The physiology of the female athlete – performance, health, and recovery

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The physiology of the female athlete – performance, health, and recovery

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Editorial: The physiology of the female athlete—performance, health, and recovery

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Editorial on the Research Topic
The physiology of the female athlete—performance, health, and recovery

Females are historically underrepresented in sports science research, with relatively few studies investigating female physiology in relation to exercise and sports performance, health, and recovery. This knowledge gap also extends to training planning, periodization, and peaking, where previous research has predominantly focused on male participants. Consequently, female athletes and their coaches have had to rely on scientific knowledge derived from male-centric research and adapt these findings based on their intuition. The exclusion of females from sports science research also carrier broader societal implications and consequences. In a society striving for equal opportunities in training, participation in activities, and the ability to pursue a career as a professional athlete, this marginalization and deprioritization of female-focused research signal that needs of female athletes are unfortunately undervalued and disregarded, undermining the inclusivity and integrity of sports science as a whole.

The percentage of females participating in international championships has increased in recent years, and they compete for approximately the same number of medals and in the same disciplines as men. Similarly, the commercial activity around women's sports has also increased in recent years with a significant rise in sponsorship revenues, advertising revenues, and revenues from televised events. However, in contrast to this progress observed in sports, research on female athletes unfortunately remains relatively scarce.

Many female athletes have significantly contributed by openly discussing various challenges related to training, sport performance, menstruation, potential use of hormonal contraception (HC), and communication with coaches. For example, the natural hormone fluctuations and potential symptoms associated with the menstrual cycle are distinct to females and underscores the importance of undertaking further research focused exclusively on the female athlete. Females are more likely than males to enter a vicious cycle of disordered eating behavior, reduced energy availability, and accompanying disruptions in the menstrual cycle, particularly in endurance sports. This can have both short- and long-term negative health effects for females engaged in chronic/habitual vigorous physical activity/exercise and/or elite sport.

In this article series "*The Physiology of the Female Athlete—Performance, Health, and Recovery*", we have received a significant number of submitted manuscripts, and after thorough peer reviews, we have accepted 13 manuscripts for publication in this special edition. Methodologically, the articles cover various approaches: reviews, qualitative interviews, observations, questionnaire surveys, interventions, cross-sectional and longitudinal studies.

Nutritional and hormonal influences play intricate roles in the health and performance of physically active premenopausal females, and particularly athletes. The interplay between menstrual blood loss, iron deficiency, and hormonal regulation has attracted attention due to its impact on female-specific health issues. Concurrently, studies linking energy availability to reproductive health and athletic performance shed light on the nuanced connections between nutrition, hormonal balance, and physical well-being. In this context, the studies by Badenhorst et al. Castellanos-Mendoza et al. Fahrenholtz et al. and Kettunen et al. presented here in this Research Topic, collectively illuminates key aspects of this complex relationship, offering insights into potential interventions and strategies to optimize the health and performance of this population. By understanding these interactions, we can better address the unique needs of premenopausal females, especially athletes, in achieving their full potential while maintaining optimal health.

Engseth et al. study on HC use among competitive cross country skiers and biathletes highlights HC's multifaceted role beyond contraception, particularly in managing menstrual challenges that could affect training and competition schedules. Despite some athletes reporting negative symptoms, the majority noted either positive or neutral effects on athletic performance, underscoring the importance of HC choices for athletic women. In a separate study, Mathy et al. investigated the impact of oral contraceptive pill intake on endurance parameters in female first-division handball players. They found significant differences in peak VO_2 and submaximal respiratory equivalents for VO_2 and VCO_2 between phases, shedding light on how pill intake can affect athletes' physiological responses during exercise. Concurrently, addressing menstruation-related symptoms (MRSs) is crucial, as emphasized by Masuda and Okada study linking MRS severity to menstrual phases, physical activity, and sleep timing. Lifestyle adjustments, such as optimized sleep timing and increased physical activity during the luteal phase, could aid in managing MRSs effectively. Bergström et al. research further emphasizes the need to bridge communication gaps regarding menstrual health in sports environments, especially among coaches and female athletes, to promote better understanding and support for menstrual health and its impact on performance and well-being. Integrating these insights into athlete support frameworks can contribute significantly to optimizing female athletes' health, performance, and overall athletic experience.

This research topic also includes a series of studies investigating the impact of physical activity and training on various markers of health and performance. For example, Grazioli et al. exploration of workplace physical activity programs for menopausal women highlights the positive impact on bone health and fall prevention, crucial factors in maintaining quality of life during menopause. Elite athletes, as studied by DeBlauw et al. benefit from monitoring heart rate variability to gauge physiological and psychological stress levels, aiding in performance optimization. Gaamouri et al. investigation into plyometric training showcases its effectiveness in enhancing various physical abilities among youth female handball players, translating to improved game-related performance. Izadi et al. research underscores the need for customized load recommendations for women in athletic training programs, given the distinct load-velocity relationships observed in bench press motions. Finally, Solli et al. longitudinal study on a world-class female biathlete provides insights into training evolution across performance levels, offering valuable guidance for elite athlete development strategies. These collective studies underscore the multidimensional approach required to optimize female athletes' well-being, training, and competitive outcomes in various stages of their athletic careers.

To conclude, this Research Topic includes high-level research papers focusing on the physiology of the female athlete, including papers on performance, health, and recovery. We hope that these will generate new, testable hypotheses that would advance the field of sport research on women further!

Author contributions

BW: Writing – original draft, Writing – review & editing. JK: Writing – original draft, Writing – review & editing. RL: Writing – original draft, Writing – review & editing. BM: Writing – original draft, Writing – review & editing. JO: Writing – original draft, Writing – review & editing. ØS: Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Prevalence and Self-Perceived Experiences With the Use of Hormonal Contraceptives Among Competitive Female Cross-Country Skiers and Biathletes in Norway: The FENDURA Project

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Purpose: To investigate the prevalence of hormonal contraceptive (HC) use by female cross-country (XC) skiers and biathletes competing at a national and/or international level, their reasons for HC use, and to compare negative symptoms related to the HC-/menstrual cycle in HC users and non-HC users. Additionally, to characterize the self-perceived influence of HC use on training and performance.

Methods: A total of 113 Norwegian competitive XC skiers and biathletes completed an online questionnaire including both closed and open-ended questions. The questions were designed to assess the type of HC, reasons for use, self-reported negative symptoms related to HC-/menstrual cycle, as well as athletes' experiences regarding how HC use affects training and performance.

Results: In total, 68% of all the athletes used HC, with 64 and 36% of them using a progestin-only and combined type HC, respectively. Non-contraceptive reasons for HC use were reported by 51% of the progestin-only HC users vs. 75% of the combined HC users ($P = 0.039$), with reduction of negative menstrual-related symptoms as the most common reason. Of the athletes reporting regular withdrawal bleedings in connection to HC use, 80% of the progestin-only and 86% of combined HC users experienced negative menstrual-related symptoms, which was comparable to the non-HC group (86%). The majority (81%) of HC users experienced solely positive, or no effect, of HC use on training and performance, with no differences between progestin-only and combined HC users ($P = 0.942$).

Conclusions: In total, 68% of the XC skiers and biathletes used HC, with the highest proportion (64%) using a progestin-only HC. Many athletes used HC to manipulate their menstrual cycle due to perceived negative menstrual-related symptoms that interfered with their training sessions and/or competitions.

Keywords: combined hormonal contraceptives, endurance, female athletes, hormonal contraceptives, progestin-only hormonal contraceptives

INTRODUCTION

Hormonal contraceptives (HCs) are exogenous hormones used to alter endogenous sex hormone concentrations to prevent pregnancy or for medical and/or health-related purposes (Davis and Westhoff, 2001; Bitzer and Simon, 2011; Burke, 2011; Shulman, 2011). HCs can be classified into two main types, progestin-only or combined, based on their concentration of synthetic estrogen and progestin (Burke, 2011; Shulman, 2011). Progestin-only HCs include oral contraceptives (OCs; mini pills), implants, injections, and intrauterine systems (IUSs) (Burke, 2011), while combined HCs contain both synthetic estrogen and progestin and include OCs, transdermal patches, and vaginal rings as delivery methods (Shulman, 2011).

Approximately 40% of women (15–49 years) in the general Nordic population have been reported to use HCs, with the most common delivery method being combined OCs (Lindh et al., 2017). However, recent data from the Norwegian Prescription Database shows a rapid increase in the use of long-acting progestin-only HCs (i.e., implants and IUS) during the past 3 years (Furu et al., 2021). In the athlete population, approximately half of the respondents from various sports in the United Kingdom, Ireland, Denmark, and Norway reported hormonal contraceptive (HC) use (Martin et al., 2018; Oxfeldt et al., 2020; Solli et al., 2020; Nolan et al., 2022), with athletes in technical sports (e.g., golf, table tennis etc.) showing a higher proportion of HC use (80%) compared to athletes competing in endurance sports (50%) (Oxfeldt et al., 2020). Overall, combined HCs were more commonly used than progestin-only HCs (Martin et al., 2018; Oxfeldt et al., 2020; Solli et al., 2020). Although these studies were recently published, it is unclear if the rapid shift toward the use of long-acting progestin-only HCs, as reported by Furu et al. (2021), is also present in the athletic population.

Although HCs are mainly used to prevent pregnancy, both types of HCs are also used for other medical or health-related purposes, such as reducing premenstrual syndrome, premenstrual dysphoric disorder, anemia, mild-to-moderate acne, and other negative menstrual-related symptoms (Burke, 2011; Shulman, 2011). In addition, hormonal contraceptives are widely used to manipulate menstruation in recreationally active and competitive women (Schaumberg et al., 2018). In athletes, the consensus from previous research is that prediction and manipulation of menstruation are the most common non-contraceptive reasons, and positive side-effects, for using HCs (Martin et al., 2018; Armour et al., 2020; Elliott-Sale et al., 2020; Oxfeldt et al., 2020; Clarke et al., 2021), even though a meta-analysis showed that OC use results in a slightly lower performance compared to a natural menstrual cycle (Elliott-Sale et al., 2020). When Armour et al. (2020) investigated why Australian team-sports athletes chose to manipulate their menstruation, "convenience" and "reducing the impact on sporting events" were the main reasons. Furthermore, athletes from various sports reported that a reduction in negative menstrual-related symptoms is a positive side-effect of using HC (Martin et al., 2018; Clarke et al., 2021). However, negative side-effects such as weight gain, irregular periods, and mood swings are also reported with HC use and are mentioned as reasons for disuse of HCs (Martin et al., 2018; Clarke et al., 2021). Although the above-mentioned studies provide initial insights into the self-reported reasons and side-effects experienced by athletes using HCs, information about the self-perceived influence of HC use on endurance training among athletes of different age groups and performance levels, and whether their experiences differ from non-HC users, is lacking.

In the previous study by Solli et al. (2020), 17% of HC using athletes reported HCs to have a positive effect on their physical fitness or performance, while only 5% experienced negative effects. Since this study provided limited insights into differences between types of HC preparations, reasons for HC use, and how the HC affected training and performance, the present study aimed to build upon the work of Solli et al. (2020), by investigating HC users in more detail. Therefore, the aims of the current study were to: (1) investigate the prevalence of different types of HC (progestin-only vs. combined HCs) used by female cross-country (XC) skiers and biathletes competing at a national and/or international level; (2) explore the athletes' reasons for HC use; (3) compare negative symptoms related to the HC-/menstrual cycle experienced by HC users and non-HC users; and (4) characterize the self-perceived influence of HC use on training and performance.

MATERIALS AND METHODS

The current study is part of The Female Endurance Athlete (FENDURA) project, which is led by the School of Sport Sciences at UiT The Arctic University of Norway, in collaboration with the Norwegian University of Science and Technology, the Norwegian Olympic Committee (Olympiatoppen), the Norwegian Ski Federation, and the Norwegian Biathlon Federation. The overall aim of the FENDURA project is to increase the knowledge on how female-specific aspects, such as the menstrual cycle and HC use, influence training and performance among female endurance athletes.

Participants

From May 2020 to September 2020, 221 Norwegian competitive female XC skiers and biathletes were invited to complete a questionnaire about their menstrual cycle and HC use. The recruitment process was performed in collaboration with coaches and staff members of the Norwegian Ski Federation and the Norwegian Biathlon Federation, and by approaching athletes directly. The inclusion criteria were as follows: (1) competing at a national or international level; (2) above 18 years of age; (3) training systematically in their sport for at least 3 years. Of the athletes invited, 115 athletes completed the questionnaire. Of these, one athlete was excluded from the analysis due to missing consent form and one athlete was excluded due to low age (<18 years). Thus, 113 responses were included in the analysis; of these, 50 athletes were biathletes and 63 were XC skiers. Overall, the total sample included 51 juniors (born ≥2000) with an average age of 19 (range from 18 to 20) years and 62 seniors (born ≤1999) with an average age of 24 (range from 20 to 32) years. Based on the framework by McKay et al. (2022),

TABLE 1 | Participant characteristics stratified by type of hormonal contraceptive (HC), and by use and non-use of HC (mean and SD).

Characteristics information	Progestin-only HC users (*n* = 49)	Combined HC users (*n* = 28)	All HC users (*n* = 77)	Non-HC users (*n* = 36)	Seniors (*n* = 62)	Juniors (*n* = 51)	National team athletes (*n* = 30)	Non-national team athletes (*n* = 83)
Age (y)	21.3 (2.7)	22.2 (4.0)	21.6 (3.2)	20.8 (3.1)	23.5 (2.9)**	18.8 (0.7)	23.2 (3.9)**	20.7 (2.6)
Body height (cm)	168.6 (5.0)	168.1 (4.3)	168.4 (4.7)	169.3 (6.4)	168.4 (5.3)	169.0 (5.4)	169.8 (4.9)	168.3 (5.4)
Body mass (kg)	61.5 (5.3)	60.3 (3.3)	61.1 (4.7)**	63.5 (4.7)	61.2 (4.6)	62.6 (4.9)	63.2 (5.0)	61.4 (4.7)
Annual training volume (hr/year)	611.5 (113.1)*	667.8 (124.8)	632.2 (119.8)	644.7 (122.4)	679.3 (122.5)**	582.8 (93.9)	725.9 (120.6)*	603.4 (102.7)

*Significant differences between groups (*P <0.05 and **P <0.01).*

30 athletes (10 juniors and 20 seniors) belonging to a national team (recruit, junior, elite) were classified as Tier 4 and 5, while 83 athletes were classified as national level athletes (Tier 3) based on their training volume being within 20% of the international athletes (**Table 1**). The study was evaluated by the Regional Committees for Medical and Health Research Ethics (REK) and approved by the Norwegian Social Science Data Services (NSD). All participants were given oral and written information about the study, before providing their written informed consent to participate.

Questionnaire

The questionnaire used in the current study was based on the previously published questionnaire by Solli et al. (2020) but modified based on consultations with an expert panel, including former athletes, physiologists, medical experts, a gynecologist, and coaches. The current questionnaire was validated in Norwegian for use among Norwegian athletes (**Supplementary Table 1**) and contained several questions, both closed- and open-ended, about HC use, reasons for use, and how HCs influence self-perceived training quality and performance. The questionnaire was designed to take ∼15–30 min to complete. All participants reported age, body height, body mass, and total training volume over the preceding season (May 1st 2019, to April 30th 2020) (**Table 1**). Additionally, HC users reported information about which HC delivery method and brand they used (**Figure 1**). Athletes were grouped as either "current HC users" or "non-HC users," and each group completed different sections of the questionnaire. Current HC users reported if they used HC for non-contraceptive reasons, and athletes who used HC for non-contraceptive reasons were also asked to state these reasons by answering an open-ended question. HC users were asked to report if they experienced any positive and/or negative influence of HC use on training and performance. All HC users who experienced a positive or negative influence on training/performance were then asked to specify these experiences through answering open-ended questions. Both HC users and non-HC users were asked if they had regular withdrawal bleedings/menstruation. Athletes with regular bleedings were asked if they had any negative menstrual-related symptoms (i.e., experienced negative symptoms in relation to their HC-/menstrual cycle), and to specify these symptoms by answering an open-ended question.

Data Analysis

Responses to the open-ended questions were analyzed by the content comparative methods of analysis (Postholm, 2019). By employing an abductive analysis approach, these questions were coded and categorized by two authors of the current study (TE and GS), which were further discussed until a consensus was reached. Frequency analyses from the open-ended questions on symptoms and reasons for HC use were completed by counting codes in the different categories. A selection of responses, representing each category from the open-ended questions about "reasons for HC use" and "self-perceived influence of HC use on training/performance," are provided as examples of the interpretation of the responses to the various open-ended questions (**Tables 3**, **5**). Quantitative data are presented as mean (SD), frequencies, or prevalence, and the statistical significance level was set at $P < 0.05$. The athletes were categorized based on HC use vs. non-HC use, as well as the type of HC (progestin-only vs. combined). We also stratified athletes based on age (senior vs. junior) and national team athletes (Tier 4 and 5) vs. non-national team athletes (Tier 3). Categorical and numerical data were analyzed using the Statistical Package for the Social Sciences (SPSS 26, IMB Corp, Armonk, NY, USA). Data were examined for normality distribution before analysis using a Shapiro–Wilk test and visual inspection of Q–Q plots and histograms. Independent sample t-tests and Mann-Whitney U-tests were used to examine between-group differences, with the latter test being used for data that were considered as non-normally distributed. The relationship between categorical variables was examined with Pearson's chi-square analysis, with Fisher's exact tests being used when any expected cell counts were <5, i.e., using a conservative approach (Field, 2013).

RESULTS

Detailed information about the athletes' characteristics are presented in **Table 1**.

HC Use

Of all participants, 68.1% were currently using HC, of whom 63.6% used a progestin-only HC (IUS: 51%; implants: 28.6%; mini-pills: 20.4%), while 36.4% used combined HC (combined-OC: 96.4%; one patch-user). A lower proportion of progestin-only vs. combined users (30.6 vs. 75.0%, $P < 0.001$) reported having a regular withdrawal bleeding in connection with their

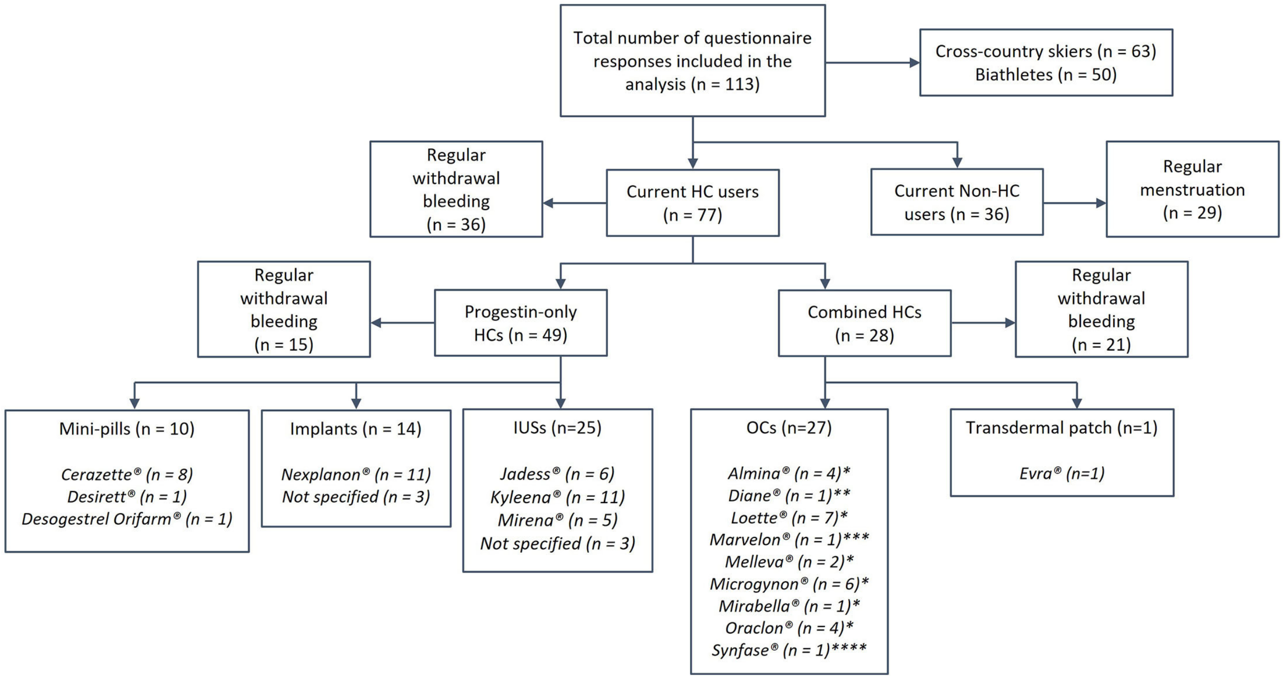

FIGURE 1 | The prevalence of type, delivery methods, and preparations of hormonal contraceptives (HCs) used. Concentration of synthetic hormones in the different delivery methods: Mini-pills, 75 µg Desogestrel; Implants, ~40 µg Etonogestrel in 24 h over 3 y; Intrauterine devices (IUSs), 6–15 µg Levonorgestrel in 24 h over 3–6 y; Oral contraceptives (OCs), * 20–30 µg Ethinyl estradiol (EE) and 100–150 µg Levonorgestrel, **35 µg EE and 2,000 µg Cyproterone acetate, ***30 µg EE and 150 µg Desogestrel, ****35 µg EE and 500–1,000 µg Noretisterone; Transdermal patch, 33.9 µg EE and 203 µg Norelgestromin in 24 h.

HC use. Furthermore, 19.5% of the HC users had previously stopped using another HC due to experiencing a negative influence on physical fitness and/or performance. No difference in the prevalence of HC use or the type of HC were found between junior and senior athletes (HC use: $P = 0.054$; Type of HC: $P = 0.596$) or between national and non-national team athletes (HC use: $P = 0.799$; Type of HC: $P = 0.074$, for details see **Supplementary Table 2**).

Reasons for HC Use

Detailed information about reasons for HC use is presented in **Table 2** and a selection of statements is presented in **Table 3**. For detailed information about the juniors vs. seniors and national- vs. non-national team athletes see **Supplementary Table 3**). Among the 77 HC users, 59.7% reported that they used HC for non-contraceptive reasons, including 51% of the progestin-only HC users vs. 75% of the combined HC users ($P = 0.039$). For these athletes, menstrual symptoms were the most common reason (60% of the progestin-only HC users vs. 67% of the combined HC users, $P = 0.762$), while practical reasons were reported by 40% of the progestin-only HC users vs. 52% of the combined HC users ($P = 0.553$). Furthermore, 16% of the progestin-only HC users vs. 43% of the combined HC users ($P = 0.056$) reported compound reasons (i.e., symptoms influencing performance and/or for avoiding symptoms during competitions). Health-related reasons were reported by 8% of the progestin-only HC users vs. 19% of the combined HC users. When stratified based on age and performance level, non-contraceptive reasons for HC use were reported by 76.7% of the juniors vs. 48.9% of the seniors ($P = 0.016$), and by 61.9% of the national and by 58.9% of the non-national team athletes ($P = 0.813$). No differences were detected between junior vs. senior and between national vs. non-national team athletes when comparing the different non-contraceptive reasons for using HCs.

Symptoms Related to HC- and Menstrual Cycle

A detailed overview of the athletes' self-reported menstrual-related symptoms is presented in **Table 4**. Of the HC users with regular withdrawal bleeding, 80 and 85.7% of the respective progestin-only and combined HC users reported having menstrual-related symptoms. By comparison, 86.2% of the non-HC users who reported regular menstruation experienced menstrual symptoms. There were no significant differences between progestin-only and combined HC users or between all HC users and non-HC users (**Supplementary Table 4**).

HC Use and Self-Perceived Influence on Training and Performance

Detailed information about HC use and self-perceived influence on training and performance is presented in **Supplementary Table 3**. Of all HC users, 49.4% experienced a solely positive influence on training and/or performance, including 51% of the progestin-only HC users and 46.4%

TABLE 2 | Frequency and prevalence for hormonal contraceptive (HC) use.

Reported reasons for HC use	Progestin-only HC users (*n* = 25)		Combined HC users (*n* = 21)		All HC users (*n* = 46)	
	Frequency (*n*)	Prevalence, %	Frequency (*n*)	Prevalence, %	Frequency (*n*)	Prevalence, %
Menstrual symptoms	15	60	14	67	29	63
Unspecified pain	11	44	13	62	24	52
Heavy bleeding	7	28	2	10	9	20
Reduced physical fitness/emotional feelings	3	12	0	0	3	7
Practical	10	40	11	52	21	46
Avoid menstruation during competitions	6	24	9	43	15	33
Cessation of menstruation	4	16	0	0	4	9
Control of the cycle	2	8	3	14	5	11
Regular menstrual cycle	0	0	1	5	1	2
Compound reasons	4	16	9	43	13	28
Symptoms influence performance	4	16	4	19	8	17
Avoid symptoms during competitions/training camps	2	8	5	24	7	15
Health-related reasons*	2	8	4	19	6	13

**No detailed information due to low n.*
No significant differences progestin-only HC users and combined HC users when comparing reasons (symptoms: P = 0.762, Cramer's V = 0.069; practical: P = 0.553, Cramer's V = 0.124; compound reasons: P = 0.056, Cramer's V = 0.297).
No significant differences between juniors and seniors when comparing reasons (symptoms: P = 0.760, Cramer's V = 0.045; practical: P = 0.139, Cramer's V = 0.218; compound reasons: P = 0.743, Cramer's V = 0.048).
No significant differences between national and non-national team athletes when comparing reasons (symptoms: P = 0.739, Cramer's V = 0.080; practical: P = 0.203, Cramer's V = 0.188; compound reasons: P = 0.469, Cramer's V = 0.142).

