scientific reports

OPEN

Effects of integrated exercise approach on total testosterone levels in eumenorrheic women: a randomized controlled trial

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Testosterone modulated by exercise plays a pivotal role in maintaining the overall health of both males and females. Therefore, this study aimed to determine the effects of an integrated exercise approach on total testosterone levels during different phases of the menstrual cycle in eumenorrheic females. This was a two-armed parallel design, single-blinded, randomized controlled trial held from March 14, 2023, to February 21, 2024, in Aadil Hospital Defense Lahore. Forty eumenorrheic females within the age range of 20 to 40 years, with a BMI ranging from 18.5 to 24.9, who were able to maintain sitting balance without the need for upper limb support or who had a minimum score of 25 on the trunk control test were recruited for the study. They were then divided into 2 groups using a random table generator and concealed envelope allocation. The treatment group was given an exercise plan 3 times per week for 16 weeks along with an awareness program for menstrual hygiene and maintaining an active lifestyle, while the control group was given an awareness program to maintain menstrual hygiene and an active lifestyle along with a recommendation to walk for 30 min 3 times a week for 16 weeks. The testosterone levels were calculated pre-intervention, mid-intervention, and post-intervention. Mixed model ANOVA was used for within- and between-group analyses. The data were analyzed using SPSS v21. The educational backgrounds of the participants were diverse, with 17.5% having completed matric, 47.5% holding a bachelor's degree, and 17.5% having a master's degree or PhD. Regarding occupation, 35% were students, 32.5% were housewives, and 32.5% were working professionals. Marital status varied, with 37.5% married, 45% unmarried, and 17.5% divorced. Total testosterone levels (ng/dl) were measured at different menstrual cycles for the experimental and control groups. During the follicular phase, the experimental group showed pre-exercise levels of 25.80 ± 2.57 (95% CI: 24.24–27.35) and post-intervention levels within 15 min of exercise of 33.04 ± 8.67 (95% CI: 28.85–37.23). In the mid-cycle phase, the pre-exercise level was 36.48 ± 2.80 (95% CI: 33.47–37.48), and the post-intervention level was 40.80 ± 7.12 (95% CI: 37.15–44.46). The luteal phase showed pre-exercise levels of 31.10 ± 3.44 (95% CI: 29.90–34.31) and post-intervention levels within 15 min of exercise of 34.97 ± 5.60 (95% CI: 31.95–38.00). Compared with the experimental group, the control group exhibited consistent testosterone levels with minor variations across all phases. The mixed model ANOVA results for the between-group effect were highly significant, with *p* = 0.00 and an effect size of 0.99. Integrated exercise leads to an increase in testosterone levels in females immediately after exercise, which decreases below pre-exercise levels within 24 h of exercise, with the testosterone level peaking in the mid-cycle phase of the menstrual cycle. This immediate increase in testosterone levels can lead to increased strength, cognition and sexual functions in females.

Trial registration number. **This clinical trial was submitted by Dr. Rabiya Noor on clinicaltrials.gov for registration with ID: NCT05460741 first posted on 31/05/2022, last updated on 03/04/2024, and last verified on 29/04/2024.**

Keywords Hormones, Eumenorrheic, Integrated exercises, Physical activity, Menstrual cycle, Testosterone

The menstrual cycle, a fundamental aspect of female reproductive physiology, encompasses a series of cyclic events orchestrated by intricate hormonal interplay. Typically lasting approximately 28 days, although varying

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among individuals, the menstrual cycle can be broadly divided into the follicular, ovulation, and luteal phases[1]. During the follicular phase, which spans approximately the first 14 days of the cycle, follicle-stimulating hormone (FSH) prompts ovarian follicles to mature, leading to the release of estrogen[2]. Estrogen promotes thickening of the uterine lining in preparation for potential implantation[3]. Ovulation marks the midpoint of the cycle and is characterized by the release of a mature egg from the ovary triggered by a surge in luteinizing hormone (LH). Following ovulation, during the luteal phase, the ruptured follicle transforms into a temporary endocrine structure known as the corpus luteum, which secretes progesterone and estrogen to maintain the uterine lining in preparation for potential embryo implantation[4]. If fertilization does not occur, the corpus luteum degenerates, leading to a decrease in hormone levels and subsequent menstruation, initiating a new cycle[5].

Hormonal balance, including optimal testosterone levels, is pivotal in preserving overall health and well-being across various physiological systems. Testosterone, predominantly recognized as a male sex hormone, is also present in females, albeit in smaller quantities, exerting multifaceted effects on both genders[6]. In males, testosterone is integral to the development of secondary sexual characteristics, such as facial hair growth and deepening of the voice, as well as supporting reproductive function. Moreover, testosterone influences mood regulation, cognitive function, and bone density, contributing to psychological and skeletal health[7,8]. In females, while estrogen and progesterone are primary sex hormones, testosterone contributes to libido, energy levels, and overall vitality[9]. Thus, maintaining an optimal testosterone balance is crucial for sustaining physiological equilibrium and promoting general well-being. Maintaining an optimal testosterone balance is vital for muscle health, metabolic function, sexual health, mood regulation, and overall quality of life in both men and women[10,11].

Adequate testosterone levels are essential for optimizing physical performance and muscle strength, regardless of sex[12]. Testosterone is pivotal in protein synthesis, facilitating muscle growth and repair processes. Moreover, it enhances muscle mass and strength by stimulating the proliferation of satellite cells and promoting muscle fiber hypertrophy. Consequently, individuals with optimal testosterone levels often exhibit greater muscle mass, increased muscle strength, and improved athletic performance. Furthermore, testosterone influences energy metabolism, favoring fats as an energy source during exercise, and enhancing endurance and stamina. Maintaining hormonal balance, including adequate testosterone levels, optimizes physical performance, improves muscle strength, and promotes overall health and vitality[13].

The concept of exercise as a modulator of hormonal levels underscores the dynamic interplay between physical activity and endocrine function, offering insights into the intricate mechanisms by which exercise influences hormonal regulation. Previous research has revealed compelling evidence suggesting that exercise exerts profound effects on testosterone levels in both men and women[6,14]. In males, acute bouts of resistance training and high-intensity interval training (HIIT) have been shown to transiently increase serum testosterone levels, which peak shortly after exercise cessation. Conversely, prolonged endurance exercise, such as long-distance running or cycling, may lead to transient decreases in testosterone levels, possibly attributed to increased cortisol secretion and metabolic stress. Moreover, chronic exercise training, particularly resistance training, has been associated with long-term elevations in resting testosterone levels, indicative of exercise-induced adaptations within the endocrine system[6,15].

