EXERCISE AND SPORT: THEIR INFLUENCES ON WOMEN'S HEALTH ACROSS THE LIFESPAN, VOLUME II

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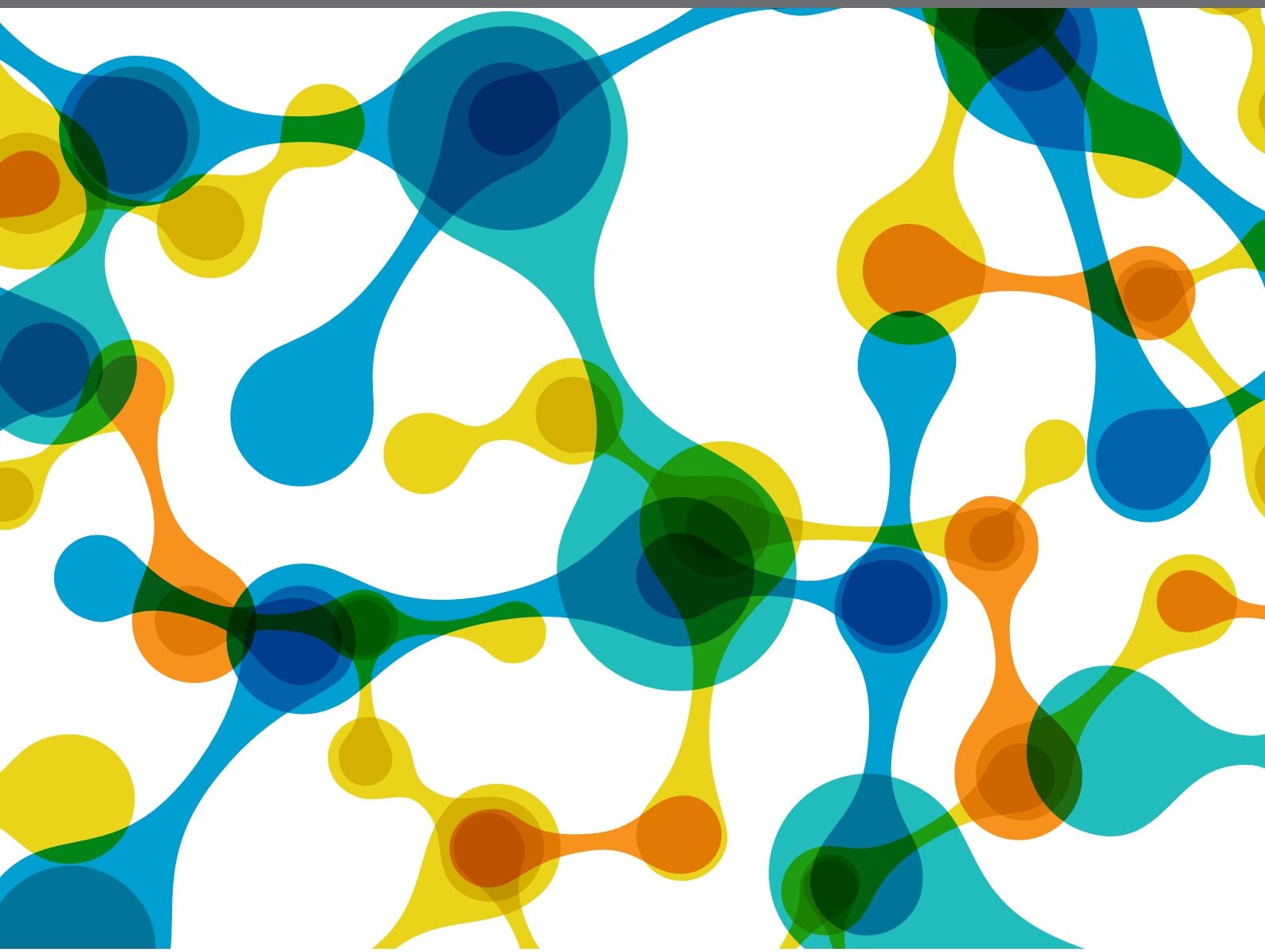

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EXERCISE AND SPORT: THEIR INFLUENCES ON WOMEN'S HEALTH ACROSS THE LIFESPAN, VOLUME II

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Effectiveness of a Virtual Exercise Program During COVID-19 Confinement on Blood Pressure Control in Healthy Pregnant Women

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Background: The situation caused by COVID-19 has led to movement restrictions for the majority of the population due to the confinement established by the health authorities. This new situation has changed people's habits and significantly affected the pregnant population. Decreased exercise and increased psychophysical stress are associated with excessive weight gain, diabetes, and gestational cardiovascular complications that affect the mother, fetus, and newborn. Recent research shows that the dynamics of maternal blood pressure is one of the most important control factors during pregnancy. Thus, prevention of these type of pathologies through interventions without maternal-fetal risks is important.

Objectives: To examine the influence of a virtual exercise program on maternal blood pressure during pregnancy.

Materials and Methods: A randomized clinical trial design was used (NCT04563065). Data from 72 pregnant women without obstetric contraindications under confinement conditions in the Madrid area were collected. Women were randomly assigned to the intervention (IG) or control group (CG). They previously signed informed consent forms. A moderate exercise program was performed as an intervention from 8–10 to 38–39 weeks of pregnancy. Systolic (SBP) and diastolic (DBP) maternal blood pressure were measured during the first, second and third trimesters of pregnancy, as well as before and immediately after delivery in both study groups.

Results: No differences in systolic and diastolic blood pressure during the first, second and third trimesters were found between groups. Significant differences in SBP were found immediately before delivery (IG = 119.83 ± 10.16 vs. CG = 125.6 ± 10.91; $p = 0.047$) and immediately after delivery (IG = 115.00 ± 11.18 vs. CG = 122.24 ± 15.71; $p = 0.045$).

Conclusions: Results show lower SBP values for the IG during delivery than CG. A virtual exercise program throughout pregnancy during COVID-19 confinement can help to control systolic blood pressure before and immediately after delivery in healthy pregnant women.

Keywords: exercise, pregnancy, maternal, gestational hypertension, pandemic

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INTRODUCTION

Given the quantity and quality of modifications in a woman's body that are involved in pregnancy, there is no physiological process like pregnancy or childbirth in the life of the human (Barakat et al., 2015). Pregnancy is distinguished by a multitude of physiological, mental and emotional adjustments. Every organ system in the expectant mother is intimately involved in this complex process, to create an optimal environment for fetal development (Artal et al., 1991). From the circulatory/hemodynamic point of view, the changes begin during the fifth week of gestation and last until approximately 1 year after delivery (Clapp and Capeless, 1997). Cardiac output increases by approximately 40% (due to an increased stroke volume and heart rate [HR]). There is a 13% increase in body mass supplied by the maternal blood (Geva et al., 1997). Important changes in the blood volume, systemic vascular resistance and vascular tone exist during pregnancy (Gilson et al., 1997; Carbillon et al., 2000; Bamfo et al., 2007; Zentner et al., 2012).

An increasing number of women in Western countries develop cardiovascular disease during pregnancy. The estimation is a rise of risk about 0.2–4% (Perales et al., 2016). Hypertension is one of the most common gestational complications with a prevalence of 10% depending on factors such as country and population studied, and the criteria used to establish the diagnosis (Acog Committee on Practice Bulletins–Obstetrics, 2002; Obstetrics and Gynecology, 2013, 2019). The consequences of cardiovascular disorders affect the mother, fetus, and newborn. Indeed, hypertensive complications during pregnancy remain a leading cause of maternal and neonatal morbidity and mortality (Amro and Sibai, 2020) and may remain 5–15 years following delivery (Bellamy et al., 2007). Many studies have shown that a sedentary lifestyle and decreased movement are determinants of multiple deleterious complications, especially cardiovascular complications (Rand et al., 2020; Yong et al., 2020).

Due to the serious health problem presented by the current global pandemic, the movement restrictions and confinement caused by the COVID-19 pandemic have significantly affected the lifestyles of the pregnant population and can become a risk factor for different alterations and even pathologies (Ayaz et al., 2020; Juan et al., 2020; Moyer et al., 2020; Zaigham and Andersson, 2020). In the current sanitary situation, the new confinement and environment of decreased physical mobility can increase the risks of cardiovascular disease, such as gestational hypertension, with several associated complications (Alomari et al., 2020; Justman et al., 2020; Magee et al., 2020).

Furthermore, the social isolation experienced by pregnant women during confinement has kept them away from their family members and other networks, and consequently this has led to an increase in prenatal depressive symptoms and anxiety (Ammar et al., 2020a,b; Chivers et al., 2020; Durankuş and Aksu, 2020); this implies an essential need for strategies to support both physical and mental health of pregnant women.

The prevention of these pathologies should be a basic column in the sanitary planning of health institutions, and it is necessary to use innocuous interventions that achieve this prevention without adverse effects for the mother, fetus, and newborn. The use of exercise as one of those preventive columns in general populations is sufficiently supported by scientific evidence; however, the efficacy of exercise in this prevention strategy during pregnancy is still poorly investigated (Davenport et al., 2018). In that sense, several scientific studies confirm the relationship between exercise during pregnancy and improved pregnancy outcomes. Therefore, international guidelines for exercise during pregnancy recommend an active pregnancy for pregnant women without obstetric complications (Mottola et al., 2018; Barakat et al., 2019; Obstetrics and Gynecology, 2020). In this sense, with the continuity of the pandemic and lack of face-to-face activities, one strategy that could be is delivery of physical activity interventions through virtual modalities.

The main objective of this study was to examine the influence of a virtual supervised exercise program throughout pregnancy on maternal systolic blood pressure (SBP) and diastolic blood pressure (DBP). We hypothesize that a supervised, moderate, and regular exercise program throughout pregnancy may be a helpful factor in controlling maternal blood pressure.

MATERIALS AND METHODS

Study Design

This study was developed by the collaboration between the Obstetrics and Gynecology Department of the Hospital Universitario Severo Ochoa (Madrid) and Universidad Politécnica de Madrid. A randomized clinical trial (NCT04563065) was approved by the Ethical Commission of Research of Universidad Politécnica de Madrid. Women were randomly assigned to the intervention group (IG) or control group (CG). The Selene program at the Hospital Universitario Severo Ochoa in Leganés (Madrid) was used to collect the personal, labor, and medical data of the participants.

Participants and Randomization

A total of 206 Spanish-speaking pregnant women from hospital obstetric consults (**Figure 1**) were assessed for eligibility. Women aged between 18 and 45 years with singleton and uncomplicated pregnancies, with no history or risk of preterm delivery and not participating in any other trial or exercise program were invited to participate. The following conditions were excluded of the study: not planning to give birth in the same obstetric hospital, not being under medical follow-up throughout pregnancy having any serious contraindicated conditions for practicing safe exercise (Mottola et al., 2018; Barakat et al., 2019; Obstetrics and Gynecology, 2020).

For the randomization of participants, a process of allocation concealment by random number blocks was used. Assessment staff was blinded to the assignments. Randomization

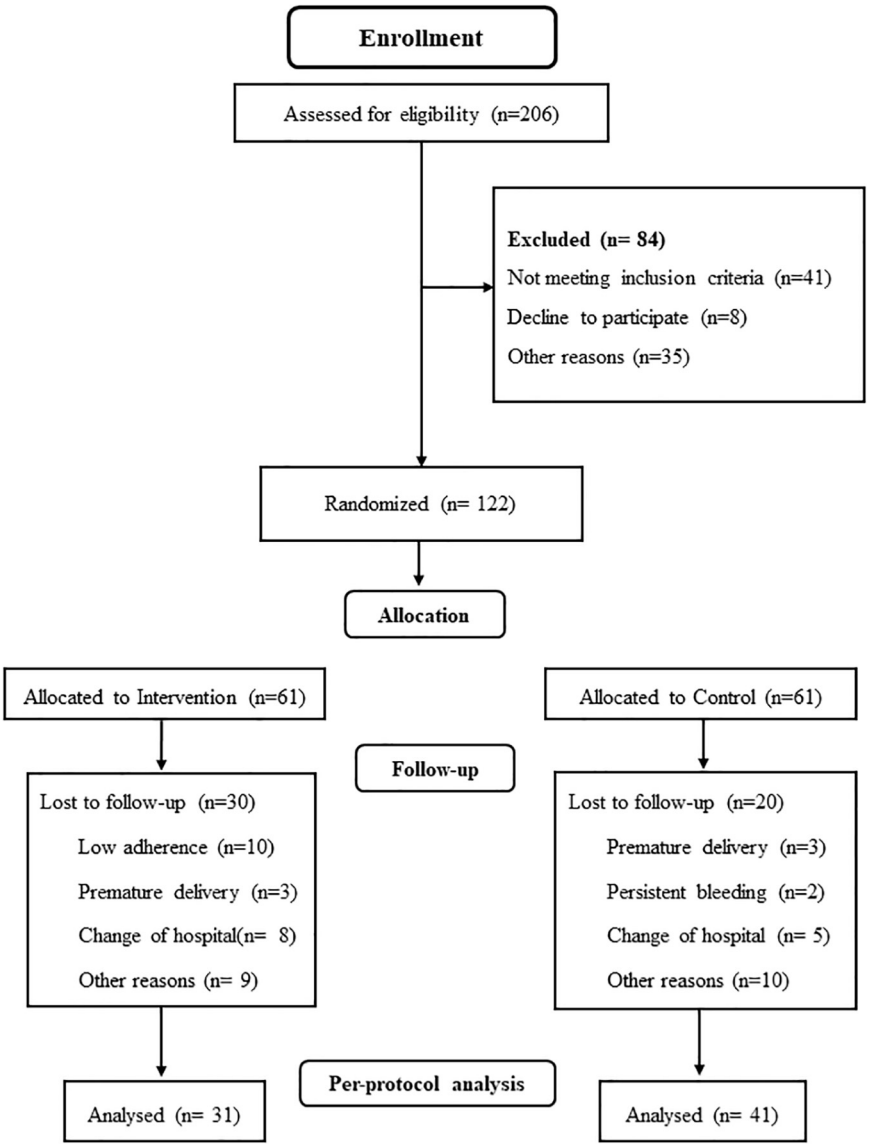

FIGURE 1 | Study population flow chart.

process (sequence generation, allocation concealment, and implementation) was conducted by three different individuals.

Intervention

Women assigned to the intervention group (IG) adhered to a virtual supervised exercise program between 8–10 and 38–39 weeks of pregnancy. An average of 80–85 training sessions were originally planned for each participant, and a minimum of 80% adherence to the exercise program was required to be included in the analysis of the results.

The virtual supervised exercise program involved 3 weekly sessions of 55–60 min of varied activities following a model established by our research group. Regarding the moderate intensity of the workload and due to the non-face-to-face nature of the program, pregnant women were previously informed for self-control through two mechanisms: Maternal Heart Rate (MHR) and perception of effort. Therefore, women used a heart rate monitor (Accurex Plus, Finland) during the training sessions (MHR was consistently 55–65% of heart rate reserve using the Karvonen formula) and a range of 12–14 of Borg Rate of Perceived Exertion Scale (Somewhat Hard) (Barakat, 2020).

From the methodological point of view, the session was divided into seven parts (Barakat, 2020):

1. Warm-up with general exercises of 5–7 min: Range of motion varied, but impact activities were not included (avoidance of jumps and falls).
2. Aerobic exercises of 8–10 min: Exercises were performed to increase the intensity up to that of moderate activities.

3. Muscle strengthening and general toning exercises of the whole body of 10–12 min: Exercises for the lower extremities (calf, quadriceps, hamstrings, adductors, abductors) and torso (abdominal, pectoral, shoulders, paravertebral musculature) were included. The muscle groups to train were distributed into the 3 weekly sessions. During each session, one or two sets of 10–12 repetitions were performed from each muscle group using barbells (2–3 kg/exercise) or low-to-medium resistance (elastic) bands (Therabands). Exercises for the most weakened muscle groups during pregnancy were also included, as the aim was to avoid muscular decompensation.
4. Coordination and balance exercises of 5–8 min: Simple eye-hand and eye-foot coordination tasks were performed with sports equipment, as well as body axis balance exercises.
5. Strengthening the pelvic floor muscles of 8–10 min: Kegel exercises were performed.
6. Cool down session of 5–7 min: The aim was to gradually lower the intensity of work with flexibility, stretching, and relaxation exercises.
7. Final discussion of 7–8 min: The aim of this session was for the pregnant women to clearly and openly express their sensations and perceptions experienced during the training session. This part was conducted only during the group virtual session.

Exercises in the supine position were not performed for more than 2 min and impact activities were not included in the sessions. Adequate hydration before and after exercise was recommended and high humidity and temperature environment for exercise was avoided in order to prevent maternal hyperthermia.

The exercise program was provided by two modalities:

1. Individual work (2 weekly sessions): These were recorded sessions, with complete visual information and indications regarding the exercises to be performed. These sessions were designed so that pregnant women could follow very easily and intuitively, and the participants had simple and agile access for downloading.
2. Group work (1 weekly session): Classes were supervised online through the Zoom Video platform.

Usual Care (Control) Group

Women assigned to the CG received general advice from their health care providers, including positive effects of physical activity or nutritional recommendations, and the usual monitoring health care sessions, which were equal to the exercise group. They also were asked about their exercise once each trimester; to this end, a "decision algorithm" (by telephone) was used (Barakat et al., 2016).

Algorithm:

Question 1: Since the beginning of pregnancy, have you exercised in your leisure time, in a supervised program or on your own?
a. Answer: No.
b. Answer: Yes.

Question 2: (if the previous response was "b"): Given 7 days a week, how many days per week did you exercise?
a. Answer: Less than 3 days.
b. Answer: 3 days or more.

Question 3: (if the previous response was "b"): Considering the total duration of physical exercise continuously, how long did you exercise every day?
a. Answer: Less than 20 min each day.
b. Answer: 20 min or more each day.

Interpretation of the "Decision Algorithm": Pregnant women in the CG who reached level b of these three questions, were excluded from the study.

Outcomes

Primary Outcome

Data corresponding to SBP and DBP were obtained from protocol obstetric visits and delivery records following established hospital protocols. Therefore, data from the first, second and third trimesters as well as previous and immediate deliveries were collected. Blood pressure during pregnancy was recorded at week 10 ± 2 for the first trimester, at week 22 ± 1.5 for the second, and at week 36 ± 1.5 for the third trimester. Data collection of SBP and DBP of delivery was performed during the period of dilation, about an hour before childbirth and the postpartum measurement was taken immediately after delivery, in the "immediate puerperium."

Secondary Outcomes

Gestational weight gain was calculated from the data of pregravid and last clinic visit before delivery weights. It was classified according to the 2009 Institute of Medicine (IOM) guidelines (Institute of Medicine (US) and National Research Council (US) Committee to Reexamine IOM Pregnancy Weight Guidelines, 2009). Excessive body weight gain was determined by IOM guidelines for pre-pregnancy BMI categories for each woman; >18 kg for underweight; >16 kg for normal; >11.5 kg for overweight; and >9 kg for obese women.

Data on maternal gestational age, birth weight, Apgar scores and pH of the umbilical cord blood were obtained from hospital perinatal records. Newborns were classified as having macrosomia when birth weight was $>4{,}000$ g and low birth weight was defined as $<2{,}500$ g.

Statistical Analysis

Version 25.0 of IBM SPSS for Windows (IBM Corporation, Armonk, NY, United States) was used for all data analyses. Preliminary assessments were conducted using the Kolmogorov-Smirnov test to screen for violations of normality.

Pearson's chi-square test was used to compare the obtained frequencies of maternal BMI, smoking, previous miscarriages, parity and employment occupation between the IG and CG.

Independent *t*-tests were used to assess the differences in age, gestational age, weight and height between the intervention and control groups.

In addition, this same test was used to examine SBP and DBP data in the first, second and third trimesters, previous delivery time and immediately after delivery between study

groups. Besides, one Factor Repeated Measure ANOVA used to assess changes in SBP and BPD throughout pregnancy within the IG and CG and between both groups. Multiple comparisons were made using the Bonferroni test. The effect size was obtained using the partial index η^2.

The data for continuous variables are presented as the means and standard deviations, and those of the nominal variables are presented as frequencies and percentages. The level of statistical significance was set at $p < 0.05$.

RESULTS

A total of 206 women over 18 years of age were randomized (ratio 1:1) in the study, and 84 were excluded: 41 did not meet the inclusion criteria, 8 declined to participate, and 35 declined for other reasons. Then, 122 women were randomized into IG ($n = 61$) and CG ($n = 61$). In the IG, 30 women were lost to follow-up: 3 had premature deliveries, 10 had low adherence, 8 had changes in hospital stay, and 9 had other reasons. The minimum adherence required has been attendance to 80% of the classes within the intervention group. In the CG, 20 women were lost to follow-up: 2 had persistent bleeding, 3 had premature delivery, 5 had changes in hospital stay, and 10 had other reasons. Finally, 31 women in the IG and 41 in the CG were analyzed (**Figure 1**).

Table 1 shows the general characteristics of the pregnant women in the study groups. No significant differences ($p > 0.05$) in maternal characteristics were found between the groups.

There were no significant differences in systolic and diastolic blood pressure at the first, second and third trimesters between the IG and CG ($p > 0.05$) (**Table 2**).

No significant intergroup differences were found (IG vs. CG) comparing the evolution of SBP [$F_{(3.78)} = 0.639$; $p = 0.551$; $\eta^2 = 0.018$] and DBP [$F_{(3,10)} = 0.684$; $p = 0.564$; $\eta^2 = 0.020$] during the whole process of pregnancy.

Performing an intragroup analysis, there were significant differences within the IG in the SBP value depending on the time of pregnancy [$F_{(2.36)} = 3.086$; $p = 0.046$; $\eta^2 = 0.128$]. Multiple comparisons show that SBP in the third trimester was significantly higher compared to the second trimester ($p = 0.022$). However, no differences were found between the first trimester in relation to the second and third. On the other hand, within the CG, no significant differences were found in the evolution of SBP [$F_{(1.30)} = 1.65$; $p = 0.210$; $\eta^2 = 0.089$].

In relation to DBP, significant differences were found depending on the time of pregnancy within the IG [$F_{(2.42)} = 4.34$; $p = 0.019$; $\eta^2 = 0.171$] as of the CG [$F_{(2.34)} = 4.37$; $p = 0.020$; $\eta^2 = 0.205$].

Within the IG, DBP showed a significant increase in the third trimester of pregnancy compared to the second ($p = 0.036$). No significant differences were found between the first compared to the second and third trimesters of pregnancy ($p > 0.05$).

Finally, within the CG, multiple comparisons revealed that DBP presented a significant decrease in the second trimester of pregnancy compared to the first ($p = 0.007$). At last, no significant differences were observed between the third trimester compared to the first and second.

According to the analysis, significant differences in SBP were found immediately before delivery ($t_{56} = 2.034$; IG = 119.83 ± 10.16 vs. CG = 125.6 ± 10.91; $p = 0.047$) and immediately after delivery ($t_{60} = 2.046$; IG = 115.00 ± 11.18 vs. CG = 122.24 ± 15.71; $p = 0.045$). There were no significant differences in DBP between the IG and CG either immediately before delivery (IG = 72.82 ± 8.20 vs. CG = 73.94 ± 9.88; $p = 0.63$) or immediately after delivery (IG = 65.71 ± 9.14 vs. CG = 68.47 ± 13.62; $p = 0.329$).

DISCUSSION

We examined the effects of virtual exercise programs during pregnancy on the control of blood pressure in healthy pregnant women. This novel approach used an integration of light resistance, toning, aerobic dance, stretching, and pelvic floor exercises in the virtual training program, which were easily incorporated into a structured exercise regime. Although not analyzed in this study, this new program includes special care for the emotional aspect of pregnancy; this aspect is generally not available to the pregnant population in exercise programs. It seems that this program was equally liked by all BMI categories, as indicated by the high adherence rate.

Our intervention exercise during pregnancy does not affect the values obtained from the protocol for obstetric controls of blood pressure in the first, second and third trimesters; however, in the data prior to and immediately after delivery, a decrease in SBP of the IG was found. Despite finding statistically significant differences in SBP and BPD within the IG and CG, these differences are within the normal levels of obstetric control and the evolution of this parameter during pregnancy.

Both time points (before and after delivery) are especially relevant due to the hemodynamic stress to which the pregnant woman is subjected to. These results may suggest a "decompression" effect of the maternal circulatory system during stressful events. From a hemodynamic point of view, the decrease in SBP in the IG group at delivery, but not in the 1st, 2nd, or 3rd trimesters, could be due to effects based on the adaptation to the acute requirements of the maternal circulatory system that exercise training during pregnancy may perpetuate (Haakstad et al., 2016).

No cause was identified for cardiovascular disorders, such as pregnancy-induced hypertension. However, it seems to be developed early in gestation, appearing symptoms in the mid- to late part of the pregnancy (Roberts and Lain, 2002). Important advances in this field have been published recently, including the identification of long-term maternal and fetal risks conferred by pre-eclampsia (Phipps et al., 2019). This could be enhanced by the complex situation caused by COVID-19 (confinement and movement restrictions, social isolation, etc.).

The benefits of an exercise program throughout pregnancy on SBP before and after delivery in healthy pregnant women (also inducing a healthy lifestyle) could demonstrate the need to promote new strategies (an

TABLE 1 | Maternal characteristics.

Variable	Intervention group (*n* = 31)	Control group (*n* = 42)	*p*-values
Age (years)	32.29 ± 6.36	33.93 ± 4.59	0.205
Maternal height (m)	1.63 ± 0.05	1.63 ± 0.07	0.816
Maternal weight (kg)	60.37 ± 9.73	66.34 ± 14.12	0.059
BMI (*n*/%)	22.61 ± 3.22	23.06 ± 7.80	0.776
<18.5	2/7.1	4/9.8	0.190
18.5–24.9	22/78.6	22/53.7	
25–29.9	3/10.7	11/26.8	
>30	1/3.6	4/9.8	
Parity[†] (*n*/%)			
None	25/80.6	27/65.9	0.333
One	5/16.1	10/24.4	
Two or more	1/3.2	4/9.8	
Smoking during pregnancy			
No	30/96.8	35/83.3	0.069
Yes	1/3.2	7/16.7	
Occupation (*n*/%)			
Active job	15/48.4	19/45.2	0.063
Sedentary job	13/41.9	11/26.2	
Homemaker	2/6.5	12/28.6	
Previous miscarriage (*n*/%)			
None	25/80.6	26/63.4	0.069
One	6/19.4	9/22.0	
Two or more	0/0	6/14.6	

[†]*Parity: children until current pregnancy.*

TABLE 2 | Systolic blood pressure and diastolic blood pressure at first, second, and third trimester in IG and CG.

		Intervention group (*n* = 31)	Control group (*n* = 42)	*p*-values
First trimester	SBP	110.55 ± 12.13	110.76 ± 13.30	0.949
	DBP	71.00 ± 7.41	72.95 ± 8.07	0.317
Second trimester	SBP	108.81 ± 14.41	113.96 ± 15.46	0.220
	DBP	68.96 ± 9.58	70.15 ± 7.97	0.628
Third trimester	SBP	115.45 ± 11.86	116.43 ± 12.76	0.753
	DBP	74.55 ± 6.53	73.77 ± 10.01	0.720

exercise program) and might be key issues to prevent chronic disease risk.

From a scientific point of view, epidemiological studies suggest that women who are physically active are less likely to develop gestational hypertension (Sorensen et al., 2003; Saftlas et al., 2004; Rudra et al., 2008; Martin and Huber, 2010; Barakat et al., 2016), although new data regarding the relationship between exercise during pregnancy and maternal blood pressure in the current COVID-19 pandemic situation are urgently needed.

From our point of view, this is the first study linking an early exercise intervention with high adherence to reduce the values of maternal SBP before and after delivery.

The scientific literature confirms that maternal exercise during pregnancy has important benefits; it has been associated with lowering blood pressure (Yeo, 2010), and the effects of exercise include an increase in aerobic and cardiovascular conditioning (Ruchat et al., 2012). Exercise may protect against preeclampsia by reducing maternal byproducts of oxidative stress, preventing endothelial dysfunction, and stimulating vascularity and placental growth (Falcao et al., 2010).

In addition, exercise has been linked to beneficial fetal and pregnancy outcomes (Wang et al., 2015; Pelaez et al., 2019; Vargas-Terrones et al., 2020), including mental and emotional aspects, which affect maternal blood pressure.

Our large RCT confirms the beneficial effects of exercise during pregnancy on the control of maternal blood pressure and demonstrates the importance of the use of the lifestyle factors of pregnant women for the prevention of risk factors and cardiovascular diseases during the pandemic state. In summary, a healthy lifestyle intervention with high adherence could be a relevant preventive element in the health of the pregnant population. It is important to note that the results could be affected by the nature of the follow-up used and the exclusions in both study groups.

CONCLUSION

A virtual exercise program throughout pregnancy during COVID-19 confinement can help to control systolic blood pressure before and immediately after delivery in healthy pregnant women.

Strengths and Weaknesses

The main strengths of our study are the design and development of a large virtual RCT with high adherence (≥80% attendance) in our exercise group, while also examining the activity of the pregnant women in the CG. We believe that this is especially relevant in the current pandemic situation.

Assessment of the exercise activity of CG (excluding highly active women) and evaluation of adherence (attendance) to a virtual exercise program of IG must be considered when examining the effects of RCTs to provide relevant information from the primary outcome to clinical practice.

Although we did not specifically evaluate women with elevated hypertension or preeclampsia, the fact that women who exercised had lower SBP values (a precursor to the development of preeclampsia) also suggests that maternal exercise may prevent cardiovascular complications.

A possible limitation of our study has been the exclusion from the final analysis of women who were non-adherent (IG) and women who were physically active (CG). Besides, other limitation of our study is the lack of nutrition or energy intake assessment; however, all participants received standard care and information about a healthy lifestyle during pregnancy in their formal obstetric consults.

In addition, the supervision of a virtual program is not identical to a face-to-face session; however, this is due to the current pandemic situation and thus using online technologies is the only way in which health authorities will allow this type of intervention with a pregnant population (program exercise). Finally, another limitation could have been the of the lack of data collection for family history of hypertension, and this could be an interesting variable to analyze in future studies.

Therefore, due to the complex healthcare environment caused by COVID-19, future studies should examine the preventive effects of interventions in alterations that the current pandemic situation will cause.

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 Ethical Commission of Research of Universidad Politécnica de Madrid. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CS-J and MS-P were responsible for the design and development of the virtual supervised program and contributed to the data analysis and the preparation of the manuscript. ÁD-B contributed to the recruitment and randomization of pregnant women, clinical data collection, registration, and analysis. JC and RB contributed to the experimental design, data analysis, and preparation of the manuscript. IR oversaw the experimental design, data analysis, and manuscript writing. All authors contributed to the article and approved the submitted version.

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Absent Exercise-Induced Improvements in Fat Oxidation in Women With Polycystic Ovary Syndrome After High-Intensity Interval Training

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Background: Polycystic ovary syndrome (PCOS) and metabolic inflexibility are linked to insulin resistance, and women with PCOS appear to be metabolic inflexible in the rested, insulin-stimulated state. Exercise training is a primary lifestyle intervention in PCOS. Exercise training improves whole-body fat oxidation during submaximal exercise in healthy women, yet little is known about the effect on this outcome in women with PCOS.

Methods: We measured whole-body fat oxidation rates during sub maximal exercise before and after 16 weeks of high-intensity interval training (HIT) in women with PCOS randomly allocated to either: low- or high-volume HIT ($n = 41$; low-volume HIT, 10 × 1 min work bouts at maximal, sustainable intensity and high-volume HIT, 4 × 4 min work bouts at 90–95% of maximal heart rate) or non-exercise control ($n = 23$), and in women without PCOS (Non-PCOS) allocated to low- or high volume HIT ($n = 15$). HIT was undertaken three times weekly. In a subset of women with and without PCOS, we measured mitochondrial respiration in abdominal and gluteal subcutaneous adipose tissue using high-resolution respirometry, as well as fat cell sizes in these tissues.

Results: At baseline, women with PCOS had lower whole-body fat oxidation and mitochondrial respiration rates in abdominal adipose tissue compared to Non-PCOS. Peak oxygen uptake (mL/min/kg) increased in women with PCOS (∼4%, $p = 0.006$) and Non-PCOS (∼6%, $p = 0.003$) after 16 weeks of HIT. Whole-body fat oxidation only improved in Non-PCOS after HIT. No changes were observed in mitochondrial respiration and cell size in abdominal and gluteal adipose tissue after HIT in either group of women.

Conclusion: We observed exercise-induced improvements in whole-body fat oxidation

during submaximal exercise in Non-PCOS, but not in women with PCOS, after 16 weeks of HIT, suggesting metabolic inflexibility in women with PCOS.

Clinical Trial Registration: www.clinicaltrials.gov, identifier NCT02419482 and NCT02943291.

Keywords: endocrinology, metabolic flexibility, exercise, cardiorespiratory fitness, insulin resistance, mitochondrial respiration, adipose tissue, cell size

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in reproductive-age women, affecting up to 13% of women globally (Bozdag et al., 2016). PCOS is associated with increased risk of infertility, insulin resistance, type 2 diabetes and cardiovascular diseases (Moran et al., 2010; Teede et al., 2010; De Groot et al., 2011). Despite the high prevalence and adverse health complications of PCOS, the underlying mechanisms and optimal treatment are still unclear. Insulin resistance and the compensatory hyperinsulinemia is proposed to play a central role in the pathophysiology, contributing to the metabolic and reproductive features of PCOS (Diamanti-Kandarakis and Papavassiliou, 2006).

Metabolic flexibility is the ability to alter substrate use in response to a physiological stimulus, including the transition from fasting to fed states/insulin stimulation or exercise (Goodpaster and Sparks, 2017). Metabolic inflexibility, characterized by distorted nutrient sensing, blunted substrate switching, and impaired energy homeostasis, is linked to insulin resistance (Goodpaster and Sparks, 2017). Women with PCOS appear to have higher metabolic inflexibility in the rested, insulin-stimulated state compared to unaffected women (Rimmer et al., 2020). Previous studies have suggested that the insulin resistance observed in PCOS may be linked to aberrant adipose tissue morphology and function including enlarged adipocytes and decreased insulin-stimulated rates of glucose utilization in adipocytes (Dunaif et al., 1992; Manneras-Holm et al., 2011).

Lifestyle modification including regular physical activity is regarded as first-line therapy in women with PCOS (Teede et al., 2018). Exercise training induces a multitude of positive, health-related outcomes in women with PCOS (Kite et al., 2019), and superior effects of vigorous intensity exercise compared to moderate intensity training, have been observed among women with PCOS (Greenwood et al., 2016; Patten et al., 2020).

The aims of this study were first to compare whole-body fat oxidation during submaximal exercise, cell size and mitochondrial respiration of adipose tissue in women with and without PCOS, and second to assess the response to 16 weeks of HIT.

MATERIALS AND METHODS

Study Design

The present study is a secondary analysis of a two-center, randomized controlled trial; IMProving Reproductive function in women with Polycystic OVary syndrome with high-intensity Interval Training [IMPROV-IT (Kiel et al., 2020); ClinicalTrials.gov identifier: NCT02419482] conducted at the Norwegian University of Science and Technology (NTNU) in Trondheim, Norway and the Australian Catholic University (ACU) in Melbourne, VIC, Australia, and a randomized, uncontrolled trial undertaken at NTNU [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 detailed study design for the IMPROV-IT trial has been published elsewhere (Kiel et al., 2020). In brief, after stratification for BMI $<$ or $\geq$ 27 kg/m^2 and study center, women with PCOS were randomized in a 1:1:1 manner to 16 weeks of semi-supervised HIT or a no-exercise control group: (1) Low-volume HIT (LV-HIT), (2) High-volume HIT (HV-HIT), or (3) Non-exercise (Non-Ex).

In the HIT-FAT trial, women without PCOS were selected as a control group, and individually matched by age ($\pm$ 5 years) and BMI ($\pm$ 2 kg/m^2) to women with PCOS in the IMPROV-IT trial. Women without PCOS were randomly allocated (1:1), after stratification for BMI $<$ or $\geq$ 27 kg/m^2, to 16 weeks of semi-supervised LV-HIT or HV- HIT. For the purpose of the analyses in this report and because no differences were observed between the LV-HIT and HV-HIT groups, we pooled the LV-HIT and HV-HIT groups into one group for women with PCOS (PCOS HIT) and one group for women without PCOS (Non-PCOS HIT).

Ethical Approval

The studies were 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 in writing before their written consent was obtained.

Participants

Sixty-four previously inactive women with PCOS and 15 previously inactive women without PCOS were included in this study. PCOS was defined according to the Rotterdam criteria (Rotterdam, 2004), with at least two of the following three features present: polycystic ovary morphology (12 or more 2–9 mm follicles or $>$10 ml in volume in at least one ovary), hyperandrogenism (either clinical signs such as acne or hirsutism, or biomedical), and/or oligo/amenorrhea. Hirsutism was defined as a modified Ferriman-Gallwey score of $\geq$8 (Ferriman and Gallwey, 1961). The PCOS diagnosis was ruled out in all the women without PCOS as they were

normally menstruating, with no evidence of hyperandrogenism or polycystic ovaries.

To be eligible for inclusion, the women had to be between 18 and 45 years old and were excluded if they were undertaking regular endurance training ≥ 2 sessions/week, had any cardiovascular diseases or endocrine disorders, were pregnant, had been breastfeeding within the last 24 weeks, or if they were using hormonal contraceptives, insulin sensitizers or drugs known to affect gonadotropin or ovulation (with a washout period of 3 months prior to inclusion).

Interventions

The exercise training was semi-supervised during the 16 weeks intervention period, and participants attended at least one weekly supervised training session, with the opportunity to perform the two remaining weekly sessions either supervised at the study centers or unsupervised (total of three exercise sessions per week).

The exercise training protocols have been described previously (Kiel et al., 2020). Briefly, participants walked or ran on treadmills during the supervised exercise sessions, while they could choose to perform the unsupervised exercise sessions walking or running on treadmills or outdoors. The LV-HIT protocol consisted of 10 × 1 min work bouts at the maximal intensity the participants could sustain, interspersed by 1 min of passive recovery or low-intensity walking. The HV-HIT protocol comprised 4 × 4 min work bouts at an intensity corresponding to 90–95% maximal heart rate (HR_{max}) interspersed by 3 min active recovery at ∼70% of HR_{max}. All training sessions included 10 min warm-up and 3 min cool-down. Participants wore HR monitors (Polar M400) during all exercise sessions, and registered their exercise sessions via an online exercise-training diary (Polar Flow) which the researchers had access to. Thereby, the researchers could supervise adherence to the protocols.

Women with PCOS assigned to the Non-Ex group were advised to continue their habitual physical activity and informed about the current recommendations of at least 150 min weekly of moderate intensity physical activity. All participants were instructed to maintain their habitual diet throughout the intervention period, and dietary intake was controlled using a 4-day diet recall at baseline and in the last week of the intervention. Physical activity level was monitored using activity monitors (Sensewear Armband, APC Cardiovascular, UK) for 5 days at baseline and during the last week of the intervention period.

Outcomes

Figure 1 displays an overview of the study protocol. Outcomes were assessed at baseline and after 16 weeks of intervention. All assessments were performed in the early follicular phase (day 1–7 after first bleeding) of the participants' menstrual cycle in women with a regular menstrual cycle while women with oligo/amenorrhea were tested independent of their cycle day.

Participants visited the laboratory on three separate occasions at baseline and after 16 weeks. On the first visit, participants performed an incremental test to exhaustion on a treadmill. We used an individualized protocol to measure peak oxygen uptake (VO_2peak) and maximal heart rate (HR_{max}); after 10 min warm-up and 3 min at moderate intensity, the treadmill speed or inclination was increased every 1–2 min by 0.5–1.0 km/h or 1–2% until volitional exhaustion. The recorded HR_{max} was used to calculate the intensity for the HIT sessions (Berglund et al., 2019).

On the second visit, participants returned to the laboratory after an overnight fast (≥12 h) and refraining from exercise >48 h prior. In Norway, body composition was estimated using bioelectrical impedance analysis (InBody 720, Biospace CO, Korea) while Dual-energy X-ray absorptiometry (DXA; GE Lunar iDXA Pro, Encore software version 16, General Electric, Boston, MA, USA) was used in Australia. Body composition was estimated using the same method in each individual at each timepoint. Waist and hip circumference were measured in duplicate to the nearest 0.5 cm. A resting blood sample was collected in a 5 mL serum tube and rested for 30 min in room temperature before it was spun at 2,200 rpm at 20°C for 10 min. Serum was collected and stored at −80°C for further analysis. On the same day, abdominal and gluteal subcutaneous adipose tissue biopsies were obtained using a 14-gauge needle under local anesthesia (1% Xylocaine) excising ∼300–500 mg of tissue from each depot. The adipose tissue was washed on gauze with saline, and capillaries and connective tissue were removed. Approximately 80 mg was allocated for immediate analysis of mitochondrial respiration using high-resolution respirometry (Oxygraph-2K, Oroboros, Innsbruck, Austria). Some of the remaining tissue (∼200–300 mg) was snap-frozen in liquid nitrogen and stored at −80°C for later analysis, whereas the rest was immediately fixed in phosphate-buffered formalin for subsequent fat cell size analysis. We were not always able to obtain adipose tissue biopsies from both depots and/or unable to excise enough adipose tissue for all analyses, and this is why the number of samples is lower in mitochondrial respiration and morphology measurements.

On the third visit and following an overnight fast, participants performed a submaximal test on a treadmill. The protocol included 20 min warm-up without gas sampling, followed by 20 min of steady-state workload at 60% of VO_2peak with sampling of the expired gas. Participants recorded their dietary intake the day prior to baseline testing and repeated this diet before the subsequent measures at 16 weeks. Fat oxidation rates (g/min) was calculated from 5 min of steady oxygen uptake during the last 10 min of the test; $1.695 \times VO_2\text{-}1.701 \times VCO_2$, where VO_2 is oxygen uptake and VCO_2 is expired carbon dioxide (Jeukendrup and Wallis, 2005).

Blood Analyses

Plasma glucose concentrations were determined using a Roche Moduclar P (Roche, Switzerland) and serum insulin concentrations were measured in duplicate using an enzyme-linked immunosorbent assay (ELISA; IBL-International, Germany). We estimated insulin resistance using the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR); fasting serum insulin (μIU/mL) × fasting plasma glucose (mmol/L) divided by 22.5 (Matthews et al., 1985). HOMA-IR < 2.5 was considered normal while HOMA-IR ≥ 2.5 indicated insulin resistance. These cut-off points for insulin resistance have been used previously in the PCOS literature

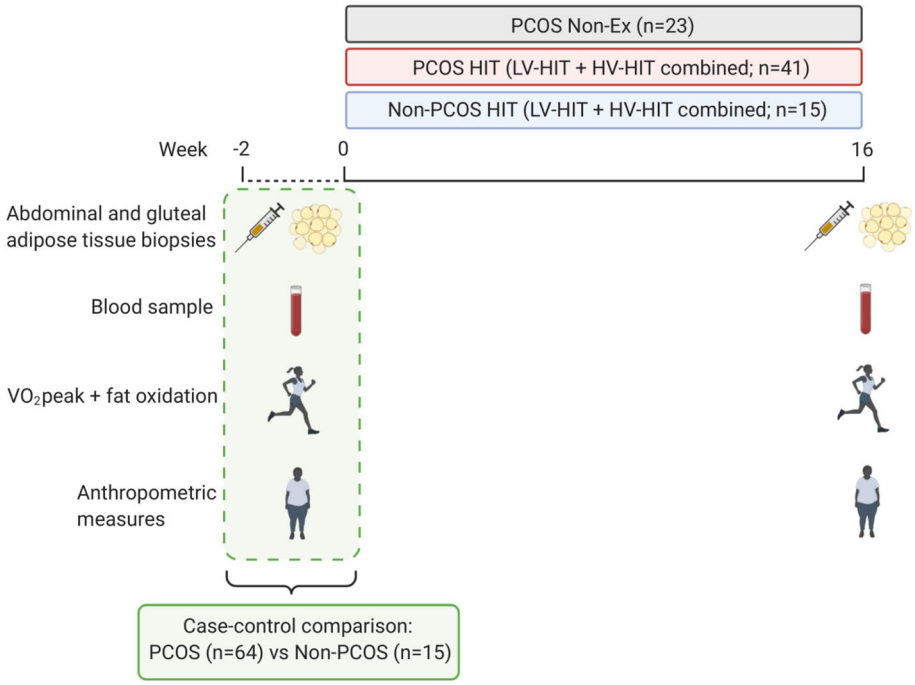

FIGURE 1 | Study protocol. The testing days included: abdominal and gluteal subcutaneous adipose tissue biopsies, fasting blood sampling, VO_2peak, submaximal exercise test to measure fat oxidation rates, body composition measures, and waist and hip circumference. The adipose tissue was used to measure mitochondrial respiration and cell size. The testing days were repeated after a 16 weeks intervention period of either performing high-intensity interval training or no exercise. PCOS, Polycystic ovary syndrome; Non-Ex, no exercise; HIT, high-intensity interval training; LV-HIT, low-volume high-intensity interval training; HV-HIT, high-volume high-intensity interval training; VO_2peak, peak oxygen uptake. Created with BioRender.com.

(Chuang et al., 2015). HbA1c was analyzed on Tosoh Automated Glycohemoglobin Analyzer HLC-723G8 version 5.24 in Norway and using Cobas b 101 system (Roche Diagnostics) in Australia. We measured testosterone and sex-hormone-binding globulin (SHBG) concentrations in women with PCOS. Total testosterone concentrations were analyzed with Agilent 1290 with 6410 Triple Quad LC/MS-MS detector (Agilent, Santa Clara, United States) SHBG concentrations were analyzed with Advia Centaur XPT (Siemens, Erlangen, Germany).

Mitochondrial Respiration

Mitochondrial respiration in abdominal and gluteal subcutaneous adipose tissue was measured in a subset of participants. We measured mitochondrial respiration in the abdominal adipose tissue from 10 non-PCOS women and 16 women with PCOS, and in gluteal adipose tissue from 14 non-PCOS women and 18 women with PCOS.

Mitochondrial respiration measurements were carried out in duplicate in ∼40 mg abdominal and gluteal subcutaneous adipose tissue using high-resolution respirometry (Oxygraph-2K respirometer; Oroboros, Innsbruck, Austria). Each of the two chambers of this instrument contained 2 mL Buffer Z (1 mM EGTA; 5 mM $MgCl_26H_2O$; 105 mM K-Mes; 10 mM KH_2PO_4; 5 mg/mL BSA; pH 7.1 at 37°C). All measurements were performed at 37°C and oxygen concentrations > 100 nmol/mL.

We used a protocol modified from Kraunsoe et al. (2010). Amplex Ultra Red (10 μM), Peroxidase from horseradish (HRP; 1 U/mL) and Superoxide dismutase (SOD; 5 U/mL) were titrated into the chambers and stabilized before ∼40 mg adipose tissue was added. Digitonin (2 μM) was added to the chambers to permeabilize the cells and baseline respiration was measured before substrates and inhibitors were added. Malate (2 mM) and Octanoyl carnitine (1.5 mM) were added together for measurement of a stable respiration with electron input through complex I and from ß-oxidation. Thereafter, Adenosine diphosphate (ADP; 5 mM) was added to stimulate phosphorylation and obtain maximum electron flow through electron transporting flavoprotein. Pyruvate (5 mM) and Glutamate (10 mM) were then added to measure state 3 respiration specific to complex I, followed by addition of Succinate (10 mM) for measurements of the maximal coupled state 3 respiration. Oligomycin (2.5 mM) was added as an Adenosine 5'-triphosphate (ATP)-synthase inhibitor, and thereafter Carbonyl cyanide m-chlorophenylhydrazone (CCCP) was titrated (starting with 2 μM and followed by steps of 1 μM) to obtain maximal uncoupled respiration. Chambers were then opened slightly for 2–3 min to increase the oxygen concentration in the chamber and avoid oxygen concentrations < 100 nmol/mL for the rest of the protocol. Rotenone (0.5 μM) was added to inhibit the flow of electrons through complex I, followed by Malonic acid (MnA; 5 mM) to inhibit complex II, and later

Antimycin A (2.5 μM) to inhibit complex III. Finally, Ascorbate (2 mM) and Tetramethyl-p-phenylenediamine dihydrochloride (TMPD; 0.5 mM) were added together to donate electrons directly to cytochrome c oxidase (COX), and shortly after Sodium azide (≥100 mM) was added as a COX inhibitor. Hydrogen peroxide (H_2O_2; 0.1 μM) was added before the following steps: adipose tissue, digitonin, ADP, Oligomycin, Rotenone and Ascorbate to measure H_2O_2 flux. All substrate and inhibitor concentrations are final concentrations.

