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Effects of Integrated Exercise Approach on Total Testosterone Levels during Different Phases of Menstrual Cycle in Eumenorrheic Females; A Randomized Controlled Trial

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Keywords

Hormones, eumenorrheic, females, physical activity, menstrual cycle

Abstract

Background: *Testosterone plays pivotal role in maintaining the overall health of males as well as females and it is modulated by the exercise. So, the objective of the study was to see the effects of integrated exercise approach on total testosterone levels during different phases of menstrual cycle in eumenorrheic females. Methods: It was a Randomized Controlled Trial in which 30 females fulfilling the inclusion criteria were recruited using Non- Probability Convenience Sampling and were divided in 2 groups using random table generator and by concealed enveloped allocation. The treatment group was given exercise plan for 3 times per week for 16 weeks along with the awareness program of menstrual hygiene and to maintain an active life style while the control group had just awareness program to maintain menstrual hygiene and active lifestyle. The testosterone levels were calculated at pre-intervention, mid-intervention and post-intervention. The data was analyzed using SPSS version 21. Results: For within group analysis the level of testosterone were higher in the mid-cycle phase of menstrual cycle and increases after the exercise which decreases below baseline level after 24 hours of exercise. For between group analyses the experimental group had significant effect on the testosterone levels. Conclusion: The level of the testosterone shows significant effect by the phases of exercise as well as by the integrated exercise approach.*

Summary box

- ***What is already known on this topic*** – *The studies suggested that exercise can lead to a transient elevation in testosterone levels, attenuate age-related decline in testosterone, and optimize acute testosterone response, with effects varying based on exercise duration, intensity, and individual factors such as obesity.*
- ***What this study adds-*** *The effects of the integrated exercise on testosterone levels was not known despite the fact that in modern era these exercises are being followed by females. So an integrated exercise approach is formulated and followed to see it effects and it showed transient increase in testosterone levels followed by decrease in it below baseline levels during recovery phase.*
- ***How this study might affect research, practice or policy****- it is seen by this clinical trial that the effects of integrated exercise on females mimics with those of males and as testosterone plays a significant role in the physiology of females too so these effects may be used for the health benefits of the females.*

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Introduction

The pursuit of a healthier, more active lifestyle has led to extensive research into the profound interplay between physical activity and hormonal fluctuations within the human body. Among many hormones affected by exercise, testosterone, primarily considered a male hormone, plays a significant role in both male and female physiology (1).

Testosterone, often recognized for its pivotal role in muscle development and sexual function, is not exclusive to men. In women, this hormone also plays a crucial role in maintaining overall health and vitality, although in significantly lower quantities(2).Recent studies have indicated that exercise, when performed in a well-balanced, integrated manner, can induce transient increases in testosterone levels in females(3, 4). This intriguing phenomenon has opened up exciting possibilities for the optimization of exercise routines tailored to the unique needs of women.

The worldwide involvement of women in fitness activities, such as gyms, home exercise plans and integrated exercise programs has grown exponentially over the last few decades. Integrated exercise program involves combining different exercises that target different body parts, such as butt/hips, legs, calves and shins, thighs, shoulders, and abs. Examples of full body/integrated exercises include ladder agility, anti-rotation reverse lunge, barbell jammers, bear crawl exercise, and resistance bands/cables(5). Due to testosterone's known connection with muscle hypertrophy resulting from exercise, numerous studies have focused on investigating how it 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 clearly points to the necessity of testosterone in the physiological adaptations of endurance-based activities. (6, 7) whereas, the effect of integrated exercise on the testosterone levels and how it is effected by the phases of menstrual cycle is not studied till date so the purpose of the study was to have comprehensive understanding of the influence of integrated exercise on testosterone dynamics and its potential implications for eumenorrheic females.