Similarly, exercise has been demonstrated to influence testosterone levels in females, albeit to a lesser extent than in males. Studies such as Bottaro et al.[16] and Patel et al.[17] have reported that acute bouts of resistance training can elicit transient elevations in serum testosterone levels in women, with the intensity and volume of exercise exerting significant effects. Moreover, regular exercise participation, particularly resistance training, has been associated with favorable alterations in sex hormone profiles in women, including increased testosterone levels and improved androgen-to-estrogen ratios. Notably, the menstrual cycle may also influence the response of testosterone to exercise, with fluctuations observed across different phases of the cycle. Overall, the burgeoning body of evidence highlights exercise as a potent modulator of testosterone levels in both men and women, underscoring the importance of physical activity in shaping hormonal balance and promoting overall health[18].

Due to the known connection of testosterone with muscle hypertrophy resulting from exercise, numerous studies, such as those by Tsampoukos et al.[19] and McNulty et al.[20], have focused on investigating how testosterone responds to resistance and strength-based workouts. In contrast, there has been relatively less emphasis on exploring the impact of extended endurance exercise on hormones, including testosterone, in both males and females, even though the evidence points to the necessity of testosterone in the physiological adaptations of endurance-based activities[21–23]. However, the effect of integrated exercise on testosterone levels and how testosterone is affected by the phases of the menstrual cycle have not been studied until recently; therefore, the purpose of this study was to obtain a comprehensive understanding of the influence of integrated exercise on testosterone dynamics and its potential implications for eumenorrheic females.

Emphasizing the need for further investigation into the effects of integrated exercise approaches on testosterone levels across different menstrual cycle phases is paramount for advancing our understanding of the complex interplay between exercise, hormonal fluctuations, and female physiology. Although existing research has provided valuable insights into exercise's acute and chronic effects on testosterone levels in women, a comprehensive understanding of how integrated exercise regimens impact hormonal dynamics throughout the menstrual cycle remains elusive. Given the potential influence of the menstrual phase on hormonal responses to exercise, elucidating the nuanced interactions between exercise modalities, timing, and menstrual cycle phases is essential for optimizing exercise prescription and tailoring interventions to maximize health benefits in women. Additionally, investigating the potential synergistic effects of integrated exercise approaches, encompassing resistance training, aerobic exercise, and flexibility training, on testosterone levels across menstrual phases holds promise for informing evidence-based exercise guidelines tailored to the unique physiological needs of women.

So, the integrated exercise regimen was designed that does not require a gym setup or expensive equipment, yet delivers the benefits of a structured workout. Each exercise in the regimen was carefully selected for its ability to engage multiple muscle groups while remaining accessible and cost-effective. The regimen focuses on key aspects of functional fitness, including strength, flexibility, balance, and coordination—essential components for enhancing overall muscular strength in women. For instance, squats and tandem walks improve lower body strength and balance[24], while arm swings with loads and crunches enhance upper body and core stability[25]. Additionally, bending and roll-ups promote flexibility and spinal mobility[26]. This structured combination ensures a well-rounded approach to fitness while minimizing resource requirements and supporting broader applicability across diverse populations. Additionally, this protocol addresses potential biases arising from variations in protocols used in previous studies and shortcomings in study design and randomization techniques, as documented in a systematic review[27]. Therefore, further research endeavors are warranted to unravel the complexities of this multifaceted relationship and foster the development of personalized exercise strategies to enhance hormonal balance and promote overall well-being in women.

Materials and methods

Ethical approval of study

This study received approval from the Research Ethics Committee of Riphah International University (ID REC/Lhr/22/1101) and is registered at http://clinicaltrials.gov (registration no. NCT05460741) where it was first posted on 31/05/2022, last updated on 03/04/2024, and last verified on 29/04/2024. All the experiments were performed in accordance with their relevant guidelines and regulations. This randomized control trial was devised following the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) 2013 guidelines[28] and reported following Consolidated Standards of Reporting Trials (CONSORT) 2010 guidelines[29]. All the study participants signed a formal consent for their participation.

Selection of study participants

A sample size of 40 eumenorrheic females was calculated by G* power[30]. Participants of the study were selected using random sampling methods. The participants were recruited for the survey of the following inclusion criteria:

- Eumenorrheic menstrual cycles were defined as consistent cycles spanning 24 to 35 days[31].
- Females with an age range of 20 to 40 years.
- BMI between 18.5 and 24.9 (normal).
- They could maintain a sitting balance without requiring upper limb support or had a minimum score of 25 on the trunk control test[32].
- These participants had refrained from any exercise regimen over the preceding six months.

Exclusions from the study were made for.

- women who were currently taking oral contraceptives (as they may alter the natural surge of hormones),
- women who were pregnant, lactating.
- women who had undergone a cesarean section within the past six months. Furthermore, individuals with menstrual irregularities such as endometriosis, ovarian cysts, or other comorbidities, including a history of cardiac events or seizures, were not included[33].

Development of sampling frame

A finite list of respondents fulfilling the criteria was not available. Therefore, researchers decided first to conduct a benchmark survey to develop the sampling frame. For this purpose, a six-month survey was organized to identify the targeted populations. Benchmark surveys assess the coverage of the sampling frame by comparing the frame to external data sources[34]. Snowball sampling was used to reach the potential study participants, as researchers had contact with a few females who fulfilled the criteria. Based on their recommendations, researchers reached out to universities, hospitals, schools, and some females who were educated but were living in their homes. After they consented to participate, their names were included, and the total population reached 120 potential participants. From the identified population, 40 were chosen as study participants using a sample size calculating formula G* Power. A post hoc power analysis was conducted to determine whether the sample size ($N = 40$) was sufficient to detect meaningful differences. Using an effect size of 0.38, α level of 0.05, and f test, the achieved power was 0.96, indicating that the study had adequate power to detect significant effects. The selected sample size is also endorsed by the Schober and Vetter (2019) as they stated that the minimum sample size in health studies is chosen to ensure low error probabilities and a power of 0.8 or 0.9, depending on the effect size and assumed variability of the data[35]. In this study, power emerged at 0.96, which is also higher than the power threshold of 80% and is commonly used for detecting clinically meaningful effects in sample size[36]. The 120 females served as the sampling frame, and 40 respondents were chosen using simple random sampling. Selecting a sample frame for a survey is important, as it determines the extent to which the target population is covered, the accuracy of contact information, and the cost[37]. Moreover, probability sampling, such as simple random sampling, improves convergence speed and reduces bias by shifting the focus from bias potentials to probability distribution reconstruction. Random sampling was employed to select the respondents to minimize bias. Of the total 120 potential participants, each was given an equal chance of being chosen as study participants using random table generators.