Adipose Tissue Cell Size

Adipose tissue was fixated in phosphate-buffered formalin immediately after sampling, before it was embedded in paraffin and cut into 4 μm sections. These sections were mounted on glass slides and dried at 37°C overnight in an incubator. The sections were stained with CD68 [Mouse monoclonal anti-CD68 (Dako, M0814)] in a Dako Autostainer [EnVision™ + Systems HRP (DAB) Mouse (Dako, K4007)]. Digital images were captured with an EVOS FL Auto 2 Imaging System (ThermoFisher Scientific, USA) at x10 objective. Images from the EVOS were analyzed with Fiji (Schindelin et al., 2012). The investigators were blinded for group (PCOS/Non-PCOS, HIT/Non-Ex) and time-point (baseline/16 weeks). The area of ≥200 adipocytes was measured per sample. Fat cell sizes in abdominal adipose tissue was measured from 7 Non-PCOS women and 22 women with PCOS, and in gluteal adipose tissue from 13 Non-PCOS women and 29 women with PCOS.

Statistical Analysis

We calculated the sample size in the IMPROV-IT trial based on the primary outcome; menstrual frequency (Kiel et al., 2020). A one-way analysis of variance test with three groups with a 5% level of significance, a standard deviation of 2 menstrual cycles/year and statistical power of 0.80 gave a target study population of 48 women to detect an increase of three menstrual cycles during a 12-month period. We added 15% to the sample size owing to the non-normality of menstrual frequency, and another 15% to allow for expected dropout, and aimed to include 64 women with PCOS. We did not perform an *a priori* sample size calculation for the outcomes reported in the present paper, but the number of participants in the groups included in our study corresponds to previous studies with similar outcomes (Despres et al., 1984; Talanian et al., 2007; Perry et al., 2008; Larsen et al., 2015; Dohlmann et al., 2018). The number of Non-PCOS women from whom we measured abdominal fat cell size ($n = 7$) was lower compared with previous studies (Despres et al., 1984; Manneras-Holm et al., 2011).

To determine between-group differences in the LV-HIT and HV-HIT groups (for PCOS and Non-PCOS women, respectively) in all reported outcomes, we used linear mixed models with participants as random factor and the effect of time and group allocation as fixed effects with these levels: PCOS baseline, Non-PCOS baseline, PCOS Non-Ex post intervention, PCOS LV-HIT post intervention, PCOS HV-HIT post intervention, Non-PCOS LV-HIT post intervention, and Non-PCOS HV-HIT post intervention. Since there were no between-group differences between the two HIT groups, we pooled these groups to increase statistical power, leaving us with 3 groups post intervention; (1) a PCOS Non-Ex group ($n = 23$), (2) a PCOS HIT group ($n = 41$), and (3) a Non-PCOS HIT group ($n = 15$).

We used linear mixed models with participants as random factor and the effect of time and group allocation as fixed effects with these levels: PCOS baseline, Non-PCOS Baseline, PCOS Non-Ex post intervention, PCOS HIT post intervention, and Non-PCOS HIT post intervention. We adjusted for baseline values as recommended by Twisk et al. (2018). Descriptive statistics at baseline are reported as mean ± SD, and comparisons within and between groups are reported as estimated means with 95% confidence intervals. Normality of residuals was evaluated using Q-Q plots. For two of the dependent variables, the residuals were not normally distributed (insulin concentration and HOMA-IR) and logarithmically transformed to obtain normality. As the results were substantially the same after log-transformation, we report the results for the non-transformed variables to improve interpretation. Group means for weekly exercise training sessions for PCOS HIT and Non-PCOS HIT were compared using Student's *t*-test for independent samples, and presented as mean ± SD.

We considered two-sided *P*-values < 0.05 as statistically significant. All analyses were carried out using SPSS version 25.0 (SPSS Inc., United States).

RESULTS

The distribution of the four PCOS phenotypes (Azziz et al., 2009) were as follows for the Non-Ex and PCOS HIT groups, respectively: 39 and 24% with phenotype A (oligo/amenorrhea + hyperandrogenism + polycystic ovaries), 0 and 15% with phenotype B (oligo/amenorrhea + hyperandrogenism), 39 and 25% with phenotype C (hyperandrogenism + polycystic ovaries), and 22 and 37% with phenotype D (oligo/amenorrhea + polycystic ovaries).

Comparisons Between Women With and Without PCOS at Baseline

Table 1 shows baseline characteristics of the Non-PCOS women and women with PCOS. Women with PCOS had greater waist/hip ratio and lower fat oxidation rates compared with Non-PCOS women (**Figures 2A,B**). When we compared fat oxidation rates between the 15 women with PCOS who were individually matched with the 15 Non-PCOS women, there were no difference in fat oxidation rates; 7.81 ± 2.14 vs. 8.52 ± 1.26 mg/FFM/min ($p = 0.28$) for women with PCOS and Non-PCOS women, respectively. There was no statistically significant difference in HOMA-IR between the groups, although 43 women with PCOS (68%) vs. five Non-PCOS women (42%) had HOMA-IR ≥ 2.5. Abdominal adipose tissue oxygen flux was higher in Non-PCOS women compared with women with PCOS (**Figure 2C**). No differences were observed in gluteal adipose tissue oxygen flux (**Figure 2D**). Nor were there any differences in metabolic outcomes, or abdominal and gluteal adipose tissue cell size between these groups (**Figures 2E,F**).

TABLE 1 | Baseline characteristics of PCOS and non-PCOS women.

	PCOS		Non-PCOS		*P-values*
	n	Mean ± SD	*n*	Mean ± SD	
Age (years)	64	30 ± 5	15	31 ± 6	0.50
Body weight (kg)	64	85.1 ± 19.6	15	81.2 ± 17.1	0.48
BMI (kg/m^2)	64	30.5 ± 6.5	15	28.4 ± 5.6	0.24
FFM (kg)	64	50.7 ± 6.8	15	51.0 ± 5.6	0.89
Body fat percentage (%)	64	39.2 ± 8.9	15	34.6 ± 9.7	0.08
Waist circumference (cm)	63	101 ± 17	14	92 ± 13	0.22
Hip circumference (cm)	63	113 ± 14	14	112 ± 12	0.81
Waist/Hip Ratio	63	0.90 ± 0.09	14	0.82 ± 0.05	**0.007**
HbA1c (mmol/mol)	64	32.4 ± 3.2	13	31.3 ± 4.0	0.30
VO_2peak (mL/min/kg)	64	33.1 ± 7.2	15	36.0 ± 6.8	0.17
VO_2peak (L/min)	64	2.7 ± 0.4	15	2.9 ± 0.3	0.21
Glucose (mmol/L)	63	5.0 ± 0.5	13	4.8 ± 0.4	0.40
Insulin (pmol/L)	64	117 ± 89	12	84 ± 61	0.28
HOMA-IR	63	4.5 ± 3.8	12	3.0 ± 2.3	0.24
Total testosterone (nmol/L)	64	1.5 ± 0.6		-	
SHBG (nmol/L)	64	43 ± 23		-	

Values are mean ± SD. PCOS, Polycystic ovary syndrome; BMI, body mass index; FFM, Fat free mass; VO_2peak, peak oxygen uptake; HOMA-IR, Homeostatic model assessment of insulin resistance; SHBG, Sex Hormone Binding Globulin. Testosterone and SHBG concentrations were only measured in women with PCOS. Statistically significant P-values are in bold.

Physiological, Metabolic, and Adipose Tissue Responses to High-Intensity Interval Training

On average, women in the PCOS HIT group completed 2.2 ± 0.5 weekly sessions while women in the Non-PCOS HIT group completed 2.6 ± 0.4 weekly sessions (p = 0.025), which corresponds to ∼6 exercise sessions less in total over the 16 weeks. Seven women with PCOS and one woman in the Non-PCOS group did not register with Polar Flow, therefore we were unable to include their exercise training data.

Table 2 shows the changes in anthropometric measures, metabolic variables, cardiorespiratory fitness and adipose tissue fat cell size from baseline to after 16 weeks of HIT. The PCOS Non-Ex group reduced body weight and body mass index (BMI) more than PCOS HIT ($p = 0.021$ and $p = 0.031$, respectively), despite no differences in the monitored physical activity level or self-reported daily energy intake (data not shown). Non-PCOS women reduced their body weight and BMI after 16 weeks of HIT, with no between-group differences compared with the PCOS HIT group. Waist circumference and waist/hip ratio decreased in the PCOS HIT group ($p = 0.011$ and $p = 0.007$, respectively), with no between group-differences.

Cardiorespiratory fitness increased by ∼6% in the Non-PCOS HIT group ($p = 0.003$) and ∼4% in the PCOS HIT group (p = 0.006) after 16 weeks of HIT. The PCOS HIT group improved their absolute VO_2peak (L/min), but not relative (mL/min/kg), significantly more than the PCOS Non-Ex group ($p = 0.002$). Fat oxidation rates increased in the Non-PCOS HIT group, with no change in the PCOS HIT or PCOS Non-Ex group (**Figures 3A,B**). There were no between-group differences in changes in oxidation rates after 16 weeks.

We observed no between-group differences in metabolic outcomes, abdominal and gluteal adipose tissue oxygen flux (**Figures 3C,D**), or cell size (**Figures 3E,F**) after 16 weeks of HIT.

DISCUSSION

Our findings provide new data on whole-body fat oxidation during submaximal exercise, and gluteal and abdominal adipose tissue mitochondrial respiration and cell size in women with PCOS and Non-PCOS women, along with the effects of 16 weeks of HIT on these outcomes. We report that 16 weeks of HIT improved fat oxidation rates during submaximal exercise in Non-PCOS women, but not in women with PCOS, despite similar improvements in VO_2peak. Furthermore, women with PCOS had lower mitochondrial respiration with substrates for complex I + II in subcutaneous abdominal adipose tissue compared to Non-PCOS women. Mitochondrial respiration in both adipose tissue sites were unaffected by 16 weeks of HIT in women with and without PCOS. Finally, subcutaneous gluteal or abdominal adipose tissue cell size did not differ between women with PCOS and Non-PCOS women and 16 weeks of HIT did not alter adipocyte sizes in any group of women.

We report novel findings on absent exercise-induced improvements in fat oxidation during submaximal exercise in women with PCOS, despite improved VO_2peak, suggesting metabolic inflexibility in women with PCOS. Conversely, fat oxidation rates improved in Non-PCOS women after 16 weeks of

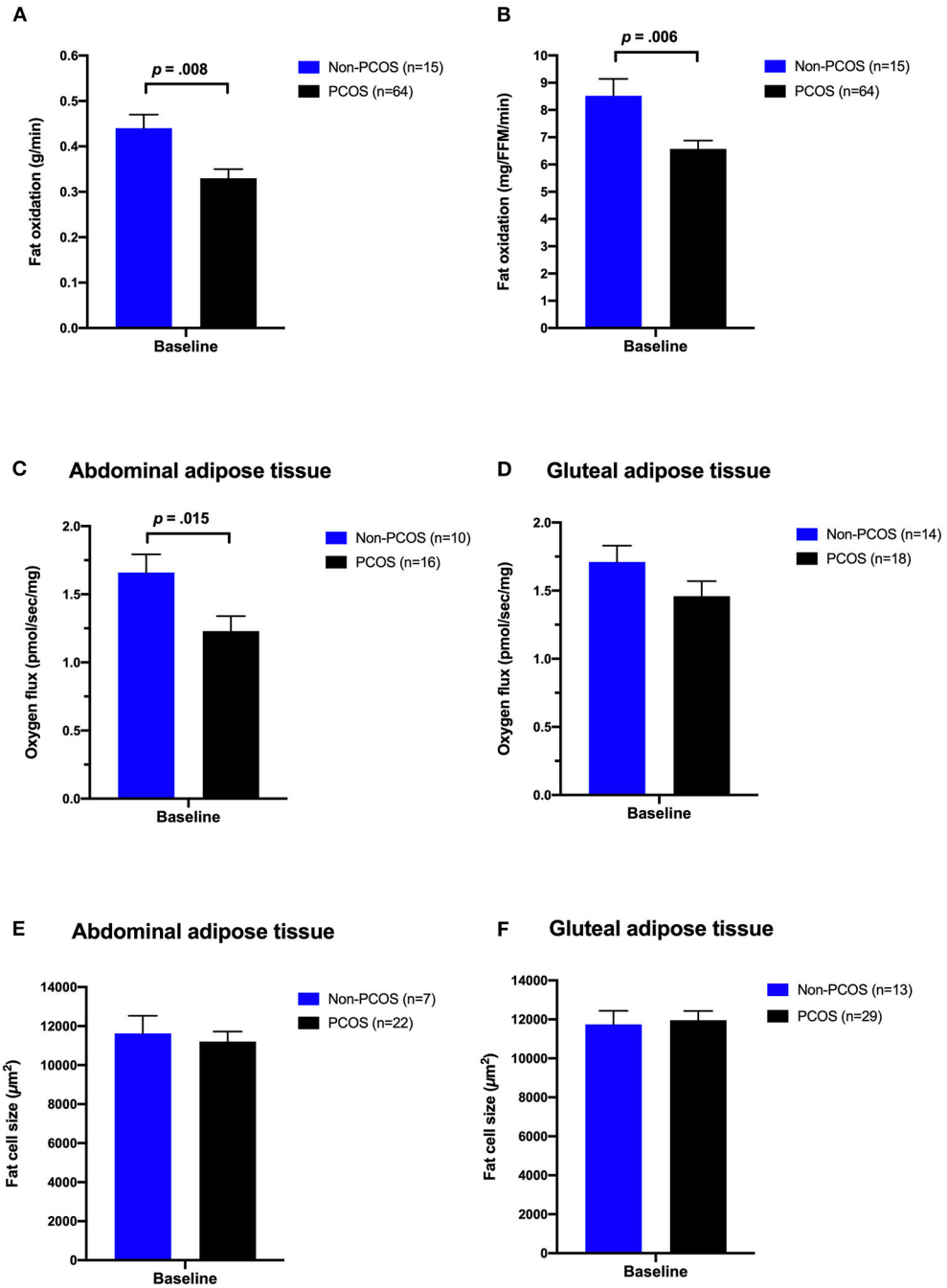

FIGURE 2 | Comparisons between women with PCOS and non-PCOS women at baseline. Whole-body fat oxidation during submaximal exercise **(A,B)**, oxygen flux with complex I + II linked substrates in subcutaneous abdominal **(C)** and gluteal adipose tissue **(D)**, and abdominal **(E)** and gluteal **(F)** adipose tissue cell size in non-PCOS (blue bars) and women with PCOS (black bars). The bars and error bars represent estimated means and SE based on linear mixed models.

HIT. A recent systematic review reported metabolic inflexibility in the rested, insulin-stimulated state in women with PCOS compared with healthy women (Rimmer et al., 2020). Metabolic inflexibility has been linked to insulin resistance and type 2 diabetes (Goodpaster and Sparks, 2017), and Broskey and colleagues found that women with PCOS and obesity (28.8 ± 4.7 years) were as metabolically inflexible as middle-aged women with type 2 diabetes and obesity (58.2 ± 9.9 years) (Broskey et al., 2018). Metabolic inflexibility has also been reported in lean women with PCOS (Hansen et al., 2019) and adolescent girls with

TABLE 2 | Effects of 16 weeks of non-exercise or high-intensity interval training.

	Non-PCOS HIT		PCOS Non-Ex		PCOS HIT		Between PCOS group difference (group x time interaction)		Between HIT group difference (group x time interaction)	
	Estimate (CI)	***P***	**Estimate (CI)**	***P***	**Estimate (CI)**	***P***	**Estimate (CI)**	***P***	**Estimate (CI)**	***P***
Body weight (kg)	−1.52 (−2.88 to −0.17)	**0.028**	−2.19 (−3.35 to −1.03)	**<0.001**	−0.44 (−1.36 to 0.49)	0.35	1.75 (0.27 to 3.24)	**0.021**	−1.09 (−2.73 to 0.55)	0.19
BMI (kg/m^2)	−0.54 (−1.00 to 0.06)	**0.029**	−0.79 (−1.20 to −0.37)	**<0.001**	−0.20 (−0.53 to 0.13)	0.13	0.58 (0.06 to 1.11)	**0.031**	−0.34 (−0.92 to 0.25)	0.25
FFM (kg)	0.32 (−0.36 to 1.00)	0.35	−0.28 (−0.86 to 0.30)	0.33	−0.09 (−0.55 to 0.37)	0.70	0.19 (−0.55 to 0.93)	0.60	0.41 (−0.41 to 1.23)	0.32
Body fat percentage (%)	−0.46 (−1.60 to 0.69)	0.43	−1.03 (−2.01 to −0.05)	**0.04**	−0.29 (−1.07 to 0.49)	0.47	0.74 (−0.51 to 1.99)	0.24	−0.19 (−1.58 to 1.20)	0.79
Waist circumference (cm)	−0.62 (−4.11 to 2.88)	0.73	−0.89 (−3.72 to 1.95)	0.53	−2.96 (−5.19 to −0.72)	**0.011**	−2.07 (−5.65 to 1.52)	0.25	2.35 (−1.81 to 6.51)	0.26
Hip circumference (cm)	−0.46 (−3.05 to 2.14)	0.73	−0.49 (−2.59 to 1.61)	0.64	−0.64 (−2.30 to 1.02)	0.44	−0.15 (−2.82 to 2.51)	0.91	0.15 (−2.93 to 3.24)	0.92
Waist/Hip Ratio	−0.00 (−0.03 to 0.03)	0.92	−0.01 (−0.04 to 0.01)	0.20	−0.03 (−0.04 to −0.01)	**0.007**	−0.01 (−0.04 to 0.02)	0.47	0.02 (−0.01 to 0.06)	0.15
HbA1c (mmol/mol)	0.67 (−0.80 to 2.15)	0.37	0.43 (−0.69 to 1.55)	0.45	1.75 (0.81 to 2.68)	**<0.001**	1.32 (−0.10 to 2.73)	0.07	−1.12 (−2.88 to 0.63)	0.21
VO_2peak (mL/min/kg)	2.20 (0.76 to 3.65)	**0.003**	0.35 (−0.85 to 1.55)	0.56	1.41 (0.43 to 2.39)	**0.006**	1.06 (−0.48 to 2.59)	0.17	0.81 (−0.94 to 2.56)	0.36
VO_2peak (L/min)	0.13 (0.02 to 0.23)	**0.016**	−0.06 (−0.15 to 0.02)	0.15	0.11 (0.04 to 0.18)	**0.002**	0.17 (0.06 to 0.28)	**0.002**	0.01 (−0.10 to 0.14)	0.82
Glucose (mmol/L)	0.10 (−0.13 to 0.32)	0.38	−0.09 (−0.26 to 0.08)	0.30	−0.11 (−0.25 to 0.03)	0.11	−0.03 (−0.24 to 0.19)	0.81	0.20 (−0.06 to 0.47)	0.13
Insulin (pmol/L)	−11.5 (−41.3 to 18.3)	0.44	−25.1 (−47.2 to −3.0)	**0.026**	−13.6 (−31.6 to 4.4)	0.14	11.5 (−16.5 to 39.4)	0.42	4.1 (−30.8 to 39.1)	0.81
HOMA-IR	−0.35 (−1.72 to 1.01)	0.61	−1.10 (−2.11 to −0.10)	**0.032**	−0.66 (−1.48 to 0.16)	0.11	0.44 (−0.83 to 1.71)	0.49	0.39 (−1.21 to 1.99)	0.63

Estimated mean difference from baseline values(95% confidence interval) based on linear mixed models.

PCOS, Polycystic ovary syndrome; CI, Confidence interval; BMI, body mass index; FFM, Fat free mass; VO_2peak, peak oxygen uptake; HOMA-IR, Homeostatic model assessment of insulin resistance. Statistically significant P-values are in bold.

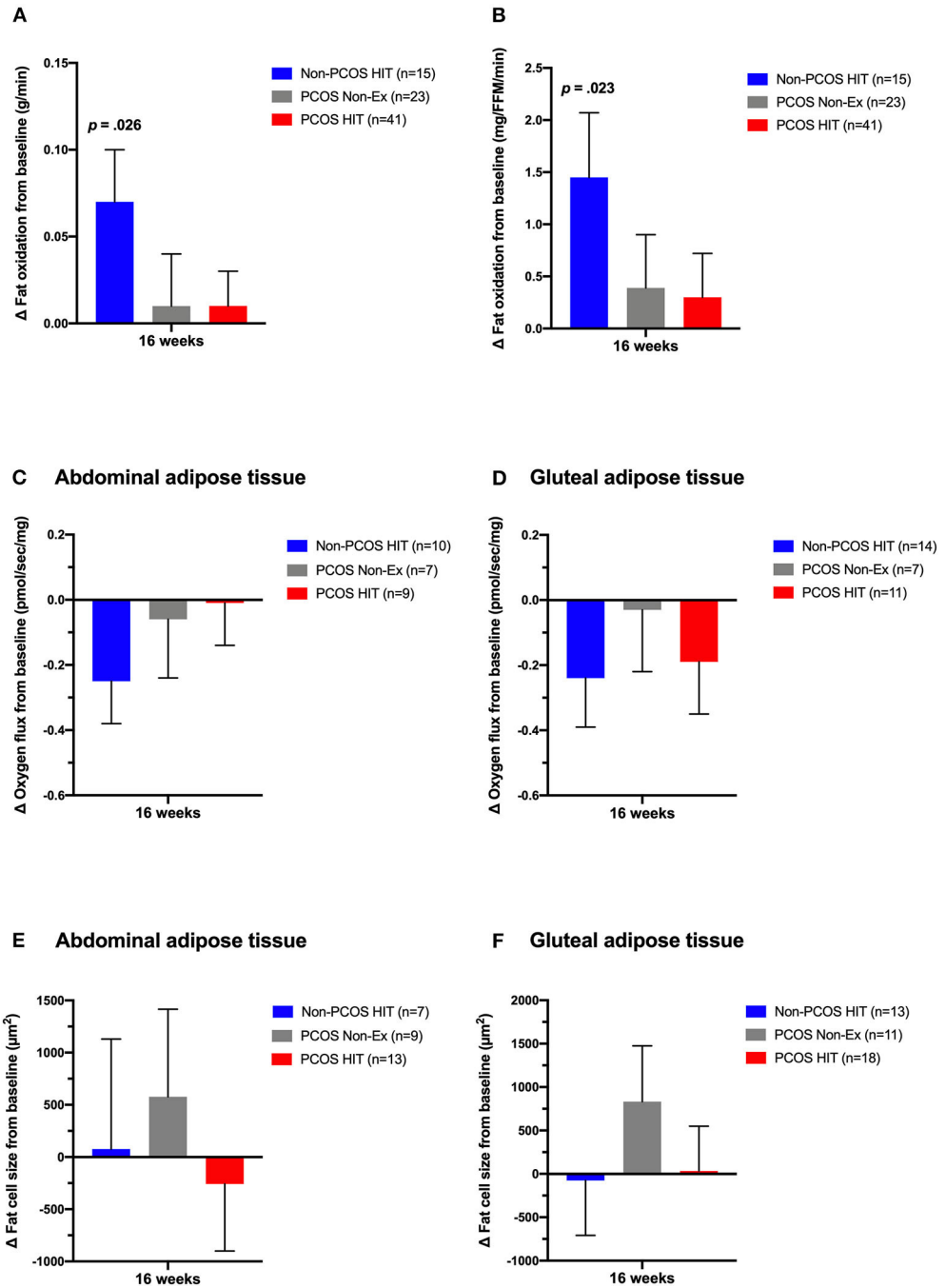

FIGURE 3 | Effects of 16 weeks of non-exercise or high-intensity interval training. Whole-body fat oxidation during submaximal exercise **(A,B)**, oxygen flux with complex I + II linked substrates in subcutaneous abdominal **(C)** and gluteal adipose tissue **(D)**, and abdominal **(E)** and gluteal **(F)** adipose tissue cell size in Non-PCOS HIT (blue bars), PCOS Non-Ex (gray bars), and PCOS HIT (red bars) after the 16 weeks intervention. The bars and error bars represent estimated means and SE based on linear mixed models. *P*-values are for within-group comparisons after 16 weeks of high-intensity interval training.

PCOS (Kim et al., 2018), suggesting that metabolic inflexibility is associated with PCOS independent of BMI and age.

Similar to our findings in Non-PCOS women, previous studies reported improvements in whole-body fat oxidation during submaximal exercise at 60% of VO_2peak in healthy recreationally active women after seven sessions of HIT (10 x 4 min bouts at 90% of VO_2peak separated by 2 min of rest) (Talanian et al., 2007), and in untrained recreationally active men and women after 6 weeks of HIT (using the same HIT protocol as Talanian et al., 2007 but for 3 days/week for 6 weeks) (Perry

et al., 2008). In our study, the Non-PCOS women exercised significantly more on a weekly basis compared to the women with PCOS (2.6 ± 0.4 vs. 2.2 ± 0.5 weekly HIT sessions, corresponding to ∼6 HIT sessions more in total over the 16 weeks), which may explain the difference observed in training-induced improvements in fat oxidation rates. However, we report similar improvements in VO_2peak in women with PCOS and Non-PCOS women after 16 weeks of HIT, which suggests that the six fewer HIT sessions performed by women with PCOS cannot fully explain the difference in training-induced improvements in fat oxidation rates. Absent training-induced improvements in women with PCOS have been reported previously; Hansen and colleagues observed improved insulin sensitivity measured by the gold-standard hyperinsulinaemic-euglycemic clamp in healthy women without PCOS, but not in women with PCOS after 14 weeks of exercise training (three weekly exercise sessions; two aerobic HIT sessions and one strength training session) (Hansen et al., 2020). Similarly, Hansen and colleagues reported improved incremental area under the oral glucose tolerance test curve for plasma glucose and insulin after exercise training in healthy women, but not in women with PCOS. Their data suggested that the lack of improvements in insulin action after exercise training were due to impaired ability to upregulate glucose uptake in skeletal muscle.

Skeletal muscle and adipose tissue are crucial tissues in energy metabolism and play a major role in metabolic flexibility in humans. However, little research has been undertaken on metabolic flexibility of white adipose tissue. Metabolic flexibility is driven by cellular processes that may be linked to the mitochondria (Goodpaster and Sparks, 2017). We are the first to explore subcutaneous abdominal and gluteal adipose tissue mitochondrial respiration in women with and without PCOS and the responses to HIT. In our study, we observed higher mitochondrial respiration through complex I + II in subcutaneous abdominal adipose tissue from Non-PCOS women compared to women with PCOS at baseline, no such differences were seen in gluteal adipose tissue. Low-grade inflammation may be one mechanism causing the lower mitochondrial respiration in women with PCOS compared to Non-PCOS women at baseline. There is a proposed link between inflammation and mitochondrial dysfunction in adipocytes, in which the proinflammatory response of macrophages may promote mitochondrial dysfunction in adipocytes (Woo et al., 2019). No changes were observed in mitochondrial respiration in either of the adipose tissue depots in either group of women after 16 weeks of HIT, which suggests that mitochondrial respiration through complex I + II in adipose tissue cannot explain the improved fat oxidation rates and metabolic flexibility observed in Non-PCOS women. Our findings are supported by data from Larsen and colleagues, who showed increased VO_2peak but no change in mitochondrial respiration in subcutaneous abdominal adipose tissue in overweight but otherwise healthy men and women after 6 weeks of LV-HIT (3 days/week with 5 × 60 s maximal effort work-bouts) (Larsen et al., 2015). However, there are some indications in the literature for exercise-induced changes in adipose tissue mitochondrial respiration. Dohlmann et al. (2018) found decreased mitochondrial respiration in subcutaneous abdominal adipose tissue in healthy men and women after 6 weeks of LV-HIT (18 HIT sessions with 7 × 1 min exercise bouts at ∼100% of VO_2peak), while Mendham et al. (2020) reported increased mitochondrial respiration in subcutaneous abdominal adipose tissue in black South-African women with obesity after 12 weeks of combined aerobic and resistance exercise training (4 days/week of 40–60 min with aerobic exercise at 75–80% HR_{peak} and resistance training that included upper- and lower-body exercises at 60–70% HR_{peak}). We are not sure of the reasons for these divergent findings, but they may be explained by different exercise modalities and intensities.

We did not observe changes in mitochondrial respiration in adipose tissue after 16 weeks of HIT. We speculate that changes may have occurred in skeletal muscle mitochondrial function as Larsen and colleagues have previously reported increased mitochondrial respiration in skeletal muscle, but not in abdominal adipose tissue, after 6 weeks of HIT in men and women who were overweight but otherwise healthy (Larsen et al., 2015). Possible gains, or lack of gains, in skeletal muscle mitochondrial function could explain the improved whole-body fat oxidation during submaximal exercise in Non-PCOS women, and lack of improvement in women with PCOS after 16 weeks of HIT. However, we did not obtain any muscle biopsies in our study.

We observed no differences in subcutaneous abdominal or gluteal fat cell size between Non-PCOS women and women with PCOS, nor do we report changes in cell size after 16 weeks of HIT in either group of women. A case-control study on women with PCOS and age- and BMI-matched women without PCOS reported enlarged abdominal adipocyte volumes in women with PCOS, and an association between hypertrophic adipocytes and insulin resistance (Manneras-Holm et al., 2011). The divergent findings in our study and the study by Manneras-Holm may be explained by higher power in their study (27 women in both groups).

Similar to our findings, previous studies have reported no changes in subcutaneous abdominal adipose tissue volume and size after 12 weeks of exercise training in men with overweight or obesity (3 days/week; aerobic exercise twice/week cycling for 30 min at 70% maximal watt and resistance training once weekly) (Stinkens et al., 2018), or after 16 weeks of exercise in women with PCOS (3 days/week for 30 min at an intensity of faster than normal walking pace) (Stener-Victorin et al., 2012). A longer and more intense training program may be required to change adipose tissue structure. Després and colleagues observed a sex difference in the sensitivity to exercise training, with less responsiveness in female adipose tissue. Twenty weeks of endurance training for 40 min at 80% HR_{max} 4–5 times weekly did not influence fat percentage or adipose cell weight in women, whereas males showed reductions in both fat percentage and adipose cell weight (Despres et al., 1984).

Our data indicate that women with PCOS allocated the non-exercising control group most likely introduced some lifestyle changes during the study period as we observed reductions in body mass, BMI and body fat percentage and improvements in fasting insulin concentration and HOMA-IR in this group.

Although we detected no changes in self-reported daily energy intake or physical activity level, these were only recorded for a brief period of time (4–5 days at each timepoint). At study inclusion, women with PCOS assigned to the control group were informed about the current recommendations of at least 150 min weekly of moderate intensity physical activity, which could have affected their physical activity behavior. Furthermore, individuals who volunteer for exercise studies usually hope to be allocated to the exercise intervention, and women allocated to the control group have most likely been motivated to introduce lifestyle changes.

Subgroup analyses from a systematic review and meta-analysis on the effectiveness of exercise compared to control in women with PCOS showed greater improvements in cardiometabolic outcomes in supervised vs. unsupervised exercise interventions (Kite et al., 2019), and a supervised rather than semi-supervised exercise protocol in our study could possibly have resulted in greater gains for women with (and without) PCOS. Furthermore, including a more varied exercise protocol with a combination of LV-HIT and HV-HIT sessions could have induced larger improvements, as shown previously (Almenning et al., 2015).

Major strengths of our study are the pair-wise age- and BMI-matching of women with PCOS and Non-PCOS women, the rigorous inclusion and exclusion criteria, and that we explored two adipose tissue depots in women with and without PCOS. We also acknowledge study limitations. We used different methodologies to estimate body composition at the two study centers, limiting the baseline comparison for these outcomes between women with and without PCOS. The Inbody 720 scale that we used for participants in Norway underestimates body fat and overestimates FFM compared with DXA (Mclester et al., 2018). Due to limited adipose tissue biopsy volumes, we were unable to compare mitochondrial respiration and fat cell size between the women with PCOS and Non-PCOS women who were individually age- and BMI-matched. Furthermore, due to limited adipose tissue volumes and statistical power, we were unable to run meaningful statistical analyses for LV-HIT and HV-HIT groups, which made us unable to detect differences in the effects of LV-HIT and HV-HIT on mitochondrial respiration and fat cell size. Instead, data for LV-HIT and HV-HIT were pooled to increase the statistical power and to investigate the effect of HIT.

In conclusion, we observed exercise-induced improvements in whole-body fat oxidation during submaximal exercise in Non-PCOS women but not in women with PCOS after 16 weeks of HIT. These findings suggest metabolic inflexibility in women with PCOS. Mitochondrial respiration was lower in subcutaneous abdominal, but not gluteal, adipose tissue in women with PCOS compared to Non-PCOS women, and 16 weeks of HIT did not alter adipose tissue mitochondrial respiration in either group of women. Further studies are required to ascertain the underlying mechanisms behind the absent exercise-induced improvements in fat oxidation and metabolic inflexibility observed in women with 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 and The ACU Human Research Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SLi drafted the manuscript. SLi, RR, and SLa analyzed the data. SLi and SLy performed statistical analyses. SLi, IK, and TM were responsible for study conception and design, coordinated the studies at the two sites, performed measurements on testing days, and supervised the exercise training. All authors provided feedback and approved the final manuscript.

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SYSTEMATIC REVIEW
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Different Methods of Physical Training Applied to Women Breast Cancer Survivors: A Systematic Review

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Objective: The objective of this systematic review was to identify the effects of different training methods in women who have survived breast cancer (WSBC).

Data Sources: Studies were identified by searching SportDiscus, Web of Science, PubMed, Scopus, Scielo, and Bireme.

Study Selection: The inclusion criteria were articles that addressed only breast cancer in women, were randomized clinical trials, and interventions involving physical training with Consort ≥80.

Data Extraction: The PICO and CONSORT strategies were used for the selection of articles and quality assessment of randomized clinical trials, respectively. Two independent reviewers searched for articles among the databases. Disagreements were discussed, and in the case of an impasse, a third reviewer was consulted.

Data Synthesis: Evidence that demonstrated the beneficial effects of physical exercise programs carried out by WSBC. Moderate or high-intensity exercise sessions have been shown to benefit women survivors of breast cancer. Among the modalities, the resistance exercise showed effects from 55% of one-repetition maximum (1 RM), exclusively or associated with other training regimes, such as aerobic (from 48% of heart rate), high-intensity interval training (HIIT), or impact. The main benefits include increased muscle strength, promoted by the practice of resistance exercise in combination with other types of exercises or alone; decreased fatigue; improved quality of life; improved psychosocial effects, and increased leisure time.

Conclusions: Physical training performed at a moderate or high intensity (aerobic or anaerobic) can reduce fatigue, improve quality of life, improve sleep quality, and increase bone mineral density in women survivors of breast cancer.

Keywords: breast tumor, women, physical activity, physical exercise, quality of life

INTRODUCTION

Cancer is one of the main public health problems in different countries. This disease was responsible for the death of ∼9.5 million people in 2018 (excluding non-melanoma skin cancer) and is considered the second leading cause of death in the world (Gray et al., 2017; Lewandowska et al., 2019; Wilson et al., 2019). Among the different types of cancer, breast cancer is second most common in the world; it is more common in women and is the type of cancer that causes the most deaths in this population (Harbeck et al., 2019; López-Cortés et al., 2020); moreover, by 2040, there are expected to be ∼2,833,941 new cases (Willams et al., 2019; Wilson et al., 2019; Wild et al., 2020).

The incidence of breast cancer is associated with risk factors, such as genetic predisposition, the consumption of alcoholic beverages and tobacco, exposure to estrogen during the use of hormone therapy, and the early use of oral contraceptive methods (Sun et al., 2017). However, other factors, such as older age, benign proliferative breast disease, increased breast density, and radiation exposure, as well as obesity and low levels of physical activity, can also contribute to the development of this pathology (Rojas and Stuckey, 2016; Wild et al., 2020).

Among the different risk factors, it is estimated that sedentary behavior, obesity, and physical inactivity in particular account for 20–40% of all cancer cases. However, some authors suggest that these factors are modifiable since regular physical activity prevents the occurrence of several types of cancer, including bladder, colon, endometrium, esophagus, kidney, stomach, and breast cancer (McTiernan et al., 2019; Patel et al., 2019).

For the treatment of breast cancer, some patients undergo chemotherapy, radiation therapy, or hormone therapy. Despite these treatments being effective, they can cause physiological and psychological impairments that affect the quality of life of patients (Kaltsatou et al., 2010; Schmitz et al., 2019). Of these impairments, the most common are pain, decreased cardiac function, body weight gain, sarcopenia, psychological stress, and cancer-related fatigue (Carayol et al., 2019).

Among the different types of interventions, regular physical exercise performed by cancer survivors can be beneficial for physical function, cancer-related fatigue, pain, and muscle strength (Buffart et al., 2018; Mijwel et al., 2018). Such effects are due, in part, to physical exercise leading to improvements in physical fitness, cardiorespiratory function, muscular endurance, and body composition (Campbell et al., 2019).

According to the American College of Sports Medicine (ACSM) guidelines, aerobic training performed by cancer patients can decrease cancer-related fatigue, increase health-related quality of life and physical function, in addition to reducing anxiety, depression and improve sleep quality. In this population, resistance training proved to be beneficial in decreasing fatigue levels, increasing health-related quality of life and physical function, attenuating lymphedema, and improving aspects related to bone health. The combination of aerobic and resistance exercises decreased fatigue, anxiety, and depression, in addition to increasing health-related quality of life and physical function (Campbell et al., 2019).

Currently, the ACSM recommends that aerobic training is the most effective and safe adjuvant for cancer treatment. The recommendation is that aerobic exercise is performed at moderate intensity for 30 min at least three times a week for a minimum period of 8 to 12 weeks. In comparison with aerobic training, resistance training showed similar effects when it was performed at an intensity of at least 60% of a maximum repetition for a minimum of two sets, including 8–15 repetitions, at least twice per week (Campbell et al., 2019).

However, studies investigating the effects of physical training in women with breast cancer are still scarce. Thus, the present study aimed to gather the scientific evidence that demonstrates the effects of different continuous/regular exercise programs in women who have survived breast cancer (WSBC).

MATERIALS AND METHODS

Literature Research Strategy

The study is a systematic review for which the PICO (**P**atient, **I**ntervention, **C**omparison and **O**utcomes) where: **P** was equivalent to women who survived breast cancer; **I** were interventions based on different training methods; **C** were the comparisons between control and intervention groups and; **O** the outcomes and/or results on the clinical aspects in the health of women survivors of breast cancer. The **PICO** strategy covers a larger number of articles and is recommended when searches are made in a variety of databases (Methley et al., 2014), this strategy was used according to the methodology of Preferred Report Items for Systematic Reviews and Meta-analyzes (PRISMA), considered relevant for the construction of systematic reviews (Moher et al., 2009b).

The SportDiscus, Web of Science, PubMed, Scopus, Scielo, and Bireme databases were searched for relevant articles by two researchers. Only articles published in English, Portuguese or Spanish were included; the keywords used were "aerobic exercise," "breast cancer," "breast tumor," "breast," "endurance exercise," "physical exercise," "females," "girl," "interval exercise," "isometric exercise," "physical activity," "resistance exercise," "strength exercise," "woman" and "women," which were crossed with the Boolean operators AND, OR or both operators. Articles published before September 2019 were included.

Studies that included WSBC who performed regular physical exercise and assessed its effects on health were searched, and only randomized clinical trials were selected.

Inclusion and Exclusion Criteria

The exclusion criteria were as follows: case-control studies, cross-sectional studies, cohort studies, meta-analyses, studies that did not exclusively involve breast cancer, studies that included men in the study population, studies involving specific minorities, studies that did not include physical exercise as an intervention, and studies of experimental models. The inclusion criteria were articles that addressed only breast cancer, included only women, were randomized clinical trials and included interventions involving physical training.

This research assessed the differences between intervention and control groups in terms of bone mineral density, muscle

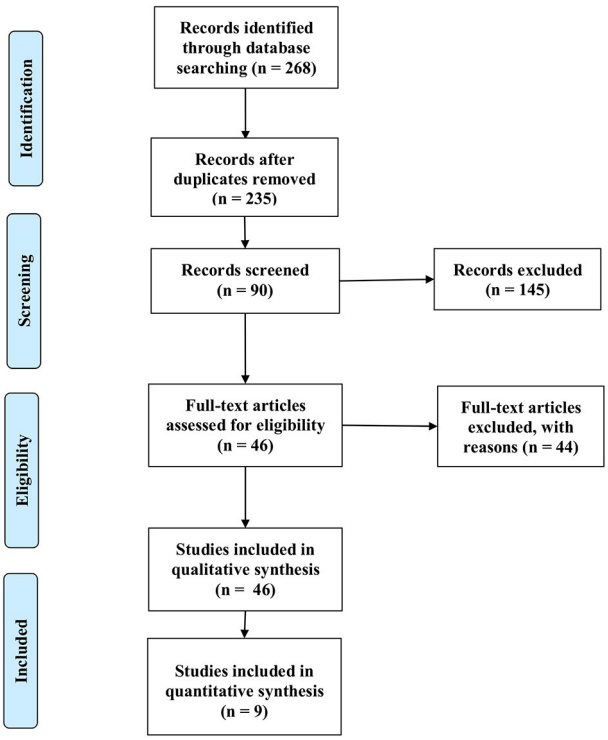

FIGURE 1 | Flow of identification, screening, and eligibility of articles in this systematic review. From Moher et al., 2009a .

strength, fatigue, role function, quality of life and sleep quality, the maintenance of peak oxygen consumption (peak VO_2), body composition, and body weight.

Data Extraction

The Kappa test was used to verify the level of agreement between the reviewers. Two independent reviewers searched for articles among the databases, screened the titles and abstracts, and evaluated the full texts of articles and their eligibility for inclusion in this systematic review. Disagreements were discussed, and in the case of an impasse, a third reviewer was consulted.

Spreadsheets were created according to CONSORT guidelines, and the reviewers used these spreadsheets to extract information about the characteristics, study population, eligibility criteria, intervention methods, and the results reported (Shulz et al., 2010; Falci and Marques, 2015). Each article was reviewed twice by the researchers to guarantee the reliability of the results (Moher et al., 2009a).

Quality Assessment of Individual Studies

The quality of original works was assessed carefully based on the physical training results in WSBC. Articles with a low risk of bias were used (percentual $\geq$ 80), as determined by the CONSORT guidelines; methodological quality, as well as the inclusion and exclusion criteria, statistical data, and results, was also verified[1] (Shulz et al., 2010; Falci and Marques, 2015).

[1]http://www.consort-statement.org.

RESULTS

Inclusion of Studies

After the databases were searched, articles were identified, of which were excluded based on the titles; thus, articles remained eligible for abstract screening. Of these articles, articles were eligible for full-text screening. Finally, nine articles were remaining thatmet the CONSORT inclusion criteria ($\geq$ 80) (**Figure 1**). The articles were organized in alphabetical order by the surname of the first author (**Table 1**).

Project and Study Population

The eligible studies (n = 09) reported the experimental design, the randomization of individuals for group allocation, an intervention, and control groups; three studies included two intervention groups and a control group.

The participants' age range was 51 to 62 years. The stage of breast cancer ranged from 0 to 4 as a follow: 0 = Cancer has not grown beyond the point of instigation; 1 = Increased in size and spread to the breast fat tissue; 2 = Affects up to three lymph nodes; 3 = Spread to the chest wall; and 4 = From the breast or the lymph nodes it already reaches other organs or bones (metastatic phase) (Nargis et al., 2019). Not all women had lymphedema, but all women were undergoing cancer treatment (chemotherapy or radiation), except for those included in one study (treatment ended more than a year ago) (Winters-Stone et al., 2011).

Duration and Types of Training That Have to Affect WSBC

Regular physical activity is related to a lower risk of mortality (Kikuchi et al., 2018) and the development of several chronic diseases, such as cardiovascular diseases, diabetes mellitus, high blood pressure (Hansen et al., 2018), cervix cancer, and breast cancer (Warburton and Bredin, 2017).

However, it is necessary to be aware of the purposes of specific physical exercise prescriptions regarding the modality, frequency, intensity, and duration of training. In addition, an individual's general health condition and disease stage must be observed so that the exercise can be individualized, and the intensity should be gradually adjusted to achieve the desired effect (Luan et al., 2019).

In the present systematic review, we found that the duration of the interventions varied from 8 weeks (Hagstrom et al., 2015), 12 weeks (Schmidt et al., 2014,?; Bloomquist et al., 2019), 16 weeks (Mijwel et al., 2017; Campbell et al., 2019), and 3 months (Cormie et al., 2013; Rogers et al., 2015) to 12 months (Winters-Stone et al., 2011). Among the studies, the physical exercise regimes were classified as aerobic, anaerobic, or a combination of these two types of physical exercise.

Among the different types of training regimes, an article used multimodal sessions for 6 weeks as initial training, consisting of low- and high-intensity exercises. After that period, for the following 6 weeks, six resistance exercises were implemented with a load starting at 70% of one-repetition maximum (1 RM) and gym equipment, targeting the main muscle groups associated

TABLE 1 | Main characteristics of the studies included in this review.

