rationale would explain the greater endurance time during high levels of estrogen (i.e., luteal phase) for a knee extensors task performed with minimal impact on blood flow (Ansdell et al., 2019), whereas for the upper extremity minimal levels of estrogen paralleled a negligible (Hoeger Bement et al., 2009; Jarvis et al., 2011) or a greater (Petrofsky et al., 1976, 2007) endurance time. In this review, each table also indicates the training status of the individuals tested, as it can influence substrate availability in women (Ruby and Robergs, 1994; Carter et al., 2001). Another potential metabolism-associated factor driving the large variability in fatigability across the menstrual cycle between individuals, and perhaps explaining the lack of agreement between studies, is the estrogen-to-progesterone concentration ratio. In brief, progesterone is typically associated with increased catabolism whereas estrogen suppresses catabolism (Lamont et al., 1987; Bailey et al., 2000). Studies conducted in individuals with a lower estrogen to progesterone ratio typically fail to show differences in motor performance between the follicular and luteal phases (for a detailed review, see Oosthuyse and Bosch, 2010). For example, in presence of a larger estrogen-to-progesterone concentration ratio, cycling and running endurance times were longer (Jurkowski et al., 1981; Nicklas et al., 1989) compared with lower ratios (Beidleman et al., 1999; Bailey et al., 2000) (∼18–21 vs. 8–12 Pmol/nmol, respectively). Other factors influencing glucose availability, such as nutritional status and exercise intensity, may explain the conflicting findings in fatigability across the menstrual cycle (Lebrun, 1993; Oosthuyse and Bosch, 2010; Isacco et al., 2012), and perhaps should be considered when designing future studies addressing the menstrual cycle effects on the exercise-induced fatigability.

## Temperature

Fluctuations in the concentration of ovarian hormones may have consequences on exercise-induced fatigability because of changes in the core temperature. Progesterone acts in the hypothalamus increasing the body set point temperature (Stephenson and Kolka, 1993). Consequently resting body temperature is slightly higher (˜0.3–0.5°C) during the luteal phase compared with the follicular phase (Marshall, 1963; Nakayama et al., 1975). The greater body temperature during the luteal phase was shown to alter the perceptual and physiological responses during the exercise. For example, during a 60 min. cycling exercise, the greater core temperature paralleled the higher heart rate and ratings of perceived exertion during the luteal phase compared to the follicular phase, but only in the women who showed a large rise in serum progesterone concentration during the luteal phase (Pivarnik et al., 1992). During a sustained isometric contraction with the hand muscles, immersing the arm in warm water (37°C) decreased the time to task failure compared to the exercise performed at 24°C (Petrofsky et al., 1976, 2007). Both these results suggest the progesterone-induced increase in the body temperature could explain the increased fatigability in the luteal phase. However, other observations showed less exercise-induced fatigability at the luteal phase during isometric intermittent contractions with the lower extremity muscles, and whole body exercise and therefore do not agree with this hypothesis (Jurkowski et al., 1981; Bandyopadhyay and Dalui, 2012; Ansdell et al., 2019; **Tables 1**, **4**, **5**). Perhaps the menstrual cycle–induced alterations in metabolism in the arm and leg muscles (detailed above) have greater impact on performance fatigability than temperature.

Regulations in body temperature can also have implications for fatiguing exercises performed in hot environments. More specifically, if adequate body thermoregulation during exercise cannot account for the greater baseline temperature showed in the luteal phase, hot and humid environmental conditions may have a strong impact on the exercise-induced fatigability. Accordingly, individuals cycling in a hot environment (32°C, 60% humidity), had reduced time to exhaustion (˜6%) during the luteal phase compared to the follicular phase (Janse et al., 2012). The menstrual cycle phase, however, had no influence on the distance run during a repeated sprint test performed in a less extreme condition (31°C, 23% humidity) (Sunderland and Nevill, 2003). This latter conflicting result may be a consequence of the task performed as well as the ratio of progesterone to estrogen in the participants (Stephenson and Kolka, 1993). Although increased concentrations of progesterone can increase the core temperature, estrogen administration can attenuate these thermoregulatory effects, and a balance between the two hormones may influence the response to thermoregulation during exercise (Oosthuyse and Bosch, 2010).

## Limitations

This review has some limitations inherent to the studies retrieved. One of them is the classification of the menstrual cycle phase.

Some early studies used a somewhat arbitrary criteria (e.g., ovulatory vs. pre-menstrual vs. post-menstrual) assuming fertility in a regular 28-day cycle, which may not correspond to the phases determined by modern hormonal documentation or the presence of anovulatory cycles. Because of the variability in how follicles grow within the ovaries or presence of anovulatory cycles, which results in considerable discrepancies in the production of ovarian hormones among women, steps to better determine the phase of the cycle were recently proposed. They include a three-step method that comprise the evaluation of serum concentration, cycle mapping, and urinary ovulation prediction (Schaumberg et al., 2017; Sims and Heather, 2018). Future studies using this strategy can provide valuable information regarding the influence of the estrogen to progesterone concentration ratio on the exercise-induced reduction in force. Others have investigated the inconsistent results across the studies with emphasis on the technique used to identify the phase of the menstrual cycle (i.e., serum concentration vs. other methods such as day of menses or body temperature) and found out that only 44% of the studies actually measured the concentration of the female hormones (Janse et al., 2019). The early studies may also be influenced by the self-expectancy of individuals performing an exercise during the menstruation, as myths and cultural restrictions were perhaps more evident leading to negative attitude toward menstruation (Lebrun, 1993). To account for the above-mentioned limitations, the current review chose to present the effect size, the statistical significance and the menstrual cycle phase of each study separately and not compiled in a meta-analysis. Another limitation is the small sample size and presence of type I and type II errors in the retrieved studies. For example, a typical error of 10%, independent of the cycle phase, was found across visits when measuring the knee extensors maximal strength (Ansdell et al., 2019) or running endurance time (Bryner et al., 1996). Caution should be used when menstrual cycle related changes in motor output are below the error of the measurement. Future studies should consider using a control group to determine the error in the measurement independent of the hormonal fluctuations.

## SUMMARY

This review indicates the effects of the menstrual cycle phase on performance fatigability has mixed results. Although several studies did not indicate a difference between the classical definitions of luteal and follicular phases, some report greater fatigability during the luteal phase whereas others show greater fatigability during the follicular phase. Disagreement across studies may be a consequence of the limb (upper vs. lower) and task differences (dynamic vs. isometric), as well as inconsistencies in the definitions of the phase of the menstrual cycle and the relative concentration of progesterone to estrogen. As the number of retrieved studies was limited, there is an ample opportunity for addressing the impact of the regular menstrual cycle phase on the exercise-induced fatigability. Future studies should consider quantifying the measurement error and using a prospective design that allows carefully mapping the menstrual cycle, quantifying the estrogen to progesterone concentration ratio, and verifying the presence of the ovulatory and anovulatory cycles, as they may modify the hormonal fluctuations responsible for changes in the exercise-induced fatigability.

## AUTHOR CONTRIBUTIONS

HP designed the study. All authors interpreted the original studies included in this review, contributed to the drafting, and revised the manuscript.

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frontiers in Physiology

SYSTEMATIC REVIEW
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# Exercise Interventions in Polycystic Ovary Syndrome: A Systematic Review and Meta-Analysis

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**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. Intervention duration ranged from 6 to 26 weeks. A total number of 777 women were included in the meta-analysis. 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, 18.5–30.1), 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 may have the greatest impact on cardiorespiratory fitness,

body composition, and insulin resistance. Our results indicate that, a minimum of 120 min of vigorous intensity per week is needed to provide favourable health outcomes for women with PCOS with studies of longer duration required to evaluate outcomes with sustained exercise.

**Keywords: lifestyle intervention, physical activity, resistance training, exercise intensity, cardiorespiratory fitness, metabolic health, insulin resistance, high-intensity interval training**

# INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most common endocrine conditions, affecting 8–13% of reproductive aged women (Teede et al., 2018a). 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 (Rotterdam EA-SPCWG, 2004). Although not currently recognised in the diagnostic criteria, insulin resistance is a key aetiological factor contributing to the severity of reproductive and metabolic features (Poretsky et al., 1999; Altuntas et al., 2003), with obesity known to exacerbate the severity of clinical symptoms (Lim et al., 2012). 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 (Moran et al., 2010).

Exercise is well-established as a therapy for preventing and managing chronic diseases in the general population (Booth et al., 2000; Roberts and Barnard, 2005), and in women with PCOS (Wild et al., 2010; Teede et al., 2018a; Stepto et al., 2019). The beneficial effects of exercise in women with PCOS have been summarised in several recent systematic reviews and meta-analyses (Benham et al., 2018; Kite et al., 2019). 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 (Teede et al., 2018a). 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 (Teede et al., 2018b; Stepto et al., 2019). 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.

# METHODS

## 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.

## Search Strategy, Study Selection, and Data Extraction

We performed a systematic search of the literature to May 2019 inclusive 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.

## Eligibility Criteria

The Participant, Intervention, Comparison, Outcomes, and Studies (PICOS) framework was used for this systematic review (**Table 1**). 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. (2010), 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

**TABLE 1 |** Eligibility criteria for study inclusion.

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

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 1**).

## Assessment of Risk of Bias in Included Studies

The Downs and Black Checklist for the Assessment of Methodological Quality (Downs and Black, 1998) 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 (Cassar et al., 2016) 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.

## 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 estimate 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 (Yang, 2003). 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 *SD*s 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* (Hopkins et al., 2009). This *SD* was derived by combining (via variances) the mean of the study *SD*s 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**) (Cassar et al., 2016). 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 (Hopkins et al., 2009). The thresholds were halved for evaluation of the magnitude of the random-effect *SD*s (Smith and Hopkins, 2011). The meta-analysis also provided predicted

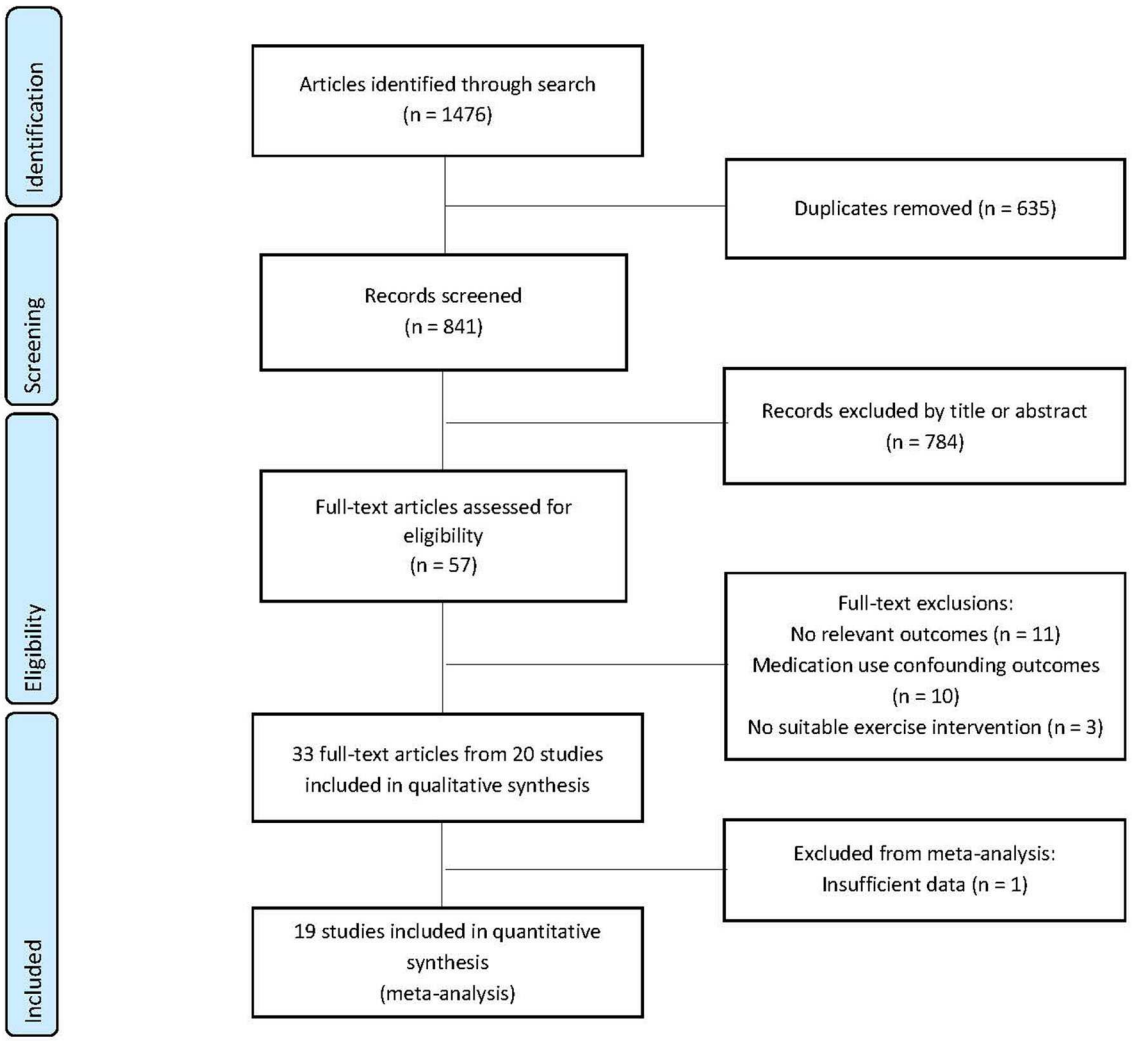


**FIGURE 1 |** Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) study selection flow diagram.

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 (Lakens et al., 2018). 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 *SD*s, and predicted population mean effects, hypotheses of substantial decrease, and increase were rejected if their respective *p*-values were <0.05. For predicted effects in individual settings, hypotheses of harm, and benefit were rejected if the respective *p*-values were <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 (Hopkins and Batterham, 2018; Hopkins, 2019). 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 (Hopkins et al., 2009). 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.

# RESULTS

The combined searches identified 1,476 papers for review. Of these, 635 were excluded due to duplication, 784 were removed by title and abstract screening, with 57 full-text articles reviewed. Twenty-four of these were excluded due to medication use, lack of data on outcomes of interest and intervention type, with 33 publications deemed suitable for inclusion in the systematic review. Multiple publications were identified for six exercise intervention studies and thereafter amalgamated with the largest reported number (*N*) for outcome measures of interest carried forward for meta-analysis. The remaining 20 articles were included in the systematic review with one article excluded from the meta-analysis due to using a non-parametric analysis (Brown et al., 2009) (**Figure 1**). A summary of study and participant characteristics, exercise intervention, and study outcomes is reported in **Table 2** and results of the methodological quality are presented in **Table 4** (for full results see **Supplementary Table 5**).

## Summary of Articles

Of the 20 trials included in the systematic review, 10 were RCTs (Bruner et al., 2006; Vigorito et al., 2007; Thomson et al., 2008; Brown et al., 2009; Jedel et al., 2011; Ladson et al., 2011; Nybacka et al., 2011; Almenning et al., 2015; Orio et al., 2016; Costa et al., 2018), five non-randomised uncontrolled trials (Randeva et al., 2002; Giallauria et al., 2008; Hutchison et al., 2012; Covington et al., 2016; Miranda-Furtado et al., 2016), one randomised parallel trial (Curi et al., 2012), and one non-randomised parallel trial (Orio et al., 2008), one randomised cross-over trial (Roessler et al., 2013), one single arm trial (Sprung et al., 2013a), and one non-randomised controlled trial (Sprung et al., 2013b). The mean age of participants ranged from 22 to 32 years. Baseline BMI ranged from 26.1 to 38.3 kg/m$^2$. Participants from 16 studies were diagnosed according to the Rotterdam criteria (Randeva et al., 2002; Bruner et al., 2006; Vigorito et al., 2007; Giallauria et al., 2008; Orio et al., 2008; Thomson et al., 2008; Jedel et al., 2011; Nybacka et al., 2011; Curi et al., 2012; Roessler et al., 2013; Sprung et al., 2013a,b; Almenning et al., 2015; Covington et al., 2016; Miranda-Furtado et al., 2016; Costa et al., 2018) and the remaining four studies used the NIH diagnostic criteria (Brown et al., 2009; Ladson et al., 2011; Hutchison et al., 2012; Orio et al., 2016). Sample sizes ranged from 5 to 62. Of the 20 reported interventions, 13 had full intervention supervision (Bruner et al., 2006; Vigorito et al., 2007; Giallauria et al., 2008; Orio et al., 2008, 2016; Brown et al., 2009; Nybacka et al., 2011; Hutchison et al., 2012; Sprung et al., 2013a,b; Covington et al., 2016; Costa et al., 2018), two had partial supervision (at least one supervised session per week; Ladson et al., 2011; Almenning et al., 2015), three had no supervision (Randeva et al., 2002; Jedel et al., 2011; Curi et al., 2012), and two did not report on supervision (Thomson et al., 2008; Roessler et al., 2013). Practitioners that supervised the exercise intervention included exercise physiologists, physiotherapists and physical activity educators.

Fourteen studies involved only a continuous aerobic intervention (Randeva et al., 2002; Vigorito et al., 2007; Giallauria et al., 2008; Orio et al., 2008, 2016; Brown et al., 2009; Jedel et al., 2011; Ladson et al., 2011; Nybacka et al., 2011; Curi et al., 2012; Sprung et al., 2013a,b; Covington et al., 2016; Costa et al., 2018), three had a high intensity aerobic interval training group (Hutchison et al., 2012; Roessler et al., 2013; Almenning et al., 2015), three had a resistance training group (Thomson et al., 2008; Almenning et al., 2015; Miranda-Furtado et al., 2016) and two had a combined resistance training and aerobic group (Bruner et al., 2006; Thomson et al., 2008). Of the interventions that included an aerobic exercise component, three included high intensity aerobic intervals (Hutchison et al., 2012; Roessler et al., 2013; Almenning et al., 2015), five included vigorous intensity exercise (Bruner et al., 2006; Vigorito et al., 2007; Giallauria et al., 2008; Orio et al., 2008, 2016), two included moderate to vigorous intensity exercise (Thomson et al., 2008; Costa et al., 2018) and nine included moderate intensity exercise (Randeva et al., 2002; Brown et al., 2009; Jedel et al., 2011; Ladson et al., 2011; Nybacka et al., 2011; Curi et al., 2012; Sprung et al., 2013a,b; Covington et al., 2016). 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 min.