Material and Methods

Study Participants A total of 40 eumenorrheic females were recruited for the study through a non-probability convenience sampling technique. For participant characteristics, please refer to Table 1 and 2. Eumenorrheic menstrual cycles were defined as consistent cycles spanning 24 to 35 days(8). Eligible participants were within the age range of 20 to 40 years (9) and having a BMI within the normal range of 18.5 to 24.9. They were able to maintain sitting balance without the need for upper limb support or had a minimum score of 25 on the trunk control test(10). These participants had refrained from any exercise regimen over the preceding six months. Exclusions from the study were made for females who were currently taking oral contraceptives, pregnant, lactating, or had undergone a C-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(9). The study received approval from the Research Ethics Committee of Riphah International University, and all participants provided written informed consent. This study is registered on clinicaltrials.gov (ID: NCT05460741).

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Table 1: Characteristics of participants in percentage and frequency (n=40)

	frequency	percentage
gender		
females	40	100
males	0	0
Education		
matric	5	12.5
bachelors	20	50
masters	10	25
PhD	5	12.5
Occupation		
student	12	30
House wives	16	40
working	12	30
Marital status		
married	17	42.5
unmarried	17	42.5
divorced	6	15
BMI category		
normal	40	100
Under weight		
Over weight		
obese		

Table 2: Characteristics of participants in mean and S.D (n=40)

	mean	SD
age	29.85	6.11
Weight in kg	57.95	4.70
Height in cm	159.95	2.80
BMI	22.65	1.67

*S.D= Standard deviation

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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 day of the menstrual cycle, corresponding to the follicular, mid-cycle, and luteal phases of the menstrual cycle, respectively. This allowed us to establish the normal hormonal ranges for the participants and monitor any deviations from these baseline levels (11).

Study Protocol

It was a Randomized Controlled Trial conducted at Aadil Hospital Defence, Lahore, where participants underwent initial screening and blood profiling. After this initial assessment, the participants were randomly divided into 2 groups using random table generator. Both groups were given the awareness program related to the hygiene of menstrual cycle and maintaining an active lifestyle. The control group was asked to do 30 min of walking three times a week on alternate days for 16 weeks along with a standard dietary plan to be followed. Whereas, the experimental group reported to the hospital's Physiotherapy department where the physiotherapist made them perform an integrated exercise protocol of 50 min on alternative days, totaling three sessions per week over a period of 16 weeks. In this protocol different types of exercises, resistance, endurance, balance, were integrated with proper warm ups, cool downs and rest intervals which required minimal of the setup to perform them (with just handheld dumbbells). Prior to 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., following a breakfast consumed at least two hours before the session. A nutritionist provided dietary guidance to ensure that participants followed these recommendations for 48 hours before each session, minimizing potential dietary 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 the hormonal surges through the day.

The levels of the testosterone in the blood serum were checked through Chemiluminescence Microparticle Immunoassay Technique (Abbott- Alinity Ci) within 30 min of the exercise and then 24hrs after the exercise. 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 took place during the 6th to 8th week (mid-intervention) of the program, again on the 4th, 14th, and 24th days of the menstrual cycle. The final set of readings occurred during the 14th to 16th week (post-intervention) of the study plan. For a visual representation of the study's flow, refer to Figure 1 (Pienado et al., 2021) in accordance with STROBE guidelines (12).