TABLE 3 | Selection of responses: reasons for hormonal contraceptive (HC) use.

Participants' reasons for using HC

Symptoms	Practical	Compound reasons	Health-related
"I use it to reduce severe menstrual cramps" "I suffered from prolonged, heavy, frequent bleeding that disturbed my everyday life. It became easier to handle with combined oral contraceptives. My menses became lighter, and my bleedings shorter with longer duration between each bleeding" "To reduce the amount of bleeding, pain and a consistent bad feeling" "Heavy bleedings, and some menstrual cramps" "Severe menstrual cramps" "Bloating, ailments, and abdominal pain" "To reduce menstrual pain"	"To get continuity in my menstruation, the ability to skip menstruation and as contraception" "To avoid menstruation at competitions/training camps etc." "Have control/avoid menstruation during training and competition" "Loss of menstruation" "Can postpone my period when I am competing or at training camps" "The possibility to skip menstruation, but the main reason is contraception" "To prevent menstruation and therefore make it more convenient, such that I do not need to think about it"	"To regulate when I have menstruation because I get painful cramps that I feel reduce my performance. Because of this, I can avoid having my period during important competitions" "To avoid bleeding at training camps and during training as it is both annoying and painful. The intrauterine system reduces my menstrual pains" "… Also had very strong menstrual cramps, and needed to be able to control my menstruation in relation to competitions etc…" "To avoid menstrual cramps during important competitions" "Severe and unpredictable menstrual cramps especially the first four days, which reduce my performance extremely" "[I] had so much menstrual pain before I started on combined oral contraceptives that I could not train" "Abdominal pain. I have struggled a lot with stomach cramps due to menstruation, which sometimes affected my performance"	"Both for contraception and due to low production of estrogen" "… polycystic ovary syndrome. I had a too low estrogen level" "Iron deficiency"

of the combined HC users. In total, 31.2% of the HC users experienced neither a positive nor a negative influence (i.e., "neutral") on training and/or performance, while 14.3 and 5.2% experienced a mixed and a solely negative influence, respectively. There were no significant differences between progestin-only and combined HC users when investigating

TABLE 4 | Prevalence of reported menstrual symptoms for progestin-only and combined hormonal contraceptive (HC) users, and for all HC users and non-HC users.

Symptoms	Progestin-only HC users (*n* = 12)		Combined HC users (*n* = 18)		All HC users (*n* = 30)		Non-HC users (*n* = 25)	
	Frequency (*n*)	Prevalence, %	Frequency (*n*)	Prevalence, %	Frequency (*n*)	Prevalence, %	Frequency (*n*)	Prevalence, %
Physical								
Stomach cramps/abdominal pain	6	50	16	89	22	73	13	52
Back pain	4	33	5	28	9	30	11	44
Heavy bleeding	4	33	4	22	8	27	4	16
Tiredness/fatigue/lethargy	2	17	1	6	3	10	4	16
Nausea/sickness/vomiting	0	0	2	11	2	7	4	16
Unspecified pain/cramps	2	17	0	0	2	7	3	12
Bloating/Other stomach problems	2	17	0	0	2	7	3	12
Reduced physical fitness	0	0	0	0	0	0	2	8
Muscle- and/or joint ache	2	17	2	11	4	13	2	8
Sweating/hot flushes	0	0	1	6	1	3	1	4
Hunger/increased appetite	1	8	0	0	1	3	1	4
Sore breasts	1	8	0	0	1	3	1	4
Headache	1	8	1	6	2	7	0	0
Emotional								
Mood changes/swings	4	33	3	17	7	23	9	36
Demotivated/sad/depressed	1	8	1	6	2	7	2	8
Flustered/Unfocused	0	0	0	0	0	0	2	8

the self-perceived influence on training and performance ($P = 0.942$). No differences were detected between junior vs. senior ($P = 0.788$) and national vs. non-national team athletes ($P = 0.379$) when investigating the athletes' perception of how HCs influenced training and performance.

DISCUSSION

The current study explored the prevalence and self-perceived experiences with the use of hormonal contraceptives in Norwegian competitive endurance athletes. Our main findings were as follows: (1) 68% of the examined athletes used HC, with 64% of these using a progestin-only type; (2) 60% of the HC users reported non-contraceptive reasons for HC use, with menstrual symptoms as the main reason (i.e., 60% of the progestin-only HC users and 67% of the combined users; non-significant difference); (3) of the HC users with regular withdrawal bleeding, 80% of the progestin-only and 86% of the combined HC users experienced negative menstrual-related symptoms (non-significant difference), which was similar to the non-HC users (non-significant); (4) there were no significant differences in how the use of HC was perceived to influence training and/or performance between progestin-only and combined HC users, and the majority (81%) of all HC users experienced a solely positive or no influence, of using HC on training and/or performance.

HC Use

The 68% prevalence of HC use in this study is higher than the ∼50% prevalence reported in earlier studies on athletes from various sports (Martin et al., 2018; Oxfeldt et al., 2020; Solli et al., 2020; Clarke et al., 2021; Nolan et al., 2022). Furthermore, over 60% of HC users in the current study employed a progestin-only type of HC, which differs from previous studies where most (61–74%) athletes used a combined type (Martin et al., 2018; Oxfeldt et al., 2020; Solli et al., 2020). This prevalence of progestin-only HC use (64%) was substantially higher than the 38% reported in Solli et al. (2020), who investigated the same population of Norwegian XC skiers and biathletes. The reason for this difference could be the general increase in long-acting contraceptives, including implants and IUS, found in the general Norwegian population (Furu et al., 2021). Based on the Norwegian Prescription Database, the investigation by Furu et al. (2021) showed an increase in the use of implants and IUS from 8 to 26% from 2015 to 2020 in women aged 20–24 years. The reason for this increase may be that public health nurses and midwives were authorized to prescribe all hormonal contraceptives for women 16 years and older from 2016, including implants and IUS. Furthermore, the reason for the higher prevalence of progestin-only HC could be the ease of use, which is reported as the most common reason for athletes' choice of type/delivery method in the study by Martin et al. (2018). Accordingly, long-acting HCs as implants and IUS, have been preferred because of their high efficacy and ease of use (Burke, 2011). However, there is limited research examining how different types of HC, especially progestin-only types, affect athletic performance (Martin and Elliott-Sale, 2016; Clarke et al., 2021). The rapid increase in the use of progestin-only HCs, particularly long-acting HCs, in endurance athletes is interesting since a significantly higher incidence of negative side-effects has been reported in progestin-only compared to combined HCs (Martin et al., 2018). However, the positive effects of long-acting HCs on athletes' training quality and performance may potentially outweigh any negative side-effects. Future studies should seek to answer this question, in addition to directing more attention to the effects of long-acting HCs on training and performance.

Reasons for HC Use

Sixty percent of the HC users reported that they used HCs for non-contraceptive reasons. Interestingly, this included a significantly higher proportion of combined HC users (75%) compared to progestin-only HC users (51%). The reason for the disagreement is unclear since no differences were detected in the reported reasons for HC use between the two groups. Reduction in negative menstrual-related symptoms was the main reason for HC use in both groups, with athletes stating (**Table 3**) to use HC "*To reduce the amount of bleeding, pain and a consistent bad feeling*," and to reduce "*Bloating, ailments, and abdominal pain*." Such reduction of menstrual symptoms, as well as "practical reasons" such as the ability to predict and/or change the HC-/menstrual cycle, and cessation of menstruation are all positive effects of HC use reported in previous studies (Martin et al., 2018; Oxfeldt et al., 2020; Clarke et al., 2021). However, these latter studies have mainly reported practical reasons as the most common non-contraceptive reason for HC use. Our findings of athletes reporting compound reasons (**Table 3**), highlight that some athletes use HC to reduce or avoid negative menstrual-related symptoms because they perceive this to interfere negatively with their training and performance.

Symptoms Related to HC- and Menstrual Cycle

In the current study, only HC users with regular withdrawal bleeding were asked about having negative menstrual-related symptoms, which excluded nearly 70% of the progestin-only HC users. Our findings indicate that a large portion of the progestin-only HC users experiences irregular or cessation of bleeding, which is a known reported side-effect of using progestin-only HC (Burke, 2011). Of the athletes with regular withdrawal bleeding, negative menstrual-related symptoms similar to the experiences of non-HC users, occurred in both progestin-only and combined HC users (with no differences between groups), which is in line with previous studies (Solli et al., 2020; Clarke et al., 2021). However, Clarke et al. (2021) speculated that although HC users experience negative menstrual-related symptoms, they may experience decreased duration or severity of symptoms. In addition, Findlay et al. (2020) highlighted that some athletes in their study used HCs to manage their negative menstrual-related symptoms. In the current study, HC users were not asked if they had fewer/lighter menstrual symptoms compared to before they started using HC. However, responses such as "*[I] had so much menstrual pain before I started on combined oral contraceptives that I could not train*" (**Table 3**), "*[I have] slightly less pain than I had without contraception. This makes it easier to complete workouts…*" (**Table 5**) suggest that athletes' negative menstrual-related symptoms may have reduced in severity as a result of HC use.

HC Use and Self-Perceived Influence on Training and Performance

No differences between progestin-only and combined HC users were detected when we examined the self-perceived influence of HC use on training and performance. Furthermore, nearly half of the athletes (51% of the progestin-only HC users and 46% of the combined users) experienced only a positive influence of HC use on training and/or performance. This is much higher than the 17% reported in Solli et al. (2020). While Solli et al. (2020) did not provide any further explanations for these positive effects, several of the athletes in our study stated that their perceived positive influence on training and/or performance was due to lighter, or absence, of negative menstrual-related symptoms (**Table 5**), e.g., "*I do not have as much menstrual pain with HC, which makes it easier to train and compete*" and "*[I] struggled a lot with menstrual pain before, but after I started on the implant, this has decreased. This means that I am not knocked out when I have my period and can train as normal.*" Based on the athletes' experiences in this study, it appears that a reduction of menstrual-related symptoms increases the perceived training quality, which might be an important positive effect from HC use in many athletes. Still, ∼30% of the athletes did not experience any influence (i.e., "neutral") of HC use on training and performance, while 14% experienced a mixed (i.e., both positive and negative) influence by using HC. In addition, 5% had solely negative experiences, which is in line with Solli et al. (2020). The negative influence reported by athletes in the current study were mostly related to perceived side-effects such as irregular bleeding or stronger negative menstrual-related symptoms.

Overall, the findings in this study emphasize that athletes use HC to improve their perceived training quality due to complex negative menstrual-related symptoms. Furthermore, our findings indicate large individual variations in response to HC use, probably due to the athletes' unique hormonal profile and their reaction to the composition of synthetic hormones in different types and brands of HCs (Elliott-Sale et al., 2021). The individual response to HC use also highlights the importance of proper communication between coaches, athletes, and medical staff, as well as the need to monitor health and performance when starting on a new HC to detect potential changes (Findlay et al., 2020; Solli et al., 2020; Höök et al., 2021).

Strengths and Limitations

A strength of the current study is the inclusion of national team athletes, which in XC skiing and biathlon includes athletes of world-class level. The high athletic level of these participants is valuable for generalization of the findings across different groups of elite endurance athletes. No differences were found between the national and non-national team athletes, suggesting that these results are representative of competitive endurance athletes of different

TABLE 5 | Selection of responses: how hormonal contraceptives (HCs) influence training and/or performance.

Participants' experiences about how HCs influence training and/or performance		
Perceived a solely positive influence	**Perceived a mixed influence**	**Perceived a solely negative influence**
Influence on training: "I do not have as much pain and heavy bleeding anymore. What's more, I have not been as exhausted or tired as before I started on combined OC, therefore I feel better when training" **Influence on performance:** "I have no pain and I'm not nauseous on competitions days anymore, feelings that I used to have before" **Influence on performance:** "Not directly on performance, but due to my previous menstrual problems, hormonal contraception has made it easier for me because I can avoid the negative menstrual symptoms" **Influence on training:** "I do not have as much menstrual pain with HC, which makes it easier to train and compete" **Influence on performance:** "HC causes less bleeding and less pain during menstruation, which has a positive effect related to my performance. It is easier to give maximum at competitions when I do not have severe pain. I do not have to take paracetamol before competitions" **Influence on training:** "To a positive degree, so that I could postpone menstruation until after a competition or reduce pain to perform better without too many distractions" **Influence on performance:** "I avoid pain, performs better. No need to think about changing sanitary pads/tampons or about bleeding through them" **Influence on training:** "[I have] slightly less pain than I had without contraception. This makes it easier to complete workouts. In addition, I bleed less. When I used OC, I had no pain or bleeding, but was often in a bad mood" **Influence on training:** "[I] struggled a lot with menstrual pain before, but after I started on the implant, this has decreased. This means that I am not knocked out when I have my period and can train as normal"	**Positive influence on training:** "I avoid menstruation, as well as menstrual pain" **Positive influence on performance:** "To avoid the menstrual cycle which can affect the hormone balance in the body. For example, it prevents me feeling very emotional for some periods, etc., which I can imagine could affect both training and performance" **Negative influence on performance:** "I do not get the natural answer that my body functions properly as it should during a normal menstrual cycle" **Positive influence on training:** "Higher iron levels, more stable body and fewer mood swings" **Negative influence on training:** "Easier to get nauseous" **Positive influence on training/performance:** "Predictable menstruation and the possibility to postpone it if it does not fit with competitions" **Negative influence on training/performance:** "Weight gain and irregular bleeding the first couple of months" **Negative influence on training:** "I can experience menstrual symptoms of pain and intermittent bleeding to varying degrees" **Positive influence on performance:** "In the period after menstruation, I feel stronger and fresher" **Positive influence on training:** "I got less bleeding and pain during menstruation when I first started on OC. In recent years no bleeding" **Negative influence on training:** "No bleeding in recent years has been practical in terms of training and competition performance. However, without (regular) menses, it is more difficult to confirm that I am not pregnant and that I have an adequate energy intake" **Positive influence on performance:** "No bleeding is practical"	**Influence on training:** "Somewhat more pain during menstruation" **Influence on training:** "I have negative experiences with mini-pills, but now when I use an implant, I have some intermittent bleeding and sometimes pain. When I get menstrual cramps, I think it is extra difficult to exercise and I am not always in shape to train" **Influence on performance:** "The days I have menstrual pain or heavy bleeding are much harder and makes it more difficult to perform optimally" **Influence on training:** "Irregular menstruation due to IUS. When I used OC, it was regular. What's more, stronger menstrual pain now than with using OC" **Influence on performance:** "When I used OC, I skipped menstruation a few times to avoid menstruation around important competitions. I cannot control this with IUS, so I think that is a negative point. At the same time, it is now very unpredictable when I will get my period" **Influence on training:** "Lethargic, and physically heavy. Harder to train. I lose the feeling of being 'light' in my body" **Influence on performance:** "Loses the feeling of being in shape, strong/fast and 'light' in my body"

levels. However, the sole focus on only biathletes and XC skiers might limit the generalizability of these findings to women competing in other endurance sports. Furthermore, the analysis of the open-ended questions provides complementary qualitative insight when analyzing reasons for HC use and the athletes' perception of how HCs may influence their training and performance.

There are also some limitations of this study. First, only current HC users were asked about previous HC use and the perceived influence of HCs on training and performance. This may have excluded important information about non-HC users' previous experiences of HC use and reasons for discontinuation. Second, HC users were not asked about perceived side-effects related to HC use, which reduces the possibility to understand the relationship between a specific type of HC and perceived side-effects. Third, the questionnaire was originally designed for two main groups (HC users and non-HC users) where only athletes with regular menstruation (and withdrawal bleedings) were asked about negative menstrual-related symptoms. Optimally, all athletes should have been asked to state if they have symptoms. In addition, associations between the duration of HC use and negative menstrual-related symptoms experienced could not be conducted with our dataset. However, since this would have been interesting information in the discussion on how HC use influence negative menstrual-related symptoms, we recommend future studies to investigate this. Forth, it is a possibility that the lack of significant differences found in this study could be caused by the low proportion of combined HC users or athletes at an international level (Tier 4–5). Thus, further studies should aim to include even more athletes in their sample. Similar to the data presented in the current study, most of the results from previous studies are limited by the descriptive and comparative analyses.

CONCLUSIONS

This study provides new insight into the prevalence and reasons for using HCs among Norwegian female endurance athletes, with in-depth knowledge on athletes' perceptions of how different types of HC influence their training and performance. Overall, we found 68% HC use in XC skiers and biathletes, which is a higher proportion then reported previously in other athlete populations. The highest proportion (64%) of athletes in the current study used a progestin-only HC, which follows the increasing trend in the general Norwegian female population. The most common non-contraceptive reason for using HC was to reduce negative menstrual-related symptoms. This substantiates the fact that many athletes use HC to avoid menstrual-related negative symptoms interfering with their training and/or competitions. These perspectives, alongside the observation of the high proportion of progestin-only HC users, provide important information for the development of specific guidelines and the direction for future research in this area.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because the dataset generated for this study is not publicly available due to privacy concerns. Requests for assessing the dataset should be directed to the corresponding author. Requests to access the datasets should be directed to TE, tina.engseth@uit.no.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by REK, Regionale komiteer for medisinsk og helsefaglig forskningsetikk (Project ID: 135555). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

TE, GS, BM, TT, DN, ØS, and BW designed the study whereas TE collected data. TE, EA, GS, and BW performed the data and statistical analyses, and interpreted the results. TE wrote the first draft of the manuscript. All authors jointly revised the manuscript and approved the final version.

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SUPPLEMENTARY MATERIAL

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A contemporary understanding of iron metabolism in active premenopausal females

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Iron metabolism research in the past decade has identified menstrual blood loss as a key contributor to the prevalence of iron deficiency in premenopausal females. The reproductive hormones estrogen and progesterone influence iron regulation and contribute to variations in iron parameters throughout the menstrual cycle. Despite the high prevalence of iron deficiency in premenopausal females, scant research has investigated female-specific causes and treatments for iron deficiency. In this review, we provide a comprehensive discussion of factors that influence iron status in active premenopausal females, with a focus on the menstrual cycle. We also outline several practical guidelines for monitoring, diagnosing, and treating iron deficiency in premenopausal females. Finally, we highlight several areas for further research to enhance the understanding of iron metabolism in this at-risk population.

KEYWORDS
menstrual cycle, menstruation, hepcidin, iron regulation, iron status

Introduction

Research in exercise-related iron deficiency has transitioned from treatment-focused research that primarily addresses dietary iron intake and supplement effectiveness, to a more prevention-focused approach that has addressed causes of iron deficiency and the influence of the iron regulatory hormone, hepcidin, on iron homeostasis (Nemeth et al., 2004; Peeling et al., 2009; Ganz, 2011; Pasricha et al., 2011; Sim et al., 2019). Iron deficiency is particularly prevalent in active females (∼15–50%) (Fallon, 2004; Parks et al., 2017) compared with their less active counterparts (∼10–14%) (Marx, 1997; WHO, 2019). The increased prevalence of iron deficiency in active females is associated with adverse health outcomes including fatigue, poor mood, decreased cognitive function, increased risk of illness and injury, impaired thermoregulation, and reduced exercise tolerance (Pasricha et al., 2010; Sim et al., 2019). Identified contributors to exaggerated iron losses and inadequate iron intakes include low total dietary iron intake and/or bioavailability (Craig, 1994; Castell et al., 2019), dietary intake patterns, iron lost through menstrual blood loss (Napolitano et al., 2014; Bruinvels et al., 2016), and exercise-related mechanisms (i.e., sweating, hemolysis, gastrointestinal bleeding). In addition, increased hepcidin activity ∼3–6 h after exercise may induce a transient reduction in iron recycling and absorption thereby reducing the uptake of iron from iron rich meals consumed post-exercise. This is likely to increase a premenopausal female athlete's risk of developing an iron deficiency if they are unable to replenish their daily iron losses (Peeling et al., 2009; Sim et al., 2019).

Given hepcidin's crucial role in controlling iron uptake kinetics after exercise, research has sought to clarify how lifestyle factors (e.g., dietary intake/patterns) (Badenhorst et al., 2015a,b, 2016; McKay et al., 2019, 2020) and external environments (e.g., altitude and heat) (Badenhorst et al., 2014; Govus et al., 2017; Hayashi et al., 2020; McKay et al., 2021; Sumi et al., 2021) influence hepcidin kinetics in active individuals. Research has also investigated the changes in hepcidin kinetics in response to exercise training, the timing of iron-rich foods and oral iron supplements which has provided translatable research outcomes detailing when iron could be ingested by active individuals to enhance its uptake (Moretti et al., 2015; Stoffel et al., 2017; McCormick et al., 2019, 2020).

Most iron metabolism research in active populations has been conducted in male-only or mixed cohorts. Consequently, few guidelines (Pedlar et al., 2018; Sim et al., 2019) provide nuanced recommendations about how to manage iron status in active premenopausal females. Furthermore, only two investigations to date have measured changes in the activity of the master iron-regulatory hormone, hepcidin, throughout the menstrual cycle (Angeli et al., 2016; Lainé et al., 2016), and only one research program has investigated changes in hepcidin activity acutely after exercise in premenopausal females (Peinado et al., 2021). This clear lack of research into understanding the regulation of iron parameters and hepcidin in active premenopausal females throughout their menstrual cycle has limited the creation of clear practical recommendations to improve the diagnosis, management, and treatment of iron deficiency in this at risk cohort. This review therefore discusses iron status and regulation from a female-specific perspective, examining the changes in iron parameters and hepcidin kinetics that are expected throughout the menstrual cycle in eumenorrheic premenopausal females (i.e., defined as natural menstruating females, with menstrual cycles that are 21–35 days, exhibiting a luteinizing hormone surge and a correct hormone profile with no influence from exogenous hormones for >3 months; Elliott-Sale et al., 2021) or premenopausal females presenting with menstrual dysfunction (i.e., anovulation or luteal phase defects). Finally, we provide practical recommendations for sports scientists, health and medical practitioners, to guide the diagnosis and treatment/s of iron deficiency in premenopausal females.

Iron requirements for active females

Iron is an essential dietary nutrient that forms the functional component of several heme and non-heme proteins involved in oxygen transport (hemoglobin), oxygen storage (myoglobin), and energy production *via* oxidative and glycolytic enzymes (Beard et al., 1996). For iron to support physiological functions, the primary function being erythropoiesis, iron absorption from dietary iron intake must balance daily iron losses (Beard and Han, 2009). In sedentary individuals, ∼2–3 mg of iron is absorbed daily from dietary iron intake to replenish iron losses and maintain iron homeostasis (Beard and Han, 2009). In eumenorrheic females, menstrual bleeding largely contributes to iron losses (Arens, 1945; Hallberg and Nilsson, 1964), with ∼1 mg of iron lost per day during menstrual bleeding (Hallberg et al., 1966). In active females, an additional 3–4 mg/day of iron may be required to replenish exercise-related iron losses (e.g., hemolysis, hematuria, gastrointestinal bleeding, sweating, and dermal losses) (Nielsen and Nachtigall, 1998). The iron demands of exercise, in addition to daily iron losses and menstruation, may result in a negative iron balance, which if not compensated for by either dietary means and/or iron supplementation, increases the risk of iron deficiency with or without anemia in active premenopausal females.

A brief overview of systemic iron regulation: The role of hepcidin

Systemic iron homeostasis is coordinated by the body's iron regulatory hormone, hepcidin, a 25-amino acid peptide hormone that is produced by the liver (Hare, 2017). Hepcidin inhibits excessive systemic iron levels by internalizing and proteolytically degrading the body's only known iron transporter, ferroportin, which is expressed on the basolateral surfaces of reticuloendothelial macrophages in the spleen, liver, and enterocytes (Hare, 2017). Hepcidin also acts on divalent metal transporter-1 (DMT-1) channels on the apical surface of duodenal enterocytes to limit intestinal iron absorption (Brasselagnel et al., 2011). When hepcidin levels are high, less iron can combine to apotransferrrin and be transported as transferrin (an iron transport glycoprotein) around the body to target cells such as erythroid precursor cells in the bone marrow, which support erythropoiesis. Several mechanisms influence hepcidin activity *in vivo,* including factors directly related to iron status and iron utilization within the body, such as plasma iron concentration, iron stores (ferritin), and the rate of erythropoiesis (Nemeth and Ganz, 2009). Additionally, hepcidin expression increases in response to infection and inflammation as part of the body's acute phase response, with the cytokine Interleukin-6 (IL-6) the main stimuli responsible for limiting iron availability for bacterial growth and viral replication (Ganz and Nemeth, 2015). The increase in hepcidin activity in response to inflammation has been identified as a potential contributor to altered iron utilization in active individuals (Peeling et al., 2009). Research in iron replete individuals (serum ferritin >35 μg/L; (Sim et al., 2019) has consistently demonstrated that elevations in IL-6 concentration immediately after exercise, in an intensity- and duration-dependent manner, promotes an increase in hepcidin activity ∼3–6 h following exercise cessation (Peeling et al., 2014). Altered iron utilization (recycling and absorption) kinetics post-exercise potentially align with post-exercise dietary intake, and in combination with the previously mentioned iron losses may jeopardize iron status in active females, increasing their risk of iron depletion and iron deficiency diagnosis.