Menstrual cycle monitoring

A gynecologist determined the menstrual cycle phase of the participants using the calendar method, tracking their cycles for the previous three months to identify any irregularities. Baseline measurements of estrogen (estradiol), progesterone, and total testosterone levels were obtained on the 4th, 14th, and 24th days of the menstrual cycle, corresponding to the follicular, mid-cycle, and luteal phases of the menstrual cycle, respectively. This allowed researchers to establish the normal hormonal ranges for the participants and monitor any deviations from these baseline levels[38].

Randomization
Participants were randomly allocated using a computerized random table generator. Each participant was assigned a number, which was then randomly drawn by the computer to obtain a random sample. Throughout the trial, a 1:1 ratio was maintained between the two groups.

Blinding
This trial was single-blinded, with the assessor blinded to the group assignments. Due to the nature of the intervention, it was impossible to blind the patients or the principal investigator. The statistician, however, was blinded by coding the data into categories such as A and B to ensure unbiased analysis.

Study protocol
This was a two-armed parallel design, single-blinded, randomized controlled trial conducted at Aadil Hospital Defense, Lahore, from 14th March 2022 to 21st February 2024. The participants were asked to report at the Aadil Hospital, where they underwent initial screening and blood profiling by a gynecologist. After the initial assessment, the participants were randomly divided into 2 groups using a random table generator. Participants were then asked to visit the physiotherapy department of Aadil Hospital, where the principal investigator (a senior physiotherapist) provided an awareness program to both groups related to hygiene during the menstrual cycle and maintaining an active lifestyle. The control group was advised to walk for 30 min three times a week on alternate days for 16 weeks along with a standard dietary plan. The experimental group was subjected to an integrated exercise protocol (within the physiotherapy department) of 50 min on alternative days, totaling three weekly sessions for 16 weeks under the close supervision of the principal investigator. In this protocol, different types of exercises, resistance, endurance, and balance, were integrated with proper warm-ups, cool-downs, and rest intervals, which required minimal setup (with only handheld dumbbells), as shown in Table 1.

This integrated exercise plan is a relatively new concept in the literature, with limited direct evidence supporting its effectiveness. However, existing research highlights the significant impact of exercise-based interventions on improving quality of life[27,39–43]. The primary objective was to ensure participants remained engaged in core-strengthening exercises for the entire 5-minute session, even if they needed brief pauses or modified movements. The total number of repetitions or the duration of continuous exercise was recorded for each participant, with any modifications duly noted.

If a participant was unable to complete crunches for the full 5 min, they were encouraged to take short breaks when experiencing fatigue or discomfort. These rest periods were limited to 10–15 s, enabling them to resume the exercise without losing momentum.

Before each session, participants were instructed to abstain from alcohol, caffeine, or strenuous physical activities or sports for a full day. The exercise protocols were initiated between 10 a.m. and 12 p.m., followed by breakfast consumed at least two hours before the commencement of the session. A nutritionist provided dietary guidance to ensure participants followed these recommendations for 48 h before each session, minimizing potential nutritional influences on the study's primary outcomes. Furthermore, participants had a standardized breakfast before each session, regardless of their menstrual cycle phase, and all blood samples were taken within 15 min after the protocol to avoid hormonal surges throughout the day.

The testosterone levels in the blood serum were checked through a chemiluminescence microparticle immunoassay technique (Abbott-Alinity Ci) within 30 min of exercise and 24 h after exercise. This assessment was blinded, as the assessor (a lab technologist) did not know about allocating patients into groups or the

High marching (warm-ups)	5 min	3 times a week on alternate days for 16 weeks
Trunk Bending (forward and side bends)	5 min	
Rest interval	2.5 min	
Roll-ups	5 min	
Rest interval	2.5 min	
Arm swings with resistance	5 min	
Rest interval	2.5 min	
Crunches	5 min	
Rest interval	2.5 min	
Tandem walk	5 min	
Rest interval	2.5 min	
Squats	5 min	
Rest interval	2.5 min	
High marching (Cool downs)	5 min	
Total duration: 50 min		

Table 1. Exercise protocol along with duration.

intervention given to patients. These readings were taken at the beginning of the intervention on the 4th, 14th, and 24th days of the menstrual cycle for all participants. The second set of readings occurred during the program's 6th to 8th week (mid-intervention), again on the 4th, 14th, and 24th days of the menstrual cycle. The final set of readings occurred during the study's 14th to 16th week (post-intervention). The CONSORT guidelines[29] followed in the study are presented in Fig. 1.

Equity, diversity, and inclusion

To ensure the diversity of our study population, we enrolled females of various ages, socioeconomic backgrounds, and professions. However, to maintain inclusivity and equity, we established specific inclusion criteria at the outset. Additionally, all participants were closely monitored to adhere to a uniform diet plan. The participants were instructed to follow the same exercise regimen at the same time of day, with samples collected in the morning to mitigate the impact of natural hormone fluctuations throughout the day. We are dedicated to furthering the causes of diversity, equity, and inclusion in science, research, and healthcare. We believe that by fostering an inclusive approach, we can contribute to a better understanding of the issues surrounding female health and hormonal changes and ultimately enhance the quality of care and information available to all individuals.