References	Participants	Intervention	Results	% Of quality according to consort
Bloomquist et al. (2019)	153 women after mastectomy, x̄ age 51,7 years old, ongoing chemotherapy, cancer stage: 1-3	**Control group–low** • *Intervention:* walking program, based on a pedometer and an individual consultation. • *Duration:* 12 weeks **Intervention group - high** • *High – until the 6th week*: • *Intervention:* combination of aerobic and resistance exercises • *Intensity:* low and high • *Duration:* 12 weeks *High – until the 12th week*: • *Intervention:* aerobic warm-up, followed by resistance exercises and 15 to 30 min of interval cardiovascular training on stationary bikes • *Intensity:* moderate to high • *Duration:* 12 weeks	↓ Of the BMI ↓ In breast symptoms and in the arms ↑ MS at the extremity of the upper limbs ↓ of pain = Lymphedema = Volume between arms = QV	84
Cormie et al. (2013)	62 women, most underwent mastectomy age 56.1 years, radiation therapy and chemotherapy in ongoing cancer stage	**Control group – UC** • *Intervention:* usual care **Intervention group - High-load** • *Intervention:* resisted exercises • *Intensity:* 75 to 85% of 1 RM using 10 to 6 maximum repetitions • *Frequency:* twice a week (60 min) • *Duration:* 3 months **Intervention group - Low-load** • *Intervention: resisted exercises* • *Intensity:* 55 to 65% of 1 RM using 15 to 20 repetitions • *Frequency:* twice a week (60 min) • *Duration:* 3 months	↑ Physical functioning (QV) = Body pain (QV) = General health (QV) = Vitality (QV) = Social functioning (QV) = Emotional role (QV) = Mental health (QV) = Physical health compound (QV) ↑ Shoulder range of motion = MGF ↑ MS ↑ Muscle endurance = Extent of swelling (lymphedema) = Severity of lymphedema symptoms = Physical function	92
Hagstrom et al. (2015)	39 women after mastectomy x̄ age 51.9 years, chemotherapy, or radiotherapy ongoing cancer stage: 1–3	**Control group:** • Usual medical care. **Intervention group – RT** • *Intervention:* Resistance training • *Intensity:* high • *Frequency*: 3 times a week • *Duration:* 8 weeks	↑ Leisure time ↓ Fatigue ↑ Upper and lower body MS ↑ General QV ↑ Physical well-being (QV) = Functional well-being (QV) = Social well-being (QV) = Emotional well-being (QV)	81

(Continued)

TABLE 1 | Continued

References	Participants	Intervention	Results	% Of quality according to consort
Mijwel et al. (2017)	206 women x̄ age of groups: HIIT = 52.7 years; AT-HIIT = 54.4 years; UC = 52.6 years cancer stage: 1–3, ongoing chemotherapy	**Control group – UC** • *Intervention:* written information about physical activity • *Duration:* 16 weeks **Intervention group - RT-HIIT** • *Intervention:* resistance training and HIIT on a cycle ergometer. • *Intensity:* high • *Duration:* 60 min • *Frequency:* twice a week / 16 weeks **Intervention group - AT-HIIT** • *Intervention:* 20 min of aerobic on a cycle ergometer, elliptical ergometer, or treadmill, followed by HIIT. • *Intensity:* moderate and high • *Duration:* 16 weeks • *Frequency:* twice a week/16 weeks	↑ CRF in the UC group Maintenance of CRF levels in Other groups. ↑ Role function (QV) ↑ Of HRQL ↓ Load of breast cancer symptoms	89
Mijwel et al. (2018)	206 women after mastectomy x̄ age of 52.6 years, cancer stages: 1–3 ongoing chemotherapy	**Control – UC** • *Intervention:* written information about physical activity • *Duration:* 16 weeks **Intervention group - RT-HIIT** • *Intervention:* resistance training directed to the main muscle groups and HIIT in a cycle ergometer. • *Intensity:* high • *Duration:* 60 min • *Frequency:* twice a week/16 weeks **Intervention group - AT-HIIT** • *Intervention:* 20 min aerobic on a cycle ergometer, elliptical ergometer, or treadmill, followed by HIIT. • *Intensity:* moderate and high • *Duration:* 16 weeks • *Frequency:* twice a week/16 weeks	↑ MS ↑ MGF ↓ Threshold PPT Maintaining the CF Maintaining body weight Prevented hyperalgesia ↓ Hemoglobin in all groups Weak inverse R between change in self-reported CRF and change in lower limb strength Inverse R between the change in SRF and the change in PPT in the gluteal muscle' No association between change in SRF and change in handgrip strength No association between change in SRF and self-reported change in CF. R between changes in MS of the lower limbs and changes in PPT in the trapezius and glutes, as well as between changes in handgrip and change in PPT in the trapezius. SRF was associated with self-reported pain.	84
Rogers et al. (2015)	42 women after mastectomy x̄ age of 56.2 ongoing chemotherapy or radiation therapy cancer stage: 0–2	**Control group** • Accelerometer monitoring **Intervention group** • *Intervention:* walking and resistance bands • *Intensity:* moderate • *Duration:* 3 months • *Frequency:* twice a week	↓ Daytime sleepiness. ↑Of sleep duration (hours per night) = Sleep quality = Sleep disorder = Accelerometer efficiency and PSQI scale = Accelerometer latency and PSQI scale = Sleeping medications = Global PSQI = Sleep dysfunction = Inflammatory markers (interleukins)	84

(Continued)

TABLE 1 | Continued

References	Participants	Intervention	Results	% Of quality according to consort
Schmidt et al. (2014)	95 women after mastectomy x̄ age of 52.7 chemotherapy in ongoing cancer stages: 1–4	**Control group – RC** • *Intervention*: progressive muscle relaxation • *Duration:* 12 weeks • *Frequency:* twice a week/60 min. **Intervention group – RE** • *Intervention:* Resisted exercises • *Intensity:* 60–80% of 1 RM • *Duration:* 12 weeks • *Frequency:* twice a week/60 min.	↑ Functional function (QV) Improvement of psychosocial effects (QV) ↑ Dry mouth feeling (QV) in the EX group = Physical function (QV) = Cognitive function (QV) = Social function (QV) ↓ Total fatigue in patients without social depression (QV) ↓ Physical fatigue in patients without depression = Affective fatigue = Cognitive fatigue = Physical fatigue = Total fatigue = depression ↑ Cognitive performance on EX only ↑ Total, physical, and affective fatigue in the RC group and maintenance in the EX group (thyroxine users) = Cognitive fatigue (thyroxine users) = Fatigue (not thyroxine users) ↓ Fatigue (smokers)	86
Steindorf et al. (2014)	155 women x̄ age of 55.8 years and cancer stage 0–3 with ongoing radiotherapy after mastectomy.	**Control group: RC** • *Intervention:* progressive muscle relaxation. • *Frequency:* twice a week (60 min/session) • *Duration:* 12 weeks **Intervention group - RE** • *Intervention:* resistance exercise • *Intensity:* 60–80% of 1 RM • *Frequency:* twice a week (60 min/session) • *Duration:* 12 weeks	= Global QV ↑ Functional function (QV) ↓ Pain = Emotional function = Social function = Body image = Depression score = Cognitive performance ↓ Reduce total fatigue ↓ Physical fatigue = Affective fatigue = Cognitive fatigue ↑ MS = Frequency of lymphedema reported	81
Winters-Stone et al. (2011)	106 women x̄ age of 62.3 (POWIR) and 62.2 (FLEX) > 1 year after chemotherapy or radiation therapy. cancer stage: 0–3	**Control group – FLEX** • *Intervention:* stretching and relaxation exercises for the entire body in a sitting or lying position. **Intervention group – POWIR** • *Intervention*: resistance exercises and impact training • *Intensity:* 60–70% of 1 RM • *Frequency:* 2 supervised sessions + 1 home session / week (45–60 min) • *Duration:* 12 months	Favorable changes in bone renewal. Maintenance of BMD in the lumbar spine. = BMD at the hip = body fat and % fat ↑ lean mass in the POWIR group that used AI ↑ Osteocalcin in FLEX and stable in POWIR ↓ Deoxypyridinoline cross-links in POWIR = Effect of using AI or SERM on BMD or fat	84

↑, increase; ↓, decrease; = no significant difference; x̄, mean; QV, component of the quality of life scale; AI, aromatase inhibitor; BMD, bone mineral density; SERM, selective estrogen receptor modulator; vs, "when compared"; HIIT-High-Intensity Interval Training; UC, Usual Care; RT, Resistance Training; AT, Aerobic Training; RC, Relaxation Control; RE, Resistance Exercise; FLEX, Flexibility Training; POWIR, Prevent Osteoporosis With Impact + Resistance; 1 RM, one-repetition maximum; min, minutes; R, correlation; MS, Muscle strength; CRF, Cancer-Related Fatigue; SRF, Self-Reported Fatigue; Assoc, Association; PPT, Pressure Pain Threshold; CF, Cardiorespiratory Fitness; HRQL, Health-Related Quality of Life; MGF, Manual Grip Force.

with other aerobic activities, which were performed on stationary bikes with peak loads equivalent to 85 to 95% of maximum heart rate (Bloomquist et al., 2019).

Two studies used two combinations of training modalities: a. aerobic exercises of moderate-intensity combined with high-intensity interval training (HIIT); b. resistance training combined with HIIT in WSBC (Mijwel et al., 2017; Campbell et al., 2019). Another study used resistance exercises combined with impact training, which consisted of jumps performed with moderate intensity loads (Winters-Stone et al., 2011).

Moderate to high-intensity resistance exercises were also used as an intervention (Steindorf et al., 2014; Hagstrom et al., 2015; Cešeiko et al., 2019). Additionally, two types of interventions based on resistance exercises were implemented, which differed only in terms of the intensity (low and high); they were compared with each other, and the intensity was inversely proportionally related to the number of repetitions (Cormie et al., 2013).

Another intervention employed was guided aerobic exercise (walking) at moderate intensity combined with strength exercises (resistance bands) and unsupervised walking sessions (Rogers et al., 2015).

Concerning the primary objective, there was little variation in the outcomes, which were as follows: the effects of physical exercise on cancer-related lymphedema (Cormie et al., 2013; Bloomquist et al., 2019); the changes in muscle mass and bone mass after an exercise regime (Winters-Stone et al., 2011); sleep quality after physical training (Rogers et al., 2015); beneficial adjustments in muscle strength, cardiorespiratory fitness, pain, and pressure thresholds and body mass in patients with breast cancer during chemotherapy (Campbell et al., 2019); cancer-related fatigue (Mijwel et al., 2017); and cancer-related fatigue and quality of life (examined by three studies) (Schmidt et al., 2014; Steindorf et al., 2014; Hagstrom et al., 2015).

Among the nine selected articles, six presented the following secondary outcomes: muscle strength (Steindorf et al., 2014; Hagstrom et al., 2015; Bloomquist et al., 2019); quality of life (Cormie et al., 2013; Mijwel et al., 2017); lymphedema symptoms (Cormie et al., 2013; Bloomquist et al., 2019); symptoms related to cancer treatment (Mijwel et al., 2017); leisure time (Hagstrom et al., 2015); psychosocial factors (Rogers et al., 2015); depressive symptoms, cognitive function and cardiorespiratory resistance (VO_2 peak) (Steindorf et al., 2014); the extent of swelling in the treated arm and physical function (Cormie et al., 2013). It is noteworthy that there were no reports of severe adverse effects after the training regimes in any of the studies analyzed.

Outcomes and Intervention Measures

Outcome and intervention measures were used to verify whether there were changes in the outcomes after the interventions based on physical exercise were performed; thus, they were categorized for better analysis and understanding.

Methods Used to Measure Lymphedema, Bone Mineral Density, and Quality of Sleep

The tools used to assess lymphedema included dual-energy X-ray absorptiometry (DXA) and bioimpedance spectroscopy, and the measurements used included the circumference of body segments and the difference in the volume of the affected and unaffected arms, which reflected the amount of extracellular fluid in the arm (Cormie et al., 2013; Bloomquist et al., 2019). The self-reported symptoms of cancer-related lymphedema were obtained by the Numeric Rating Scale (NRS) (Bloomquist et al., 2019), Disability of the Arm, Shoulder, and Hand (DASH) questionnaire, and Brief Pain Inventory (BPI) questionnaire, in addition to the morbidity subscale of the Functional Assessment of Chronic Illness Therapy (FACT-B +4) questionnaire for breast cancer survivors with lymphedema (Cormie et al., 2013).

Bone mineral density, lean bone mass, and fat mass were assessed by DXA, bone turnover was assessed by serum osteocalcin (ng/mL), and demographic and clinical characteristics were obtained by self-report. For chronic medical conditions, the Charlson Comorbidity Index and the concentration of follicle-stimulating hormones (FSHs) were evaluated to determine whether the patients were undergoing menopause (Winters-Stone et al., 2011).

Sleep quality was assessed using the Pittsburgh Sleep Quality Index (PSQI) and Patient-Reported Outcomes Measurement Information (PROMIS®) scale; the latter scale was also used to assess depression, anxiety, and fatigue. These same authors verified the inflammatory mediators (interleukins (IL): −6, −8, −10 and TNF-alpha) in serum samples from fasting patients; body fat was estimated with bioelectrical impedance; social support and enjoyment for physical activity were assessed by a 5-point Likert scale (Rogers et al., 2015).

Methods Used to Measure Fatigue, Quality of Life, Depression, Symptoms Caused by Breast Cancer, and Food Intake

Cancer-related fatigue was assessed using the following questionnaires and scales: Functional Assessment of Cancer Therapy – Fatigue scale (FACIT fatigue) (Hagstrom et al., 2015); Fatigue Assessment Questionnaire (FAQ) (Schmidt et al., 2014; Steindorf et al., 2014); and the Swedish version of the 22-item Piper Fatigue Scale (PFS) (Mijwel et al., 2017; Campbell et al., 2019).

To assess the quality of life, the following questionnaires were used: the European Organization for Research and Treatment of Cancer questionnaire (EORTC-QLQ-C30 version 3.0) (Schmidt et al., 2014; Steindorf et al., 2014; Mijwel et al., 2017; Campbell et al., 2019), the European Organization for Research and Treatment of Cancer questionnaire (EORTC QLQ BR23 version 3.0) (Schmidt et al., 2014; Bloomquist et al., 2019), one of the subscales of these questionnaires (Mijwel et al., 2017), the Functional Assessment of Cancer Therapy – general questionnaire (FACT-G) (Hagstrom et al., 2015) and the Medical Outcomes Study 36-item short-form survey (SF-36) (Cormie et al., 2013).

The symptoms resulting from breast cancer were verified by the Memorial Symptom Assessment Scale (MSAS), which consists of 32 items (Mijwel et al., 2017). Additionally, for the evaluation of depressive symptoms, the 20-item Center for Epidemiologic Studies Depression (CES-D) scale was used.

For cognitive function, the trail-making test was used (Schmidt et al., 2014; Steindorf et al., 2014); leisure time was measured by the Godin leisure-time exercise questionnaire, which categorizes leisure time by three levels of intensity (strenuous, moderate, and light) and evaluates the level of physical activity of the participant over the last 7 days (Hagstrom et al., 2015).

Carbohydrate intake was assessed according to a three-day diet recall (FoodWorks 13) (Rogers et al., 2015); the usual calcium intake (dietary - supplementary) and the total energy intake were assessed using the 2005 Block food frequency questionnaire (Winters-Stone et al., 2011).

Methods Used to Measure Patterns of Physical Activity, Muscle Strength, Pain Perception, and Breathing Capacity

An accelerometer was used by the participants to record their physical activity patterns (Rogers et al., 2015; Campbell et al., 2019). The Community Health Activity Model Program for Seniors (CHAMPS) questionnaire for older adults was used (Winters-Stone et al., 2011).

The handgrip strength was verified using a manual hydraulic dynamometer (Cormie et al., 2013; Campbell et al., 2019), muscle strength was measured as the isometric muscle capacity of the thigh and isokinetic strength of representative muscle groups in the upper and lower limbs (Schmidt et al., 2014; Steindorf et al., 2014).

The maximum strength of the upper and lower parts of the body was assessed using 1 RM protocols involving chest presses, seated rows, and leg press exercises (Cormie et al., 2013; Hagstrom et al., 2015; Rogers et al., 2015; Bloomquist et al., 2019). A dynamometer (back and leg dynamometer) was used to evaluate the extensor force of the legs (Rogers et al., 2015).

Hemoglobin and pain were measured, and the latter was assessed bilaterally in the middle trapezius and gluteus muscles with an electronic algometer (Campbell et al., 2019); these same authors assessed cardiorespiratory fitness by the Åstrand-Rhyming submaximal cycle test. However, other authors assessed cardiorespiratory fitness using the submaximal treadmill test (modified Naughton protocol) (Rogers et al., 2015) and resistance performance by the peak VO_2 and spiroergometric measures (Schmidt et al., 2014) during exercises performed on an exercise bike (Steindorf et al., 2014). The range of motion assessments for the wrist, elbow, and shoulder joints was performed using standard goniometric procedures (Cormie et al., 2013).

Quality of the Studies

In the evaluation of the individual studies, the agreement between the reviewers was 100% in the analysis of the titles ($k = 1.00$, $p < 0.001$) and 88% in the analysis of abstracts ($K = 0.88$, $p < 0.001$). According to the CONSORT guidelines, out of a total of 46 studies, 13 had scores of <49.9%, 24 studies had scores of 50–79.9%, and nine studies had scores of ≥80%.

Of the 46 articles analyzed, 48% indicated the study model in the title, 35% indicated how the sample size was calculated, 59% described the method used for randomization, 28% reported the adverse effects of the interventions, 35% reported the limitations of the studies, and 47% reported the sources of study funding.

DISCUSSION

Overall Outcomes

In this review, evidence that demonstrated the beneficial effects of physical exercise programs carried out by WSBC was gathered from studies considered to be of high quality, so the risk of bias was low (Shulz et al., 2010; Falci and Marques, 2015). Moderate or high-intensity exercise sessions have been shown to benefit WSBC.

The main benefits include increased muscle strength, promoted by the practice of resistance exercise in combination with other types of exercises (Cormie et al., 2013; Hagstrom et al., 2015; Campbell et al., 2019) or alone (Bloomquist et al., 2019); decreased fatigue (Schmidt et al., 2014; Steindorf et al., 2014; Hagstrom et al., 2015); improved quality of life (Steindorf et al., 2014; Mijwel et al., 2017; Bloomquist et al., 2019); improved psychosocial effects (Schmidt et al., 2014) and increased leisure time (Hagstrom et al., 2015).

Specific Outcomes

Changes Promoted by Physical Exercise in Quality of Life, Muscle Strength, and Fatigue in WSBC

Different studies have demonstrated that aerobic exercises combined with resistance exercises at moderate or high intensity are efficient in improving quality of life (Hong et al., 2019), muscle strength (Buffart et al., 2018), and fatigue (Dieli-Conwright et al., 2018).

Muscle function is affected by cancer treatment, in part due to the loss of muscle mass as a consequence of movement limitations and reduced force-generating capacities of muscles (Klassen et al., 2017). However, physical exercises, when practiced during treatment, are effective in maintaining muscle strength (Methley et al., 2014). The exclusive practice of resistance exercise led to significant improvements in muscle strength in the upper body (Cormie et al., 2013) and lower limbs (Hagstrom et al., 2015).

In similar studies, an increase in the muscle strength of WSBC was found after 12 weeks of high-intensity resistance exercise (Cešeiko et al., 2019; Santagnello et al., 2020). Similar results were observed in elderly survivors of BC who performed 16 weeks of resistance exercise at a high intensity (Serra et al., 2018).

When it was performed in combination with other exercises of moderate (walking) and/or high (HIIT) intensities, resistance exercise also improved muscle strength in the upper and lower body (Travier et al., 2015; van Waart et al., 2015).

Cancer-related fatigue is different from that experienced by healthy individuals daily, as it is not relieved with rest nor is it proportional to the level of physical activity performed; thus, it affects patients' quality of life (Berger et al., 2015). In cancer patients, fatigue is a distressing, constant, and subjective symptom of physical, emotional, and/or cognitive tiredness or exhaustion (Bower, 2019). Also, fatigue is one of the symptoms resulting from cancer or its treatment that affects patients with BC and gynecological cancer and is considered one of the main

factors for the poor quality of life (Wang and Woodruff, 2015; van Vulpen et al., 2016). Some authors suggested that cancer-related fatigue is due in part to muscular and mitochondrial dysfunction, peripheral immune activation and inflammation dysfunction, as well as central nervous system (CNS) disorder (Yang et al., 2019).

The mechanisms which explain how physical training attenuates cancer-related fatigue are still not entirely clear (Juvet et al., 2017). However, one of the hypotheses suggests that physical training increases functionality, causing a decrease in the physical effort employed and consequently decreasing fatigue (Furmaniak et al., 2016). Another study attests that women breast cancer survivors who participated in a physical training program reported an increased feeling of energy and vigor, which are central aspects of fatigue; thus, physical exercise would be able to decrease the levels of fatigue in this population (Johnsson et al., 2019).

Resistance training, when it is performed in combination with aerobic training, reduced fatigue in women with BC during treatment (van Waart et al., 2015). Similarly, another study (Dieli-Conwright et al., 2018) reported that after 16 weeks of resistance training (intensity 80% of 1 RM for the lower body and 60% for the upper body at 65–80% of the maximum heart rate) reduced the fatigue of WSBC with obesity or overweight who were physically inactive (Dieli-Conwright et al., 2018). In addition, 12 weeks of high-intensity resistance training also decreased fatigue in WSBCs (Santagnello et al., 2020). The same result was detected in elderly survivors of BC when they performed resistance training but at moderate intensity (Serra et al., 2018).

In another study, fatigue levels were higher in sedentary women who were undergoing treatment for BC than in patients who underwent 18 weeks of training involving both resistance and aerobic exercises immediately after treatment for BC (Travier et al., 2015).

Previous meta-analyses, which analyzed physical exercise in different prescription parameters (regardless of whether it was aerobic or anaerobic), demonstrated that training a. reduced fatigue in WSBC (Juvet et al., 2017) who underwent adjuvant therapy (radiation therapy) for the treatment of cancer (Lipsett et al., 2017) and b. improved their quality of life (Meneses-Echávez et al., 2015).

Quality of life can be defined as an individual's well-being, concerning his or her state of mental and physical health, as well as social relationships and economic and environmental factors (Kolotin and Andersen, 2017). In WSBC, this variable can be assessed during the treatment of the disease (Shafaee et al., 2018). Quality of life is strongly affected by the treatment of BC (Chrischilles et al., 2019). However, some authors have suggested that physical exercise promotes significant positive changes in the quality of life and well-being of WSBC (Duncan et al., 2017; Möller et al., 2019).

A resistance training regime of moderate-intensity, not performed in combination with other types of physical exercise, improved the quality of life of elderly survivors of BC (Serra et al., 2018). The combination of both resistance and aerobic training performed at intensities above 60% of the 1 RM and maximum heart rate improved the quality of life of WSBC who were overweight or obese (Dieli-Conwright et al., 2018). In addition, 9 weeks of resistance exercises combined with aerobic exercises positively affected the quality of life and body composition increased the lean mass and decreased the percentage of fat and BMI of WSBC who were being treated with aromatase inhibitors (IAs) (Thomas et al., 2017; Paulo et al., 2018, 2019).

Changes in Body Composition and Sleep Quality Promoted by Physical Exercise in WSBC

It is common for individuals affected by BC to become obese after diagnosis, and obesity is associated with worse survival than is the normal weight (Linge et al., 2018; Trestini et al., 2018). However, physical exercise can improve the body composition of cancer survivors (Schwartz et al., 2017).

In one study, sedentary women who underwent BC treatment showed increased body fat and decreased lean mass (Freedman et al., 2004). However, combined training (aerobic exercise and resistance exercise) enhanced lean body mass associate with a reduced percentage of body fat (Thomas et al., 2017). In another study, an increase in muscle strength was found in the extremities of the upper limbs (Bloomquist et al., 2019). Combined training at moderate intensity alleviated symptoms caused by cancer treatment, such as nausea, vomiting, pain, and constipation, in WSBC (van Waart et al., 2015).

The lean mass of WSBC increased after 12 weeks of high-intensity resistance training (Santagnello et al., 2020). After an 11-week intervention involving aerobic and resistance exercises combined with various activities, which involved hypopressive exercises, the BMI of WSBC stabilized, their percentage of body fat decreased (Leclerc et al., 2017).

Lymphedema is the result of the exacerbated retention of lymphatic fluid in the interstitial compartment associated with deficient lymphatic drainage, which can be caused by lymphatic vascular changes, an underlying disease, trauma, or systemic surgery (Grada and Phillips, 2017). Lymphedema impairs the quality of life of WSBC, as it decreases the function of the affected limb (Nelson, 2016; Shah et al., 2016).

Studies have shown that resistance exercise does not increase the extent of swelling and did not worsen symptoms in WSBC with lymphedema (Cormie et al., 2013; Bauman et al., 2018). However, another study also found that resistance exercises both decrease symptoms related to lymphedema and reduces the volume of the WSBC arm (Panchik et al., 2019).

Especially in advanced stages of cancer, patients' sleep and wake cycles are affected (Bernatchez et al., 2017), suggesting that sleep quality may be altered in WSBC. On the other hand, studies have reported that physical exercise can improve sleep quality in WSBC (Matthews et al., 2018; Fang et al., 2019; Kreutz et al., 2019). Accordingly, combined training is known to be more efficient in improving the quality of sleep and the number of hours of sleep per night, as combined training helps reduces the severity of sleep disorders and daytime sleepiness (Rogers et al., 2015). Also, resistance training for 12 weeks, at an intensity of 60–80% of 1 RM, decreased sleep disorders in WSBC (Steindorf et al., 2017).

Changes in Cardiorespiratory Fitness Promoted by Physical Exercise in WSBC

Cardiorespiratory fitness is considered both an indicator of an individual's health and the prognoses of diseases (Ozemec et al., 2018). Some authors have suggested that physical exercise is an effective means of improving cardiorespiratory fitness and decreasing cardiometabolic risk in individuals with pathological conditions (Ruegsegger and Booth, 2018).

In WSBC, physical exercise can improve cardiorespiratory responses and reduce fatigue (Ruegsegger and Booth, 2018). In a previous study (Campbell et al., 2019), WSBC showed an increase in muscle strength and handgrip strength and maintained the same level of cardiorespiratory fitness and body weight after resistance and combined training, both of which include components of HIIT. On the other hand, in the same study (Campbell et al., 2019), in the control group, such responses were not observed. In another study (Mijwel et al., 2017), WSBC maintained similar fatigue scores and showed improved role function scores on the quality of life scale (EORTC-QLQC30) after combined training; such positive responses were not observed in the control group (Mijwel et al., 2017). Nevertheless, combined training maintained the cardiorespiratory fitness of WSBC; however, the sedentary group showed worse cardiorespiratory fitness (van Waart et al., 2015).

Eighteen weeks of combined training, including HIIT components, improved the submaximal cardiorespiratory fitness of WSBC (Travier et al., 2015). Another work (Dieli-Conwright et al., 2018) showed that overweight/obese WSBC who underwent 16 weeks of combined training (60% of the 1 RM for the upper body and 80% for the lower body in resistance exercises and 65–80% of the maximum heart rate for aerobic exercises) showed improvement in cardiorespiratory fitness.

In addition, the combination of combined training and flexibility exercises for 12 weeks improved pain perception, maximum VO_2, flexibility, and muscle strength in WSBC (Reis et al., 2018); similar results, as well as improvements in shoulder pain, were observed in another study after combined training (Möller et al., 2019).

Changes in Bone Mineral Density Promoted by Physical Exercise in WSBC

In women diagnosed with BC, treatment with AIs is considered the standard pharmacological method to increase patient survival (Geisler, 2011). However, these drugs have the side effects of increased bone resorption (Baker et al., 2018), accelerated bone loss, and, consequently, an increased risk of fractures (Ramchand et al., 2019).

One method used to stimulate bone remodeling and increase bone mineral density is the practice of impact physical training, which must be performed with loads greater than that experienced in day-to-day life (Kirkham et al., 2016). In addition to the appropriate intensity, adherence to the physical exercise program is essential to improve bone mineral density in WSBC (Kemmler et al., 2016).

In the other study, women with BC being treated with IAs showed favorable changes in bone renewal, the maintenance of bone mineral density in the spine and lumbar spine as well as an increase in lean mass after 12 months of impact training combined with resistance exercises (Winters-Stone et al., 2011). In another study (Zaidi et al., 2018), women with BC using IAs were able to maintain their total bone mineral density after performing aerobic training; in the group that performed impact training combined with resistance exercise, the bone mineral density in the patients' spine was preserved (Zaidi et al., 2018).

Weaknesses Detected During the Analysis of the Articles and Suggestions for Future Studies

Before listing the gaps found in some studies, we must first emphasize that each selected work has important strengths that contributed to improving the understanding of the effects of physical training in women with BC for the scientific and academic community. Thus, at no time did we aim to undermine the efforts of the authors of the scientific articles referenced in this systematic review.

The most common problems were related to the data collection of several parameters inherent for the control groups in the studies. Among the articles selected for this systematic review, we detected the absence of a sedentary group, as the control groups did not undergo any interventions and/or performed stretching exercises (Winters-Stone et al., 2011). Also, another important point was that aerobically active women were not excluded, 62% of whom adhered to the interventions. In this same study, the training program lasted 12 months, with the ideal period being longer for the completion of bone remodeling cycles.

The daily activities of the participants who did not undergo a physical exercise intervention also involved an individualized walking program (monitoring by a pedometer) and were encouraged to progressively increase the number of steps they took each day until they reached 10,000 steps per day (Bloomquist et al., 2019). Furthermore, in the same study (Bloomquist et al., 2019), lymphedema of the upper limbs after resistance exercise was not assessed, and the women who did not undergo training had more baseline-related lymphedema than did those who underwent physical training; nevertheless, we noticed the absence of data on the volume between the arms and the measurements of extracellular volume in all patients (Bloomquist et al., 2019).

In another study, the participants were not evenly distributed among groups during the training period, and we also detected (a) the use of broad recruitment criteria; (b) a lack of limits to restrict the variation in the time after the treatment of BC; (c) the failure to perform power calculations for secondary purposes and assess the effects on fatigue and muscle strength in the treated limb; (d) insufficient data from the intervention group to allow an analysis of how social interactions improved due to physical exercise (Hagstrom et al., 2015). We emphasize that the motivational aspect of patients participating in the study can be a limiting agent of the research since it does not represent the behavior of all women with lymphedema related to BC or even the use of compression clothes.

In other studies, the sample sizes were relatively small for subgroup analyzes (Cormie et al., 2013), and a large number of patients randomized to the control group did not participate

in the research (Campbell et al., 2019) and had a high rate of abandonment of physical training (Mijwel et al., 2017).

In another study, limitations such as a small sample size, short exercise sessions, and a lack of assessments for more detailed aspects of sleep in patients with BC were detected; moreover, in that same study, it was not clear whether the results can be generalized to other types of cancer, and the effects of exercise on psychosocial factors were not distinguished (Rogers et al., 2015).

Concerning the research participants, not all studies verified their daily physical activity routines (Cormie et al., 2013; Steindorf et al., 2014; Hagstrom et al., 2015; Rogers et al., 2015; Bloomquist et al., 2019), and information on food, alcohol intake, and/or tobacco use was not evaluated (Winters-Stone et al., 2011; Cormie et al., 2013; Schmidt et al., 2014; Hagstrom et al., 2015; Mijwel et al., 2017; Bloomquist et al., 2019; Campbell et al., 2019).

Study Limitations

This systematic review has important strengths, such as a low risk of bias. However, some limitations must be mentioned, such as a small number of articles and strict criteria for the analysis of specific aspects of each type of physical training (load, intensity, and volume). It was found that there is strong evidence showing that regular physical exercise performed at a moderate or high intensity, regardless of whether it is aerobic or anaerobic, alone or in combination with other exercise modalities, can benefit WSBC. Among these benefits, we highlight increased muscle strength, improved quality of life, and decreased fatigue. In addition, positive effects on sleep quality as well as the maintenance of bone density and the bone turnover rate were observed. However, in future studies, it is necessary to individualize the WSBC regarding the type of treatment, stages of the disease and time after the diagnosis of breast cancer, and what is the best type of training that they should carry out to improve health and quality of life.

CONCLUSIONS

We recommend that the regular practice of physical exercise, supervised and prescribed by the doctor of the patient, is important for the maintenance and/or recovery of the health and quality of life of WSBC.

Physical training programs that include resistance exercise exclusively at an intensity of 55–80% performed at least twice a week and targeted at the main muscle groups have proven to be efficient in improving parameters related to the quality of life, muscle strength, endurance, physical function, cognitive performance, and leisure time; also, decreased levels of fatigue and maintenance of lymphedema status can be verified.

The combination of physical exercises, such as aerobic (85–95% of maximum heart rate) associated with resistance (70–90% of 1 RM) decreased BMI, breast and arm symptoms, and pain, in addition to increasing muscle strength. HIIT associated with resistance training, with an intensity of 70–80% of 1 RM; or HIIT plus aerobic exercise (with a score of 13–15 on Borg's perceived exertion scale) provided positive effects on quality of life, muscle strength, and handgrip, body weight stabilization and fatigue in addition to reducing pain and symptoms related to breast cancer.

A physical exercise program that made use of resistance bands associated with walking (48–52% of heart rate) for 3 months brought benefits on the quality of sleep of breast cancer survivors, such as decreased daytime sleepiness and increased number of hours of sleep per night. Impact exercises (jumping with heavy vests) combined with resistance exercises, with an intensity of 60–70% of 1 RM, promoted favorable bone changes as well as an increase in lean mass in women survivors of breast cancer who used IAS.

DATA AVAILABILITY STATEMENT

The original contributions generated for the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

SS and RS: conceptualization, data treatment, and wrote – original draft. SS, NS, and AM: formal analysis. SS, RS, and FV: investigation. NS: methodology. NS and AM: project administration. FA, JdS, FV, RV, NS, and AM: wrote – review and editing. All authors contributed to the article and approved the submitted version.

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frontiers in Physiology

SYSTEMATIC REVIEW
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Exercise During Pregnancy and Prenatal Depression: A Systematic Review and Meta-Analysis

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Background: Prenatal depression is associated with an increased risk of physical, physiological, cardiovascular, and psychological diseases for mothers and future newborns. Prenatal depression and depressive symptoms could have negative effects on the cognitive, emotional, social, and behavioral development of children.

Objective: This study aimed to examine the influence of exercise during pregnancy on the prevalence of prenatal depression and depressive symptoms in the scientific literature.

Data Sources: A search was carried out examining different online databases up to November 2020.

Methods of Study Selection: A systematic review with random effects meta-analysis was performed. Only randomized controlled trials published in English or Spanish with pregnant populations and interventions with exercise programs carried out during pregnancy were included. The scores obtained by the tools that measured the emotional state and depressive symptoms as well as the number and percentage of depressed women of the study groups were analyzed.

Tabulation, Integration, and Results: We analyzed 15 studies and found a negative association between moderate exercise during pregnancy and prenatal depression (ES = −0.36, 95% CI = −0.58, −13, I^2 = 80.2%, $P_{heterogeneity}$ = 0.001). In addition, the studies also showed that women who were inactive during pregnancy had a 16% higher probability of suffering prenatal depression [RR = 0.84 (95% IC = 0.74, 0.96) I^2 = 61.9%, $P_{heterogeneity}$ = 0.010].

Conclusion: Supervised exercise during pregnancy may be useful for the prevention and reduction of prenatal depression and depressive symptoms.

Systematic Review Registration: Registered in PROSPERO (Registration No. CRD42020164819).

Keywords: exercise, pregnancy, prenatal depression, model, fetus

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INTRODUCTION

Depression (major depressive disorder) is an important, common, and serious medical illness that negatively affects how a person feels, thinks, and acts. Depression causes different symptoms, such as feelings of sadness, loss of interest/energy, difficulty thinking/concentrating, and/or thoughts of death or suicide. All symptoms can vary from mild to severe (Bienvenu et al., 2013).

During the last 20 years, the World Health Organization (2004) has monitored diseases associated with depressive symptoms and the dangerous growth rate of the prevalence of these diseases within developed countries. Pregnant women are not exempt from depressive symptoms, and pregnancy is a particularly vulnerable time for depression to occur compared with other periods of a woman's life (Campagne, 2004).

Indeed, the physical and psychological changes that occur in pregnant women are the largest promoters of this type of emotional lability (Lederman et al., 1997). Symptoms that alter emotional balance and lead to sickness (in some women) usually appear at the beginning of pregnancy; these symptoms, together with the fear of not being able to cope with the baby, hormonal changes, transformation of the female body, and the possible presence of a history of psychological disorders, lead to a complex condition and may be responsible for the well-known emotional lability during pregnancy (Barakat, 2006; Austin et al., 2008).

It seems that the relationships between gestational alterations of a psychological and emotional nature (depression, changes in self-esteem, anxiety, stress, insecurity, etc.) and physiological parameters (longer deliveries and more instrumental labors, altered birth weights, etc.) have been scientifically confirmed (Field et al., 2006; Rahman et al., 2007; Grote et al., 2010). This negative association extends beyond the gestational period, causing postnatal complications for both mothers and offspring (Hammond and Crozier, 2007; Deave et al., 2008; Hay et al., 2010; Field, 2011). Undoubtedly, the intrauterine environment is decisive for the life of future humans.

Recent studies have estimated the prevalence of depression during pregnancy to be between 10 and 30% (Teixeira et al., 2009). It is closely associated with depression in the postpartum period, which has a prevalence of between 17 and 17.7% (Hahn-Holbrook et al., 2018; Shorey et al., 2018).

Drug treatment during pregnancy is difficult and often questioned due to the possible side effects of antidepressants in the mother and fetus (Hammond and Crozier, 2007). Depression is a difficult complication to control because it is necessary to implement an intervention that avoids the possible negative effects on the fetus and the mother, such as altered brain development (O'Connor et al., 2002; Lee et al., 2007), an increased risk of preterm birth or intrauterine growth restriction (Field et al., 2006; Li et al., 2009; Field, 2011). Notwithstanding the aforementioned difficulty, it is clear that it is necessary to establish strategies that prevent the already proven, dangerous, and increasing association between prenatal depression and postpartum depression, which is a more well-known type of depression that involves a series of related complications, such as mother–child bonding difficulties (Wisner et al., 2009), infant feeding difficulties and infant overweight problems (Ertel et al., 2010), low birth weight and long hospital stays.

This situation constitutes an interesting incentive to investigate alternative treatments for depression (Hammond and Crozier, 2007). According to a review of studies about the effects of exercise on the non-pregnant population, previous studies report beneficial antidepressant effects (Barbour et al., 2007; Blumenthal et al., 2007; Martinsen, 2008). Furthermore, the results of previous investigations, involving psychological variables during an exercise program carried out in pregnant women are encouraging (Koniak-Griffin, 1994; Goodwin et al., 2000; Nordhagen and Sundgot-Borgen, 2002; Orr et al., 2006).

Over the last 30 years, exercise has been shown to have many benefits for pregnant women without a risk of adverse effects for maternal–fetal well-being provided that the activity is of a moderate intensity and is supervised by a professional. Although the scientific literature is not fully conclusive, there are many investigations indicating the positive effects of moderate exercise on the prevention of complications, including the adequate control of certain maternal, fetal, and newborn parameters (Barakat et al., 2015, 2016; Klein et al., 2018). However, due to fear, ignorance, or other factors, the prevalence of women who achieve the minimum weekly recommendation of exercise during pregnancy is ∼15–20% (Mottola et al., 2018; Barakat et al., 2019).

The purpose of this systematic review and meta-analysis was to synthesize the literature and determine the effect of exercise during pregnancy on the prevalence of prenatal depression.

METHODS

This review was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2015) and was registered with PROSPERO, the International Prospective Register of Systematics Reviews (Registration No. CRD42020164819).

Eligibility Criteria

For this systematic review, the PICOS framework (population, intervention, comparison, outcome, study design) was used, and the nature of the interventions included in this research (Moher et al., 2015) was analyzed. Only articles written in English or Spanish and published between 2010 and 2020 were selected.

Population

The chosen population was pregnant women without any contraindication during pregnancy to undergo an exercise intervention during this period. Women suffering any absolute (e.g., heart failure, multiple pregnancy, or premature labor) or relative (e.g., essential arterial hypertension, cardiac arrhythmia, or anemia) contraindication were excluded from analyses.

Intervention

The following characteristics of the interventions were analyzed: (i) intensity: except for one study, all of the included studies had a light-to-moderate intensity of the load, which was prescribed using 55–65% of maximum maternal heart rate and in some cases by means of the perception of effort (range 12–14 of the

Borg Scale); (ii) duration of the program; (iii) type of exercise (e.g., aerobic, strength, balance, or pelvic floor training); (iv) weekly frequency of the sessions; (v) duration of the sessions; (vi) whether the exercise program was supervised by a professional; (vii) adherence of the sample to the exercise program; and (viii) in some studies analyzed, a complementary intervention with different outcomes was carried out, and so this was classified as "exercise + cointervention" as shown in **Table 1**.

Comparison

Women who engaged in exercise or physical activity were compared with those who did not. Additionally, the intervention characteristics were reviewed (shown in **Table 1**) to enrich our understanding of each study: no exercise intervention; weekly frequency, duration (both the program and sessions) or supervision of the program by a professional; mode of exercise; or if depression was a primary or secondary variable.

Outcomes

Target outcomes were the number of pregnant women with depression in both groups (to compare both the control and intervention groups) and the prenatal depression score of each depression questionnaire administered. The questionnaires dealing with prenatal depression among the selected articles were, in order of relevance, the Center for Epidemiologic Studies Depression Scale (CES-D), Edinburgh Postnatal Depression Scale (EPDS), The Short Form 36 Health Survey (SF-36) and Hospital Anxiety and Depression Scale (HADS).

Study Design, Information Sources, and Search Strategy

To perform this review, the SPORTDiscus, ClinicalTrials.gov, and MEDLINE (PubMed) databases were searched at the Universidad Politécnica de Madrid. The search began in October 2019 and the study was last updated between February and March 2020.

- English: exercise OR physical activity OR sport OR fitness AND pregnancy OR prenatal depression OR depression OR emotional OR emotional factors AND randomized clinical trial.
- Spanish: ejercicio O ejercicio físico O actividad física O deportes Y embarazo O depresión prenatal O depresión O emocional O factores emocionales Y ensayo clínico aleatorizado.

Study Selection

The inclusion criteria of the studies were randomized clinical trials whose intervention involved measurable or quantifiable activity or exercise (studies in which only advice to have an active pregnancy was provided were not selected), prenatal depression outcomes (depression symptoms or diagnosed depression) measured, and different characteristics of an exercise program provided. The selection process followed for the reviewed articles is captured in **Figure 1** (Page et al., 2021).

Regarding the parameters studied, prenatal maternal depression expressed quantitatively (scale) and/or categorically (maternal depression yes/no) was the primary outcome extracted from the included studies. To determine the extent of the effects of exercise on the health of pregnant women, other pregnancy outcomes were examined as secondary outcomes (sociodemographic and physiological maternal variables and newborn outcomes, among others).

The data extracted from each of the included studies were the author(s) and publication year, country in which the study was conducted, number of participants, details of the type of exercise program, primary and secondary variable(s) analyzed and cointervention, if applicable (**Table 1**).

Statistical Analysis

Meta-analysis was performed separately by a different expression of depression variable. First, when the depression variable was expressed as a continuous variable, such as a score obtained by a questionnaire, the overall confidence interval (CI) was calculated using the standardized mean difference (Hedges et al., 2010).

Second, when depression was expressed as a categorical variable (yes/no), the number of events present in each study group and its relative risk (RR) were recorded, and the total sum of the RR was calculated using a random effects model (Higgins and Thompson, 2002).

In both analyses (dichotomous and continuous) the compensated average was established by assigning each study a weight relative to its sample size or number of events that contributed to the entire study (weight) or, in short, the information burden that each of them contributes. To quantify the heterogeneity present in the results, the I^2 statistic was used, which indicates the proportion of variability observed in the effect of the intervention (between studies) that is due to the heterogeneity between studies and is not random. The following thresholds were used for the I^2 statistic: low = 25%, moderate = 50%, and high = 75% (Daley et al., 2018).

One approach previously used to solve the problem of high heterogeneity has been to split the studies into subgroups based on some characteristics that could explain the variability of the studies (Ferreira González et al., 2011). However, in our case, given the limited number of the studies identified for the review, we have opted to present all of the examined studies in each analysis as we understand that this approach provides a more comprehensive view of the study.

Quality of Evidence Assessment and Risk of Bias

Despite being often mistaken as interchangeable terms, "quality" and "risk of bias" are, in fact, distinct but related concepts that should, thus, be differently addressed (Gunnell et al., 2020). In the present study, the assessment of quality (i.e., the degree to which studies are conducted in alignment with the highest possible standards) was performed by means of the Grading of Recommendations Assessment, Development and Evaluation (GRADE) technique (Guyatt et al., 2008), and studies that were rated as having high or moderate quality were included.

On the other hand, risk of bias (i.e., the degree to which potential biases may have led to underestimation or overestimation of an effect) was assessed following the Cochrane Handbook (Higgins et al., 2020).

TABLE 1 | Characteristics of the studies analyzed.

Author, year, and country	N; IG; CG	Intervention Physical exercise program							Main variables analyzed	Secondary variables analyzed	Co-intervention
		W Freq.	Int.	Time	Type	Sup.	Duration	Adh.			
Daley et al. (2018) (United Kingdom)	784; 391; 393	2	Mod	30 min	Aerobic	Yes	8 weeks	–	Prenatal and postnatal depression	Maternal parameters and smoking	Smoking cessation and mental health treatment
Vargas-Terrones et al. (2019) (Spain)	124; 70; 54	3	Mod	60 min	Aerobic + strength + balance + pelvic floor + stretching + relaxation	Yes	22–26 weeks	69.3%	Prenatal depression and maternal parameters	Habits before pregnancy	Basic prenatal care
Haakstad et al. (2016) (Norway)	105; 52; 53	2	Mod	60 min	Aerobic + strength + relaxation	Yes	12 weeks	80%	Prenatal depression and regular physical exercise	Sociodemographic variables, habits, and complications during pregnancy	No
Perales et al. (2016) (Spain)	241; 120; 121	3	Mod	55–60 min	Aerobic + strength + stretching + relaxation	Yes	∼30 weeks	90 ± 8%	Maternal variables, hypertension, and excessive weight gain	Prenatal depression and diabetes	No
Uebelacker et al. (2016) (United States)	20; 12; 8	1	Mod	75 min	Yoga	Yes	9 weeks	80%	Quality of life and prenatal depression	Sociodemographic variables and maternal parameters	Yoga practice at home
Davis et al. (2015) (United States)	46; 23; 23	1	Mod	75 min	Yoga	Yes	8 weeks	–	Prenatal depression and anxiety	Maternal parameters	No
Perales et al. (2015) (Spain)	106; 52; 54	3	Low–Mod	55–60 min	Stretching + aerobic + relaxation	Yes	∼30 weeks	85%	Prenatal depression and maternal parameters	Habits in pregnancy and sociodemographic variables	No
Ussher et al. (2015) (United Kingdom)	785; 392; 393	2	Mod	30 min	Aerobic	Yes	8 weeks	88.5%	Prenatal depression	Maternal parameters	No
Perales et al. (2014) (Spain)	167; 90; 77	3	Low–Mod	55–60 min	Aerobic + strength + stretching + relaxation	Yes	∼30 weeks	85%	Prenatal depression	Sociodemographic and maternal variables and new-born outcomes	No
Field et al. (2013b) (United States)	92; 46; 46	1	Mod	20 min	Yoga	No	12 weeks	–	Prenatal, postnatal depression, and anxiety	Cortisol, estriol, and progesterone levels	No
Field et al. (2013a) (United States)	92; 46; 46	1	Mod	20 min	Tai chi/Yoga	Yes	12 weeks	–	Depression and anxiety during pregnancy	Psychotic disorders and sleep complications in pregnancy	No
Satyapriya et al. (2013) (India)	96; 51; 45	7	Mod	60 min	Yoga	Yes	16 weeks	-	Maternal parameters, prenatal depression, and anxiety	Sociodemographic variables	No
Field et al., 2012 (United States)	84; 28; 28-28	2	Mod	20 min	Yoga	Yes	12 weeks	80%	Depression and anxiety during pregnancy	Legs and back pain	No
Robledo-Colonia et al. (2012) (Colombia)	80; 40; 40	3	Mod–High	60 min	Aerobic + stretching + relaxation	Yes	12 weeks	–	Depressive symptoms	Sociodemographic variables	Physiotherapy treatment
Mosquera-Valderrama et al. (2012) (Colombia)	74; 37; 37	3	Mod	50 min	Aerobic + stretching	Yes	12 weeks	–	Depressive symptoms	Sociodemographic variables	Walks twice a week unsupervised

Rf, reference; RCT, randomized controlled trial; IG, intervention group; CG, control group; W freq., weekly frequency; Int., intensity; Mod, moderate; Sup., supervised sessions; Adh., adherence.