Several other regulators of hepcidin activity have been identified including growth factors (e.g., myonectin) (Goodnough et al., 2012; Halon-Golabek et al., 2019), the mechanistic target of rapamycin (mTOR) (Guan and Wang, 2014), testosterone (Bachman et al., 2010), estrogen (E2) (Hamad et al., 2020), progesterone (P4) (Li et al., 2015), growth hormone (Vihervuori et al., 1994), leptin (Yamamoto et al., 2018), and insulin (Fillebeen et al., 2020). Since female sex hormones (progesterone and estrogen) regulate hepcidin activity, we next discuss the influence of female sex hormones on hepcidin and systemic iron metabolism.

The influence of sex hormones on iron regulation

The menstrual cycle is defined by biphasic fluctuations in E2 and P4, with each cycle lasting approximately 28 days, but may range from 21 to 35 days (Elliott-Sale et al., 2021). Estrogen, and in particular 17β-estradiol (E2), is the most abundant endogenous form of E2 in human females. Estrogen is considered the primary female sex steroid, and elevated concentrations of E2 have been positively correlated with iron demand and the release of iron into the systemic circulation, and negatively correlated with hepcidin concentration (Hamad et al., 2020), however, the exact mechanism by which E2 influences iron regulation is still to be determined. *In vitro* studies in breast (Bajbouj et al., 2018), ovarian SKOV3 (Yang et al., 2012), liver cells (HUH7 and Hep-G2) (Hou et al., 2012) and rodent models suggest E2 may support the upregulation of genes involved in iron metabolism (e.g., ferroportin, lactotransferrin, ferroxidase ceruloplasmin, lipocalin 2) (Stuckey et al., 2006; Bajbouj et al., 2018; Hamad et al., 2020), likely by downregulating hepcidin activity. In ovarian cells (E2-S and SKOV-3), E2-induced upregulation of HIF-1α has also been shown to downregulate hepcidin gene (*HAMP)* expression, subsequently reducing hepcidin concentration (Hou et al., 2012). Additionally, in human liver cells treated with E2, reduced hepcidin levels were observed to occur due to the binding of E2 on E2 responsive elements (ERE) on the *HAMP* gene, suppressing the *HAMP* gene expression (Hou et al., 2012). *In vivo* studies have demonstrated a marked suppression in serum hepcidin levels from 4.85 to 1.43 ng/mL (a decline of ∼40%) (Lehtihet et al., 2016) in females treated with large doses of E2 (0.15 to 3.99 ng/mL an E2 increase of ∼25%) during *in vitro*-fertilization (IVF).

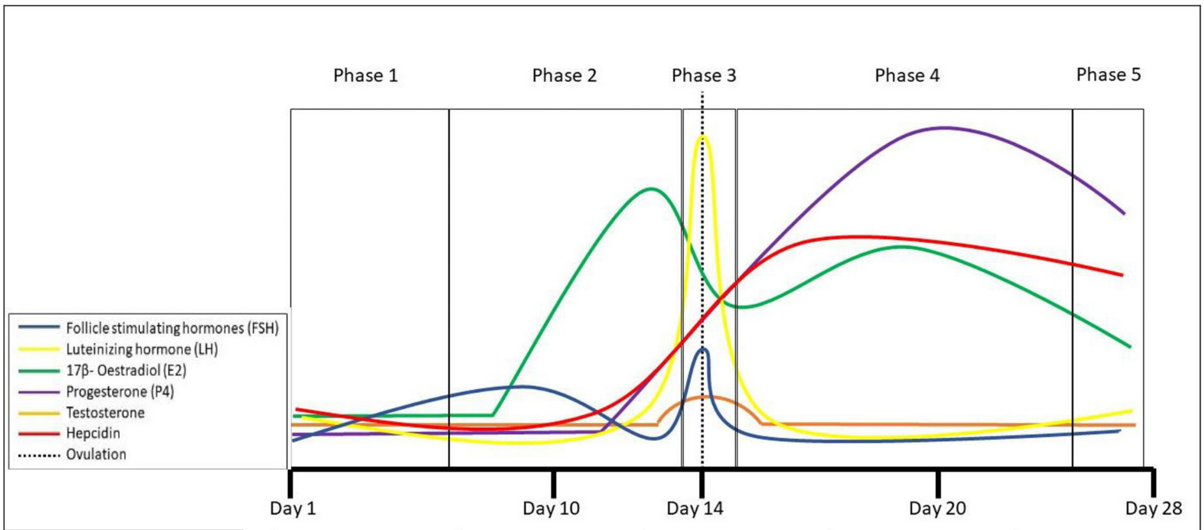
While most available research suggests an inverse relationship between E2 and hepcidin, two studies have reported an increase in *HAMP* gene expression and hepcidin synthesis in response to E2 treatment (Ikeda et al., 2012; Bajbouj et al., 2020). In ovariectomized mice, E2 appeared to increase serum and liver iron through increased *HAMP* gene expression which occurred *via* a GPR30 (G-protein coupled receptor 30), the 7-transmembrane E2 receptor (Ikeda et al., 2012). Further, treatment with E2 and G1 (GPR30 agonist) reduced BMP6 and hepcidin expression, suggesting E2 may be involved in hepcidin expression *via* a GPR30-BMP6-dependent mechanism (Ikeda et al., 2012). The response of E2 on *HAMP* gene expression may thus depend on the differential expressions of E2 receptors, such as membrane-anchored E2 receptors GPR30, and their co-regulators in various cell types (Ikeda et al., 2012).

The relationship between E2 and iron homeostasis may be cell-type specific. In monocytes, E2 may differentially alter iron metabolism in an IL-6-dependent manner. *In vitro* exogenous treatment with E2 in human cell lines, including U937 cells, initiated a cascade of effects commencing with an increase in IL-6 synthesis, a reduction in TNF-α, HIF-1α, and ERα gene expression followed by an increase in hepcidin levels (Bajbouj et al., 2020). However, in uterine cells, E2 in uterine cells, E2 may play an important role in reducing hepcidin expression and supporting iron turnover during E2-induced cell growth and development (i.e., in the proliferation phase that occurs during the mid-late follicular phase of the menstrual cycle, days 5–14). Conversely, in immune cells, increases in E2 mid-cycle may enhance the pro-inflammatory response in macrophages and dendrites, increasing hepcidin and iron sequestration as part of the anti-inflammatory response (Bajbouj et al., 2020; Hamad et al., 2020).

Two research publications to date have monitored changes in basal hepcidin concentration throughout the menstrual cycle (Angeli et al., 2016; Lainé et al., 2016). During the early follicular phase, defined by the presence of menstrual bleeding and low sex hormone concentrations (low E2 and P4), hepcidin levels appear at their lowest (Lainé et al., 2016). During this phase, iron is actively lost through menstrual bleeding, thus low hepcidin levels during the early follicular phase may reflect the regulation of hepcidin *via* systemic iron levels, compensating for menstrual iron loss by facilitating a physiological state that supports efficient recycling of iron and iron uptake from the diet. This proposal is supported by total iron-binding capacity (TIBC) being at its highest (Kim et al., 1993) during the early follicular phase. There is a graded increase in E2 after menstruation, produced by the developing follicle in the ovaries, that peaks in the late follicular phase (days 10–14 at ∼200–250% above early follicular baseline E2 levels) before ovulation in an eumenorrheic female (Constantini and Hackney, 2013; Prior, 2018). Hepcidin levels rebound mid-cycle, which may align with the drop in E2 following the pre-ovulation surge. Additionally, during mid-cycle ovulation, a peak in testosterone levels (increase by ∼40%) has been observed in eumenorrheic females (Cook et al., 2021). Testosterone has consistently been shown to potently suppress hepcidin in both males and females (Bachman et al., 2010; Latour et al., 2014). Rodent models demonstrate that hepcidin down-regulation by testosterone occurs due to testosterone-dependent upregulation of epidermal growth factor (EGF) receptors in the liver (Latour et al., 2014). In both younger (19–35 years) and older (59–75 years) males, testosterone suppresses hepcidin in a dose-dependent manner, with these changes being strongly associated with increases in hemoglobin and hematocrit (Bachman et al., 2010). Testosterone levels in females range from 0.4–2.0 nmol/L, ∼4–5 times lower than the concentration in men (Kanakis et al., 2019). Females with excess androgens, as seen in Polycystic Ovarian Syndrome (PCOS), have testosterone levels above the physiological range in healthy females (∼0.34–5.5 nmol/L), although their testosterone concentrations are still less than age-matched males (Kanakis et al., 2019). A mild iron overload is a common comorbidity in females with PCOS, possibly due to a testosterone-dependent suppression of hepcidin and a large influx of iron into the systemic circulation (Escobar-Morreale, 2012). However, in eumenorrheic females, the suppressive effect of testosterone and E2 on *HAMP* gene expression in some cell types may result in a progressive increase in systemic iron levels during the mid-late follicular phase. After ovulation, the drop in both E2 (E2 drops to ∼100% above baseline early follicular E2 levels) and testosterone (which are hepcidin suppressors), coinciding with the increase in systemic iron levels before ovulation may contribute to the rebound in hepcidin during the latter half of the cycle.

Progesterone is the other key steroid sex hormone that is produced by the corpus luteum in a pulsatile manner. Peaks in P4 occur in the luteal phase following successful ovulation, with concentrations of P4 being roughly over 1,000% compared to baseline P4 in the early follicular phase (Constantini and Hackney, 2013; Prior, 2018). Despite P4 being the primary hormone of the luteal phase in eumenorrheic premenopausal females, the effect of P4 on iron metabolism has not been examined in detail. *In vitro* studies have identified P4 as a hepcidin-inducing steroid (HIS) that increases hepcidin biosynthesis in human hepatoma (Hep-G2) cells, independent of inflammation (Li et al., 2015). *In vivo* results in females undergoing IVF who received daily intramuscular injections of 50 mg of P4 had 3-fold and 2-fold higher hepcidin levels on days 6 and 15 of P4 treatments respectively (Li et al., 2015). The induction of hepcidin *via* the P4-mediated pathway appears to be delayed compared to the fast-acting BMP-signaling pathway. Increased hepcidin expression occurred ∼4 h following the peak in P4 concentration and peaked ∼12 h following the commencement of P4 administration. The delayed rise in hepcidin levels following exogenous P4 administration may suggest that HIS require a secondary intracellular messenger or metabolite to reach a critical threshold before triggering downstream effects that result in increased hepcidin expression. Within the luteal phase, lower TIBC and higher hepcidin levels may be indicative of stable iron utilization (Kim et al., 1993; Angeli et al., 2016; Lainé et al., 2016). These changes may be due to the direct influence of P4 on hepcidin activity, or following the rebound in systemic iron levels around ovulation, hepcidin levels may increase to regulate iron homeostasis and prevent iron excess (Nemeth and Ganz, 2009; Li et al., 2015). With a single research study examining the effects of P4 on hepcidin activity, further research is needed to clarify the *in vivo* effects of P4 on hepcidin levels in eumenorrheic females.

Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) are two hormones that are part of the reproductive axis in both males and females. In females, FSH peaks in the late luteal phase, stimulating the recruitment and development of ovarian follicles in the ovary (Reed and Carr, 2018). Within granulosa cells in the ovary, FSH stimulates aromatase enzyme that aids the conversion of androgens to E2. FSH levels drop at the start of menses as a result of the negative feedback from E2 and inhibin B produced by the developing follicle in the ovary (Groome et al., 1996). The decline in FSH in the early follicular phase is associated with an increase in the androgenic environment within the ovary (Reed and Carr, 2018). During the follicular phase, the increase in size in the dominant follicle and granulosa cells supports the graded increase in E2 from the mid to late follicular phase. In the presence of increasing E2 in the follicular phase, FSH stimulates the formation of LH receptors on the granulosa cells, which results in the secretion of small quantities of P4 and 17-hydroxyprogesterone (17-OHP), both of which have a feed-forward effect on the pituitary gland to start secreting LH. Within the ovary, LH initiates E2 production *via* the conversion of androstenedione to E2 in the thecal and granulosa cells. To a lesser degree, LH also stimulates the production of testosterone in theca cells (Reed and Carr, 2018). LH levels are low during the early follicular phase but will begin to rise in the mid-late follicular phase due to the positive feedback of E2 on the pituitary gland. For LH to be released from the pituitary gland and initiate ovulation, E2 needs to surge above a threshold of 200 pg/mL for 50 h. LH is secreted by the anterior pituitary in a pulsatile manner, with pulses roughly occurring every 60–90 min at a stable amplitude in the early follicular phase, which then increases in frequency and amplitude toward ovulation (Reame et al., 1984). To date, no research has investigated whether FSH or LH exert any influence over *HAMP* gene expression or hepcidin activity. However, the



	Phase 1	**Phase 2**	**Phase 3**	**Phase 4**	**Phase 5**
Factors that may regulate hepcidin throughout the menstrual cycle	Early follicular phase Low E2 and P4	Mid-Late follicular phase Exponential increases in E2	Ovulation Surge LH, peak in testosterone, declining E2	Luteal phase High P4, moderate E2 levels	Late luteal phase Withdrawal of P4 and E2, rise in FSH
Systemic iron levels (serum iron)	↓	↓	↑	↑	↑
Iron stores (serum ferritin)	↓	↓	↑	↑	↑
Estrogen (E2)	↓	↓			
Progesterone (P4)				↑	↑
Inflammatory cytokines			↑	↑	↑

FIGURE 1
Phases of the menstrual cycle and factors that collectively may contribute to the variability in hepcidin expression throughout the menstrual cycle in healthy eumenorrheic iron sufficient (serum fcrritin > 35 µg/L) females.

activity of these two gonadotrophic hormones is intrinsically linked to the production of E2 and P4 throughout the menstrual cycle in naturally menstruating females. Therefore, their effect on hepcidin activity and iron regulation is likely to be indirect and associated with the production of E2 and P4.

Hepcidin activity throughout the menstrual cycle in eumenorrheic premenopausal females and its alignment with sex steroid hormones (E2, P4, and testosterone) and their subsequent influence on systemic iron levels requires further clarification. An overview of sex hormone changes throughout the menstrual cycle and the influence on hepcidin and iron parameters discussed in this sector are presented in Figure 1.

Inflammation throughout the menstrual cycle

The ovaries and endometrium display repeated inflammation throughout the menstrual cycle, with increases in inflammatory cytokines typically occurring at ovulation and upon the commencement of menstruation (Maybin and Critchley, 2015). Within premenopausal females, the withdrawal of P4 initiates menstrual bleeding and acute inflammatory changes including an influx of leucocytes, an increase in inflammatory cytokines, prostaglandins, and destructive enzymes of the extracellular matrix

(Hapangama and Bulmer, 2016). In premenopausal females, ~2-fold increases in IL-6 levels have been observed during menses, with a lesser increase occurring around ovulation (Whitcomb et al., 2014).

Estrogen has a minor influence on lymphocyte proliferation, with some research showing increases in E2 within the menstrual cycle are accompanied by increases in IL-6 (Angstwurm et al., 1997), while others suggest an inverse relationship between E2 and IL-6 (Whitcomb et al., 2014). This inverse relationship may be explained by the timing of data collection that may have aligned with a drop in E2 at ovulation and at the start of menstruation after stimulating IL-6 production. Conversely, P4 has been shown to downregulate cytokine synthesis (Angstwurm et al., 1997), with research consistently demonstrating an inverse relationship between P4 and IL-6 (Whitcomb et al., 2014). The proinflammatory cytokine IL-1β, also observed to have a key role in the progression of inflammation and hepcidin activity (Cannon and Dinarello, 1985; Kanamori et al., 2019), increases post-ovulation during the luteal phase in premenopausal females. The post-ovulation increase of IL-1 from macrophages is potentially due to increased inflammation following follicle rupture and may be induced by E2 and P4 increases in the luteal phase (Cannon and Dinarello, 1985). The changes in inflammatory cytokines do not appear to align with changes in hepcidin in the follicular phase of the menstrual cycle. Hepcidin regulation in the early-late follicular phase may be the result of changes in systemic iron levels (declines with menstrual bleeding), E2 and testosterone activity (peaks before ovulation). During ovulation, elevated systemic iron levels (cessation of menstrual bleeding), declines in E2, and increases in proinflammatory cytokines may contribute to the rebound in hepcidin activity mid-cycle (Figure 1). Whilst during the luteal phase, elevated systemic iron levels, and P4 could contribute to the observed elevated hepcidin levels (Hapangama and Bulmer, 2016).

During the late luteal phase, ~81.1% of females are likely to present with premenstrual syndrome (PMS), which may be induced by, and increase, in response to: inflammatory cytokines, the reduction in reproductive hormones (P4 and E2), or the increase in prostaglandins (Bruinvels et al., 2021). Typical PMS symptoms include fatigue, headaches, mood changes, sleep disruption, and poor concentration/memory (Bruinvels et al., 2021), all of which are commonly associated symptoms with iron deficiency. The association between the severity of PMS and iron stores has not been investigated, and future research may seek to clarify if PMS symptoms are exacerbated when females present with depleted iron stores. Ferritin and hepcidin are both considered acute phase reactants, yet research to date has not clarified if there is an increase in either of these iron parameters in the ~3–5 days before menstruation. With large between-female variability and low within-female variability in inflammation throughout the menstrual cycle (Whitcomb et al., 2014), practitioners should be mindful of the timing of iron parameter collection in females and be sure to collect markers of inflammation to ensure the correct determination of iron status in active females.

Changes in iron parameters throughout the menstrual cycle in eumenorrheic females

To the best of the authors' knowledge, eight studies have measured the changes in iron parameters and regulation throughout the menstrual cycle (Table 1), albeit with high intra- and inter-individual variability. Serum iron and transferrin saturation have both been reported to be lower in the early follicular phase compared to the mid-late follicular phase and mid-luteal phase in a group of premenopausal females with stage 1 iron deficiency (serum ferritin of <35 μg/L) (Alfaro-Magallanes et al., 2022). Collectively, this prior research has reported decreased serum iron, transferrin, and occasionally hemoglobin, and transferrin saturation (Kim et al., 1993) during menses, followed by a gradual increase in these iron parameters during the mid-to-late-follicular phase, and a plateau during the luteal phase, mirroring the changes in hepcidin observed throughout the cycle.

Mean serum ferritin levels appear to remain stable across the menstrual cycle (Belza et al., 2005; Angeli et al., 2016; Lainé et al., 2016; Suzuki et al., 2018; Zheng et al., 2021; Alfaro-Magallanes et al., 2022), with some investigations reporting no change in iron parameters throughout the menstrual cycle (Belza et al., 2005; Alfaro-Magallanes et al., 2022) especially in iron-depleted individuals (serum ferritin: <12–35 μg/L). Research in athletes has demonstrated the magnitude of hepcidin activity ~3–24 h after exercise (Peeling et al., 2014) is largely influenced by their pre-exercise serum ferritin levels with minimal changes typically seen in post-exercise iron parameters and hepcidin levels in iron-deplete (serum ferritin: <35 μg/L) compared to iron replete athletes (serum ferritin >35 μg/L) (Peeling et al., 2014, 2017). Therefore, the magnitude of the changes in iron parameters and hepcidin throughout a female's menstrual cycle may also largely depend upon her current/basal serum ferritin levels.

Acute exercise and menstrual cycle phase effect on hepcidin and iron status

Only recently have acute changes in iron parameters and hepcidin been examined before and after exercise (40 min run at 75% VO_{2peak}) in three distinct phases of the menstrual cycle (early, mid-follicular phase, and luteal phase). Here, pre-exercise serum iron and IL-6 levels were lower in the early follicular phase compared to the luteal phase (Barba-Moreno et al., 2020). Despite serum hepcidin levels increasing after exercise,

TABLE 1 Studies that have investigated iron status and hepcidin at different phases of the menstrual cycle in premenstrual females.

Authors	Study ranking for methodological control in female cohorts (Smith et al., 2022)	Population	Age	Blood markers measured	Iron deficiency prevalence	Measure of the menstrual cycle	Comments
Zilva and Patston (1966)	Bronze	$n = 11$	22–38 years	SI	Not noted	Mean values for SI calculated for each day of the cycle, excluding readings for 6 days around the onset of the menstrual cycle. Average deviation from the mean of SI was calculated for each day of the cycle	Fall in SI 2–3 days into menses, lowest point reached 3rd day of menses. SI increased on the 4th day of the cycle and reached mean SI values around ovulation (∼ day 14). Increased slightly and stabilized in the luteal phase.
Kim et al. (1993)	Bronze	$n = 1{,}712$	18–44 years	Hb, MCV, SI, TIBC, TS, EP, SFer Analyzed in 1/3rd of the females	Defined by SFer and MCV models. SFer model: highest incidence iron deficiency in menstrual phase vs luteal phase (23 vs 8%) MVC model: iron deficiency prevalence decreases with each successive phase of the menstrual cycle. Highest in menstrual (11%) vs luteal (4%) and late luteal (4%)	Collected by interview: • When did you last period or menstrual cycle end? Phases of cycle were operationally defined as: • Menstrual phase: currently menstruating at time of survey • Follicular phase: days 1–9 after menstruation ended • Luteal phases: days 10–16 after menstruation ended • Late Luteal phase: days 17–30 after menstruation ended	Hb, SI and TS values significantly associated with menstrual phase. Lowest in the menstrual phase and highest in luteal phase TIBC: highest in menstrual phase and lowest luteal phase EP: highest menstrual and follicular phases and lowest in luteal and late luteal phase SFer: highest in luteal and late luteal phase MCV: slightly lower in follicular phase vs menstrual phase Serum albumin (hemodilution): higher luteal phase.
Lainé et al. (2016)	Ungraded	$n = 90$	18–45 years	Hb, TS, Fer, SI, SH	29% presented with low iron stores in menses	Serum samples at 6 time points within a cycle: Day 0: start menses 3 visits during menses and in following day Last 2 visits toward the end of the cycle	SH, SI and TS dropped during menses, increasing mid cycle and stabilizing toward the end of the cycle. Higham Score (intensity of blood loss in menses) positively correlated with magnitude of SH and iron variations

(Continued)

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TABLE 1 Continued

Authors	Study ranking for methodological control in female cohorts (Smith et al., 2022)	Population	Age	Blood markers measured	Iron deficiency prevalence	Measure of the menstrual cycle	Comments
Belza et al. (2005)	Bronze	$n = 13$	23–30 years	Hb, RDW, SFer, sTfR, LH, α-ACT	Required to be iron depleted but not deficient Fer: 12–30 ug/L Hb <119 g/L	Monitored timing and duration of menstrual cycle and irregularities. Measured LH in each blood sample Menstrual cycle divided into phases: follicular and luteal. Luteal phase determined by estimation of ovulation based on LH levels.	No change in iron status across the menstrual cycle within iron depletion.
Angeli et al. (2016)	Ungraded	$n = 90$	19–44 years	Hb, TS, SFer, SI, SH		Measured blood samples at 6 time points. First sample collected on day 2 of menses. 3 others in menses and late follicular phase, 2 in luteal phase.	TS, SI and SH show variations throughout the menstrual cycle. SFer, Transferrin and Hb showed minimal variation but showed high degree of inter-variability TS highly correlated with SI
Zheng et al. (2021)	Silver	$n = 8$	25–45 years	Hb, Fer, Tf, SI, SH	Participant requirement for SFer to be >30 ug/L, non-deficient. Mean SFer: 59 ug/L	Measured blood samples in the early follicular (days 3–7) and mid-luteal phase (days 20–22). Repeated measures trial, samples compared here are baseline (pre-exercise) in thermoneutral conditions. Menstrual phase confirmed with retrospective serum hormonal analysis, and confirmation of ovulation with ovulatory urine test.	No difference in SH, SFer and Tf between menstrual phases throughout the trial SI appeared to be unaffected my menstrual phase.
Suzuki et al. (2018)	Ungraded	$n = 4$	19–20 years	Hb, SI, SFer, TS	1 out of 4 had SFer <35 ug/L	Blood samples collected on days 2, 10 and 22 of the menstrual cycle. Self-reported normal cycles, but no hormonal clarification	Hb and SFer peaked on day 10 of the cycle, SI and TS peaked in the luteal phase High inter- and intra-variability in participants iron status markers

(Continued)

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TABLE 1 Continued

Authors	Study ranking for methodological control in female cohorts (Smith et al., 2022)	Population	Age	Blood markers measured	Iron deficiency prevalence	Measure of the menstrual cycle	Comments
Alfaro-Magallanes et al. (2022)	Silver	*n* = 36	18–40 years	SI, SFer, TS	Mean SFer for naturally menstruating females < 35 ug/L, thus majority of the participant cohort would be defined as stage 1 iron deficiency	Blood samples collected in the early follicular phase (days 2–5), mid-late follicular phase (days 7–12), mid-luteal phase (days 19–24). Phase retrospectively confirmed with serum hormonal analysis	TS and SI significantly lower in early follicular vs mi-late follicular and mid-luteal phases. Iron deficiency erythropoiesis (TS <16%) greatest in early follicular phase. No association or variation in menstrua phase and iron stores

Hb, Hemoglobin; MCV, mean corpuscular volume; SI, serum iron; TIBC, total iron binding capacity; TS, transferrin saturation; EP, erythrocytes protoporphyrin; SFer, serum ferritin; SH, Serum Hepcidin; RDW, Red cell distribution width; α-ACT, acute phase protein; sTfR, Soluble transferrin receptor; LH, Luteinizing hormone; Tf, Transferrin.

basal hepcidin levels showed no variation throughout the cycle (Table 2). Furthermore, neither exercise-induced changes in serum iron nor IL-6 appeared to stimulate large variations in hepcidin activity after exercise. The blunted post-exercise hepcidin response was most likely due to participants' low mean basal serum ferritin levels, ranging from 25.4 ng/mL during the early follicular phase to 29.2 ng/mL during the luteal phase, which is lower than the 35 µg/L cut off for stage 1 iron deficiency (Barba-Moreno et al., 2020). Therefore, large variations in hepcidin concentration would not be expected in this cohort since hepcidin activity has likely been suppressed to promote iron absorption in response to depleted iron stores (Galetti et al., 2021). To account for participants' low pre-exercise iron status, participants were divided into females with basal serum ferritin levels <20 and >20 µg/L and hepcidin and iron parameter responses were again reviewed before and after exercise in the early, mid-follicular, and luteal phases of the menstrual cycle. Pre-exercise hepcidin levels were significantly lower in the <20 µg/L ferritin group in the early follicular and mid-follicular phases of the cycle, but were not significantly different from the >20 µg/L ferritin group in the mid-luteal phase (Alfaro Magallanes et al., 2021a). Post-exercise, no significant difference in hepcidin levels was observed between the two groups. The results for this second study should be interpreted with caution, due to several analytical and methodological considerations. Firstly, the authors did not complete an ANOVA but rather conducted their analysis through a series of *t*-tests between the groups and time points within this study. This method may not have appropriately adjusted for the Familywise Error Rate, which could affected the statistical significance of the results they obtained (Alfaro Magallanes et al., 2021a). Secondly, basal ferritin or iron status was not included as a covariate when analyzing the effect of hepcidin activity between menstrual cycle phases or pre-and post-exercise. Therefore, the hepcidin response could have been influenced by participants' low pre-exercise iron stores, especially when considering the mean serum ferritin for the >20 µg/L was not greater than 50 µg/L, which has been suggested as a healthy serum ferritin cut-off for females (Galetti et al., 2021). Thirdly, the small sample size in each cohort ($n = 7$–8) of the studies reduces the statistical power to infer statistical differences in iron status between each menstrual cycle phase within this study.