Statistical analysis

The data are presented in tables as the mean ± standard deviation (SD). The assumptions of normality and homogeneity of variances were tested. Normality was assessed using the Shapiro-Wilk test, and the homogeneity of variances and sphericity were evaluated using Levene's and Mauchly's tests, respectively. The results indicated the assumptions were met ($p > 0.05$ for all tests). In cases where sphericity was violated, Greenhouse-Geisser

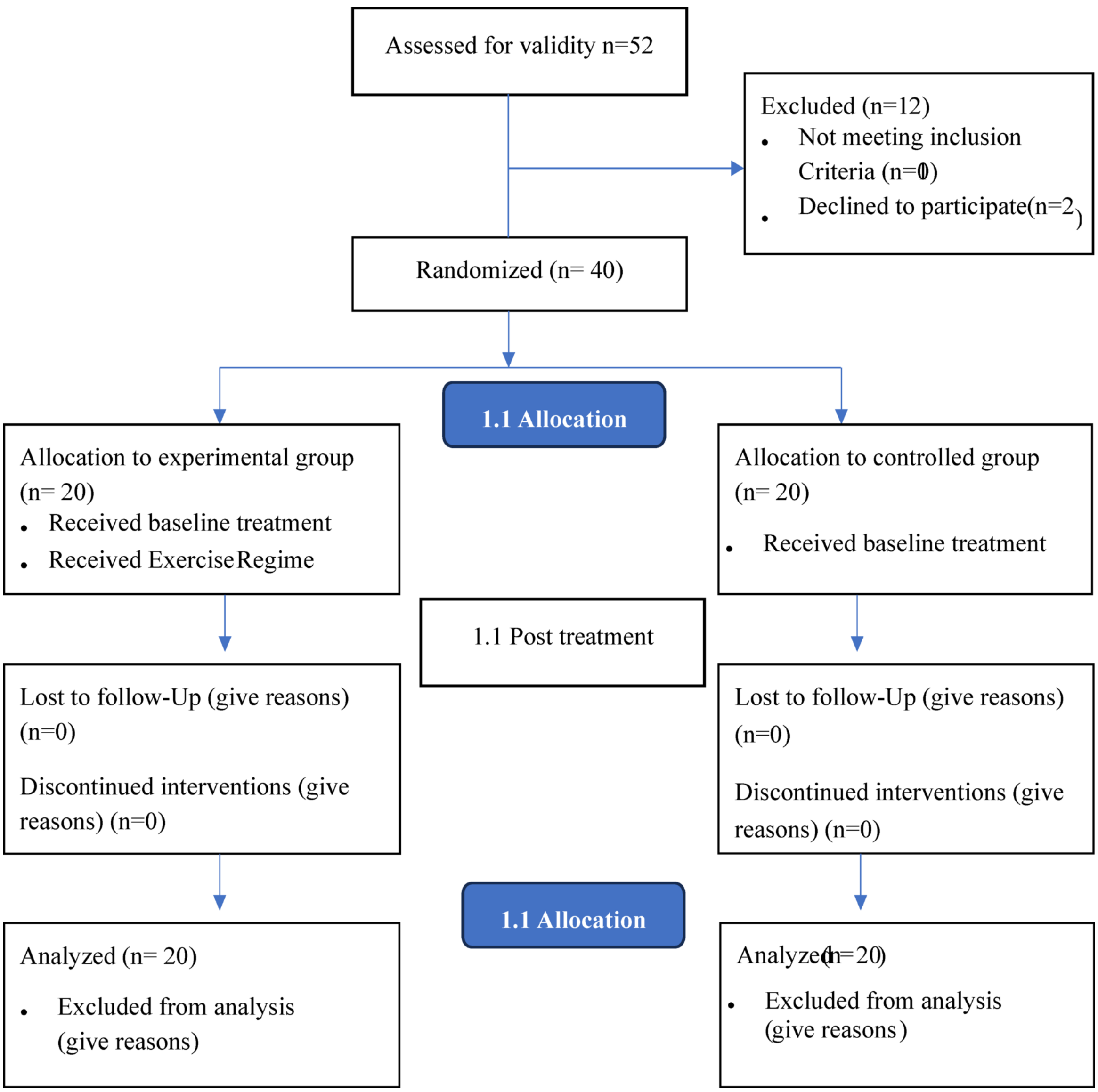

Fig. 1. Flow diagram of study participants according to CONSORT guidelines[29].

corrections were applied to adjust the degrees of freedom. An analysis of variance with a mixed model ANOVA was employed to examine the influence of menstrual cycle days (4th, 14th, and 24th) and the interaction between menstrual cycle phases and testosterone levels at times mentioned earlier. Partial eta squared values were calculated to gauge the effect sizes and determine the significance of the observed changes. Effect sizes were classified as small, medium, or large, with threshold values typically falling at approximately 0.01, 0.06, and 0.14, respectively.

Additionally, 95% confidence intervals (CIs) were computed, and statistical significance was established when p values were less than 0.05. An effect size was considered meaningful if its confidence interval did not include zero. All the statistical analyses were conducted using SPSS software, version 25 (IBM Corp., Armonk, NY, USA)[44].

Results

Characteristics of study participants

Half (50%) of the participants were bachelor's degree holders, and 12.5% had completed their PhD in different disciplines. In the context of occupation, 30% were students, followed by 40% housewives and 30% working ladies. This indicates that study participants were diverse in their education and occupation. Of the total respondents, 42.5% were married whereas 42.5% were single. However, 15% had confirmed that they were divorced. All the study participants had normal BMI (8.5 to 24.9) (Fig. 2).

The average age of respondents was 29.85 years, regarded as the productive age group. As far as the average of weight of study participants was concerned, the average was 57.95 kg. Average height was recorded at 159.95, followed by an average BMI of 22.65, which was within the normal BMI range (Fig. 3).

Table 2 shows that 20 participants who received the integrated exercise (M = 30.47, SD = 3.03) compared to the participants in the control group (M = 24.95, SD = 2.03) demonstrated statistically highly significant mean difference at mid-intervention levels within 15 min exercise at follicular phase, t = 4.766, $P = 0.000$. There was also a statistically significant mean difference between the experimental and control groups (t= -1.057, $P < 0.05$), which indicates that testosterone levels in eumenorrheic females in the experimental group decreased at mid-intervention levels after 24 h. Post-intervention levels within 15 min of exercise (t = 2.842, $P < 0.05$) and pot intervention levels after 24 h of exercise (t = -1.119, $P < 0.05$) had a statistical mean difference. The testosterone level of study participants decreased for the experimental group at post-intervention levels after 24 h of exercise.