Seventeen studies measured $VO_{2peak}$ (Randeva et al., 2002; Bruner et al., 2006; Vigorito et al., 2007; Giallauria et al., 2008; Orio et al., 2008, 2016; Thomson et al., 2008; Brown et al., 2009; Jedel et al., 2011; Ladson et al., 2011; Hutchison et al., 2012; Roessler et al., 2013; Sprung et al., 2013a,b; Almenning et al., 2015; Covington et al., 2016; Costa et al., 2018). Of these studies, 16 reported significant improvements following an exercise intervention (Randeva et al., 2002; Bruner et al., 2006; Vigorito et al., 2007; Giallauria et al., 2008; Orio et al., 2008, 2016; Thomson et al., 2008; Brown et al., 2009; Jedel et al., 2011; Hutchison et al., 2012; Roessler et al., 2013; Sprung et al., 2013a,b; Almenning et al., 2015; Covington et al., 2016; Costa et al., 2018). Nineteen studies measured metabolic outcomes (Randeva et al., 2002; Bruner et al., 2006; Vigorito et al., 2007; Giallauria et al., 2008; Orio et al., 2008, 2016; Thomson et al., 2008; Brown et al., 2009; Jedel et al., 2011; Ladson et al., 2011; Nybacka et al., 2011; Curi et al., 2012; Hutchison et al., 2012; Sprung et al., 2013a,b; Almenning et al., 2015; Covington et al., 2016; Miranda-Furtado et al., 2016; Costa et al., 2018), 11 of which reported significant changes in at least one marker of metabolic health (Bruner et al., 2006; Vigorito et al., 2007; Giallauria et al., 2008; Orio et al., 2008, 2016; Thomson et al., 2008; Ladson et al., 2011; Hutchison et al., 2012; Sprung et al., 2013b; Almenning et al., 2015; Covington et al., 2016). Four studies reported significant decreases in HOMA-IR (Thomson et al., 2008; Hutchison et al., 2012; Almenning et al., 2015; Orio et al., 2016), eight studies reported decreases in fasting insulin levels (Bruner et al., 2006; Vigorito et al., 2007; Giallauria et al., 2008; Orio et al., 2008, 2016; Thomson et al., 2008; Hutchison et al., 2012; Almenning et al., 2015), three reported significant improvements in glucose infusion or glucose disposal rates (Hutchison et al., 2012; Covington et al., 2016; Orio et al., 2016), five studies reported positive increases in HDL (Orio et al., 2008, 2016; Ladson et al., 2011; Almenning et al., 2015; Covington

**TABLE 2 |** Summary of studies identified for systematic review detailing participants, intervention characteristics and main outcomes measures.

| Study | Study design | QA score | Exercise *N* (total *N*) | Participant characteristics | PCOS diagnostic criteria | Exercise intervention characteristics | CRF outcomes | Cardiometabolic outcomes | Hormonal and reproductive outcomes | Body composition outcomes |
|---|---|---|---|---|---|---|---|---|---|---|
| **Included in meta-analysis** | | | | | | | | | | |
| Almenning et al. (2015) | RCT | 17 | HIIT = 8<br>RT = 8<br>(25) | Age = 27.2 ± 5.5<br>RT: BMI = 27.4 ± 6.9<br>HIIT: BMI = 26.1 ± 6.5 | Rotterdam | Type: Aerobic Intervals or RT<br>Frequency: 3/weeks<br>Intensity: Vigorous-High (70–95% $HR_{max}$)<br>Duration: 10 weeks<br>Supervision: Partial | HIIT:<br>↑ VO2peak<br>RT: No change | HIIT:<br>↓ HOMA-IR,<br>↓ Fasting Insulin,<br>↑ HDL<br>RT: No change | HIIT: No change<br>RT:<br>↓ AMH<br>↑ SHBG,<br>↓ FAI | HIIT:<br>↓ Fat mass,<br>↓ BF%<br>RT:<br>↓ BF%,<br>↑ Fat free mass |
| Bruner et al. (2006) | RCT | 10 | 7 (12) | Age = 32.3 ± 1<br>BMI = 36.2 ± 2 | Rotterdam | Type: Aerobic and RT<br>Frequency: 3/weeks<br>Intensity: Vigorous (70–85% $HR_{max}$)<br>Duration: 12 weeks<br>Supervision: Full | ↑ $VO_{2peak}$ | ↓ Fasting Insulin | No change | ↓ WC |
| Costa et al. (2018) | RCT | 17 | 14 (27) | Age = 27.6 ± 4.5<br>BMI = 25-39.9 | Rotterdam | Type: Aerobic<br>Frequency: 3/weeks<br>Intensity: Moderate-Vigorous (60–85% $HR_{max}$)<br>Duration: 16 weeks<br>Supervision: Full | ↑ $VO_{2peak}$ | No change | Not measured | ↓ BMI,<br>↓ WC |
| Curi et al. (2012) | Randomised parallel trial | 11 | 12 (27) | Age = 26.3 ± 1.4<br>BMI = 31.8 ± 1.6 | Rotterdam | Type: Aerobic<br>Frequency: N/R<br>Intensity: Moderate<br>Duration: 26 weeks<br>Supervision: None | Not measured | No change | No change | ↓ BMI,<br>↓ WC |
| Giallauria et al. (2008) | Uncontrolled trial | 17 | 62 (124) | Age = 22.8 ± 3.7<br>BMI = 29.2 ± 2.9 | Rotterdam | Type: Aerobic<br>Frequency: 3/weeks<br>Intensity: Vigorous (60–70% $VO_{2peak}$)<br>Duration: 12 weeks<br>Supervision: Full | ↑ $VO_{2peak}$ | ↓ Fasting Insulin,<br>↓ $AUCi_{ns}$,<br>↑ $AUC_{glu}/AUC_{ins}$ | ↑ SHBG | ↓ BMI,<br>↓ W/H |
| Hutchison et al. (2011)<br>Moran et al. (2011)<br>Hutchison et al. (2012)<br>Harrison et al. (2012) | Uncontrolled trial | 15<br>13<br>13<br>14 | 13 (21) | Age = 29.75 ± 1.4<br>BMI = 35.6 ± 5.8 | NIH | Type: Aerobic Intervals<br>Frequency: 3/weeks<br>Intensity: Vigorous (75–100% $HR_{max}$)<br>Duration: 12 weeks<br>Supervision: Full | ↑ $VO_{2peak}$ | ↑ GIR,<br>↓ Fasting Insulin,<br>↓ HOMA-IR. | ↓ AMH<br>No other changes | ↓ BMI |
| Ladson et al. (2011) | RCT | 17 | 59 (114) | Age = 28.8 ± 4.6<br>BMI = 38.3 ± 8 | NIH | Type: Aerobic<br>Frequency: ≥2/weeks<br>Intensity: Moderate<br>Duration: 26 weeks<br>Supervision: Partial | No change | ↑ $AUC_{glu}$,<br>↑ HDL | No change | ↓ WC |

*(Continued)*

**TABLE 2** | Continued

| Study | Study design | QA score | Exercise *N* (total *N*) | Participant characteristics | PCOS diagnostic criteria | Exercise intervention characteristics | CRF outcomes | Cardiometabolic outcomes | Hormonal and reproductive outcomes | Body composition outcomes |
|---|---|---|---|---|---|---|---|---|---|---|
| Miranda-Furtado et al. (2016)<br>Kogure et al. (2016)<br>Kogure et al. (2018) | Uncontrolled trial | 13<br>15<br>14 | 45 (97) | Age = 28.1 ± 5.4<br>BMI = 28.4 ± 6 | Rotterdam | Type: RT<br>Frequency: 3/weeks<br>Intensity: % of 1RM<br>Duration: 16 weeks<br>Supervision: Full | Not measured | No change | ↓ T,<br>↓ FAI,<br>↑ SHBG | ↓ WC |
| Moro et al. (2009)<br>Redman et al. (2011)<br>Covington et al. (2015)<br>Covington et al. (2016) | Uncontrolled trial | 10<br>10<br>9<br>9 | 8 (16) | Age = 25.6 ± 3.1<br>BMI = 32.1 ± 5.2 | Rotterdam | Type: Aerobic<br>Frequency: 5/weeks<br>Intensity: Moderate (55% $VO_{2peak}$)<br>Duration: 16 weeks<br>Supervision: Full | ↑ $VO_{2peak}$ | ↑ GDR,<br>↑ HDL | No change | No change |
| Nybacka et al. (2011)<br>Nybacka et al. (2013) | RCT | 10<br>12 | Exercise = 17, Diet and exercise = 12 (43) | Age = 31.8 ± 4.9<br>BMI = 34.9 ± 5.3 | Rotterdam | Type: Aerobic<br>Frequency: 2–3/weeks<br>Intensity: Moderate<br>Duration: 16 weeks<br>Supervision: Full | Not measured | No change | ↑ Menstrual Cyclicity.<br>No change in hormonal markers. | ↓ BMI |
| Orio et al. (2016) | RCT | 18 | 39 (136) | Age = 25.9 ± 2.7<br>BMI = 26.7 ± 2.8 | NIH | Type: Aerobic<br>Frequency: 3/weeks<br>Intensity: Vigorous (60–70% $VO_{2peak}$)<br>Duration: 24 weeks<br>Supervision: Full | ↑ $VO2_{2peak}$ | ↓ Fasting Insulin,<br>↓ HOMA-IR,<br>↑ GIR,<br>↓ $AUC_{ins}$,<br>↓ Total Cholesterol,<br>↑ HDL,<br>↓ LDL | No change | ↓ BMI,<br>↓ W/H |
| Orio et al. (2008) | Non-randomised parallel study | 14 | 32 (64) | Age = 18-40<br>BMI = 28.9 ± 3 | Rotterdam | Type: Aerobic<br>Frequency: 3/weeks<br>Intensity: Vigorous (60–70% $VO_{2peak}$)<br>Duration: 24 weeks<br>Supervision: Full | ↑ $VO_{2peak}$ | ↓ Fasting Insulin,<br>↓ AUCins,<br>↑ AUCglu/ins,<br>↑ HDL,<br>↓ LDL | No change | ↓ BMI<br>↓ WC,<br>↓ W/H |
| Randeva et al. (2002) | Uncontrolled trail | 12 | 12 (21) | Age = 29.7 ± 6.8<br>BMI = 33.9 ± 4.5 | Rotterdam | Type: Aerobic<br>Frequency: 3/weeks<br>Intensity: Moderate<br>Duration: 26 weeks<br>Supervision: None | ↑ $VO_{2peak}$ | No Change | Not measured | ↓ W/H |
| Roessler et al. (2013) | Randomised crossover trial | 15 | 8 (17) | Age = 31 (SEM−3)<br>BMI = 34.8 (SEM-2.5) | Rotterdam | Type: Aerobic and aerobic intervals<br>Frequency: 3/weeks<br>Intensity: Vigorous-High (70–75 and 80–100% $HR_{max}$)<br>Duration: 8 weeks<br>Supervision: N/R | ↑ $VO_{2peak}$ | Not measured | Not measured | ↓ Weight,<br>↓ BMI,<br>↓ WC |

*(Continued)*

**TABLE 2 |** Continued

| Study | Study design | QA score | Exercise *N* (total *N*) | Participant characteristics | PCOS diagnostic criteria | Exercise intervention characteristics | CRF outcomes | Cardiometabolic outcomes | Hormonal and reproductive outcomes | Body composition outcomes |
|---|---|---|---|---|---|---|---|---|---|---|
| Sprung et al. (2013a) | Single-arm trial | 15 | 6 (12) | Age = 28 (25 – 31)<br>BMI = 31 (28 – 34) | Rotterdam | Type: Aerobic<br>Frequency: 3–5/weeks<br>Intensity: Moderate (30–60% HRR)<br>Duration: 16 weeks<br>Supervision: Full | ↑ $VO_{2peak}$ | No change | No change | No change |
| Sprung et al. (2013b) | Non-RCT | 14 | 10 (17) | Age = 29 ± 7<br>BMI = 34 ± 6 | Rotterdam | Type: Aerobic<br>Frequency: 3–5/weeks<br>Intensity: Moderate (30–60% HRR)<br>Duration: 16 weeks<br>Supervision: Full | ↑ $VO_{2peak}$ | ↓ Total Cholesterol,<br>↓ LDL<br>No change in insulin sensitivity | No change | No change |
| Jedel et al. (2011)<br>Stener-Victorin et al. (2009)<br>Stener-Victorin et al. (2012) | RCT | 16<br>13<br>16 | 30 (74) | Age = 30.2 ± 4.7<br>BMI = 27.7 ± 6.4 | Rotterdam | Type: Aerobic<br>Frequency: 3/weeks<br>Intensity: Moderate<br>Duration: 16 weeks<br>Supervision: None | ↑ $VO_{2peak}$ | No change | ↑ SHBG,<br>↓ Free T,<br>↓ Estradiol | ↓ Weight,<br>↓ BMI |
| Thomson et al. (2008)<br>Thomson et al. (2012)<br>Thomson et al. (2016) | RCT | 11<br>13<br>10 | Diet and aerobic exercise = 18, Diet and combined exercise = 20 (52) | Age = 29.3 ± 6.8<br>BMI = 36.1 ± 4.8 | Rotterdam | Type: Aerobic, RT or combined aerobic and RT<br>Frequency: 5/weeks<br>Intensity: Moderate-Vigorous (60–80% $HR_{max}$ and 50–75% of 1RM)<br>Duration: 20 weeks<br>Supervision: N/R | ↑ $VO_{2peak}$ | ↓ HOMA-IR,<br>↓ Fasting Glucose,<br>↓ Fasting Insulin,<br>↓ Lipids | ↓ T,<br>↓ FAI,<br>↑ SHBG,<br>↑ Menstrual Cyclicity | ↓ Weight,<br>↓ WC,<br>↓ Fat mass,<br>↓ BF% |
| Vigorito et al. (2007) | RCT | 15 | 45 (90) | Age = 21.7 ± 2.3<br>BMI = 29.3 ± 2.9 | Rotterdam | Type: Aerobic<br>Frequency: 3/weeks<br>Intensity: Vigorous (60-70% $VO_{2peak}$)<br>Duration: 12 weeks<br>Supervision: Full | ↑ $VO_{2peak}$ | ↓ Fasting Insulin,<br>↓ AUCins,<br>↑ AUCglu/AUCins | ↑ Menstrual Cyclicity.<br>No change in hormonal markers. | ↓ WC,<br>↓ BMI,<br>↓ W/H |
| **Included for systematic review only** | | | | | | | | | | |
| Brown et al. (2009) | RCT | 17 | 8 (20) | Age = 36.5 (5)<br>BMI = 37.9 (9.4) | NIH | Type: Aerobic<br>Frequency: 3–5/weeks<br>Intensity: Moderate (40–60% $VO_{2peak}$)<br>Duration: 20–24 weeks<br>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-Mullerian 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.*

**TABLE 3 |** Meta-analysed effects on peak oxygen uptake ($VO_{2peak}$), body mass index (BMI) and waist circumference expressed as population mean effects in control and exercise groups, and as modifying effects of exercise duration, baseline, and dietary co-intervention.

| | $VO^{b}_{2peak}$ | | | 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 standardisation. 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.*

et al., 2016) and eight studies reported no changes in markers of metabolic health following an exercise intervention (Randeva et al., 2002; Brown et al., 2009; Jedel et al., 2011; Nybacka et al., 2011; Curi et al., 2012; Sprung et al., 2013a; Miranda-Furtado et al., 2016; Costa et al., 2018). Sixteen studies measured changes in hormonal markers and reproductive health (Randeva et al., 2002; Bruner et al., 2006; Vigorito et al., 2007; Giallauria et al., 2008; Orio et al., 2008, 2016; Thomson et al., 2008; Jedel et al., 2011; Ladson et al., 2011; Nybacka et al., 2011; Curi et al., 2012; Hutchison et al., 2012; Sprung et al., 2013a,b; Almenning et al., 2015; Covington et al., 2016; Miranda-Furtado et al., 2016). Five studies reported a significant increase in sex hormone binding globulin (SHBG) levels (Giallauria et al., 2008; Thomson et al., 2008; Jedel et al., 2011; Almenning et al., 2015; Miranda-Furtado et al., 2016), three studies reported significant decreases in FAI (Thomson et al., 2008; Almenning et al., 2015; Miranda-Furtado et al., 2016), two studies reported significant decreases in anti-mullerian hormone (AMH) levels (Hutchison et al., 2012; Almenning et al., 2015), three studies reported improvements in menstrual cyclicity (Vigorito et al., 2007; Thomson et al., 2008; Nybacka et al., 2011) and eight studies reported no changes in reproductive outcomes post exercise intervention (Bruner et al., 2006; Orio et al., 2008, 2016; Ladson et al., 2011; Curi et al., 2012; Sprung et al., 2013a,b; Covington et al., 2016). All 20 studies measured changes in body composition following exercise intervention. Sixteen reported significant changes in at least one measure of body composition (Randeva et al., 2002; Bruner et al., 2006; Vigorito et al., 2007; Giallauria et al., 2008; Orio et al., 2008, 2016; Thomson et al., 2008; Jedel et al., 2011; Ladson et al., 2011; Nybacka et al., 2011; Curi et al., 2012; Hutchison et al., 2012; Roessler et al., 2013; Almenning et al., 2015; Miranda-Furtado et al., 2016; Costa et al., 2018). Ten studies reported significant decreases in BMI (Vigorito et al., 2007; Giallauria et al., 2008; Orio et al., 2008, 2016; Jedel et al., 2011; Nybacka et al., 2011; Curi et al., 2012; Hutchison et al., 2012; Roessler et al., 2013; Costa et al., 2018), nine reported decreases in waist circumference (Bruner et al., 2006; Vigorito et al., 2007; Thomson et al., 2008; Ladson et al., 2011; Curi et al., 2012; Roessler et al., 2013; Miranda-Furtado et al., 2016; Orio et al., 2016; Costa et al., 2018), five reported decreases in waist to hip ratio (Randeva et al., 2002; Vigorito et al., 2007; Giallauria et al., 2008; Orio et al., 2008, 2016), three report decreased weight (Thomson et al., 2008; Jedel et al., 2011; Roessler et al., 2013). The remaining four studies reported no significant changes in any measure of body composition (Brown et al., 2009; Sprung et al., 2013a,b; Covington et al., 2016).

## Meta-Analysis

The results from the meta-analysis of the effect of exercise characteristics on cardiorespiratory fitness measured by $VO_{2peak}$, body composition (BMI and 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 2**, **3**) and predicted effects of exercise across various durations and baseline values (**Figures 2**–**4**).