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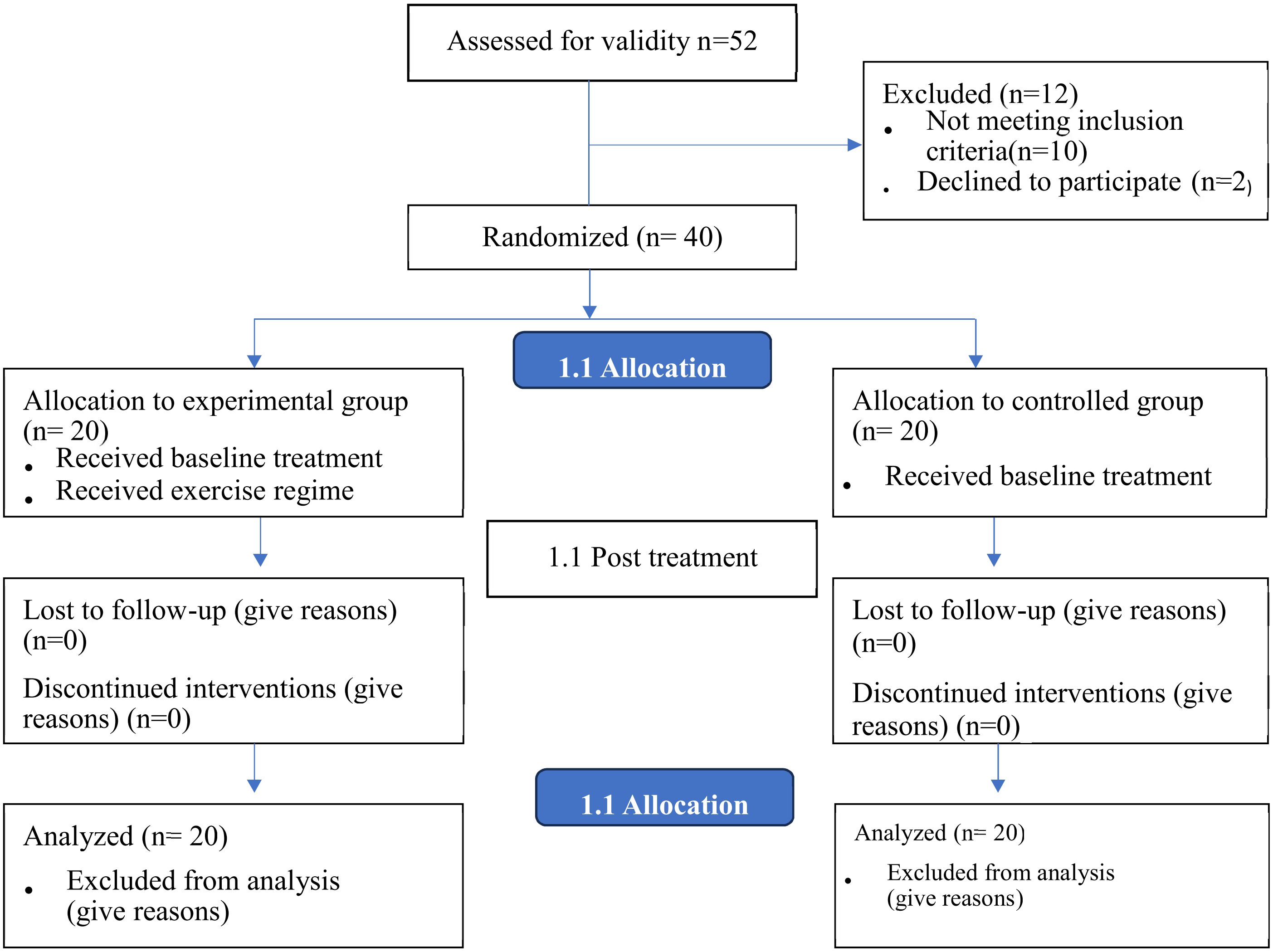

Figure 1 Flow diagram of study participants modified from Peinado et al. (2021)(12) according to STROBE guidelines.

To ensure the diversity of our study population, we enrolled females of various ages, socioeconomic backgrounds, and professions. However, in order to maintain inclusivity and equity, we established specific inclusion criteria at the outset. Additionally, all participants were closely monitored to adhere to a nearly uniform diet plan. They 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 that occur throughout the day. We are dedicated to furthering the cause 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.

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Statistical Analysis

The data were presented in tables as the mean ± standard deviation (SD). To assess the normal distribution of the variables, a Shapiro-Wilk test was conducted. 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 the levels of testosterone at aforementioned times. In cases where Mauchly's test indicated a violation of the assumption of sphericity, the Greenhouse-Geisser correction was applied. To gauge the effect sizes and determine the significance of observed changes, partial eta squared was calculated. Effect sizes were classified as small, medium, or large, with threshold values typically falling around 0.01, 0.06, and 0.14, respectively. Additionally, 95% confidence intervals (CI) 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 statistical analyses were conducted using SPSS software, version 25 (IBM Corp., Armonk, NY, USA) (13).

Results

Table 3 shows the mean values of testosterone levels both 15 min after exercise and 24hrs after exercise at follicular, mid-cycle and luteal phases of menstrual cycle and at pre intervention, mid intervention and post intervention. The testosterone levels are maximum during mid-cycle phase and surge during 15 min post exercise and keeps on decreasing during recovery phase.