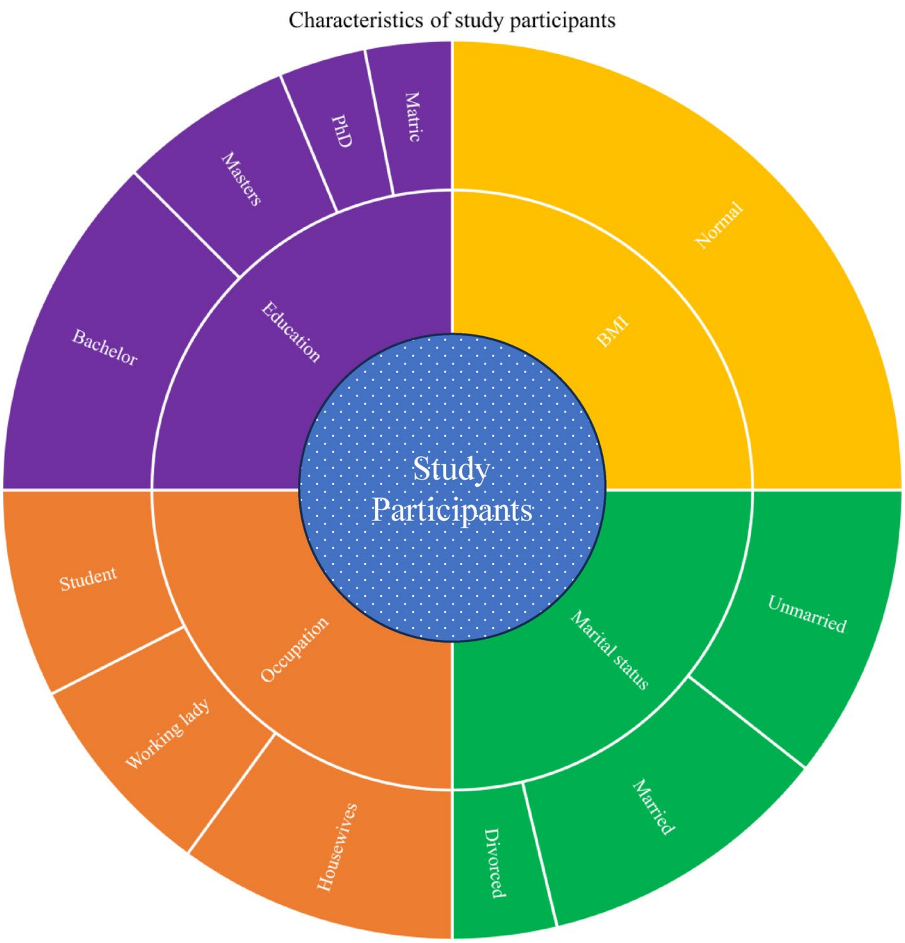

Fig. 2. Sunburst chart for the characteristics of the study participants.

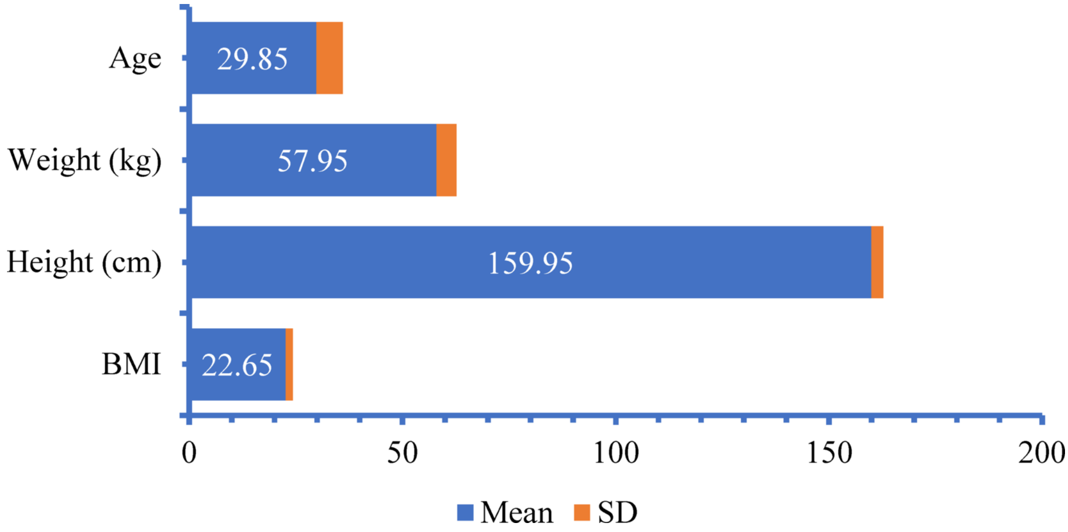

Fig. 3. BMI, height, weight, and age of the participants.

Stages of the menstrual cycle	Time	Study group	Mean ± S. D	95% CI Lower	95% CI Upper	T values
Follicular phase	Pre exercise	Experimental	25.80 ± 2.57	24.24	27.35	0.868^{NS}
		Control	24.89 ± 2.08	23.33	26.44	
	Mid intervention levels within 15 min of exercise	Experimental	30.47 ± 3.03	28.75	32.19	4.766**
		Control	24.95 ± 2.03	23.23	26.67	
	Mid intervention levels after 24 h of exercise	Experimental	21.93 ± 2.32	22.48	25.38	-1.057*
		Control	24.96 ± 2.04	23.51	26.42	
	Post intervention levels within 15 min of exercise	Experimental	33.04 ± 8.67	28.85	37.23	2.842*
		Control	25.02 ± 2.10	20.82	29.21	
	Post intervention levels after 24 h of exercise	Experimental	20.58 ± 7.03	20.41	25.28	-1.119*
		Control	24.99 ± 2.08	21.54	28.43	
Mid-cycle phase	Pre exercise	Experimental	36.48 ± 2.80	33.47	37.48	0.714^{NS}
		Control	36.66 ± 3.21	34.65	38.66	
	Mid intervention levels within 15 min of exercise	Experimental	41.93 ± 8.87	37.51	46.34	1.810*
		Control	36.55 ± 3.11	32.13	40.96	
	Mid intervention levels after 24 h of exercise	Experimental	22.74 ± 2.50	20.79	24.69	-10.446**
		Control	36.45 ± 3.31	34.50	38.40	
	Post intervention levels within 15 min of exercise	Experimental	40.80 ± 7.12	37.15	44.46	1.712*
		Control	36.59 ± 3.12	32.93	40.25	
	Post intervention levels after 24 h of exercise	Experimental	23.66 ± 1.10	22.10	25.23	-12.286**
		Control	36.58 ± 3.13	35.01	38.14	
Luteal phase	Pre exercise	Experimental	31.10 ± 3.44	29.90	34.31	1.124^{NS}
		Control	30.54 ± 3.17	28.23	32.64	
	Mid intervention levels within 15 min of exercise	Experimental	34.89 ± 3.70	32.59	37.20	2.935*
		Control	30.34 ± 3.21	28.04	32.64	
	Mid intervention levels after 24 h of exercise	Experimental	23.76 ± 1.31	22.11	25.40	-5.926**
		Control	30.32 ± 3.24	28.67	31.97	
	Post intervention levels within 15 min of exercise	Experimental	34.97 ± 5.60	31.95	38.00	2.212*
		Control	30.47 ± 3.17	27.44	33.49	
	Post-intervention levels after 24 h of exercise	Experimental	23.75 ± 5.10	23.93	29.58	-1.940*
		Control	30.45 ± 3.19	27.62	33.27	