### 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 was seen after vigorous intensity exercise (**Table 3**). Across all conditions, the modifying effects

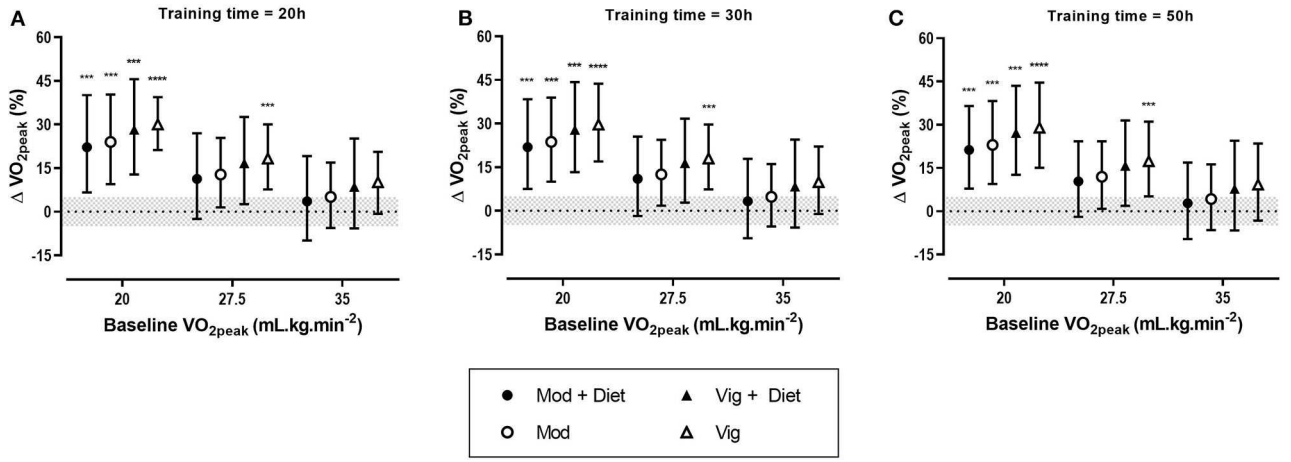


**FIGURE 2 |** Predicted effects of exercise alone or exercise plus diet versus a control group on peak oxygen uptake ($VO_{2peak}$) after 20 h **(A)**, 30 h **(B)**, and 50 h **(C)** of moderate (Mod), or vigorous (Vig) intensity exercise in an individual study setting. Clear effects are shown with the probability of a true substantial change (***very likely, ****most likely). Magnitudes in bold are clear with 99% compatibility intervals.

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 2**). 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.

## Effects 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 3**). 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 3**).

## Effects 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 3**).

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 3**). 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 h of exercise in comparison to 20 h of exercise (**Figure 3**).

## Effects 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, three included a resistance training intervention. Our analysis showed that the greatest improvements in FAI occurred after resistance training (**Table 3**). 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 3**).

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 4**). 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 h of exercise.

## 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 4**). 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 4**).

The predicted effects analysis on the effects of exercise on HOMA-IR show that clear improvements in HOMA-IR were

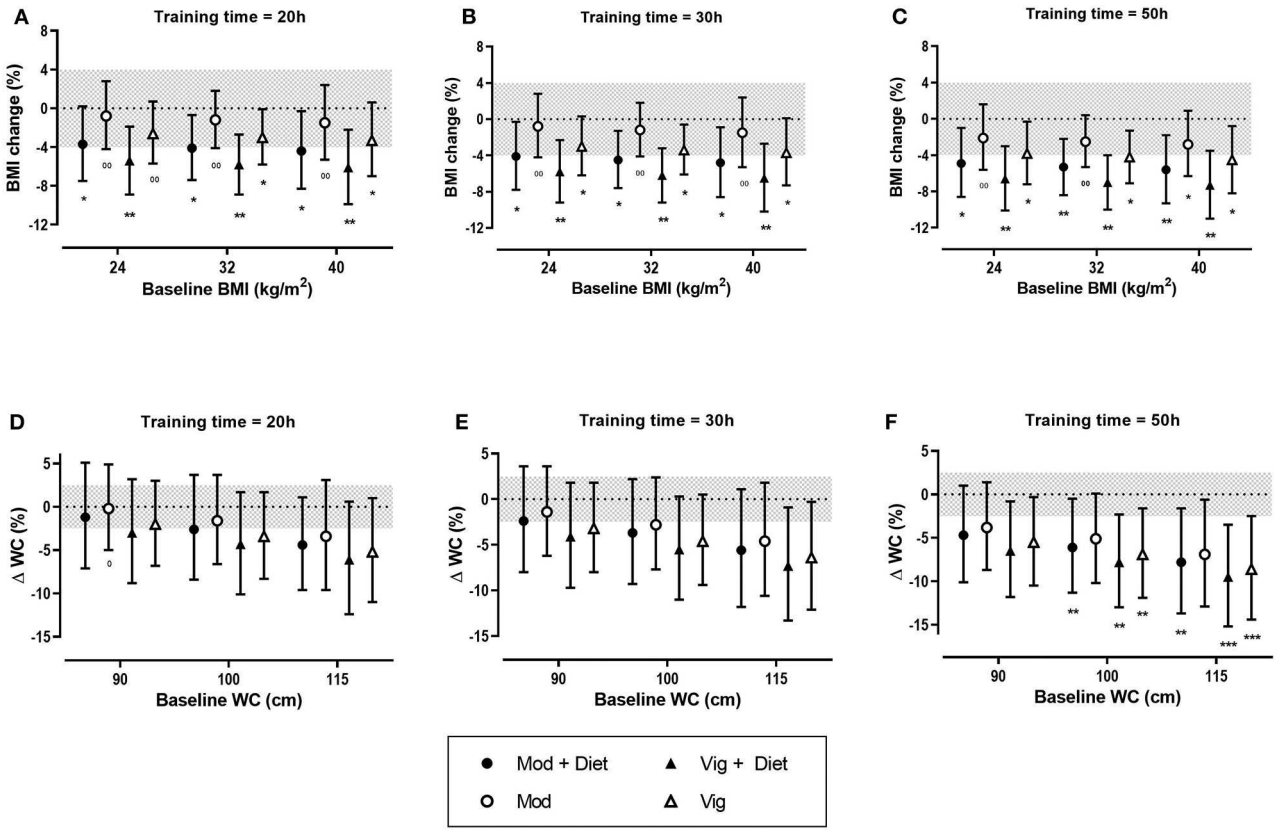


**FIGURE 3 |** Predicted effects of exercise alone or exercise plus diet versus a control group on body mass index (BMI) and waist circumference (WC) after 20 h **(A,D)**, 30 h **(B,E)**, and 50 h **(C,F)** of moderate (Mod), or vigorous (Vig) intensity exercise in an individual study setting. Clear effects are shown with the probability of either a true substantial change (*possibly, **likely, ***very likely) and/or a true trivial change ($^{0}$possibly, $^{00}$likely). Magnitudes in bold are clear with 99% compatibility intervals.

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 4**).

## 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 (Teede et al., 2018a; Stepto et al., 2019) and meta-analyses (Benham et al., 2018; Kite et al., 2019) 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 (Haqq et al., 2015; Kite et al., 2019; Stepto et al., 2019). $VO_{2peak}$ is a measure of cardiorespiratory fitness and is an important indicator of health and mortality (Blair et al., 1989). 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 (Barry et al., 2014). To illustrate this point, with each 3.5

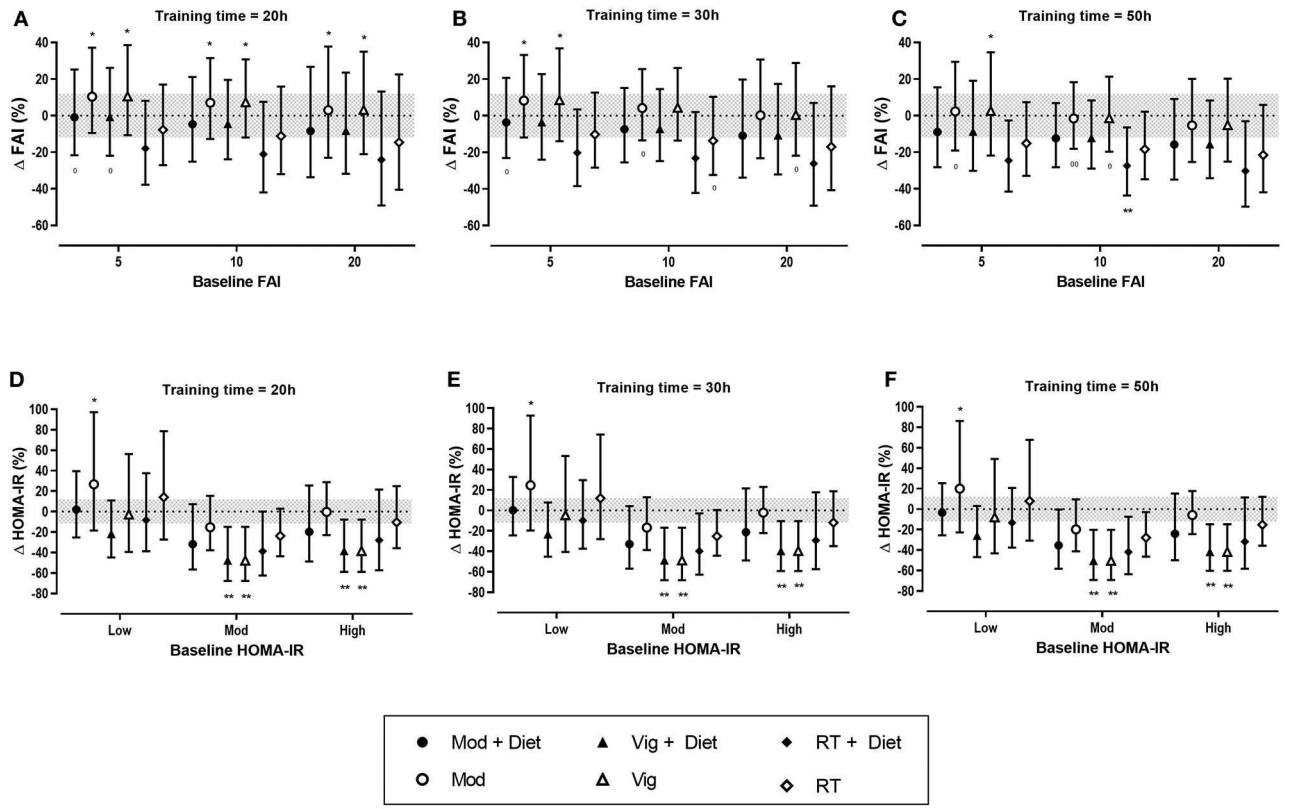


**FIGURE 4 |** Predicted effects of exercise alone or exercise plus diet versus a control group on free androgen index (FAI) and homeostatic model assessment of insulin resistance (HOMA-IR) after 20 h **(A,D)**, 30 h **(B,E)**, and 50 h **(C,F)** of moderate (Mod), vigorous (Vig) intensity exercise, 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.

mL/kg/min increase in $VO_{2peak}$, there is an associated 13% risk reduction from all-cause mortality (Kodama et al., 2009). 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 h 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 (Harrison et al., 2012; Roessler et al., 2013; Almenning et al., 2015; Costa et al., 2018) 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% (Lim et al., 2012). 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 (Teede et al., 2018a). However, it is important to note that health benefits can occur without significant weight loss (Hutchison et al., 2011; Sprung et al., 2013b; Covington et al., 2016). 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. (2008) 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. (2006) 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 (Rothman, 2008). 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 (Janssen et al., 2004). It is possible that exercise training alone

**TABLE 4 |** 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 ($^{00}$likely, $^{000}$very likely). Magnitudes in bold are clear with 99% compatibility intervals.*

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 (Dunaif et al., 1989; Diamanti-Kandarakis and Papavassiliou, 2006). Although not currently included in the diagnostic criteria, insulin resistance determined from insulin clamps is prevalent in 56–95% of women with PCOS (Stepto et al., 2013; Tosi et al., 2017; Li et al., 2019). 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 (Ishii et al., 1998; Holten et al., 2004; Ibañez et al., 2005), however, there is limited evidence to support the benefits of such training in PCOS (Thomson et al., 2008). We identified moderate decreases in HOMA-IR after resistance training interventions when compared to a control group. Resistance training is yet to be 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 (Pattyn et al., 2014; Weston et al., 2014; Jelleyman et al., 2015; Cassidy et al., 2017). Results from a study conducted by Greenwood et al. (2016) support the superior health benefits of vigorous exercise compared to moderate exercise in women with PCOS. They reported that 60 min of vigorous intensity exercise per week was associated with a 22% reduced odds of metabolic syndrome. In addition, Harrison et al. (2012) reported a 16% improvement in insulin sensitivity in women with PCOS following a 12-weeks vigorous intensity exercise intervention, as determined by the gold-standard euglycaemic-hyperinsulinaemic clamp method (DeFronzo et al., 1979). 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 (Tosi et al., 2017). 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 (Teede et al., 2011). Current research that measures FAI prior to and following an exercise intervention show contradictory results (Giallauria et al., 2008; Thomson et al., 2008; Stener-Victorin et al., 2012). 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 (Kite et al., 2019). 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% (Miranda-Furtado et al., 2016). In addition, a study comparing a 10-weeks 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 (Almenning et al., 2015). Although resistance training shows promising results, reductions in FAI have also been reported after aerobic exercise (Randeva et al., 2002; Giallauria et al., 2008; Covington et al., 2015). 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).

## 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 (Jones and Podolsky, 2015; Frieden, 2017) 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 (Jelleyman et al., 2015). 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.

# 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 h of vigorous intensity exercise over 10–12 weeks, equating to 120 min 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, and 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 of longer duration required to provide definitive exercise prescription recommendations in women with PCOS.

# DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: **Supplementary Table 3.**

# AUTHOR CONTRIBUTIONS

RP and RB conducted the literature search and data extraction. RP, RB, WH, and NS contributed to data analysis. RP, RB, and NS designed the figures and tables. All authors contributed to study design, data interpretation, and to writing and reviewing of the manuscript.

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# SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2020.00606/full#supplementary-material

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# MAMMA MIA! Norwegian Midwives' Practices and Views About Gestational Weight Gain, Physical Activity, and Nutrition

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**Objectives:** Most studies regarding prevalence of prenatal lifestyle counseling are based on patient report of provider advice. The aim of the present study was to describe midwives' practice and views in promoting three distinct, but importantly related lifestyle factors: gestational weight gain (GWG), regular physical activity (PA), and nutrition.

**Design:** A cross-sectional study.

**Setting:** Healthcare clinics in Oslo and Akershus County, Norway.

**Participants:** Clinics that expressed interest to participate provided an email list of the midwives. Of 107 midwives invited to participate, 65 completed the 15-min electronic survey (SurveyXact), giving a response rate of 60.7%.

**Outcome Measures:** We developed a new questionnaire based on questions and results from similar studies, as no validated questionnaires existed when we initiated this project in 2014. The final electronic questionnaire included a mix of close-ended questions, semi–close-ended questions, and 11-point Likert scales and covered demographics, personal health behaviors, counseling practice, views, and self-perceived role in lifestyle counseling.

**Results:** Mean workload in prenatal care was 78%, and mean years practicing was 8.9 (±7.5). Across all three health topics, most (74–95%) reported to give advice on the first meeting, with a mean frequency of 2.2 (±1.4), 2.7 (±1.8), and 2.7 (±2.0) for GWG, PA, and nutrition counseling, respectively. Approximately 40% did not report advice on GWG or give advice discordant with the Institute of Medicine (IOM) recommendations (2009) for at least one prepregnancy body mass index (BMI) category. GWG was rated as more unpleasant to talk about than PA (3.0 ± 2.8 vs. 1.1 ± 2.5, $p < 0.001$) and nutrition (3.0 ± 2.8 vs. 1.2 ± 2.5, $p = 0.002$). Also, regarding the importance of giving lifestyle advice, PA (9.6 ± 0.9 vs. 8.3 ± 2.2, $p < 0.001$) and nutrition (9.9 ± 0.4 vs. 8.3 ± 2.2, $p < 0.001$) were rated as more important than advice about GWG. Postpartum, nearly 40% gave advice about PA, whereas only two (3.1%) reported to discuss weight/weight retention ($p < 0.001$).

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**Conclusion:** While most midwives gave advice on GWG, PA, and nutrition at the first meeting and rated lifestyle counseling as an important topic, the advice on GWG was often discordant with IOM recommendations, and the topic was viewed as more unpleasant to talk about than PA and nutrition.

**Keywords: gestational weight gain, lifestyle counseling, midwives, nutrition, physical activity, pregnancy, prenatal care**

## INTRODUCTION

It is suggested that important life transitions, including medical diagnosis or other naturally occurring events, such as pregnancy and entering parenthood, may motivate individuals to adopt, change, and maintain health-enhancing behaviors (McBride et al., 2003). Having a baby is a major life event, and for the majority of women, pregnancy is a period when they have sustained contact with a healthcare provider (Shieh et al., 2010). In Norway, prenatal care is free of charge and generally provided through 8–12 alternating visits with midwives and general practitioners, including an ultrasound scan (Backe, 2001; The Norwegian Directorate of Health, 2019)[1]. This regular counseling throughout gestation, and that women may be motivated to make healthy lifestyle changes, has suggested pregnancy to be a "window of opportunity" (ACOG, 2015). A recently published systematic review and meta-analysis of individual participant data from 36 trials ($n$ = 12,526) showed that women randomized to lifestyle intervention and exercise had slightly lower average gestational weight gain (GWG) (−0.70 kg) compared to women in the control group (International Weight Management in Pregnancy (i-Wip) Collaborative Group, 2017). This is in accordance with others reviews (da Silva et al., 2017; Goldstein et al., 2017), favoring the intervention group compared with the controls (−0.9 to −1.1 kg). Similar evidence was found in overweight or obese populations (Choi et al., 2013; Farpour-Lambert et al., 2018). In addition, in high-income nations, 80–90% of women have at least one child during their reproductive years (Rostad et al., 2006; Martinez et al., 2012). Hence, healthcare providers may have a widespread opportunity to capitalize on this increased motivation by promoting positive behavior change.

The Institute of Medicine (IOM) (Institute of Medicine [IOM], 2009) has summarized substantial literature on GWG, including several cohort studies assessing GWG on pregnancy outcomes for women who are overweight or obese. The results show that, compared with higher and lower weight gain, GWG within the recommended range is associated with a reduced risk of maternal and infant complications (Institute of Medicine [IOM], 2009). Still, there are no formal, evidence-based guidelines from Norway or other European countries, and it is important to highlight that the IOM guidelines are largely based on observational data (Siega-Riz et al., 2009).