Table 3: Mean values of testosterone levels at 3 phases and 3 times

Stages of the menstrual cycle	Total testosterone levels (ng/dl)	study group of the females	Mean ± S. D
follicular phase	Pre exercise	experimental	25.80±2.57
		control	24.89±2.08
	Mid intervention levels within 15 min of exercise	experimental	30.47±3.03
		control	24.95±2.03
	Mid intervention levels after 24hrs of exercise	experimental	23.93±2.32
		control	24.96±2.04
	Post intervention levels within 15 min of exercise	experimental	33.04±8.67
		control	25.02±2.10
		experimental	27.58±7.03

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	Post intervention levels after 24hrs of exercise	control	24.99±2.08
Mid-cycle phase	Pre exercise	experimental	35.48±2.80
		control	36.66±3.21
	Mid intervention levels within 15 min of exercise	experimental	41.93±8.87
		control	36.55±3.11
	Mid intervention levels after 24hrs of exercise	experimental	22.74±2.50
		control	36.45±3.31
	Post intervention levels within 15 min of exercise	experimental	40.80±7.12
		control	36.59±3.12
	Post intervention levels after 24hrs of exercise	experimental	23.66±1.10
		control	36.58±3.13
Luteal phase	Pre exercise	experimental	32.10±3.44
		control	30.44±3.17
	Mid intervention levels within 15 min of exercise	experimental	34.89±3.70
		control	30.34±3.21
	Mid intervention levels after 24hrs of exercise	experimental	23.76±1.31
		control	30.32±3.24
	Post intervention levels within 15 min of exercise	experimental	34.97±5.60
		control	30.47±3.17

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	Post intervention levels after 24hrs of exercise	experimental	26.75±5.10
		control	30.45±3.19

**S.D= Standard Deviation*

Table 4 presents the testosterone level changes during different phases, times and it interactions between different phases and groups, time and groups and lastly phases, times and groups. All of these interactions shows significant difference ($p < 0.05$).

Table 4: Mixed model ANOVA for within group analyses

	df	Mean Square	Sig.	Partial Eta Squared	Observed Power
phase	2	2163.864	.000	.848	1.000
phase * group	2	257.137	.000	.399	.991
time	5	396.069	.000	.568	1.000
time * group	5	392.389	.000	.566	1.000
phase * time	10	55.089	.000	.379	.991
phase * time * group	10	57.498	.000	.389	1.000

Table 5 presents the testosterone level changes across the experimental and control group which shows significant difference ($p < 0.05$).

Table 5: Mixed model ANOVA for between group analyses

	df	Mean Square	Sig.	Partial Eta Squared	Observed Power
group	1	337309.956	.00	.994	1.00

After these significant increases immediately post-exercise, all measurements gradually decreased and at the 24hr post-exercise time, total testosterone reached significantly lower levels than their resting, pre-exercise values ($p < 0.05$) and these changes are maximum during mid-cycle phase.

The effect size for all these changes are also reported in Table 4and 5. This analysis demonstrates that for the significant changes in our measures, the effect size ranged from medium to large in magnitude.

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Discussion

This study contributes to the existing body of knowledge by shedding light on the impact of exercise on testosterone levels, with a particular focus on the often underrepresented female population (14). Testosterone, despite its traditionally male-centric reputation, plays a significant role in female physiology (1). Previous research predominantly examined the effects of resistance and endurance exercises on testosterone levels. However, in the modern era, there is a growing emphasis on the 'Integrated Exercise Approach,' which combines various exercise types into a single plan to maximize benefits (15).

In this novel study, we formulated an integrated exercise approach and investigated its effects on testosterone levels during different phases of the menstrual cycle: follicular, mid-cycle, and luteal phases. The primary finding of this research is that the integrated exercise plan transiently increases testosterone levels immediately after exercise, followed by a decrease below baseline levels within the first 24 hours during the recovery phase. This phenomenon aligns with studies conducted by Kujala, U,(16) and Nindl BC (3) which suggests that exercise-induced suppression of serum testosterone is associated with a reduced testicular capacity to secrete testosterone during the recovery period. Other factors attributed are the result of a feedback regulatory mechanism triggered by the initial substantial increase in response to exercise (17). Alternatively, it could be due to a delayed enhancement in the metabolic clearance of the hormone, or possibly a stress-related response, such as the inhibition induced by cortisol, which could be seen as a dysfunctional reaction (17, 18). On the contrary, Kraemer et al. (19) have suggested that such changes are indicative of homeostatic mechanisms involved in the repair and recovery process.