Table 2. Within group analyses of levels of total testosterone (ng/dl) at 3 phases and 3 time points.

	df	Mean Square	F	*P*	Effect size	Observed Power
Phase	2	2163.864	100.58	0.000	0.848	1.000
Phase * group	2	257.137	11.95	0.000	0.399	0.991
Time	5	396.069	23.66	0.000	0.568	1.000
Time * group	5	392.389	23.44	0.000	0.566	1.000
Phase * time	10	55.089	10.98	0.000	0.379	0.991
Phase * time * group	10	57.498	11.46	0.000	0.389	1.000

Table 3. Mixed model ANOVA for main effects and interaction effects of phases, time and groups.

	df	Mean Square	F	Sig.	Effect size	Observed Power
Group	1	337309.956	3115.26	0.00	0.994	1.00

Table 4. Mixed model ANOVA for between-group analyses.

At the Mid-cycle phase and luteal phase, it was confirmed that testosterone levels in study participants were found to decrease at mid-intervention levels within 15 min and post-intervention levels after 25 h of exercise, defying the alternate hypothesis that there was no statistical mean difference between the experimental and control group ($P<0.05$). This is synthesized to mean testosterone levels 15 min after exercise and 24 h after exercise at the menstrual cycle's follicular, mid-cycle, and luteal phases and at pre-intervention, mid-intervention, and post-intervention. The testosterone levels reach a maximum during the mid-cycle phase, increase 15 min post-exercise, and decrease during the recovery phase.

All the data are reported as the mean ± standard deviation.

Table 3 presents the changes in testosterone levels during different phases and times and the interactions between different phases and groups, between time and groups, and between phases, times, and groups. These interactions were statistically significantly different ($p=0.000$). This implies that within the phase, the effect on testosterone levels varied. Empirically, that effect was around 84% across the phases, whereas within time, the effect was 56%.

Table 4 shows the changes in testosterone levels between the experimental and control groups, which were significant ($p<0.05$). As shown in Table 2, there were significant increases in testosterone levels in the experimental group, as compared to the control group, immediately post-exercise, for which they gradually decreased. At 24 h post-exercise, total testosterone reached significantly lower levels than the corresponding resting, pre-exercise values ($p<0.05$), and these changes were greatest during the mid-cycle phase. The effect sizes for all these changes are also reported in Tables 3 and 4. This analysis demonstrated that the effect size ranged from medium to large in magnitude for the significant changes in our measures.

Figure 4 illustrates the group-wise Minimal Clinically Important Differences (MCID) between the follicular, mid-cycle, and luteal phases of the menstrual cycle at three time points: pre-exercise, mid-intervention (15 min, 24 h), and post-intervention (15 min, 24 h). The results indicate a positive MCID 15 min after both mid-intervention and post-intervention exercises, while a negative MCID is observed 24 h after both mid-intervention and post-intervention exercises.

Discussion

This study investigated the effects of an integrated exercise approach on total testosterone fluctuations across different phases of the menstrual cycle in eumenorrheic females. The key outcome of the study is that total testosterone levels transiently increase immediately after exercise but decline below baseline within 24 h during recovery. The discussion of the key results is appended below under different subsections.

Comparison of testosterone levels in males and females

Testosterone levels in healthy adult males and females differ significantly. Adult males typically produce approximately ten times more testosterone than females[45]. This hormonal disparity plays a key role in the distinct physiological characteristics observed between the sexes, particularly in terms of muscle mass, strength, and metabolic functions[46].

In males aged 19 to 39 years, the harmonized normal range of total testosterone is 264 to 916 ng/dL[47], with age-specific median levels ranging from 409 to 478 ng/dL in those aged 20 to 44 years[48]. In contrast, premenopausal women typically exhibit total testosterone levels ranging from 15 to 46 ng/dL[49]. These differences highlight the need to interpret testosterone fluctuations in women within the context of their naturally lower baseline levels.

Although women have lower testosterone concentrations, this hormone remains critical for various physiological functions, including muscle development, bone density maintenance, and overall metabolic health[50](Clark et al., 2018). Resistance exercise has been shown to induce acute increases in testosterone levels in both sexes, potentially contributing to improved musculoskeletal adaptations over time[46]. Therefore, understanding the dynamics of testosterone in females, particularly in response to physical activity, is essential for designing effective, sex-specific exercise interventions.