Regular physical activity (PA) and healthy eating may lead to adequate GWG and prevent postpartum weight retention and women's future risk of overweight and obesity, also with reference to women who already have a high body mass index (BMI) and their weight management (ACOG, 2013). In addition, a healthy lifestyle may reduce the risks of adverse pregnancy and birth outcomes, including gestational diabetes mellitus, gestational hypertension and preeclampsia, depressive symptoms, preterm birth, macrosomia, small for gestational age infants, and cesarean deliveries (ACOG, 2015; Mottola et al., 2018; Dipietro et al., 2019). Hence, it is important that women receive consistent and updated counseling on these topics during pregnancy (ACOG, 2015).

The American College of Obstetricians and Gynecologists issued a committee opinion report in 2013 (ACOG, 2013), emphasizing that healthcare providers should consider prepregnancy BMI at the first prenatal visit, with the aim to advice on appropriate GWG, PA/exercise, and nutrition throughout gestation. Correspondingly, The Norwegian Directorate of Health (2019) recommends that all pregnant women should receive lifestyle counseling on these topics. Still, studies indicate that provider–patient communication regarding maternal health behavior is inadequate, largely limited by lack of knowledge or skills to undertake this type of counseling, in addition to be of low priority (Stengel et al., 2012; Willcox et al., 2012; Wilkinson et al., 2013; Morris et al., 2017; McGee et al., 2018). Current data show a wide range in numbers receiving GWG advice (20–60%) and that women with obesity were more likely than normal-weight women to receive such guidance (Whitaker et al., 2016; Morris et al., 2017; Vinturache et al., 2017; Wilkinson et al., 2017). The factors influencing GWG counseling are many-sided, and it is important to also acknowledge the psychological aspects (such as affect, cognition, and personality) that could explain the dialog between the healthcare provider and the pregnant women (Kapadia et al., 2015). Hitherto, no studies have explored these associations.

McGee et al. (2018) reported that fewer than 25% of obstetricians regularly discussed exercise during prenatal care, whereas others have found somewhat higher numbers (30–60%) with respect to combined PA/nutrition counseling (Whitaker et al., 2016; Santo et al., 2017; Vinturache et al., 2017). Up to date, most studies regarding the prevalence of prenatal lifestyle counseling are based on the women's report of provider advice and the vast majority are done in the United States or Canada (Whitaker et al., 2016; Morris et al., 2017; Santo et al., 2017; Vinturache et al., 2017; Emery et al., 2018; McGee et al., 2018), except from a body of work on the management of GWG from Australia (Wilkinson et al., 2013; de Jersey et al., 2018, 2019). In many European countries, like Norway, midwives provide approximately 50% of the antenatal care (Backe, 2001; The Norwegian Directorate of Health, 2019, see text footnote 1),

[1]https://www.helsedirektoratet.no/retningslinjer/svangerskapsomsorgen/konsultasjoner-i-svangerskapsomsorgen

whereas this is far less prevalent especially in the United States (Vedam et al., 2018). Women report receiving different advice from obstetricians, general practitioners, and midwives (Ferrari et al., 2013), and the time of the visits varies, typically 10, 15, and 30 min, respectively (McDonald et al., 2012). Hence, the duration of these appointments may influence the amount of time available for counseling on lifestyle topics. Considering the above and that little is known about midwife-based prenatal care in Norway, including to what extent pregnant women are advised on GWG, PA, and nutrition, the primary aim of the present cross-sectional study was to describe midwives' practice and views about these three health topics.

# MATERIALS AND METHODS

## Study Design and Participants

This was a cross-sectional study design, using an electronic questionnaire (a paper version was also available) to investigate midwives' practice and views about GWG, PA/exercise, and nutrition, conducted in Oslo and Akershus County, Norway.

Oslo and Akershus are highly populated counties in Norway, comprising both urban and rural settings, with 45 healthcare clinics (Oslo: 17 and Akershus: 28) and 130 midwives working with prenatal care. The project manager called all healthcare clinics in Oslo and Akershus and talked to the head of the clinics. Midwives were considered eligible if they were seeing prenatal woman at the time of study enrollment. The clinics that expressed interest to participate received additional information about the project and responded back with an email list of the midwives working at the respective clinic. Because of restrictions of privacy, not all health clinics ($n = 12$) agreed to distribute email contact information. Of 107 invited midwives, 65 completed the questionnaire, giving a response rate of 60.7%. Unfortunately, we do not have any data or explanations about why certain health clinics did not want to participate in the study and are aware that this may have given intrinsic bias.

## Ethical Approval

The study was reviewed by the Regional Committee for Medical and Health Research Ethics (REK 2015/1941 A), who concluded that, according to the Act on medical and health research (the Health Research Act 2008), the study did not require full review by REK. The study was approved by the Norwegian Social Science Data Services (NSD 560627). Following the Helsinki Declaration, the first survey page included in-depth information about the study's purpose, procedures, and outcomes. It was emphasized that participation was voluntary and that they could withdraw from the project at any time with no explanation required. Hence, to complete the online survey, the midwives had to confirm that they had received adequate information and wanted to participate by checking the associated box. No financial support was given.

## Data Collection

The electronic survey (SurveyXact) was sent by e-mail directly from the project manager to the midwives and then automatically forwarded back to us after completion. After approximately 2 weeks, one reminder was sent to those who had not responded. The respondent could not change the answers after completion. One clinic wanted to use a paper version of the survey, and the head of the clinic handed out questionnaires to the midwives ($n = 10$). Two weeks later, the project manager picked up four completed surveys from these clinics.

## Outcome Measures

To our knowledge, no validated questionnaires on practices of the healthcare provider existed when we initiated this project in 2014. Hence, we developed a new questionnaire based on questions and results from similar studies (Entin and Munhall, 2006; Bauer et al., 2010; Chang et al., 2013). Both the electronic questionnaire and paper version included 72 questions, required approximately 15 min to complete, and were a mix of close-ended questions, semi–close-ended questions, and 11-point Likert scales, divided into seven subcategories. No validation was performed for this questionnaire; however, all question-and-answer options were piloted for comprehensibility among six midwives, as well as among the research group, and were revised accordingly. We will also highlight that future studies addressing midwives' practices and views about GWG, PA, and nutrition should include more information about personal lifestyle choices, including height and body weight for calculation of participants' BMI, as well as parity, valuable in assessing if this influenced lifestyle advice.

Following is a presentation of the four subcategories, 26 questions, and three statements used to answer the present research questions.

### Participants' Demographics

Participants' demographics addressed age, gender, number of years practicing antenatal care, and percentage of workload consisting of antenatal care.

### Personal Health Behaviors

Providers' personal behaviors regarding PA, diet, and smoking were assessed with the following questions (Haskell et al., 2007; Nordic Nutrition Recommendations, 2014):

- All adults are recommended to perform moderate-intensity PA (activities that make you breathe somewhat harder than normal, such as brisk walking, housework, etc.) for a minimum of 30 min 5 days a week, equal to 2.5 h. With this in mind, would you characterize yourself as physically active?
  Response options: "yes," "no," or "I don't know."
- The Norwegian Directorate of Health[2] recommends a balanced and varied diet, comprising whole-grain products, vegetables, fruits and berries, lean dairy products, fish, legumes, and nuts, while also limiting the amount of processed meats, red meat, and foods high in saturated fat, sugar, and salt. With this in mind, how would you characterize your diet in the current week?

[2]https://helsedirektoratet.no

The participants rated their diet on a scale from 0 to 10, where 0 represented "very bad," and 10 represented "excellent." According to these levels, the responses were also divided into two categories: healthy eating habits (excellent and good diet: ≥8) and unhealthy eating habits (average, bad and very bad diet: <8).

- Do you smoke daily?
  Response options: "yes" or "no".

### Counseling Practice

Whether or not the midwives gave advice to their pregnant women on GWG, PA, and nutrition was assessed using a simple yes or no question for each topic. Providers answering yes were asked to elaborate on what they based their advice on. The categorical responses developed for this study were as follows: "Recommendations from the Norwegian Directorate of Health (see text footnote 2)," "Research articles/scientific evidence," "Supplementary education/conferences," and "Own experiences." Participants had the opportunity to choose more than one response category.

Frequency ("How often do you give advice/inform about GWG, PA, and nutrition?") and when such advices were given throughout gestation (for each of the three health topics) were also examined. For the latter, the midwives could choose more than one categorical option: "first visit," "first trimester," "second trimester," "third trimester," "postpartum," and/or "at all occasions."

To evaluate if the advice regarding GWG was coherent with the IOM recommendations (2009), we asked the following questions: "Based on a women's prepregnancy BMI category, how much (total GWG in kg) would you recommend her to gain during pregnancy?: (1) underweight, (2) normal weight, (3) overweight, and (4) obese." Responses were given as one value.

### Views

If the midwives answered no to counseling on GWG, PA, and nutrition, the reasons why were investigated. The categorical responses were as follows: "I do not have the time to address lifestyle behaviors," "Physical activity/nutrition/weight gain is of low priority in the context of a typical prenatal visit," "I do not have sufficient knowledge/skills regarding physical activity/nutrition/weight gain," and "The women are not interested in talking about these topics."

Further, to explore self-perceived role in promoting lifestyle factors, the participants were asked to rate three different statements for each health topic:

- "To give pregnant women advice on weight gain/physical activity/nutrition/is a very important part of prenatal care."
- "It is uncomfortable/difficult to talk to pregnant women about physical activity/nutrition/weight gain."
- "For pregnant women and her baby, appropriate weight gain/physical activity/a healthy diet/is of great importance."

Responses indicated level of agreement with each statement on a scale from 0 (strongly disagree) to 10 (strongly agree). Response options and statements were based on results from similar studies (Chang et al., 2013; Morris et al., 2017).

## Statistical Analysis

All statistical analyses were conducted with SPSS software version 24 for Windows. Data were presented as numbers with percentages or means with standard deviation and $p$ values. $\chi^2$ analysis was used to compare categorical data and two-sided independent-sample $t$ test for continuous data. Differences in mean scores for self-perceived role in lifestyle counseling were compared using one-way analysis of variance.

All descriptive data were explored for normality and determined by skewness, histograms, and significance level (Kolmogorov–Smirnov test for normality) in SPSS. The histogram was emphasized if the three variables showed both normality and skewness. Even though some of the data were not normally distributed, we chose after discussion with professor in biostatistics (Morten Vang Fagerland, Head of Section for Biostatistics and Epidemiology, Oslo University Hospital, Norway), to compare differences using parametric tests because of the sample size ($n \geq 30$) (Pallant, 2013).

As most respondents reported a healthy lifestyle with respect to PA (88%) and healthy eating habits (68%), the numbers would have been too small to investigate the associations between poor personal health behavior and whether they provided counseling on GWG, PA, and nutrition. In addition, we did not ask about providers' body weight and are therefore not able to assess this as a as predictor of GWG advice.

# RESULTS

## Participant Characteristics

Of the 65 midwives participating in the study, 64 were women, and mean age was 47.5 years (±8.9). Mean workload in prenatal care was 78%, and mean years practicing was 8.9 (±7.5). Eighty-eight percent reported to be regularly active according to guidelines; 68% had high adherence to nutritional recommendations, and none used tobacco daily (**Table 1**).

## Practice and Views About Lifestyle Variables and Health Behaviors

All the midwives reported giving advice on PA at least once throughout gestation, whereas five (7.7%) and four (6.2%) reported no counseling on nutrition and GWG, respectively (**Table 2**). One answered that GWG advice is of low priority in the context of a typical prenatal visit; otherwise, no other reasons for not providing advice were reported.

Across all three health topics, most midwives reported to give advice on the first visit, with approximately one-quarter following up this counseling throughout gestation. Frequency of counseling was approximately twice for GWG, and three times for PA and nutrition (**Table 2**). Postpartum, nearly 40% gave advice about PA, whereas only two (3.1%) reported to talk about weight/weight retention ($p < 0.001$).

When viewing all three lifestyle factors as one, the vast majority of providers reported basing their advice on recommendations from the health authorities and a brochure

**TABLE 1 |** General characteristics and personal health behaviors of the participants ($n$ = 65).

| Variable | |
|---|---|
| Age in years [Mean (SD*)] | 47.5 (8.9) |
| Gender [n (%)] | |
| Women | 64 (98.5) |
| Years practicing [Mean (SD)] | 8.9 (7.5) |
| <10 years [n (%)] | 37 (57.8) |
| ≥10 years [n (%)] | 27 (42.2) |
| Proportion of prenatal care [n (%)] | |
| <50% | 6 (9.4) |
| ≥50% | 58 (90.6) |
| Location of health clinic [n (%)] | |
| Oslo | 36 (55.4) |
| Akershus | 29 (44.6) |
| Current daily smoker [n (%)] | 0 |
| Units of alcohol weekly [n (%)] | |
| None | 11 (17.2) |
| 1–5 (light drinking) | 49 (76.6) |
| 6–13 (moderate drinking) | 3 (4.7) |
| ≥14 (heavy drinking) | 1 (1.6) |
| Healthy eating habits [n (%)] | 44 (67.7) |
| Regular PA active** [n (%)] | 57 (87.7) |

**SD; Standard Deviation, **Moderate-intensity physical activity (PA) ≥ 2.5 h weekly.*

**TABLE 2 |** Practice and views about lifestyle variables and health behaviors ($n$ = 65).

| Variable | GWG | PA | Nutrition |
|---|---|---|---|
| Advice ≥ once throughout gestation [n (%)] | 61 (93.8) | 65 (100) | 60 (92.3) |
| Frequency of counseling [Mean (SD)] | 2.2 (1.4) | 2.7 (1.8) | 2.7 (2.0) |
| 1–3 talks | 35 (53.8) | 48 (80.0) | 42 (64.6) |
| 4–6 talks | 9 (13.8) | 9 (15.0) | 6 (9.2) |
| ≥7 talks | 0 (0) | 3 (5.0) | 5 (7.7) |
| Counseling was given at. *[n (%)] | | | |
| First visit | 48 (73.8) | 62 (95.4) | 53 (81.5) |
| 1st trimester | 14 (21.5) | 21 (32.3) | 15 (23.1) |
| 2nd trimester | 18 (27.7) | 27 (41.5) | 17 (26.2) |
| 3rd trimester | 14 (21.5) | 15 (23.1) | 13 (20.0) |
| Postpartum | 2 (3.1) | 24 (36.9) | 11 (16.9) |
| At all occasions | 18 (27.7) | 17 (26.2) | 21 (32.3) |
| Basis/source for the advice* [n (%)] | | | |
| Health authorities | 59 (90.8) | 63 (96.9) | 63 (96.9) |
| Research/scientific evidence | 23 (35.4) | 37 (56.9) | 29 (44.6) |
| Supplementary education/conferences | 7 (10.8) | 6 (9.2) | 13 (20.0) |
| Own experiences | 2 (3.1) | 21 (32.3) | 8 (12.3) |
| Handing out information pamphlets | 26 (40.0) | 40 (61.5) | 49 (75.4) |
| Self-perceived role in lifestyle counseling ** [Mean (SD)] | | | |
| *". to give advice is an important part of prenatal care"* | 8.3 (2.2) | 9.6 (0.9) | 9.9 (0.4) |
| *". it is uncomfortable to talk about"* | 3.0 (2.8) | 1.1 (2.5) | 1.2 (2.5) |
| *". GWG/PA/nutrition is important for a healthy pregnancy"* | 7.1 (3.0) | 9.9 (0.4) | 9.8 (0.6) |

**Selection of more than one response was allowed, and the participants were instructed to tick one or more alternatives that best matched their practice, **Responses indicated level of agreement with each statement on a scale from 0 (strongly disagree) to 10 (strongly agree).*

published by the Norwegian Directorate of Health[3] (**Table 2**). In addition, regarding PA counseling, 32.3% based their advice on personal sport/exercise experiences, whereas 3.1% and 12.2% used their own experiences to give advice on GWG and nutrition, respectively ($p = 0.006$). We do not have any data about numbers of midwives who had been pregnant and cannot further investigate if their own pregnancy experiences were important for the information provided.

The midwives rated dialog about GWG as more uncomfortable/difficult than discussions about PA ($p < 0.001$) and nutrition ($p = 0.002$). Also, regarding the importance of giving lifestyle advice during consultations, PA ($p < 0.001$) and nutrition ($p < 0.001$) were rated as more important subjects than giving advice about GWG (**Table 2**).

## Gestational Weight Gain

Nearly 40% of the midwives did not report or give advice on GWG discordant with the IOM recommendations (2009) for at least one prepregnancy BMI category. The proportion of providers giving advice consistent with the guidelines did not differ between the prepregnancy BMI categories, and mean values were within the recommended range for all groups (**Table 3**).

# DISCUSSION

Most midwives reported counseling pregnant women about GWG, PA, and nutrition at first prenatal visit. Still, repeated lifestyle counseling is not met as recommended by ACOG (2013) and the Norwegian health authorities (The Norwegian Directorate of Health, 2019). In the present study, few routinely provided women with advice about GWG, PA, and nutrition, and merely one-quarter gave advice about these topics more than once throughout gestation. GWG was viewed as more uncomfortable to talk about than PA and nutrition. Also, regarding the importance of giving lifestyle advice, PA and nutrition were rated as more important than advice about GWG, and 40% did not report or give advice on GWG discordant with the present recommendations for at least one prepregnancy BMI category.

The low rates of midwives answering the specific section about recommended total weight gain are of concern, because it is likely that non-respondents are those who do not give advice or are not familiar with the IOM guidelines. Postpartum, only two reported talking about weight/weight retention, compared with 36.9% and 16.9% having a dialog with the women about PA and nutrition, respectively. Hence, more midwives used this opportunity to talk about lifestyle choices, PA and healthy eating,

[3]https://helsedirektoratet.no/publikasjoner/gode-levevaner-for-og-i-svangerskapet

**TABLE 3 |** Midwives recommendations of GWG, using pre-pregnancy BMI groupings recommended by the Institute of Medicine (IOM) ($n$ = 65).

| Variable | Below | Within | Above | No response |
|---|---|---|---|---|
| Underweight women (pre-pregnancy BMI < 18.5) | 11 (16.9) | 35 (53.8) | 2 (3.1) | 17 (26.2) |
| Recommended GWG in kg [Mean (SD)] | | 14.8 (2.3) | | |
| Normal weight women (pre-pregnancy BMI 18.5–24.9) | 9 (13.8) | 39 (60.0) | 0 | 17 (26.2) |
| Recommended GWG in kg [Mean (SD)] | | 12.7 (1.7) | | |
| Overweight women (pre-pregnancy BMI 25.0–29.9) | 5 (7.7) | 41 (63.1) | 1 (1.5) | 18 (27.7) |
| Recommended GWG in kg [Mean (SD)] | | 8.8 (1.7) | | |
| Obese women (pre-pregnancy BMI ≥ 30) | 6 (9.2) | 37 (56.9) | 4 (6.2) | 18 (27.7) |
| Recommended GWG in kg [Mean (SD)] | | 6.1 (2.5) | | |

*Results are given in numbers (%), unless otherwise is specified.*

which may lead to healthy body weight, rather than attention on weight management.