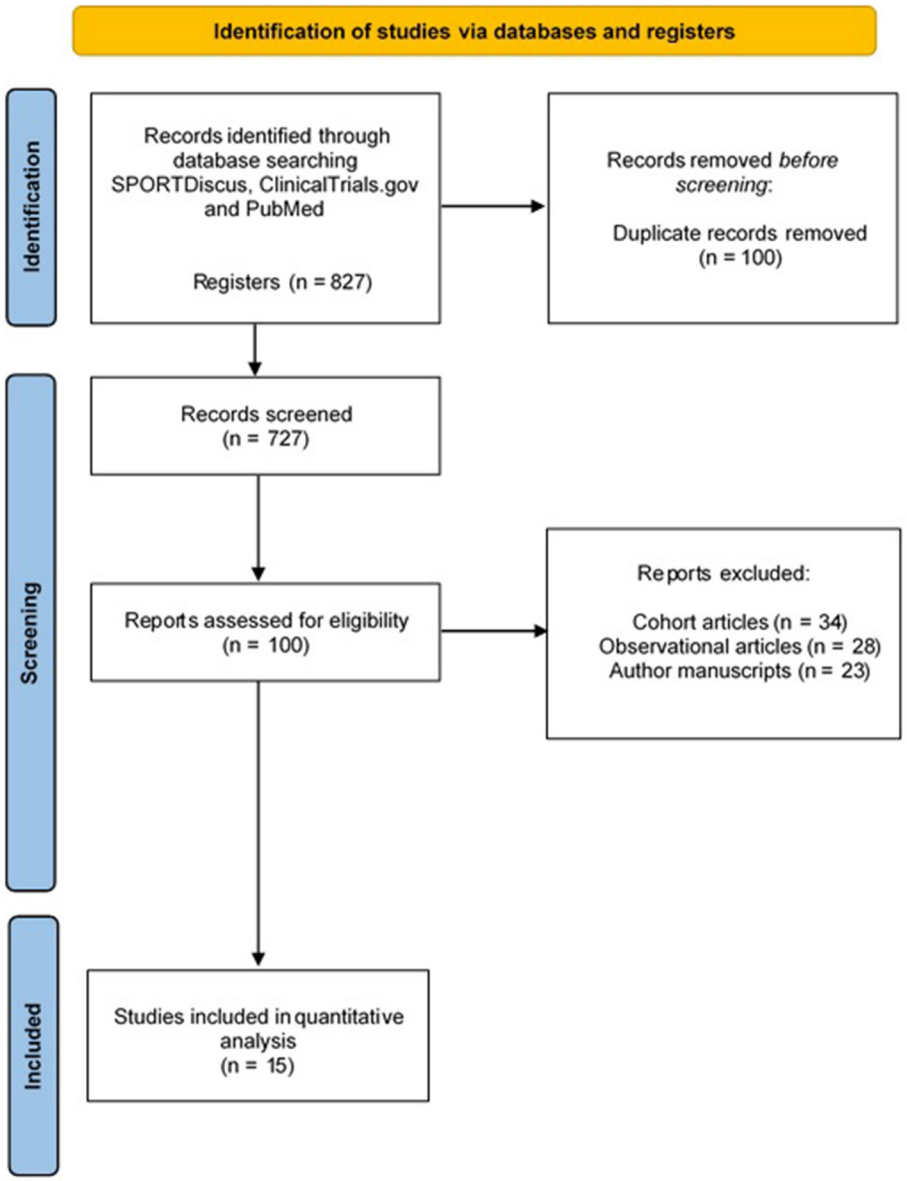

FIGURE 1 | Flow chart of the retrieved and analyzed articles.

RESULTS

From a total of 827 retrieved articles, 727 were excluded for not meeting any of the inclusion criteria (**Figure 1**). In addition, 85 articles were excluded because they were not completely randomized clinical trials (i.e., they lacked a randomization process). Finally, 15 studies were included for analysis. Thirteen of them reported participants' depression or depression symptoms as a continuous variable (i.e., a score obtained in a questionnaire), and these studies are presented in **Figure 2**. On the other hand, in eight of them, depression was treated as a categorical variable reporting the number of women showing depression symptoms in each group (control and intervention). These studies are displayed in **Figure 3**. Last, six studies reported information about depression as both a continuous variable (score obtained in a questionnaire) and a dichotomous variable (suffering/not suffering depression). These works have, thus, been included in both **Figures 2**, **3** (Perales et al., 2014, 2015; Ussher et al., 2015; Haakstad et al., 2016; Uebelacker et al., 2016; Daley et al., 2018).

Among the 15 articles included in the meta-analysis, 14 included sessions supervised by professionals in the field. The exercise sessions (both supervised and not) were designed for low-to-moderate intensity or moderate-to-high intensity exercise and were not performed more than three times per week. The session durations varied from 20 to a maximum of 75 min for a session.

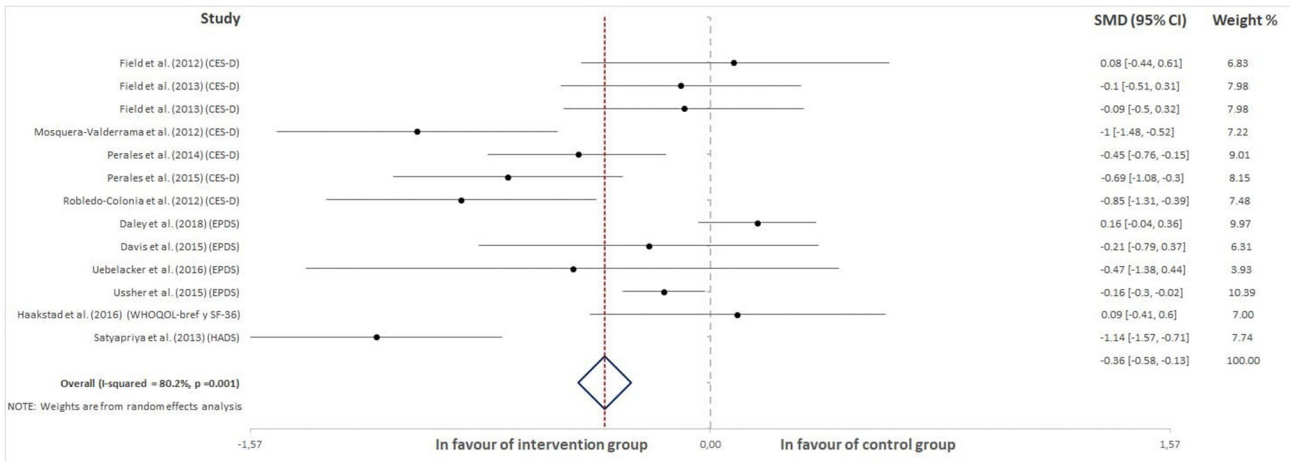

FIGURE 2 | Effect of exercise on the score obtained for the participants on the administered questionnaires.

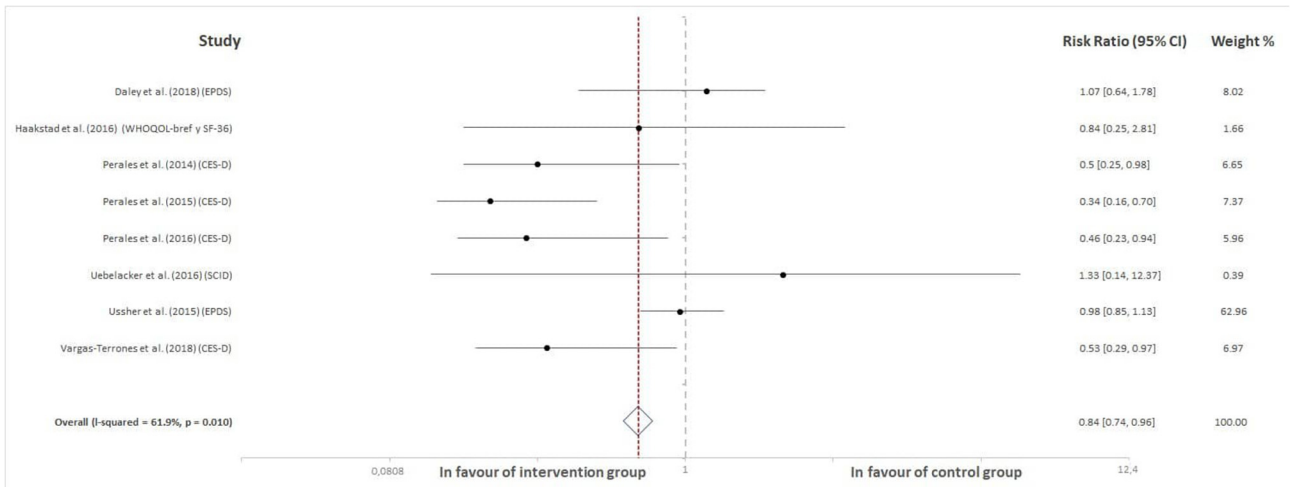

FIGURE 3 | Effect of exercise programs during pregnancy on the probability of experiencing prenatal depression.

Effect of Exercise on the Score Obtained for the Participants on the Administered Questionnaires

Thirteen studies were included in this analysis. The results revealed a negative association between exercise practice during pregnancy and the scores obtained for the questionnaires that were used to measure depression in pregnant women (ES = −0.36, 95% CI = −0.58, −13, $I^2 = 80.2\%$, $P_{heterogeneity} = 0.001$). **Figure 2** shows the forest plot corresponding to the present meta-analysis.

Effect of Exercise on the Number and Percentage of Depressed Pregnant Women in the Study Groups

A total of eight studies were included in this analysis. The global effect of exercise was evident in the number and percentage of pregnant women when those who were considered depressed in the intervention groups were compared with those in the control groups. Specifically, the total RR compensated was 0.84 (95% CI = 0.74, 0.96, $I^2 = 61.9\%$, $P_{heterogeneity} = 0.010$). These outcomes indicate that women who remain inactive present a 16% greater probability of experiencing prenatal depression. **Figure 3** shows the forest plot corresponding to the present meta-analysis.

Risk of Bias Assessment

Overall, the quality of evidence ranged from low to high (see **Figure 4**; **Table 2**).

Most of the studies exhibited low selection and attrition bias. There was a reasonable number of studies whose risk of detection bias was found to be unclear because studies reported whether outcome assessors had not been blinded to participants. The most common sources of risk of bias were (a)

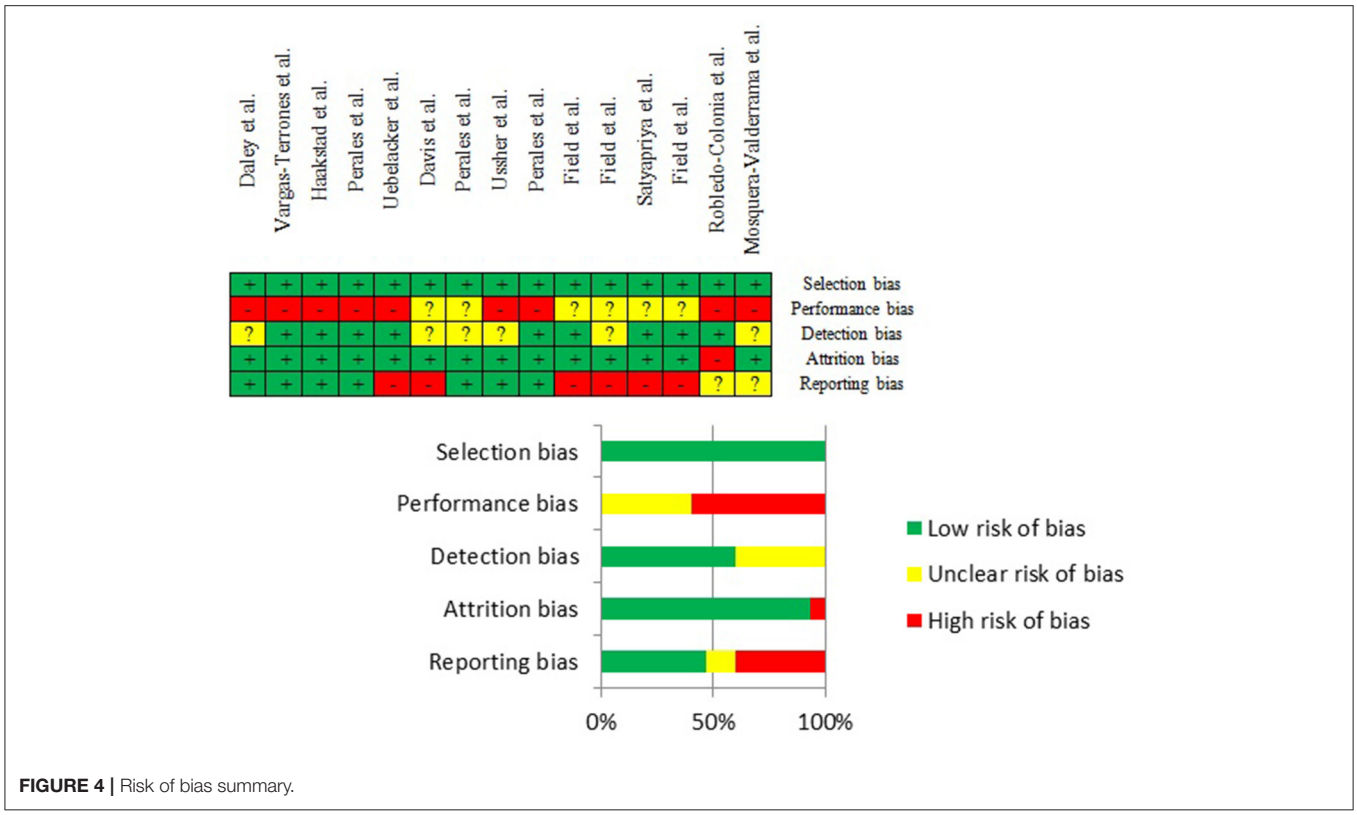

FIGURE 4 | Risk of bias summary.

the low likelihood that either study participants or personnel were blinded to the experimental condition (performance bias) and (b) the fact that findings of some outcomes appearing in the protocol of the studies were not published in the analyzed studies (reporting bias).

Despite the risk of bias findings, it was decided to not disregard any study in our analyses. First, with regard to performance bias, because it is acknowledged in the Cochrane Collaboration's Tool (Higgins et al., 2020), blinding is not possible in certain situations (it is usually impossible to blind people to whether a PA program has been followed). Second, relating to the reporting bias, based on data extracted from the included studies, it is unlikely that the remaining outcomes of interest (biological rather than social data) were significantly associated with depression.

DISCUSSION

The present study aimed to determine the effects of exercise during pregnancy on maternal prenatal depression. The results show a trend toward a small reduction in both the scores of the instruments (questionnaires) that measured the prevalence of depressive symptoms in pregnant women and the number and percentage of women with diagnosed prenatal depression in the intervention groups (physical exercise). It is important to clarify that, during pregnancy, a woman may present depressive symptoms and increased emotional lability but not be diagnosed with prenatal depression although obviously both conditions are closely associated.

Because all the studies investigated healthy pregnant women, the results obtained in these studies are related to depressive symptoms rather than to diagnosed depression. Therefore, from a global perspective, the findings of the present study could be used and have an important clinical application to prescribe groups and supervised exercise during pregnancy as a preventive factor against emotional lability and associated complications. The results of the present study allow us to conclude that supervised exercise during pregnancy constitutes a powerful tool for preventing and reducing prenatal depression.

Regarding the impact of exercise during pregnancy, the results suggest that there is a positive association between an active pregnancy and a more balanced and adequate emotional state. Because depressive symptoms begin early in pregnancy (first trimester), the supervised exercise program designed for healthy pregnant women should also begin early, once it is known that there are no obstetric contraindications to exercise during pregnancy.

The study designed by Perales et al. (2014) deserves particular attention; the article included 167 pregnant women and noted that, although depression levels were similar in both groups (slightly higher in the intervention group) at the beginning of pregnancy, by the end of pregnancy there was a significant difference (reduction) in favor of the intervention group (exercise) compared with the control group in relation to both the questionnaire values (CES-D questionnaire) and

TABLE 2 | Risk of bias of reviewed studies.

Articles/Bias	Selection bias		Performance bias		Detection bias		Attrition bias		Reporting bias	
	Risk	**Text where it is located**	**Risk**	**Text where it is located**	**Risk**	**Text where it is located**	**Risk**	**Text where it is located**	**Risk**	**Text where it is located**
Daley et al. (2018)	Low	"At the first session, participants were randomly assigned (based on a computer-generated code)…"	High	"It was not feasible to mask participants or researchers to group allocation."	Unc.	"…higher self-reports of activity in the physical activity group compared with the control group may be biased by knowledge of allocation."	Low	"When using an intention to treat approach it is acceptable to exclude patients' data, without risking bias…"	Low	The study protocol is available and all of the study's pre-specified (primary and secondary) outcomes that are of interest in the review have been reported.
Vargas-Terrones et al. (2019)	Low	"A simple randomization was performed with the Epidat V.3.1 program to allocate the participants into two groups in order of entry: intervention group (IG) and control group (CG)."	High	The nature of the intervention prevented the study from blinding participants.	Low	"Participants were not involved in the design, recruitment, and conduct of the study."	Low	"An analysis by intention-to-treat was also performed using two different methods."	Low	The study protocol is available and all of the study's pre-specified (primary and secondary) outcomes that are of interest in the review have been reported.
Haakstad et al. (2016)	Low	"Allocations were sealed in opaque numbered envelopes following a simple computer-based randomization program."	High	The nature of the intervention prevented the study from blinding participants.	Low	"In order to treat the two groups identically apart from for the experimental intervention, the controls underwent all tests and completed the same interview as the exercise group, also with respect to assessment of total physical activity level and exercise habits."	Low	"The principal analysis was done on an intention to treat basis…"	Low	The study protocol is available and all of the study's pre-specified (primary and secondary) outcomes that are of interest in the review have been reported.
Perales et al. (2016)	Low	"Thereafter, they were randomly assigned to a standard care (control, initial $n = 121$) or intervention group (exercise, $n = 120$) using a computer-generated list of random Numbers."	High	"The study participants and the qualified fitness instructors who supervised the exercise sessions were not blinded to the group allocation."	Low	"The researchers responsible for assessing eligibility, baseline measures, or outcome assessment were blinded to the group allocation.	Low	"All the analyses were performed using the Statistical Package for Social Sciences program version 22.0, and were adhered to the intention-to-treat principle…"	Low	The study protocol is available and all of the study's pre-specified (primary and secondary) outcomes that are of interest in the review have been reported.
Uebelacker et al. (2016)	Low	"Once we received clearance, we re-contacted the participant and randomized her to the prenatal yoga program (PYP) or a perinatal health education control condition."	High	"Because this is a study of behavioral interventions, participants could not be blind to which intervention they received."	Low	"Study groups did not differ on any variables."	Low	No missing data.	High	Cannot locate protocol.

(Continued)

TABLE 2 | Continued

Articles/Bias	Selection bias		Performance bias		Detection bias		Attrition bias		Reporting bias	
	Risk	**Text where it is located**	**Risk**	**Text where it is located**	**Risk**	**Text where it is located**	**Risk**	**Text where it is located**	**Risk**	**Text where it is located**
Davis et al. (2015)	Low	"Participants in both conditions completed a clinical interview and baseline self-report questionnaires prior to randomization."	Unc.	"Interrater reliability for the Yoga Adherence Scale was 98% for the four classes that both research assistants evaluated."	Unc.	There is no evidence about blinding of outcomes, but everything suggests that it is done.	Low	No missing data.	High	Cannot locate protocol.
Perales et al. (2015)	Low	"A computer-generated list of random numbers was used to allocate the participants into the groups."	Unc.	"The randomization blinded process (sequence generation, allocation concealment, and implementation) was performed by three different authors."	Unc.	There is no evidence about blinding of outcomes, but everything suggests that it is done.	Low	No missing data.	Low	The study protocol is available and all of the study's pre-specified (primary and secondary) outcomes that are of interest in the review have been reported.
Ussher et al. (2015)	Low	"An independent statistician generated a randomization list using Stata, with random permuted blocks of random size stratified by recruitment cente, in a 1:1 ratio."	High	"It was not feasible to mask participants or researchers to group allocation."	Unc.	There is no evidence about blinding of outcomes, but everything suggests that it is done.	Low	"Analysis was on an intention to treat basis…"	Low	The study protocol is available and all of the study's pre-specified (primary and secondary) outcomes that are of interest in the review have been reported.
Perales et al. (2014)	Low	"For allocation of the participants, a computer-generated list of random numbers was used."	High	"…due type of intervention blinding of participants was not possible."	Low	"Randomization process (sequence generation, allocation concealment, and implementation) was made for three different authors in order to facilitate blinding of process and outcomes assessment."	Low	No missing data.	Low	The study protocol is available and all of the study's pre-specified (primary and secondary) outcomes that are of interest in the review have been reported.
Field et al. (2013a)	Low	"…the depressed pregnant women were randomly assigned to a yoga or a social support group based on a random numbers table."	Unc.	"…by trained researchers who were blinded to the group assignment and the study hypotheses.	Low	"The groups did not differ on demographic variables and baseline measures.	Low	No missing data.	High	Cannot locate protocol.
Field et al. (2013b)	Low	"The participants were clinically depressed pregnant women who were randomly assigned to either a tai chi/yoga treatment or a control group."	Unc.	The nature of the intervention prevented the study from blinding participants.	Unc.	There is no evidence about blinding of outcomes, but everything suggests that it is done.	Low	No missing data.	High	Cannot locate protocol.

(Continued)

TABLE 2 | Continued

Articles/Bias	Selection bias		Performance bias		Detection bias		Attrition bias		Reporting bias	
	Risk	**Text where it is located**	**Risk**	**Text where it is located**	**Risk**	**Text where it is located**	**Risk**	**Text where it is located**	**Risk**	**Text where it is located**
Satyapriya et al. (2013)	Low	"…the subjects were allocated to two groups (yoga and control) using a computer generated random number…"	Unc.	As this was an interventional study, the participants or the trainer could not be blinded. Attempts were made to mask wherever feasible to reduce the bias.	Low	"The team who did the assessments was not involved in administering the intervention. The statistician who did the randomization and analysis was blind to the source of the data."	Low	No missing data.	High	Cannot locate protocol.
Field et al. (2012)	Low	"The women were then randomly assigned to a yoga, massage therapy or standard prenatal care control group."	Unc.	"All assessments were conducted by the trained research associates who were blind to the study' hypotheses and to the group assignment."	Low	"In addition, the yoga and massage therapy groups did not differ on neonatal outcomes including gestational age and birthweight."	High	There are missing data (more than 10% of sample).	High	Cannot locate protocol.
Robledo-Colonia et al. (2012)	Low	"Randomization was performed using a permuted block design with a block si e of 10 and exp:con ratios of 5:5, 6:4 or 4:6."	High	"Participants and therapists administering the intervention were not blinded."	Low	"The investigators responsible for outcome assessment were blinded to group allocation."	Low	"Analysis was according to the principle of intention-to-treat."	Unc.	The variables don't coincide in the protocol and in the article methodology.
Mosquera-Valderrama et al. (2012)	Low	"Después de la realización de estas pruebas, las pacientes fueron asignadas aleatoriamente."	High	"La principal limitación es que los terapeutas de campo y las participantes no pueden ser cegados a las intervenciones con el entrenamiento físico aeróbico."	Unc.	There is no evidence about blinding of outcomes, but everything suggests that it is done.	Low	"…destacamos la validez de los hallazgos debido al diseño del estudio, que incorpora varias características que minimizan la posibilidad de sesgo en los resultados, tales como la aleatorización, y el análisis de intención de tratamiento."	Unc.	The variables don't coincide in the protocol and in the article methodology.

Unc., unclear.

the number and percentage of pregnant women considered depressed. Overall, this result confirms the positive impact of a supervised exercise program during pregnancy on the emotional state of pregnant women.

It is interesting to analyze what factors present in exercise programs can be associated with the emotional response of pregnant women and determine an improvement in emotional state. In this sense, we find that both the supervision of exercise by a professional and the adherence of the participants to the program were relevant factors to increase the positive impact of exercise on improving emotional state. This positive association between exercise and the prevention of maternal prenatal depression has been demonstrated by some studies with supervised exercise programs and high adherence of the participants (Field et al., 2012; Perales et al., 2014, 2015, 2016; Ussher et al., 2015; Haakstad et al., 2016; Uebelacker et al., 2016; Vargas-Terrones et al., 2019).

The other important aspects seem to include the type of exercise and the activities implemented in the sessions. Thus, some studies include a combined practice with mixed activities (aerobic resistance, muscle strengthening, coordination, pelvic floor, flexibility) and show good results (Mosquera-Valderrama et al., 2012; Robledo-Colonia et al., 2012; Perales et al., 2014, 2015, 2016; Haakstad et al., 2016; Vargas-Terrones et al., 2019). Additionally, there was a reduction in prenatal depression in studies that used yoga (Davis et al., 2015) or tai-chi (Field et al., 2013a) as an intervention program; however, it is important to highlight that there is no absolute consensus as to which type of exercise reduces these symptoms the most.

In a recent RS-MA, Davenport et al. (2018) found a positive influence of supervised exercise on the reduction of depressive symptoms during pregnancy. Similarly, there are other recent systematic reviews that conclude that supervised exercise with low-to-moderate intensity is a preventive measure against postpartum depression as well as gestational depression (Shivakumar et al., 2011; Daley et al., 2015; Gong et al., 2015).

As it is been shown that women who were inactive during pregnancy were 16% more likely to suffer prenatal depression, future studies will focus on comparing the effect of antidepressants during pregnancy with exercise during the same period. However, more studies examining the effect of exercise during pregnancy are needed to contribute to understanding our research question.

The limitations of this study were the scarcity of scientific literature about exercise and prenatal depression because there are more papers that study postnatal depression than prenatal depression, the lack of consensus on the depression scales used (there were four different scales with four different measured scores), and the variability of the exercise programs performed in all studies. The results of the present study demonstrate that aerobic and supervised exercise during pregnancy could be a tool to prevent depressive symptoms as well as diagnosed prenatal depression.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

MS-P was responsible for the design of the review study, contributed to the data selection and analysis, and the preparation of the manuscript. EF led data selection and analysis and contributed to manuscript writing. CS-J and JG-A contributed to the data selection and analysis. JP-T contributed to the data selection and analysis and the preparation of the manuscript. RB contributed to the design of the study and the manuscript writing. IR was mainly responsible for the design of the review study and the data analysis and led the manuscript writing. All authors contributed to the article and approved the submitted version.

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Strength Performance Across the Oral Contraceptive Cycle of Team Sport Athletes: A Cross-Sectional Study

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Oral contraceptive pills (OCP) are very popular in female athletes not only for contraceptive effects but also due to the possibility of cycle manipulation. Moreover, it is debatable whether the manipulation of the menstrual cycle has a beneficial effect on exercise performance. Therefore, the aim of this study was to investigate potential differences in knee-extensor and flexor strength performance of first division team sport athletes between phases of the oral contraceptive cycle. Sixteen female handball players (age: 23.3 ± 3.1 years; body mass: 67.0 ± 8.52 kg; body stature: 1.68 ± 0.05 m) using a monophasic OCP participated in strength performance tests, once during OCP consumption (CONS) and once during withdrawal (WITH). Tests were performed on a dynamometer to measure knee-extensor and flexor maximal voluntary isokinetic and isometric torque. Prior to each test, body mass was assessed, and venous blood samples were collected. Wilcoxon signed-rank test and magnitude-based inferences have been conducted to analyze differences between WITH and CONS. Significance was accepted at $P < 0.05$. No significant differences between oral contraceptive cycle phases of knee-extensor and flexor strength parameters and body mass have been indicated (all at $P > 0.05$). Follicle-stimulating hormone (FSH) ($P = 0.001$) and luteinizing hormone ($P = 0.013$) were significantly higher in WITH, whereby estradiol and progesterone showed no significant difference between phases (both at $P > 0.05$). These results support the notion that knee-extensor and flexor isokinetic and isometric strength performance does not differ between phases of oral contraceptive cycle in well-trained team sport athletes. OCP intake is suggested to cause a stable but downregulated hormone cycle, which has no effect on knee-extensor and flexor strength when comparing oral contraceptive cycle phases. Therefore, manipulation of the female cycle using OCP in order to achieve a higher knee-extensor and flexor strength performance does not seem to be justified; however, it is currently unclear if cycle manipulation might affect other physiological systems.

Keywords: hormone pill, withdrawal bleeding, athletes, maximal voluntary force, hormonal contraceptive, female strength, exercise performance

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INTRODUCTION

Female athletes are often neglected in exercise physiological research; still, a growing body of literature recognizes the importance of research on the effects of the female cycle (Oleka, 2020). Approximately half of female elite athletes are using hormonal contraceptives, and the most commonly used type is the combined oral contraceptive pill (OCP) containing estrogen and progesterone (Prog) (Martin et al., 2018). OCP intake is popular, as it allows manipulation of the female cycle besides its contraceptive effect. The female cycle might be manipulated by extending the phase of pill intake and/or shortening the phase of withdrawal (Sulak et al., 2002). Cycle manipulation allows to control the timing of the withdrawal bleeding and potential painful side effects of menses.

The combination of estrogen and Prog in the combined OCP enables prevention of pregnancy and control of menstrual bleeding (Cooper and Mahdy, 2020). Its application is divided into two phases: 21 days of pill consumption (CONS) and 7 days without pill consumption, when withdrawal bleeding is induced (WITH) (Sims and Heather, 2018). The intake of exogenous estrogen and Prog through combined OCPs leads to changes in the cervix and a constantly low level of endogenous estrogen (E2) and Prog (Cooper and Mahdy, 2020). In detail, exogenous Prog leads to a lower level of gonadotropin-releasing hormone and consequently a minor secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). This effect inhibits the follicle development and suppresses the secretion of E2. When taking OCPs, exogenous estrogen supports this hormonal downregulation by slowing down FSH secretion (Cooper and Mahdy, 2020).

Interestingly, in physically active and competitive females, the manipulation of female cycle using OCP is widespread (Sulak et al., 2002). In elite athletes using OCPs, 72.6% reported to have their menstrual cycle manipulated at least once in the previous year. However, it is still questionable whether OCP-induced cycle manipulation is justified when it comes to maximizing athletic performance. Moreover, it is not completely clear whether consumption of exogenous hormones might affect strength performance when comparing different phases of the OCP cycle.

Previous studies assessing the effects of OCPs on exercise performance are controversial. On the one hand, it has been investigated that differences in strength performance through oral contraceptive cycle are unlikely (Elliott et al., 2005; Ekenros et al., 2013; Elliott-Sale et al., 2020). This is due to the low and stable concentrations of E2 and Prog. This might result in a constant strength performance through OCP cycles (McNulty et al., 2020). On the other hand, previous work suggests a potential benefit on sporadic cycle manipulation (Schaumberg et al., 2018). A previous study in OCP users found that drop jump performance was significantly lower in the late withdrawal phase. However, other jumping and sprinting tests of the same study did not support this notion (Rechichi and Dawson, 2009). Further, different types of exercises are suggested to bias the results and lead to controversial findings (Elliott-Sale et al., 2020). Therefore, an objective of this study was to investigate isolated thigh strength parameters using a dynamometer to reduce a potential bias of complex test performances and requirements.

Most studies investigated heterogeneously trained samples (Ekenros et al., 2013), untrained participants (Drake et al., 2003; Elliott et al., 2005; Mackay et al., 2019), or moderately trained participants (Lebrun et al., 2003; Bell et al., 2011). To provide a useful guidance, this study aims to provide new insights into a homogenously trained sample of first division team sport athletes using OCP during the general preparatory phase. Therefore, the purpose of this study was to narrow this research gap by assessing knee-extensor and flexor strength performance in team sport athletes by comparing strength performance across phases of OCP intake and withdrawal. We hypothesized non-significant differences in knee-extensor and flexor strength parameters between OCP cycles.

MATERIALS AND METHODS

Participants

A sample of 16 female team sport athletes (age: 23.3 ± 3.1 years; body mass: 67.0 ± 8.5 kg in withdrawal phase; body stature: 1.68 ± 0.05 m) were recruited for this study. Participants were not conducting additional resistance training nor basic endurance sessions besides their habitual handball-specific training sessions. Inclusion criteria were (a) to play in the first Austrian handball league, (b) training at least three times a week, (c) a handball-specific training history of more than 3 years, and (d) using a commercially available low- to middle-dose monophasic OCP for at least half a year according to the instructions of the package insert. Monophasic OCPs contained 0.020–0.035 mg of ethinylestradiol (exogenous estrogen) and 0.10–2.00 mg of gestodene (exogenous Prog) (see **Supplementary Material**). The administration of the monophasic OCP consists of 7 days of no treatment (induction of withdrawal bleeding) (WITH) followed by 21 days of pill consumption (CONS). Timing of OCP consumption was not standardized to enable a common, regular daily routine of participants. Further, participants were free from any diseases and injuries and were included in the study only after medical approval.

This study has been approved by the host institutioń s Ethics Committee (#00435) and conformed to the principles of the World Medical Association's Declaration of Helsinki (2013). After being fully informed about all procedures and risks of the study, participants provided written informed consent to participate.

Experimental Approach to the Problem

The present study is designed as a cross-sectional study analyzing knee-extensor and flexor strength performance of female team sport athletes using OCPs. Oral contraceptive cycle phases are usually identified from the first day of withdrawal bleeding to the day preceding the next withdrawal bleeding. To investigate potential effects of oral contraceptive cycle phases (i.e., different concentrations in oral contraceptive cycle hormones) on knee-extensor and flexor strength, participants of this study reported

TABLE 1 | Descriptive data of isometric and isokinetic knee-extensor and flexor parameters comparing OCP withdrawal and consumption (n = 16).

		Isokinetic strength			Isometric strength		
		PT extension (Nm)	**PT flexion (Nm)**	**H–Q ratio (%)**	**PT (Nm)**	**PRTD (Nm/s)**	**TTPT (ms)**
Right leg	WITH	147 (130; 169)	108 (97; 126)	74 (69; 77)	192 (158; 210)	936 (767; 1,445)	410 (387; 465)
	CONS	148 (130; 180)	105 (92; 117)	72 (68; 79)	188 (160; 198)	1,218 (858; 1,481)	414 (351; 468)
	z	–0.08	–0.78	–0.39	–0.62	–1.45	–0.48
	P	0.938^{T}	0.438^{T}	0.697^{T}	0.535^{T}	0.148^{S}	0.629^{S}
	% change WITH/trivial/CONS	0.0/99.9/0.0	0.7/86.7/12.6	11.7/80.0/8.3	44.5/46.6/8.9	64.5/34.6/0.9	0.7/94.5/4.8
	Qualitative inference	No menstruation phase favored	Ambiguously favors CONS	Unclear	Unclear	Ambiguously favors WITH	No menstruation phase favored
Left leg	WITH	142 (121; 177)	105 (86; 124)	70 (63; 76)	183 (156; 209)	944 (675; 1,256)	449 (431; 468)
	CONS	150 (117;174)	105 (88; 117)	71 (65;75)	182 (152; 211)	1,314 (782; 1,384)	425 (388; 460)
	z	–0.05	–0.28	–0.10	–0.26	–1.65	–1.17
	P	0.959^{T}	0.776^{T}	0.917^{T}	0.796^{T}	0.100^{S}	0.244^{S}
	% change WITH/trivial/CONS	0.0/100.0/0.0	1.0/98.6/0.3	3.4/83.5/13.0	8.0/88.8/3.2	20.2/79.8/0.0	80.7/12.0/7.3
	Qualitative inference	No menstruation phase favored	No menstruation phase favored	Unclear	Unclear	Ambiguously favors WITH	Unclear

Descriptive data are presented as med (IQR).
WITH, withdrawal; CONS, consumption; PT, peak torque; PRTD, peak rate of torque development; TTPT, time to peak torque; H–Q ratio, hamstrings-quadriceps ratio; T, trivial effect size; S, small effect size; OCP, oral contraceptive pill; IQR, interquartile range.

twice to the laboratory to perform isokinetic and isometric maximal strength tests. Participants were tested a) at day 2 or 3 of the oral contraceptive cycle during withdrawal of OCP intake (WITH) and b) at day 16 or 17 of the oral contraceptive cycle during phase of OCP consumption (CONS), when OCP intake was reinitiated since at least 10 days. The tests were performed at the same time of the day, and the order of lab visits (i.e., WITH vs. CONS) was alternated to avoid potentially negative effects. Athletes were asked to avoid strenuous exercise 24 h prior to each testing session and to arrive at the laboratory in a fed and fully hydrated state. Moreover, they were required to refrain from alcohol the preceding 24 h and from caffeine or sports drink intake the preceding 3 h. Furthermore, subjects had to control their diet 48 h prior to testing and replicate the food intake for the second test. This was verbally verified prior to testing.

TABLE 2 | Descriptive data of lateral deficit in isokinetic measurement comparing OCP withdrawal and consumption (n = 16).

	LD (%)	
	Hamstrings	**Quadriceps**
WITH	11.6 (5.5 14.1)	5.9 (2.3-10.9)
CONS	11.2 (3.5;14.4)	3.7 (8.1; 15.1)
Z	–0.72	–0.93
P	0.469^{T}	0.352^{S}
% change WITH/trivial/CONS	11.5/29.5/50.0	62.8/30.6/6.6
Qualitative inference	Unclear	Unclear

Descriptive data are presented as med (IQR).
WITH, withdrawal, CONS, consumption, LD, lateral deficit; T, trivial effect size; S, small effect; OCP, oral contraceptive pill.

Procedures

Body mass was measured during both visits to examine potential changes in body mass due to oral contraceptive cycle phases. Venous blood samples were collected on both test days in order to analyze for menstrual cycle hormones. The serum gel tube was centrifuged for 10 min at relative centrifugal force of 3,500 × g (Rotina 420R, Hettich, Vienna, Austria) after resting for 30 min. Samples of serum were frozen at –40°C until all samples were collected and were subsequently analyzed in a certified laboratory. Blood samples were collected to analyze for several female cycle hormones (i.e., FSH, LH, E2, and endogenous Prog) using Beckman Coulter Access Immunoassays (Beckman Coulter Inc., Brea, CA, United States). Prior to strength assessment, participants completed a standardized warm-up on a stationary ergometer (Racer 9, Kettler Freizeit GmbH, Ense-Parsit, Germany) for 10 min at a pre-defined workload of 0.75 W/kg body mass. To measure maximal voluntary knee-extensor and flexor strength performance, a dynamometer (ISOMED2000, Ferstl GmbH, Regensburg, Germany) with a sampling rate of 200 Hz was used. Participants were given a sufficient amount of time to familiarize with the device and the specific movements using submaximal contractions (Potzelsberger et al., 2015). Prior to formal data collection, maximal sessions for familiarization purposes were carried out. Unpublished data for maximal contractions from our laboratory for isokinetic and isometric strength on this dynamometer have demonstrated intraclass correlation coefficients of $0.88 < r < 0.99$ and coefficients of variation (CV%) between 8.7 and 19.5%. However, if the difference between the first and second repetitions is greater than the CV%, an additional repetition would have been performed, but this was not required for any participant (n = 0). The device was combined with a unilateral knee attachment in order to

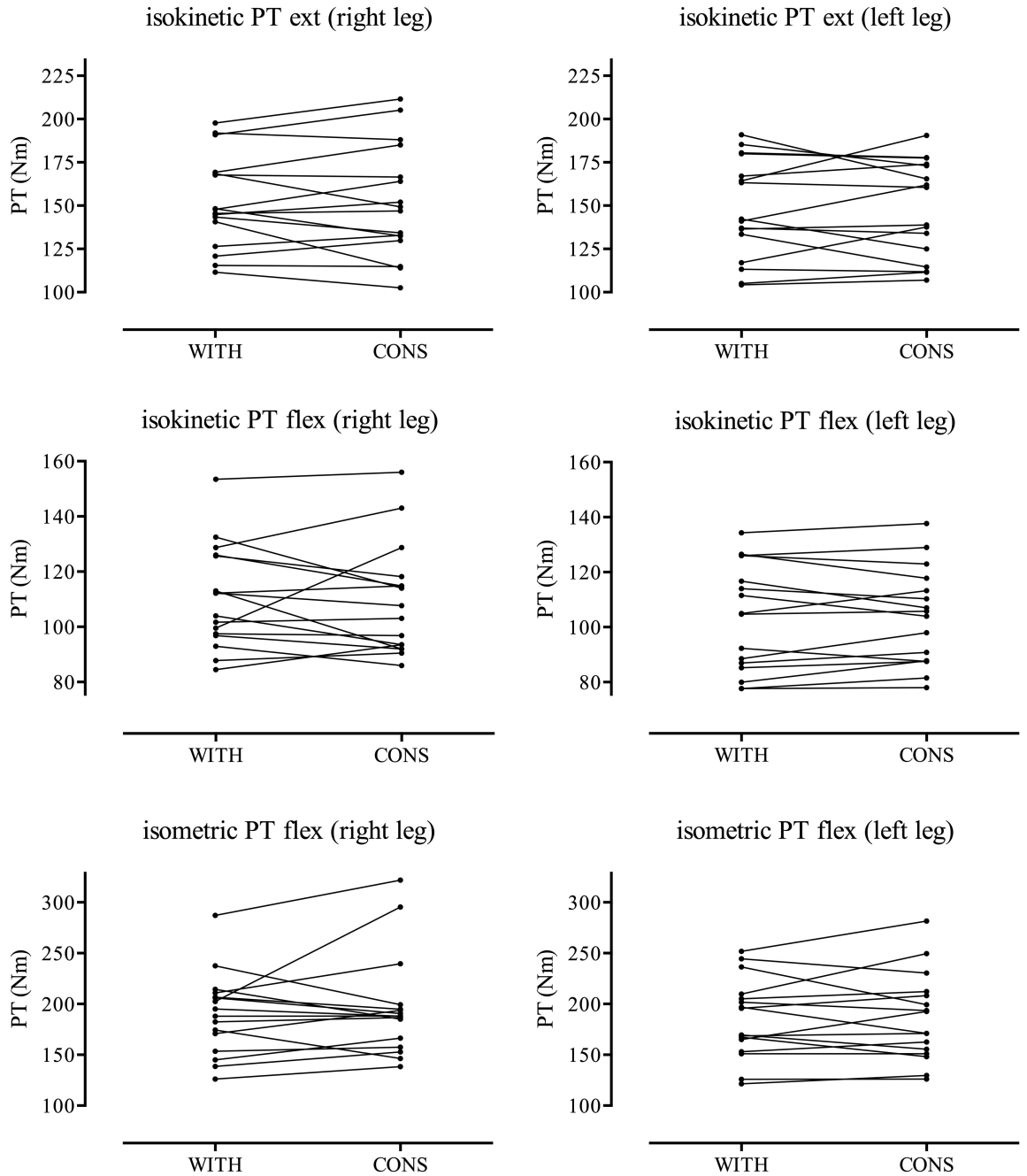

FIGURE 1 | Individual changes of PT for extension and flexion for right and left legs during withdrawal phase (WITH) and consumption phase (CONS).

measure knee-extensor and flexor strength. Maximal isokinetic and isometric torque were assessed in a seated position with the backrest at 75° for right and left legs separately. Participants were instructed to use the handles during contractions, and the straps were applied to minimize movement of the upper body during the test. All settings were recorded and replicated for the second visit.

Isokinetic strength was assessed by two or three identical concentric repetitions of knee joint flexion and extension for each leg separately. Angular velocity was set at 60°/s. The range of motion for the isokinetic tests was between 8° and 90°. A painless range of motion was ensured prior to each test. Passive rest for 3 min was provided between the repetitions. Analyzed parameters for isokinetic measures were (a) peak torque (PT) of extension, (b) PT of flexion, (c) H-Q ratio, and lateral deficit for (d) quadriceps muscles (LD_Q) and (e) hamstring muscles (LD_H). After another passive rest of 3 min, maximal isometric strength for extension was assessed. Therefore, two identical

TABLE 3 | Descriptive data of body mass and blood parameters comparing OCP withdrawal and consumption (n = 16).

	Body mass (kg)	FSH (IU/L)	LH (IU/L)	E2 (pg/ml)	Prog (ng/ml)
WITH	66.7 (59.7; 68.9)	5.65 (1.38; 9.73)	3.30 (0.40; 4.75)	<15	0.25 (0.10; 0.60)
CONS	66.6 (60.5; 69.7)	1.85 (0.25; 3.30)	1.05 (0.20; 2.68)	<15	0.25 (0.09; 0.60)
Z	0.31	–3.31	–2.48	n/a	–0.16
P	0.754^{S}	0.001^{L}	0.013^{L}	n/a	0.875^{S}

Descriptive data are presented as med (IQR).
WITH, withdrawal; CONS, consumption; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, estradiol; Prog, progesterone; S, small effect size; L, large effect size; OCP, oral contraceptive pill.

static repetitions of a 5-s maximal extension at 60° knee joint angle were performed, interspersed by another 3-min passive rest. Analyzed isometric parameters were (f) isometric PT, (g) peak rate of torque development (PRTD), and (h) time to PT (TTPT). The same procedures have been repeated on the other leg interspersed by 5-min passive rest. Temperature and humidity in the air condition-controlled laboratory were between 20 and 22°C, and between 45 and 55%, respectively.

Statistical Analyses

Normality was assessed using the procedures of Shapiro–Wilk. Due to violation of normal distribution, a non-parametric Wilcoxon signed-rank test was applied to analyze differences in knee-extensor and flexor strength parameters and concentrations of cycle hormones between different phases of the oral contraceptive cycle. The effect sizes (r) were calculated by dividing the z-value by the square root of numbers of participants (trivial $r < 0.01$; small $0.01 \leq r < 0.3$; moderate $0.3 \leq r < 0.5$; large $r \geq 0.5$). Magnitude-based inferences (MBIs) were conducted to determine the potential beneficial or harmful effects of OCP intake using a spreadsheet with compatibility limits set at 95%. Cohen effect size of 0.2 was used as the smallest worthwhile change (Hopkins, 2000). Quantitative changes of performance effects are presented through a qualitative descriptor, which has been assigned as follows: 0.5–5%, very unlikely; 5–25%, unlikely; 25–75%, possibly; 75–95%, likely; 95–99.5%, very likely; and > 99.5%, most likely (Batterham and Hopkins, 2006). Descriptive data are reported as median and interquartile range. Significance was accepted at an alpha level of $P < 0.05$. The analyses were conducted using SPSS statistical software package 26 (IBM SPSS Statistics, SPSS Inc., Chicago, IL, United States).

RESULTS

Strength Parameters

Descriptive data, results of the Wilcoxon rank test, and MBI calculations are presented in **Tables 1**, **2**. The individual changes are displayed in **Figure 1**. Isokinetic parameters were unaffected by oral contraceptive cycle phases. PT extension, PT flexion, and H-Q ratio of right and left legs did not indicate significant differences with *trivial* effect sizes between cycle phases (**Table 1**). No significant differences were observed in LD_Q and in LD_H between oral contraceptive cycle phases demonstrating *trivial* to *small* effect sizes (**Table 2**). For isometric parameters, no significant differences between cycle phases have been detected. PT, PRTD, and TTPT right and left legs demonstrated no significant differences with *trivial* to *small* effect sizes (**Table 1**). MBI indicated no favor for any cycle phase, or effects were unclear, despite values for PT flexion right leg (uncertainly favors CONS) and PRTD right and left legs (uncertainly favors WITH).

Hormones and Body Mass

Descriptive data of blood parameters are depicted in **Table 3**. There were significant differences between oral contraceptive cycle phases in FSH and LH, both indicated by a *large* effect size. No significant differences between cycle phases were observed in Prog reflected by a *small* effect size. Concentrations for E2 were below the detection limit of 15 pg/ml in both phases. Furthermore, no significant differences in body mass have been found, and the effect size was *small*.

DISCUSSION

The aim of the present research was to examine potential differences in knee-extensor and flexor strength of female team sport athletes between oral contraceptive cycle phases. This study has not identified any physiologically meaningful differences in parameters reflecting knee-extensor and flexor strength performance between oral contraceptive phases. Another finding was that significantly higher concentrations of FSH and LH were found during OCP withdrawal, but no notable differences for E2 and Prog. The present results support our hypothesis that knee-extensor and flexor strength performance is not influenced by different oral contraceptive cycle phases in first division team sport athletes.