Another potential limitation of the previous two studies is their use of microplate ELISA (Elabscience Human Hep25 ELISA kit) to measure hepcidin, which is limited compared to the mass spectrometry method. ELISAs may not identify the active, full length of the 25-amino acid hormone from truncated variants that are present due to biological breakdown that occurs during sample collection and sample analysis (Hare, 2017). Hepcidin measured *via* matrix-assisted laser desorption ionization weak cation exchange time of flight (MALDI-TOF) mass spectroscopy is currently considered the gold standard measurement method. A round-robin investigation on hepcidin

TABLE 2 Studies that have investigated iron status response pre and post exercise at various phases in the menstrual cycle in premenopausal females.

Author	Study ranking for methodological control in female cohorts (Smith et al., 2022)	Population characteristics and classification of level of training (McKay et al., 2022)	Exercise intervention	Phase of Cycle	Results on iron status and metabolism	Comments
Roecker et al. (2005)	Ungraded	$n = 14$ Marathon runners 26–45 years 52–75 kg Marathon time: 3:59–4:55 No medication that would affect iron status Tier 2	Assessment of iron stores pre, post, 1 day post and 3 days post a marathon	No phase of menstrual cycle or OCP reported	Urinary hepcidin increased after marathon in 10/14 females Max hepcidin 1 day after marathon Responders (iron sufficient stores): increase in hepcidin 4–27-fold of pre-race value	No dietary control No indication of volume of training No monitoring of iron stores prior to marathon
Newlin et al. (2012)	Ungraded	$n = 12$ Female runners 19–32 years VO_2max: 52.1 mL/kg/min Iron sufficient (Fer >30 µg/L) Tier 1	Trial 1: 60 min at 65% vVO_2peak Trial 2: 120 min at 65% vVO_2peak	All tests completed 7–10 days after the onset of menses. Phase likely to be late FP but no hormonal confirmation	Increase Hct and Hb post exercise Increase SH 3 h post exe for both trials, but was greater in 120 min trial SI increased post and 3 h post, lowest at 9 h post SFer increased post exercise in both trials	24 and 48 h dietary control Changes in SI and SH followed each other post exercise Responders for SH had higher SI (78.8 ug/L) vs non-responders (61.5 ug/L)
Ishibashi et al. (2017)	Ungraded	$n = 16$ Long distance runners Mean age: 20.5 years Tier 3	Blood sample collection Low training period (~499 km/month) High training period (~622 km/month)	Self-reported regular menstrual cycles in 25% in low training period vs. 19% in high training period 2 participants with amenorrhea No further clarification of menstrual phase	SFer Low: 30.9 ug/L SFer High: 28.1 ug/L SI Low: 55 ug/L SI High: 65 ug/L CK higher in high training period 31% during low were iron deficient 37% during high were iron deficient SH greater in high training period Positive correlation between SH and SFer in both training periods	0% use supplements in low training period 44% used supplements in high training period

(Continued)

TABLE 2 Continued

Author	Study ranking for methodological control in female cohorts (Smith et al., 2022)	Population characteristics and classification of level of training (McKay et al., 2022)	Exercise intervention	Phase of Cycle	Results on iron status and metabolism	Comments
Barba-Moreno et al. (2020)	Bronze	$n = 15$ Female athletes VO_2max:50.3 mL/kg/min Mean age: 35.6 years Tier 1	Baseline testing in early FP (days 2–5 of cycle), followed by 3 trials • Early FP: days 2–5 and low hormone • Mid FP days 7–10, high E2 and low P4 • LP days 19–21, high P4 and E2 5 min warm up at 60% vVO_2, 40 min running at 75% of vVO_2peak, 5 min recovery at 30% vVO_2peak.	Natural cycles, occurring between 24–24 days in length Blood sample taken upon arrival at the lab prior to exercise and retrospectively analzsed for E2, P4, LH and FSH. No LH surge to confirm ovulation Deficient luteal phase defined as P4 < 16 nmol/L in single measurement	Effect of time differences found for transferrin, SFer, IL-6 and SH. Significant main effects were found for SI and menstrual cycle, while CRP and IL-6 showed a trend toward significance. No significant changes in SH, SFer and transferrin throughout the menstrual cycle.	Only significant interaction effect for menstrual cycle and time was for IL-6. With an increase from pre to 0 h and further to 3 h post-exercise. Increase significantly greater in LP vs Early FP and Mid FP. Peak in SH at 0 h post exe vs. 3 h post.

BM, Body mass; VO_{2peak}, Maximal aerobic capacity; vVO_{2peak}, Velocity at maximal aerobic capacity; Hb, Hemoglobin; Hct, Haematocrit; SI, Serum iron; SH, Serum Hepcidin; SFer, Serum ferritin; TS, Transferrin Saturation; CK, Creatine kinase; IL-6, Interleukin-6; OCP, Oral contraceptive pill; FP, Follicular phase; LP, Luteal phase; MHR, Maximal heart rate; LH, Luteinizing hormone; FSH, Follicle stimulating hormone; E2, estrogen; P4, Progesterone; CRP, C-reactive protein; BMI, Body mass index.

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analysis (mass spectrometry and ELISA's) showed significant variation in hepcidin levels between eight different laboratories for a single analysis (Kroot et al., 2009). Several competitive and sandwich-based ELISA's for hepcidin analysis are available, however, when compared to mass spectrometry the results obtained by ELISA's may lack the sensitivity of detecting hepcidin in biological fluids (i.e., plasma, serum, or urine) (Kroot et al., 2009; Hare, 2017). For example, in male and female athletes who presented with iron deficiency, their mean serum ferritin of ∼20 µg/L resulted in suppressed mean serum hepcidin of 20–30 ng/mL (Burden et al., 2015). Following treatment with IV iron (500 mg Ferinject; Vifor Pharma Ltd., Opfikon, Switzerland), serum ferritin has been shown to increase to ∼100–120 µg/L, with subsequent increases in serum hepcidin to ∼100–150 ng/mL (Burden et al., 2015). In the aforementioned studies, the eumenorrheic female participants had mean serum ferritin levels of 25.4 ng/mL, yet their hepcidin levels ranged from 60 to 80 ng/mL (Barba-Moreno et al., 2020). The serum hepcidin levels reported by Barba-Moreno et al. (2020) and Alfaro Magallanes et al. (2021a), appear to be inconsistent with prior research in iron depleted individuals. Rather, the reported hepcidin levels appear equivalent to those measured in athletes after IV iron treatment (Burden et al., 2015). Such discrepancies in the results may be due to the variation in hepcidin ELISA measurements, therefore we recommend thorough reviews of hepcidin ELISA values against mass spectroscopy and existing literature to ensure consistency in results published within this research area.

Few studies have investigated changes in iron status in active females over a prolonged period (weeks-months) (Table 3). None of the available research has considered monitoring menstrual cycle status while recording changes in iron status in the female participants. This prior research lacks consideration of confounding variables in females that may affect iron status. Hence, we encourage researchers looking to complete prolonged investigations of iron status in females to ensure menstrual cycle length, bleeding, and symptoms are monitored to allow a more nuanced analysis.

The effects of acute exercise and oral contraceptive pill phase on hepcidin and iron status

The oral contraceptive pill (OCP) is a widely and commonly used contraceptive method for menstruating females. In a cohort of 430 female athletes, ∼50% used a form of hormonal contraception, of which ∼68% used an OCP (Martin et al., 2018). Forms of OCP include, the combined monophasic pill providing a low dose of both ethinylestradiol and synthetic progestin (levonorgestrel, norethisterone, gestodene, desogestrel, drospirenone) consistently for 21 days, the biphasic pill providing consistent doses of ethinylestradiol and higher doses of synthetic progestins in the second half of the active pill cycle and the triphasic pill with synthetic ethinylestradiol and progestin doses changing every 7 days during the active pill phase. Typically, most OCPs will have a 21-day active or consumption phase and a 7-day pill-free/withdrawal phase (Elliott-Sale et al., 2020). Doses of the exogenous E2 and progestins within combined OCPs may range from ∼20 to 40 mg of ethinylestradiol and ∼70–300 mg of synthetic progestins (Elliott-Sale et al., 2013). An alternative to the combined OCP is the progesterone-only pill, providing doses of ∼35–75 mg of synthetic progestins (Elliott-Sale et al., 2013). The provision of exogenous E2 and progestins have a negative feedback on gonadotrophic hormones, prolonging the downregulation of the hypothalamic-pituitary-ovarian axis, subsequently resulting in significant reductions of endogenous E2 (∼60 pmol/L for 21 days) and P4 (consistently ∼5 nmol/L) within premenopausal females (Rechichi et al., 2008; Elliott-Sale et al., 2020; Alfaro-Magallanes et al., 2022). One hour following daily ingestion of the OCP, synthetic E2, and P4 peak, and during the 21-day active pill period the basal values of the synthetic hormones may slightly increase (Elliott-Sale et al., 2020). During the seven pill-free or placebo pill days, endogenous E2 levels may rise to ∼140 pmol/L, equivalent to E2 levels in the early follicular phase (Elliott-Sale et al., 2020).

In general, the magnitude of the hepcidin response following exercise does not seem to differ between OCP users and non-users (Table 4). Two studies have investigated the hepcidin response pre-and post-exercise in female athletes during the withdrawal and active pill phases. Both research studies showed the typical increase in hepcidin post-exercise, however, no significant difference between the active and withdrawal trials on hepcidin activity pre-and post-exercise was observed. The low sample sizes in the research investigating the influence of the OCP on iron metabolism makes it difficult to draw clear conclusions (Sim et al., 2015, 2017; Alfaro-Magallanes et al., 2021b). Aligning with previous research, hepcidin kinetics are likely to have responded to participants' iron status, with higher hepcidin levels reported in the participants of Sim et al. (2015) (43–47 µg/L) who had higher iron stores, and inflammatory increases post-exercise rather than steroid sex hormone fluctuations associated with OCP use. Neither of these studies included a control group (non-OCP, basal iron status matched) to compare the post-exercise hepcidin response between OCP users and non-users. Additionally, while both research investigations tried to capture a hormone-free period and an active hormone phase, only one study (Alfaro-Magallanes et al., 2021b) attempted to account for the increase in endogenous E2 in the withdrawal phase, while one did not (Sim et al., 2015), which may have influenced hepcidin kinetics and iron parameters during this data collection period.

More recently, basal iron status has been compared between three phases of the menstrual cycle in eumenorrheic females,

TABLE 3 Studies that have investigated changes in hepcidin and iron parameters pre and post chronic training periods in premenopausal females.

Author	Study ranking for methodological control in female cohorts (Smith et al., 2022)	Population characteristics and classification of level of training (McKay et al., 2022)	Exercise intervention	Phase of Cycle	Results on iron status and metabolism	Comments
Auersperger et al. (2013)	Ungraded	$n = 14$ Female runners Divided into 2 groups N: Fer>20 µg/L D: Fer<20 µg/L Tier 1	2-week prep phase 8-week intensified training 2 × 3-week progressive overload periods followed by 1 week taper 3–4 sessions per week 1–2 interval sessions at 88–100% MHR) 2 aerobic runs per week (70–85% MHR)	No recording of menstrual cycle or changes	SH decreased in recovery weeks vs baseline Prevalence of Fer <20 µg/L increased to 71% (10/14) in 8 weeks	N group had a 4.8% improvement in performance D group had a 3% improvement in performance IL-6 in detectable ranges at all time points No significant group effects for CRP
Ma et al. (2013)	Ungraded	$n = 20$ Female runners Control n=10 age and BMI matched females Age 18–23 years Tier 4	Weekly running volume in runners ranged from 56.3–104.6 km Average run volume 24 h prior to the blood test in runners was 10.5 km Run pre-blood test was continuous, moderate effort (~12.9–13.7 kph)	Blood collections between 15 and 19th day of cycle. Phase likely to be early LP. No hormonal confirmation of reproductive hormones or LH for ovulation	SFer tended to be higher in runners SI tended to be lower in runners SH tended to be higher in runners	Regular menstrual cycles reported in 80% controls and 70% runners Exercise was completed prior to the morning sample. Run length ranged from 0 to 19.3 km, likely to contribute to the high SH and SFer in the runners

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TABLE 3 Continued

Author	Study ranking for methodological control in female cohorts (Smith et al., 2022)	Population characteristics and classification of level of training (McKay et al., 2022)	Exercise intervention	Phase of Cycle	Results on iron status and metabolism	Comments
Buyukyazi et al. (2017)	Ungraded	$N = 30$ $N = 8$ control group and no exercise Age: 43.5 years* VO_2peak: 23.9 mL/kg/min* $N = 22$ experimental group BWG =11 brisk walking group Age: 41.0 years* VO_2peak:34.1 mL/kg/min* MWG = 11 moderate tempo walking group Age: 38.0 VO_2peak:31.4 mL/kg/min* Not taking iron supplements, regular length menstrual cycle for 12 months and not taking any other medication Tier 0	Week 1: low intensity for all to gain familiarity with protocols 8-week walking intervention for 2 groups at 2 different intensities. Start at 30 min and build to 40 min in duration in first 4 weeks. 3 × a week on a 400 m track BWG: weeks 1–4 70% MHR, weeks 5–8: 75% MHR MWG: weeks 1–4 50% MHR, weeks 5–8: 55% MHR Intensity determined by Karvonen Equation	No recording of menstrual cycle	Baseline iron status: BWG: 10.3 ng/mL* MWG: 10.7 ng/mL* CG:10.7 ng/mL* SH increased post exercise intervention in BWG, MWG and CG. IL-6 decreased in the BWG and MWG. No change SI TIBC increased BWG Increase in Hct and RBC in BWG No change SFer, TS	Baseline iron parameters (serum ferritin and iron) were not statistically different between groups Morning rested blood collection pre and post exercise intervention. Bloods collected ~24-h post and VO_2peak ~48 h post No screening of iron status Measured pro-hepcidin a prohormone form. Minimal comparisons to gold standard and other studies in exercise-based studies. ~Stage 2 iron deficiency, degree of change expected in 8 weeks is minimal. Hepcidin levels likely to be low in iron depleted individuals.

BM, Body mass; VO_{2peak}, Maximal aerobic capacity; vVO_{2peak}, Velocity at maximal aerobic capacity; Hb, Hemoglobin; Hct, Haematocrit; SI, Serum iron; SH, Serum Hepcidin; SFer, Serum ferritin; TS, Transferrin Saturation; CK, Creatine kinase; IL-6, Interleukin-6; OCP, Oral contraceptive pill; FP, Follicular phase; LP, Luteal phase; MHR, Maximal heart rate; LH, Luteinizing hormone; FSH, Follicle stimulating hormone; E2, estrogen; P4, Progesterone; CRP, C-reactive protein; BMI, Body mass index; BWG, Brisk walking group; MWG, Moderate tempo walking group. *Median presented in report and in table.

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with the withdrawal and active pill phases of females on the OCP (Alfaro-Magallanes et al., 2022). Regardless of the menstrual cycle or OCP phase, serum ferritin, hemoglobin and IL-6 did not display any variations between phases (Alfaro-Magallanes et al., 2022), consistent with research suggesting there is no difference in the proportion of menstruating females and females on the OCP who presented with iron deficiency (Casabellata et al., 2007). The average serum ferritin for this study appears to have been ∼30–35 µg/L, which we have previously noted may limit the magnitude of change in iron parameters between phases. Lower transferrin saturation, iron, and transferrin were noted in the early follicular phase in eumenorrheic females when compared to the withdrawal and active pill phases. In addition, these same iron parameters were lower in the mid-late follicular phase and mid-luteal phase when compared to the active pill phase. Elevated transferrin and serum iron (∼1.2 fold higher) have been reported in OCP females, from the first to the third generation of OCP pills (Casabellata et al., 2007), with females on these OCPs more likely to have transferrin saturation levels >45% and be considered at risk of hemochromatosis (McKnight et al., 1980). These results are likely due to the hepatic effects of synthetic estrogens (McKnight et al., 1980), offering a potential explanation for the difference in iron parameters noted between eumenorrheic and OCP users (Alfaro-Magallanes et al., 2022). Therefore, the influence of variations in synthetic estrogens and progestin in the first through to the third generation of OCPs on iron parameters will need to be verified, as these synthetic sex hormones may be confounding our interpretation of iron status in OCP users (Casabellata et al., 2007).

Interestingly, different brands of the monophasic pill result in different levels of endogenous hormones. Research has demonstrated that 11 out of 30 OCP brands tested (∼37%) will produce significantly different levels of endogenous E2 and P4 in both the active and withdrawal phases (Elliott-Sale et al., 2013). The different E2 and P4 hormone concentrations between women may arise due to different doses of exogenous E2 and P4 in each pill (brand and type), differential metabolism of exogenous hormones which is influenced by an individual's genetics, and the subsequent effects of these different endogenous hormone levels on hypothalamic-pituitary-gonadal feedback loops, and their interactions with catecholamines and prostaglandins (Yen, 1977; Elliott-Sale et al., 2013). Without controlling the OCP brand used by participants in research studies, there will likely be large variations in endogenous hormone levels, which may then be summated to calculate the mean differences between OCP users and non-users. This raises several methodological concerns (Type II errors) for the accurate interpretation of how changes in endogenous hormone concentrations in OCP users affect iron parameters and hepcidin activity.

Behavioral OCP use including the timing of OCP ingestion (morning or evening and peak of synthetic hormones post-ingestion) relative to the measurement of iron parameters will need to be considered in study designs and when interpreting iron status in athletic females, given reproductive hormones can affect iron parameters. Additionally, considerations for athletic females to avoid the 7-day withdrawal pills in favor of continuation of the active pills to reduce bleeding incidence and manipulation of their cycle length (Schaumberg et al., 2018), have not been considered in prior research and may contribute to the lower prevalence of iron depletion in these females. Therefore, researchers and practitioners should take note of the method of OCP use in females and how this may influence the iron parameters that are being assessed.

A final consideration for both researchers and practitioners is that no research to date has investigated the effect of the progestin-only pill or other forms of hormonal contraceptives (hormonal IUD, Depo Provera injection) on hepcidin kinetics. The synthetic dose of progestin is in some cases equivalent to the P4 doses provided daily in IVF treatment. Women receiving P4 during IVF have significantly increased hepcidin levels after 6–15 days (Li et al., 2015), therefore a similar hepcidin response may be observed in iron sufficient females on the progestin only pill.

Changes to the diagnosis of iron depletion in premenopausal females

Recent reviews have set out a framework for regular iron status monitoring of athletes (Sim et al., 2019), however, there is large variability in the optimal ferritin levels that have been suggested. Whilst stage 1 iron deficiency has been defined as serum ferritin <35 µg/L (Peeling et al., 2014), some authors advocate for a higher serum ferritin cut offs, such as 50 µg/L, for performance, health, and to aid adaptation to training loads (Peeling et al., 2014; Galetti et al., 2021). This elevated serum ferritin threshold is based on sustained increases in hepcidin following exercise (1–3 sessions per day), in conjunction with exercise-induced iron loss mechanisms each session increasing the risk of developing a negative iron balance (Fensham et al., 2022). Subsequently this then increases the risk of being diagnosed with an iron deficiency and experiencing the adverse health and performance outcomes associated with this diagnosis.

Recent research has provided physiological support for a higher serum ferritin threshold for the diagnosis of iron deficiency based on the up-regulation of iron absorption and recycling (Galetti et al., 2021). Results from this investigation demonstrated an exponential relationship between serum ferritin and hepcidin, with 41.8% of the variation in fractional iron absorption in healthy premenopausal females explained by variations in hepcidin. Key results indicated that fractional iron absorption increased from 7.2% to 33.2% when serum hepcidin was ≤3.09 nmol/L. The corresponding serum ferritin was ∼51.1 µg/L, and similarly, fractional iron absorption increased when serum ferritin concentration was <51.1 µg/L

TABLE 4 Studies that have investigated basal iron status and pre to post exercise iron status changes during the withdrawal and active phases of the oral contraceptive pill (OCP).