In Australia, 66% reported that they needed more training in counseling and that insufficient time was a main barrier to conversing with women (de Jersey et al., 2019). In addition, our results are in contrast to what women state that they want during prenatal consultations, as studies conclude that most women would prefer GWG advice early, with regular follow-ups and discussions during the course of pregnancy, as well as postpartum (de Jersey et al., 2013; Nikolopoulos et al., 2017). Studies have also shown that prenatal nutrition and related lifestyle counseling throughout gestation may lower GWG and neonatal macrosomia, especially in a high-risk population, such as women with overweight or obesity (Mitchell et al., 2017; Peccei et al., 2017). Hence, healthcare providers have the potential to influence and be a reliable source of evidence-based health information during an important period of a woman's life (Weeks et al., 2018). de Jersey et al. (2018) found that a brief education session integrated into an existing mandatory training program has shown positive results in improving the knowledge and confidence of midwives in delivering advice and support for healthy GWG. Midwifery training could potentially also be a solution to this problem in Norway and better prepare midwives to discuss issues related to weight management during pregnancy and postpartum (Premji et al., 2019).

## Strengths and Weaknesses of the Study

One strength of the present study is the response rate and sample size, comprising a large proportion (50%) of all midwives working in Oslo and Akershus, two highly populated counties in Norway, comprising both urban and rural settings. Hence, our results have good generalizability, compared with most other research in this area, using qualitative methods with a small sample size (Willcox et al., 2012; Chang et al., 2013; Wennberg et al., 2014; Whitaker et al., 2016; Arrish et al., 2017). Also compared to other email surveys (Lutsiv et al., 2012; Ferraro et al., 2013; McGee et al., 2018), our sample size and response rate may be considered high. Furthermore, we adapted already validated questions and questions used in similar populations (Chang et al., 2013; Morris et al., 2017), and pilot-tested the survey before use.

This is the first study in Norway to describe midwives' practice with respect to lifestyle counseling on three distinct, but importantly related topics: GWG, PA, and nutrition, and also one of the first to report on midwives' knowledge of appropriate GWG, in conjunction with IOM guidelines (Institute of Medicine [IOM], 2009). Still, the results may only be generalizable to Norway.

While we were able to describe whether the midwives reported giving lifestyle advice, we could not evaluate the quality of the dialogue and information presented to the pregnant women. In addition, all data were self-reported, and the participants were aware of study aims; hence, we cannot rule out social desirability bias, or the "open book strategy," meaning that the responders checked the IOM weight gain guidelines before giving responses. Nevertheless, if this was the case, the results of the present study provide a conservative calculation of midwives' practice and views of GWG, PA, and nutrition.

## Discussion of Results

In high-income nations, the average number of prenatal consultations is 8–10, with only a slight difference between nulliparous and multiparous women (Backe, 2001; Stephansson et al., 2018). This frequent counseling throughout gestation and also that women may be more receptive and motivated to make changes for their own and their babies health have suggested pregnancy to be a particular "teachable moment" (ACOG, 2015). Still, and in line with the present results, qualitative research and surveys have shown that provider–patient communication regarding GWG, PA, and nutrition is infrequent and that, unless requested, few pregnant women receive regular guidance about these health topics (de Jersey et al., 2012, 2013; Stengel et al., 2012; Willcox et al., 2012; Whitaker et al., 2016; Morris et al., 2017; Wilkinson et al., 2017; Emery et al., 2018; McGee et al., 2018; Dalhaug and Haakstad, 2019). Hence, this evidence signals a lack of focus on lifestyle behavior in pregnancy and highlights the importance of improving healthcare provider's knowledge, confidence, and skills in giving such guidance. On the other side, most of the studies regarding prevalence of prenatal lifestyle counseling are based on the pregnant women's report of provider advice (Stengel et al., 2012; Whitaker et al., 2016; Emery et al., 2018; Dalhaug and Haakstad, 2019), and some have shown little congruence between patient and healthcare providers perceptions of counseling practice (Lutsiv et al., 2012; Ferraro et al., 2013). This discrepancy may be due to the healthcare providers giving socially desirable responses or the pregnant women not recalling having received advice from their healthcare provider.

Compared with women counseled by obstetricians or general practitioners, a higher proportion seeing midwives reported having discussed GWG, PA, and nutrition (35–39% vs. 64%) (McDonald et al., 2012; Premji et al., 2019). Also, midwives' self-report in promoting lifestyle counseling shows more frequent lifestyle advice compared to other healthcare providers (Lutsiv et al., 2012; Ferraro et al., 2013; Morris et al., 2017). Hence, there seems to be some evidence that midwives as a group discuss health-enhancing behaviors more often than other prenatal providers do. There is, however, a need for educational input to enhance the quality of information and confidence in delivering such advice for all healthcare providers, including the midwives.

Our results are consistent with the results of McDonald et al. (2012), showing that approximately 60% of pregnant women had received advice about appropriate GWG as recommended by IOM from their midwife. Still, a significant proportion of pregnant women do not receive evidence-based advice about appropriate GWG, and this lack of information may drive several pregnant women to seek other information sources such as internet, blogs, online forums, books, parenting magazines, family, and friends, often being less reliable (McDonald et al., 2012; Dalhaug and Haakstad, 2019). In addition, health professionals who view lifestyle counseling as important are likely those who respond to a survey related to GWG, PA, and nutrition, hence overestimating the rate of giving such advice in the present study, and as such being a potential source of bias (Morris et al., 2017).

## Explanations and Implications

There are many competing interests during prenatal consultations. Providers are required to assess medical, familial, pregnancy, and psychological history, as well as provide information, antenatal tests, procedures, and bookings (Willcox et al., 2012). Stengel et al. (2012) found that pregnant women often only received lifestyle advice from their provider at the initial prenatal visit and that it sometimes was limited to written education. On the other side, information leaflet may be valuable and a source that can be referred to and used by the women in the future.

The midwives in our study gave, on average, advice on GWG, PA, and nutrition between two and three times, including the postpartum visit. Also, a large proportion handed out information pamphlets, especially for healthy eating during pregnancy. However, approximately only 23% followed up advice on GWG and nutrition in all trimesters. We do not know why the midwives in the present study did not prioritize lifestyle counseling on a regular basis, as only one participant gave response to the question addressing this: "Why do you not prioritize to advice on lifestyle variables: GWG, PA, and nutrition?" Hence, this is a flaw in the questionnaire, and if other researchers should address the same issue, the question must be rephrased to address potential barriers (e.g., "What do you perceive as the most important barriers with respect to give lifestyle counseling?"). Others have reported that lack of time and not perceiving it as important are key barriers to advising pregnant women on GWG, PA, and nutrition (Willcox et al., 2012; Whitaker et al., 2016).

Providers in our study rated PA and nutrition as more important for a healthy pregnancy than favorable weight gain. These results agree with the studies of Willcox et al. (2012) and Chang et al. (2013), both showing that although management of GWG was given a low priority, most providers recognized the importance of diet and exercise. Sensitivity of the topic and the providers feeling uncomfortable when talking to women about GWG, knowing that some women may feel offended or embarrassed, could also be a barrier to discussing weight-related issues. Consistent with previous research (Chang et al., 2013; Whitaker et al., 2016), the midwives in our study reported GWG to be slightly unpleasant to talk about. Another study also found that midwives have expressed concern for the trend that many pregnant women are worried about putting on too much weight, and in order not to make women more anxious, midwives choose to avoid the topic (Willcox et al., 2012). It can be discussed whether it is acceptable that midwives avoid dialog about GWG and regardless of whether the women are afraid of gaining too much or, on the other side, need to limit GWG. Either way, given the importance of appropriate GWG, this should be properly addressed. Hence, responses to our survey, as well as other research (Willcox et al., 2012), indicate missed opportunities in information exchange, as well as a need to improve midwives' confidence and knowledge in giving GWG guidance.

## Future Research

Upcoming studies should evaluate more in-depth the quality of lifestyle counseling, including knowledge, beliefs, and attitudes of healthcare providers, as well as elucidate the effectiveness of different intervention approaches to increase the number of women who are accurately and effectively advised about GWG, PA, and nutrition during pregnancy. Research is also needed to evaluate midwife-based prenatal care compared with general practitioners.

## Conclusion

Few midwives routinely provided advice about GWG, PA, and nutrition, and merely one-quarter addressed these topics more than once throughout gestation. Hence, lifestyle counseling seems to be of low priority in the context of a typical prenatal visit. In addition, a high percentage did not provide data or give values on GWG discordant with the present IOM guidelines, as well as viewed GWG as more unpleasant to talk about than PA and nutrition. Given the importance of appropriate GWG, regular PA, and a healthy diet during pregnancy, as well as frequent and sustained contact between providers and pregnant women, the present results support the "window of opportunity" hypothesis. We further highlight that midwives need guidelines and education to play a more active role in lifestyle counseling. Studies are also warranted to increase our understanding of psychological factors associated with GWG guidance.

## DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Regional Committee for Medical and Health Research Ethics (REK 2015/1941 A), who concluded that, according to the Act on medical and health research (the Health Research Act 2008), the study did not require full review by REK. The study was approved by the Norwegian Social Science Data Services (NSD 560627). The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

LH and ED conceived the idea for the study and developed the survey questionnaire. JM and ED were responsible for participant follow-up and data collection. LH supervised the project and outlined the manuscript. All authors read and corrected draft versions of the manuscript and approved the final version.

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# Circulating and Adipose Tissue miRNAs in Women With Polycystic Ovary Syndrome and Responses to High-Intensity Interval Training

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MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression post-transcriptionally. In women with polycystic ovary syndrome (PCOS), several miRNAs are differentially expressed compared to women without PCOS, suggesting a role for miRNAs in PCOS pathophysiology. Exercise training modulates miRNA abundance and is primary lifestyle intervention for women with PCOS. Accordingly, we measured the expression of eight circulating miRNAs selected *a priori* along with miRNA expression from gluteal and abdominal adipose tissue (AT) in 12 women with PCOS and 12 women matched for age and body mass index without PCOS. We also determined the miRNA expression "signatures" before and after high-intensity interval training (HIT) in 42 women with PCOS randomized to either: (1) low-volume HIT (LV-HIT, 10 × 1 min work bouts at maximal, sustainable intensity, $n$ = 13); (2) high-volume HIT (HV-HIT, 4 × 4 min work bouts reaching 90–95% of maximal heart rate, $n$ = 14); or (3) non-exercise control (Non-Ex, $n$ = 15). Both HIT groups trained three times/week for 16 weeks. miRNAs were extracted from plasma, gluteal and abdominal AT, and quantified via a customized plate array containing eight miRNAs associated with PCOS and/or exercise training responses. Basal expression of circulating miRNA-27b (c-miR-27b), implicated in fatty acid metabolism, adipocyte differentiation and inflammation, was 1.8-fold higher in women with compared to without PCOS ($P$ = 0.006) despite no difference in gluteal or abdominal AT miR-27b expression. Only the HV-HIT protocol increased peak oxygen uptake ($VO_2$peak L/min; 9%, $P$ = 0.008). There were no changes in body composition. In LV-HIT, but not HV-HIT, the expression of c-miR-27b decreased (0.5-fold, $P$ = 0.007). None of the remaining seven circulating miRNAs changed in LV-HIT, nor was the expression of gluteal or abdominal AT miRNAs altered. Despite increased cardiorespiratory fitness, HV-HIT did not alter the expression of any circulating, gluteal or abdominal AT miRNAs. We conclude that women with PCOS have a higher basal

expression of c-miR-27b compared to women without PCOS and that 16 weeks of LV-HIT reduces the expression of this miRNA in women with PCOS. Intense exercise training had little effect on the abundance of the selected miRNAs within subcutaneous AT depots in women with PCOS.

**Keywords: exercise, miRNA-27b, insulin resistance, epigenetic modifications, cardiorespiratory fitness, female**

## INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in reproductive-aged women, affecting up to 13% of females globally (Bozdag et al., 2016). Beyond causing infertility (Teede et al., 2011), PCOS is associated with whole-body insulin resistance and metabolic disorders such as obesity and type 2 diabetes mellitus (Anderson et al., 2014; Cassar et al., 2016; Orio et al., 2016). Despite the high prevalence and adverse health implications of PCOS, the etiology and optimal treatments for women with PCOS are unclear. Insulin resistance is an underlying feature of PCOS (Teede et al., 2011). Indeed, insulin sensitivity is 27% lower in women with PCOS compared to healthy controls (Cassar et al., 2016), with up to 85% of women with PCOS being insulin resistant (Stepto et al., 2013). Lifestyle interventions, including exercise training, are recommended as first-line therapy for women with PCOS (Teede et al., 2011, 2018). In this regard, a growing body of evidence demonstrate that high-intensity interval training (HIT) confers greater health benefits compared to moderate intensity exercise in clinical cohorts (Weston et al., 2014; Cassidy et al., 2017). Such superior effects of vigorous intensity exercise are also evident among women with PCOS (Patten et al., 2020). Thus, a greater knowledge of the molecular mechanisms underlying the efficacy of exercise interventions such as HIT on metabolic health may help to understand the pathophysiology of PCOS.

MicroRNAs (miRNAs) are small non-coding RNAs that act as post-transcriptional regulators by binding with specific mRNA transcripts and preventing protein and gene translation or by degrading the target mRNA (Chen X. et al., 2012). miRNAs can function within the tissue of origin or adjacent tissues (Chen X. et al., 2012), thus playing crucial regulatory roles in many biological processes. Several studies in women with PCOS have reported altered expression of miRNAs in the circulation (Murri et al., 2013, 2018; Long et al., 2014; Sorensen et al., 2014, 2019; Ding et al., 2015; Sathyapalan et al., 2015; Arancio et al., 2018; Chen et al., 2019), adipose tissue (AT) (Chen et al., 2013, 2019; Sorensen et al., 2014; Chuang et al., 2015), and follicular fluid and granulosa cells (Sorensen et al., 2014; Butler et al., 2019; Chen et al., 2019), suggesting a potential role for miRNAs in the pathophysiology of this condition. Specifically, abnormal expression of miRNAs with putative roles in regulating glucose metabolism in adipocytes in insulin resistant women with PCOS have been observed (Chen et al., 2013; Wu et al., 2014; Chuang et al., 2015). Furthermore, studies have reported significantly abnormal expression of miRNAs in subcutaneous abdominal AT in insulin resistant women, regardless of PCOS status (Wu et al., 2014; Chuang et al., 2015).

Women with PCOS have larger adipocytes and reduced adipocyte insulin sensitivity compared to women matched for body mass index (BMI) without PCOS (Dunaif et al., 1992; Manneras-Holm et al., 2011). As such, miRNAs could play a role in these AT abnormalities in women with PCOS. Abdominal obesity is associated with increased risk of insulin resistance and cardiovascular disease (Karpe and Pinnick, 2015) and women with PCOS have greater abdominal fat (Fauser et al., 2012). AT from different depots express unique molecular, cellular and metabolic properties and respond in distinct ways to exercise and nutritional challenges (Tan et al., 2004; You et al., 2014; Tsiloulis et al., 2017). Therefore, in order to understand these differential expression profiles, interrogation of miRNAs from different AT depots in women with PCOS is necessary. However, to the best of our knowledge, such profiles are currently lacking. Accordingly, the primary aim of the present investigation was to compare the expression of selected miRNAs in the circulation, gluteal and abdominal AT in women with PCOS to an age- and BMI-matched control group of women without PCOS. Secondary aims were to investigate the effects of two chronic protocols of HIT on the "expression signature" of these miRNAs in women with PCOS, and to assess differences in the selected miRNA expressions in gluteal and abdominal AT and whether the two AT depots responded in distinct ways to HIT. Our main hypotheses were that there would be differential expression patterns in circulating and AT miRNA's in women with, compared to those without PCOS, and that the expression signature would be modulated by chronic exercise training in women with PCOS.

## MATERIALS AND METHODS

### Participants and Study Design

Forty-two women with PCOS and 12 women without PCOS (Non-PCOS) were recruited for this study, which was part of the Improving Reproductive Function in Women with Polycystic Ovary Syndrome with High-Intensity Interval Training (IMPROV-IT) trial (Kiel et al., 2020) (ClinicalTrials.gov Identifier: NCT02419482) and of the Adipose Tissue Function and Response to Exercise Training in Women With and Without Polycystic Ovary Syndrome trial (HIT-FAT; ClinicalTrials.gov Identifier NCT02943291). The IMPROV-IT trial is a two-center randomized controlled trial undertaken at The Norwegian University of Science and Technology (NTNU) in Trondheim, Norway and at The Australian Catholic University (ACU) in Melbourne, Australia. The study protocol for the IMPROV-IT trial has been published previously (Kiel et al., 2020). Non-PCOS participants were recruited only in Norway. We selected 12

women without PCOS (Non-PCOS) as a control group, who were individually matched to 12 women with PCOS. Women with and without PCOS were individually matched by age and BMI with an age difference of 5 years and BMI difference within 2 kg/m$^2$. PCOS was defined according to the Rotterdam criteria, where a minimum of two of the following three conditions must be present: (1) polycystic ovary morphology (12 or more 2–9 mm follicles or >10 mL in volume in at least one ovary), (2) hyperandrogenism (either clinical signs such as acne or hirsutism, or biomedical) and/or oligo/amenorrhea (Rotterdam, 2004). Hirsutism was defined with a Ferriman Gallwey score ≥8 (Ferriman and Gallwey, 1961). The cut-off values for biochemical hyperandrogenism were defined as a testosterone concentration of >3.0 nmol/L, calculated free testosterone concentration of >32 pmol/L, sex hormone binding globulin (SHBG) concentration of <30 nmol/L, or free androgen index (FAI as 100× testosterone concentration (nmol/L)/SHBG concentration (nmol/L) >5% (Al Kindi et al., 2012). Oligo/amenorrhea was defined as an intermenstrual interval >35 days and ≤9 menstruations in the past year. Amenorrhea was defined as absent menstruations in the past 90 days.

To be eligible for inclusion into the study, women had to be aged between 18 and 45 years. Exclusion criteria were; if women were undertaking regular endurance training ≥2 sessions/week, cardiovascular diseases or other endocrine disorders, pregnancy or breastfeeding within the last 24 weeks, physical ailments or injuries that would hinder exercise performance, or undergoing concurrent treatments with hormonal contraceptives, insulin sensitizers or drugs known to affect gonadotropin or ovulation (with a wash-out period of 3 months prior to inclusion). Women were not excluded based on dietary intake and were encouraged to continue with their habitual diet during the study period. For women without PCOS, inclusion and exclusion criteria were the same as for women with PCOS, but they were normally menstruating women with no evidence of hyperandrogenism or polycystic ovaries.