Additionally, we observed significant effect sizes (ES) for hormonal responses, particularly in terms of testosterone. The magnitude of the ES ranged from medium to very large among our study participants (20). Importantly, ES values are independent of sample size, indicating that the significance levels alone may not fully capture the strength of our findings. In other words, the effect sizes suggest that our results are more substantial and meaningful than the significance levels might imply. Furthermore, we found that testosterone levels were highest during the ovulation phase, suggesting that the menstrual cycle phases can influence testosterone levels (20, 21).

Most of the literature on female sex steroid hormones is characterized by heterogeneity, which can be attributed to variations in ethnicity, lifestyle, dietary habits, and a lack of a common exercise regime that incorporates proper warm-ups and cool-downs. In our study, we addressed these issues by rigorously screening participants with the assistance of a gynecologist to ensure that baseline hormonal levels were within the normal range. We provided dietary guidelines, recommended a meal at least two hours before exercise, implemented a well-structured exercise plan with proper warm-up and cool-down routines, and ensured that samples were collected in the morning to minimize hormone fluctuations throughout the day.

Clinical Implications:

Although females have lower levels of testosterone compared to males, it 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 like arthritis and other bone-related diseases in females by boosting bone metabolism.

Limitations of the study:

This study has some limitations that encourages the future researchers to work on it. The demographic factors like age and occupation of the females may have effect on the sex steroid hormones as documented by study conducted by Davis (22). So, it should be documented that how these demographical factors play role in modulation of testosterone levels after exercise.

Conclusion

In conclusion, our study on physically active women revealed that testosterone levels remained elevated in the initial stages of recovery after integrated exercise, but declined significantly 24hrs later and it is effected by the phases of menstrual cycle, the levels of testosterone being highest in mid cycle phase of menstrual cycle. These findings are consistent with responses seen in

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men undergoing similar types of exercise, once we account for sex-related concentration variations.

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Ethical Consideration:
The ethical consideration for the study was taken from Ethical Committee Riphah International University with the Protocol ID REC/Lhr/22/1101.

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Author's Contribution
All authors have made significant contributions to this work, encompassing various aspects of the research process. These contributions include involvement in the conception and design of the study, participation in data analysis and interpretation, drafting of the manuscript, critical revision of the manuscript for substantial intellectual content, and granting their final approval for the version of the manuscript intended for publication. Furthermore, all authors have reviewed and consented to the publication of the manuscript in its present form.

Competing interests
The authors declare they have no competing interests.