Author	Study ranking for methodological control in female cohorts (Smith et al., 2022)	Population characteristics and classification of level of training (McKay et al., 2022)	Exercise intervention	Phase of OCP	Results on iron status and metabolism	Comments
Sim et al. (2015)	Silver	$n = 10$ Mean age 26 years Mean BM: 57 kg Mean VO_2max: 50.3 mL/kg/min Tier 1	40 min run at 75% vVO_2peak Withdrawal: Day 2–4: hormone free period Active: Day 12–14: end of week 1 of active hormone therapy	On OCP for a minimum of 3 months. 3 brands: 1. Leven 2. Yasmin 3. Estelle OCP ethinyl estradiol ranges 0.03–0.035 mg OCP progestogen ranges 0.15–3 mg	SI increased immediately post exercise and remained elevated 3 h post in withdrawal trial. SFer increased post exercise in both trials and remained high 3 h post in withdrawal, only trending to remain high in active trial. Increase in SH in both trials with no difference between trials. No correlation between IL-6 and SH Moderate relationship between SH and SFer in active trial SI and SH not related 3 h post exercise	Time course of hormones post ingestion or cumulative levels after 1 week of active pill ingestion not quantified All sessions completed in the morning, when there is likely to be a spike in exogenous hormones Testing in withdrawal phase likely to occur at a time point when there is a rebound in endogenous estrogen equivalent to early FP
Sim et al. (2017)	Silver	$n = 15$ Mean age: 25 years Mean BM: 56.4 kg Tier 1	-	On OCP for a minimum of 3 months. 3 brands: 1. Leven 2. Yasmin 3. Estelle OCP ethinyl estradiol ranges 0.03–0.035 mg OCP progestogen ranges 0.15–3 mg	No difference in SI, SH, TS between withdrawal and active pill phases Serum ferritin significantly higher in active pill phase compared to withdrawal phase (69.4 vs 61.1 ug/L respectively)	Time course of hormones post ingestion or cumulative levels after 1 week of active pill ingestion not quantified All sessions completed in the morning, when there is likely to be a spike in exogenous hormones Testing in withdrawal phase likely to occur at a time point when there is a rebound in endogenous estrogen equivalent to early FP

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TABLE 4 Continued

Author	Study ranking for methodological control in female cohorts (Smith et al., 2022)	Population characteristics and classification of level of training (McKay et al., 2022)	Exercise intervention	Phase of OCP	Results on iron status and metabolism	Comments
Alfaro-Magallanes et al. (2021b)	Silver	$n = 16$ Female athletes: VO_2peak 47.4 mL/kg/min Mean age: 25.3 years Not iron deficient Tier 1	3 visits over 2 OCP phases. 1. Baseline in withdrawal phase 2. Trial in withdrawal phase (∼day 4.9) 3. Trial in active pill phase (∼ day 22.1) Exercise task: 5 min warm-up at 60% vVO_2peak, 8 x 3 min at 85% vVO_{2peak}, with 90 s recovery at 30% vVO_2peak. 5 min cool down at 30% vVO_2peak Serum samples at pre-exercise, 0 h, 3 h post and 24 h post exercise. Completed between 8 and 10 a.m.	All previously used OCP for 6 months prior Different formulas of OCP used and not controlled for. OCP ethinylestradiol ranges 0.02–35 mg OCP progestogen ranges 0.075–250 mg OCP use for study: 7 days (Days 1–7) no hormonal load/withdrawal phase 21 days of exogenous hormone pills/active phase	No significant difference in SH, IL-6, CRP and SFer between OCP phases No significant differences between the OCP phases at any time point pre and post exercise for SH and SFer Trend for hepcidin to be higher at baseline in withdrawal phase Trend for CRP to be higher in the withdrawal phase TNF-α significantly higher in active phase SI significantly higher at all time points in active pill phase Transferrin significantly lower in active phase at 0, 3, and 24 h post exercise.,	17β-estradiol, LH and FSH were significantly lower in active pill phase vs withdrawal phase Time of SH measurement affected results. SH was significantly higher at 0 h and 3 h post-exercise vs pre-exercise in withdrawal phase. IL-6 was significantly higher at 0 h, 3 h and 24 h post-exercise in both phases. SFer higher levels 0 h post-exercise vs preexercise in both withdrawal and active phases. TNF-α higher 0 h post-exercise vs pre-exercise in withdrawal phase Transferrin significantly higher 0 h post exercise vs pre-exercise and 24 h post exercise in active phase No note of when the OCP was ingested by participants and if this may have influenced the results

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TABLE 4 Continued

Author	Study ranking for methodological control in female cohorts (Smith et al., 2022)	Population characteristics and classification of level of training (McKay et al., 2022)	Exercise intervention	Phase of OCP	Results on iron status and metabolism	Comments
Alfaro-Magallanes et al. (2022)	Silver	n=24 Mean age: 27 years Mean BM: 57.25 kgs VO_2peak 49.4 mL/kg/min Tier 1	-	All previously used OCP for 6 months prior Different formulas of OCP used and not controlled for. Pill ingested every night for 21 days OCP ethinylestradiol ranges 0.02–0.03 mg OCP progestogen ranges 0.01–3 mg Tested late withdrawal phase ~day 5 to coincide endogenous estrogen peak Active pill phase tested on ~day 20 to coincide reduced ovarian functioning and peak in exogenous hormone concentrations	Ts and SI significantly higher in the active pill phase. Tf was lower in the active pill phase	Samples collected in rested and fasted state Endogenous 17β-estradiol in withdrawal and active pill phases were 31.51 and 9.81 pg/ml, respectively Endogenous progesterone in withdrawal and active pill phases were 0.37 and 0.36 ng/ml respectively Blood samples were collected in the morning (8–10 a.m.) to reduce the influence of diurnal variation. Timing was greater than 6 h and potentially coincided with reduced levels due to metabolic breakdown following ingestion.

BM, Body mass; VO_{2peak}, Maximal aerobic capacity; vVO_{2peak}, Velocity at maximal aerobic capacity; Hb, Hemoglobin; Hct, Haematocrit; SI, Serum iron; SH, Serum Hepcidin; SFer, Serum ferritin; TS, Transferrin Saturation; Tf, Transferrin; CK, Creatine kinase; IL-6, Interleukin-6; OCP, Oral contraceptive pill; FP, Follicular phase; LP, Luteal phase; MHR, Maximal heart rate; LH, Luteinizing hormone; FSH, Follicle stimulating hormone; E2, estrogen; P4, Progesterone; CRP, C-reactive protein; BMI, Body mass index.

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but remained relatively stable when hepcidin and serum ferritin levels were >3.09 nmol/L and >51.1 µg/L, respectively. Raising the serum ferritin threshold for iron depletion to ~50 µg/L in active females may increase the sensitivity in detecting iron depletion. The benefit of early detection of low iron levels affords early correction through dietary means and oral iron supplementation if required (for a review of iron treatment strategies see McCormick et al., 2020).

The wide confidence intervals for both hepcidin and serum ferritin in the study by Galetti et al. (2021) represent the large inter-individual variability for both serum ferritin and hepcidin measurements that exists within the females. While population-derived serum ferritin cut-offs provide some information about an individual's iron status and risk of iron depletion/deficiency, regular medical and iron status screening is still strongly advised for active females to determine the typical intra-individual variation in hematological and iron parameters throughout the competitive season or sporting career.

Heavy menstrual bleeding in premenopausal females-implications and considerations for iron status

Sequential exposure of the E2 primed endometrium to P4 and subsequent withdrawal of P4 and E2 in the late luteal phase initiates spontaneous decidualization of the endometrium resulting in menstrual bleeding (Hapangama and Bulmer, 2016). Mean menstrual blood loss in premenopausal females may range from 6.55 to 178.7 mL, with ~63% of women losing between 1 and 40 mL of menstrual blood (Hallberg et al., 1966). Many females with normal menstrual blood loss (1–40 mL), may present with a healthy and normal iron status (Hb >12 g/100 mL, serum iron >104 µg/100 mL; Hallberg et al., 1966), suggesting that normal menstruation may not be a root cause of iron deficiency in premenopausal eumenorrheic females.

However, endocrine irregularities (structural and non-structural) that prevent these sequential events have the potential to result in heavy menstrual bleeding (HMB) (Hapangama and Bulmer, 2016). Heavy menstrual (or uterine) bleeding, formerly called menorrhagia, is defined as a total menstrual fluid loss that regularly exceeds 80 mL (Hapangama and Bulmer, 2016). Females who present with HMB may have iron losses that are approximately 5–6 times higher during menstruation, with >80 mL of menstrual fluid loss equating to ~5.2 µg/L of iron loss (Napolitano et al., 2014). As such, HMB has consistently been suggested to increase the risk of iron deficiency (Hallberg et al., 1966; Napolitano et al., 2014; Bruinvels et al., 2016). A handful of research has attempted to quantify the incidence of HMB in sedentary females (Hallberg et al., 1966; Napolitano et al., 2014) and active females (Bruinvels et al., 2016), however, most of this research has based its results on the participants' subjective (survey) opinion or perception of their menstrual bleeding volume (Fraser et al., 2001; Bruinvels et al., 2016). There is no validated survey for the detection of HMB, and many females are unable to accurately estimate the volume of blood lost during menstruation, and appear to have low awareness of what constitutes as a healthy/normal compared to a heavy volume of menstrual blood lost during menstruation (Hallberg et al., 1966; Mansour et al., 2021). In a cohort of females classified with HMB (>80 mL), 37% considered this to be only moderate menstrual blood loss, while 4% considered this scant menstrual blood loss. In contrast, 14% of females that presented with 20 mL of menstrual blood loss considered this to be HMB (Hallberg et al., 1966). Given the current lack of validated HMB measures, the prevalence of HMB within athletic premenopausal females may have been underestimated.

The relationship between iron deficiency and HMB prevalence has been, to date, limited to premenopausal females with a healthy cycle (Bruinvels et al., 2016; Mansour et al., 2021). Minimal consideration has been given to how variations in menstrual cycle status (non-structural cause/s of HMB), and reproductive hormone profiles affect bleeding characteristics (volume, duration). Alterations to menstrual bleeding patterns/characteristics may occur in response to the adaption of the hypothalamic-pituitary-ovarian axis, typically presenting as ovarian suppression and resulting in changes in reproductive hormone concentration with subsequent effects on endometrial proliferation (Brown and Thomas, 2011). Changes to cycle length (oligomenorrhea), cycle characteristics (HMB) and sex hormone levels (subclinical ovulatory disturbances e.g., luteal phase defects or anovulation) can occur at any age in healthy premenopausal women in response to physiological [regular exercise (Shangold et al., 1979; De Souza et al., 1998; De Souza, 2003); or altered dietary intake (Bedford and Barr, 2010)] and psychological stressors (Barr, 1999).

Initial changes to menstrual cycle status may manifest as a subclinical ovulatory disturbances (SOD) inclusive of luteal phase defects (LPD), which may present as short luteal phases (<10 days) or inadequate/low P4 levels (<16 nmol/L), or anovulation (failure to ovulate and subsequently low P4 levels, <16 nmol/L). A SOD cycle in healthy premenopausal females typically remains undiagnosed as the menstrual cycle length (number of days) does not change (De Souza, 2003). However, the changes in reproductive hormone concentrations within SOD cycles, specifically E2 excess and low/lack of P4 are associated with compromised differentiation of the endometrial lining, resulting in poor quality of the endometrium and infertility in that cycle (De Souza, 2003). The role of P4 in the endometrium is to reduce the biologic activity of E2, transitioning the endometrium from the proliferation phase to the secretory phase, with menstrual bleeding then initiated by P4 withdrawal. However, in the presence of low P4 (e.g., LDP or anovulatory cycle), the reduction of E2-induced proliferation is likely to be low, resulting in excessive endometrial growth or delayed initiation of menstrual bleeding. Therefore, during the early follicular phase, following the withdrawal of E2 and P4 and spontaneous decidualization of the endometrial tissue, females presenting with LPD or anovulatory cycles, may have altered menstrual bleeding patterns, possibly including either HMB or prolonged menstrual bleeding ≥7 days. Evidence to support the relationship between endocrine irregularities and HMB prevalence has been reported in obese females (but not diagnosed with PCOS) and females diagnosed with PCOS (who may or may not be obese) (Hapangama and Bulmer, 2016). In females diagnosed with PCOS (obese and not obese), excess androgens are converted to estrone in peripheral tissues, with prolonged periods of excessive and unopposed E2 action on the endometrium resulting in HMB (Hapangama and Bulmer, 2016). In obese females, with no current PCOS diagnosis, the conversion of androstenedione secreted by the adrenal glands to estrone in adipose tissue provides an additional source of E2 that supports excessive endometrial growth and HMB (Hapangama and Bulmer, 2016).

The prevalence of SOD cycles in healthy females with normal length menstrual cycles (21–35 days) in 2 and 6 month prospective studies was 16 and 22.9% respectively (Bedford et al., 2010; Schliep et al., 2014). Within active females, the prevalence of anovulatory and LPD cycles are relatively common, with a 48 and 79% prevalence respectively in a 3-month study (De Souza et al., 1998). Within this study, ∼46% of females had consistent length cycles but had inconsistent cycle-to-cycle hormonal variations with intermittent presentations of ovulatory, anovulatory, and LPD cycles. Approximately 33% of the recreationally active females presented with consistent LPD cycles (De Souza et al., 1998). In a cohort of 120 healthy non-active females, 16% experienced HMB that was caused by a SOD cycle (Khan et al., 2016). Self-reported HMB in 54 and 36% in active females (n = 789) and marathon runners (n = 1,073), respectively have been reported in survey-based data (Bruinvels et al., 2016). The prevalence of previous iron deficiency in those who reported HMB was 43.1 and 38.1% in active females and marathon runners, respectively (Bruinvels et al., 2016). A limitation of this study was that no time frame was set for when participants may have experienced HMB before the marathon or data collection. Therefore, we are unable to determine if HMB occurred closer to the marathon when exercise and psychological stress may have been high and may have increased the likelihood of a SOD cycle occurring. Additionally, the age or other characteristics/factors (other menstrual disorders e.g., PCOS, body mass, nutritional intake, past pregnancy) of the active females or marathon runners were not presented. Regardless, the high prevalence of SOD cycles and association with HMB in active females may provide some insight into why active females may be more prone to iron deficiency than their sedentary counterparts.

Historically OCPs have been used to treat HMB and may reduce menstrual bleeding by ∼50% (Larsson et al., 1992; Mansour et al., 2021). The suppressed endogenous E2 and P4 levels that occur with OCP use are known to result in atrophy of the endometrium, poorly developed or absent spiral arterioles in the endometrium, and affect coagulation, fibrinolysis and prostaglandin synthesis (Larsson et al., 1992). These changes help to minimize menstrual blood loss in OCP users and reduce the risk of iron deficiency in these females. Thus, variations in endogenous E2 and P4 production, either suppressed by OCPs, or high E2 and low P4 in LPD and anovulation cycles in naturally menstruating females may affect menstrual bleeding patterns in premenopausal females and the relative risk of iron deficiency.

Altered hypothalamic-pituitary-ovarian axis and sex hormones function has been widely considered in the clinical sequelae of Relative Energy Deficiency in Sport (RED-S) syndrome in female athletes (Mountjoy et al., 2014; Williams et al., 2019). Research has consistently shown a reduction in triiodothyronine (T_3), within athletes presenting with RED-S symptoms, a result that is proposed to occur due to the adaption of the hypothalamic-pituitary-thyroid axis to reduced energy intake (McCall and Ackerman, 2019). Changes in circulating T_3 appear to be correlated with both the induction and reversal of ovulatory disturbances in females (Williams et al., 2001). The suppression of the hypothalamic-pituitary-ovarian axis during a hypometabolic state has been significantly associated with T_3 levels and not weight loss in monkeys (Williams et al., 2001). Therefore, restoring energy balance, either by reducing energy expenditure or increasing energy intake, may provide a sufficient stimulus to reverse the hypometabolic adaptation of the hypothalamic-pituitary-ovarian axis, aiding the recovery of a fully eumenorrheic cycle. Endometrium quality and thickness have also been correlated with energy availability, reproductive hormonal environment, and iron status, such that females with optimal iron status and ovulatory function present with both increased endometrial thickness and quality (Clancy et al., 2006). During intense training periods, when the incidence of HMB may increase in addition to exercise accelerating iron loss, female athletes should be encouraged to regularly check their iron status, ensure they are ingesting sufficient fuel to support training and health (achieving optimal energy availability) and track their menstrual cycle length, bleeding length and intensity of blood loss (e.g., by using smartphone application/s).

Practical recommendations to maintain iron status in premenopausal females

The following practical recommendations are aimed at improving iron status in active, premenopausal females:

1. Consider raising the serum ferritin threshold to 50 μg/L.
2. Ensure screening every 2–3 months for individuals considered at risk of iron deficiency, including athletes,

vegans, vegetarians, and individuals traveling to or training at altitude).

3. Consider food first interventions with an accredited Sports Dietitian, providing nutritional interventions/support aimed at increasing the overall amount of iron within the diet and adequate energy and carbohydrate availability. However, iron supplements should be provided and are recommended when required (e.g., if iron depleted or deficient).
4. Regular and consistent tracking of the menstrual cycle for all menstruating females is recommended, specifically cycle length, menstrual bleeding length, relative heaviness of a menstrual bleed, and change or prevalence of premenstrual symptoms (de Paula Oliveira et al., 2021).

   – For simplicity, we suggest athletes use smartphone-based applications, currently there are several commercial options available to athletes, recreationally active individuals, and coaches that can be used for this purpose (e.g., Clue™, WILD AI™, FitrWoman™, and Garmin ™).

5. Education of athletes, coaches, and sport science support staff on the menstrual cycle, cycle to cycle variations, menstrual cycle dysfunctions, and the burden of iron deficiency on health and exercise performance and training ability.

   – This may include nutritional education, psychological support (e.g., information about managing pressures on body image, competition pressure, work, and life stress).

6. We recommend that the individual's phase of the menstrual cycle, and any hormonal (OCP type and brand, hormonal IUD) or non-hormonal contraceptive (copper IUD) be recorded when performing hematological screening to enable the correct determination of iron status.

Directions and recommendations for future research

Future research may consider the following to help improve our understanding of iron regulation in premenopausal females.

1. In naturally menstruating females, research should seek to clarify the *in vivo* influence of sex steroid hormones, inflammatory cytokines, and systemic iron levels on hepcidin throughout a eumenorrheic cycle. To date, there is minimal research on females that would allow us to comprehensively state the effects of endogenous hormones in the menstrual cycle on iron status. More research of this nature is required in iron sufficient individuals.
2. Seek to clarify if increased inflammation in the late luteal phase, aligning with the initiation with menstrual bleeding is associated with an inflammatory-driven increase in hepcidin prior to the decrease that occurs during menstrual bleeding (early follicular) phase.
3. An examination of the interactions between the severity of PMS, inflammatory cytokine levels, and iron status may better establish the role of iron status in premenopausal females exhibiting PMS.
4. Researchers should consider the female menstrual cycle status before and during study participation and consider including females that present with ovulatory disturbances (LPD or anovulatory cycles) within research trials. This research may provide insight into the risk of iron deficiency with changes in sex steroid hormone concentration and menstrual bleeding characteristics. To date, iron regulation has been predominantly investigated in normally menstruating or eumenorrheic females. Any female participants presenting with ovulatory (e.g., luteal phase defects, anovulation) and cycle disturbances (e.g., oligomenorrhea, and secondary amenorrhea) that result in differing concentrations of sex hormones within their menstrual cycle, or changes to menstrual cycle bleeding (i.e., heavier, or prolonged bleeding) characteristics, have either been removed from a data set or have not been identified in research.
5. Few studies have investigated the changes in iron status in active females while also considering their menstrual cycle over a prolonged training period. According to recent guidelines, almost all the existing research conducted on active females is of a low quality (Table 3). This is an area that requires additional research to enhance our understanding of the changes in iron status over a prolonged training period. Such research may benefit our understanding of if there is an increase in the incidence of LPD or anovulatory cycles and whether this then exacerbates the negative iron balance and risk of iron deficiency. The recent reviews by Elliott-Sale et al. (2021) and Smith et al. (2022) provide a good discussion of how to improve the quality of research conducted with female athletes.
6. Female athletes are considered an at-risk population for low energy availability, RED-S, HMB, and iron deficiency. Research may seek to clarify any interrelationship between these conditions based on the evidence presented within this review.
7. With only four research trials to date investigating the effect of OCP use on iron status in active females, two during exercise and two throughout the respective phases

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TABLE 5 Expected changes to iron parameters throughout the menstrual cycle in active females.

Commonly measured iron variable	What does it measure	Early Follicular Phase: Menses	Mid to late Follicular phase	Luteal phase	Factors that will upregulate iron parameters	Factors that will downregulate iron parameters
Serum Iron	Measure of how much iron is in your plasma	Low	Low/ Gradual increase	High	Previous exercise session, exogenous/synthetic estrogens, iron supplements, diurnal variation (afternoon), dietary intake	Menstrual bleeding, diurnal variation (morning), dietary intake, inflammation, E2
Total iron binding capacity (TIBC)	Measure of the blood capacity to bind with iron	High	High	Low	Menstrual bleeding, dietary intake, serum iron, basal iron status	Dietary intake, serum iron, basal iron status
Transferrin Saturation	Measure of a percentage of iron bound to transferrin. Calculated as Serum iron/TIBC	Low	Low/ Gradual increase	High Early luteal phase it is not uncommon for females to present with > 45%	Mirrors changes in serum iron	Mirrors changes in serum iron
Hemoglobin	Measure of free levels of hemoglobin in the blood	Low/ Normal	Increasing/No change	High/ No change	Previous exercise, dehydration, decrease in plasma volume (e.g., post exercise shifts, changes with posture)	Haemodilution, hypovolemia with training or heat adaptation, increase fluid retention due to P4 in luteal phase,
Serum Ferritin	Measure of the body's iron stores	Low/ no change	Increasing/no change	High/no change	Previous exercise, infection/illness, inflammation, iron infusion/injection, prolonged suppression of serum hepcidin	Prolonged elevation in serum hepcidin, iron supplements or iron rich food in iron sufficient individuals
Serum Hepcidin	Measure of the concentration of hepcidin in the blood	Low/No change	Increasing/No change	High/No change	Previous exercise, dehydration, decrease in plasma volume, P4, energy availability status, inflammation/illness/ infection, iron supplements, iron fortified foods, high-normal iron status	Haemodilution, hypovolemia, hydration status, E2, carbohydrate availability, altitude exposure, enhanced erythropoiesis, deficient iron status

of OCP ingestion, more research is required to determine whether the provision of exogenous reproductive hormones has an impact on iron status and regulation in premenopausal females. Considerations for these studies include; comparisons to iron status matched naturally menstruating females (control group), iron status in respective phases (i.e., withdrawal phase and menses or active pill and mid-luteal) in iron replete females, the brand and dose of exogenous hormones provided, the impact of progestin-only OCP on iron status, and the impact of other hormonal contraceptives (e.g., hormonal IUD, Depo Provera injection, contraceptive implant), prior behavioral use of OCP (skipped placebo pills) on iron status and hepcidin.

8. Future research may also need to give some consideration to genetic variability in hepatic cytochrome p450 (CYPs), which is responsible for the heterogeneity in OCP metabolism observed between females (Lynch and Price, 2007). It is probable that inter-individual differences in CYP expression affects the rate of metabolism of exogenous hormones from the OCP, impacting the rate of disappearance from the individual's system and possibly the subsequent effects that the OCP may have on iron regulation. To date, genetic variability in OCP metabolism and exogenous hormones has not been considered in research and may require future investigations.
9. Additionally, exogenous E2 induces CYP expression (Zhang et al., 2018). Future research may seek to investigate the impact of varying doses of E2 in the OCP and the genetic expression of CYP in females on iron regulation.
10. Research is required to update HMB prevalence rates using validated subjective tools in healthy premenopausal females. Validated menstrual blood loss tools, such as the menstrual pictogram (Magnay et al., 2014, 2018), may be used to educate premenopausal females on HMB. Enabling them to recognize changes in menstrual bleeding that may increase their risk of iron deficiency diagnosis and seek medical support when required.

When conducting research on iron status in premenopausal females, the following statistical analysis approaches are advised:

1. Employ a generalized linear or non-linear mixed model (see Gałecki and Burzykowski, 2013) or generalized additive mixed model (see Wood, 2017) that suitably estimates the between- and within-participant variability throughout the menstrual cycle by treating participants as a random variable. Given the changes in iron parameters across the menstrual cycle are rarely linear, a spline-based model may provide a more flexible way to analyse their relationship to sex steroid hormone responses.
2. Where multiple OCP brands have been used throughout a study, then if the sample size allows it, the OCP brand may be either fit as a random intercept (in addition to participant ID), and/or a heterogeneous residual variance-covariance structure fit to allow a different, within-participant variance to be estimated per OCP brand.
3. Given the repeated measures nature of menstrual cycle research, autocorrelation should be accounted for by fitting a suitable variance-covariance matrix (e.g., autoregressive, autoregressive moving average, or exponential spatial). In our experience, serum ferritin and hepcidin are often right-skewed, which is often partially addressed *via* log-transformation, or less accurately, by using a non-parametric, rank-based approach (e.g., Wilcoxon signed-rank test). However, ferritin and hepcidin data are often more accurately modeled using a Gamma distribution with a log link function, allowing the response variable to be modeled on its raw unit scale.
4. Baseline serum ferritin level should be included as a covariate in randomized, controlled pre vs. post repeated measures designs to estimate the treatment effect more accurately. We advise against modeling either raw unit or percentage differences in these designs since they do not appropriately account for the dependent nature of the data (see Vickers and Altman, 2001). Serum ferritin is a continuous, zero bound variable that is often unnecessarily categorized into "cut scores" for diagnostic or analysis purposes, whilst this may be attractive from a decision-making perspective, categorization of serum ferritin during statistical modeling may lead to a loss of information, power, and efficiency (Altman, 2014).

de Paula Oliveira et al. (2021) recently employed an excellent statistical approach (i.e., a state-space model) to model the menstrual cycle length in women using a smartphone application to monitor their menstrual symptoms. Future investigations may consider similar statistical approaches to understand how sex steroid hormone fluctuations throughout the menstrual cycle affect iron parameters in female OCP users and non-users.

Conclusion

Despite active females having a higher risk of developing an iron deficiency than males, only ∼11% of iron regulation research has been conducted in female only cohorts, with ∼35–40% conducted in male only cohort. Research into iron regulation in females often does not consider the phase of the menstrual cycle (fluctuation in sex hormones), hormonal contraceptive use, or menstrual irregularities. This sex disparity is not exclusive to the research conducted in iron regulation

(Costello et al., 2014). In this review, we have provided an in-depth analysis and summary of the current female-specific iron metabolism research, and detailed practical recommendations for iron monitoring, diagnosis, and treatment in female athletes (Figure 1; Table 5). In addition, we have highlighted key areas for future research that are required to better understand iron metabolism in physically active premenopausal females.