## Ethical Approval

The study was performed according to the Helsinki declaration and approved by The Regional Committee for Medical and Health Research Ethics in Central Norway (REK-midt 2015/468 and 2016/545) and the ACU Human Research Ethics Committee (2017-260H). Participants were informed about the experiments and potential risks verbally and their written consent was obtained prior to study entry.

## Pre- and Post-intervention Testing

**Figure 1** displays an overview of the experimental protocol. Participants with a regular menstrual cycle were tested during the early follicular phase (day 1–7 of the menstrual cycle) whereas women with oligo/amenorrhea were tested independent of the time of their cycle. Participants performed an incremental test to exhaustion on a treadmill to measure peak oxygen uptake ($VO_2$peak) and estimate maximal heart rate ($HR_{max}$) using an individualized protocol. Following a 10 min warm-up and 3 min at moderate-intensity, the treadmill speed or incline was increased every 1–2 min by 0.5-1.0 km/h or 1–2% until volitional fatigue. After an overnight fast (≥12 h) and refraining from exercise for ≥48 h, participants returned to the laboratory and body fat percentage (BF%) was estimated. In Australia, the BF% was estimated using dual-energy X-ray absorptiometry (DXA; GE Lunar iDXA Pro, Encore software version 16, General Electric, Boston, MA, United States). In Norway, the BF% was estimated using bioelectrical impedance analysis (InBody720, Biospace CO, South Korea). Waist and hip circumference were also measured to the nearest 0.5 cm in duplicate. A resting blood sample was collected in a 4 mL EDTA and a 5 mL serum tube from the antecubital vein. The EDTA tube was immediately spun at 2,200 rpm at 20°C for 10 min while the serum tube rested 30 min before it was spun with the same protocol. Plasma was collected and stored at −80°C for subsequent analysis. On the same day, abdominal and gluteal subcutaneous AT biopsies (300–500 mg) were collected using a 14-gauge needle under local anesthesia with 1% xylocaine and washed on gauze with saline. Blood and connective tissue were removed before approximately 100 mg was snap-frozen in liquid nitrogen and stored at −80°C for subsequent miRNA analyses. Non-PCOS women were tested for all the measures described at baseline, whereas all women with PCOS were tested at baseline and after the 16 weeks exercise intervention (**Figure 1**). To avoid any residual effects of the last exercise session on miRNA expression, resting plasma and AT were sampled ≥48 h following the last exercise session.

## Training Protocols

Women with PCOS ($n$ = 42) were randomized to 16 weeks of HIT or a non-exercising control group. Participants were stratified for a BMI < or ≥27 kg/m$^2$ and randomly assigned in a 1:1:1 manner to one of three groups: (1) LV-HIT ($n$ = 13); (2) HV-HIT ($n$ = 14); (3) Non-exercise (Non-Ex, $n$ = 15). The training program has been described previously (Kiel et al., 2020). Briefly, exercise training was performed on a treadmill or as outdoor running/walking three times/week. Women assigned to the LV-HIT group completed 10 × 1 min work bouts at the maximal intensity they could sustain, separated by 1 min of passive recovery or low-intensity walking. Women in the HV-HIT group completed 4 × 4 min work bouts reaching 90–95% $HR_{max}$ during the first two minutes of each bout, separated by 3 min of active recovery at ∼70% of $HR_{max}$. All training sessions included a 10 min warm-up and a 3-min cool-down period. At least one weekly session was supervised at the laboratory, and the participants wore HR monitors (Polar M400) during all sessions to ensure compliance with both exercise protocols. Women in the Non-Ex group were advised to continue their habitual physical activity and informed about the current recommendations for physical activity in adults.

## Circulating miRNA Extraction and Reverse Transcription

After centrifuging thawed plasma samples for 10 min at 4°C in order to remove cellular debris, circulating miRNAs (c-miRs) were extracted using the miRNeasy Serum/Plasma Advanced Kit (217204; Qiagen, Australia), which allows for extraction and purification of small (<200 nt) cell-free RNA. A total of 200 μL

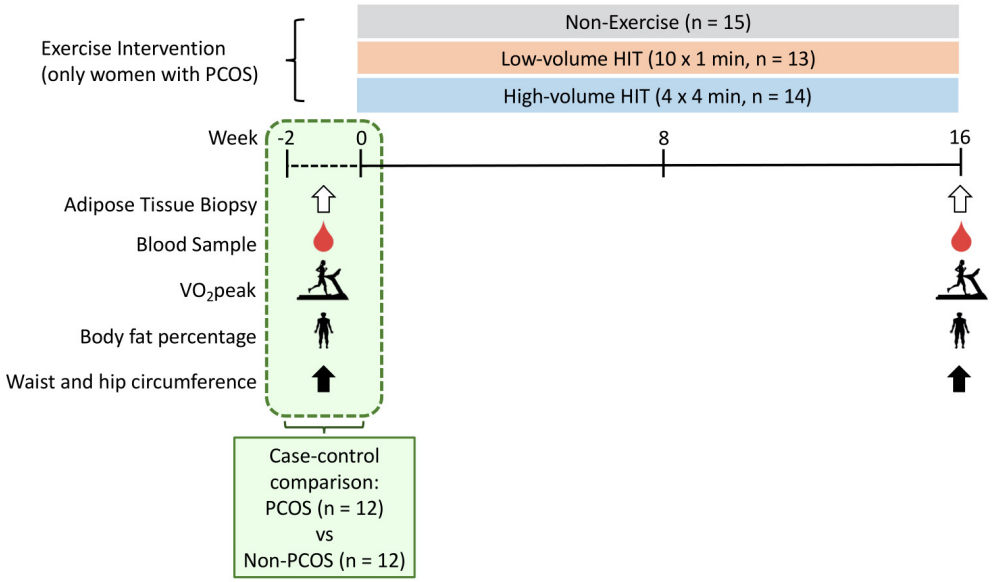


**FIGURE 1 |** Study protocol. The testing days included: $VO_2$peak, body fat percentage, waist and hip circumference measurements as well as blood and adipose tissue sampling. Non-PCOS ($n$ = 12) women were only tested at baseline, whereas all women with PCOS ($n$ = 42) were tested at baseline and after the 16 weeks exercise intervention. The 12 PCOS women in the case-control comparison also took part in the exercise intervention. Non-PCOS were age- and BMI-matched to these 12 women with PCOS.

supernatant was then transferred to a new sterile tube. To assist with determining the yield of template recovered, 3.5 μL of a miRNeasy Serum/Plasma Spike-In Control (219610; Qiagen, Australia) was added to all samples prior to extraction of RNA. Input amounts of RNA were standardized using the same initial volume (2 μL) for all samples following the manufacturer's instructions. RNA was reverse transcribed into cDNA by using the miRCURY LNA RT kit (339340; Qiagen, Australia) in a BioRad thermal cycler (BioRad, Australia). The resulting cDNA was stored at −20°C.

## Adipose Tissue miRNA Extraction and Reverse Transcription

MicroRNAs were extracted from frozen abdominal and gluteal AT samples using the miRNeasy Mini (217004; Qiagen, Australia) and RNeasy MinElute Cleanup (74204; Qiagen, Melbourne, VIC, Australia) Kits according to manufacturer's instructions. Approximately 100 mg of AT was homogenized in QIAzol Lysis Reagent and phase separated with chloroform. Samples were then loaded into spin columns, washed with ethanol and eluted in 14 μL of RNAse-free water. Extracted miRNAs were quantified on a Qubit 4 Fluorometer using the Qubit microRNA Assay Kit (Q32881; Life Technologies, Australia). Samples were then equilibrated with RNase-free water and reverse transcribed to cDNA using the miRCURY LNA RT Kit (339340; Qiagen, Australia) in a BioRad thermal cycler (BioRad, Australia). The resulting cDNA was stored at −20°C.

## Targeted MicroRNA Quantification

Quantification of both circulating and AT miRNAs were performed on a Qiagen customized 96-well miScript miRNA PCR Array (339330; Qiagen, Australia) with miScript SYBR using a BioRad CFX96 (BioRad, Australia) following manufacturer's instructions. The array contained eight miRNAs selected *a priori* based on previous reports of altered c-miRs profiles in women with PCOS and/or in response to exercise training (Murri et al., 2013; Sorensen et al., 2014; Improta et al., 2018; Da Silva et al., 2019). The selected c-miRs were hsa-miR-21-5p, hsa-miR-27b-5p, hsa-miR-93-5p, hsa-miR-146a-5p, hsa-miR-155-5p, hsa-miR-222-3p, hsa-miR-223-3p, and hsa-miR-103a-3p. These miRNAs have been shown to be implicated in hormone secretion and metabolism, inflammation, adipogenesis, and lipid and glucose metabolism (Chen W. et al., 2012; Murri et al., 2013; Sorensen et al., 2014; Chuang et al., 2015). The same eight miRNAs were investigated in the subcutaneous abdominal and gluteal AT ($n$ = 8 for each group) to explore any "cross-talk" between the circulation and a potential target tissue, as accumulating evidence demonstrates that c-miRs can be transferred to and taken up by recipient cells and tissues where they can regulate their specific targets (Fabbri et al., 2012; Russell and Lamon, 2015).

PCR arrays were run using a miScript SYBR Green PCR Kit (339346; Qiagen, Australia). A quantification cycle above 40 in >50% of the samples was considered as absence of expression of the miRNA. Moreover, samples that exhibited low abundance (relative threshold cycle above 40) were excluded from analysis as indicated in **Table 3**. The array also contained an inter-plate calibrator, along with UniSp3 and UniSp6 RNA Spike-in controls, RNU1A1 and SNORD44. Due to low circulating expression of SNORD44, c-miR expression was normalized to the geometric mean of UniSp3, UniSp6, and RNU1A1. In contrast, AT miRNAs were normalized to the geometric mean of SNORD44, UniSp3, UniSp6, and RNU1A1. The $2^{\Delta\Delta}$CT method of relative

quantification was used to calculate the relative abundance of miRNAs in plasma and AT (Livak and Schmittgen, 2001).

## Blood Biochemistry and Insulin Sensitivity

Plasma glucose and serum insulin concentrations and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) were determined for the case-control study (PCOS and Non-PCOS). Plasma glucose concentration was measured using a Roche Moduclar P (Roche, Switzerland), while serum insulin concentration was measured in duplicates using an enzyme-linked immunosorbent assay (ELISA; IBL-International, Germany). HOMA-IR was used as a method for quantifying insulin resistance; fasting serum (μU/mL) × fasting plasma glucose (mmol/L) divided by 22.5 (Matthews et al., 1985).

## Statistics

Descriptive statistics are presented as means ± SD. Group means for PCOS vs Non-PCOS were compared by Student's two-tailed *t*-test for independent samples. We used linear mixed models with participant as random factor, and with two dummy variables to uniquely identify the two exercise groups (LV-HIT and HV-HIT) and their interactions, with time as fixed factors. We adjusted for the baseline values for the outcome variables as recommended by Twisk et al. (2018). In this model, the coefficients for the interaction terms give the estimated exercise training effects in LV-HIT and HV-HIT compared to the Non-Ex group. Paired *t*-tests were used to test for differences in miRNA expression in AT depots (gluteal versus abdominal) at baseline. To test if miRNA expression in the two AT depots would respond in distinct ways to the two HIT protocols, changes in miRNA expression were calculated by using post-intervention minus pre-intervention values (corresponding to the ΔmiR expression in **Table 6**). We used linear mixed models as detailed but with the two exercise groups' interaction with AT depots as fixed factors. Due to previously observed abnormal expression of miRNAs in subcutaneous AT in insulin resistant women regardless of PCOS status, we also analyzed the gluteal and abdominal subcutaneous AT miRNA expression based on insulin resistance. Insulin resistance was estimated as HOMA-IR, with similar cut-off points as previously reported (Chuang et al., 2015); HOMA-IR value <2.5 was considered normal whereas HOMA-IR ≥ 2.5 indicated insulin resistance. Group means for insulin resistant and non-insulin resistant women were compared by Student's two-tailed *t*-test for independent samples. Normality of residuals was evaluated using visual inspection of Q-Q plots and logarithmic transformation (log10) of the dependent variable was performed when necessary to obtain normality. Due to multiple hypotheses, we considered *P-values* <0.01 as statistically significant. Pearson correlation between miRNAs different between groups (PCOS and Non-PCOS) and baseline $VO_2$peak, BMI, fat percentage or waist/hip ratio was calculated using GraphPad Prism version 8.1.2 (GraphPad Software, United States). All other analyses were carried out using SPSS version 25.0 (SPSS Inc., United States).

# RESULTS

## Characteristics: PCOS vs Non-PCOS Women

Characteristics of the participants included in the case-control comparison are presented in **Table 1**. Apart from greater waist/hip ratio in women with PCOS (0.90 ± 0.06 versus 0.83 ± 0.05, $P = 0.005$), there were no differences between groups (**Table 1**). There was no difference in HOMA-IR between the groups, although nine of the women with PCOS versus five women without PCOS had HOMA-IR ≥ 2.5 (**Table 1**).

## Physiological Responses to High-Intensity Interval Training

There were no changes in body weight, BMI, body fat percentage, waist, and hip circumference or waist/hip ratio for either LV-HIT or HV-HIT post intervention (**Table 2**). Although not significant, it is worth noting that the mean fat percentage decreased by 1.4% in the Non-Ex group. This is not a systemic decrease in fat percentage in the Non-Ex group but a result of two participants in this group decreasing by 6% in fat percentage. Following 16 weeks of HIT, absolute, but not relative, $VO_2$peak increased by 9% ($P = 0.008$) in HV-HIT (**Table 2**).

## miRNA Expression Profile in PCOS vs Non-PCOS

Basal expression of circulating miRNA-27b (c-miR-27b) was higher in women with PCOS compared to Non-PCOS (1.8-fold, $P = 0.006$; **Figure 2B**), with no significant differences in the expression of c-miR-21, −93, −103a, −146a, −155, −222, and −223 (**Figures 2A–H**). No differences in basal gluteal and

**TABLE 1 |** Baseline characteristics of PCOS and Non-PCOS women.

| | Non-PCOS | PCOS | *P-value* |
|---|---|---|---|
| *n* | 12 | 12 | |
| Age (years) | 30 ± 7 | 30 ± 7 | 0.98 |
| Body weight (kg) | 83.0 ± 18.5 | 85.2 ± 19.7 | 0.78 |
| BMI (kg/m$^2$) | 29.3 ± 5.8 | 29.8 ± 6.5 | 0.85 |
| Body fat percentage (%) | 36.4 ± 9.2 | 36.8 ± 9.0 | 0.91 |
| Waist circumference (cm) | 94 ± 14 | 102 ± 14 | 0.16 |
| Hip circumference (cm) | 113 ± 13 | 113 ± 11 | 0.99 |
| Waist/Hip ratio | 0.83 ± 0.05 | 0.90 ± 0.06 | **0.005** |
| $VO_2$peak (L/min) | 2.83 ± 0.38 | 2.73 ± 0.38 | 0.54 |
| $VO_2$peak (mL/min/kg) | 34.9 ± 7.0 | 33.2 ± 6.1 | 0.53 |
| Glucose (mmol/L) | 4.9 ± 0.4 | 5.0 ± 0.6 | 0.52 |
| Insulin (μU/mL) | 14.2 ± 10.6 | 15.7 ± 6.9 | 0.71 |
| HOMA-IR | 3.1 ± 2.3 | 3.6 ± 1.8 | 0.58 |

*Values are mean ± SD. PCOS, polycystic ovary syndrome; BMI, body mass index; $VO_2$peak, peak oxygen uptake; HOMA-IR, homeostatic model assessment of insulin resistance. In the Non-PCOS group, not enough blood was collected for analyzing insulin concentration, so n = 11 for insulin concentration and HOMA-IR in Non-PCOS. The meaning of a bold value is that the P-value is statistically significant.*

**TABLE 2 |** Participant characteristics before and after 16 weeks of high-intensity interval training.

| | Pre | | Post | | Difference (time × group) | | |
|---|---|---|---|---|---|---|---|
| | *n* | Mean ± SD | *n* | Mean ± SD | Estimate | 95% CI | *P-value* |
| **Age (years)** | | | | | | | |
| Non-Ex | 15 | 28 ± 5 | | | | | |
| LV-HIT | 13 | 31 ± 5 | | | | | |
| HV-HIT | 14 | 30 ± 5 | | | | | |
| **Body weight (kg)** | | | | | | | |
| Non-Ex | 15 | 86.2 ± 20.1 | 15 | 84.1 ± 19.3 | | | |
| LV-HIT | 13 | 84.6 ± 19.1 | 13 | 81.8 ± 20.2 | −0.75 | −5.28 to 3.78 | 0.74 |
| HV-HIT | 14 | 92.6 ± 22.6 | 14 | 91.9 ± 23.4 | 1.66 | −2.78 to 6.11 | 0.45 |
| **BMI (kg/m$^2$)** | | | | | | | |
| Non-Ex | 15 | 31.4 ± 6.9 | 15 | 30.6 ± 6.5 | | | |
| LV-HIT | 13 | 28.9 ± 6.6 | 13 | 28.8 ± 6.7 | 0.65 | −0.08 to 1.38 | 0.08 |
| HV-HIT | 14 | 33.1 ± 7.4 | 14 | 32.8 ± 7.6 | 0.59 | −0.13 to 1.30 | 0.11 |
| **Body fat percentage (%)** | | | | | | | |
| Non-Ex | 15 | 41.0 ± 8.3 | 15 | 39.6 ± 8.2 | | | |
| LV-HIT | 13 | 35.5 ± 10.7 | 13 | 35.3 ± 10.4 | 0.98 | −0.75 to 2.72 | 0.26 |
| HV-HIT | 14 | 41.8 ± 7.9 | 14 | 41.2 ± 9.0 | 0.82 | −0.88 to 2.52 | 0.34 |
| **Waist circumference (cm)** | | | | | | | |
| Non-Ex | 15 | 101 ± 16 | 15 | 99 ± 16 | | | |
| LV-HIT | 13 | 98 ± 17 | 12 | 95 ± 18 | −0.63 | −5.83 to 4.56 | 0.81 |
| HV-HIT | 14 | 110 ± 17 | 14 | 105 ± 16 | −2.46 | −7.44 to 2.53 | 0.33 |
| **Hip circumference (cm)** | | | | | | | |
| Non-Ex | 15 | 115 ± 14 | 15 | 114 ± 12 | | | |
| HV-HIT | 13 | 112 ± 14 | 12 | 111 ± 12 | 0.01 | −3.32 to 3.35 | 0.99 |
| LV-HIT | 14 | 117 ± 14 | 14 | 115 ± 15 | −1.22 | −4.42 to 1.98 | 0.45 |
| **Waist/Hip ratio** | | | | | | | |
| Non-Ex | 15 | 0.88 ± 0.07 | 15 | 0.87 ± 0.09 | | | |
| LV-HIT | 13 | 0.87 ± 0.07 | 12 | 0.85 ± 0.09 | −0.005 | −0.05 to 0.04 | 0.81 |
| HV-HIT | 14 | 0.94 ± 0.06 | 14 | 0.91 ± 0.08 | 0.01 | −0.03 to 0.05 | 0.65 |
| **$VO_{2peak}$ (L/min)** | | | | | | | |
| Non-Ex | 15 | 2.73 ± 0.49 | 15 | 2.63 ± 0.45 | | | |
| LV-HIT | 13 | 2.85 ± 0.29 | 13 | 2.85 ± 0.39 | 0.13 | −0.07 to 0.32 | 0.20 |
| HV-HIT | 14 | 2.81 ± 0.34 | 13 | 2.94 ± 0.38 | 0.27 | 0.07 to 0.46 | **0.008** |
| **$VO_{2peak}$ (mL/kg/min)** | | | | | | | |
| Non-Ex | 15 | 32.2 ± 6.9 | 15 | 32.5 ± 7.5 | | | |
| LV-HIT | 13 | 34.4 ± 8.6 | 13 | 35.5 ± 8.0 | 0.88 | −1.45 to 3.20 | 0.45 |
| HV-HIT | 14 | 31.7 ± 6.5 | 13 | 34.6 ± 8.0 | 1.97 | −0.36 to 4.30 | 0.10 |

*The difference (time × group) is the exercise training effect in LV-HIT and HV-HIT compared to the Non-Ex group. n, number of participants; SD, standard deviations; CI, confidence interval; Non-Ex, non-exercising group; LV-HIT, 10 × 1 min exercise group; HV-HIT, 4 × 4 min exercise group; BMI, body mass index; $VO_{2peak}$, peak oxygen uptake. The meaning of a bold value is that the P-value is statistically significant.*

abdominal AT miRNA expression between women with PCOS and non-PCOS were observed (**Figures 3A–H**, **4A–H**). No correlations were observed between basal c-miR-27b expression and baseline $VO_2$peak, BMI, fat percentage, or waist/hip ratio (data not shown).