References
1. Handelsman DJ, Hirschberg AL, Bermon S. Circulating Testosterone as the Hormonal Basis of Sex Differences in Athletic Performance. Endocr Rev. 2018;39(5):803-29.
2. Davis SR, Wahlin-Jacobsen S. Testosterone in women—the clinical significance. The Lancet Diabetes & Endocrinology. 2015;3(12):980-92.
3. Nindl BC, Kraemer WJ, Gotshalk LA, Marx JO, Volek JS, Bush JA, et al. Testosterone Responses after Resistance Exercise in Women: Influence of Regional Fat Distribution. International Journal of Sport Nutrition and Exercise Metabolism. 2001;11(4):451-65.
4. Hackney AC, Willett HN. Testosterone Responses to Intensive, Prolonged Endurance Exercise in Women. Endocrines. 2020;1(2):119-24.
5. Kondo R, Tani Y, Sugimoto M, Inami M, Kitazaki M. Scrambled body differentiates body part ownership from the full body illusion. Sci Rep. 2020;10(1):5274-.
6. Hackney AC. The male reproductive system and endurance exercise. Medicine &amp Science in Sports &amp Exercise. 1996;28(2):180-9.
7. Hackney AC. Molecular and Physiological Adaptations to Endurance Training. Concurrent Aerobic and Strength Training: Springer International Publishing; 2018. p. 19-34.
8. Elliott-Sale KJ, Minahan CL, de Jonge XAKJ, Ackerman KE, Sipilä S, Constantini NW, et al. Methodological Considerations for Studies in Sport and Exercise Science with Women as Participants: A Working Guide for Standards of Practice for Research on Women. Sports Med. 2021;51(5):843-61.
9. McNulty KL, Elliott-Sale KJ, Dolan E, Swinton PA, Ansdell P, Goodall S, et al. The Effects of Menstrual Cycle Phase on Exercise Performance in Eumenorrheic Women: A Systematic Review and Meta-Analysis. Sports Med. 2020;50(10):1813-27.
10. La Porta F, Franceschini M, Caselli S, Cavallini P, Susassi S, Tennant A. Unified Balance Scale: An activity-based, bed to community, and aetiology-independent measure of balance calibrated with Rasch analysis. Journal of Rehabilitation Medicine. 2011;43(5):435-44.
11. Peinado AB, Alfaro-Magallanes VM, Romero-Parra N, Barba-Moreno L, Rael B, Maestre-Cascales C, et al. Methodological Approach of the Iron and Muscular Damage: Female Metabolism and Menstrual Cycle

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during Exercise Project (IronFEMME Study). Int J Environ Res Public Health. 2021;18(2):735.
12. Ekenros L, Hirschberg AL, Heijne A, Fridén C. Oral Contraceptives Do Not Affect Muscle Strength and Hop Performance in Active Women. Clinical Journal of Sport Medicine. 2013;23(3):202-7.
13. Nakagawa S, Cuthill IC. Effect size, confidence interval and statistical significance: a practical guide for biologists. Biological Reviews. 2007;82(4):591-605.
14. Mujika I, Taipale RS. Sport Science on Women, Women in Sport Science. International Journal of Sports Physiology and Performance. 2019;14(8):1013-4.
15. Marchesini G, Melchionda N, Apolone G, Cuzzolaro M, Mannucci E, Corica F, et al. The metabolic syndrome in treatment-seeking obese persons. Metabolism. 2004;53(4):435-40.
16. Kujala UM, Alen M, Huhtaniemi IT. GONADOTROPHIN-RELEASING HORMONE AND HUMAN CHORIONIC GONADOTROPHIN TESTS REVEAL THAT BOTH HYPOTHALAMIC AND TESTICULAR ENDOCRINE FUNCTIONS ARE SUPPRESSED DURING ACUTE PROLONGED PHYSICAL EXERCISE. Clinical Endocrinology. 1990;33(2):219-26.
17. Hackney AC, Hooper DR. Reductions in testosterone are not indicative of exercise performance decrement in male endurance athletes. The Aging Male. 2019;23(1):33-4.
18. Dote-Montero M, Carneiro-Barrera A, Martinez-Vizcaino V, Ruiz JR, Amaro-Gahete FJ. Acute effect of HIIT on testosterone and cortisol levels in healthy individuals: A systematic review and meta-analysis. Scandinavian Journal of Medicine & Science in Sports. 2021;31(9):1722-44.
19. Kraemer WJ, Ratamess NA, Nindl BC. Recovery responses of testosterone, growth hormone, and IGF-1 after resistance exercise. Journal of Applied Physiology. 2017;122(3):549-58.
20. Durlak JA. How to Select, Calculate, and Interpret Effect Sizes. Journal of Pediatric Psychology. 2009;34(9):917-28.
21. Schulze J, Suominen T, Bergström H, Ericsson M, Björkhem Bergman L, Ekström L. Urinary steroid profile in relation to the menstrual cycle. Drug Test Anal. 2021;13(3):550-7.
22. Davis SR, Bell RJ, Robinson PJ, Handelsman DJ, Gilbert T, Phung J, et al. Testosterone and Estrone Increase From the Age of 70 Years: Findings From the Sex Hormones in Older Women Study. The Journal of Clinical Endocrinology & Metabolism. 2019;104(12):6291-300.

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