Maximum isokinetic and isometric strength performance in the present study did not significantly differ between oral contraceptive cycle phases with *trivial* to *small* effect sizes for all strength parameters analyzed. Further, these findings are supported by the MBI calculation, which indicated *unclear* effects or *no favor* for any oral contraceptive phase for H–Q ratio, TTPT, LD, and PT flexion left leg. In accordance with previous studies (Lebrun et al., 2003; Elliott et al., 2005; Rechichi and Dawson, 2009; Ekenros et al., 2013; Elliott-Sale et al., 2020), the present results indicate no physiologically meaningful differences between oral contraceptive cycle phases in knee-extensor and flexor strength performance of female athletes using OCPs. Interestingly, an *uncertain favor* was demonstrated for right leg PRTD and PT flexion for CONS and WITH, respectively. An explanation for this uncertain favor in PRTD might be caused by changes in muscle control patterns. Neuromuscular variation through contraceptive cycle has also been a suggested explanation for the differences in reactive strength of various drop jump heights in OCP users (Rechichi and Dawson, 2009). As the favor in the present results is uncertain and additionally no effects have been found in TTPT, we follow the suggestion that there is no physiologically meaningful effect on neuromuscular

function by contraceptive cycle phases. However, it has to be taken into account that TTPT and PRTD are practical and functional parameters of neuromuscular function and of force production capacity, and no measures using the methods of electromyography or electrically stimulated contractions at different torque percentages of maximal voluntary contraction were carried out.

It is common knowledge that female cycle hormones have various physiological effects on athletes (e.g., Rechichi et al., 2009; Thompson et al., 2020). For example, E2 is suggested to cause an anabolic effect on skeletal muscle and lower blood lactate levels (Oosthuyse and Bosch, 2010). In contrast, Prog is suggested to have a synergetic catabolic effect (Oosthuyse and Bosch, 2010; McNulty et al., 2020) and may increase resting heart rate (Sedlak et al., 2012). Unsurprisingly, in the present work, concentrations of female cycle hormones have been low due to the downregulation of OCP. Concentrations of E2 and Prog were downregulated and were similar to OCP users in a previous study (Elliott et al., 2005). Unsurprisingly, E2 was even below the detection limit in some of the participants. Significantly lower concentrations of FSH and LH have been found in CONS compared with WITH, represented by a *large* effect size. It is, however, noteworthy that hormonal concentrations in both phases were apparently low enough to cause a concomitant suppressing effect on E2 and Prog secretions. In short, the analyzed endogenous hormones were merely low and stable; and in knee-extensor and flexor strength performance, no notable changes have been found through oral contraceptive cycle phases. Consequently, these findings confirm prior works that there are no influences of endogenous or exogenous hormones, which differently influence strength performance through an oral contraceptive cycle in elite athletes (Lebrun et al., 2003; Elliott et al., 2005; Rechichi and Dawson, 2009; Ekenros et al., 2013; Elliott-Sale et al., 2020).

For body mass, no significant differences between contraceptive cycle phases and merely a *small* effect size were found. Therefore, no changes in relative knee-extensor and flexor strength performance are evident. Participants of the present study were using OCPs for at least half a year. This finding goes along with several previous studies with participants consuming OCP regularly. Previous studies investigated weight gain and an increase in sum of skinfold only when females started a regular intake of OCPs (Lebrun et al., 2003).

Results of this study are limited to the fact that athletes were not using the same OCP product. This potential bias is alleviated since only athletes using a low- to middle-dose monophasic OCP with comparable concentration of ethinylestradiol and gestodene were recruited. Furthermore, monitoring more than one contraceptive cycle would have provided more detailed results. Due to the training routine and time issues of first division athletes, additional visits to the laboratory did not seem feasible for this study.

In conclusion, the current findings demonstrate no notable difference in knee-extensor and flexor strength performance through oral contraceptive phases. OCPs are a desirable option for many athletes due to the possibility to eliminate unpredictable menstruation and pain associated with eumenorrheic menstrual cycles (Elliott-Sale et al., 2020). Therefore, pain release and feeling more comfortable in competition cycle manipulation might have a beneficial influence on the individuals. From the perspective of isolated knee-extensor and flexor strength, there is no evidence of a benefit in female cycle manipulation in OCP users; however, our findings are limited to the effects of OCP on these muscle groups. Therefore, coaches and practitioners can take an informed decision that female cycle manipulation by using OCP does not seem to be justified when a higher knee-extensor and flexor strength performance is desired.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University of Vienna. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AR conducted testing, analyzed the data, and wrote parts of manuscript. BW conducted statistical analysis and wrote parts of the manuscript. PH conducted testing and recruitment of participants. HT wrote parts of the manuscript and conceptualized the study. CT conceptualized the study and wrote parts of the manuscript. All authors contributed to the article and approved the submitted version.

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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.2021.658994/full#supplementary-material

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frontiers in Physiology

SYSTEMATIC REVIEW
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HIIT'ing or MISS'ing the Optimal Management of Polycystic Ovary Syndrome: A Systematic Review and Meta-Analysis of High- Versus Moderate-Intensity Exercise Prescription

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Introduction: Polycystic Ovary syndrome (PCOS) is a metabolic disorder associated with increased cardiovascular disease risk. Exercise is an effective treatment strategy to manage symptoms and reduce long-term health risk. High-intensity interval training (HIIT) has been suggested as a more efficient exercise mode in PCOS; however, it is not clear whether HIIT is superior to moderate intensity steady state exercise (MISS).

Methods: We synthesized available data through a systematic review and meta-analysis to compare the effectiveness of isolated HIIT and MISS exercise interventions. Our primary outcome measures were cardiorespiratory fitness and insulin resistance, measured using $\dot{V}O_{2max}$ and HOMA-IR respectively.

Results: A total of 16 studies were included. Moderate-quality evidence from 16 studies identified significant improvements in $\dot{V}O_{2max}$ following MISS ($\Delta = 1.081$ ml/kg/min, $p < 0.001$, $n = 194$), but not HIIT ($\Delta = 0.641$ ml/kg/min, $p = 0.128$, $n = 28$). Neither HIIT nor MISS improved HOMA-IR [($\Delta = -0.257$, $p = 0.374$, $n = 60$) and ($\Delta = -0.341$, $p = 0.078$, $n = 159$), respectively].

Discussion: A significant improvement in $\dot{V}O_{2max}$ was evident following MISS, but not HIIT exercise in women with PCOS. This contrasts with previous literature in healthy and clinical cohorts that report superior benefits of HIIT. Therefore, based on available moderate-quality evidence, HIIT exercise does not provide superior outcomes in $\dot{V}O_{2max}$ compared with MISS, although larger high-quality interventions are needed to fully address this. Additional dietary/pharmacological interventions may be required in conjunction with exercise to improve insulin sensitivity.

Keywords: PCOS, exercise, moderate-intensity, high-intensity, insulin resistance, cardiorespiratory fitness, cardiometabolic risk

INTRODUCTION

Polycystic Ovary syndrome (PCOS) is the most common endocrine condition, affecting between 5 and 21% of the premenopausal population (Teede et al., 2010; Azziz et al., 2016; Lizneva et al., 2016), and is the leading cause of anovulatory infertility (Moran et al., 2017). Criteria for diagnosis include 2 or more of: biochemical or clinical hyperandrogenism, irregular or absent menses, and the presence of morphological polycystic ovaries (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). In addition to its reproductive sequelae, PCOS is recognized as a metabolic disorder that increases the prevalence of cardiovascular risk factors including hypertension and type 2 diabetes mellitus (Kakoly et al., 2019), which may increase the likelihood of developing cardiovascular disease (CVD) (Talbott et al., 2004; Teede et al., 2018; Berni et al., 2021). A key alteration in PCOS is insulin resistance (IR), which is central to disease pathogenesis and intrinsic to the condition (Cassar et al., 2016). The intrinsic IR experienced by women with PCOS has the potential to exacerbate or be affected by risk factors such as obesity (Cassar et al., 2016) and hyperandrogenism (Burghen et al., 1980; Diamanti-Kandarakis and Dunaif, 2012).

Management decisions are driven by symptomatic need. Lifestyle and diet modification, and pharmacological interventions are commonly utilized. However, adherence to treatment interventions, including lifestyle and pharmacological methods, is often poor in this population, and has been reported as low as 21% (Hoeger, 2008; Kim et al., 2020; Parker et al., 2020). Exercise, alone and in conjunction with concurrent interventions, has recently been reviewed (dos Santos et al., 2020; Patten et al., 2020). Studies of moderate-intensity steady state (MISS) exercise prescription in PCOS have shown improvements in body composition (Aye et al., 2018; Costa et al., 2018; Kirk et al., 2019; dos Santos et al., 2020), insulin sensitivity (Al-Eisa et al., 2017; Aye et al., 2018; Kirk et al., 2019) and hormonal profile (Al-Eisa et al., 2017; Aye et al., 2018). Thus, international guidelines recommend that individuals with PCOS achieve 150-mins of MISS exercise, or 75-mins of vigorous-intensity activity per week (Teede et al., 2018). However, these PCOS-specific guidelines are based on general population data due to a lack of high-quality controlled trials in this population (Stepto et al., 2019). Consequently, the optimum exercise prescription for the management of PCOS is currently unknown.

Emerging data suggest that high-intensity interval exercise (HIIT) may improve cardiometabolic risk factors in individuals with PCOS and may improve exercise adherence (Almenning et al., 2015; Greenwood et al., 2016). However, interpretation of these data is hampered by inconsistency in the interventions utilized, incorporation of diet and/or pharmacological interventions, widely varied modalities, intensities and prescriptions, and small participant numbers. It is therefore challenging to establish the true effects of HIIT on outcomes and thus its role in PCOS management (Stepto et al., 2019). The primary aim of this systematic review and meta-analysis was to establish the impact of both MISS and HIIT exercise interventions on cardiorespiratory fitness and insulin resistance. Our secondary aim was to investigate the influence of both prescriptions on anthropometric and lipid profiles.

METHODS

Protocol and Registration

This meta-analysis was approved and registered with PROSPERO (registration number: CRD42021255461).

Ethical Approval, Search Strategy and Data Extraction

We performed a systematic search of the literature in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (**Figure 1**) of all publications up to 14th April 2021 utilizing the Pubmed, Scopus, EBSCO and ovidMEDLINE databases. Search terms were modified when required for the purpose of each database and consisted of the terms *Polycystic Ovary syndrome, exercise, fitness, insulin, body mass index and hyperandrogenism* (**Supplementary Material A**). Restrictions on search limits where possible included research in humans, females and studies written in the English language. Following the removal of all duplicates, two reviewers (CTR and RNL) independently screened all identified titles and abstracts, and full texts. Any disagreements throughout this process were discussed and consensus reached by a third reviewer (VLM). The reference list of all included studies following full-text review were manually screened to identify any other potential studies to include within the analysis.

Two authors (CTR and RNL) completed the extraction of all relevant data from eligible studies. Where reported, anthropometric, lipid profile, cardiometabolic profile, cardiorespiratory fitness data and sample sizes were extracted through the Covidence software (Covidence, RRID:SCR_016484, version 1) into a predesigned form (Microsoft Excel, RRID:SCR_016137, version 16.49). Where a trial produced multiple publications, results were merged and the largest participant number for each outcome was used in the quantitative synthesis. Where data were unclear or unable to be extracted as presented in the manuscript, the authors were contacted via email twice. If no response was received within 14 days of the second email, or raw data was unable to be provided, the study was excluded from the meta-analysis.

Participants, Eligibility and Interventions

Utilizing the Participant, Intervention, Comparison, Outcome and Studies framework, our systematic review consisted of females diagnosed with PCOS through any recognized criteria between the ages of 18–50 years (**Table 1**). Inclusion in the meta-analysis was under the premise that the participants completed an isolated HIIT or MISS exercise intervention that did not include any concurrent treatment, including dietary manipulation, drug interventions or resistance training. Control data were collected from eligible studies that utilized PCOS controls who stated the following: participants were not provided with an exercise intervention; participants were not eligible to participate if they were exercising more than twice per week; were told to maintain their normal lifestyle with no change; and/or continue with standard care offered by their GP. Odds ratios (OR) comparing exercise vs. usual care for primary outcomes were calculated. The British Association for Cardiovascular

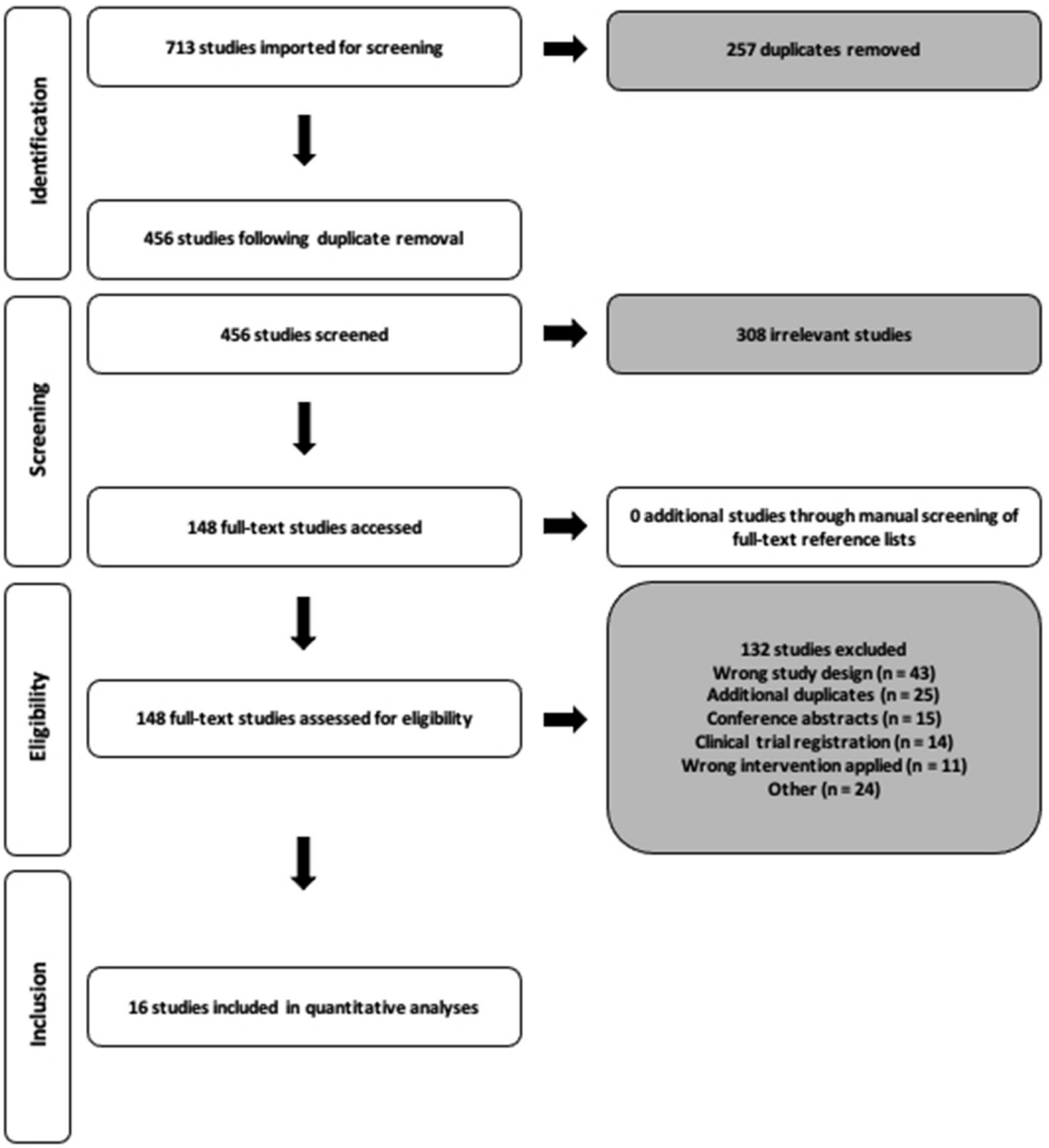

FIGURE 1 | PRISMA study selection flow diagram.

Prevention and Rehabilitation (BACPR, 2019) guidelines were utilized for the categorization of MISS [40–70% $\dot{V}O_{2max}$ /heart rate reserve (HRR) or 60–80% maximal heart rate (HRmax)]. HIIT was defined as exercise that was repetitive and intermittent in nature, with intensities exceeding 90% HRmax, 85%HRR or 85% $\dot{V}O_{2max}$, in accordance with Norton et al. (2010). Exercise interventions that began within one threshold and traversed into another during the intervention period were excluded.

The primary outcomes of the meta-analysis were measures of insulin resistance through homeostatic model assessment of insulin resistance (HOMA-IR) and cardiorespiratory fitness, as measured by relative maximal oxygen consumption ($\dot{V}O_{2max}$). Secondary outcomes included anthropometrics (body mass, body mass index [BMI] and waist circumference), cardiometabolic indices such as lipid profile (high-density lipoprotein cholesterol [HDL-C], low-density lipoprotein cholesterol [LDL-C], total cholesterol [TC] and triglycerides), fasting glucose and fasting insulin. Data on exercise adherence and fidelity was extracted from studies where provided. Adherence was calculated as the number of sessions completed divided by the number of sessions prescribed. Exercise fidelity was reported as a % achievement of target exercise intensity.

TABLE 1 | Population, interventions, comparators, outcomes and study designs framework.

Participant	Intervention	Comparison	Outcome
PCOS diagnosis by any established criteria	Exercise intervention of moderate-intensity exercise (40–70% $\dot{V}O_{2max}$/%HRR or 60–80% HR_{max}) or high-intensity exercise (repetitive bouts of exercise above the maximum threshold of moderate intensity exercise, interspersed by active/rest periods)	HIIT vs. MISS	Primary: Insulin resistance (HOMA-IR) and cardiorespiratory fitness ($\dot{V}O_{2max}$)
Premenopausal women aged 18–50			Secondary: anthropometrics (body mass, BMI and WC), lipid profile (HDL-C, LDL-C, TC and triglycerides), fasting insulin and fasting glucose
No weight restrictions			

PCOS, polycystic ovary syndrome; HRR, heart rate reserve; HR_{max}, maximal heart rate; HIIT, high-intensity interval training; MISS, moderate-intensity interval training; BMI, body mass index; WC, waist circumference; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol.

Data Analysis

The mean ± standard deviation (SD) and sample size were input for each variable where provided. Where standard error of the mean (SEM) was presented, SD was calculated by:

$$\text{SEM x } \sqrt{\text{N}}.$$

where 95% confidence intervals (CI) were presented, the SD was calculated by:

$$\sqrt{\text{N}} \text{ x (Upper limit of CI } \quad \text{Lower limit of CI)}/3.92$$

All outcome variables were input into the analysis software (Comprehensive Meta-analysis software (V.2.0), Biostat, Englewood, NJ, USA). To establish the effect of exercise in PCOS compared with usual care non-exercising PCOS controls, random effects OR were calculated on primary outcomes for HIIT and MISS exercise interventions, and reported as [OR (95% CI = lower [lwr.] to upper [upp.]), p = x]. Random-effects meta-analyses were run on each individual outcome variable in order to account for heterogeneity within the sample. The random-effects model provides a buffer for the individual variation that is inevitable between studies due to effect sizes and sample variation, and allows for a more comparable estimate of the true effect. Using the DerSimonian and Laird (1986) method, weighted means (Δ), standard error (SE), variance, and 95% confidence intervals (CI; lwr. to upp.) were calculated for each outcome variable and reported as [Δ, (95% CI = lwr. to upp.), p = x]. Forest plots of the standard difference in means ± 95% confidence intervals were created for each individual meta-analysis. Analyses were grouped to allow comparisons between the impact of HIIT exercise vs. MISS exercise interventions on each individual outcome variable.

Risk of Bias and Quality of Evidence Assessment

Publication bias was assessed through funnel plots on primary outcomes and was reported for grouped analyses. The weighted sum of squared differences between individual study effects and the pooled effect across the studies (Q), and the percentage of variation across the studies due to heterogeneity (I^2) was reported as (Q, I^2, p = x).To assess the quality and validity of the included studies, the Tool for the assEssment of STudy quality and reporting in EXercise (TESTEX) was utilized (Smart et al., 2015). This scale utilizes a points system of a maximum of 15 points awarded for quality and reporting, and is specialized for the use in exercise interventions. Studies scored between 0–5 were classified as low-quality evidence, between 6–10 as moderate-quality evidence, and 11–15 as high-quality evidence. Two authors (CTR and RNL) independently assessed study quality using the TESTEX checklist, and any conflicts were resolved by a third reviewer (VLM).

RESULTS

Search Outcomes

The systematic search of the literature returned 713 studies (**Figure 1**). Following title/abstract and full-text screening, 16 studies were included within the final analysis. For the included 16 studies, the total sample size for our primary outcomes of HOMA-IR and $\dot{V}O_{2max}$ was 219 (HIIT = 60; MISS = 159) and 222 (HIIT = 28; MISS = 194), respectively. The number of studies included in the individual analyses varied due to inconsistency of reported outcomes across the literature. Study quality score and characteristics, including intervention exercise prescription, adherence and fidelity are reported in **Table 2**.

High-Intensity Exercise Interventions

A total of five publications utilized HIIT as their method of intervention. Intervention duration ranged from 8 to 24 weeks (14.0 ± 6.3) with a session frequency of 3 sessions per week. Exercise modality varied between cycle ergometer (Roessler et al., 2013) treadmill walking/running and outdoor walking/running (Roessler et al., 2013; Faryadian et al., 2019; Ribeiro et al., 2020). Two studies (Almenning et al., 2015; Benham et al., 2021) allowed for participants to select their desired aerobic equipment to complete the exercise. Two studies reported partial supervision of the exercise intervention (at least 1 session supervised)

TABLE 2 | Study characteristics and quality assessment scores.

Author	Participant characteristics (Age [years] and BMI [kg/m^2])	Quality assessment score	PCOS diagnostic criteria	Exercise intervention	Modality	Duration of intervention	Session frequency	Exercise intensity	Session duration	Supervised	Adherence	Fidelity
Al-Eisa et al. (2017)	Age = 27.9 ± 4.1:BMI = 33.5 ± 2.8	5	Rotterdam	MISS	Treadmill walking	12 weeks	3× p/w	65–75% HRR	45 mins	Yes		
Almenning et al. (2015)	BMI = 23.8 ± 4.8	11	Rotterdam	HIIT	Treadmill or outdoor walking/running and/or cycling (self-selected)	10 weeks	3× p/w	2× (90–95% HR_{max}) 1× "maximal intensity"	2× ∼40 mins 1× ∼35 mins	Partial	80%	
Aye et al. (2018)	Age = 28.3 ± 6.5:BMI = 29.4 ± 25.5	7	Rotterdam	MISS	Treadmill	8 weeks	3× p/w	60% $\dot{V}O_{2max}$	60 mins	Yes		
Benham et al. (2021)	Age = 29.1 ± 4.1:BMI = 31.4 ± 8.6	9	Rotterdam	HIIT	Aerobic exercise equipment of choice	24 weeks	3× p/w	10× (30secs:90secs) @ 90% HRR or 9/10 Borg rating	∼ 20–25 mins	Partial	81%	65% (51%, 85%)*
	Age = 29.5 ± 4.6:BMI = 31.2 ± 9.0			MISS	Aerobic exercise equipment of choice	24 weeks	3× p/w	50–60% HRR or 4–6/10 Borg rating	40 mins	Partial	79%	81% (56%, 85%)*
Covington et al. (2016)	Age = 25.6 ± 3.1:BMI = 32.1 ± 5.2	6	Rotterdam	MISS	Treadmill walking/running	16 weeks	5× p/w	55% $\dot{V}O_{2max}$	∼ 23 mins (W1-4) → ∼ 58 mins (W13-16)	Yes		
Faryadian et al. (2019)	Age = 34.3 ± 4.7:BMI = 21.2 ± 1.7	4	Rotterdam	HIIT	Running	12 weeks	3× p/w	2× [4× (4mins:3mins) @ 90–95% HR_{max}] 1× [10× (1min:1min) @ "maximal intensity"]	2× ∼35–40 mins 1× ∼30–35 mins			
Giallauria et al. (2008)	Age = 22.8 ± 3.7:BMI = 29.2 ± 2.9	9	Rotterdam	MISS	Cycle ergometer	12 weeks	3× p/w	60–70% $\dot{V}O_{2max}$	40 mins	Yes	100%	67%
Jedel et al. (2011)	Age = 30.2 ± 4.7:BMI = 27.7 ± 6.4	7	Rotterdam	MISS	Self selected (walking/cycling/ aerobic exercise)	16 weeks	3× p/w	HR above 120bpm	∼ 30 mins	No	73%	

(Continued)

TABLE 2 | Continued

Author	Participant characteristics (Age [years] and BMI [kg/m^2])	Quality assessment score	PCOS diagnostic criteria	Exercise intervention	Modality	Duration of intervention	Session frequency	Exercise intensity	Session duration	Supervised	Adherence	Fidelity
Kirthika et al. (2019)		6	Rotterdam	MISS	Treadmill	12 weeks	3× p/w	6km/h	45 mins	Yes	93%	
Orio et al. (2016)	Age = 25.9 ± 2.7:BMI = 26.7 ± 2.8	8	Rotterdam	MISS	Cycle ergometer	24 weeks	3× p/w	60–70% $\dot{V}O_{2max}$	45 mins	Yes	78%	
Randeva et al. (2002)	Age = 29.7 ± 6.8:BMI = 34.0 ± 4.5	7	NIH	MISS	Walking	24 weeks	∼ 3× p/w	Above 120bpm	∼20–60 mins	No		
Ribeiro et al. (2020)	Age = 29.0 ± 4.3:BMI = 28.7 ± 4.8	6	Rotterdam	HIIT	Treadmill	16 weeks	3× p/w	70% HR_{max} → 85–90% HR_{max}	30 mins → 50 mins	Yes	83%	97%
	Age = 29.1 ± 5.3:BMI = 28.4 ± 5.6			MISS	Treadmill	16 weeks	3× p/w	65% HR_{max} → 75–80% HR_{max}	30 mins → 50 mins	Yes	76%	85%
Roessler et al. (2013)	Age = 31.0 ± 4.9:BMI = 36.7 ± 4.7	7	Rotterdam	HIIT	Cycle ergometer and walking/running	8 weeks (2 week ramp)	3× p/w	(Cycle) 20s−3 mins work @ 80–100% HR_{max} : 25s−3 mins rest @ 45–65% HR_{max} (Walking/Running) 3–5 mins work @ 80–90% HR_{max} : 1 min rest @ 50–60% HR_{max}	55 mins		82%	67%
Sprung et al. (2013)	Age = 28.0 ± 4.8:BMI = 33.0 ± 3.2	6	Rotterdam	MISS	NA	16 weeks	3× p/w → 5× p/w	30% HRR → 60% HRR	30 mins → 45 mins	Yes	100%	91%
Tiwari et al. (2019)	Age = 24.5 ± 4.8:BMI = 26.3 ± 3.7	9	Rotterdam	MISS	Marching	24 weeks	3× p/w	HR above 120 bpm	30 mins	Partial		
Wu et al. (2021)	Age = 32.7 ± 3.2:BMI = 23.8 ± 3.0	6	Rotterdam	MISS	Cycle ergometer	12 weeks	4× p/w	Individualized $\dot{V}O_{2AT}$	60 mins	Yes		

** Data were reported as median and IQR.*
HR, heart rate; HRR, heart rate reserve.
Progression throughout the intervention is depicted by → .

(Almenning et al., 2015; Benham et al., 2021), one reported full supervision (Ribeiro et al., 2020) and two studies did not report supervision status (Roessler et al., 2013; Faryadian et al., 2019). Exercise intensity was prescribed using HR_{max} in four studies (Roessler et al., 2013; Almenning et al., 2015; Faryadian et al., 2019; Ribeiro et al., 2020), and HRR was utilized in a single study (Benham et al., 2021). Session duration ranged between 20 and 55 mins. Adherence to HIIT across these studies was 82 ± 1% and exercise fidelity was 82 ± 21%.

Moderate-Intensity Exercise Interventions

A total of 12 publications utilized MISS as their method of exercise intervention. Intervention duration ranged from 8 to 24 weeks (16.6 ± 5.6), with session frequency ranging from 3 to 5 sessions per week. Exercise modality across MISS interventions varied, with cycle ergometer (Giallauria et al., 2008; Orio et al., 2016; Wu et al., 2021) and treadmill (Covington et al., 2016; Al-Eisa et al., 2017; Aye et al., 2018; Kirthika et al., 2019; Ribeiro et al., 2020) most frequently utilized. Two studies allowed participants to select their desired modality to complete the exercise (Jedel et al., 2011; Benham et al., 2021), while one reported using marching on the spot (Tiwari et al., 2019) and one did not report modality (Sprung et al., 2013). Eight studies indicated full supervision of the exercise intervention (Giallauria et al., 2008; Sprung et al., 2013; Covington et al., 2016; Orio et al., 2016; Aye et al., 2018; Kirthika et al., 2019; Wu et al., 2021), two studies indicated partial supervision (Tiwari et al., 2019; Benham et al., 2021) and two studies indicated no formal supervision (Randeva et al., 2002; Jedel et al., 2011). Exercise intensity was prescribed using $\%\dot{V}O_{2max}$ in four studies (Giallauria et al., 2008; Covington et al., 2016; Orio et al., 2016; Aye et al., 2018), HRR in three studies (Sprung et al., 2013; Al-Eisa et al., 2017; Benham et al., 2021) and $\%HR_{max}$ in one study (Ribeiro et al., 2020). Two studies utilized a minimum working heart rate of 120 bpm (Randeva et al., 2002; Tiwari et al., 2019), one study using a set treadmill speed of 6 km/h (Kirthika et al., 2019) and one used the individuals $\dot{V}O_2$ achieved at anaerobic threshold ($\dot{V}O_{2AT}$) (Wu et al., 2021). Session duration across the MISS intervention ranged from 20 to 60 mins. Adherence to MISS was 67 ± 9% and exercise fidelity was 86 ± 14%.

Publication Bias

There was significant heterogeneity in overall reported $\dot{V}O_{2max}$ scores (Q = 24.43, I^2 = 59%, p = 0.007) which, when grouped for HIIT (Q = 1.43, I^2 = 0%, p = 0.490) and MISS (Q = 20.22, I^2 = 65%, p = 0.005), was only evident in the MISS studies. There was also significant heterogeneity in overall reported HOMA-IR scores (Q = 23.39, I^2 = 49%, p = 0.025), which was evidenced in only the MISS (Q = 18.71, I^2 = 57%, p = 0.017) studies when grouped for exercise type (HIIT, Q = 4.10, I^2 = 27%, p = 0.251). Analyses were not corrected for publication bias, and are shown below (**Figures 2–5**).

Quality of Evidence

The evidence was rated as moderate-quality. Two studies (Al-Eisa et al., 2017; Faryadian et al., 2019) were of low-quality evidence, 13 studies (Randeva et al., 2002; Giallauria et al., 2008; Jedel et al., 2011; Roessler et al., 2013; Sprung et al., 2013; Covington et al., 2016; Orio et al., 2016; Aye et al., 2018; Kirthika et al., 2019; Tiwari et al., 2019; Ribeiro et al., 2020; Benham et al., 2021; Wu et al., 2021) were of moderate-quality evidence, and a single study (Almenning et al., 2015) was of high-quality evidence.

Meta-Analyses

Odds Ratios

HIIT exercise did not statistically reduce HOMA-IR [1.641, (0.86–3.12), p = 0.131] or increase $\dot{V}O_{2max}$ [1.899, (0.34–10.66),

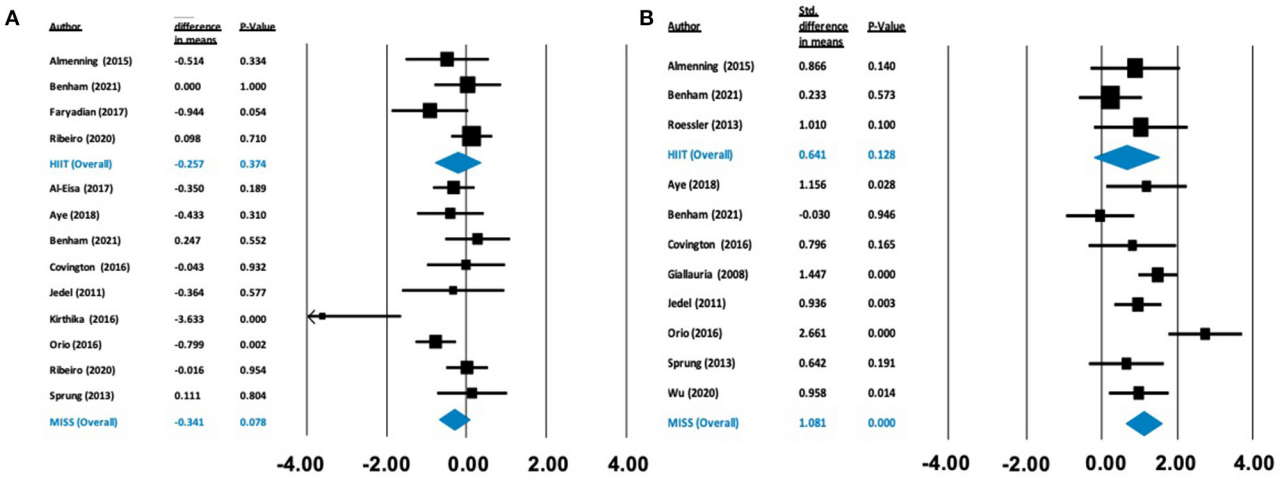

FIGURE 2 | Forest plots of standard difference in means 95% ± confidence intervals for the effect of high-intensity interval training (HIIT) and moderate intensity steady state exercise (MISS) on **(A)** HOMA-IR as a measure of insulin resistance, and **(B)** maximal oxygen consumption (VO_{2max}) as a measure of cardiorespiratory fitness, in polycystic ovary syndrome. Filled squares represent study outputs. Lines represent 95% confidence intervals. Filled blue diamonds represent the weighted mean determined through meta-analyses.

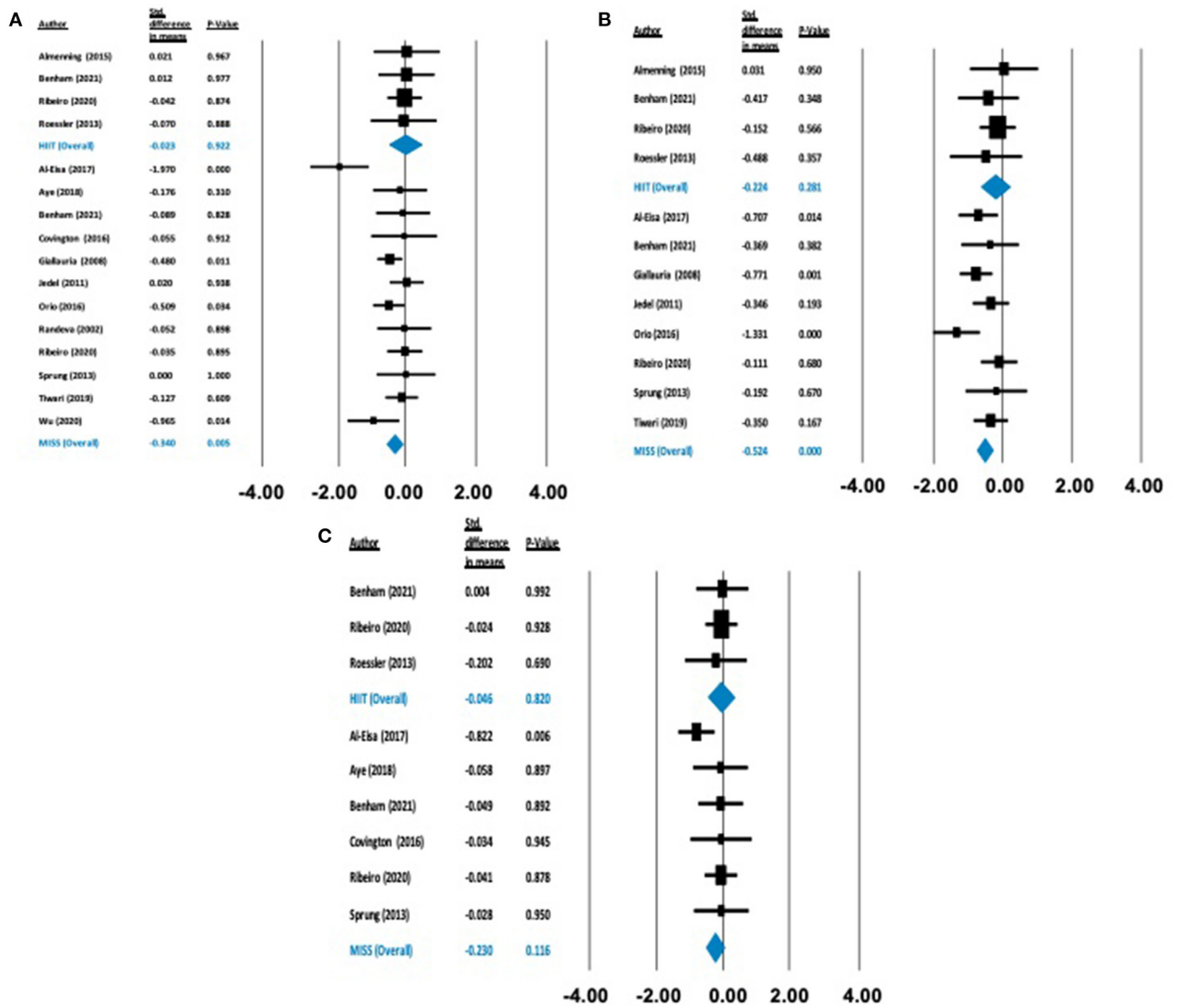

FIGURE 3 | Forest plots of standard difference in means 95% ± confidence intervals for: **(A)** BMI, **(B)** waist circumference, and **(C)** body mass as measures of anthropometric profile. Filled squares represent study outputs. Lines represent 95% confidence intervals. Filled blue diamonds represent the weighted mean determined through meta-analyses.

$p = 0.466$] compared with PCOS controls. Conversely, MISS exercise statistically, significantly reduced HOMA-IR [(1.727, 1.04–2.87), $p = 0.035$] and statistically increased $\dot{V}O_{2max}$ [4.683, (1.92 – 11.43), $p = 0.001$] compared with PCOS controls [**Supplementary Figures B** and **C** (https://doi.org/10.6084/m9.figshare.c.5437518)].

Primary Outcomes

There was no effect on HOMA-IR following HIIT [$\Delta = -0.257$ (-0.822 to 0.309), $p = 0.374$] or MISS exercise [$\Delta = -0.341$ (-0.721 to 0.038), $p = 0.078$]. In contrast, there was a statistically significant increase in $\dot{V}O_{2max}$ following MISS exercise [$\Delta = 1.081$ ml/kg/min (0.624–1.537), $p < 0.001$], but not following HIIT [$\Delta = 0.641$ ml/kg/min (-0.185 to 1.466), $p = 0.128$] (see **Figure 2**).

Anthropometric Outcomes

There was no statistically significant effect on BMI [$\Delta = -0.026$ kg/m^2 (-0.397 to 0.344), $p = 0.890$], body mass [$\Delta = -0.046$ kg (-0.447 to 0.354), $p = 0.820$] or waist circumference [$\Delta = -0.224$ cm (-0.634 to 0.183), $p = 0.281$] following HIIT exercise. Conversely, following MISS exercise, there were statistically significant reductions in BMI [$\Delta = -0.332$ kg/m^2 (-0.505 to -0.160), $p = 0.000$] and waist circumference [$\Delta = -0.524$cm (-0.751 to -0.297), $p = 0.000$]. There was no statistically significant effect of MISS exercise [$\Delta = -0.230$ kg (-0.517 to 0.057), $p = 0.116$] on body mass (**Figure 3**).

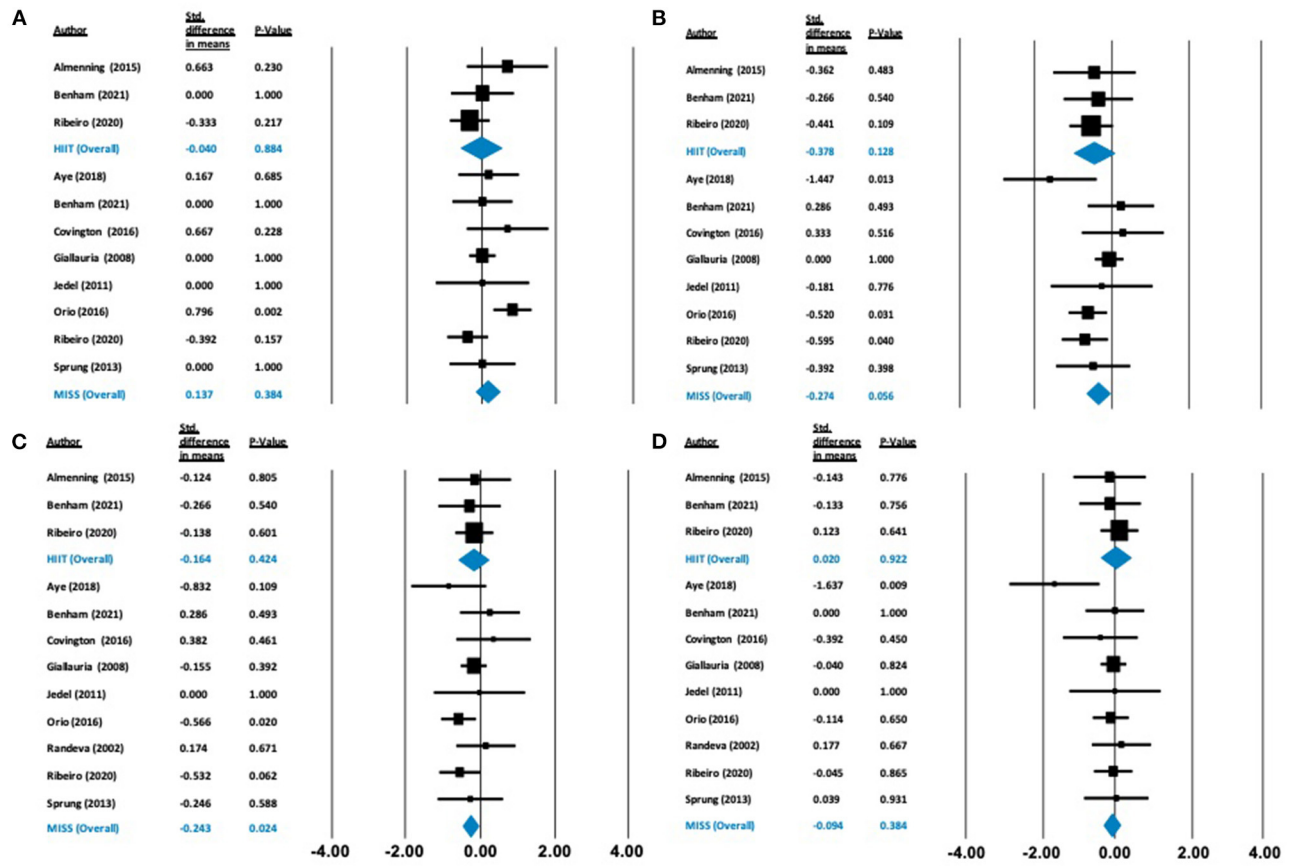

FIGURE 4 | Forest plots of standard difference in means 95% ± confidence intervals for: **(A)** HDL-C, **(B)** LDL-C, **(C)** T-C, and **(D)** triglycerides as measures of lipid profile. Filled squares represent study outputs. Lines represent 95% confidence intervals. Filled blue diamonds represent the weighted mean determined through meta-analyses.

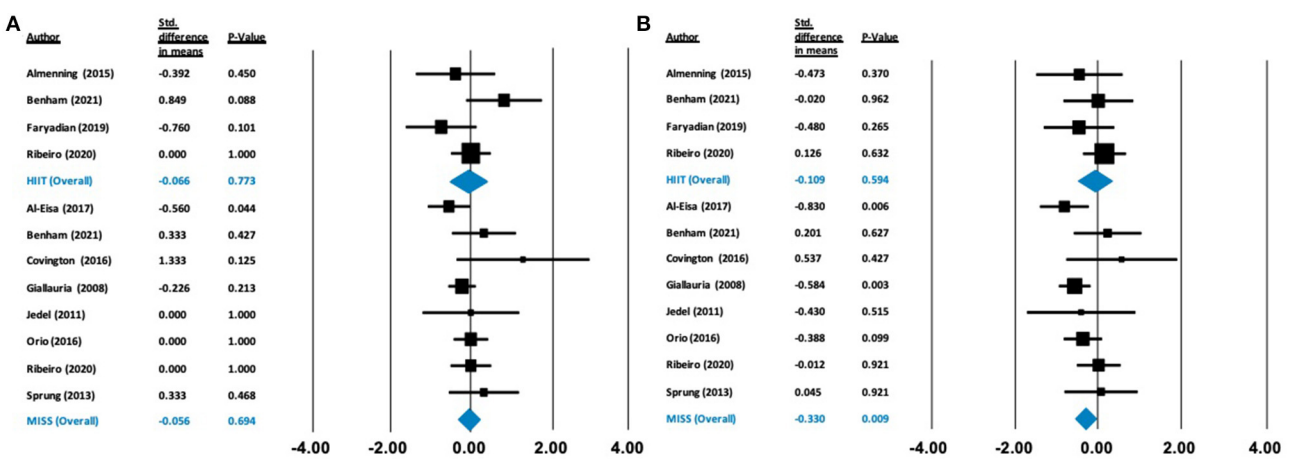

FIGURE 5 | Forest plots of standard difference in means 95% ± confidence intervals for: **(A)** fasting glucose, **(B)** fasting insulin as measures of cardiometabolic profile. Filled squares represent study outputs. Lines represent 95% confidence intervals. Filled blue diamonds represent the weighted mean determined through meta-analyses.

Cardiometabolic Outcomes

Our analysis showed no effect of either HIIT or MISS exercise on HDL-C ([$\Delta = -0.040$ mmol/L (-0.574 to 0.495), $p = 0.884$] and [$\Delta = -0.137$ mmol/L (-0.172 to 0.447), $p = 0.384$], respectively), LDL-C ([$\Delta = -0.378$ mmol/L (-0.864 to 0.108), $p = 0.128$] and [$\Delta = -0.274$ mmol/L (-0.555 to 0.007), $p = 0.056$], respectively) or triglycerides ([$\Delta = 0.020$ mmol/L (-0.382 to 0.422), $p = 0.922$; and $\Delta = -0.094$ mmol/L (-0.304 to 0.117), $p = 0.384$, respectively]) (**Figure 4**). Total cholesterol was not impacted by HIIT [$\Delta = -0.164$ mmol/L (-0.567 to 0.239), $p = 0.424$], but was statistically, significantly reduced following MISS exercise [$\Delta = -0.243$ mmol/L (-0.454 to -0.032), $p = 0.024$]. Fasting glucose remained unchanged following both HIIT [$\Delta = -0.066$ mmol/L (-0.518 to 0.385), $p = 0.773$] and MISS exercise [$\Delta = -0.056$ mmol/L (-0.332 to 0.221), $p = 0.694$] (**Figure 5**). In contrast, there was a statistically significant reduction in fasting insulin following MISS exercise [$\Delta = -0.330$ pmol/L (-0.577 to -0.083), $p = 0.009$] but not HIIT [$\Delta = -0.019$ pmol/L (-0.510 to 0.292), $p = 0.594$] (**Figure 5**).

DISCUSSION

Summary of Main Findings

The aim of this analysis was to determine the effects of an isolated exercise intervention of HIIT or MISS exercise on measures of cardiorespiratory fitness and insulin resistance in individuals with PCOS from previously published data. We also sought to investigate the impact of HIIT and MISS exercise on anthropometric and cardiometabolic indices. The key findings from this analysis are (1) Only MISS exercise interventions improved $\dot{V}O_{2max}$, (2) Neither exercise type improved HOMA-IR, (3) Only MISS exercise improved anthropometric profile, and (4) MISS exercise interventions decreased TC, but neither exercise type had any effect on HDL-C, LDL-C or triglycerides. Based on our analyses of the current moderate-quality evidence, MISS exercise appears to be a superior approach in improving cardiorespiratory fitness and BMI in women with PCOS, and should be prescribed as part of the comprehensive package of care for this condition. However, there is not enough high-quality evidence to disregard HIIT as a potential method of management of the condition, and further research is needed to understand the impact of HIIT exercise on outcomes in PCOS.