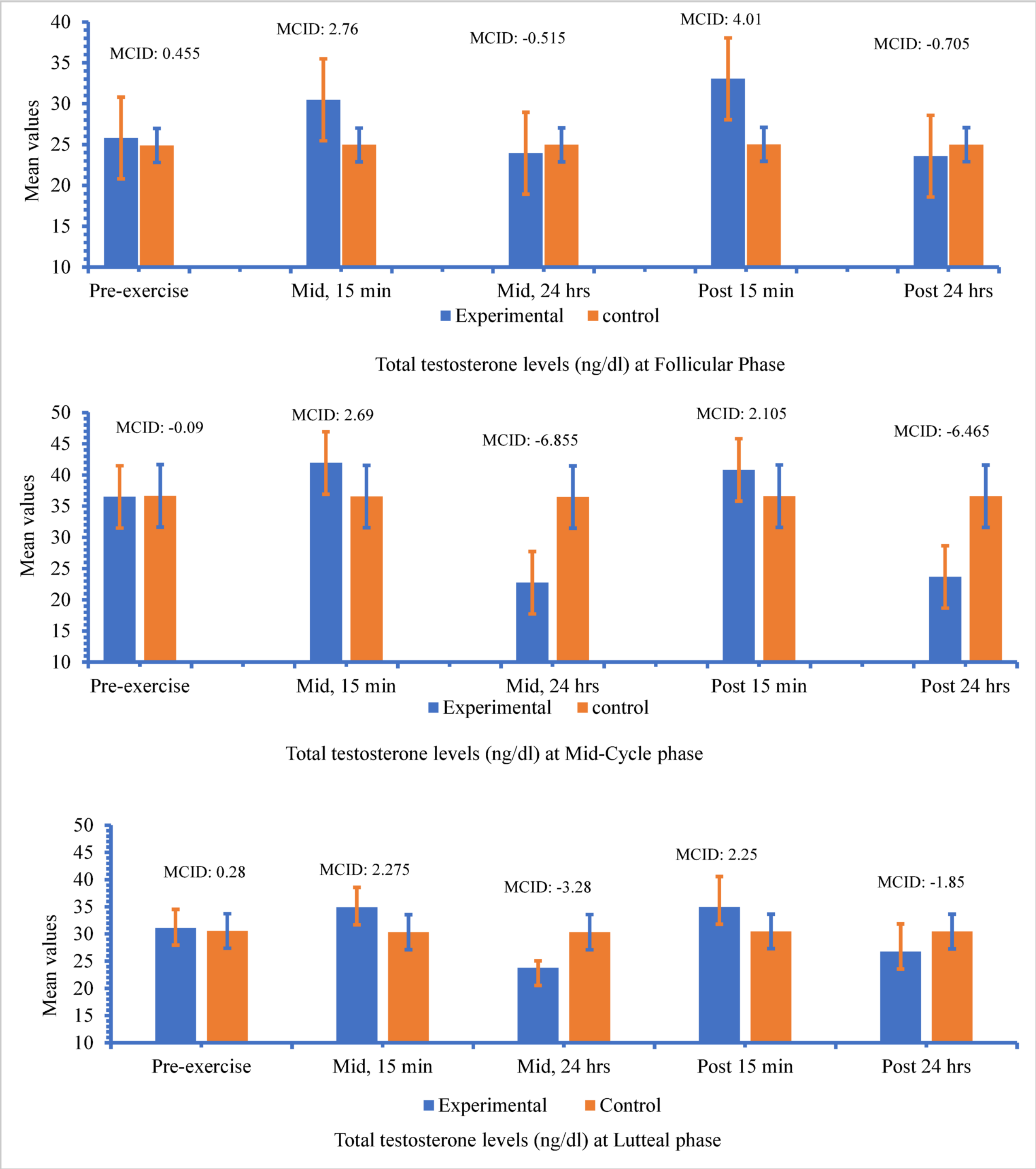

Fig. 4. Group-wise minimal clinically important differences (MCIDs) between testosterone (ng/dl) in the follicular, mid-cycle, and luteal phases of the menstrual cycle (pre exercise levels; mid-intervention levels 15 min after exercise; mid-intervention levels 24 h after exercise; post-intervention levels 15 min after exercise; post-intervention levels 24 h after exercise).

Sources of testosterone in women

In women, testosterone is naturally produced by two main sources: the ovaries and the adrenal glands[51]. Approximately 50% of circulating testosterone in healthy women originates from the ovaries, with the remaining half derived from the adrenal glands[52,53]. Once produced, testosterone is immediately released into the bloodstream to exert its physiological effects.

Despite lower concentrations than in men, testosterone plays a crucial role in women's musculoskeletal health, cognitive function, and cardiovascular well-being[54]. Women naturally secrete higher total amounts of androgens compared to estrogens, with testosterone being a key androgen and dihydrotestosterone (DHT) forming through peripheral metabolism[51].

In specific clinical scenarios—such as menopause or surgical removal of the ovaries (oophorectomy)—endogenous testosterone production may decrease significantly. In such cases, external testosterone supplementation may be considered to restore physiological levels. For example, testosterone therapy has shown benefits in improving sexual function in postmenopausal women with low sexual desire, with non-oral delivery methods preferred to minimize adverse effects on lipid profiles[55].

Testosterone fluctuations in response to exercise

The findings indicate that total testosterone levels transiently increase immediately after exercise but decline below baseline within 24 h during recovery. These results are consistent with previous studies[56], which reported that testosterone levels peak in the early recovery period following exhaustive endurance exercise but significantly decrease after 24 h. Similarly, Lane et al.[14] observed acute testosterone elevations in response to physical activity in women, suggesting that this transient increase may be linked to physiological adaptations enhancing performance and recovery. Additionally, Baydil[57] reported that exhausting exercise affects the total testosterone profile in females, further supporting the observed fluctuations in our study.

Mechanisms underlying hormonal changes

The underlying mechanisms for these hormonal changes may involve increased metabolic clearance of testosterone, reduced secretion capacity during recovery, and feedback regulatory mechanisms triggered by the initial spike in response to exercise. Kujala et al. and Nindl et al.[58,59] suggested that exercise-induced suppression of serum testosterone is primarily associated with decreased testosterone production during recovery, which aligns with our findings. Furthermore, the decline could be attributed to cortisol-induced inhibition, a known physiological response to exercise-induced stress[60]. In contrast, other studies have proposed that such fluctuations are part of a homeostatic mechanism involved in tissue repair and recovery[60].

Despite the initial rise in testosterone, our study highlights that hormone levels do not remain elevated long-term and may experience a rebound effect, dropping below pre-exercise values within 24 h. Kvorning et al.[61] emphasized that individual differences in hormonal regulation and exercise volume influence the magnitude of post-exercise testosterone responses. This aligns with findings by Kraemer et al.[60], who demonstrated that total workout volume significantly impacts post-exercise testosterone levels.