Author contributions

CB and AG conceived the project and drafted the initial manuscript. The work was reviewed and critically reviewed by all authors before all agreed to the final manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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"I hardly have a problem […] I have my period quite rarely too": Female football players' and their coaches' perceptions of barriers to communication on menstrual cycle

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Introduction: Symptoms related to the menstrual cycle (MC) affect the performance level and health of female athletes in various ways. Previous research has reported MC symptoms such as pain, mood disturbance, reduced coordination and competition distraction as well as diminished performance levels and an increased injury risk among female elite athletes. Despite this, the coach-athlete communication related to the female hormonal cycle is limited. The aim of the present study was to explore the perceptions of MC communication in a group of junior elite football players and their male coaches in a case study of one youth football team in a specific club in Norway.
Methods: The study used a qualitative approach with semi-structured interviews. In total, 8 female junior elite players (aged 16-20) from a Norwegian football team and 2 of their male coaches participated in the study.
Results: The data analysis revealed two main communication barriers: 1. interpersonal barriers (e.g., false assumptions about the coach/athletes and social discomfort) and 2. knowledge barriers (e.g., unaware/perceptions of insufficient knowledge levels).
Discussion: As the players seemed to be unaware of their insufficient MC knowledge (e.g., failed to see a connection between the MC and their health and performance level), the coaches perceived their knowledge as insufficient and coped mainly by outsourcing MC communication to female staff and apps. Hence, the MC communication was hindered by both the athletes and the coaches (e.g., mutual avoidance). In line with previous research, this study supports that there is a need for developing effective strategies to overcome the interpersonal barriers and knowledge gaps.

KEYWORDS
barriers, player development, junior-to-senior transition, avoidance, one-day seminar

Introduction

The junior-to-senior transition (JST) (1) is a critical stage in the talent development process of many athletes (2, 3). Players' career progression to a senior elite level in football is affected by both social (e.g., player–coach–teammate interactions) and psychosocial (e.g., wellbeing) factors (4). The JST is often associated with increasing demands in several life areas (e.g., sport and academic), perceived stress, and an increased risk for injuries (5). Previous research suggest that most coaches are men, both in elite sports (6, 7) and even more within team sports compared to individual sports (8). Since the coaches' gender and their own coaching experience have an impact on their training philosophies and expectations, the coaches' gender has been found to be influential on the athletes (7, 9). Additionally, compared to male players, female footballers are underrepresented in the literature (10). Since coaches are known to be the most central socializing agents for young athletes, their views and thoughts are assumably very important for the athletes. This gender hierarchy in sport may affect coaches to view masculine features as the norm, which can influence their coaching methods and understanding for female athletes negatively (7). Hence, female-specific needs during the JST (e.g., the menstrual cycle, MC) might be overlooked (6, 11), seen as a problem, or weakness (7). Furthermore, this may not only inhibit female athletes in their sportily development but also risk their health and wellbeing (11, 12).

Previous research has shown that symptoms related to the MC affect the performance level and wellbeing of many female athletes in various ways (6, 13–16). For example, MC disturbance is one of the most well-known signs of Relative Energy Deficiency in Sport (RED-S), which can impair athlete performance negatively (e.g., increased risk of injury, decreased level of strength and endurance, and reduced training response) (17). Other studies have reported symptoms such as pain, mood disturbance, reduced coordination, and competition distraction (e.g., worry) among senior elite athletes from various sports (16, 18–20). Additionally, Read et al. (15) reported symptoms such as decreased appetite, reduction in sleep quality, recovery and perceived self-confidence, as well as diminished performance levels among elite footballers. Previous research has also shown an association between hormonal fluctuations and anterior cruciate ligament (ACL) injury (21, 22). For example, Martin et al. (23) reported a significant increase (88%) in muscle and tendon injuries (e.g., rupture, tear, strain and cramps) in the late follicular phase compared to other phases of the MC among international footballers. McNamara et al. (19) showed that as much as 65% of 195 female Australian individual sports athletes preparing for the Olympic and Paralympic Games 2020 perceived that their MC affected their performance. To manage their MC, many athletes use hormonal contraceptives (HC) (11, 14, 18, 24). For example, Ekenros et al. (13) reported that 63% from a group of 1,086 athletes used various HC, from which 40% perceived a variety of side effects. Although HCs may affect athlete performance in multiple ways (e.g., weight gain, tiredness, depression, and a decrease of maximal aerobic capacity) (18, 25, 26), the HC knowledge among elite athletes is reported to be low (23). Not surprisingly, only a minority of female athletes considered MC or HC issues when planning their training and competitions (13).

Several recent studies have reported that the athlete–coach communication related to the female hormonal cycle is limited [e.g., (6, 8, 16, 18)]. To improve the current situation, previous studies have urged for educational interventions including, for example, basic terminology and organized discussion forums (6, 11, 18, 27). Such measures are believed to help athletes and their training staff to relate the female hormonal cycle to sport performance and thereby putting it in the same light as other physical functions (18). This can hopefully lower existing communication barriers by increasing awareness and openness (6, 11, 16) as well as correct existing misinformation and misconceptions (12, 18, 20). Höök et al. (11) described this as interpersonal barriers in their study of elite female cross-country athletes and their coaches.

The limited athlete–coach communication related to MC could be explained by the social discomfort of talking about the topic, again related to the gender of the coach (often men) and a lack of specific MC knowledge (11, 27, 28). For example, von Rosen et al. (8) reported that female athletes perceive the knowledge acquired by their male coaches as poor or very poor compared to female coaches. Solli et al. (6) showed that 92% of 140 female athletes (in individual sports) felt that they had insufficient knowledge related to how the hormonal cycle affects athletic performance. Although many of these athletes experienced several MC-related symptoms, only 27% communicated with their coach about their MC (6). Similar findings have been reported in other studies [e.g., (12, 14)]. Additionally, Verhoef et al. (12) suggested five main reasons among female athletes who avoid reporting MC abnormalities (e.g., amenorrhea) to their coaches and training staff: (1) normalization of amenorrhea in elite sport, (2) not expecting the absence of an MC as a problem, (3) shame and taboo, (4) prioritization of sports performance, and (5) denial of the problem. Similarly, Höök et al. (11) found that some elite athletes failed to recognize MC disturbance as a potential health risk, as others believed that there was no need to discuss their MC with their coaches, since they used HC. It was worth noticing that previous research has reported limited MC knowledge levels among coaches as well [e.g., (11, 27)]. Höök et al. (11) described these as knowledge barriers in a study of elite female cross-country athletes and their coaches. Consequently, the combination of social discomfort and insufficient knowledge among female athletes and their coaches may hinder effective MC communication and the incorporation of the MC into the sport-specific practice in both individual and team sports (8).

Given that many previous studies have urged for educational efforts, there is a lack of research that have explored the results of such interventions. Furthermore, previous studies have mainly focused on senior athletes [e.g., (15, 18, 19)] and athletes in individual sports [e.g., (6, 11, 20)]. Since the JST is a critical stage in the talent development process (2, 3) and that the MC may affect the performance level and wellbeing (6, 13–16), it is crucial to explore the experiences of MC communication among junior athletes and their coaches in team sports as well. Knowledge about junior athletes is important since we might expect that they would consider talking about MC more difficult compared to senior athletes. Furthermore, the end of the teens might be a critical period since symptoms related to MC might be more severe for younger athletes compared to senior athletes. Therefore, the aim of the present study was to explore the perceptions of MC communication in a group of junior elite football players and their male coaches in a case study of one youth football team in a specific club in Norway. The team had 6 months earlier been a part of a one-day seminar on MC organized by the club. The importance of the seminar will be discussed in our discussion.

Methodology

Participants and data collection

To gain an in-depth understanding of the perceptions and experiences of junior elite female athletes and their coaches' barriers to MC communication, a qualitative approach was conducted. In the present study, the *Conceptual map of the social support services* model of Bianco and Eklund (29) was applied for the understanding of communication. The model highlights the distinctions between social support activities (e.g., measures/actions done) and social support messages (e.g., the meaning of such measures/actions). Furthermore, the model illustrates the complex processes of how intended support (e.g., from the coach) is perceived by the receiver (e.g., the athlete) depending on the individual's expectations and perceived needs of social support (e.g., instrumental or relational). Hence, the social support actions and messages may not always match the expectations and perceived needs of the receiver or be interpreted differently from what the sender intended (29), which may affect further actions and communication on specific topics (e.g., the MC). Therefore, exploring how athletes and coaches perceive MC communication as well as their actions taken may help understand potential barriers (or the opposite).

Potential participants were contacted through the team's assistant coach (coaches) and team captain (players). In total, eight female junior elite footballers (age 18 ± 2 years) and two of their male coaches (assistant coach and physical coach) from the team agreed to participate. The informants were playing or coaching on the highest junior level in Norway. To ensure that the informants would refer to the same case, they were recruited from one football team. The number of participants was considered sufficient in relation to the study aim, sample specificity, and quality of the dialog. This is in line with the concept of "Information power" by Malterud et al. (30). For example, Malterud et al. (30) stated that, "*Information power indicates that the more information the sample holds, relevant for the actual study, the lower amount of participants is needed*" (p. 1753). Our study is positioned within a social interactionist ontology and utilizes an interpretivist approach (31). The focus of this study is on the everyday interactions that occur between individuals, and how the meanings associated with these interactions are managed and transformed through peoples' interpretative processes as they try to make sense of, and adjust to, their social worlds. The female researcher on the project conducted the interviews, which was intentional since the topic has been considered a taboo topic, especially female athletes talking to male coaches (11). With our social interactionist ontology approach, we considered it lightly that the interviewer and the interviewed would empathize with and identify each other in some way [see (32)].

The data were collected through two semi-structured focus group interviews (players and coaches separately) and three individual interviews (players). The variations in the interview technique were determined by the players' and coaches' schedules and availability to participate in the study. Before conducting the interviews, all participants were appraised with the study aim and given all necessary information before obtaining their written consent. All participants were informed that their contribution was voluntary and that they could withdraw from the study at any time during the research process until the article was published. To ensure confidentiality, the participants were given pseudonyms. The interviews took place in the team's sport arena in February and March 2022 and lasted from 25 to 60 min and were held by the second author. The interview guide was inspired by Höök et al. (11) and was organized around the following themes: (1) menstruation and sport, (2) communication, (3) contraceptives and sport, (4) knowledge, and (5) coach–athlete relationship. The interviewer functioned as a facilitator to encourage everyone to contribute, as well as keeping the discussions relevant to the study aim. Since the female hormonal cycle is perceived as a tabooed topic by many athletes and coaches (8, 11, 12), the interviewer used probes in line with Patton (33) to encourage the participants to share their own thoughts and experiences. Examples of such probes were (a) the interviewer started the discussion by sharing her own MC experiences and (b) pointing to recent media coverage of the MC in elite sports. At the end of each probe, the interviewer added relevant questions for each theme such as: (1) "Can you describe how the MC affects you in your athletic career?" (menstruation and sport); (2) "How do you feel about discussing the MC with your coach/athletes?" "Can you recall a specific situation?" (communication); (3) "Do you have any experience with HC?" "How did/does it affect you as an athlete?" (contraceptives and sport); (4) "What additional knowledge would you need about the MC?" (knowledge); and (5) "Tell me about your relationship with your coach/athletes in general" (coach–athlete relationship).

Data analysis

The aim of the analysis was to identify the different barriers athletes and coaches had encountered in their MC communication. To analyze the interview data, the present study used the six steps of thematic analysis: (1) familiarizing yourself with the data, (2) generating initial codes, (3) searching for themes, (4) reviewing themes, (5) defining and naming themes, and (6) producing the report (34). The first and second authors worked closely with categorizing the raw data. After transcribing the interviews, the first and second authors read the text to get a general sense of the material (step 1). Next, interesting features were bunched into main themes (step 2) (e.g., knowledge) and subthemes (step 3) (e.g., assumptions), based on a deductive analysis based on interpersonal and knowledge barriers. In step 4, all authors reviewed and discussed the themes and subthemes from different research angles and implications. No specific framework for classifying communication barriers were used for the analysis. However, when the authors explored the perceptions of MC communication among the informants, several barriers were discovered from the dataset, also in line with the study of barriers by Höök et al. (11). In the next step, the themes were then refined and labeled into two main themes [(1) interpersonal barriers and (2) knowledge barriers] (step 5). In the final stage of the analysis (step 6), several quotes were chosen to reflect these themes in relation to the study aim and previous research. Additionally, to ensure peer validity, the authors discussed various perspectives and interpretations of the themes throughout data analysis. This is in line with previous literature recommendations (33).

Ethical statement

The study was conducted in line with the Declaration of Helsinki and approved by Norwegian Social Sciences Data Services (reference nr. 613821).

Results

The qualitative analysis revealed two main barriers faced between female junior footballers and their coaches. The first barrier, *interpersonal barriers*, reflects the players' and coaches' perceived challenges for discussing the MC with each other and how this led to the avoidance of MC communication (see **Figure 1**). The second barrier, *knowledge barriers*, reflects how limited knowledge limits both players and coaches to understand how the MC can affect the players' sport performance and their health.

Theme 1: interpersonal barriers

Six months prior to the interviews, both players and coaches had attended a 1-day seminar organized by the club. The seminar was focused on training measures, nutrition, and injury prevention in relation to the MC. The aim of the seminar was to increase the MC knowledge levels and the initiative had been appreciated by both players and coaches. Despite this, they still struggled to communicate with each other about the MC at the time of the interviews. It was worth noticing that both the players and the coaches described to have a close coach–athlete relationship. Yet, discussing the MC was perceived as something private compared to other performance-related factors. Interestingly, the interpersonal barriers were in many cases based on false assumptions from both the players and the coaches. For example, the players assumed that their male coaches were not interested in knowing anything about their MC and that they would not have sufficient knowledge or understanding. Other players believed that the coaches would see them as weak if they would approach them with their MC symptoms:

> You don't feel that they [men] care about it, somehow. One thinks that they are not interested, and then we leave it at that (**A5**).

> If you have so much pain and hurt or a lot of discomfort, I think it's difficult to tell a coach because I think he thinks it's strange. You feel that he is not going to respect you (**A7**).

Consequently, this made the players await the coaches to take the initiative, even though some may have felt a need to discuss their MC. The coaches, however, interpreted the silence from the players as if they had no wish or need to share information about their MC with them, even if they had at least a basic understanding about the MC, as expressed by **C1**:

> I have a twin sister, I have a wife, I know. We cannot force them [*the players*]. We cannot make them come up to us and let us know when they have their time of the month. They have to feel comfortable to be able to do it without, I think, us saying it.

Hence, this created a situation in which both players and coaches expected the other to take the initiative and where silence was interpreted as if there was no need to talk to each other about the MC. In addition to the players' and coaches'

	Players´ perspective	Coaches´ perspective
Interpersonal barriers	- **False assumptions about the coaches** *(e.g., Lack of interest; Little knowledge and understanding; Expecting the coach to take initiative; Fear of being seen as weak by the coach)* - **Private (female) topic** *(e.g., Social discomfort discussing MC with men)*	- **False assumptions about the athletes** *(e.g., The players have no wish/need to discuss MC with them; Expecting the players to take initiative)* - **Private (female) topic** *(e.g., Perception of taboo, outsourcing MC talk to female staff or apps)*
Knowledge barriers	- **Unaware of insufficient MC knowledge** *(e.g., Not knowing what´s normal; Diminishing/comparing own MC symptoms; Little/no understanding for how the MC affects performance and health)*	- **Perception of insufficient MC knowledge** *(e.g., Challenges to implement MC into the sport-specific practice; No insight in the players´ MC)*

FIGURE 1
Description of the barriers experienced by the players and coaches based on the thematic analysis.

assumptions about each other, the MC was perceived as a private topic. The MC was associated with taboo and social discomfort. For example, the players perceived the MC as a difficult subject to discuss with men in general. Hence, the interpersonal barriers might not have been unique between them and their present coaches. For example, the two following quotes show that the players had experienced MC-related problems (e.g., having to change sanitary napkin or vomiting) but chose to conceal them from their coaches with what they perceived as less discomforting excuses:

> At least I've had mostly boys' and men's coaches who haven't said anything about this at all. And then it becomes a bit unpleasant to say "I have to go to the bathroom," or I don't really have to go to the bathroom, I just have to change tampons. You don't like to say that to your coach if he's a man (**A3**).

> When we played on another team earlier, we also had a male coach. That's when I threw up. The next practice when I came and he asked how it went and that I had thrown up, I just said that I must have eaten something bad because I didn't want to say that the reason was my period (**A8**).

The coaches coped by outsourcing MC talks to female staff or MC apps. Their coping strategies may indicate that discussion of MC directly with the players was associated with social discomfort but also it reflects a need to help their athletes:

> I feel that it is easier for the players to talk to her [*the female coach*]. We also have a female fitness coach, and that is important … so we try to manage that [*having female staff*], and prioritize that [the MC] (**C1**).

> […] if they want to report it to us [*about their MC*], they can *via* that app. That goes to our physical coach, who is female. We could possibly log the cycle of every player. That is a lot of work, but if they want they can do it (**C2**).

Interestingly, both the players and the coaches seemed to be aware that the MC was relevant to the sport practice at least to some extent but still struggled to find ways to initiate conversations about it. Hence, this left them waiting for each other. For example, as mentioned in the second quote from **C2**, he says: "…*if they want, they can do it*," thereby leaving the initiative to the players. However, since the players seemed to conceal MC-related symptoms from their coaches, as mentioned by **A3** and **A8**, this also hindered MC communication by leaving the coaches unknowing.

Theme 2: knowledge barriers

The second barrier that inhibited MC communication was limited MC knowledge levels among both the players and the coaches. For example, some players failed to recognize MC disturbance as a symptom relevant to their performance and health, as expressed by **A4**:

> I hardly have a problem […] I have my periods quite rarely too … So I am bothered very little compared to many others, but I, who am bothered so little, have had no need to talk about it. I get through a week where I'm a bit tired without needing to put anything up after that.

Additionally, **A4** compares her own symptoms to what she thinks her teammates might experience and concludes that they probably are worse off than her. Because of her perception of just having minor or no MC problems, she sees no need to share the information with the coaches. Other athletes seemed to believe that the MC did not concern them anymore, since using HC. Instead, she diminishes the perceived MC symptoms and continues with the training as normal. This is similar to **A6,** who describes her experiences with MC symptoms:

> I am very lucky that I have not suffered from pain. I can feel it occasionally in my stomach and lower back, but it has not been a problem […] You are afraid of bleeding, especially during training and the like. Now I don't think about it too much. It's only if my back hurts that I get a little stiffer after training. Right after […] we fortunately have black shorts […] We have white away shorts, and then I think more about it.

A6 starts with expressing a contradiction. In the first sentence she says that she is lucky because she does not have any MC-related pain, but still admits it in the next sentence. Just like **A4, A6** seemed to believe that her problems were not big enough or irrelevant to share with the coach staff. Additionally, she mentions implicitly that the fear of bleeding through her white shorts affects her concentration during trainings and games. Although she was aware of the distraction this meant to her during practice and games, she failed to make the connection that this might affect her performance level. Hence, she did not approach the coaches with the information.

The coaches felt that they would not know how to implement the perceived MC symptoms in a team setting and their sport-specific practice, for example, as **C2** sees challenges in logging the MC or individualizing the training for each of the 27 players. The perception of having insufficient MC knowledge was shared by **C1**:

> I think one of the difficulties in football is that we are a team of 27 players, all individuals […] In some way we try to log their monthly cycle, but it is hard in a team setting to put menstruation cycle in to account in training (C2).

> For me as a man, the barrier must be broken down for us to get the fullest knowledge and understanding and appreciation of how to work with it [*the MC*] in the best way we can […] My knowledge is not enough, because we need to be having more conversations (C1).

Interestingly, **C1** is aware that there is a barrier between him and the players. Furthermore, he sees having MC conversations as a key to gain more knowledge and to improve his understanding in order "*to work with it in the best way*." Yet, as mentioned in theme 1, both the coaches and players avoided initiating such talks. As the players seemed to be unaware of their insufficient MC knowledge (e.g., failed to see a connection between the MC and their health and performance level), the coaches perceived their knowledge as insufficient. For example, they felt unsure how to implement the MC into the sport-specific practice. Almost contradictory, they believed, on the one hand, that discussing the MC with their players would enhance their knowledge, as expressed by **C1**. On the other hand, they seemed to avoid it because of the perceived lack of MC knowledge. Therefore, avoiding MC communication also kept them unaware of what the actual MC issues were such as fear of bleeding through their shorts. Consequently, in combination with the perceived interpersonal barriers, the *status quo* (e.g., no MC communication) was maintained by both the players and the coaches.

Discussion

The aim of the present study was to explore the perceptions of MC communication in a group of junior elite football players and their male coaches in case study of one youth football team in a specific club in Norway. The data analysis revealed two main communication barriers: (1) interpersonal barriers (e.g., false assumptions about the coach/athletes and social discomfort) and (2) knowledge barriers (e.g., unaware/perceptions of insufficient knowledge levels). Similar to Höök et al. (11), our study focused on both the athletes' and coaches' perspectives. In line with previous research showing interpersonal barriers [e.g., (6, 8, 16, 18)], the present study showed that the coach–athlete MC communication was limited even though the athletes reported several MC symptoms.

Previous studies have suggested educational interventions including, for example, basic terminology and organized discussion forums as an important step to enhance MC communication (6, 11, 18, 27). Interestingly, both the athletes and their coaches in the present study had attended at a 1-day MC seminar organized by the club. Even so, there was no coach–athlete MC communication or perceived changes in how the club worked with MC issues 6 months later, without discussing the quality or the length of the seminar. The data analysis did regardless reveal that the interpersonal barriers were partly a consequence of false assumptions and prejudices. As the athletes assumed that the coaches would not want to be bothered with MC-related problems or that they would not be able to understand them, the coaches interpreted their silence as if they had no will or need to discuss MC issues. The findings show that the coaches had more understanding and willingness to help than the athletes expected. In the present study, the coaches coped by outsourcing MC matters to female staff members and MC apps where they had no insight. Although previous research suggest that the MC should be monitored just like the athlete's training load, recovery, wellbeing, and injuries to promote the long-term development (35), it can be discussed if their coping strategy maintained the interpersonal barriers since it also meant avoiding direct MC communication with the athletes. For example, their lack of direct involvement might have been interpreted as a lack of interest by the athletes. Furthermore, this may also indicate a mismatch between the social support actions and the intended social support messages (28). Temm et al. (35) argued that whichever monitoring method is applied, it is crucial that it can be individualized, is affordable, and easy to implement. Here, possibly combined with other protocols, direct coach–athlete communication can be an effective and inexpensive way to prevent ACL injury and RED-S among female athletes.

A reason for the interpersonal barriers found in this study might be that elite sports is embedded in the normalized "culture of risk" (36), found both among female and male football players (37). The expectation of always striving for success and accepting health risks are internalized by both athletes and coaches (37). Within the literature, the risk of injury studies show that players are unwilling to "play hurt"—risk being stigmatized, isolated, and ignored by coaches (38), and consequently have been found to play a pivotal role in decisions whether to compete while injured (39). This could be related to the present study since some athletes feared of being seen as weak by the coaches if they would approach them with their MC symptoms. Even though our study did not focus on the risk of injury, this may indicate that they were concerned about how their development opportunities (e.g., risking getting dropped out of games) would be affected. However, ignoring or concealing MC symptoms (e.g., MC disturbance, pain or sickness) may be a counterproductive strategy in the long-term performance development perspective (e.g., reduced performance level, increased injury risk, or developing RED-S) (15, 17).

Previous research has shown that many athletes perceive that their MC and the knowledge about it affect their performance (19). Yet, only a minority discuss this with their coach (6, 12, 14). Similar to other studies [e.g., (11, 27, 28], the lack of MC knowledge and communication among the athletes and coaches was also affected by social discomfort (e.g., shame and taboo) and the gender of the coach. For example, the athletes believed that men in general (including their coaches) were not interested in the MC. This is in line with von Rosen et al. (8), who reported that female athletes perceive the knowledge acquired of their male coaches as poor or very poor compared to female coaches, indicating a knowledge barrier. However, it can also be speculated if the players perceived a gender hierarchy (7) in their sport that might have affected them to view masculine features as the norm, and thereby the MC as an abnormality or weakness. Hence, this may be another explanation for why some of the athletes feared that the coaches would see them as weak if they would approach them with MC issues.