Insulin Resistance

Insulin resistance is a common feature of PCOS independent of overweight or obesity (Burghen et al., 1980; Dunaif, 1997; Stepto et al., 2013), which can interplay with and exacerbate symptoms of the condition (Teede et al., 2007; Stepto et al., 2013). In our analysis, neither HITT nor MISS significantly improved HOMA-IR. Similar results have been reported recently, with no improvement in HOMA-IR or fasting insulin following 16 weeks of HIIT exercise (Lionett et al., 2021) and equivocal results following MISS exercise (Shele et al., 2020) in individuals with PCOS. However, we did observe a significant reduction in fasting insulin following MISS exercise, which may suggest improved insulin sensitivity, as a reduced amount of insulin is required to act upon a given concentration of glucose in order to maintain normal metabolic homeostasis (Iaccarino et al., 2021). One potential mechanism that may underpin the differences that appear within our analysis is a shift toward more oxidative and insulin-sensitive fiber type (T_1) in the skeletal muscle (Wojtaszewski and Richter, 2006; Fisher et al., 2017). Longer duration, moderate-intensity, aerobic-based exercise, but not HIIT, has been associated with an increased percentage of T_1 fibers (Wilson et al., 2012). Human and rodent studies (Fisher et al., 2017) have suggested that a greater insulin-stimulated glucose uptake in T_1 muscle fibers is related to insulin sensitivity, therefore increased T_1 muscle fibers may improve metabolic health.

A recent review has suggested that exercise volume may also play a pivotal role in controlling insulin sensitivity (Iaccarino et al., 2021). The authors reported that exercise interventions of $\sim$ 170 mins of weekly exercise showed greater improvements in insulin sensitivity than interventions of $\sim$115 mins/week. From our synthesis, the mean weekly exercise MISS interventions was around the 170 minute threshold (164 $\pm$ 59 mins/week), whereas the HIIT interventions did not meet this threshold (124 $\pm$ 31 mins/week). This may explain the improvement shown in fasting insulin following MISS interventions in this cohort compared with HIIT, and may indicate that individuals with PCOS should complete a larger volume of exercise if their aim is improving insulin sensitivity. It is also important to note that improvements in insulin sensitivity can be lost within 4 days of exercise cessation independent of exercise type (Ryan et al., 2020). Therefore, the timing of any post-intervention assessments may also explain the lack of change in HOMA-IR seen in our analysis. In addition, studies included within our analysis used HOMA-IR to measure insulin sensitivity. The euglycaemic–hyperinsulinaemic clamp is the gold standard measure of insulin sensitivity in humans and is more sensitive to small fluctuations in insulin sensitivity compared to HOMA-IR (Muniyappa et al., 2008). This may explain the lack of change in insulin sensitivity following both MISS and HIIT interventions, however few studies report insulin sensitivity using the euglycaemic–hyperinsulinaemic clamp method, and HOMA-IR is commonly used as the clinical measure of insulin sensitivity (Muniyappa et al., 2008).

Cardiorespiratory Fitness

An increase in $\dot{V}O_{2max}$ of 1-MET (equating to an $\sim$3.5 ml/kg/min increase in oxygen consumption) can reduce the risk of CVD related mortality by 15% (Kodama et al., 2009). Our synthesis suggests that MISS exercise significantly improves $\dot{V}O_{2max}$ by $\sim$3 ml/kg/min, equating to an $\sim$11% risk reduction for all-cause mortality (Kodama et al., 2009). MISS exercise also resulted in an increase in $\dot{V}O_{2max}$ four-fold greater than usual care, non-exercising PCOS controls. These increases were evident in relative $\dot{V}O_{2max}$ and are unlikely due to changes in body mass. This therefore likely reflects an improvement in absolute $\dot{V}O_{2max}$ rather than a change in body composition. Surprisingly, these significant improvements were absent following HIIT, despite a mean improvement of $\sim$2.8 ml/kg/min, which would confer similar reductions in mortality risk (Kodama et al., 2009).

Furthermore, differences in $\dot{V}O_{2max}$ outcomes may be attributed to the total volume of exercise stimulus that participants were exposed to. Our analysis showed that MISS interventions were 2.6 weeks longer in duration and included 14 more exercise sessions during the intervention period than those partaking in HIIT exercise interventions. This shorter study duration may have limited the improvements in $\dot{V}O_{2max}$ in the HIIT interventions. In addition, of the HIIT interventions assessed, two studies (Roessler et al., 2013; Benham et al., 2021) reported exercise fidelity of ∼65%. The inability to achieve the desired intensity within these interventions may also explain the lack of significant improvement in the HIIT interventions.

Our results deviate from previous studies where significant improvements in $\dot{V}O_{2max}$ were evident following HIIT interventions in obesity (Chin et al., 2020), cardiometabolic disease (de Nardi et al., 2018; Boff et al., 2019) and PCOS (Lionett et al., 2021). This deviation may be a result of a significant publication bias toward studies reporting increases in $\dot{V}O_{2max}$ in the MISS literature which was not evident within the HIIT literature included in our analyses. In addition, selection bias from participants may be impacting the improvement in $\dot{V}O_{2max}$. The HIIT participants in our analysis had a higher baseline $\dot{V}O_{2max}$ (30.3 ± 4.9 ml/kg/min) compared with the MISS group (26.3 ± 4.6 ml/kg/min). Exercise interventions typically result in the greatest improvement in $\dot{V}O_{2max}$ in those with the lowest baseline values. Therefore, the lack of improvement in $\dot{V}O_{2max}$ following HIIT interventions may be explained by baseline differences in cardiorespiratory fitness between the HIIT and MISS cohorts in our analysis, the publication bias in the MISS studies included, or differences in the duration and frequency of exercise interventions employed.

Importantly, in our analysis, MISS interventions resulted in a significant improvement in $\dot{V}O_{2max.}$ Increased mitochondrial oxidative capacity is linearly correlated with improvements in $\dot{V}O_{2max}$ (van der Zwaard et al., 2016). MISS exercise induces an increase in mitochondrial volume and density, and a subsequent increase in respiratory capacity of the mitochondria (Holloszy and Coyle, 1984), potentially mediated by a shift toward T_1 skeletal muscle fibers. Increases in mitochondria are also only evident in T_1 muscle fibers following 12 weeks of MISS exercise and are not evident following the same duration of sprint-interval training (Skelly et al., 2021). Taken together, this suggests that MISS exercise, but not HIIT exercise, may improve oxidative capacity in individuals with PCOS through changes in muscle fiber type and mitochondrial density.

Anthropometric Profile

Overweight and obesity are prevalent in more than 50% of individuals with PCOS and have the potential to exacerbate symptoms of the condition (Diamanti-Kandarakis and Dunaif, 2012). Our analyses showed that MISS exercise induced significant reductions in BMI (−3.3 kg/m^2) and waist circumference (−2.84 cm) which were absent following HIIT exercise interventions. These results are similar to previous studies, where there is a clearly established dose-response relationship between total exercise volume and reductions in weight (Slentz et al., 2004). Our analysis is also in line with previous work in healthy and diseased cohorts, where HIIT exercise has not been shown to elicit improvements in anthropometric profile, likely related to the lower exercise volume employed in HIIT interventions (Sultana et al., 2019; Viana et al., 2019). The observed reductions in waist circumference following MISS exercise may reflect important benefits for individuals with PCOS, as visceral adipose accumulation is associated with increased insulin resistance and systemic inflammation (Kojta et al., 2020), and an ∼25% increased mortality risk, independent of BMI (Koster et al., 2008). Therefore, MISS exercise should be prescribed to individuals with PCOS as a means of reducing anthropometric indices, especially visceral adiposity, which may result in metabolic health benefits.

Cardiometabolic Profile

Commonly, individuals with PCOS present with dyslipidaemia, characterized by reduced HDL-C, elevated triglycerides and increased LDL-C concentrations (Wild et al., 2011). LDL-C is established as a potent risk factor for the development of CVD (Ference et al., 2017). Lowering of LDL-C concentration through pharmacological intervention has been shown to reduce the risk of cardiovascular events (Ference et al., 2017; Johannesen et al., 2020). However, aerobic exercise alone does not appear to change LDL-C levels unless accompanied by weight loss (Katzmarzyk et al., 2001; Wang and Xu, 2017), and may not be sensitive to low-moderate intensity exercise (Albarrati et al., 2018). In accordance with this, our results showed no significant reduction in LDL-C following MISS or HIIT exercise alone, despite a significant decrease in weight following MISS. Longer-term (16 weeks) intervention has been shown to reduce LDL-C significantly following treatment with diet and exercise combined, with optimal reductions in LDL-C observed after 12 months (Varady and Jones, 2005). The mean duration of isolated exercise of both HIIT and MISS interventions were 14 and 16 weeks, respectively, neither of which induced significant change. Therefore, to reduce LDL-C, a combination of diet and exercise may be required over a longer-term duration.

Our findings following MISS exercise showed a significant reduction in TC, which incorporates both HDL-C and LDL-C and can be therefore be misleading. It is likely that a significant reduction in TC following MISS exercise can be attributed to the non-significant reduction in LDL-C given no impact of MISS exercise on HDL-C. A reduction in TC is important for long-term cardiovascular disease risk in this population, and has been previously associated with volume of exercise (Varady and Jones, 2005; Kodama et al., 2007). As exercise volume is lower in HIIT compared to MISS, this may also explain the improvement in TC evident following MISS interventions only. Intriguingly, HDL-C did not change as a result of either exercise type. Therefore, these results may also be due to the inclusion of PCOS patients with lipid profiles within normal ranges, who did not present with hyperlipidaemia. Our analysis investigated the impact of isolated exercise without concurrent intervention, such as diet or lifestyle

modification. Kodama et al. (2007) suggest that exercise alone only improves HDL-C when MISS exercise at a volume (duration and frequency) greater than exercise guideline recommendations is employed. Exercise alone may also not improve HDL-C levels in those with a higher BMI (Kodama et al., 2007) and weight loss may need to accompany exercise to increase plasma HDL-C (Nicklas et al., 1997). Therefore, while MISS exercise may have some beneficial effect on cardiometabolic profile due to a greater overall exercise volume, patients with PCOS may require additional dietary and/or pharmacological interventions to appropriately control dyslipidaemia.

Limitations

There was a significant publication bias in the analysis of MISS interventions that demands caution when interpreting these results. There are very few randomized controlled trials on the impact of exercise without concurrent intervention. Therefore, we were required to extract data from a wider range of methodological studies where there was potential for researcher bias, participant selection, small samples of participants and small study numbers. This also impacts on study quality, where most evidence synthesized was of moderate quality. Our review was also focused on the cardiometabolic aspects of PCOS and did not include analysis of androgen levels or clinical symptoms. Finally, despite PCOS having multiple phenotypes, only four studies (Jedel et al., 2011; Almenning et al., 2015; Ribeiro et al., 2020; Benham et al., 2021) explicitly reported phenotypical subgroups within their analyses. Previous data suggest that the different phenotypical presentations of PCOS may respond differently to exercise stimuli hence this is an important area for further study (Borzan et al., 2021).

Future Direction

Our analysis highlights the requirement for larger, randomized controlled trials to be conducted in order to further our understanding of PCOS, and how exercise, especially HIIT, can be utilized as a tool for disease management. Such studies should include an analysis of androgen concentrations and clinical manifestations of the condition. Future exercise studies should also report on exercise adherence, compliance and fidelity of the programme in order to further understand the optimal method of exercise to help manage this condition, in addition to analyzing the impact of these factors on exercise behavior following the intervention period. Furthermore, the impact of different exercise modalities on PCOS phenotypes is required in order to discriminate any effect of PCOS sub-type, rather than employing a "one size fits all" approach.

CONCLUSION

Our analysis is the first to compare the impact of isolated HIIT and MISS exercise intervention in individuals with PCOS. MISS exercise resulted in a four-fold increase in $\dot{V}O_{2max}$ and significant reduction in HOMA-IR compared with controls receiving usual care from their GP. A beneficial impact of MISS exercise was also evident on anthropometric indices and total cholesterol in individuals with PCOS, which supports the value of MISS exercise prescription in disease management. In contrast, HIIT did not convey these benefits, although higher-quality evidence is required to fully understand the impact of HIIT on outcomes in PCOS before this can be excluded as a potential treatment option.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: 10.6084/m9.figshare.14687526.

AUTHOR CONTRIBUTIONS

CR, DR, PJ, and RL conceived the study. CR acquired the data. CR, VM, and RL analyzed and interpreted the data. CR drafted the manuscript. All authors critically reviewed the manuscript. All authors provide final approval of the version to be published and agree to be accountable for the work.

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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.2021.715881/full#supplementary-material

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Urinary Steroid Profile in Elite Female Athletes in Relation to Serum Androgens and in Comparison With Untrained Controls

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Introduction: In female athletes, the interpretation of doping tests is complex due to hormonal variations during the menstrual cycle and hormonal contraceptive use, both influencing the urinary steroid profile. Exercise is suggested to affect circulating steroid hormone levels, and in women, the urinary steroid profile differs between in competition testing and out of competition testing. No previous study has investigated the relationship between amount of exercise and the urinary steroid profile in female elite athletes.

Purpose: To compare the urinary steroid profile between female Olympic athletes and age- and BMI-matched untrained controls, and to study the urinary steroid profile in relation to serum hormones and amount of exercise.

Methods: In this cross-sectional study conducted at the Women's Health Research Unit, Karolinska University Hospital, Stockholm, 94 female elite athletes and 86 untrained controls were included. Serum estrogens and testosterone and the urinary steroid profile were analyzed by liquid chromatography–tandem mass spectrometry and gas chromatography-tandem mass spectrometry, respectively. Exercise hours/week were evaluated by questionnaire.

Results: Although serum steroid hormones were comparable between groups, the athletes demonstrated approximately 30% lower urinary steroid metabolites of testosterone, epitestosterone, androsterone, etiocholanolone, 5α-androstan-3α,17β-diol, and 5β-androstan-3α,17β-diol compared to the controls. The urinary steroid metabolites correlated positively with serum steroid hormones. In the athletes, urinary steroid metabolites: androsterone ($r_S = -0.28$, $p = 0.007$), epitestosterone ($r_S = -0.22$, $p = 0.034$), 5αAdiol ($r_S = -0.31$, $p = 0.002$) and testosterone ($r_S = -0.24$, $p = 0.026$), were negatively correlated with amount of training (hours per week).

Conclusion: The urinary concentrations of steroid metabolites were lower in elite athletes than in sedentary controls, although serum steroids were comparable between groups. Moreover, exercise time was negatively associated with the urinary concentrations. Our findings suggest alternative excretion routes of androgens in the athletes related to training.

Keywords: serum androgens, urinary steroid profile, athlete biological passport, exercise, female athlete

INTRODUCTION

Since 2014, the athlete biological passport (ABP) has been used to identify doping with endogenous anabolic steroids, such as testosterone (T). The urinary concentrations of T, its isomer epitestosterone (E), and the T metabolites, androsterone (A), etiocholanolone (Etio), 5α-androstanediol (5αAdiol), and 5β-androstanediol (5βAdiol) are analyzed by gas chromatography-tandem mass spectrometry (GC-MS/MS). These concentrations are combined into five ABP ratios (T/E, A/Etio, 5αAdiol/E, 5αAdiol/5βAdiol, and A/T), and an adaptive Bayesian algorithm calculates individual reference thresholds (Sottas et al., 2010; Sottas and Vernec, 2012). An atypical passport finding is obtained when a sample in the passport goes outside the individually calculated reference ranges, which may trigger a confirmatory isotope ratio mass spectrometry analysis to identify if testosterone is of endogenous or exogenous origin (Wada Wada Technical Document, 2021a,b). The urinary profile is analyzed after the urine has been hydrolyzed with β-glucuronidase and consequently, it is the unconjugated, as well as the glucuronidated fractions that are quantified. Even though glucuronidation is the main excretion route of androgens, the ABP metabolites are also to a lesser extent excreted as sulfate-conjugates (Rane and Ekstrom, 2012; Schiffer et al., 2019).

We, and others, have previously shown that implementation of the ABP increases the chance to detect testosterone intake in men as compared to traditional population based cut-off values after administration of low dose of testosterone (Strahm et al., 2015; Mullen J. et al., 2017; Nair et al., 2020). Furthermore, in women the longitudinal ABP approach is superior to the population-based thresholds to detect administered T, however, not all women are identified as having atypical findings (Handelsman and Bermon, 2019; Knutsson et al., 2020; Salamin et al., 2020). It has been shown that after 10 weeks of transdermal T application in healthy women, only 40% were identified as having atypical passport findings (Knutsson et al., 2020) even though their serum T levels were elevated to concentrations associated with performance enhancing effects (Hirschberg et al., 2020). As a supplementary method, the serum concentration of T, androstenedione and dihydrotestosterone may be co-monitored (Salamin et al., 2020) and subsequently there is an interest to understand the relation between the serum steroid concentrations and urinary excretion rate of the ABP metabolites.

It is well known that hormones fluctuate during the menstrual cycle. For example, urinary E is at the highest concentrations in the luteal phase, whereas the other ABP metabolites show minor fluctuations during a menstrual cycle. These variations result in larger individual ABP-thresholds in women (Schulze et al., 2020). Other challenges associated with ABP interpretation in female athletes include the use of hormonal contraceptives (HC) (Schulze et al., 2014; Ekström et al., 2019), and the impact of genetic polymorphisms in UDP-glucuronosyltransferases (UGTs), such as UGT2B17 (Schulze et al., 2014). The time of sampling may also be pivotal for test-results. In a large compilation of ABP data from over 11,000 Swedish and Norwegian athletes, both the intra- and inter-individual variations for all ABP ratios were larger in women than men. Furthermore, women demonstrated 65% higher T excretion in competition (IC) as compared to out of competition (OOC), whereas men's urinary steroid profile did not differ to the same extent between IC and OOC testing (Mullen et al., 2020). In women, a great part of the androgen production occurs in the adrenal gland stimulated by adrenocorticotropic hormone (ACTH) from the pituitary gland (Burger, 2002; Schiffer et al., 2019). Since physiological stress stimulates ACTH secretion and in turn androgen and cortisol from the adrenal gland (Schiffer et al., 2019) this may explain the higher T excretion IC found in women.

In addition, previous studies suggest that exercise affects the concentrations of circulatory hormones (Nindl et al., 2001; Enea et al., 2011), whereas there are no studies investigating the relationship between the amount of exercise and excretion rate of androgens. Therefore, we aimed to investigate the excretion profile of glucuronides and sulfate androgen conjugates in elite female athletes in relation to matched sedentary controls, and further to study the ABP urinary steroid profiles in relation to serum hormones, genetic variation in androgen metabolism and amount of exercise.

MATERIALS AND METHODS

Study Population

The athletes participating in this study were part of a cohort of 106 Swedish female Olympic athletes, members of an Olympic team or part of the high-performance programs of the Swedish Olympic Committee (SOC). In addition, 117 healthy female controls [body mass index (BMI)- and age-matched, allowed a maximum of 2 h training/week and no prior participation in elite level sports] were recruited. A more detailed description of the study cohort is previously published (Eklund et al., 2017).

The present study population included 94 female Olympic athletes and 86 controls, from which urinary samples were available for analysis. Serum androgen levels and urinary sulphate levels were previously analyzed (Eklund et al., 2017, 2020) and are

here presented for the same 94 athletes and 86 controls included in the current study.

The participants were investigated in connection with training camps or at the Women's Health Research Unit, Karolinska University Hospital. Health status, training hours/week and gynecological data (hormonal contraceptive use, menstrual cycle data, pregnancies) were collected by a questionnaire. A blood sample and urinary samples were collected in a fasted, rested state between 07.00 and 10.00 h and stored at −20°C until further analyses. Blood and urine samples were collected randomly during the menstrual cycle.

The study was conducted according to the Declaration of Helsinki and was approved by the Regional Ethics Committee, Stockholm (EPN 2011/1426-32) and informed consent was acquired from all participants.

Serum Hormonal Analyses

Serum T, estrone (E1), and estradiol (E2) were determined by liquid chromatography tandem mass spectrometry (LC-MS/MS) at the Endoceutics laboratory, Quebec, Canada, as previously described (Ke et al., 2014, 2015). Free androgen index (FAI) was calculated, testosterone nmol/L divided by sex hormone- binding globulin (SHBG) nmol/L × 100. Follicle stimulating hormone (FSH), luteinizing hormone (LH), SHBG and cortisol were analyzed by electrochemiluminescence immunoassay (ECLIA) using commercial kits from Roche Diagnostics AG (CH 6343 Rotkreuz, Switzerland) (Cobas8000), at the Department of Clinical Chemistry, Karolinska University hospital, Stockholm (Eklund et al., 2017). Detection limits and within and between assay coefficients of variation were for FSH 0.1 IU/L, 2.6 and 3.6%, for LH 0.1 IU/L, 1.2 and 2.0%, for SHBG 0.04 μg/mL, 1.3 and 2.1%, and for cortisol 1.5 nmol/L, 1.7 and 2.2%, respectively.

Urinary Steroid Profile

The urinary levels (glucuronide and unconjugated fractions) of T, E, A, Etio, 5αAdiol, and 5βAdiol were determined with GC-MS/MS at the World Anti-doping agency (WADA) accredited anti-doping Laboratory at the Karolinska University Hospital, Huddinge, Stockholm as previously described (Mullen J.E. et al., 2017). Briefly, internal standard, phosphate buffer (pH 6.5) and β-glucuronidase from *E. coli* was added to 2 mL sample. The mix was incubated for 60 min at 50°C. Once cooled, the sample was extracted using potassium carbonate and methyl tert-butyl ether which was dried using sodium sulfate. After centrifugation the water phase was frozen, and the ether decanted into a fresh tube. The ether was evaporated under a gentle stream of nitrogen and placed in a desiccator. 100 μL derivatization reagent was added and left to react (incubated for 30 min at 50°C) to form tri-methyl silyl derivates. The sample was transferred to injection vials and injected to an Agilent 7890B gas chromatograph and 7000C Triple Quadrupole mass spectrometer.

Urinary sulphate metabolite levels [testosterone–sulphate (T–S), epitestosterone-sulphate (EpiT-S), androsterone-sulphate (ADT-S), and etiocholanolone-sulphate (Etio-S)], were analyzed using LC-MS/MS at the WADA accredited anti-doping Laboratory at the Karolinska University Hospital, Huddinge, Stockholm as previously described (Mullen J.E. et al., 2017; Eklund et al., 2020).

Specific gravity (SG) was measured with a Digital Refractometer to adjust for the dilution of the urine, using formula; $C_{\text{corrected}} = C_{\text{measured}} \times (1.020 - 1/\text{SG} - 1)$.

Genotyping

Genomic DNA was extracted from whole blood using QIAmp DNA Blood Mini Kit (Qiagen). Twenty ng was used as template in 15 μL reactions using UGT2B17 copy number assay (#Hs03185327_cn, Life Technologies, Holland), and 2 × TaqMan Universal Master Mix II, no UNG (#4440043, Life Technology, Holland). The ubiquitously expressed RNase P (#4403326, Control Reagents, Life Technologies, Holland) was used as an endogenous reference gene for reaction quality control. Samples with RNase P signals but no UGT2B17 amplification were identified as del/del.

Statistical Analyses

Continuous data was presented as mean ± SD when symmetrically distributed or as median and interquartile range (25th–75th percentile) otherwise. For comparisons between athletes and controls regarding HC use and the number of individuals with UGT2B17 del/del, the Pearson Chi-square test was applied. Mean serum hormones were compared between the groups using the student's *t*-test when approximate normal distributions could be assumed. The Mann-Whitney *U*-test was used otherwise. Urinary androgen metabolites were not normally distributed and was therefore square root-transformed (testosterone, T/E ratio, and A/Etio) or log- transformed (all remaining urinary steroid metabolites and ratios) prior to parametric statistical tests. Concerning age, BMI, training hours per week and urinary androgen metabolites, comparison between groups were performed using the student's *t*-test. Two-way ANOVA was applied to evaluate the potential impact of HC use when comparing urinary androgen metabolites and cortisol between athletes and controls (the interaction term group × HC use). In case of a significant interaction, differences between groups were tested with/and without HC use. Spearman correlation was used to evaluate association between variables. UGT2B17 del/del individuals were excluded in the Spearman correlations analyses between U-Testosterone and all serum androgens. *P*-values < 0.05 were considered statistically significant. Statistical analyses were performed using Statistica version 13 [TIBCO Software Inc. (2018)].

RESULTS

Age and BMI were comparable between groups. When comparing the frequency of HC use and UGT2B17 del/del individuals no significant differences were found between athletes and controls. As expected, the athletes had significantly higher amount of training per week (h/w) than the controls. As previously published, the athletes had significantly lower E1 levels compared to controls but no significant difference was

found for serum T levels or FAI between athletes and controls (Eklund et al., 2017). The athletes demonstrated significantly lower urinary steroid metabolites (glucuronide and sulphate metabolites) compared to controls (**Table 1**).

In the athletes and controls not using HC, similar results were found when comparing urinary steroid metabolites (**Supplementary Table 1**). For the T/E ratio, two–way ANOVA indicated an interaction for HC use. Subgroup analyses found a significantly higher T/E ratio in controls using HC compared to controls not using HC [1.0 (0.7–1.5) vs. 0.6 (0.4–0.9), $p < 0.001$].

For cortisol, two- way ANOVA indicated that the differences between groups may be dependent on HC use. Comparison between groups demonstrated that in the subgroup of participants not using HC, athletes demonstrated significantly higher cortisol than controls (474.1 ± 131.2 vs. 376.4 ± 108.0, $p = 0.004$). In the subgroup using HC, no significant difference was found. As expected, HC users had significantly higher cortisol levels than non-users (athletes: 748.0 ± 220.7 vs. 474.1 ± 131.2, $p = <0.0001$, and controls: 759.1 ± 279.0 vs. 376.4 ± 108.0, $p = <0.0001$).

TABLE 1 | General characteristics, serum hormones and urinary androgen metabolites in female Olympic athletes and controls.

Parameter	Controls	Athletes
n	86	94
Age	26.3 ± 6.0	25.8 ± 5.5
BMI	22.0 ± 2.8	22.0 ± 1.9
HC use, *n* (%)	31 (36%)	36 (38%)
UGT2B17 del/del, *n* (%)	8 (9.4)	9 (9.7)
Amount of training (h/w)	0.7 ± 0.8	18.2 ± 5.8***
Serum hormones		
n	86	94
E1 (pg/mL)	47.4 (26.5–78.7)	34.9 (21.8–58.8)*
E2 (pg/mL)	55.5 (25.8–122.8)	35.6 (14.3–87.5)
T (pg/mL)	290.0 ± 105.6	284.9 ± 116.4
T (nmol/L)	1.0 ± 0.37	0.99 ± 0.40
FSH (IU/L)	4.0 (2.4–5.7)	4.6 (2.7–6.2)
LH (IU/L)	5.8 (3.4–7.9)	5.6 (2.4–8.9)
SHBG (nmol/L)	80.5 (62.0–129.0)	82.0 (57.0–117.0)
FAI	1.1 (0.6–1.8)	1.1 (0.6–1.9)
Cortisol (nmol/L)∧	516.0 ± 263.6	579.0 ± 216.3
U-androgen metabolites		
n	86	94
U-Testosterone-G (ng/mL)	6.90 (4.27–14.30)	4.59 (2.25–8.00)***
U-Testosterone-S (ng/mL)	1.77 (1.28–3.16)	1.55 (0.94–2.56)*
U-Epitestosterone-G (ng/mL)	10.99 (6.34–19.64)	6.09 (3.60–11.41)***
U-Epitestosterone-S (ng/mL)	5.79 (3.24–9.64)	2.69 (1.69–5.83)***
U-Androsterone-G (ng/mL)	3,386 (2,390–5,627)	2,178 (1,278–3,554)***
U-Androsterone-S (ng/mL)	756 (292–1,361)	519 (263–975)*
U-Etiocholanolone-G (ng/mL)	3,647 (2,504–4,838)	2,762 (1,769–4,139)**
U-Etiocholanolone-S (ng/mL)	273 (163–461)	255 (116–479)
U-5αAdiol-G (ng/mL)	33.4 (21.3–52.7)	19.6 (12.4–30.0)***
U-5βAdiol-G (ng/mL)	86.8 (53.0–197.1)	84.5 (39.1–132.8)*
T/E ratio∧	0.7 (0.5–1.1)	0.7 (0.4–1.3)
A/Etio ratio	1.0 (0.8–1.3)	0.8 (0.6–1.1)*
A/T ratio	458 (294–767)	428 (329–696)
5αAdiol/E	3.2 (1.9–5.0)	3.6 (2.4–4.8)
5αAdiol/5βAdiol	0.3 (0.2–0.6)	0.3 (0.2–0.5)

Values presented as mean ± SD or median and interquartile range (25th–75th percentile).

A, androsterone; 5αAdiol, U-5α-Androstane-3α,17β-diol; 5βAdiol, U-5β-Androstane-3α,17β-diol; BMI, body mass index; E, epitestosterone; E1, estrone; E2, estradiol; Etio, etiocholanolone; FAI, free androgen index; FSH, follicular-stimulating hormone; h, hours; HC, hormonal contraceptive; LH, luteinizing hormone; SHBG, sex hormone-binding globulin; T, testosterone; S, sulphate metabolite; w, week.

∧Two-way ANOVA indicated that HC use interacted with the comparison between groups.

**p < 0.05; **p < 0.01; ***p < 0.001.*

Correlations Between Serum Hormones and Urinary Steroid Metabolites

Correlation analysis between the urinary androgen metabolites and serum androgens can be found in **Table 2**. In the subgroup of athletes and controls not using HC, similar correlations were found between serum hormones and urinary steroid metabolites (data not shown).

Correlations Between Training Hours per Week, Serum and Urinary Steroid Metabolites

In the athletes, significant negative correlations were found between training hours per week and U-Androsterone, U-Epitestosterone, U-5αAdiol, and U-testosterone, respectively (**Figure 1**). One of the ratios included in the ABP, A/Etio, also correlated negatively with training hours per week ($r_s = -0.22$, $p = 0.036$). No significant correlations were found between serum androgens and trainings hours per week.

DISCUSSION

For the first time, we demonstrate a difference in urinary steroid levels between female athletes and sedentary controls, i.e., the urinary levels of steroid metabolites both glucuronide and sulfate conjugated, were lower in the athlete population. Similar findings have previously been described when comparing male athletes and controls (Timon et al., 2008). Since it has been shown that exercise acutely increases the production and serum concentrations of androgens, lower levels of all the ABP metabolites may appear contradictory. Stress has been discussed as an influencing factor that may increase the excretion rate of urinary steroids IC, particularly in women (Mullen et al., 2020). There was no difference in cortisol levels between the sedentary controls and the athletes, however, when HC users were excluded, cortisol levels were higher in the athletes, and hence the influence of stress cannot explain the lower levels of steroid metabolites in the athletes.

The consistently lower urinary steroid levels in the athletes are not reflective of the serum steroids. In our participants, there were no differences in circulatory levels of any of the ABP related androgens studied between groups. In contrast, as previously published in the total cohort of Swedish female Olympic athletes,

TABLE 2 | Correlation matrix between serum androgens and urinary androgen metabolites in female Olympic athletes (n = 94) and controls (n = 86).

U-androgen metabolites	Serum androgens					
	E1	**E2**	**T**	**FAI**	**FSH**	**LH**
U-testosterone[#]						
Athletes	0.27*	ns	0.48***	0.72***	0.31**	0.26*
Controls	0.25*	0.31**	0.32**	0.48***	ns	0.37**
Epitestosterone						
Athletes	0.73***	0.65***	0.57***	0.61***	0.24*	0.54***
Controls	0.63***	0.65***	0.53***	0.65***	ns	0.55***
U-Androsterone						
Athletes	0.32**	0.22*	0.44***	0.51***	ns	0.21*
Controls	0.22*	ns	0.40***	0.32**	ns	0.22*
U-Etiocholanolone						
Athletes	0.23*	ns	0.49***	0.61***	0.26*	ns
Controls	ns	ns	0.29**	0.33**	ns	0.26*
U- 5α Adiol						
Athletes	0.35***	0.27**	0.40***	0.62***	ns	0.24*
Controls	0.32**	0.26*	0.31**	0.49***	0.30**	0.37***
U- 5β Adiol						
Athletes	0.21*	ns	0.35***	0.51***	0.21*	ns
Controls	ns	ns	ns	0.22*	ns	ns

Values reported are Spearman rank-order correlation (r_S).
E1, estrone; E2, estradiol; FAI, free androgen index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; ns, non-significant; T, testosterone; U-5αAdiol, U-5α-Androstane-3α, 17β-diol; U-5βAdiol, U-5β-Androstane-3α,17β -diol.
[#]del/del (n = 8 controls, n = 9 athletes) excluded from analyses.
<0.05; **<0.01; *<0.001.*

the serum androgen precursor dehydroepiandrosterone (DHEA) was higher in the athletes compared to controls (Eklund et al., 2017). A possible explanation may be that androgens in athletes are also eliminated by additional routes, i.e., in feces and/or sweat. However, quantification of steroids in feces have been poorly studied in humans. In eight healthy men, Colldén et al. (2019) showed that T and DHT are highly abundant in feces. Sweat might be a potential excretion route due to the lipophilic properties of steroids (Thieme, 2012). Already in 1983, it was reported that steroid metabolites included in the ABP (A and T) can be excreted as sulphate conjugates in human axillary sweat (Tóth and Faredin, 1985), whereas the glucuronide conjugated metabolites have not been evaluated in sweat. It is unlikely that different phase II metabolism explain the different urinary excretion rate of ABP markers between athletes and sedentary controls as the androgen-sulfates levels were also higher in the controls. It is therefore possible that sweat excretion might at least partly explain the negative correlations between amount of training (hours) and urinary levels observed herein.

After transdermal application of estr-4-ene diol, the metabolites nortestosterone, and estr-4-enedione were found in sweat collected after physical exercise (Thieme et al., 2003). The sweat production may depend on training intensity, sport activity, and temperature, etc. No difference between sport categories could be discerned (data not shown), possibly due to lack of power. As the ratios (particularly T/E) rather than the metabolites are monitored in the ABP, the non-urinary excretion routes may not have a direct impact on doping testing. However, it is possible that training mediated fluctuations in absolute androgen concentrations may be visible in an athlete's passport, particularly in connection to situations where large differences in training load are expected, i.e., between in and off-seasons and after injuries. A future longitudinal study with controlled training schedule and analyses of additional sample matrixes (sweat) would be of interest to understand the connection between amount of training and urinary excretion rates of androgens. Moreover, sweat has been discussed as an alternative matrix in forensic toxicology, including detection of steroid abuse (Thieme, 2012).

The knowledge on how urine and serum androgen metabolites are connected may be of interest in anti-doping since quantification of endogenous serum steroids may be a complementary approach to the urinary steroid profile method in the future (Salamin et al., 2020). The monitoring of serum T may increase the likelihood to detect T intake in female athletes as serum T is superior compared to the urinary steroid profile (Handelsman and Bermon, 2019; Börjesson et al., 2020; Knutsson et al., 2020). Our correlations between the urinary ABP metabolites and serum hormones are in agreement with previously published data (Knutsson et al., 2020; Schulze et al., 2020).

Certain limitation of the present study should be addressed. Due to the cross-sectional study design, causality cannot be concluded. Even though blood and urine samples were collected by a standardized procedure (in a fasted and over-night rested state), we acknowledge that the sampling was performed

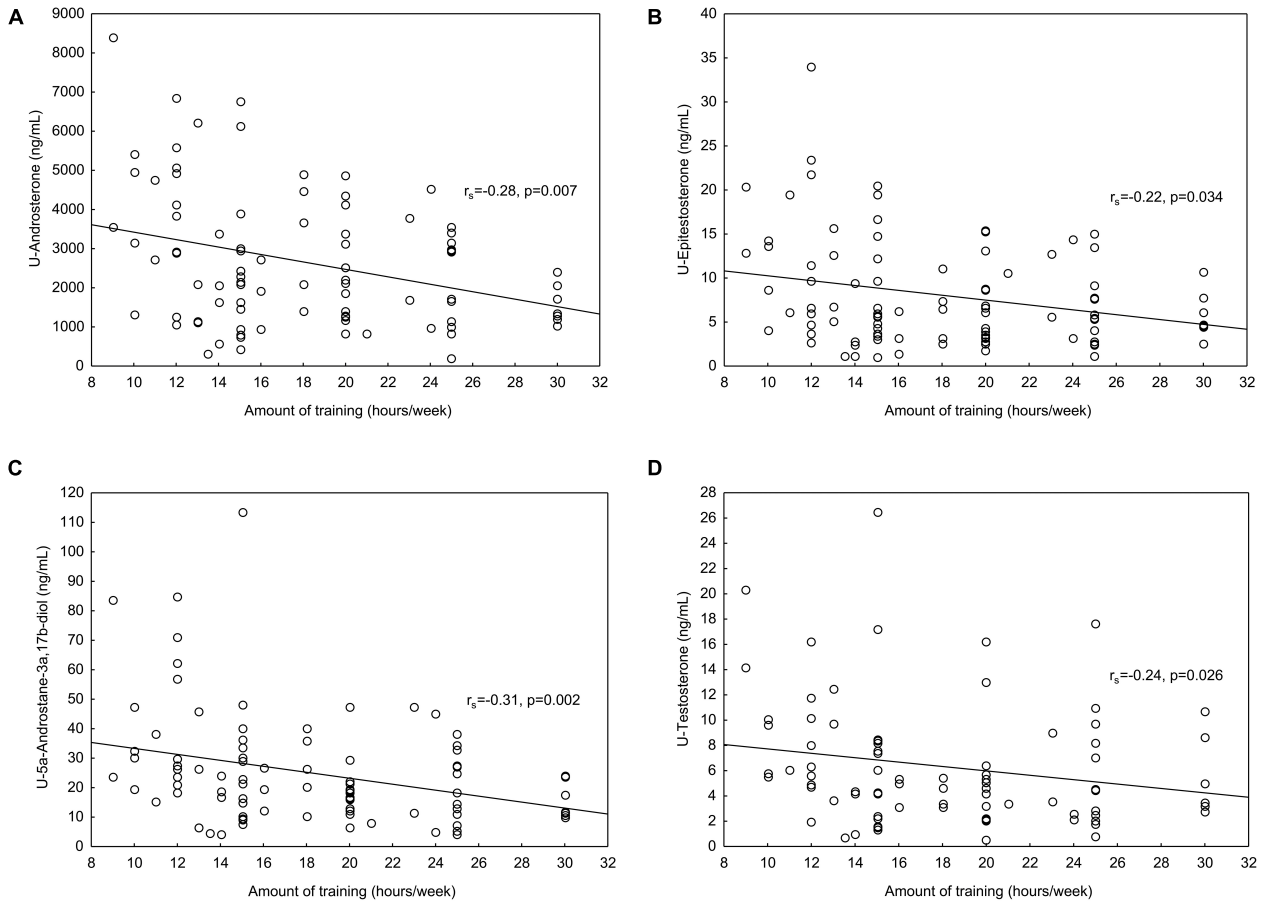

FIGURE 1 | (A) In the athletes, significant negative correlations were found between amount of training (hours/week) and **(A)** U-Androsterone, **(B)** U-Epitestosterone, **(C)** U-5αAdiol (U-5α-Androstane-3α,17β-diol) and **(D)** U-testosterone. In the U-testosterone correlations, UGT2B17 del/del individuals (n = 9) were excluded from the analyses.

randomly according to the menstrual cycle. In premenopausal women, there is a small mid-cycle increase in serum T (Handelsman et al., 2018). In addition, urinary androgens, especially urinary E, fluctuates during the menstrual cycle (Schulze et al., 2020). We found that urinary E demonstrated the strongest association with serum estrogens. Subsequently E is more sensitive to menstrual cycle fluctuations than other urinary metabolites (Schulze et al., 2020), resulting in larger individual ABP ranges in women than in men (Mullen et al., 2020). However, since doping tests are collected randomly, we believe that the results presented here provide valuable information.

It is well known that HC use affects serum T and SHBG levels (Sonalkar et al., 2000; Zimmerman et al., 2014). Furthermore, urinary androgens vary depending on HC use. In a previous study including female athletes, we showed that urinary E was suppressed in HC users (Schulze et al., 2014). These findings were confirmed in an intervention study examining the disposition of the androgen metabolites and ABP ratios in relation to HC use (Ekström et al., 2019). In the current study, the correlation analyses between serum and urinary androgens were evaluated in both HC and non-HC users. When HC-users were excluded, we did not observe any significant difference in the associations between serum- and urine androgens. Therefore, we suggest that regardless of HC use, the urinary metabolites reflect the androgenic load (serum concentrations of T, FAI, and LH) to the same degree in both athletes and controls. Furthermore, we found comparable differences in urinary steroid levels between athletes and controls in the subgroup not using HC.

It is well known that UGT2B17 exerts a large impact on the urinary concentrations of T (i.e., T-glucuronide). Therefore, we excluded the del/del subjects from the statistical analyses including urinary T. The UGT2B17 deletion polymorphism was found in same frequency in athletes and controls, i.e., approximately 10% being homozygous for the deletion allele. This allele frequency corroborates with other studies conducted in samples from athletes (Anielski et al., 2011; Choong et al., 2016). Another limitation is that training hours per week were based on self-reported data by the athletes.

In conclusion, we have shown that the urinary excretion rate of androgen metabolites monitored in ABP are higher in sedentary controls than in elite athletes, and that the amount of training is negatively associated with the urinary concentrations. Further

studies are needed to understand the association between training and urinary excretion rate of androgens in athletes.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion 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 Regional Ethics Committee, Stockholm (EPN 2011/1426-32). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AH, EE, and LE were involved in the concept and design of the study. AH and EE were responsible for the acquisition of data and in collaboration with LE and AA also the data analysis. AA performed the quantification of urinary androgens. EE, AA, LE, and AH were involved in the manuscript preparation, critical revision of the manuscript, and approval of the manuscript. All authors listed met the conditions required for full authorship.

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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.2021.702305/full#supplementary-material

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Women's Experiences of Using Anabolic Androgenic Steroids

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Anabolic androgenic steroids are used by women to increase their muscle mass and because of their performance-enhancing effects. Despite permanent/high risk of side effects, knowledge is inadequate. Our aim has been to deepen understanding about women's use of anabolic androgenic steroids. This phenomenological study is based on the reflective lifeworld research (RLR) approach. Lifeworld interviews were conducted with 12 women, aged 21–56 years, about their experiences of using anabolic steroids. The results show that women experience a sense of pride when they successfully achieve their goals. This is the driving force, triggering tension between suffering and success. Our research adds important knowledge from a reflective lifeworld perspective and shows that women's use of anabolic androgenic steroids is a complex phenomenon. Understanding and knowledge are important in order to be able to meet and support women in their fears and difficulties.

Keywords: anabolic androgenic steroids, doping, women, phenomenology, reflective lifeworld research

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INTRODUCTION

Even though the use of anabolic androgenic steroids (AAS) is considered to be a health problem, little is known about women using AAS, despite the expectedly high risk of side effects, even permanent ones. AAS are categorized as illegal substances in Sweden, but they are effective and can greatly increase strength, muscle, and fat-free mass when combined with strength training (Bhasin et al., 1996; Rogerson et al., 2007). They have also shown a performance-enhancing effect in women (Hirschberg et al., 2020). Typical AAS-induced physical side effects in men include potency problems, acne, and gynaecomastia. Typical psychiatric side effects include depression, sleep disorders and mood disturbances (Sjoqvist et al., 2008). Even though AAS use is mainly a male phenomenon, it is not limited to men. One explanation why women do not use AAS to the same extent may be that women are not as interested as men in becoming very muscular and are more vulnerable to the masculinizing effects of AAS (Kanayama and Pope, 2012) e.g., change of voice, enlargement of clitoris (Strauss et al., 1985; Malarkey et al., 1991). Women generally take fewer substances and lower doses (Borjesson et al., 2016, 2020). In females, sports performance appears to be the main reason for using AAS (Kanayama et al., 2007; Borjesson et al., 2016), especially in bodybuilding and weightlifting (Gruber and Pope, 2000; Phillips et al., 2010). Women identify themselves as competitive bodybuilders or power lifters (Phillips et al., 2010) and appear to be influenced by the men with whom they are in close relationships (Skarberg et al., 2008; Borjesson et al., 2016). Research show that women seek healthcare earlier than men for the negative effects they experience (Garevik et al., 2011; Borjesson et al., 2016).

Currently, no deeper knowledge or understanding exist of women's experiences of using AAS, therefore it is important to study this phenomenon. In this research we turn to the women themselves and to their lifeworld. This is the first study with female AAS users that has practiced the reflective lifeworld research (Dahlberg et al., 2008) (RLR) with a caring science perspective (Dahlberg, 2011). Through the RLR approach, we aimed to reach an existential dimension that is missing in previous research. The results are expected to contribute important knowledge and understanding, especially for healthcare professionals since AAS may affect an individual's health.

MATERIALS AND METHODS

Design

This study has practiced the reflective lifeworld research (RLR) approach described by Dahlberg et al. (2008) and are based on phenomenological epistemology developed by the philosopher Husserl (1970/1936). The study is influenced by a caring science perspective (Dahlberg, 2011) because it is necessary to understand the individual's health in order to be able to support and strengthen the individual in her health process. The lifeworld includes our unique existential world, our experiences and the relationship between them. Through the lifeworld perspective we seek understanding in the lived everyday world. The RLR approach is characterized by an open mind and flexibility toward the phenomenon. The phenomenon being explored and illuminated in this study is women's use of AAS. The researchers needed to have a reflective attitude described as bridling, which involves slowing down the process of understanding and not being too quick to make definite that which is indefinite (Dahlberg and Dahlberg, 2003). To be objective in a phenomenological sense, personal values, theories, and other assumptions may not impede us from acquiring a new understanding of meaning (van Wijngaarden et al., 2017). By not taking anything for granted, we can take control over our preconceptions.

Participants and Study Setting

A total of 12 women participated voluntarily in the study. All were current or former users of AAS. They were recruited in two ways: either via snowball sampling or when contacting the Anti-Doping Hot-Line. The participants had to be over 18 years of age, as this is the age of majority in Sweden, and their age range was 21–56. Participants were required to understand the Swedish language and were included in the study in the order they came in contact with the interviewer. Snowball sampling (Heckathorn, 2011) is a convenient method to get in touch and generate informants in hard-to-reach populations. It involves an initial contact, in this case AAS users, who in turn can generate new informants. The Anti-Doping Hot-Line has been organized since 1993 as an anonymous free telephone counseling service for people concerned about or affected by their non-medical use of AAS (Eklof et al., 2003). The Anti-Doping Hot-Line started after observations of the need in society for an information service about the health risks of doping. The service is managed by trained nurses and clinical pharmacologists at the Department of Clinical Pharmacology, Karolinska University Hospital Stockholm, Sweden.

Data Collection

The interviews lasted between 45–90 min and were tape-recorded and thereafter transcribed verbatim. To maintain privacy, the interviews took place upon the informant's request in a separate room at a library or in the informant's home. One of the interviews took place in a café based on the informant's request. Each interview started with a presentation of the study's aim and then continued with the main open question: "How is it to use anabolic androgenic steroids?" Follow-up questions were asked (e.g., how do you mean, can you describe more?) to capture the individual's perception and to gain deeper insight into the phenomenon.