The findings suggest that structured, alternate-day exercise routines, including squats, crunches, and high marches, may enhance muscle strength and bone mineral density in women through testosterone modulation. This supports earlier research by Ciolac et al.[62], who highlighted that resistance training is crucial in preventing musculoskeletal deterioration, improving physical function, and enhancing overall quality of life, particularly in aging populations. Given testosterone's importance in female physiology, our study provides practical implications for designing exercise regimens that optimize hormonal balance and musculoskeletal health.

Impact of low testosterone on body composition in women

Clinical evidence suggests that low testosterone levels can impact body composition and metabolic health in women, although the effects may differ from those observed in men. In oophorectomized early postmenopausal women, low testosterone has been associated with endothelial dysfunction, indicating potential cardiovascular and metabolic implications[63].

Testosterone plays a key role in skeletal muscle development and maintenance. In physically active women, moderate increases in testosterone have been shown to enhance aerobic capacity and lean body mass[64], while short-term testosterone administration results in fiber-type specific muscle hypertrophy and increased capillarization[65]. Similarly, Miller[66] reported that although low testosterone in women may not significantly alter fat mass, it can affect skeletal muscle dynamics.

Testosterone therapy has been associated with increased trunk muscle area in women with testosterone deficiency, although changes in pelvic floor musculature were minimal[67]. These anabolic effects align with findings in postmenopausal women, where resistance training increased muscle mass and strength, partly mediated by elevated IGF-1 levels[68].

While most studies on testosterone and body composition have focused on men, particularly in populations with spinal cord injury (SCI), some emerging evidence supports similar benefits in women. For instance, low-dose testosterone replacement combined with resistance exercise has been found to increase lean mass, reduce visceral fat, and improve metabolic risk factors in individuals with SCI, though these studies are largely male-focused[69].

Collectively, these findings support the view that testosterone plays a vital role in female musculoskeletal and metabolic health, and that low testosterone may compromise body composition and performance outcomes, though more female-specific clinical studies are warranted.

Methodological strengths

A notable strength of this study is its rigorous methodological approach, which addressed variability in female sex steroid hormone research. Many previous studies have been characterized by heterogeneity due to differences in ethnicity, lifestyle, dietary habits, and inconsistent exercise protocols. To minimize these confounding factors, we:

- Conducted rigorous participant screening with gynecological assessment to ensure normal baseline hormonal levels.

- Provided standardized dietary guidelines, requiring participants to consume a meal at least two hours before exercise.
- Implemented structured warm-up and cool-down routines to ensure consistency in exercise protocols.
- Collected blood samples in the morning to reduce fluctuations in hormone levels throughout the day.

As a result, we observed significant effect sizes (ESs) for hormonal responses, particularly testosterone. The ES values ranged from medium to very large, indicating the robustness of the observed effects. Importantly, effect sizes are independent of sample size, reinforcing the strength of our findings beyond conventional significance testing. Furthermore, the highest testosterone levels were recorded during ovulation, supporting prior research indicating that menstrual cycle phases influence hormonal responses to exercise[58,60]. This reinforces the importance of considering menstrual cycle phases when designing exercise programs tailored for women.

Limitations

Despite these contributions, several limitations should be acknowledged. First, the relatively small sample size may limit the generalizability of our findings. Additionally, individual hormonal variations and exercise intensity were not fully controlled, which may have influenced testosterone responses. Demographic factors such as age and occupation of females may affect sex steroid hormones, as documented by a study conducted by Davis[51]. Therefore, how these demographic factors play a role in modulating testosterone levels after exercise should be investigated. In addition, further work should also investigate the following;

- Employ larger, more diverse cohorts to enhance statistical power and generalizability.
- Utilize advanced hormonal monitoring techniques to capture real-time fluctuations more precisely.
- Investigate the long-term effects of integrated exercise approaches on hormonal regulation.
- Explore exercise responses in populations with hormonal imbalances, such as polycystic ovary syndrome (PCOS) patients, to assess broader clinical implications.

Clinical implications

Although females have lower testosterone levels than males, testosterone plays a crucial role in maintaining bone metabolism, cognition, and sexual function. Therefore, incorporating these integrated exercises, which require minimal setup and are accessible to a broad population, could have potential implications for the primary prevention of conditions such as arthritis and other bone-related diseases in females by boosting bone metabolism.

Conclusion

This study determines that testosterone levels in physically active women rise immediately after integrated exercise but decline significantly within 24 h, with fluctuations influenced by menstrual cycle phases, peaking at mid-cycle. These findings parallel hormonal responses observed in men, accounting for sex-related concentration differences. By highlighting the role of testosterone in female exercise physiology, this study underscores its significance in women's health and performance. Future research should further refine integrated exercise approaches to optimize hormonal regulation and enhance musculoskeletal health outcomes in women.

Data availability

The datasets used and/or analyzed during the current study is available from the corresponding author on reasonable request and will be provided according to the policy of Riphah International University, from which ethical approval has been obtained.

Received: 28 October 2024; Accepted: 29 April 2025
Published online: 05 May 2025

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Acknowledgements
This study was undertaken at Aadil Hospital Defense, Lahore, where patients were assessed and recruited. The authors thank the following for their contributions to this research: Mr Fahdel Sheikh: CEO of Aadil Hospital Lahore, for trusting and giving permission to gather the data.Dr. Anees Raunaq: Gynecologist Aadil Hospital for assessing the female menstrual cycle phasesMiss Makia: Nutritionist Riphah International University Lahore Campus for providing the diet chart to females.Dr. Muhammad Saleem Ashraf: University of Agriculture Faisalabad, for helping in reviewing and refining the manuscript.Mr Bilal Umar: PhD Scholar University of Lahore, for guiding the data analysis.

Author contributions
WS designed the study, conducted the experiments, collected the data and analyzed the data. RN supervised the study and critically revised the manuscript.

Funding
No funding was achieved.

Declarations

Competing interests
The authors declare no competing interests.

Ethical approval
Ethical approval for the study was obtained from the Ethical Committee of Riphah International University with the Protocol ID REC/Lhr/22/1101.

Consent to participate
Formal informed consent was obtained from all individual participants included in the study. All the individuals who participated in this study were adults, and they willingly participated in this study. They were assured that their information would be kept anonymous and that the obtained results would only be used for the research.

Consent to publish
Not applicable.

Adverse event records, reports, and treatments
Some of the participants stated that they had growth of hair on the back due to increased testosterone levels after exercise.

Additional information
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