The data analysis also revealed that lack of MC-specific knowledge limited the coach–athlete communication as well, indicating that the interpersonal barriers and knowledge barriers were impacting on each other. Although the athletes reported symptoms such as MC disturbance, pain, and sickness, they did not report this to their coaches. Two possible explanations for this phenomenon are that they (a) failed to recognize the symptoms as something abnormal and relevant to their performance and health and (b) compared their symptoms with what they believed others experienced. This is in line with Verhoef et al. (12) who reported athletes' prioritization of sport performance, normalization of amenorrhea, and denial of it as a problem that hinders MC communication, based on lack of knowledge. Additionally, some athletes using HC seemed to believe that the MC did not concern them anymore. Hence, in line with Bianco and Eklund (28), this may have affected the perceived need of MC-specific social support from their coaches. Similar findings have been seen in other studies [e.g., (11)]. Notably, the players had attended a 1-day seminar that focused on training measures, nutrition, and injury prevention in relation to the MC together with their coaches. Such educational interventions are encouraged in the literature since they have the potential to lower existing communication barriers by increasing awareness and openness (6, 11, 16) as well as correct existing misinformation and misconceptions (12, 18, 20).

Even though the players and the coaches described the seminar as a positive initiative by their football club, a limitation of this study is that exact content and working methods used in the seminar were unknown to the researchers. However, based on the findings as already stated, clearly a 1-day seminar does not seem to be enough, indicating the need for more educational interventions, even though it also depends on the content of such interventions. For example, the present study showed a need for educating athletes about how MC symptoms can affect sport performance and the potential health risks of ignoring them. Yet, the main challenge to overcome seemed to be the interpersonal barriers. In contrast to the players, who seemed to be unaware of their insufficient MC knowledge, the coaches perceived their knowledge as insufficient. In the present study, we found a paradox that exemplifies the challenges perceived by the coaches. On the one hand, the coaches believed that discussing the MC with their athletes would enhance their knowledge. On the other hand, they seemed to avoid it because of the perceived lack of MC knowledge or players sharing their experiences. Furthermore, they felt unsure how to implement the MC into the team sport setting (e.g., social support actions). Here, one critical question is how they would implement the MC into their sport practice if they did not find out what the perceived MC issues among the players were. Rather, it seemed that avoiding or outsourcing MC communication kept them unaware of MC issues, such as fear of bleeding when playing with white shorts, which would have been a relatively easy thing to "fix" (e.g., changing the color of the team shorts). Furthermore, if athletes and coaches would be able to communicate the MC, it may also help them to identify where the knowledge gaps are and plan future educational interventions together based on this. Yet, how could coaches and athletes be aware of the importance of discussing the MC without at least a basic understanding of the MC? Therefore, future studies could explore the best place to start. In line with previous research (6, 11, 12, 16, 18, 20), this study supports that there is a need for developing effective strategies to overcome the interpersonal barriers and knowledge gaps. Since our study shows that these barriers were maintained by both the players and coaches, an active engagement from both athletes and coaches, as well as support from their sport clubs and sport federations may be necessary in changing the "status quo." Even so, since coaches are known to be the most central socializing agents for young athletes and their views and thoughts are assumably very important for the athletes, one might expect the coaches to be the initiative taker on this issue. On a deeper level, this may also mean a continued work with gender hierarchies (7) and gender biases (9) in sport (e.g., in research). This will hopefully contribute to enhance sport performance and injury prevention, as well as female athletes' health and wellbeing.

Limitations

There are some limitations in the current study that must be considered. The use of both focus group and individual interviews might be considered a limitation since the focus group participants might have spoken more freely if they were interviewed individually. On the other hand, it might be that they spoke more freely when they experienced that the other players or coaches open on a difficult topic to discuss. The use of a female researcher was intentional because of the topic and the group of youth athletes. Doing qualitative research and adopting a social interactionist ontology and interpretivist epistemology used in our interviews enabled us to frame our interviews as a relational space. This meant that both the participants and the interviewers could explore themes together and co-construct knowledge (31). This might have meant that the interviewed had a strong voice if the interviewer and the interviewed empathized and identified with each other [see (32)], which we would say was the case in the present study. The taboo topic in the paper might be an obvious reason since the female researcher and the participants could talk about a topic, as shown in our results, which is considered difficult to talk about, in our study described as interpersonal barriers to talk to their male coaches. The use of probes in the interviews had the intention to let the interviewed talk about and relate to how they talked about MC was important in their everyday interactions with their coaches. Another limitation could be related to the limited education the coaches and players received through a 1-day seminar, which naturally also must be considered in terms of both the coaches' and players' knowledge on the topic. A third limitation was that more detailed information about the athletes' training and performance level, training hours, menstrual cycle lengths and use of hormonal contraceptives, the educational level of the coaches, as well as demographic data and details about the seminar are missing. This could have added information to the findings and enriched the discussion.

Conclusion

The main findings of the study indicate that the players seemed to be unaware of their insufficient MC knowledge (e.g., failed to see a connection between the MC and their health and performance level), while the coaches perceived their knowledge as insufficient and coped mainly by outsourcing MC communication to female staff and apps. Overall, it could be argued that the MC communication was hindered by both the players and the coaches (e.g., mutual avoidance). These findings are in line with previous research mostly on individual athletes, supporting that there is a need for developing effective strategies to overcome the interpersonal barriers and knowledge gaps, also within team sports. This will hopefully enhance female athletes' sport performance and injury prevention, as well as their health and wellbeing.

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 Norwegian Social Sciences Data Services (reference nr. 613821). The patients/participants provided their written informed consent to participate in this study.

Author contributions

MB, MR, and SAS conceptualized and designed the study and assisted in the planning and acquisition of data. MB and MR did the first analysis of the data, and SAS helped with the analysis and interpretation of the data later in the process, critically revising the manuscript, and adding important intellectual content. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Long-term development of performance, physiological, and training characteristics in a world-class female biathlete

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Purpose: The purpose of this study is to investigate the long-term development of performance, physiological, and training characteristics in a world-class female biathlete, with emphasis on differences between junior and senior athlete seasons.
Methods: The participant is a highly decorated female biathlete with 22 (10 gold) medals from international championships and 28 individual World Cup wins. Performance development (ages 17–33), physiological tests (ages 22–33), and day-to-day physical and shooting training (ages 17–33) were analyzed. Training data were systemized by endurance [low-intensity training (LIT), moderate-intensity training (MIT), and high-intensity training (HIT)], exercise mode, and strength training. Shooting training recorded for each session included the number of shots fired during rest, LIT, MIT, HIT, or competitions and time spent on dry fire training.
Results: The annual volume of physical training (409–792 h·season^{-1}) and number of shots fired (1,163–17,328 shots·season^{-1}) increased from the age of 17 to 28 followed by a subsequent reduction in physical training (range 657–763 h·season^{-1}) and shots fired (13,275–15,355 shots·season^{-1}) during the seasons of peak performance at ages 31–33. Maximal oxygen uptake in roller ski skating increased by 10% (62.9–69.2 ml·kg^{-1}·min^{-1}) from the age of 22 to 27. The physical training volume was 48% higher (694 ± 60 vs. 468 ± 23 h·season^{-1}, P = .030), with 175% more shots fired (14,537 ± 1,109 vs. 5,295 ± 3,425 shots·season^{-1}, P = .016) as a senior athlete than a junior athlete. In the physical training, these differences were mainly explained by higher volumes of LIT (602 ± 56 vs. 392 ± 22 h·season^{-1}, P = .032) and MIT (34 ± 1 vs. 7 ± 2 h·season^{-1}, P = .001) but less HIT (27 ± 1 vs. 42 ± 3 h·season^{-1}, P = .006) as a senior than a junior. In line with this, shooting training as a senior included more shots fired both at rest (5,035 ± 321 vs. 1,197 ± 518 shots·season^{-1}, P = .011) and during LIT (7,440 ± 619 vs. 2,663 ± 1,975 shots·season^{-1}, P = .031), while a smaller insignificant difference was observed in the number of shots fired in connection with MIT, HIT, and competitions (2,061 ± 174 vs. 1,435 ± 893 shots·season^{-1}, P = .149).
Conclusions: This study provides unique insights into the long-term development of physical and shooting training from junior to senior in a world-class female biathlete. The major differences in training characteristics between junior and senior athlete seasons were higher sport-specific volumes of LIT and MIT and less HIT. These differences were accompanied by more shooting training, particularly at rest, and in connection with LIT.

KEYWORDS

endurance training, female athlete, junior athlete, shooting, training quality, XC skiing

Introduction

Biathlon is a Winter Olympic sport that combines cross-country (XC) skiing over undulating terrain in the skating style with rifle shooting in competitions lasting ∼20–50 min. During competitions, biathletes carry a ∼3.5 kg rifle while skiing and stop two to four times to perform a five-shot series of shooting in either the prone or standing position (1). Although the physiological and technical demands of biathlon are comparable to those of XC skiing (2), the additional demands of shooting directly after high-intensity exercise separate biathlon from most other endurance sports (1). Accordingly, success in biathlon requires a well-developed aerobic endurance capacity and skiing technique combined with rapid and accurate shooting performed under high physiological strain and mental pressure (1).

To reach elite to world-class levels in endurance sports, a successful long-term development process and a progressive increase in training volume are required to ensure sustainability and gradual performance development (3). In a recent case study, Schmitt et al. (4) reported the long-term development process of a world-class male biathlete and showed a 32% increase in the overall training volume (530–700 h·season^{-1}) from the age of 21 to 31. On average, the training consisted of 86% low-intensity training (LIT), 4% moderate-intensity training (MIT), 4% high-intensity training (HIT), and 6% strength training across the annual cycles investigated. In comparison, an 80% increase in training volume (522–940 h·season^{-1}) was reported in the most successful female XC skier of all time from the age of 20 to 35 (5). Furthermore, a 27% increase in endurance training volume (462–635 h·season^{-1}) was observed in a world-class male Nordic combined athlete from the age of 19 to 23 (6). However, knowledge of the progression of endurance training when combined with a mentally challenging task such as shooting in biathlon is still limited, and no data on the long-term training characteristics of world-class female biathletes exist.

In a recent review, Laaksonen et al. (1) provided an overview of the recent advances and perspectives in Olympic biathlon. Although biathlon has developed substantially over the last decades with a corresponding increasing scientific interest, most of the available literature has its emphasis on its competitive demands and performance-determining factors (7–12). In contrast, literature on the related training characteristics of elite to world-class biathletes is rather sparse, compared with that on successful XC skiers (5, 13–15). While some of the available literature on XC skiers training is also relevant to biathlon, many features unique to biathlon have significant implications for the training performed. For example, carrying a rifle influences both the physiological responses and kinematic patterns of skiing (16, 17), and the interspersed periods of shooting lead to even more interval-based fluctuations in exercise intensity compared with XC skiing (1). Moreover, the requirement for rapid and accurate shooting in biathlon involving features such as optimal rifle stability and triggering behavior as well as a reduction of range time and shooting time has obvious implications for training, where biathletes must balance a complex interplay between both physical and shooting training.

There is a need for a better understanding of the training characteristics of biathletes and particularly the challenging balance and progression of physical- and shooting-specific training over time. In addition, previous research on elite endurance athletes has primarily focused on the athletes' senior seasons. In biathlon, no data on the differences between junior and elite-level senior training exist, where athletes are classified as juniors from the age of 17 until they transition to seniors at the age of 23. Therefore, the main aim of this case study was to investigate the long-term development of performance, physiological, and training characteristics in a world-class female biathlete, emphasizing differences between the junior and senior athlete seasons.

Methods

Participant

The participant (born in 1981) is a highly decorated female biathlete with 4 Olympic medals (two golds), 18 World Championship medals (eight golds), 28 individual World Cup wins, and 4 podiums of the overall International Biathlon Union (IBU) World Cup. The Norwegian Social Science Data Services approved the study, and the participant provided written informed consent to participate.

Overall design

To provide a comprehensive understanding and detailed insight into the participant's long-term development process, the study was divided into two parts: (1) a retrospective description of the participant's long-term performance, physiological, and training characteristics across 17 seasons from the age 17 to 33 years (1997–2014) and (2) detailed comparisons between three annual cycles as a junior athlete (18–20 years) and three annual cycles (years of peak performances) as a senior athlete (31–33 years). The senior seasons were chosen based on the performance level and further confirmed by the participant. To provide an accurate comparison with senior seasons, the first three junior seasons where the participant started prioritizing biathlon as her main sport were chosen.

Performance data

The participant's performance development was based on performance analyses, including World Cup competitions, Olympic Games, and World Championships, downloaded from results publicly available (18).

Training monitoring

The participant recorded her day-to-day training in both handwritten diaries (ages 17–22) and Microsoft Office Excel sheets (>22 years old) developed by the Norwegian Biathlon Federation. Physical training recorded for each session included the total duration of each training form (endurance and strength training), exercise mode (skiing, roller skiing, running, cycling, etc.), and intensity (LIT, MIT, and HIT). Skiing/roller skiing in the skating style was classified as specific endurance training, skiing/roller skiing in the classical style as semi-specific endurance training, and all other exercise modes (running and cycling) as non-specific endurance training. Shooting training recorded for each session included the number of shots fired during rest, LIT, MIT, HIT, or competitions and time spent on dry fire training (shooting without ammunition).

To record her physical training, the participant used a combination of the *session goal* and *time in zone* referred to as the *modified session goal approach*, as reported by Sylta et al. (19). As a junior athlete, the endurance training intensity was mainly controlled by using a combination of heart rate (HR) and rating of perceived exertion (RPE) during sessions. As a senior athlete, measurements of blood lactate were introduced and used in addition to HR and RPE and particularly in connection with MIT sessions and at altitude training camps. The five-zone intensity scale developed by the Norwegian Top Sports Center (20) was used as a framework to control and log her endurance training intensity with some adjustments throughout her career. The overall physiological boundaries between the different intensity zones used by both junior and senior athletes were LIT [zones 1–2, <2 mmol·L^{-1} blood lactate, 60%–82% of maximal HR (HR_{max})], MIT (zone 3, 2–4 mmol·L^{-1} blood lactate, 82%–87% of HR_{max}), and HIT (zones 4–5, including competitions, >4 mmol·L^{-1} blood lactate, >87% of HR_{max}). However, as a senior athlete, the participant became more accurate in her intensity control and used smaller target zones for different training intensities. Her target intensities were LIT (0.8–1.2 mmol·L^{-1} blood lactate, 65%–75% HR_{max}), MIT (2–3.2 mmol·L^{-1} blood lactate, 85%–90% of HR_{max}), and HIT (>3.2 mmol·L^{-1} blood lactate, 90%–95% of HR_{max}), respectively. In all physical training sessions, shooting time was not included. For MIT and HIT sessions performed as intervals, time in the respective intensity zones included time spent from the first interval to the end of the last interval, excluding breaks. Strength training was recorded from the start to the end of that specific part of the session, including breaks. Speed training was not included in the analyses due to the unsystematic reporting of some stages of the participant's career. The included information on speed training is, therefore, solely collected through interviews with the participant. The researchers systematically analyzed all training data session by session using the framework and periodization phases previously reported (5). The annual cycles were categorized into the phases *general preparation period one* (*GP1*, *weeks 18–30*), *general preparation period two* (*GP2*, *weeks 31–43*), *specific preparation period* (*SP*, *weeks 44–52*), and *competition period* (*CP*, *weeks 1–13*). *The 4-week transition period* (*weeks 14–17*) *between CP and GP1 was not included in the analyses of the different phases.* Shooting training was systematized by calculating the total number of shots distributed into the categories shots fired during rest, shots fired during LIT sessions, shots fired during MIT/HIT sessions and competitions, and time spent on dry fire training.

Physiological testing

Starting at the age of 22 years, the participant underwent regular physiological testing at three different test centers using similar equipment. The same standardized test protocol with measurements of the oxygen uptake (VO_2) and HR was used throughout her career with a minor modification from the age of 24. The protocol consisted of 3 × 5 min stages with 1 min breaks at fixed speeds (2.5 m/s from the age of 22 to 24 and 2.75 m/s from the age of 25 to 32) and increasing inclination (5°, 6°, and 7°, respectively) during treadmill roller ski skating. From 22 to 24 years of age, the protocol was performed one time in the G2 sub-technique (21), while from the age of 25, the protocol was performed two times (3 × 5 min in the G2 sub-technique followed by 3 × 5 min in the G3 sub-technique). In some cases, the protocol was followed by an incremental test to exhaustion to determine the maximal VO_2 (VO_{2max}), using a protocol with fixed speed (2.5 or 2.75 m/s) and increasing inclination by 0.5°–1° every minute until exhaustion. HR and respiratory recordings using open-circuit indirect calorimetry with a mixing chamber (Oxycon Pro, Jaeger GmbH, Hoechberg, Germany) were collected over the final minute of each stage.

Interviews

To gather additional information, complete missing data, ensure compliance with the training diary commentaries, and verify the training intensity of different training sessions, three semi-structured interviews with the participant were conducted during the data analysis phase of the study. The interviews were conducted face-to-face and tape-recorded.

Statistical analyses

All data from the investigated seasons (junior vs. senior athlete) and annual phases (GP1, GP2, SP, and CP) are presented as mean ± standard deviation. The assumption of normality was tested by using a Shapiro–Wilk test in addition to visual inspection of *Q*–*Q* plots and histograms. Variables with normal distribution were analyzed using a paired-sample *t*-test for junior vs. senior seasons. Otherwise, the Wilcoxon signed-rank test was used. All statistical tests were processed using IBM SPSS statistics version 24 software for Windows (SPSS Inc., Chicago, IL, USA) and Office Excel 2016 (Microsoft Corporation, Redmond, WA, USA).

Results

Long-term performance characteristics

Childhood and youth

During the interviews, the participant described an active childhood with long walks or ski touring, fishing, and hunting in the mountains. The participant started training for XC skiing at the age of 8 and biathlon at the age of 10. At the age of 12–14 years, a typical week in the winter for her and her brother (who also became a world-class biathlete) included XC skiing training on Tuesdays and Thursdays, biathlon training on Wednesdays, and traveling around for competitions on weekends. In the summer, the participant also engaged in athletics (800 m as her favorite discipline) and long sessions of road cycling or running in the mountains.

Junior athlete

The participant started at a top sports high school at the age of 17, where she specialized even more in XC skiing and biathlon. During her first junior years (age of 16–18), she competed in both biathlon and XC skiing but identified herself most as a XC skier. The participant performed at a high national level in both sports before she started to prioritize biathlon only at the age of 18. She participated in the biathlon Junior World Championship at both the ages of 19 (bronze in the sprint, 9th in the pursuit, and 13th in the individual event) and 20 (24th in the sprint, 38th in the pursuit, and 21st in the individual event).

Senior athlete

At age 21, the participant was selected for the Norwegian senior biathlon national team and participated in her first World Cup competition. However, this was the start of a 2-year period characterized by stagnation and a lack of performance development. The participant confirmed this during interviews, suggesting it was caused by frequent changes of coaches and corresponding training philosophies, which she uncritically performed without paying attention to her body's signals. This probably led to a state of underperformance (non-functional overreaching or overtraining syndrome), and she had to take a break from training and competitions to regain her balance. The participant decided to transfer to the national development team, where she was encouraged by her coach to take greater ownership of her training process. From this point, she took more responsibility for both the planning and adjustment of her training. During this time, the participant also discovered that she had to increase her prioritization of shooting-specific training.

In the subsequent season (age 24), the participant had her international breakthrough with two podiums in the World Cup. She achieved her first World Cup win at age 27 and her first Olympic gold medal at age 29. During the 3 years from 31 to 33 (defined as the seasons of peak performances), she was at the podium in the overall IBU World Cup (3rd, 1st, and 1st in 2011, 2012, and 2013, respectively) and won a total of 16 World Cup victories and 12 medals (eight golds) from international championships (World Championships and Olympic Games). The participant retired after winning three Olympic medals during her final season (age 33). The participant's performance development and associated training characteristics (physical and shooting) across the 17 seasons analyzed are presented in **Figures 1A–C**.

Long-term physiological characteristics

The participant's body mass remained stable across the seasons including physiological testing (58.9 ± 0.5 kg). VO_{2max} in the G2 sub-technique was measured during GP2 in three of the seasons and increased from 62.9 ml·kg^{-1}·min^{-1} at the age of 22 to 64.6 ml·kg^{-1}·min^{-1} at the age of 23, and further to 69.2 ml·kg^{-1}·min^{-1} at the age of 27. Moreover, there was an increase in VO_2 and a corresponding decrease in HR in both the G2 and G3 sub-techniques over the 3 × 5 min stages performed from ages 25 to 32 (**Figure 2**).

Long-term training characteristics

The annual volume of physical training increased by 95% (409–798 h·season^{-1}) from the age of 17 to 27. This is a progression of 40 ± 50 h·season^{-1} and an increase from ∼8 to 15 h week^{-1}. The training volume remained stable at the age of 28 before decreasing by 15% at the age of 29 and thereafter remained relatively stable (range of 657–763 h·season^{-1}) from the age of 29 to 33 (**Figure 1B**). The annual number of shots fired increased substantially from the age of 17 to 28 (1,163–17,328 shots·season^{-1}). On average, this constituted a progression of 1,473 ± 1,508 shots·season^{-1} and an increase from ∼22 to 333 shots week^{-1}. From the age of 28 to 29, the annual number of shots fired decreased by 2,272 shots·season^{-1} before remaining relatively stable (range of 13,275–15,355 shots·season^{-1}) from the age of 29 to 33 (**Figure 1C**).

Comparison between junior and senior athlete seasons

Total training

Comparisons between three annual cycles as a junior athlete (18–20 years) and the three years of peak performances as a senior athlete (31–33 years) showed that the annual training volume was 48% higher as a senior than a junior (694 ± 60 vs. 468 ± 23 h·season^{-1}, $P = .030$), while the number of sessions was 51% higher (464 ± 35 vs. 307 ± 28 sessions·season^{-1}, $P = .004$). This included 50% more endurance training (662 ± 57 vs. 441 ± 19 h·season^{-1}, $P = .028$) and 26% more strength training (31 ± 3 vs. 25 ± 3 h·season^{-1}, $P = .046$). The volume of endurance training was 110% higher as a senior than a junior athlete in specific exercise modes (328 ± 30 vs. 155 ± 20 h·season^{-1}, $P = .026$) and 71% higher in semi-specific exercise modes, although not significant (118 ± 42 vs. 69 ± 10 h·season^{-1},

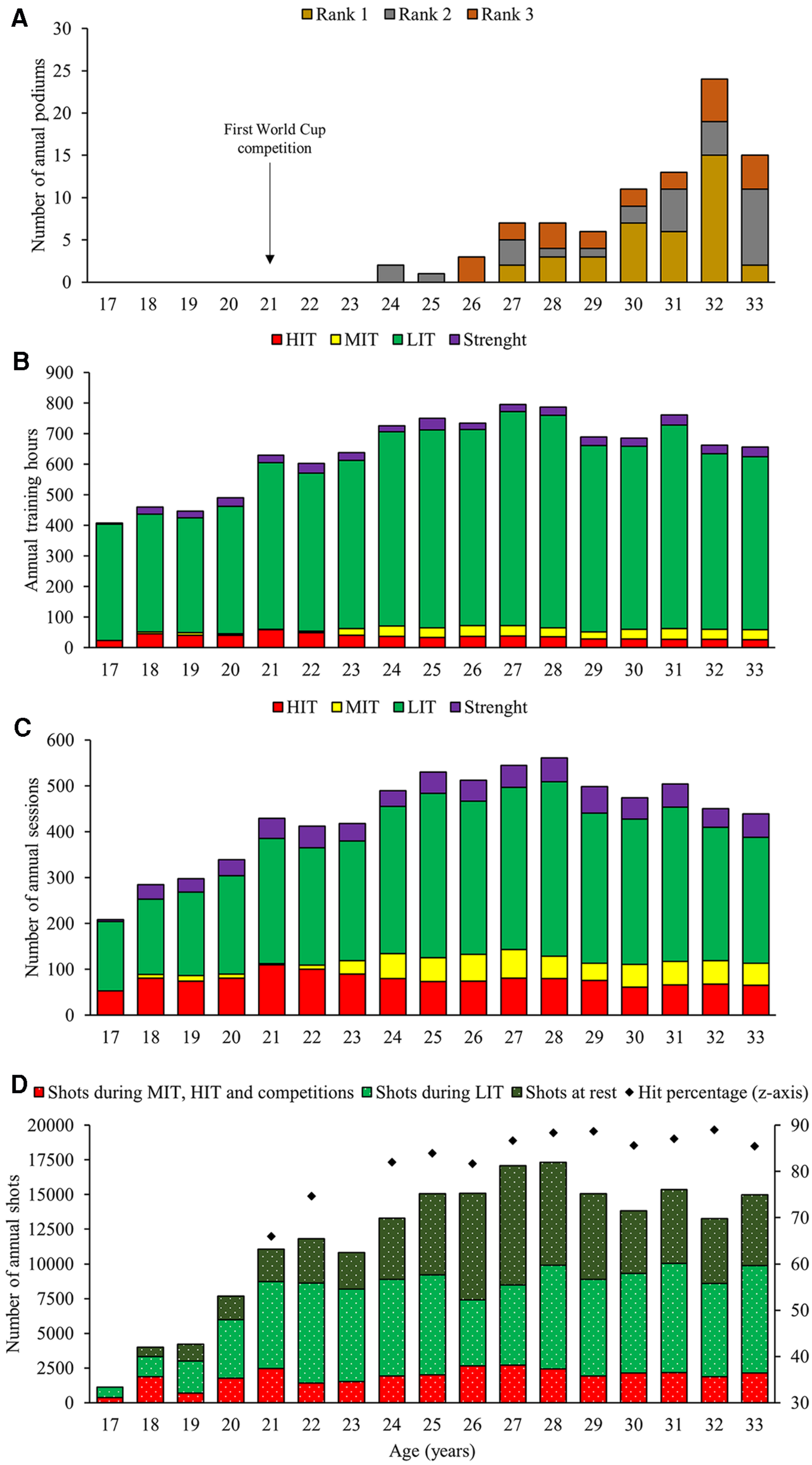

FIGURE 1
Annual top 3 performances in international competitions (A), the annual distribution of physical training distributed as endurance [low-intensity training (LIT), moderate-intensity training (MIT), and high-intensity training (HIT)] and strength training presented as training volumes (B), training sessions (C), and shooting training (shots at rest, shots during LIT, and shots during MIT, HIT, or competitions) and the total hit percentage of all international competitions (D) across 17 seasons in a world-class female biathlete.