Data Analysis

The analysis followed the phenomenological approach in accordance with the guidelines for RLR (Dahlberg et al., 2008). Analyzing data according to RLR is about understanding the phenomenon and finding its meaning, and abstraction is carried out by referring back and forth between the whole and its parts, and then reconstructing the whole. This is a process to understand different abstract levels of meaning when seeking for the essence of the phenomenon. The focus on the phenomenon is of crucial importance in this process. The informants had described their experiences in the interviews, which means that they had delivered data to be analyzed. A rich material in meanings and variations of these meanings is necessary to be able to reach the essential description of the phenomenon. In the analysis process, however, we concentrate on the phenomenon, which means that the analysis is not subject-orientated, but phenomenon-orientated. In other words, we seek for meanings in the experiences collected, that constitute the phenomenon. The essence presents the meanings of the phenomenon on an abstract and general level. Thus, the total result of the study describes the phenomenon in a general sense, which is not solely related to the informants in the study. In order to find the essential meaning of the phenomenon, the analysis work must be carried out with a reflective and bridling attitude according to the RLR approach (Dahlberg et al., 2008). According to Brinkmann and Kvale (2014), the data must be divided, organized, and simplified to get a clear picture. The analysis began with listening and reading the interviews in their entirety with an open mind to facilitate an initial understanding. After repeated reading, the transcript was divided into meaning units to search for meanings. From a phenomenological perspective and validity research should be meaning-oriented (van Wijngaarden et al., 2017). When the interviews were emptied of all meaning, the meanings were clustered together to find similarities and differences. A pattern of meanings slowly emerged and shaped a meaningful structure that constitutes the essence of the phenomenon. The aim is then to describe the variations and nuances of the phenomenon, which means the constituents. This means that the focus is still on the phenomenon, but the nuances are illustrated with quotes from the informants.

Ethics

Ethical approval was obtained from the Regional Ethics Committee at the Karolinska Institutet, Stockholm (nr. 2016/1762-31/5). Before and on the occasion for the interview, the informants received oral and written information about the purpose of the study, stating that participation was voluntary and confirming their right to withdraw if they so wished, without explanation. Information was also given about the confidentiality of the interviews. Written consent was given by the participants. After the interviews, the participants in the study were offered care and support if needed.

RESULTS

The results are first presented by the essential structure of meanings, followed by its five constituents.

Living with AAS can be hard in many ways and difficult to endure. It involves existential challenges to achieve the perfect body. Body dissatisfaction creates anxiety, which is mastered by hard training, strict diet and the use of AAS. The ambition is to use training, diet, and AAS as the means to acquire a perfect body as well as recognition and social acceptance. The experience of succeeding through their achievements creates a sense of pride, which is the driving force, triggering tension between suffering and success. Lack of self-esteem contributes to the experience of the body's imperfection. Low self-esteem is compensated for by self-control, discipline and performance. Using AAS means living with feelings of fear, guilt, shame, and vulnerability. It is an arduous endeavor to balance the substances' side effects with desired femininity. Existing standards of femininity casts a permanent shadow over existence. Constant search for knowledge leads to insights about the use of AAS. A self-preoccupation is shown as emotional coldness toward and distanced from people around. The use of AAS also means living with lies and the fear of being discovered, because AAS are illegal.

Striving for the Perfect Body

In striving for the perfect body, women live with body anxiety, which means experiencing that their bodies are not perfect. To manage this anxiety, they begin strength training in order to build muscles. This allows them to eat more without gaining weight. Eating disorders, previously part of their lives, have made them aware that exercise helps to avoid the problem of weight gain.

One woman recalled:

> *"The first times? Well, I had bulimia and so on before…that was perhaps also part of the reason for starting this training thing, and also generally that I realized that I could then eat without having to puke. Instead I had to eat to gain muscle, and then it became like periods of building up when it was okay to eat and exercise hard and I didn't put on weight because I gained muscle".*

Despite hard training and the use of AAS, women may still feel that they are not achieving what they want. The feeling of dissatisfaction persists even though their muscles are getting bigger. Their distorted body image makes it difficult for them to perceive their own bodily changes realistically and also to receive positive comments from others. To gauge their progress, they ask selected people for advice, look at photographs of themselves, use tape measures or try on clothes. Even when they perform well, feelings persist of their results not being good enough, and they start to focus on the next set or new goals.

One woman described her feeling of dissatisfaction with her bodily changes:

> *"We were all influenced by our idols, and how they looked on stage. We read magazines to see ourselves in that role or in that situation and perhaps someone felt that her shoulders weren't good enough. If her shoulders were okay, then perhaps her legs were wrong. All the time, our aim was the perfect physique with all the muscles in harmony with each other. Genetically, everybody has a muscle group or a body part that genetically doesn't develop as quickly".*

A strictly controlled diet is helping the women in their achievements of reaching the perfect body. Scheduled eating days are planned when eating more freely is permitted, i.e., food that is not normally allowed. It is easy to eat more than planned and sometimes food intake goes out of control. A feeling then arises of bodily collapse, creating body anxiety and resulting in compensatory training. This can be particularly difficult in periods, especially after bodybuilding competitions. Therefore, these women constantly continue with the same strict diet. Prior to competitions, small changes in diet can be perceived as crucial, as one woman noted:

> *"I came second in the Swedish championship and I had cheated by eating 4 grapes two weeks before the competition. It wasn't really cheating because everyone else did it too, but I felt it was cheating and I came second in that competition. In other words, could I have won if I hadn't eaten those grapes? is going round in my head. Before, I couldn't even use lip balm because it contained fat and I was scared of getting it into my body. I was so scared of everything that could sabotage a diet or a commitment, because it meant my whole life to me".*

Before women start using AAS, they need to have a basic physique. When this stage is reached and the body can no longer develop naturally, they feel that a careful use of AAS is justified.

Increasing Self-Esteem Through Performance

Women control their lack of self-esteem through their performance. It is important to be successful to counteract early fears of not being good enough. A life-history with eating disorders, bullying, negative comments about appearance, lack of recognition, and lack of love is common.

Lack of self-esteem is regulated through self-control and discipline and this is achieved mainly by following strict dietary and hard exercise routines. Building one's body provides the opportunity to demonstrate skills and value. People recognize and look up to bodybuilders as individuals, making them feel successful and strengthening their experience of being determined, disciplined and healthy.

One woman reflected:

"I think many people come from a very destructive background, so many have pushed themselves hard before with eating disorders or other destructive things. Because it's not really healthy to push yourself so hard…so you have to be hard-headed and that comes from somewhere. Either your upbringing was tough, or you're prepared to fight even though it hurts. I don't know, perhaps pushing yourself or punishing yourself makes it clearer in some way. Because afterwards you get rewarded a little by time in the limelight and attention for all your hard work".

Physical development thus leads to approval and attention from other people. This increases personal status and motivates further and better performance. Living healthily creates a feeling of superiority to others. However, there are also thoughts that the result has not come quite naturally.

Maintaining One's Femininity

To avoid masculinising side effects and over-large muscles, the intake of AAS needs to be balanced. Women are uncertain about being able to handle this balancing act and live in a fear of losing their femininity. They have an inner limit for acceptable side effects, so they struggle to maintain the balance between desirable muscle development and acceptable side effects. Not being able to get pregnant, and permanent side effects such as clitoral enlargement, increased body hair or a deeper voice frighten them. However, in order to develop in training and to have a realistic chance of meeting other people in the bodybuilding sport, certain risks must be taken. If muscle development is too slow, thoughts may appear of increasing the dose or switching to a more potent substance. However, if side effects occur, the dose may be reduced or discontinued.

One woman commented:

"But nothing happened. So then I thought, well, I can just keep going. Every time I had the injection, I felt such anxiety in my body. I repeatedly checked my clitoris, I searched on Google and read about clitoris enlargement about 100 times".

Knowledge about AAS and how to use the substances is required in order to hide the use of AAS from others. Being well-informed and critical of one's sources reduces the risk of both side effects and being deceived into making the wrong choices. The concern about and fear of incorrect advice and the authenticity of the substances make it impossible to trust advices from others. The women are usually led by men who give them advice based on how men use AAS. They request first-hand information about how AAS works in a female body but rarely exchange experiences with each another. The women think that societal information from a female perspective where not only the negative effects are described would increase credibility. People's views on femininity are affected by traditions and societal norms in terms of appearance and appropriate clothing. Women with large muscles are questioned by others.

One woman described her own experience of not fitting in:

"When my body got muscles, they roared with laughter at me and said that the men's department is on the other side of the street…you feel a bit….divided about how to dress yourself if you're very muscular. If I go in dresses or skirts and stuff, then I feel like people are looking at me like I'm a transvestite. For a while I thought it was really hard because…..it was like this, I was almost…I don't know…not hurt but I thought it was hard to constantly have to stand up for myself and I had to fight all the time to….do I need to prove that I am a girl or what….or do I look like a guy".

A muscular appearance makes women vulnerable. Disgusting comments and shameful suggestions to women with muscular looks come from men in social media who are fascinated by women with strong, muscular bodies. Unwanted confirmation in daily life occurs too: unknown people pinch and feel women's bodies without warning and without asking for permission. One woman described her experience of comments in social media:

"Is it okay to behave like this, what if someone had been fat – why are you so fucking fat, is it okay to say things like that? – and besides, I have a fan page on facebook, a page where you go to look at pictures of me, why the hell should they go there and look at pictures of me and then make negative comments? They should shut up and scroll on if they don't like a picture".

Self-Preoccupation Impedes Social Life

These women need to focus to be able to attain their goals, and this need tends to exclude family and friends. Their self-centered behavior mainly revolves round routines related to food and exercise. A bodybuilder's lifestyle is tough and time-consuming. In addition to foodplanning and several workouts per day, most are employed and have to work. It is important to get enough sleep, and time for the family's activities as well as socializing is thus limited. One driving force is being able to show themselves and their families that they can succeed and that sacrifices have not been in vain. Having one's own experience and understanding of what it means to disappear into the "bubble" where only exercise and food exist also makes it easier to live with a bodybuilder.

One woman related what sometimes took place:

"…uh but we can do this tomorrow instead, let's go to the cinema tomorrow, I'm too tired today. So we plan to go tomorrow and then didn't make it…or you'd like to go to the park and then you feel too tired, maybe we can watch TV instead. So it was like, early mornings with the children when they were very small, a 1-year-old and a 2-year-old, it was winter and I'd go out on my morning walk with a twin stroller, putting two children in a stroller each with a comic at 4 in the morning and then going out and ploughing through the snow like. It's not much fun for two small children…"

Another woman told a different story:

"I didn't care very much about what happened to my ex and that the children chose to move. It became less important and didn't bother me that much anymore. I started to sleep well at night, and I was only living for my workouts and substances. In a way, it was like going into a "bubble" and completely ignoring what was happening outside the "bubble". Of course, I was a selfish person, 100 % selfish because I only thought of myself and maybe even ignored how the children were doing. I thought it was more important to exercise

than have time with my children, it was scary that it became so emotionless".

When empathy is lacking, conflicts, and/or an agitated mood can easily result. Sometimes, people get in the way of performance, and it is usually planned routines that are disturbed by others e.g., when exercising. Relationships may be perceived as demanding under stressful conditions. Daily routines such as controlling one's body can be time-consuming when one is preoccupied with oneself.

One woman compared her past with AAS and the present:

"In other words, nowadays I feel that I only need to look at myself in the mirror before leaving in the morning and then again when I get home. Nowadays, I might examine my face for specks during the day, but at that time it was totally insane. I mean, before that time, I've never ever liked to see myself naked or in lingerie, but then I almost had to, I wanted to take off most of my clothes so I could see every part of my body. I studied my body for about one hour every day, which is completely insane. And I liked what I saw, it was a strange feeling to love looking at yourself. Narcissus in other words, it was like a moment of love every day to be able to see myself naked".

Traveling, education, and time with the family get excluded when the focus is on bodybuilding. This conscious sacrifice may make these women wonder if it is worth missing "normal life." However, it may be difficult to create a new identity with less focus on muscles and to stop admiring what one has looked up to for so many years.

Living With Lies

The secret use of AAS requires women to live with lies. The fear of this secret use being revealed is constant, if the physical changes and side effects were to be noticed by others and lead to social consequences and penalties.

One woman admitted her concerns:

"I actually had nightmares that the Police would take me and my children would be alone. Then I stopped at once. That fear was horrible. But after a couple of years, I started again".

Women often ignore any side effects and hope that others will not notice them either. They try to protect themselves by hiding their physical development and disguising visible and invisible side effects e.g., they hide their bodies in larger clothes and/or try to avoid doping tests.

The use of AAS is not only illegal but also charged with taboo and must therefore take place in secret to reduce the risk of being exposed. Honesty is not possible when using AAS, because society is judgemental and condemns the use of AAS as cheating. Therefore, certain social situations have to be avoided. It is important to keep one's self-image as a good person untarnished. Being a good person means being physically fit and well-trained, disciplined and healthy, and not being a person who uses forbidden or illegal substances or has an "artificially built body." Exposure for using illegal or forbidden substances can generate severe feelings of shame. Lying is a tool to escape shame and avoid being confronted by disapproval and rejection. Only a few people, among the closest and most trusted, know about a person's use of AAS.

One woman confessed:

"After all, I was a personal trainer and role model for many people who admired my physique. I was a role model for them, and I know that everything would have collapsed if it had become widely known that I have injections regularly to look like I do".

Women sometimes withdraw to avoid questioning and non-accepting people who do not share the same values. For example, they hide with their lunch box in the toilet to follow their special diet in secret. The choice to spend time with people who share the same lifestyle is made easier by friends who do not understand the need to take one's own food to a party or decline to participate in activities because of the need to exercise.

One woman listed some of the questions she hears from other people:

"Can't you just eat normally?" or "Are you always going to do that?" or "Are you going to live like that for the rest of your life?, haven't you got a life?, aren't you going to live normally?, are you going to keep on like this forever?".

DISCUSSION

The women described perfectionist traits showing their expectations of achieving the perfect body. They were hard on themselves, driven by unhealthy ideals, sought confirmation, were self-critical and constantly saw their bodies' faults and shortcomings. Perfectionist behavior often involves excessively high demands (overcompensation, excessive control, correction) and the pursuit of flawlessness. These characteristics are also typical for elite athletes (Lemyre et al., 2008). According to Stoltz and Ashby (2007), there is a satisfaction and nothing wrong in trying to reach perfection (adaptive perfectionism). It is when a healthy striving gives way to self-imposed demands, self-critical evaluations of achievements and concerns about negative assessments (maladaptive perfectionism) that it can become unhealthy. The unhealthy part is not being able to accept oneself unless one is perfect, and constantly considering that one could do better (Lundh, 2004). Perfectionism has increased in recent decades. This may be due to individualistic and materialistic environments with greater competition, higher demands, and unrealistic expectations (Curran and Hill, 2019). International valuation surveys show that Sweden ranks high on the scale of values for individualism and self-expression (World Values Survey, 2015). Individuals in the Western world are positively committed to increasing their physical strength and to setting high goals (Lo et al., 2011).

In our research, the women described that achievement was important to them. The women had experiences of not feeling loved, not being good enough or not receiving recognition. Their concern for and fear of not peaking in performance made them strive even more and even harder. They needed to show others and themselves that they were self-disciplined

women. However, they never felt that they attained their goal even though they looked perfect to others. This feeling of non-attainment caused lack of self-esteem. Performance-based self-esteem (PBSE) (Hallsten et al., 2005) is a variant of low self-esteem that is based on the individual proving her human value through performance. It is based on a concern for not being good enough and the results achieved are an important measure of ability (Hallsten et al., 2005; Makower, 2018). PBSE indicates how self-esteem is created and how it is maintained, and is a concept linked to perfectionism (Hallsten et al., 2005). Individuals with high PBSE are often ambitious and base their value on external factors such as success and personal status. External confirmation becomes a compensation for their lack of self-esteem (Hallsten et al., 2005). Chasing achievements and positive feed-back from other people may lead to negative consequences such as stress and exhaustion (Svedberg et al., 2016). Individual characteristics as well as inadequate support in working life and from family have been showed to trigger PBSE (Blom, 2012).

The results show body dissatisfaction and strong focus on increasing muscle mass in the individuals interviewed. Body anxiety occurred when routines around exercise or diet were disturbed. Muscle dysmorphia (MD) is a form of body image disorder characterized by a preoccupation with muscularity and body image (Phillips et al., 2010). Individuals with MD describe a dissatisfaction with their bodies and a desire to be more muscular. Male bodybuilders report a greater incidence of MD where the focus is on strict diet, extremely heavy weight training and the use of AAS (Pope et al., 1997; Mitchell et al., 2017). Female bodybuilders probably tend to have the same risk of developing MD as men (Hale et al., 2013) but have been investigated to a very small extent (Gruber and Pope, 2000). Men with MD experience symptoms of anxiety when exposed to environments where the body can be seen (Olivardia et al., 2000).

Women live with lies because the use of AAS is forbidden and illegal and occurs in secrecy. Its exposure would therefore feel shameful. Also, people generally are unable to understand the sacrifices needed to reach the goals of bodybuilding. They hid their bodies or avoided certain social situations. In MD hiding a body are described as a part of the symptomatology (Pope et al., 1997) to not experience anxiety. This kind of anxiety was not seen in our results. Our view, however, leans toward that women are concealing their bodies due to fear of being exposed for illegal activity rather than fear of being judged for their appearance.

Developing muscles requires adequate, monitored food intake. Eating was itself a major reason why women started with weight training. A previous eating disorder made it easier for them to handle a strict diet. Diet increased their control over their bodies and reduced their anxiety. Unlike their previous experiences, eating became justified. Eating disorders have been described in male bodybuilders with MD (Pope et al., 1993; Mitchell et al., 2017), in female bodybuilders (Gruber and Pope, 2000; Phillips et al., 2010), in maladaptive perfectionism (Dahlenburg et al., 2019), and in athletes in weight control sports (Thompson and Sherman, 2014). Despite this, the fear existed of not eating right or being able to handle a controlled diet, as has been described previously in female bodybuilders (Gruber and Pope, 2000).

The women in this study were trying to create a balance for themselves with regard to their physical attributes, somewhere in the border area between what is considered masculine and what is considered feminine. Their distorted body perception made it difficult for them to apprehend how muscular they were. It is not easy to know the limit for side effects and when they will occur, a fear exists of being masculinised (Sverkersson et al., 2020). The advantages and disadvantages of AAS regarding masculinisation need to be weighed against each other and evaluated. This must be done because women are more vulnerable than men to the negative effects of AAS and are more susceptible to side effects (Strauss et al., 1985; Gruber and Pope, 2000). It was important for the women in this study to maintain their femininity and regulate the size of their muscles. Strong, muscular women are not perceived as feminine and are not an accepted norm in society. According to the social constructivist perspective, we are born into a society that constantly influences us where we relate to existing norms and conceptual frameworks (Goffman, 1959). We are brought up in different gender roles, for example, how we should dress, look and behave. Traditionally, the hegemonic understanding of the female body is weak and fragile in contrast to men's which is both big and strong. In addition, women's appearance should also appear natural (Goffman, 1977). When people are of the same opinion, a general perception is formed that leads to the creation of ideals. Body perception is influenced by the appearance ideals that exist in society (Goffman, 1959). Bodybuilding women have the potential to both accept and subvert the ideal (Tajrobehkar, 2016).

The results showed that women were concerned and had a fear of getting incorrect advice from others and being deceived into making the wrong choices. They seldom exchanged experiences with each another and were led by men who gave them advice on how to use AAS. The women only trusted their own knowledge and had a critical approach to information. The women's networks are smaller and newer when it comes to AAS, but it has been shown that they follow a similar path for acquiring knowledge as men (Henning and Andreasson, 2019). The discussions at the online forums, where women are also present, are usually dominated by men who share their experiences and give advice. Women prefer first-hand experiences from other women (Sverkersson et al., 2020) and not advice from men, this makes it more difficult to sort among information (Henning and Andreasson, 2019). Online communities only for women are though emerging (Andreasson and Henning, 2021), which enables for women to discuss their use of AAS from a female perspective (Sverkersson et al., 2020). There is a need for credible societal information from several perspectives, i.e., not only the negative aspects. It is also important to take women's experiences into account when designing targeted prevention strategies, especially when it comes to the fear of exposing their use.

Women with muscles challenge what is considered to be the sphere of men (Sverkersson et al., 2020). The women in this study are potential role models who, through their practice, go against the hegemonic view of femininity and norms of how women should be. They crush the notion of femininity as something fragile (Tajrobehkar, 2016) and possess a strength with

their efforts to change their bodies, beyond stereotypical gender configurations (Sverkersson et al., 2020).

There are not many women using AAS and it is a hard-to-reach population. A total of 12 interviews were included in this study. This may be seen as too few to allow any conclusions to be drawn or to be representative of all women using AAS (Polit and Beck, 2017). However, through the use of a phenomenological research approach with a lifeworld perspective, the data collection has contributed with meanings forming the basis for an analysis and generating an essential description of the phenomenon: women's use of AAS. The results thus constitute knowledge development for understanding women in general who use AAS.

The informants gave their descriptions based on their lived experiences. However, since the intake of AAS is illegal under Swedish law, there is an increased risk that the informants may have chosen their answers to protect themselves. One of the interviews took place in an undisturbed part of a café, this may also have affected the informant's way of answering. However, with the help of in-depth interviews and support for reflection, substantive meanings have emerged. In this way we believe that the material is truthful. In-depth interviews as a method have been shown to be a strength in order to gain a deeper understanding. The method is time-consuming which is a weakness and requires knowledge in interview technology.

CONCLUSIONS AND CLINICAL IMPLICATIONS

The overall knowledge in society about women's use of AAS is very low. Men's use of AAS is known to healthcare, but no deeper understanding or knowledge exist of the phenomenon in women. Women's use of AAS is a complex phenomenon. The results show that women experience a sense of pride when they are successful in their achievements. This is their driving force, triggering tension between suffering and success.

Understanding and knowledge are important in order to be able to meet women in their fears and difficulties. This research contributes knowledge that provides support to the development and improvement of prevention and treatment strategies, not only medically but also psychologically and socially. Gender needs to be considered when disseminating information. The law in Sweden imposes barriers since use is illegal. Because of this, anonymous telephone counseling is a good solution for providing support and advice.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethical approval was obtained from the Regional Ethics Committee at the Karolinska Institutet, Stockholm (nr. 2016/1762-31/5). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AB and VV designed the study. The interviews were carried out and transcribed by AB. All the authors have read and approved the final manuscript, and analyzed the data.

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Influence of Menstrual Cycle or Hormonal Contraceptive Phase on Physiological Variables Monitored During Treadmill Testing

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Purpose: To examine the influence of menstrual cycle (MC) and hormonal contraceptive (HC) cycle phases on physiological variables monitored during incremental treadmill testing in physically active women (eumenorrheic, EUM = 16 and monophasic HC-users, CHC = 12).

Methods: Four running tests to exhaustion were performed at bleeding, mid follicular (mid FOL)/active 1, ovulation/active 2, and mid luteal (mid LUT)/inactive. HC and MC phases were confirmed from serum hormones. Heart rate (HR), blood lactate (Bla), and $\dot{V}O_2$ were monitored, while aerobic (AerT) and anaerobic (AnaT) thresholds were determined. $\dot{V}O_{2peak}$, maximal running speed (RUN_{peak}), and total running time (RUN_{total}) were recorded.

Results: No significant changes were observed in $\dot{V}O_2$ or Bla at AerT or AnaT across phases in either group. At maximal effort, absolute and relative $\dot{V}O_{2peak}$, RUN_{peak}, and RUN_{total} remained stable across phases in both groups. No significant fluctuations in HR_{max} were observed across phases, but HR at both AerT and AnaT tended to be lower in EUM than in CHC across phases.

Conclusion: Hormonal fluctuations over the MC and HC do not systematically influence physiological variables monitored during incremental treadmill testing. Between group differences in HR at AerT and AnaT underline why HR-based training should be prescribed individually, while recording of MC or HC use when testing should be encouraged as phase may explain minor, but possibly meaningful, changes in, e.g., Bla concentrations or differences in HR response.

Keywords: endurance testing, menstrual cycle, hormonal contraceptives, aerobic testing, female physiology

INTRODUCTION

Incremental aerobic treadmill testing is an essential tool for determining cardiorespiratory fitness and/or monitoring training adaptations. Accordingly, test results are commonly used to evaluate, prescribe, and adjust training for athletes. Among commonly investigated physiological variables are peak oxygen uptake ($\dot{V}O_{2peak}$) as well as heart rate (HR), blood lactate concentration (Bla), respiratory exchange ratio (RER), ventilation rate (VE), peak running speed (RUN_{peak}), total running time (RUN_{total}), and rating of perceived exertion (RPE). Aerobic (AerT) and anaerobic (AnaT) thresholds are often determined (Aunola and Rusko, 1984, 1986) due to their importance in exercise prescription. Several factors can affect physiological variables and testing outcomes, such as training status, psychological state, glycogen stores, level of recovery, etc. Moreover, some level of biological variation is expected between repeated tests that may not be directly attributed to training or detraining (Bagger et al., 2003). For female athletes, it can be hypothesized that the fluctuation of endogenous sex hormones (e.g., estrogen, E2, and progesterone, P4) associated with the menstrual cycle (MC) or hormonal contraceptive (HC) use (i.e., exogenous hormones) might influence physiological variables and/or performance due to their non-reproductive actions on the cardiovascular system and substrate metabolism as described in the following paragraph.

A eumenorrheic MC is typically divided into two basic phases: the follicular phase (FOL), which is characterized by low concentrations of E2 and P4, and the luteal phase (LUT), which is characterized by high concentrations of E2 and P4 (Davis and Hackney, 2017). Ovulation commonly occurs between FOL and LUT and is marked by a surge in luteinizing hormone (LH) and E2, as well as a smaller surge in follicle-stimulating hormone (FSH). Monophasic HCs suppress hypothalamic-pituitary-ovarian (HPO) axis function (Elliott-Sale et al., 2013). In the skeletal muscle and cardiac tissues, E2 may cause vasodilation (Mendelsohn and Karas, 1999) that increases in parallel to E2 concentrations at rest (Kawano et al., 1996). During exercise between 40-100% maximal oxygen consumption (VO_{2max}), a tendency for increased plasma volume, and a concomitant increase in pulmonary diffusion capacity associated with an increase E2 may also be observed (Smith et al., 2015). Progesterone appears to attenuate the effects of E2 (Mendelsohn, 2002). Higher levels of P4 (i.e., mid LUT) in eumenorrheic women have been linked to increased HR, VE, and core temperature at rest, although MC phase (mid FOL versus mid LUT) does not appear to affect VE (MacNutt et al., 2012), HR, O_2 uptake, or CO_2 output during either submaximal or strenuous exercise (Jurkowski et al., 1981). Metabolically, E2 has been shown to spare glycogen and increase fat oxidation (Hackney, 1999) (reflected as lower Bla and RER values), although conditions in which P4 is also high (i.e., mid LUT) may mitigate these effects (D'Eon et al., 2002). Monophasic HCs do not appear to influence VO_{2max} or RUN_{total} (Bryner, 1996), however, they may induce a glycogen-sparing effect (Bonen et al., 1995). In rowers, power output, HR, $\dot{V}O_2$, CO_2 production, VE, mean RER, and ventilatory equivalents of O_2 and CO_2 did not differ between active and inactive HC phases (Vaiksaar et al., 2011); however, the active phase significantly increased VE, breathing frequency, and VE for O_2 and CO_2 in endurance trained women (Barba-Moreno et al., 2019). In theory, these observations regarding the effects of endogenous and exogenous reproductive hormones on skeletal muscle and cardiac tissues, metabolism, and cardiovascular responses suggest that MC phase and/or HC use could affect physiological variables monitored during incremental exercise testing. Indeed, the flux of hormones during the MC has been reported to affect cardiorespiratory function, training responses and adaptations, recovery from exercise (Ihalainen et al., 2019), and performance (Davies et al., 1991). In the context of exercise testing, Smekal et al. (2007) reported no effect of the MC on $\dot{V}O_{2peak}$, while Lebrun et al. (1995) reported a higher absolute $\dot{V}O_{2peak}$ in the early FOL than mid LUT, although these differences may be attributed, in part, to methodological approaches.

At present, the quality of evidence regarding the variation in physiological variables monitored during incremental treadmill testing and/or performance outcomes across the MC is relatively low (McNulty et al., 2020). This topic requires more research with improved methodological quality, including the confirmation of MC phases and reporting HC types and dosages used by participants. Similarly, the majority of research focusing on HC use is of low quality and investigates and compares the differences between HC use and non-use (Elliott-Sale et al., 2020a), rather than examining potential changes in physiological variables monitored during testing and/or performance over the HC. Although recent meta-analyses found trivial changes in performance across the MC (McNulty et al., 2020) and minor effects of HC use on performance, in general (Elliott-Sale et al., 2020a), it is essential to consider the large between-study variance as well (Elliott-Sale et al., 2020a; McNulty et al., 2020). Considering the contradictory findings described above as well as the importance of the physiological variables evaluated during incremental testing, in the context of sports training and monitoring, the purpose of this study was to investigate the influence of MC phase, and comparable time-points in the HC-cycle, on physiological variables monitored during incremental treadmill testing in physically active eumenorrheic and monophasic HC-using women. Based on previous observations, we hypothesized that small but possibly meaningful fluctuations in physiological parameters, such as decreased RER and lactate in the luteal phase, might be observed in eumenorrheic women. We also hypothesized that the measured physiological variables would remain relatively stable in women using monophasic HCs.

MATERIALS AND METHODS

Participants

Healthy women, age 18–40 years, were recruited by advertisements in the local newspaper and *via* social media. Before inclusion in the study, each prospective participant was asked to complete a health questionnaire and a Low Energy Availability in Females Questionnaire (LEAF-Q)

(Melin et al., 2014). Inclusion criteria required that participants be physically active (strength training 3 times·week^{-1} and endurance training 3 times·week^{-1}) with a BMI of 18–25 kg·m^{-2} and a LEAF-Q score <8. Participants were excluded if they were pregnant or lactating, if they had conditions affecting ovarian function, amenorrhea, endocrine disorders, or chronic diseases, or if they were taking medication that may have affected exercise responses. Participants received detailed information about the study design, measurements, and procedures before signing an informed consent document. Participants were aware that they could withdraw from the intervention at any time. The data presented are part of a larger endogenous and exogenous hormones and performance in women (MEndEx) study, which was approved by the Ethical Committee at the University of Jyväskylä, Finland on October 22, 2018.

A total of 33 women were enrolled in the study. Five participants dropped out prior to the completion of the study due to personal reasons or schedule conflicts. Data were ultimately analyzed and are presented for n = 28. Descriptive data (gathered at bleeding, see study design), including participant characteristics, are presented in **Table 1**. Participants included women who had an MC classified as eumenorrheic and had not used a HC for at least one year (EUM = 16) and women who had used a monophasic combined synthetic estrogen and progestin HC for at least one year (CHC = 12). The monophasic contraceptives used by participants are listed in **Table 2**.

Study Design

Four experimental testing sessions were completed by each participant over an individual MC or HC-cycle with the timing of testing illustrated in **Figure 1**. The phase of the MC or HC-cycle in which testing commenced was randomized. Procedures were performed according to current recommendations for best practice (Elliott-Sale et al., 2020b). Ovulation was identified using daily urine tests completed by the participant at home, starting mid FOL, to identify the LH surge (Dipro, LH Ovulation Strip, Aidian Oy, Finland). Ovulation was detected in all EUM participants and MC phases were retrospectively confirmed by analysis of serum hormones. Tests were scheduled 1–2 days after ovulation was detected. Cycle length (**Table 1**) was within clinical norms for all participants (Hampson, 2020). Menstrual bleeding and withdrawal bleeding are simply referred to as "bleeding" throughout the manuscript. For practical purposes, MC and HC-cycles were compared at bleeding, mid FOL/active 1, ovulation/active 2, and mid LUT/the inactive phase.

TABLE 1 | Participant information for the eumenorrheic (EUM) women and the monophasic hormonal contraceptive users (CHC).

	EUMn = 16	CHCn = 12
Age (years)	26 ± 4	23 ± 2
Body mass (kg)	67.9 ± 7.0	62.8 ± 5.1
Height (cm)	167.1 ± 5.6	170.0 ± 5.6
Body fat (%)	21.8 ± 6.6	19.2 ± 3.2
LEAF-Q (points)	3.8 ± 2.7	5.1 ± 1.8
Length of menstrual cycle (days)	28.3 ± 2.3	28

TABLE 2 | Brand and dosage of hormonal contraceptives used by participants in the monophasic hormonal contraceptive user group (CHC).

N	Brand name	Dose
5	Yaz, Tasminetta, Stefaminelle	0.02 mg ethinyl estradiol/3 mg drospirenone
3	Yasmin	0.03 mg ethinyl estradiol/3 mg drospirenone
1	Zoley	2.5 mg nomegestrol/1.5 mg estradiol
2	Vreya	0.035 mg ethinylestradiolum/2 mg cyproteron acetate
1	Nuvaring	0.120 mg etonogestrel/0.015 mg ethinyl estradiol

Incremental Treadmill Running Testing

A treadmill test was performed to assess physiological variables associated with aerobic capacity using a standard incremental protocol (Mikkola et al., 2007). A resting fingertip blood sample was taken for the analysis of resting Bla (EKF diagnostic, C-line system, Biosen, Germany). Treadmill incline remained constant at 0.5° for the entire test. Treadmill velocity was 6 km·h^{-1} for the first 3-min stage of the test and was increased by 1 km·h^{-1} every third min until volitional exhaustion. Fingertip blood samples, for the subsequent analysis of submaximal Bla, were taken between each stage when the treadmill was briefly stopped for 30-s. HR was recorded continuously using a HR monitor (Polar 800, *Polar Electro*, Kempele, Finland). Mean HR and $\dot{V}O_2$ values from the last 30-s of each stage were used for analysis. The $\dot{V}O_2$ was measured breath-by-breath using a portable gas analyzer (calibrated according to manufacturer instructions, Oxycon Mobile®, Jaeger, Hoechberg, Germany) and $\dot{V}O_{2peak}$ was defined as the highest average 30-s $\dot{V}O_2$ value. The AerT and AnaT were determined from Bla and gas exchange variables according to Aunola and Rusko (1986) a method previously shown to be reproducible and reliable (Aunola and Rusko, 1984). The RUN_{peak} was defined as the highest treadmill speed maintained for >30 s and RUN_{total} was measured from the start of the first stage until the participant reached exhaustion, when the treadmill was subsequently stopped. HR_{max} was recorded as the highest 5-s HR value. Testing was completed at the same time of day ±1 h to avoid the possible confounding effects of e.g., circadian rhythms. Furthermore, participants were instructed to refrain from strenuous exercise for the 24 h prior to testing.

Nutrition

Participants were instructed to maintain their typical diet throughout the study and to continue eating as they normally would, *ad libitum*. A 3-day food diary including the day before, day of, and day after aerobic testing was collected for each phase. Analysis of food diaries using software (Fineli, National Institute for Health and Welfare, Helsinki, Finland) indicated no significant differences in total energy or macronutrient intake between tests (see Ihalainen et al., 2021). Prior to testing, participants were instructed to avoid caffeine and to eat a typical light meal or snack roughly 3 h before the test. Strength testing, including maximal voluntary contractions and a loading of explosive leg press (2 × 10 at 60%1RM load with 2 min recovery between sets) was completed prior to endurance testing and was followed by 15-min rest. While strength testing may induce some fatigue, participants were accustomed to physical exertion

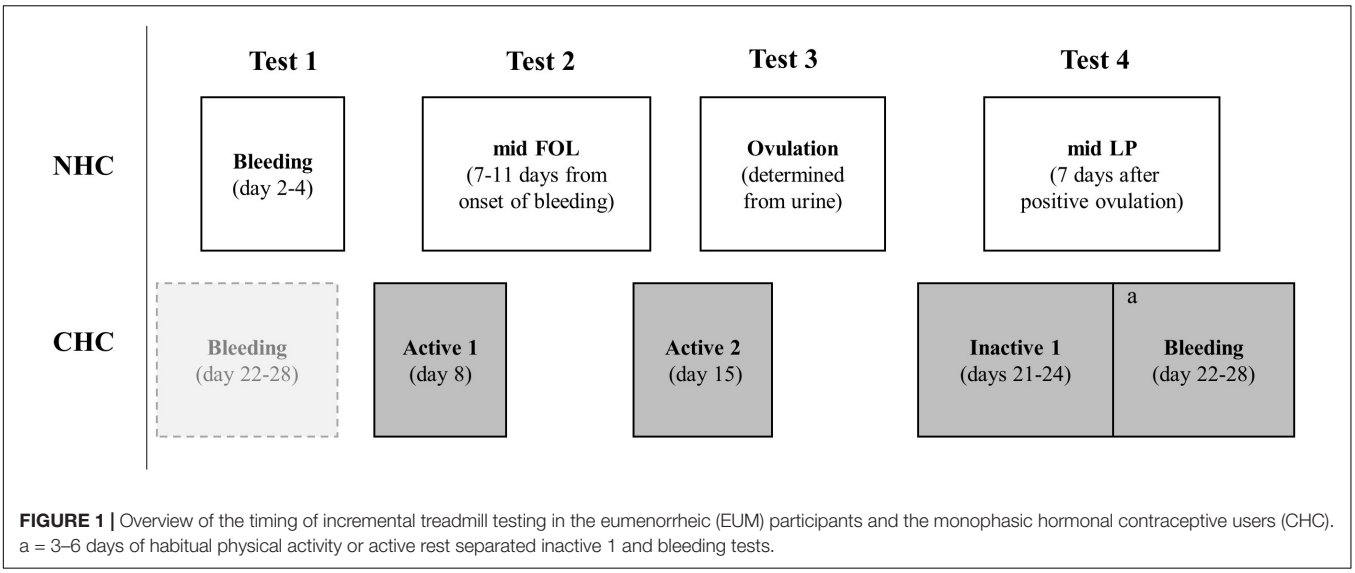

FIGURE 1 | Overview of the timing of incremental treadmill testing in the eumenorrheic (EUM) participants and the monophasic hormonal contraceptive users (CHC). a = 3–6 days of habitual physical activity or active rest separated inactive 1 and bleeding tests.

were adequately recovered upon commencement of endurance testing (lactate at pre was 1.37 ± 0.46 mmol·L^{-1} in EUM and 1.43 ± 0.41 mmol·L^{-1} in CHC). Participants were offered an energy bar (Isostar High Energy Sport Bar, multi fruit) and 1.5 dl of water prior to aerobic testing. If the participant chose to eat the energy bar (or part of the energy bar) prior to aerobic testing, this was repeated during all trials.

Body Composition

Body composition was assessed in a 12-h fasted state in the morning between 07:00 and 09:00 prior to aerobic testing using a multi-frequency bioelectrical impedance analyzer (InBody 720; Biospace, Seoul, South Korea) with participants wearing only underwear. To reduce the potential for influencing eating behaviors, participants were not given feedback regarding their body composition results until the study was completed.

Blood Samples

Blood samples were collected in a 12-h fasted state in the morning between 07:00 and 09:00 prior to testing. Samples were taken from the antecubital vein into serum tubes (9 ml Venosafe Gel + Clot activator tubes, Terumo Medical Co., Belgium and 6 ml Venosafe EDTA Tubes, Terumo Medical Co., Belgium). Each participant's basic blood count (analyzed from blood samples in the EDTA tubes by Sysmex KX-21N, Kobe, Japan) was evaluated for indication of acute illness/infection. The samples in the Gel + Clot activator tubes were centrifuged for 10 min at 2000 × *g* and a refrigerated temperature of +4°C (Heraeus Megafuge 1.0 R, Thermo Scientific, Karsruhe, Germany). The serum was separated and immediately frozen at −80°C for later analysis of E2, P4, LH, and FSH. Hormonal analyses were performed using chemical luminescence techniques (Immulite 2000) with an assay sensitivity of 55.0 pmol· L^{-1} for E2, 0.3 ng·ml^{-1} for P4, 0.05 mIU·L^{-1} for LH, and 0.10 IU·L^{-1} for FSH. Inter-assay coefficients of variation were 6.7% for E2, 9.7% for P4, 4.8% for LH, and 3.4% FSH.

Statistical Analysis

Bleeding, mid FOL and active 1, ovulation and active 2, and mid LUT and the inactive phase of the HC cycle were "matched" for the sake of reporting and analysis. Mean values and standard deviations (±SD) were calculated using standard methods. Statistical analyses were completed using IBM SPSS Statistics 26.0 (IBM Corporation, IBM SPSS Statistics for Windows, Armonk, New York, United States). Data was normally distributed. A factorial mixed design ANOVA, including 1 between-subject factor (groups: EUM and CHC) × 1 within-subject factor (phase: bleeding, mid FOL/active 1, ovulation/active 2, and mid LUT/the inactive phase) was performed. In the presence of a main effect for phase or group, simple main effects (the effect of phase on EUM or CHC groups alone or the effect of group over phase) pooled for error term *via* MANOVA were completed to determine if the main effect can be justifiably interpreted. In the presence of an interaction, 2 × 2 mixed design ANOVAs were performed to identify where the interaction resides along the independent variable (phase). Mauchly's test was used to test the assumption of sphericity. Where this assumption was violated, Greenhouse–Geisser adjustments were applied. Statistical significance was set at $p \leq 0.05$. Due to the sample size being <20, effect sizes were estimated using Hedges' *g* where values of <0.25, 0.25–0.5, 0.5–1.0, and >1.0 were interpreted as trivial, small, medium, and large, respectively.

RESULTS

Female Reproductive Hormones During the Menstrual and Hormonal Contraceptive Cycles

Serum hormone concentrations measured for EUM and CHC in the present study (**Table 3**) are reflective of normal eumenorrheic MC and HC-cycles, respectively.

TABLE 3 | Serum concentrations of estradiol (E2), progesterone (P4), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) across the four cycle phases for the non-hormonal contraceptive (EUM) and hormonal contraceptive (CHC) groups.

	EUM				CHC				ANOVA		
	Bleeding	**Mid FOL**	**Ovulation**	**Mid LUT**	**Bleeding**	**Active 1**	**Active 2**	**Inactive**	**Phase**	**Group**	**Phase × group**
E2 ($pmol \cdot L^{-1}$)	285 ± 139	537 ± 381	689 ± 479 *	669 ± 233 *	300 ± 270	190 ± 138	217 ± 234 *	189 ± 108 *	$F = 2.575$ $p = 0.084$	**$F = 12.340$ $p = 0.002$**	**$F = 3.519$ $p = 0.035$**
P4 ($nmol \cdot L^{-1}$)	1.94 ± 1.64	1.02 ± 0.43	4.08 ± 2.58 *	14.77 ± 8.4 *	1.05 ± 0.52	1.00 ± 0.48	1.14 ± 0.97 *	1.18 ± 1.01 *	**$F = 16.986$ $p < 0.001$**	**$F = 21.392$ $p < 0.001$**	**$F = 15.572$ $p < 0.001$**
LH ($mIU \cdot L^{-1}$)	5.77 ± 3.32	6.85 ± 2.77 *	14.16 ± 13.04 *	4.32 ± 2.80	3.75 ± 2.87	2.97 ± 3.17 *	1.79 ± 2.08 *	2.54 ± 2.42	$F = 3.386$ $p = 0.069$	**$F = 7.813$ $p = 0.011$**	**$F = 5.000$ $p = 0.028$**
FSH ($IU \cdot L^{-1}$)	5.43 ± 2.48	6.62 ± 2.73 *	6.82 ± 2.99 *	2.81 ± 1.23	4.36 ± 2.24	2.34 ± 1.81 *	1.82 ± 1.61 *	2.86 ± 2.40	**$F = 6.048$ $p = 0.001$**	**$F = 10.458$ $p = 0.004$**	**$F = 7.523$ $p < 0.001$**

Values are presented as mean ± SD and significant ANOVA findings are denoted in bold. Simple main effects for phase were observed for E2, P4, LH, and FSH in EUM ($F = 7.62$, $p < 0.001$; $F = 41.56$, $p < 0.001$; $F = 10.42$, $p < 0.001$; and $F = 13.75$, $p < 0.001$) but not in CHC ($F = 0.11$, $p = 0.956$; $F = 0.02$, $p = 0.995$; $F = 0.19$, $p = 0.903$; and $F = 2.31$, $p = 0.085$). Simple main effects for group at each phase are denoted by "" in the table ($p < 0.05$).*

Incremental Treadmill Running Testing

Body mass, physiological and aerobic performance test variables are presented in **Table 4** for EUM and CHC. No within-group or between-group differences were observed in body mass.

Aerobic Threshold

The Bla, $\dot{V}O_2$ and HR relative to HR_{max} associated with AerT remained stable over phases in EUM and CHC and no group differences were observed between these variables. A main effect for group was observed in HR at AerT ($p = 0.026$, partial $\eta^2 = 0.205$) indicating that when HR at AerT across phase for each group is considered there may be significant difference between groups over phase. Analysis of simple main effects indicate a tendency for HR at AerT means to be lower in EUM than CHC (bleeding, $p = 0.030$, $g = 0.94$, *medium*; mid-FOL/active 1, $p = 0.020$, $g = 0.82$, *medium*; ovulation/active 2, $p = 0.044$, $g = 0.5$, *medium*; and mid LUT/inactive, $p = 0.051$, $g = 0.61$, *medium*).

Anaerobic Threshold

The Bla and $\dot{V}O_2$ associated with AnaT remained stable over phases in EUM and CHC and no differences between groups were observed. A main effect for group was observed in HR at AnaT ($p = 0.030$, partial $\eta^2 = 0.197$) indicating that when HR at AnaT across phase for each group is considered there may be significant difference between groups over phase. Analysis of simple main effects indicate a tendency for HR at AnaT means to be lower in EUM than CHC (bleeding, $p = 0.015$, $g = 1.13$, *large*; mid-FOL/active 1, $p = 0.016$, $g = 0.83$, *medium*; ovulation/active 2, $p = 0.067$, $g = 0.45$, *small*; and mid LUT/inactive, $p = 0.094$, $g = 0.59$, *medium*).

When HR was analyzed relative to HR_{max} at AnaT a significant phase x group interaction was observed ($p = 0.022$, partial $\eta^2 = 0.135$). This interaction was determined to reside between bleeding and mid LUT/inactive ($p = 0.14$, partial $\eta^2 = 0.237$). In addition, a significant main effect for group ($p = 0.017$, partial $\eta^2 = 0.232$) indicating that when HR was analyzed relative to HR_{max} at AnaT across phase for each group is considered there may be significant difference between groups over phase. Further analysis revealed a tendency for HR relative to HR_{max} at AnaT to be lower in EUM than CHC (bleeding, $p = 0.031$, $g = 1.15$, *large*; mid-FOL/active 1, $p = 0.005$, $g = 1.00$, *large*; ovulation/active 2, $p = 0.010$, $g = 1.00$, *large*, mid LUT/inactive, $p = 0.800$, $g = 0.50$, *medium*). The main effect for group observed in HR relative to HR_{max} at AnaT appears to be meaningful even in the presence of the significant interaction due to the observation that CHC generally has higher means of HR relative to HR_{max} at AnaT than EUM.

Maximal Effort

At maximal effort (just prior to volitional exhaustion), a main effect for phase ($p = 0.045$, partial $\eta^2 = 0.114$) was observed in Bla indicating that when both groups are considered there may be significant fluctuation in Bla over phase. Simple main effects for EUM ($p = 0.020$) and CHC ($p = 0.120$) indicate that this main effect could be driven by significant fluctuation in Bla in EUM, however, further evaluation of EUM over phase revealed no statistically significant differences between phases in Bla.

Heart rate values measured at maximal effort were unchanged across phases in EUM and CHC and no between-group differences were observed. Both absolute and relative $\dot{V}O_{2peak}$ remained unchanged across phases in EUM and CHC while no difference between groups was observed. No statistically significant differences between phases or groups were observed for RUN_{peak}, or RUN_{total} (**Table 4**). **Figure 2** shows individual percent change from bleeding for RUN_{total} and relative $\dot{V}O_{2peak}$.

DISCUSSION

In the present study, incremental treadmill testing was performed at four hormonally unique time-points over a MC, and comparable time points during a HC-cycle, in order to elucidate the possible influence of reproductive hormones on physiological variables typically measured and monitored during incremental treadmill testing. Our data suggest that MC and HC phases do not systematically influence physiological variables monitored during incremental treadmill testing. Likewise, testing outcomes are not influenced by MC or HC phase although recording MC phase or

TABLE 4 | Heart rate, blood lactate, and $\dot{V}O_2$ at aerobic and anaerobic thresholds as well as maximal effort across the four cycle phases for the eumenorrheic (EUM) and the hormonal contraceptive (CHC) participants.