## Effect of HIT on miRNA Expression Profile

The expression of the selected miRNAs in the circulation, gluteal and abdominal AT before and after 16 weeks of HIT in women with PCOS are summarized in **Tables 3**–**5**. In LV-HIT, the expression of c-miR-27b decreased significantly after training (0.5-fold, $P = 0.007$; **Table 3**), while the expression of c-miR-155 showed a tendency to decline ($P = 0.06$, **Table 3**). There were no changes in circulating miR-21, -93, -103a, -146a, -155, -222, and -223 in either of the groups. Chronic exercise training did not alter the expression of any of the selected miRNAs in the two AT depots in women with PCOS (**Tables 4**, **5**).

At baseline, none of the selected miRNAs were differentially expressed in gluteal compared to abdominal AT (data not shown). Furthermore, there were no differences in how the selected miRNAs changed in abdominal versus gluteal AT in response to either of the two HIT interventions (**Table 6**).

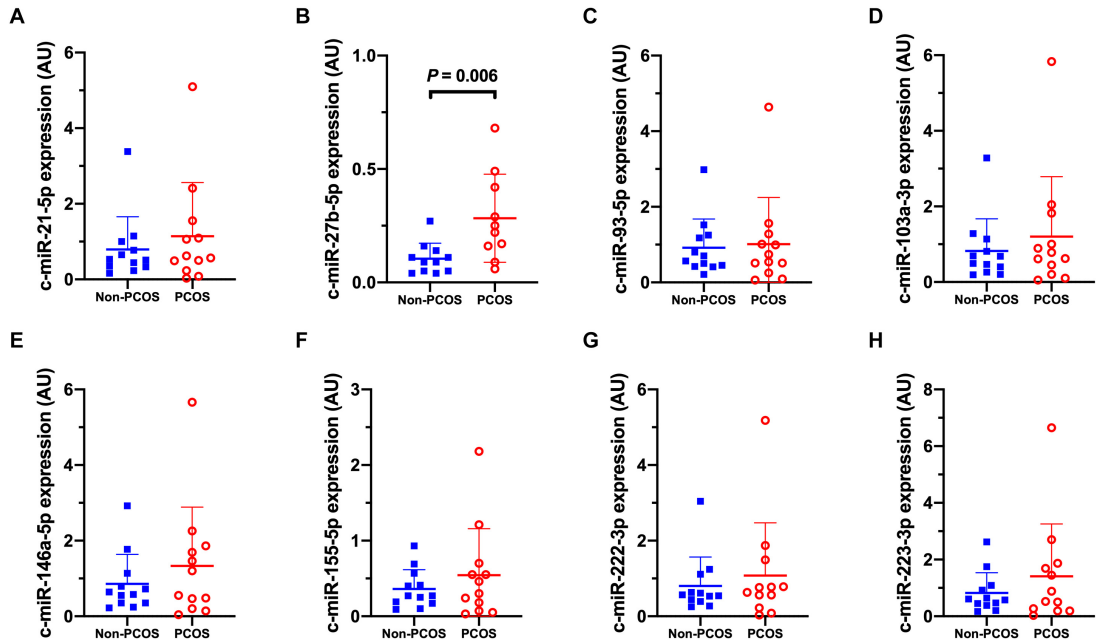


**FIGURE 2 |** Circulatory microRNA expression patterns. **(A)** c-miR-21-5p, **(B)** c-miR-27b-5p, **(C)** c-miR-93-5p, **(D)** c-miR-103a-3p, **(E)** c-miR-146a-5p, **(F)** c-miR-155-5p, **(G)** c-miR-222-3p, **(H)** c-miR-223-3p abundance in women with (PCOS; open, red circles) and without PCOS (Non-PCOS; blue squares). Values are arbitrary units expressed relative to the geometric mean of UniSp3, UniSp6 and RNU1A1. Individual data with group means and SD are displayed. PCOS, polycystic ovary syndrome; c, circulating; miR, microRNA; AU, arbitrary units.

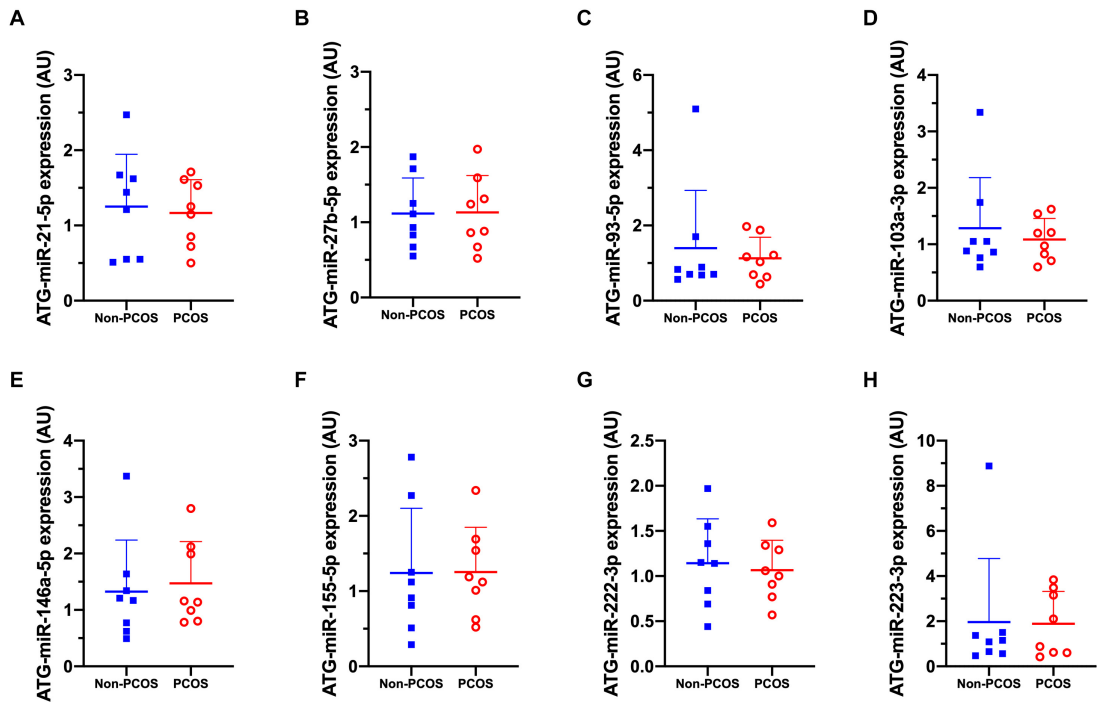


**FIGURE 3 |** Gluteal adipose tissue (ATG) microRNA expression patterns. **(A)** ATG-miR-21-5p, **(B)** ATG-miR-27b-5p, **(C)** ATG-miR-93-5p, **(D)** ATG-miR-103a-3p, **(E)** ATG-miR-146a-5p, **(F)** ATG-miR-155-5p, **(G)** ATG-miR-222-3p, **(H)** ATG-miR-223-3p abundance in women with (PCOS; open, red circles) and without PCOS (Non-PCOS; blue squares). Values are arbitrary units expressed relative to the geometric mean of SNORD44, UniSp3, UniSp6, and RNU1A1. Individual data with group means and SD are displayed. PCOS, polycystic ovary syndrome; ATG, gluteal adipose tissue; miR, microRNA; AU, arbitrary units.

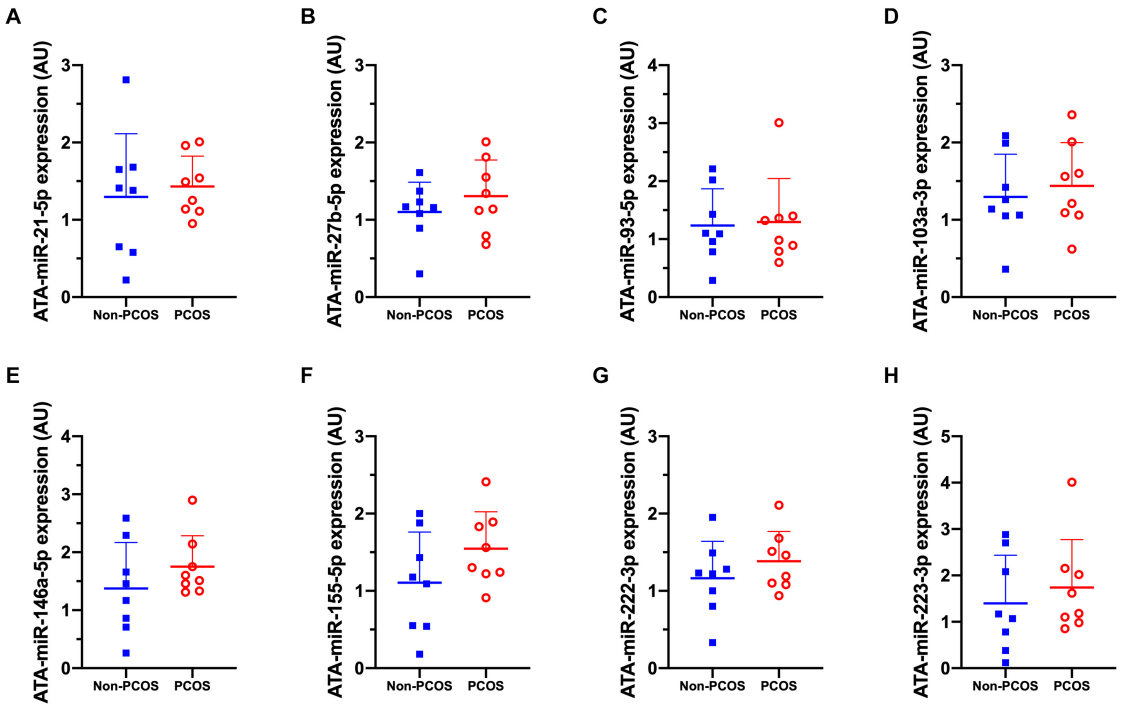


**FIGURE 4 |** Abdominal adipose tissue (ATA) microRNA expression patterns. **(A)** ATA-miR-21-5p, **(B)** ATA-miR-27b-5p, **(C)** ATA-miR-93-5p, **(D)** ATA-miR-103a-3p, **(E)** ATA-miR-146a-5p, **(F)** ATA-miR-155-5p, **(G)** ATA-miR-222-3p, **(H)** ATA-miR-223-3p abundance in women with (PCOS; open, red circles) and without PCOS (Non-PCOS; blue squares). Values are arbitrary units expressed relative to the geometric mean of SNORD44, UniSp3, UniSp6, and RNU1A1. Individual data with group means and SD are displayed. PCOS, polycystic ovary syndrome; ATA, abdominal adipose tissue; miR, microRNA; AU, arbitrary units.

## Gluteal and Abdominal AT miRNA Expression Profile in Insulin Resistant and Non-insulin Resistant Women

There were no differences in basal gluteal and abdominal AT miRNA expression between insulin resistant and non-insulin resistant women regardless of PCOS status (data not shown). miR-103a tended to be lower in insulin resistant women in gluteal subcutaneous AT ($P$ = 0.09) and miR-155 tended to be higher in insulin resistant women in abdominal subcutaneous AT ($P$ = 0.08).

# DISCUSSION

We report novel data regarding miRNA expression profiles in women with and without PCOS, along with responses to 16 weeks of HIT undertaken by women with PCOS. We show that basal expression of c-miR-27b was higher in women with PCOS compared to age- and BMI-matched women without PCOS. We also show that 16 weeks of exercise training induced a significant decrease in c-miR-27b in women with PCOS, despite no changes in the expression profile of the other targeted c-miRs. Finally, we show that miRNA expression from the two separate subcutaneous AT sites was unaffected by exercise training. Collectively, our findings provide new data regarding the effects of chronic exercise training and selected miRNAs responses in women with PCOS.

The first important finding from the current investigation was that basal c-miR-27b expression was higher in women with PCOS compared to age- and BMI-matched women without PCOS. miRNA-27b has been implicated in several cellular and metabolic processes including fatty acid metabolism, adipocyte differentiation, substrate metabolism, and inflammation (Fernandez-Valverde et al., 2011; Chen W. et al., 2012; Murri et al., 2013). Murri et al. (2013) reported that women without PCOS with a BMI > 30 kg/m$^2$ had lower basal whole blood miR-27b expression compared to women with normal weight (BMI < 25 kg/m$^2$), whereas obesity was associated with higher expression of miR-27b compared to normal weight in women with PCOS. These findings, together with our data, indicate divergent expression of basal c-miR-27b in women with and without PCOS. Previous work showed that overexpression of miR-27b attenuated the abundance of regulators of adipogenesis such as peroxisome proliferator-activated receptor-γ (PPAR-γ) and CCAAT/enhancer-binding protein α (C/EBPα) (Lin et al., 2009), with PPAR-γ confirmed as a direct target of miR-27b (Karbiener et al., 2009). Similarly, work in diabetic rats and 3T3-L1 adipocytes revealed elevated expression of the closely related miR-27a within the retroperitoneal fat pad and in the presence of high glucose, respectively (Herrera et al., 2010). While these findings indicate a role for miR-27b in the etiology of adipogenesis and obesity, we found no relationship between basal c-miR-27b expression and BMI, fat percentage or waist/hip ratio, possibly indicating the cellular effects of miR-27b are likely

**TABLE 3 |** Circulatory miRNA expression before and after 16 weeks of high-intensity interval training.

| | Pre | | Post | | Difference (group × time) |
|---|---|---|---|---|---|
| | *n* | Mean ± SD | *n* | Mean ± SD | *P-value* |
| **c-miR-21b-5p** | | | | | |
| Non-Ex | 14 | 1.22 ± 0.59 | 15 | 2.03 ± 2.83 | |
| LV-HIT | 13 | 1.68 ± 1.84 | 13 | 1.61 ± 1.75 | 0.46 |
| HV-HIT | 14 | 1.61 ± 1.76 | 14 | 1.28 ± 0.94 | 0.30 |
| **c-miR-27b-5p** | | | | | |
| Non-Ex | 14 | 0.73 ± 0.50 | 15 | 1.15 ± 1.25 | |
| LV-HIT | 12 | 0.39 ± 0.79 | 12 | 0.19 ± 0.20 | **0.007** |
| HV-HIT | 11 | 0.23 ± 0.23 | 10 | 0.20 ± 0.10 | 0.12 |
| **c-miR-93-5p** | | | | | |
| Non-Ex | 14 | 1.07 ± 0.58 | 15 | 1.46 ± 1.57 | |
| LV-HIT | 13 | 1.61 ± 1.56 | 13 | 1.49 ± 1.34 | 0.83 |
| HV-HIT | 14 | 1.44 ± 1.46 | 14 | 1.83 ± 2.44 | 0.70 |
| **c-miR-103a-3p** | | | | | |
| Non-Ex | 14 | 1.13 ± 0.63 | 15 | 1.73 ± 1.99 | |
| LV-HIT | 13 | 1.75 ± 1.86 | 13 | 1.55 ± 1.55 | 0.59 |
| HV-HIT | 14 | 1.64 ± 1.79 | 14 | 1.46 ± 1.33 | 0.45 |
| **c-miR-146a-5p** | | | | | |
| Non-Ex | 14 | 1.91 ± 0.93 | 15 | 2.90 ± 3.46 | |
| LV-HIT | 13 | 1.61 ± 1.71 | 13 | 1.45 ± 1.28 | 0.19 |
| HV-HIT | 14 | 1.58 ± 1.72 | 14 | 1.33 ± 1.05 | 0.10 |
| **c-miR-155-5p** | | | | | |
| Non-Ex | 14 | 0.85 ± 0.53 | 15 | 1.25 ± 1.41 | |
| LV-HIT | 12 | 0.94 ± 0.92 | 13 | 0.69 ± 0.82 | 0.06 |
| HV-HIT | 14 | 0.70 ± 0.76 | 13 | 0.58 ± 0.39 | 0.37 |
| **c-miR-222-3p** | | | | | |
| Non-Ex | 14 | 0.95 ± 0.45 | 15 | 1.33 ± 1.27 | |
| LV-HIT | 13 | 1.56 ± 1.61 | 13 | 1.33 ± 1.30 | 0.59 |
| HV-HIT | 14 | 1.51 ± 1.59 | 14 | 1.39 ± 1.13 | 0.54 |
| **c-miR-223-3p** | | | | | |
| Non-Ex | 14 | 2.03 ± 1.17 | 15 | 3.16 ± 3.80 | |
| LV-HIT | 13 | 1.64 ± 1.86 | 13 | 1.55 ± 1.55 | 0.17 |
| HV-HIT | 14 | 1.69 ± 2.03 | 14 | 1.41 ± 1.03 | 0.14 |

*The difference (time × group) is the exercise training effect in LV-HIT and HV-HIT compared to the Non-Ex group. n, number of participants; SD, standard deviations; Non-Ex, non-exercising group; LV-HIT, 10 × 1 min exercise group; HV-HIT, 4 × 4 min exercise group; c, circulating; miR, microRNA. The meaning of a bold value is that the P-value is statistically significant.*

**TABLE 4 |** Gluteal adipose tissue miRNA expression before and after 16 weeks of high-intensity interval training.

| | Pre | | Post | | Difference (group × time) |
|---|---|---|---|---|---|
| | *n* | Mean ± SD | *n* | Mean ± SD | *P-value* |
| **ATG-miR-21-5p** | | | | | |
| Non-Ex | 8 | 1.07 ± 0.42 | 8 | 2.18 ± 3.09 | |
| LV-HIT | 8 | 1.07 ± 0.39 | 8 | 1.03 ± 0.54 | 0.25 |
| HV-HIT | 8 | 1.41 ± 1.25 | 8 | 1.49 ± 1.52 | 0.48 |
| **ATG-miR-27b-5p** | | | | | |
| Non-Ex | 8 | 1.07 ± 0.41 | 8 | 1.43 ± 1.46 | |
| LV-HIT | 8 | 1.12 ± 0.55 | 8 | 0.96 ± 0.25 | 0.53 |
| HV-HIT | 8 | 1.02 ± 0.56 | 8 | 1.29 ± 1.28 | 0.69 |
| **ATG-miR-93-5p** | | | | | |
| Non-Ex | 8 | 1.17 ± 0.78 | 8 | 1.94 ± 2.92 | |
| LV-HIT | 8 | 1.09 ± 0.47 | 8 | 0.99 ± 0.58 | 0.41 |
| HV-HIT | 8 | 0.91 ± 0.54 | 8 | 0.89 ± 0.59 | 0.19 |
| **ATG-miR-103a-3p** | | | | | |
| Non-Ex | 8 | 1.09 ± 0.52 | 8 | 2.12 ± 3.25 | |
| LV-HIT | 8 | 1.07 ± 0.36 | 8 | 0.97 ± 0.47 | 0.18 |
| HV-HIT | 8 | 0.95 ± 0.37 | 8 | 1.05 ± 0.69 | 0.15 |
| **ATG-miR-146a-5p** | | | | | |
| Non-Ex | 8 | 1.03 ± 0.30 | 8 | 2.01 ± 2.91 | |
| LV-HIT | 8 | 1.19 ± 0.79 | 8 | 0.94 ± 0.64 | 0.20 |
| HV-HIT | 8 | 1.72 ± 1.73 | 8 | 1.52 ± 1.24 | 0.89 |
| **ATG-miR-155-5p** | | | | | |
| Non-Ex | 8 | 1.04 ± 0.33 | 8 | 2.79 ± 4.38 | |
| LV-HIT | 8 | 1.10 ± 0.56 | 8 | 1.13 ± 0.69 | 0.19 |
| HV-HIT | 8 | 1.47 ± 1.37 | 8 | 1.47 ± 1.22 | 0.43 |
| **ATG-miR-222-3p** | | | | | |
| Non-Ex | 8 | 1.06 ± 0.41 | 8 | 2.10 ± 3.13 | |
| LV-HIT | 8 | 1.07 ± 0.41 | 8 | 0.95 ± 0.35 | 0.16 |
| HV-HIT | 8 | 1.02 ± 0.55 | 8 | 1.06 ± 0.65 | 0.18 |
| **ATG-miR-223-3p** | | | | | |
| Non-Ex | 8 | 1.63 ± 2.03 | 8 | 1.87 ± 2.60 | |
| LV-HIT | 8 | 1.46 ± 1.40 | 8 | 1.21 ± 1.37 | 0.39 |
| HV-HIT | 8 | 1.31 ± 1.06 | 8 | 1.11 ± 0.79 | 0.48 |

*The difference (time × group) is the exercise training effect in LV-HIT and HV-HIT compared to the Non-Ex group. n, number of participants; SD, standard deviations; Non-Ex, non-exercising group; LV-HIT, 10 × 1 min exercise group; HV-HIT, 4 × 4 min exercise group; ATG, gluteal adipose tissue; miR, microRNA.*

to be confined more locally to within particular AT depots rather than whole body in such adipogenic models (Karbiener et al., 2009; Herrera et al., 2010).

Accumulating evidence demonstrates the capacity of vesicle-carrying miRNAs within the circulatory system to be released into the cytosol of recipient cells where they can regulate specific mRNA targets (Mittelbrunn et al., 2011; Fabbri et al., 2012). As such, we investigated miRNA expression within the subcutaneous abdominal and gluteal AT depots of women with PCOS. Somewhat surprisingly, and in contrast to one of our original hypotheses, we found no differences in miRNA expression signatures between women with and without PCOS in either of the subcutaneous adipose sites. Previously, Chuang et al. (2015) reported significantly higher miR-223 expression in subcutaneous abdominal AT in insulin resistant women regardless of PCOS status. However, when data from insulin resistant (HOMA-IR $\geq$ 2.5) and insulin sensitive (HOMA-IR $\leq$ 2.5) women were pooled, there was no difference in miR-223 expression between women with and without PCOS (Chuang et al., 2015). Similarly, the expression patterns of other subcutaneous abdominal AT miRNAs have been reported to be similar between women with and without PCOS, but to be different between insulin resistant (HOMA-IR $\geq$ 2.5) and insulin sensitive (HOMA-IR $<$ 2.5) women (Wu et al., 2014). We found no differences in either gluteal or abdominal AT miRNA expression profiles between insulin resistant and non-insulin resistant women (regardless of PCOS status). Our ability to detect

**TABLE 5 |** Abdominal adipose tissue miRNA expression before and after 16 weeks of high-intensity interval training.

| | Pre | | Post | | Difference (group × time) |
|---|---|---|---|---|---|
| | *n* | Mean ± SD | *n* | Mean ± SD | *P-value* |
| **ATA-miR-21-5p** | | | | | |
| Non-Ex | 8 | 1.18 ± 0.76 | 8 | 0.86 ± 0.39 | |
| LV-HIT | 8 | 1.34 ± 0.93 | 8 | 1.06 ± 0.56 | 0.57 |
| HV-HIT | 8 | 1.33 ± 0.62 | 8 | 1.73 ± 2.00 | 0.19 |
| **ATA-miR-27b-5p** | | | | | |
| Non-Ex | 8 | 1.05 ± 0.41 | 8 | 0.90 ± 0.37 | |
| LV-HIT | 8 | 1.23 ± 0.71 | 8 | 0.87 ± 0.33 | 0.93 |
| HV-HIT | 8 | 1.01 ± 0.42 | 8 | 1.01 ± 0.66 | 0.75 |
| **ATA-miR-93-5p** | | | | | |
| Non-Ex | 8 | 1.03 ± 0.28 | 8 | 1.10 ± 0.73 | |
| LV-HIT | 8 | 1.33 ± 1.01 | 8 | 1.15 ± 0.60 | 0.67 |
| HV-HIT | 8 | 1.08 ± 0.28 | 8 | 1.60 ± 1.37 | 0.33 |
| **ATA-miR-103a-3p** | | | | | |
| Non-Ex | 8 | 1.04 ± 0.31 | 8 | 0.96 ± 0.34 | |
| LV-HIT | 8 | 1.19 ± 0.67 | 8 | 1.18 ± 0.33 | 0.26 |
| HV-HIT | 8 | 1.23 ± 0.40 | 8 | 1.38 ± 0.66 | 0.15 |
| **ATA-miR-146a-5p** | | | | | |
| Non-Ex | 8 | 1.10 ± 0.50 | 8 | 0.94 ± 0.39 | |
| LV-HIT | 8 | 1.44 ± 1.10 | 8 | 1.38 ± 1.10 | 0.52 |
| HV-HIT | 8 | 1.47 ± 0.74 | 8 | 2.16 ± 2.00 | 0.07 |
| **ATA-miR-155-5p** | | | | | |
| Non-Ex | 8 | 1.14 ± 0.57 | 8 | 0.80 ± 0.29 | |
| LV-HIT | 8 | 1.38 ± 0.98 | 8 | 1.36 ± 1.01 | 0.22 |
| HV-HIT | 8 | 1.40 ± 0.65 | 8 | 1.56 ± 0.87 | 0.07 |
| **ATA-miR-222-3p** | | | | | |
| Non-Ex | 8 | 1.03 ± 0.27 | 8 | 1.05 ± 0.42 | |
| LV-HIT | 8 | 1.19 ± 0.55 | 8 | 1.04 ± 0.31 | 0.89 |
| HV-HIT | 8 | 1.15 ± 0.48 | 8 | 1.27 ± 0.78 | 0.49 |
| **ATA-miR-223-3p** | | | | | |
| Non-Ex | 8 | 1.11 ± 0.60 | 8 | 1.50 ± 1.22 | |
| LV-HIT | 8 | 1.75 ± 1.82 | 8 | 2.54 ± 3.60 | 0.73 |
| HV-HIT | 8 | 1.83 ± 1.16 | 8 | 1.79 ± 1.58 | 0.91 |

*The difference (time × group) is the exercise training effect in LV-HIT and HV-HIT compared to the Non-Ex group. n, number of participants; SD, standard deviations; Non-Ex, non-exercising group; LV-HIT, 10 × 1 min exercise group; HV-HIT, 4 × 4 min exercise group; ATA, abdominal adipose tissue; miR, microRNA.*

**TABLE 6 |** Adipose tissue depots differences (gluteal versus abdominal) in miRNA expression response to high-intensity interval training.

| | Gluteal AT | | Abdominal AT | | Difference (group × AT depots) |
|---|---|---|---|---|---|
| | *n* | Mean ± SD | *n* | Mean ± SD | *P-value* |
| **ΔmiR-21-5p** | | | | | |
| Non-Ex | 8 | 1.11 ± 3.13 | 8 | −0.32 ± 0.52 | |
| LV-HIT | 8 | −0.04 ± 0.52 | 8 | −0.28 ± 1.01 | 0.96 |
| HV-HIT | 8 | 0.08 ± 2.19 | 8 | 0.40 ± 1.77 | 0.73 |
| **ΔmiR-27b-5p** | | | | | |
| Non-Ex | 8 | 0.36 ± 1.72 | 8 | −0.15 ± 0.43 | |
| LV-HIT | 8 | −0.16 ± 0.57 | 8 | −0.37 ± 0.68 | 0.80 |
| HV-HIT | 8 | 0.27 ± 1.50 | 8 | 0.00 ± 0.79 | 0.78 |
| **ΔmiR-93-5p** | | | | | |
| Non-Ex | 8 | 0.77 ± 0.07 | 8 | 0.06 ± 0.57 | |
| LV-HIT | 8 | −0.10 ± 0.87 | 8 | −0.18 ± 1.26 | 0.98 |
| HV-HIT | 8 | −0.02 ± 0.88 | 8 | 0.52 ± 1.27 | 0.77 |
| **ΔmiR-103a-3p** | | | | | |
| Non-Ex | 8 | 1.03 ± 3.35 | 8 | −0.08 ± 0.32 | |
| LV-HIT | 8 | −0.09 ± 0.61 | 8 | −0.01 ± 0.86 | 0.87 |
| HV-HIT | 8 | 0.10 ± 0.87 | 8 | 0.15 ± 0.56 | 0.95 |
| **ΔmiR-146a-5p** | | | | | |
| Non-Ex | 8 | 0.97 ± 2.86 | 8 | −0.17 ± 0.43 | |
| LV-HIT | 8 | −0.26 ± 0.67 | 8 | −0.07 ± 1.19 | 0.97 |
| HV-HIT | 8 | −0.20 ± 2.34 | 8 | 0.68 ± 2.09 | 0.48 |
| **ΔmiR-155-5p** | | | | | |
| Non-Ex | 8 | 1.75 ± 4.42 | 8 | −0.33 ± 0.49 | |
| LV-HIT | 8 | 0.03 ± 0.78 | 8 | −0.03 ± 1.15 | 0.63 |
| HV-HIT | 8 | 0.00 ± 2.04 | 8 | 0.16 ± 0.91 | 0.84 |
| **ΔmiR-222-3p** | | | | | |
| Non-Ex | 8 | 1.04 ± 3.24 | 8 | 0.02 ± 0.44 | |
| LV-HIT | 8 | −0.13 ± 0.49 | 8 | −0.15 ± 0.69 | 0.71 |
| HV-HIT | 8 | 0.04 ± 1.00 | 8 | 0.13 ± 0.94 | 0.92 |
| **ΔmiR-223-3p** | | | | | |
| Non-Ex | 8 | 0.24 ± 1.09 | 8 | 0.39 ± 1.17 | |
| LV-HIT | 8 | −0.25 ± 2.02 | 8 | 0.79 ± 2.77 | 0.53 |
| HV-HIT | 8 | −0.19 ± 1.46 | 8 | −0.04 ± 1.57 | 0.52 |

*n, number of participants; SD, standard deviations; Non-Ex, non-exercising group; LV-HIT, 10 × 1 min exercise group; HV-HIT, 4 × 4 min exercise group; AT, adipose tissue; miR, microRNA. ΔmiR corresponds to the changes in miRNA expression calculated by using post-intervention minus pre-intervention values.*

significant differences between these cohorts may, in part, be due to a lack of statistical power because of low subject numbers in the present study.

Despite women with PCOS having significantly higher basal expression of c-miR-27b, there were no differences in the tissue abundance of this miRNA in either subcutaneous abdominal or gluteal AT. This "disconnect" in miRNA expression between the circulatory system and AT could be due to several reasons. First, the origin of c-miR-27b is unknown. Second, the subcutaneous AT depots may lack the specific membrane transporters to import this miRNA from the circulation (Icli and Feinberg, 2017). Third, temporal differences in miRNA expression are likely to exist between different body compartments (i.e., in the systemic circulation versus various tissues/organs). Future work will be required to underpin the mechanistic basis of miRNA "cross-talk" between the circulation and target tissues, and may provide important information linking PCOS and adipogenesis.

Another finding from the current investigation was the decrease in c-miR-27b expression in women with PCOS after 16 weeks of low-, but not high-volume, HIT. It is difficult to explain such a divergent response, especially as only the high-volume HIT protocol was associated with a significant increase in cardiorespiratory fitness. While little is known about c-miR-27b expression patterns following exercise training, Barber et al. (2019) recently reported that 20 weeks of moderate-intensity endurance training in middle aged men and non-PCOS women was associated with a two-fold increase in c-miR-27b (Barber et al., 2019). The discrepancy between our current work and the

findings by Barber et al. (2019) may be explained by differences in exercise modality. HIT versus moderate-intensity continuous endurance training can differentially influence cardiometabolic risk factors including blood pressure, low- and high-density lipoproteins, body weight and insulin sensitivity in patients with cardiometabolic diseases (Weston et al., 2014). Additionally, diverse sampling timepoint (24 h post last training session in the study by Barber et al. (2019) compared to≥48 h in our study) and study participants (middle aged men and women without PCOS compared to women with PCOS in our study) may, in part, help explain these divergent findings. Parr et al. (2016), Denham et al. (2018), Barber et al. (2019), and Da Silva et al. (2019) have previously reported that chronic exercise training programs can significantly modulate c-miRNA expression in healthy individuals and in those with overweight/obesity. The physiological significance of such responses are unclear and difficult to explain due to a variety of different miRNA quantification techniques reported, differences in sampling time points, a range of exercise protocols, and divergent clinical cohorts (Gomes et al., 2015). Time-course studies are needed to span the acute and chronic sampling points for a more comprehensive coverage of miRNA expression profiles.

We found no changes in miRNA expression in either subcutaneous abdominal or gluteal AT after high- or low-volume HIT protocols in women with PCOS. This observation is in agreement with recent data from Tsiloulis et al. (2017) who reported no effect of 6 weeks of endurance exercise training (four supervised sessions/week; three moderate intensity sessions; and one HIT session) on the expression of 526 miRNAs in abdominal and gluteal AT in overweight males. Collectively, these findings indicate miRNAs are stably expressed in both abdominal and gluteal AT and are largely unaltered in response to exercise training protocols lasting up to several months. Furthermore, at baseline, we found no difference in expression of the eight selected miRNAs in basal gluteal versus abdominal AT. Our findings are largely supported by the results of Tsiloulis et al. (2017), who reported only four out of the 526 identified miRNAs to be differentially expressed between gluteal and abdominal adipocytes in overweight males. In addition, we found no differentially expressed miRNAs between the AT depots following exercise training. Other studies have reported that AT from different depots express unique molecular, cellular, and metabolic properties and respond in distinct ways to exercise and nutritional challenges (Tan et al., 2004; You et al., 2014). However, this does not seem to be the case for selected miRNA expression.

A major strength of the present study is the concurrent investigation of miRNA expression patterns in the circulation and abdominal and gluteal AT. Some miRNAs exert specificity in tissue expression within the body and can either be released or taken up by these tissues from the bloodstream (Russell and Lamon, 2015). Thus, investigation of targeted miRNAs from both the circulation and a "target" tissue allowed us to compare expression patterns between these tissues. We also acknowledge study limitations: we used a targeted approach to selectively investigate a small number of miRNAs and therefore may have missed changes in expression patterns of other miRNAs. Finally, larger subject numbers would have conferred greater statistical power to detect small, but potential differences in miRNA expression.

In conclusion, we report that the basal expression of circulating miR-27b was higher in women with PCOS compared to women without PCOS. We were unable to detect any differences in the eight targeted miRNAs in subcutaneous abdominal and gluteal AT. While 16 weeks of low- but not high-volume HIT altered the expression of c-miR-27b in women with PCOS, the chronic exercise training protocols employed in this investigation did not induce alterations in the expression of the other targeted miRNAs within the circulation or the miRNAs in subcutaneous abdominal and gluteal AT in women with PCOS. Further studies are required to ascertain miR-27b's role and association with obesity, inflammation and adipogenesis in PCOS.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Regional Committee for Medical and Health Research Ethics in Central Norway (REK-midt 2015/468 and 2016/545) and the Australian Catholic University Human Research Ethics Committee (2017-260H). The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

SL and DC drafted the manuscript, performed the analyses, and analyzed the data. SL and SLy performed statistical analyses. SL, IK, DC, EV, JH, and TM were responsible for study conception and design. SL, IK, EP, and TM coordinated the study at the two sites, performed measurements on test-days, and supervised the exercise training. All authors provided feedback and approved the final manuscript.

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