	EUM				CHC				ANOVA		
	Bleeding	**Mid FOL**	**Ovulation**	**Mid LUT**	**Bleeding**	**Active 1**	**Active 2**	**Inactive**	**Phase**	**Group**	**Phase × group**
Body mass (kg)	67.1 ± 7.8	66.4 ± 7.9	66.7 ± 8.2	66.6 ± 7.6	63.5 ± 6.0	63.1 ± 6.1	63.3 ± 8.2	63.5 ± 5.8	$F = 1.714$ $p = 0.174$	$F = 3.869$ $p = 0.063$	$F = 0.983$ $p = 0.392$
Blood glucose (mmol·L^{-1})	5.0 ± 0.3	5.1 ± 0.4	5.0 ± 0.4	4.9 ± 0.4	4.8 ± 0.4	4.7 ± 0.4	4.9 ± 0.4	4.9 ± 0.3	$F = 0.164$ $p = 0.921$	$F = 1.081$ $p = 0.309$	$F = 2.073$ $p = 0.112$
Aerobic threshold											
Heart rate (bpm)	154 ± 14 *	155 ± 13 *	156 ± 12 *	156 ± 11 +	166 ± 11 *	165 ± 11 *	162 ± 12 *	163 ± 12 +	$F = 0.196$ $p = 0.899$	**$F = 5.678$** **$p = 0.026$**	$F = 1.022$ $p = 0.388$
% of HR_{max} (%)	81 ± 7	81 ± 6	82 ± 5	82 ± 4	86 ± 4	85 ± 4	84 ± 5	84 ± 4	$F = 0.078$ $p = 0.972$	$F = 3.705$ $p = 0.067$	$F = 1.490$ $p = 0.225$
Blood lactate (mmol·L^{-1})	1.96 ± 0.64	2.03 ± 0.62	1.92 ± 0.75	1.80 ± 0.56	1.54 ± 0.35	1.82 ± 0.46	1.65 ± 0.35	1.73 ± 0.40	$F = 1.850$ $p = 0.528$	$F = 1.072$ $p = 0.312$	$F = 0.528$ $p = 0.664$
$\dot{V}O_2$ (ml·kg^{-1}·min^{-1})	31.6 ± 6.8	32.8 ± 6.9	32.4 ± 6.9	33.0 ± 4.7	34.1 ± 3.8	34.4 ± 5.7	34.1 ± 4.5	33.2 ± 4.6	$F = 1.473$ $p = 0.246$	$F = 0.020$ $p = 0.889$	$F = 0.307$ $p = 0.717$
Anaerobic threshold											
Heart rate (bpm)	175 ± 8 *	176 ± 8 *	176 ± 8 +	178 ± 8	184 ± 8 *	183 ± 9 *	180 ± 10 +	183 ± 9	$F = 1.538$ $p = 0.213$	**$F = 5.391$** **$p = 0.030$**	$F = 1.827$ $p = 0.151$
Percentage of HR_{max} (%)	92 ± 3 *	92 ± 2 *	92 ± 2 *	93 ± 2 +	95 ± 2 *	94 ± 2 *	95 ± 2 *	94 ± 2 +	$F = 0.773$ $p = 0.513$	**$F = 6.629$** **$p = 0.017$**	**$F = 3.437$** **$p = 0.022$**
Blood lactate (mmol·L^{-1})	4.02 ± 0.85	4.09 ± 1.00	3.78 ± 0.4	4.01 ± 0.87	3.89 ± 0.83	3.52 ± 0.83	3.49 ± 0.83	3.82 ± 0.55	$F = 1.850$ $p = 0.147$	$F = 1.072$ $p = 0.312$	$F = 0.528$ $p = 0.664$
$\dot{V}O_2$ (ml·kg^{-1}·min^{-1})	39.1 ± 5.4	39.7 ± 6.8	39.8 ± 5.5	40.7 ± 4.2	40.5 ± 4.3	41.2 ± 4.9	40.4 ± 4.2	39.0 ± 4.5	$F = 0.133$ $p = 0.854$	$F = 0.328$ $p = 0.575$	$F = 1.198$ $p = 0.312$
Maximal effort											
Heart rate (bpm)	190 ± 8	192 ± 8	191 ± 8	191 ± 9	195 ± 10	194 ± 9	192 ± 10	193 ± 13	$F = 1.661$ $p = 0.184$	$F = 1.855$ $p = 0.187$	$F = 2.763$ $p = 0.065$
Blood lactate (mmol·L^{-1})	11.2 ± 3.4	11.4 ± 3.3	11.3 ± 3.1	10.4 ± 2.2	9.6 ± 2.3	10.2 ± 2.7	8.9 ± 2.7	9.7 ± 2.9	**$F = 2.828$** **$p = 0.045$**	$F = 0.879$ $p = 0.359$	$F = 2.456$ $p = 0.071$
$\dot{V}O_{2peak}$ (ml·kg^{-1}·min^{-1})	44.1 ± 5.8	46.4 ± 6.4	46.7 ± 5.3	46.1 ± 5.4	44.1 ± 4.8	47.2 ± 4.4	45.5 ± 3.6	44.9 ± 4.5	$F = 0.552$ $p = 0.649$	$F = 0.849$ $p = 0.368$	$F = 0.249$ $p = 0.862$
$\dot{V}O_{2peak}$ (L·min^{-1})	2.97 ± 0.37	3.00 ± 0.40	3.00 ± 0.45	3.02 ± 0.35	2.81 ± 0.38	2.84 ± 0.32	2.77 ± 0.30	2.80 ± 0.34	$F = 1.933$ $p = 0.135$	$F = 1.799$ $p = 0.197$	$F = 0.314$ $p = 0.815$
RUN_{peak} (km·h^{-1})	14.8 ± 1.4	15.1 ± 1.5	15.1 ± 1.4	14.9 ± 1.4	14.5 ± 1.3	14.9 ± 1.4	14.9 ± 1.3	14.8 ± 1.6	$F = 0.675$ $p = 0.570$	$F = 0.737$ $p = 0.400$	$F = 1.910$ $p = 0.136$
RUN_{total} (min:s)	27:50 ± 4:31	29:19 ± 4:29	29:06 ± 4:11	28:25 ± 4:02	27:18 ± 3:14	27:31 ± 4:00	27:26 ± 3:58	27:55 ± 4:23	$F = 0.479$ $p = 0.698$	$F = 1.257$ $p = 0.274$	$F = 1.526$ $p = 0.216$

Values are presented as mean ± SD and significant findings are denoted in bold. Simple main effects for group at each phase are denoted by "" in the table ($p < 0.05$) and trends are indicated by "+".*

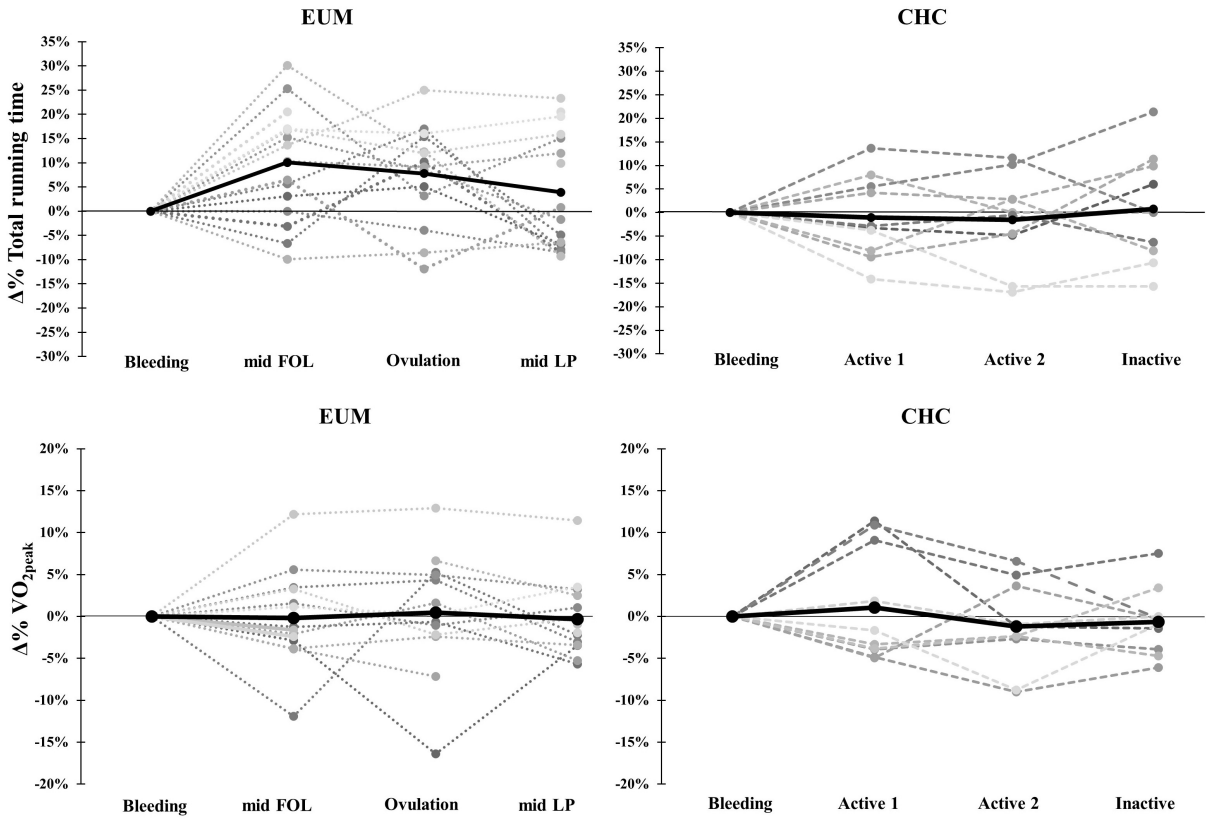

FIGURE 2 | Individual Δ% changes in total running time and $\dot{V}O_{2peak}$ in eumenorrheic (EUM) participants and monophasic hormonal contraceptive users (CHC). The solid black line represents the group mean.

HC use may be useful for interpretation of results at an individual level when performing incremental aerobic testing. In the present study a tendency for HR to be lower in EUM than CHC users at AerT and AnaT was observed.

Menstrual Cycle

The present results suggest that endogenous reproductive hormonal fluctuation characteristic of the MC in eumenorrheic women does not cause significant changes in body mass, maximal performance (RUN_{peak} or RUN_{total}) or physiological responses (HR, Bla, or $\dot{V}O_2$) at AerT, AnaT, and maximal effort. With regard to body mass fluctuations in relation to endogenous hormonal profiles, research is equivocal with reports suggesting both significant fluctuation (Tomazo-Ravnik and Jakopiè, 2006) as well as no changes.

The lack of significant MC phase-based fluctuation in absolute HR are in line with Jurkowski et al. (1981), who reported similar responses of HR, cardiac output, and stroke volume during both incremental and steady-state cycling exercise in the mid FOL and mid LUT phases, concluding that central cardiovascular response to exercise is not influenced by MC phase. Similarly, Freemas et al. (2021) did not observe any differences in HR between mid FOL and mid LUT during an 8-km cycling time-trial. These findings are, however, not consistent with Rael et al. (2021) who reported lower HR early in FOL compared to late FOL (coinciding with the LH surge indicating ovulation) during high-intensity interval training (8 × 3-min bouts at 85% of maximal aerobic speed with 90-s recovery at 30% of maximal aerobic speed) in endurance-trained women with eumenorrheic cycles. Likewise, a lower HR was observed in FOL compared to LUT during 40 min of running at 75% of individual maximal aerobic speed by Barba-Moreno et al. (2019), an observation attributed to the higher core temperature associated with LUT that is suggested to increase cardiovascular strain (Lebrun, 1993).

The stability of absolute and relative $\dot{V}O_{2peak}$ (Dean et al., 2003; Smith et al., 2015) and submaximal $\dot{V}O_2$ observed at AerT and AnaT over the MC appear to be consistent with previous research. Plasma volume and pulmonary diffusion capacity are reported to increase during exercise when E2 concentrations increase, such as in LUT, however, this does not appear to affect $\dot{V}O_{2peak}$ (Smith et al., 2015). Freemas et al. (2021) did not observe differences in absolute $\dot{V}O_2$, $\dot{V}CO_2$, or VE during an 8-km cycling time-trial between mid FOL and mid LUT. In contrast, Barba-Moreno et al. (2019) observed a higher submaximal $\dot{V}O_2$ at mid FOL versus early FOL during running at 75% of maximal aerobic running speed in endurance trained women. Similarly, they reported that tidal volume and ventilatory equivalents of O_2 and CO_2 during LUT were higher during mid FOL versus early FOL, suggesting that cardiorespiratory efficiency may be lower during mid FOL (Barba-Moreno et al., 2019). The differences between

these findings may be related to methodological approaches as physiological demands, e.g., 8 km cycling time-trial testing at mid FOL and mid LUT in Freemas et al. (2021); continuous submaximal running at early and mid FOL as well as during LUT in Barba-Moreno et al. (2019); and incremental treadmill running to volitional exhaustion in the present study differ. Furthermore, the timing of measurements within MC phases is not consistent between studies.

The stability in Bla concentrations at AerT and AnaT over the MC observed in the present study are in agreement with Mattu et al. (2020), who reported no differences in Bla after maximal exercise or 30-min constant-load cycling trials between mid FOL and mid LUT. Likewise, no changes in lactate threshold (comparable to AnaT) were observed between early FOL, mid FOL, and mid LUT phases of the MC during a graded, maximal exercise test (Dean et al., 2003) and no differences in Bla were observed between mid FOL and late LUT in eumenorrheic participants during 20-s of anaerobic exercise followed by 100-s of aerobic exercise (Lynch and Nimmo, 1998). Research indicates that fluctuations in E2 and P4 might influence metabolic substrate use including fat oxidation (Jurkowski et al., 1981; Hackney et al., 2007) where lipid metabolism during LUT may be enhanced, particularly at lower intensities (Hackney et al., 1994). This observation, generally reflected in lower RER and Bla values suggests a glycogen sparing effect (Hackney, 1999) that may be advantageous for endurance type exercise (Nicklas et al., 1989). Feeding may be a factor mediating the differences observed in substrate metabolism over phase, as carbohydrate supplementation is reported to mitigate differences observed between LUT and FOL in rates of glucose appearance and disappearance as well as total contribution of carbohydrate to energy expenditure (Campbell et al., 2001). It is possible that the sports bar consumed prior to aerobic testing (see section "Materials And Methods") influenced Bla in the present study, although our approach was standardized for each participant, and practically speaking, it is unlikely for an athlete to perform such incremental treadmill testing in a fasted state.

Hormonal Contraceptive Cycle

The present results suggest that exogenous reproductive hormone use (monophasic combined HC use) and the resulting endogenous hormonal milieu does not cause significant changes in body mass, maximal performance (RUN_{peak}, RUN_{total}) or physiological responses (HR, Bla, or $\dot{V}O_2$) at AerT, AnaT, or maximal effort, across the HC cycle. Monophasic HCs, such as those used by participants in the present study (see **Table 2**), provide a relatively stable hormonal condition for 21–24 active days followed by 4–7 hormone-free (inactive) days (Schlaff et al., 2004), although endogenous hormonal profiles may vary considerably between individuals even when HCs employ the same mechanism of action (Elliott-Sale et al., 2013).

The relative stability of submaximal (AerT and AnaT) HR across the HC-cycle is consistent with the findings of Barba-Moreno et al. (2019) and Mattu et al. (2020), who examined the difference between active and inactive phases of HC use by means of a steady-state endurance and a maximal lactate steady-state and ramp-incremental tests, respectively. The lack of significant differences in Bla at AerT and AnaT across the HC-cycle are also in agreement with Mattu et al. (2020), who observed no differences in Bla after maximal exercise between inactive and active pill phases. In contrast, Lynch and Nimmo (1998) observed a higher peak Bla within one week of taking HC compared to a test performed one week later in HC users performing intermittent exercise [20-s of anaerobic exercise followed by 100-s of aerobic exercise (Lynch and Nimmo, 1998)].

The lack of differences in submaximal HR and $\dot{V}O_2$ between active and inactive HC phases are in agreement with those of Rechichi et al. (2009), in which HC phase was not observed to have a systematic effect on 1-h cycling performance in female athletes, although cyclic variation in other ventilatory variables was noted (Rechichi et al., 2009). Likewise, Vaiksaar et al. (2011) found no differences between "phases" (HC-cycle day 8 $\pm$ 3 versus day 20 $\pm$ 2) in terms of power output, HR, $\dot{V}O_2$, CO_2 production, VE, mean RER or ventilatory equivalents of O_2 and CO_2 in rowers who were using a monophasic HC (Vaiksaar et al., 2011). In contrast, significant increases in VE, breathing frequency, and ventilatory equivalents for O_2 and CO_2 were observed in the active (hormonal) phase versus the inactive phase by Barba-Moreno et al. (2019), who suggested slightly decreased cardiorespiratory efficiency during active HC phases versus the inactive phase.

Group Differences

The primary purpose of the present study was not to compare groups, however, it is worth noting that in this relatively homogeneous population HR at AerT and AnaT (i.e., submaximal intensities) tended to be higher in CHC than EUM, a difference that was accompanied by medium to large effect sizes. These differences were also present when AnaT was analyzed relative to HR_{max}, where effect sizes were medium or large. This latter finding should be interpreted with caution as HR relative to HR_{max} is not based off a fixed HR_{max}, but testing day HR_{max}, which shows great SDs at maxial effort. The difference in absolute HR observed between CHC and EUM may be explained by E2. Increased concentrations of E2 are known to increase vasodilation and decrease vascular resistance, resulting in decreased HR and blood pressure where E2 concentrations are generally higher in EUM than in CHC. No difference in HR is observed at maximal effort, presumably because HR_{max} is determined to a large extent by age rather than sex (endogenous hormone concentrations) or even training status (Tanaka et al., 2001). These findings emphasize the necessity to prescribe HR-based training individually.

Strengths and Weaknesses

The present study included several strengths and weaknesses. Our participants were relatively homogeneous in terms of age while all participants were physically active rather than sedentary. Our inclusion of both EUM and CHC in this investigation provides additional perspectives to scientists and practitioners while our findings illustrate potential differences between EUM and CHC in terms of hormonal profiles over the MC and HC-cycles while demonstrating how endocrine fluctuations might influence variables used to monitor and assess aerobic fitness. The

study design was rigorous and included four time-points rather than the usual two or three used in many studies. Furthermore, in order to mitigate the influence of the learning effect, the order of starting the tests was randomized, although this did mean that parts of two consecutive MCs or HCs may have been used rather than a single MC or HC-cycle. Nevertheless, we incorporated both prospective determination of MC phases as well as retrospective confirmation of both MC and HC phases according to current recommendations for best practice (Elliott-Sale et al., 2020b). Furthermore, we collected nutritional data and strived to perform testing in a standardized fed state as well as at the same time of day (Ihalainen et al., 2021). We must acknowledge that different HC formulations and dosages individually affect endogenous hormonal profiles (Elliott-Sale et al., 2013), while also recognizing that eumenorrheic cycles display variation (MacNutt et al., 2012). More frequent hormonal sampling and the use of different kinds of aerobic performance testing could further help to elucidate the possible influence of endogenous hormonal profile on physiological variables monitored in testing. We also acknowledge that a plethora of other endogenous and exogenous factors including, but not limited to neuromuscular performance, nutrition, sleep, and motivation, may have a greater influence on physiological variables monitored during testing as well as test outcomes than MC or HC phase alone.

CONCLUSION

This study provides evidence that endogenous or exogenous-induced hormonal fluctuations over MC and HC-cycles do not systematically influence physiological variables used to assess aerobic fitness, and do not significantly affect the interpretation of incremental treadmill running tests of young, healthy, physically active women. The observed differences in HR at AerT and AnaT observed between EUM and CHC should be noted and used as a reminder that HR-based training be prescribed individually. Athletes, coaches, and researchers are encouraged to record MC or HC-cycle phase when completing testing and to consider MC phase particularly when analyzing or comparing physiological responses at AerT and AnaT (e.g., Bla) over a series of tests as individual differences in performance over phase are possible and subjective feelings related to MC or HC phase may be of importance. Further investigation of individual hormonal profiles and their effects on individual variables related to performance may be warranted.

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 Ethics Committee of the University of Jyväskylä, Jyväskylä, Finland. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JI, RT-M, and AH contributed to conception and design of the study. RT-M and AR performed the statistical analysis. RT-M, AR, HP, and JI wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Klotho Polymorphism in Association With Serum Testosterone and Knee Strength in Women After Testosterone Administration

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Administration of testosterone (T) is associated with increased serum T concentrations and improved physical performance in women. However, the inter-individual variation in T concentrations after T treatment is large and may in part be due to genetic variations. Serum T, as well as dihydrotestosterone (DHT), androstenedione (A) and the T/A ratio have been suggested as promising doping biomarkers for testosterone intake. Here, polymorphisms in androgen metabolic enzyme genes have been investigated in healthy women prior to and after 10 weeks administration of testosterone cream. Klotho is a protein that has been associated with anaerobic strength and here a genetic variation in klotho gene was studied in relation to performance as measured by isokinetic knee strength, as well as to serum androgen disposition. The AKR1C3 genotype (rs12529) was associated with serum T levels at baseline, whereas serum concentrations post T treatment did not differ between genotypes. The SLCO2B1 (rs12422149) and UGT2B17 deletion polymorphisms were not associated with serum concentration of either T, DHT or A. The klotho polymorphism (rs9536314) was associated with serum concentrations of both total T and T/A ratio after T administration. Individuals with the GT genotype increased T concentrations and T/A ratio more than women homozygous for the T allele. No significant difference in the association of klotho genotype with knee muscle strength was observed between placebo and T treatment. However, individuals homozygous for the T allele showed higher isometric mean torque scores at exit than GT subjects after T administration. This is the first time a genotype has been associated with androgen concentrations after T administration and muscle strength in women. Our results imply that subjects with a polymorphism in klotho may be more prone to detection using serum T and A as biomarkers.

Keywords: testosterone, doping, AKR1C3, UGT2B17, SLCO2B1, knee strength, klotho

INTRODUCTION

Androgens are considered beneficial for athletic performance (Bhasin et al., 2001) (Eklund et al., 2017) and are therefore forbidden in sport. Administration of supra-physiological doses of testosterone (T) are known to increase muscle size and strength in men, (Bhasin et al., 1996) (Rogerson et al., 2007), and recently it was shown that 10 weeks transdermal T application in young, healthy women was associated with enhanced physical performance as assessed by running time to exhaustion whereas anaerobic physical performance was unchanged (Hirschberg et al., 2020).

It is possible that additional factors rather than a direct increase in T may contribute to anaerobic physical performance as assessed by e.g., knee strength. The klotho protein, often described as an "anti-age" protein has been associated with muscle strength in middle-aged and elderly populations (Koyama et al., 2015) (Amaro-Gahete et al., 2019). A functional T>G single nucleotide polymorphism (rs9536314) in the klotho gene has been associated with loss of enzymatic activity (Arking et al., 2002). It is possible that genetic variation in the klotho gene reflects anaerobic physical strength women, but this has not been studied before. Interestingly, klotho has been associated with T and dehydroepiandrosterone (DHEA) serum levels in healthy middle-aged adults (Dote-Montero et al., 2019) indicating a connection between androgens and klotho.

To test for doping with endogenous anabolic androgenic steroids (EAAS), urinary testosterone (T) and epitestosterone (E) are quantified, and a T/E ratio >4 is an indicative marker for T administration. Since 2016, the T/E ratio, as well as four additional urinary steroid ratios, are monitored in the athlete biological passport (ABP). The implementation of the ABP passport has considerably increased the chances to detect T doping both in men (Strahm et al., 2015) (Nair et al., 2020) and women (Elings Knutsson et al., 2021) (Salamin et al., 2021). Still some athletes may escape the detection radar, particularly women, and a future complementary testing approach may consider to also monitor serum steroids in an endocrine module of the ABP (Salamin et al., 2020) (Elings Knutsson et al., 2021). The androgens T, dihydrotestosterone (DHT), androstenedione (A) as well as T/A ratio have been suggested to function as biomarkers for testosterone intake (Salamin et al., 2021).

Notably, there is large inter-individual variability in serum T levels after transdermal administration of T in healthy males (Mullen et al., 2018) and females (Elings Knutsson et al., 2021). The reason behind the large differences in absolute serum concentrations and the fold increase post T administration may partly be due to genetic variations in genes coding for proteins/enzymes involved in steroidogenesis and androgen metabolism. Aldo-keto-reductase 1C3 (AKR1C3) is a promiscuous enzyme that participates in the biosynthesis and metabolism of a variety of substrates including androgens (Penning et al., 2000). A C>G polymorphism (rs12529) resulting in a histidine to glutamine change (H5Q) has been associated with cancer risk (Vaidyanathan et al., 2017), and men homozygous for the C-allele had higher serum T levels than G-carriers after T administration (Bhasin et al., 2018). Organic anion-transporting polypeptide (OATP) 1B1, an important drug transporter that mediates the hepatic uptake of many compounds, is encoded by the SLCO1B1 gene. The G>A polymorphism (s12422149), leading to amino acid change arginine to glutamine (R312Q), has been associated with higher T serum concentrations, both at baseline and after T administration in men (Schulze et al., 2012). Regrettably, neither the AKRIC3 nor SLCO2B1 SNPs have been studied in relation to T applications in women.

It is well known that the urinary steroid profile is highly influenced by a deletion polymorphism of the uridine diphospho-glucuronosyl transferase 2B17 (UGT2B17) gene, the main enzyme involved in T glucuronidation (Jakobsson et al., 2006). Individuals devoid of UGT2B17 are not reaching the population-based T/E cut-off of four after T administration (Schulze et al., 2008). However, the absence of UGT2B17 exerts no impact on circulatory T levels, neither at baseline nor after T administration in men (Ekstrom et al., 2011). But the UGT2B17 copy number variation (CNV) polymorphism has not been studied in relation to serum T levels in women administered with T.

The aim of this study was to study the association between AKR1C3 (rs12529), SLCO2B1 (rs12422149), and UGT2B17 deletion polymorphisms and the serum levels of T, DHT and A before and after 10 weeks daily T administration in women. Furthermore, to investigate the klotho polymorphism (rs9536314) in relation to steroid serum profile, as well as if physical performance as assessed by knee strength is dependent on the klotho genotype. Additionally, all polymorphisms were analyzed in relation to urinary steroid profile.

METHODS

Study Population

The cohort includes 48 women from a randomized, double blind, placebo controlled, parallel study conducted at the Karolinska University Hospital, Stockholm, Sweden, between May 2017 and June 2018 (ClinicalTrials. gov ID: NCT03210558). Healthy women were recruited by advertisement, mainly from the Swedish School of Sports and Health Sciences. Inclusion criteria included: age 18–35 years, body mass index 19–25 kg/m^2, non-smoking, a moderate to high self-reported level of recreational physical activity, not taking hormonal contraception and willing to use highly efficient non-hormonal contraception during the study period. In order not to infringe antidoping rules, the women had to agree not to participate in any sport competition event during the study period and for 1 month after termination of the study. Exclusion criteria were the presence of cardiovascular, liver, biliary or renal disease; hyperlipidaemia; uncontrolled high blood pressure; endocrinological disorders; oligomenorrhea or amenorrhea; pregnancy; history of thromboembolic disorder; any malignancy; and use of hormonal contraception in the 2 months prior to the study.

TABLE 1 | Clinical characteristics of the women in the testosterone group and the placebo group before and after treatment.

	Testosterone		Placebo	
	Before	After	Before	After
Age	28.4		28.4	
BMI kg/m^2	23.3	23.4	23.0	23.2
Total lean mass g	47034	47773**	45418	45582
Testosterone nmol/L ±SD	0.9 (0.4)	4.3 (2.8)***	1.0 (0.4)	1.1 (0.4)

**p < *01*, ***p < *0.001*.

The study was approved by the regional ethics committee in Stockholm (2016/1485-32, amendment 2017/779–32) and was carried out in accordance with Good Clinical Practice and the World Medical Association Declaration of Helsinki—ethical principles for medical research involving human subjects. All women gave written informed consent. Participants were randomly assigned to treatment with placebo cream (1 ml) or testosterone cream 10 mg (1 ml) (AndroFeme 1) applied every evening to the upper outer thigh for 10 weeks. Demographic characteristics of the women are described in **Table 1**. A detailed description of study population has been described previously (Hirschberg et al., 2020).

Serum and Urinary Steroid Analyses and Physical Performance Analyses

The first baseline samples were all collected in the follicular phase of the menstrual cycle. Serum androgen concentrations (T, A and DHT) were quantified with LC-MS/MS method described in (Ke et al., 2014) and presented in our previous paper (Elings Knutsson et al., 2021). The urinary steroid profile including T, E, etiocholanolone (etio), androsterone (A), 5β-androstanediol (5βAdiol) and 5α-androstanediol (5αAdiol) were quantified with WADA accredited GC-MSMS method as described (Elings Knutsson et al., 2021). The physical performance tests presented in the original study (Hirschberg et al., - 2020) included knee extension torque.

Genotyping

Blood samples from 46 women were available for DNA extraction (two samples missing), of which 23 were from women in the testosterone treatment group. DNA was extracted from 200 µL whole blood samples using PureLink® Genomic DNA kit (Life Technology), and according to the manual. The DNA concentrations were determined on NanoDrop. Genotyping SNP analyses for AKRIC3 (rs12529), SLCO2B1 (rs12422149), and Klotho (rs9536314) were performed using following allelic discrimination assays; C___8723970_1_, C __3101331_10, C___2983037_20, all from Life Technology. The UGT2B17 deletion polymorphism the CNV assay ID Hs03285327_cn (Life Technology) was used. Also, the ubiquitously expressed RNaseP (assay ID 4403326, Life Technology) was used as an endogenous reference gene when calculating the presence of one or two UGT2B17 alleles with delta-delta CTT formula (Livak, 1999). Subjects with no UGT2B17 PCR signal but with RNaseP were identified as del/del. The final volume for the PCR reactions was 10 or 15 µL consisting of 20 ng DNA and 2xTaqman Fast Universal PCR Master Mix (ThermoFisher). The PCR was run on StepOne™ Real-Time PCR System with the fast program; 50°C 2 min, 95°C 20 s and 40 cycles of 95°C 3 s and 60°C 20 s. The fluorescence signals were analyzed with the StepOne software 2.3 (Applied Biosystems).

Statistical Analyses

The isometric mean torque data were analyzed using a mixed model, with subjects as a random factor, and klotho genotype (GT, TT), treatment (testosterone and placebo), time (baseline and exit) and genotype*treatment, genotype*time, treatment*time and genotype*treatment*time as fixed factors. Genotype and treatment were the between group factors and time was the within group factor. The *p*-value for the interaction genotype*time was less than 0.10, and therefore we performed simple main effects tests, i.e. the effect of factor time was tested by holding the factor genotype fixed, or vice versa, averaged over the level of the factor treatment. The analyses were carried out with the statistical program R version 4.0.0 Copyright (C) 2020 The R Foundation for Statistical Computing.

Statistical analyses comparing steroid profile were performed using GraphPrism software version 8.3 from GraphPad (San Diego, CA, United States). For androgens showing normal distribution (T and A) the comparison between genotype groups was done with non-paired ANOVA (followed by Tukey`s test), or *t*-test. As DHT showed non Gaussian distribution, Kruskal Wallis (followed by Dunns multiple comparisons test) or Mann Whitney test were used. Differences were considered significant at the level $p < 0.05$ (2-sided test). One subject was homozygous for klotho GG genotype in the testosterone treatment group and was excluded from statistical analyses except for serum baseline levels where placebo and T group were pooled.

RESULTS

Genetic Variation and Androgen Serum Profile Before and After Testosterone Administration

All genotypes distribution was in Hardy-Weinberg HW equilibrium.

The AKR1C3 polymorphism had an impact on serum T levels at baseline, individuals homozygous for the G-allele showed 35% higher serum levels than heterozygotes, $p = 0.03$ (**Table 2**). No association between the SLCO2B1, UGT2B17 and klotho deletion polymorphism and serum levels of any of the steroids were seen at baseline (**Table 2**).

For the AKRIC3, SLCO2B1 and UGT2B17 genotypes, no differences in T, A and DHT absolute levels after 10 weeks topical testosterone administration were discerned. Moreover, the fold increase in the androgen concentrations observed after testosterone treatment did not associate with any of these genotypes (data not shown).

TABLE 2 | Distribution of polymorphisms in steroidogenic genes and androgen serum concentrations prior to intervention (at baseline) in the combined groups of women in the testosterone group and the placebo group (mean ± SD). Samples were taken in the follicular phase of the menstrual cycle (n = 44 women).

AKR1C3 (rs12529)	**CC (n = 8)**	**CG (n = 21)**	**GG (n = 17)**
Testosterone nmol/L ±SD	0.89 ± 0.36	0.81 ± 0.27	1.10 ± 0.38*
Dihydrotestosterone nmol/L ±SD	0.39 ± 0.15	0.37 ± 0.16	0.45 ± 0.17
Androstenedione nmol/L ±SD	4.37 ± 0.98	3.50 ± 0.72	4.51 ± 0.81
SLCO2B1(rs12422149)	**GG (n = 12)**	**AG (n = 34)**	
Testosterone nmol/L ±SD	0.94 ± 0.37	0.91 ± 0.30	
Dihydrotestosterone nmol/L ±SD	0.41 ± 0.18	0.37 ± 0.13	
Androstenedione nmol/L ±SD	4.16 ± 0.87	3.75 ± 0.75	
UGT2B17 deletion	**Ins/ins (n = 21)**	**Ins/del (n = 19)**	**Del/del (n = 6)**
Testosterone nmol/L ±SD	0.94 ± 0.53	0.96 ± 1.03	0.89 ± 0.38
Dihydrotestosterone nmol/L ±SD	0.40 ± 0.16	0.41 ± 0.19	0.38 ± 0.13
Androstenedione nmol/L ±SD	4.07 ± 0.76	3.97 ± 0.93	4.08 ± 0.91
Klotho (rs9536314)	**GG (n = 2)**	**GT (n = 11)**	**TT (n = 33)**
Testosterone nmol/L ±SD	1.30 ± 0.23	1.00 ± 0.47	0.88 ± 0.30
Dihydrotestosterone nmol/L ±SD	0.44 + 0.21	0.43 ± 0.21	0.39 ± 0.15
Androstenedione nmol/L ±SD	5.86 + 0.26	4.07 ± 0.76	3.91 ± 0.85

The Klotho (rs9536314) genotype, however, was associated with T levels after the T treatment period, being 100% higher in heterozygous individuals as compared to individuals homozygous for the T allele, p = 0.009 (**Figure 1A**). The fold change in serum T was significantly associated with klotho genotype; women with two T alleles showed a mean T increase of 4.7-fold (SD ± 3.2) compared to G-allele carriers where a mean increase of 9.4-fold (SD ± 5.9) was noted, (p = 0.02). The A and DHT serum concentrations were not significantly associated with klotho genotype (**Figures 1B,C**).

The serum biomarker T/A did not differ between klotho genotype groups at baseline, **Figure 1D**. The mean T/A values in the testosterone group was 0.23 (range 0.19–0.36) priori to T administration. After 10 weeks of T administration, the mean T/A ratio was 140% higher in GT individuals as compared to TT subjects, p = 0.004 (**Figure 1D**). Subsequently, the fold increase in T/A was significantly higher in the heterozygotic participants, mean 7.5-fold (range 1.8–12.2), than TT mean 3.4-fold (range 0.8–9.1) p = 0.01.

As expected, no association between klotho genotype and serum steroid levels in the placebo group were found (data not shown).

Genetic Variation and Androgen Urinary Steroid Profile Before and After Testosterone Administration

The UGT2B17 copy number variation polymorphism had a large impact on the urinary T and 5βAdiol levels. At baseline, individuals expressing UGT2B17 (ins/ins and ins/del) showed higher T (median 10.7 ng/ml and 7.6 ng/ml) and 5βAdiol (median 157 ng/ml and 143 ng/ml) concentrations compared to individuals devoid of UGT2B17 gene (0.61 ng/ml and 26 ng/ml for T and 5βAdiol, respectively), p < 0.001. After the T treatment period, the same pattern remained, individuals homozygous for the deletion allele showed lower T and 5βAdiol. Subsequently the increase in T/E as well as ABP ratios including T and 5βAdiol were associated with the UGT2B17 deletion polymorphism. Individuals homozygous for the klotho rs9536314 T-allele showed significantly lower T/E and higher A/T ratios than the heterozygotes subjects after T administration. The SNPs investigated in SLCO2B1 and AKR1C3 did not influence the urinary steroid profile. **Supplementary File S1** shows the results for all urinary steroid metabolites and ABP-ratios in relation to different genotypes.

Klotho SNP and Physical Performance

Since previous studies have found an association between klotho and muscle strength we investigated if the klotho SNP (rs9536314) was associated with isokinetic knee extension mean torque.

There was no significant difference in mean torque between baseline and exit using treatment-genotype three factor interaction (p = 0.57). Furthermore, no significant interaction of klotho genotype*time was observed, even though a trend towards higher knee extension mean torque scores among TT subjects were discerned (p = 0.08). At exit, there was a significant difference in knee extension mean torque between TT and GT in the T group (p = 0.02), **Figure 2**.

DISCUSSION

This is the first time a genetic variation has been associated with serum T concentrations after T administration in healthy women. Individuals carrying one G-allele of the klotho SNP (rs9536314) had higher total testosterone serum levels after 10 weeks daily transdermal application of testosterone than homozygous for the more common T allele. However, at baseline and in the placebo group, there was no association between circulatory T and the klotho genotype suggesting different response of exogenous androgen between the genotype variants. Serum T are of interest to monitor in anti-doping testing, together with A and DHT as a complement to the urinary steroid profile. In addition to T, A and DHT, the T/A ratio has been suggested as a longitudinally stable biomarker for testosterone intake in women not influenced by the menstrual cycle (Salamin et al., 2021). After T administration, Salamin et al. observed an increase in T/A (Salamin et al., 2021)

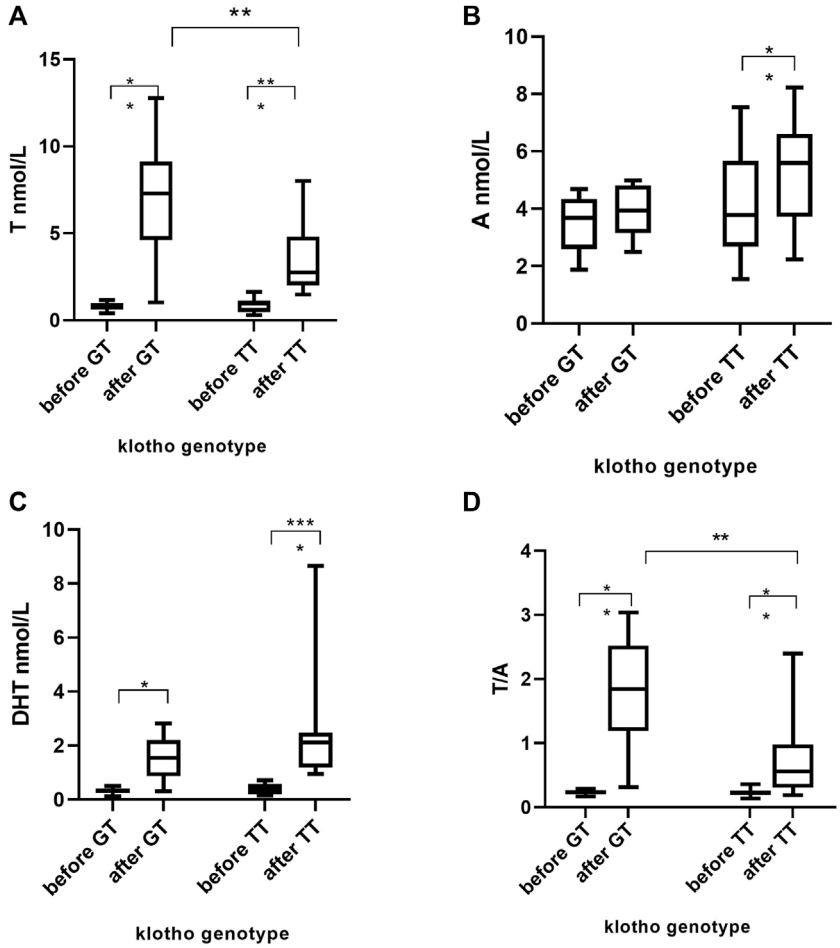

FIGURE 1 | Serum concentrations of T, A, DHT and T/A before and after 10 weeks daily administration of T in 22 women. T = testosterone, A = androstenedione, DHT = dihydrotestosterone. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

that corroborates with T/A values in our study participants, both before and after T treatment. The T/A ratio post T treatment in our study and the fold increase in T/A were associated with the klotho genotype, i.e., women homozygous for the TT allele displayed a lower T/A ratio and a smaller increase than those with the GT allel. Even though all women, regardless of genotype, increased their T/A (except one), the result indicates that women with GT klotho genotype may be more prone for detection using serum T/A as a biomarker.

The klotho SNP results in an amino acid change (F352V), that has been found to reduce the secretion of klotho in HeLa cells (Arking et al., 2002). The SNP, to our knowledge, has not been studied in relation to klotho serum levels, but theoretically reduced secretion may lead to lower klotho serum levels in individuals carrying a G-allele levels. Higher klotho serum levels have been associated with smaller decline in knee strength in older adults (Semba et al., 2016) This is in line with our hypothesis i.e., women homozygous for the T allele displayed higher scores in isokinetic muscle strength after T administration than subjects with a G-allele. Even though no significant association with treatment (placebo/testosterone) was noted, the role of T treatment can not be ruled out as genotype differences was noted in the T group but not in the placebo group. Notably, the genotype group that performed best in the muscle strength test is the variant associated with a smaller increase in serum T levels, and hence enhance our previous finding that T increase per se in serum does not mediate an effect on anaerobic strength. However, the circulatory T levels does not necessary reflect the androgen load in the muscles (Schiffer et al., 2018), and it is possible that the intracrine androgen metabolism differs between the klotho genotype groups. Another potential mechanism of the role of klotho in muscle strength might be indirectly via insulin-like growth factor I (IGF-I) signaling (Semba et al., 2016), as higher endogenous IGF-I and in insulin-like growth factor binding protein 3 (IGFBP-3) levels have been associated with klotho and androgens, as well as improved isometric knee strength (Jurimae et al., 2010). Future studies are warranted to elucidate the klotho polymorphism's influence on muscle strength in healthy women.

In addition to klotho, genetic variations in metabolic enzymes AKR1C3, SLCO2B1 and UGT2B17 were studied. The AKR1C3 C>G polymorphism (rs12529) resulting in a non-conservative

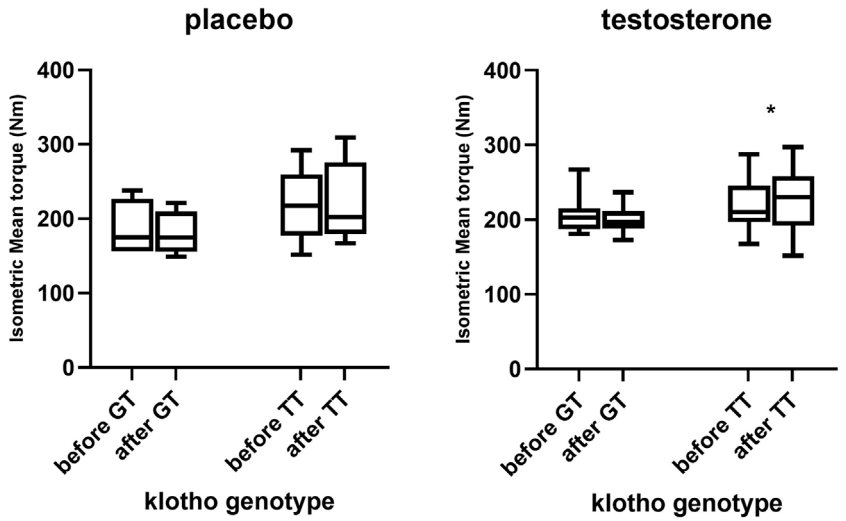

FIGURE 2 | Percentage change in knee extension mean torque and peak torque in different klotho genotype groups in participants treated with testosterone and placebo respectively (n = 22 in each group). *$p < 0.05$.

amino acid change (H5Q) has been associated for the first time with circulatory T concentrations in women. The result is in line with a study where men homozygous for GG exhibit higher baseline testosterone levels than GC/CC subjects (Shiota et al., 2020). However, after 10 weeks T administration no differences in serum T levels between the AKR1C3 genotype groups were determined, which is opposite to findings in men where individuals homozygous for the AKR1C3 (rs12529) CC allele displayed higher serum T compared G-carriers after testosterone replacement therapy (Bhasin et al., 2018). The reason may be sex differences.

Organic anion-transporting polypeptides (OATPs), which are encoded by SLCO, are a superfamily of membrane transport molecules that mediate the cellular uptake of steroid conjugates (Kinzi et al., 2021). A testosterone administration study in healthy men revealed that individuals homozygous for the SLCO2B1 (rs12422149) G-allele showed lower levels of total T prior to and 2 days after T injection, as compared to individuals expressing the A-allele (Schulze et al., 2012). However, here no association between SLCO2B1 genotype and androgen concentrations before and after T administration could be detected. A recent study indicated that OATP1B3 may be the main uptake transporter of T glucuronide, (Li et al., 2020). It is therefore possible that the involvement of OATP2B1 in T transport might be minor and exert a smaller impact in women where lower T increase are achieved.

The UGT2B17 gene deletion polymorphism is very well known to play a crucial rule in the urinary concentrations of T (Schulze et al., 2008), and to exert no impact on circulatory T levels in men (Ekstrom et al., 2011). Thirteen percent of the participants were homozygous for the UGT2B17 deletion allele and hence the genotype frequency match the phenotype frequency (i.e., T/E < 0.2) in our previous study including the same study population. The results herein confirm that the UGT2B17 CNV is not associated with serum T levels also in women neither at baseline nor after T administration. The urinary steroid profile was as expected connected to the UGT2B17 deletion polymorphism. Both T and 5βAdiol were excreted at much lower concentrations in subjects homozygous for the deletion variant in agreement with a previous study conducted in females (Schulze et al., 2014). Also, genetic variation in the klotho gene were associated with different T/E and A/T ratios post T administration, which is in line with higher serum T observed in GT subjects. However, the data interpretation was disturbed by the influence of UGT2B17 polymorphism, as del/del subjects were only represented in the TT panel, and the study cohort is too small to further analyze the contribution of klotho. Nevertheless, any putative association between the klotho SNP and the urinary steroid profile can be considered small and not of relevance in anti-doping testing.

A limitation with the study is relatively few subjects for genotype association studies and minor associations may be missed and/or risk of randomly false positive associations. The connection between circulatory androgens and klotho needs to be verified in future studies. Another drawback is that only one blood sample was taken after the last testosterone administration, and the time post last administration could vary between 8 and 12 h. Moreover, the compliance to T treatment is not known as this was not controlled for throughout the study. However, the increase in serum T at the end of treatment support good compliance. A future study should be controlled in regard of administration and sampling time-points and preferably also include more collection time points for fully pharmacokinetic assessment.

In conclusion, this is the first time a genetic variation in klotho has been linked to serum concentrations of T and muscle strength after T administration in women. This might have implications

on detection sensitivity using T (and/or T/A ratio) as biomarker in a serum steroid passport.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ebi.ac.uk/eva/, PRJEB51000.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics committe in Stockholm. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JK and AL were involved in the design of the original study. The genotyping analyses were carried out by the LE and CS. LE conducted the data analyses and wrote the manuscript in collaboration with AL. All the authors have read and approved the final 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.2022.844133/full#supplementary-material

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