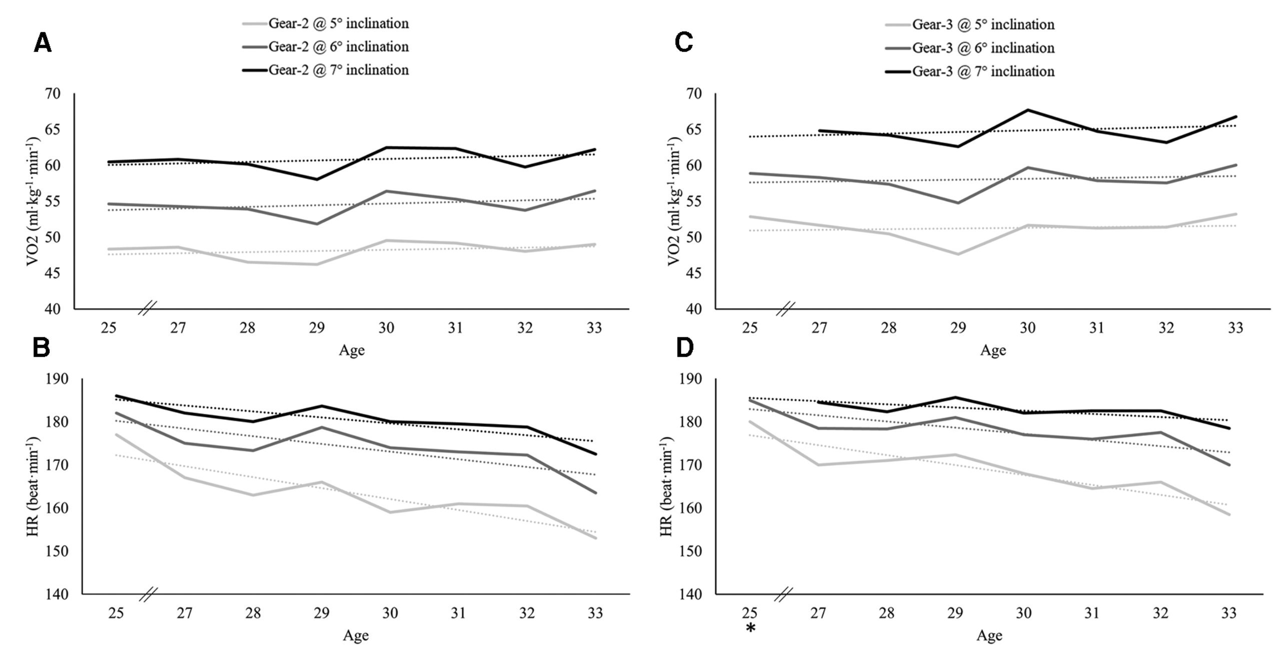

FIGURE 2
(A–D) Changes in oxygen uptake (VO_2) and heart rate (HR) during roller ski skating from age 25 to age 33 in a world-class female biathlete. *The participant did not perform the G3 stage at 7° inclination at the age of 25.

P = .245). In contrast, non-specific exercise modes remained relatively stable (216 ± 16 vs. 218 ± 24 h·season^{-1}, P = .924). The proportion of specific/semi-specific/non-specific exercise modes was 50/18/33% as a senior athlete and 35/16/49% as a junior athlete. The weekly training patterns across the annual cycle are presented in **Table 1**.

The annual volume of dry fire training (28.7 ± 9.4 vs. 12.2 ± 9.8 h·season^{-1}, P = .043) and the number of sessions including shooting (216 ± 9 vs. 75 ± 14 sessions·season^{-1}, P = .043) were higher as a senior than a junior. This difference in shooting-specific training included 175% more shots fired (14,537 ± 1,109 vs. 5,295 ± 3,425 shots·season^{-1}, P = .016) in total and 321% more shots fired at rest (5,035 ± 321 vs. 1,197 ± 518 shots·season^{-1}, P = .011) as a senior compared with a junior athlete. Further, the number of shots fired during LIT was 179% higher (7,440 ± 619 vs. 2,663 ± 1,975 shots·season^{-1}, P = .031) and during MIT, HIT, and competitions was 44% higher (2,061 ± 174 vs. 1,435 ± 893 shots·season^{-1}, P = .149) as a senior athlete.

Intensity distribution

The annual distribution of endurance training intensity is presented in **Figure 3**. As a senior athlete, 91/5/4% of the annual endurance training time was distributed as LIT/MIT/HIT, respectively (73/12/15% in number of sessions). The corresponding distribution for junior athlete seasons was 89/1/10% for LIT/MIT/HIT, respectively (69/3/28% in number of sessions).

Annual periodization

The physical training volume was 124% higher in GP1, 97% higher in GP2, 35% higher in SP, and 4% higher in CP during the senior vs. junior seasons. Accordingly, the participant's periodization of physical training differed between the junior and senior seasons. While a traditional pattern with high training volumes in GP1 and GP2 followed by a clear reduction in training volume toward CP was observed in the senior seasons, an increase in training volume from GP1 to SP was observed as a junior. The intensity distribution also developed differently from GP1 to CP between the senior and junior seasons. In the senior seasons, a progressive reduction in both the volume and proportion of LIT and MIT with corresponding increased HIT was observed toward the CP. In contrast, the junior seasons included an increased volume of LIT from GP1 to SP before a reduction to CP, while HIT volumes remained relatively stable from GP1 to SP before increasing to CP (**Table 1** and **Figure 3**).

The training volume in specific exercise modes was higher across all the annual phases in the senior vs. junior seasons. As a senior athlete, the proportion of specific exercise modes was relatively stable during GP1 and GP2 (42% and 44%, respectively) before increasing progressively to 70% in CP. As a junior athlete, the proportion of specific exercise modes was 18% during GP1 but increased progressively to 65% in CP. The proportion of semi-specific modes was relatively similar across all phases as senior vs. junior athlete, except for a higher proportion of semi-specific modes performed in GP1 as senior (20% vs. 6%).

The number of shots fired as senior was substantially higher in the GP1 (564%) with somewhat smaller differences in the GP2 (147%), SP (100%), and CP (81%) compared with junior. As a senior, the overall number of shots fired followed the same pattern across the annual phases as the volume of physical training. As a junior, an increase in the number of shots fired

TABLE 1 Weekly training distribution (mean ± SD) across the different periodization phases.

	Junior					Senior				
	Total	GP1	GP2	SP	CP	Total	GP1	GP2	SP	CP
Physical training										
Hours	8.9 ± 4.0	8.6 ± 4.1	10.2 ± 5.1	10.6 ± 3.6	8.4 ± 2.2	13.3 ± 6.2*	17.8 ± 3.9*	17.4 ± 3.6*	12.5 ± 4.5*	8.7 ± 4.0
Session	5.9 ± 2.5	5.5 ± 1.7	6.4 ± 2.8	6.8 ± 2.6	5.2 ± 2.2	8.9 ± 3.3*	9.5 ± 3.2*	8.7 ± 3.7*	9.4 ± 2.6*	9.4 ± 1.9*
Training forms										
Endurance (h·week^{-1})	8.4 ± 3.9	8.4 ± 3.9	9.6 ± 5.0	10.0 ± 3.7	8.0 ± 2.1	12.7 ± 5.9*	16.8 ± 3.7*	16.5 ± 3.3*	12.1 ± 4.3	8.5 ± 3.8
Strength (h·week^{-1})	0.5 ± 0.6	0.4 ± 0.5	0.6 ± 0.6	0.7 ± 0.5	0.4 ± 0.6	0.6 ± 0.6*	1.0 ± 0.5*	0.8 ± 0.5*	0.5 ± 0.3*	0.1 ± 0.3*
Exercise mode										
Specific (h·week^{-1})	3.0 ± 2.8	1.7 ± 1.5	2.0 ± 1.7	4.1 ± 3.4	5.4 ± 2.5	6.3 ± 2.9*	7.0 ± 2.3*	7.1 ± 1.9*	7.2 ± 2.6*	5.8 ± 2.7
Semi-specific (h·week^{-1})	1.3 ± 1.6	0.6 ± 1.0	1.1 ± 1.5	2.3 ± 1.8	1.7 ± 1.6	2.3 ± 2.0*	3.3 ± 1.9*	2.2 ± 1.6	2.4 ± 1.8	1.6 ± 2.0
Non-specific (h·week^{-1})	4.2 ± 3.5	6.1 ± 2.6	6.4 ± 3.6	3.6 ± 3.4	1.0 ± 0.9	4.2 ± 3.6	6.6 ± 2.7*	7.2 ± 3.2	2.5 ± 1.8*	1.1 ± 1.0
Specific/Semi-specific/Non-specific (%)	35/15/50	18/6/76	24/10/66	40/24/36	65/22/13	53/18/29	42/20/39	44/13/43	61/20/19	70/18/12
Intensity distribution										
LIT (h·week^{-1})	7.5 ± 3.6	7.3 ± 3.9	8.8 ± 4.6	8.9 ± 3.5	6.8 ± 1.7	11.6 ± 5.5*	15.5 ± 3.4*	15.1 ± 3.2*	10.8 ± 3.9	7.4 ± 3.4
MIT (h·week^{-1})	0.1 ± 0.3	0.3 ± 0.5	0.1 ± 0.3	0.2 ± 0.4	0.1 ± 0.2	0.6 ± 0.6*	0.9 ± 0.5*	1.0 ± 0.5*	0.6 ± 0.5*	0.3 ± 0.3*
HIT (h·week^{-1})	0.8 ± 0.8	0.8 ± 0.9	0.7 ± 0.5	0.9 ± 0.8	1.2 ± 0.8	0.5 ± 0.5*	0.4 ± 0.3*	0.4 ± 0.4	0.7 ± 0.5	0.8 ± 0.6
LIT/MIT/HIT (%)	89/1/10	85/3/12	91/1/8	89/2/9	85/1/14	91/5/4	92/6/2	91/6/3	89/5/6	87/3/10
Intensity distribution										
LIT (sessions·week^{-1})	3.6 ± 1.9	3.0 ± 1.1	4.1 ± 2.3	4.1 ± 2.0	3.1 ± 1.6	5.8 ± 2.5*	5.7 ± 2.2*	5.2 ± 2.8	6.4 ± 2.0*	6.7 ± 1.8*
MIT (sessions·week^{-1})	0.2 ± 0.4	0.2 ± 0.5	0.2 ± 0.4	0.3 ± 0.5	0.1 ± 0.3	1.0 ± 0.8*	0.8 ± 0.7*	1.0 ± 0.8*	1.2 ± 0.9*	1.0 ± 0.7*
HIT (sessions·week^{-1})	1.5 ± 1.2	1.8 ± 1.3	1.4 ± 1.2	1.4 ± 0.9	1.5 ± 1.4	1.3 ± 1.2	1.4 ± 1.3	1.2 ± 1.3	1.0 ± 1.1	1.5 ± 1.2
LIT/MIT/HIT (%)	69/3/28	62/4/35	72/2/26	70/5/25	70/2/28	73/12/16	73/10/17	71/13/15	74/15/12	72/11/16
Shooting training										
Total (shots·week^{-1})	101 ± 98	90 ± 114	153 ± 117	117 ± 79	102 ± 64	280 ± 177*	395 ± 182*	366 ± 144*	249 ± 108*	185 ± 101*
At rest (shots·week^{-1})	23 ± 49	39 ± 50	51 ± 73	11 ± 31	4 ± 15	97 ± 134*	223 ± 152*	138 ± 120*	25 ± 51	9 ± 34
During LIT (shots·week^{-1})	51 ± 62	39 ± 77	64 ± 61	73 ± 72	61 ± 55	143 ± 91*	135 ± 94*	183 ± 78*	178 ± 69*	131 ± 82*
During MIT, HIT, and competitions (shots·week^{-1})	27 ± 45	13 ± 26	38 ± 63	33 ± 51	38 ± 37	40 ± 28*	38 ± 28*	45 ± 25	46 ± 23	44 ± 29
Sessions with shooting										
Total (sessions·week^{-1})	1.7 ± 1.7	0.8 ± 1.1	1.9 ± 1.7	1.9 ± 1.5	2.6 ± 1.8	4.2 ± 2.2*	4.7 ± 2.0*	4.8 ± 1.7*	4.4 ± 1.6*	4.1 ± 2.2*
LIT shooting (sessions·week^{-1})	1.0 ± 1.1	0.6 ± 0.9	1.4 ± 1.2	1.2 ± 0.9	0.9 ± 0.9	2.1 ± 1.4*	2.7 ± 1.5*	2.7 ± 1.3*	2.0 ± 1.1*	1.5 ± 1.2*
MIT, HIT, and competitions (sessions·week^{-1})	0.8 ± 1.2	0.2 ± 0.5	0.5 ± 0.7	0.6 ± 0.9	1.7 ± 1.7	1.8 ± 1.3*	1.4 ± 0.9*	1.7 ± 1.0*	2.3 ± 1.1*	2.5 ± 1.6*
Dry fire training (sessions·week^{-1})	0.4 ± 0.8	0.4 ± 0.8	0.1 ± 0.2	1.0 ± 1.2	0.3 ± 0.7	3.2 ± 148*	3.3 ± 2.8*	4.4 ± 1.8*	3.1 ± 2.0*	2.9 ± 2.2*

GP1, general preparation period 1; GP2, general preparation period 2; SP, specific preparation period; CP, competition period; LIT, low-intensity training; MIT, moderate-intensity training; HIT, high-intensity training. *Significantly different from junior seasons ($P < .05$).

was observed from GP1 to GP2, with a subsequent reduction toward the CP. The proportion of shots fired at different intensities across the annual phases was relatively similar between the junior and senior seasons. Therefore, large differences in the annual periodization of shooting training were observed in GP1, particularly caused by more shots fired at rest and LIT in the senior compared with junior athlete seasons (Figures 3, 4).

Low-intensity training

The annual LIT volume was 53% higher as a senior athlete compared with a junior athlete (602 ± 56 vs. 392 ± 22 23 h·season^{-1}, $P = .032$), with a correspondingly higher number of sessions (301 ± 32 vs. 187 ± 25, sessions·season^{-1}, $P < .010$). Furthermore, a higher number of annual sessions including shooting both in total (301 ± 32 vs. 187 ± 25, sessions·season^{-1}, $P < .010$) and during LIT (86 ± 9 vs. 38 ± 20, sessions·season^{-1}, $P < .001$) were performed as a senior compared with a junior. A typical LIT session as a senior vs. a junior included 8–16 vs. 8–12 series of 40–80 vs. 30–60 shots with a total duration of 1.5–2.5 vs. 1.0–1.75 h. Sessions without shooting were typically 1.5–3 vs. 1–2 h as a senior vs. a junior, respectively, using different exercise modes (running, roller skiing, or skiing) but could also be longer sessions of cycling (2–3 h) or hunting days (6–8 h walking in the mountains, typically registered as half of the time in the training diary). The distribution and duration of endurance training sessions at different intensities, as well as the number of shots fired in each session across one season and representative training weeks as a junior and a senior, are presented in Table 2 and Figure 4.

Moderate- and high-intensity training

The annual MIT volume was almost four times higher (34 ± 1 vs. 7 ± 2 h·season^{-1}, $P = .001$), with a correspondingly higher number of sessions (50 ± 2 vs. 10 ± 2 sessions·season^{-1}, $P < .001$) as a senior compared with a junior athlete. As a senior, a typical MIT session included a 0.5–1 h warm-up followed by 6–7 × 6–8 min intervals in a roller ski course (the course length decided the duration of each interval). The shooting was most often

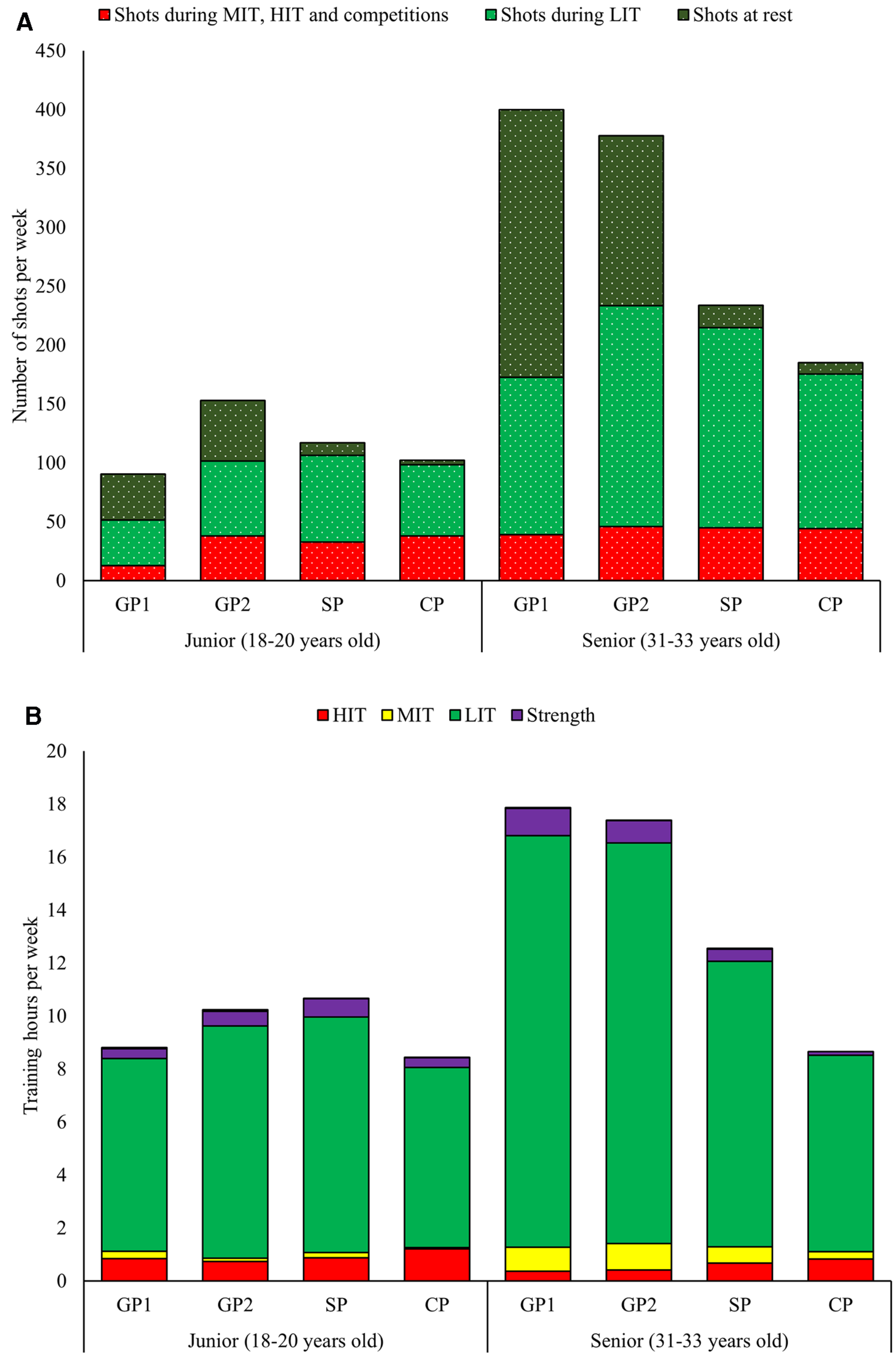

FIGURE 3
(A,B) Weekly distribution of shooting training (A) (shots at rest, shots during LIT, and shots during MIT, HIT, or competitions) and physical training (B) distributed as endurance [low-intensity training (LIT), moderate-intensity training (MIT), and high-intensity training (HIT)] and strength training across the annual phases in junior and senior seasons.

performed at the end of, although sometimes in the middle of, each interval. The proportion of MIT sessions including shooting increased toward the competition phase (GP1: 70%, GP2: 88%, SP: 89%, and CP: 94%). In the CP, MIT sessions had shorter duration and were typically performed as 3 × 5 min intervals (controlled intensity including 20 shots), for example, on Tuesdays when World Cup competitions were held on Thursday, Saturday, and Sunday. The participant mentioned during the interviews that she was very accurate with the intensity control during MIT sessions by consistently performing the first two

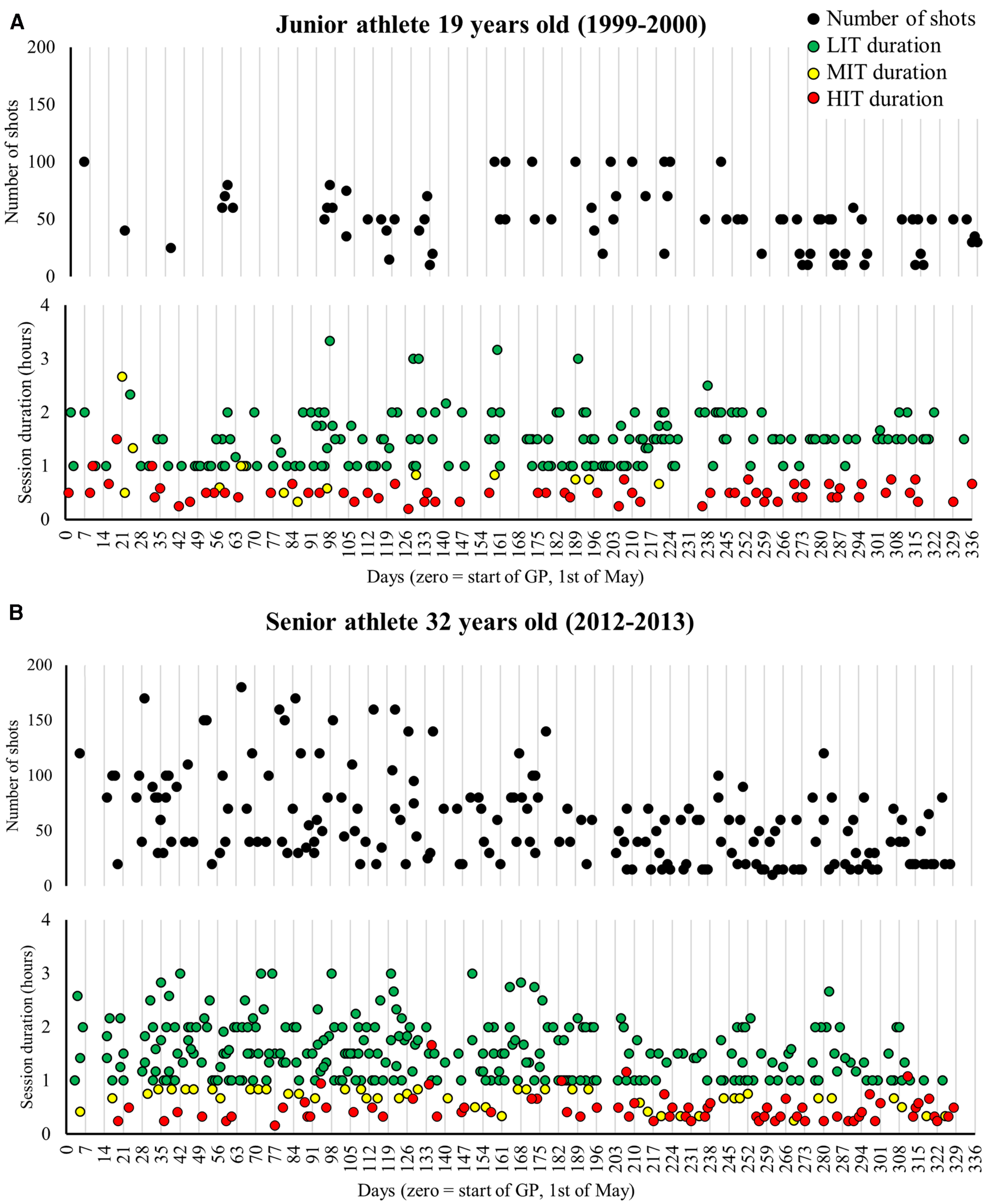

FIGURE 4
(A,B) The duration of low-intensity training (LIT), moderate-intensity training (MIT), and high-intensity training (HIT) sessions and number of shots fired across the annual cycle in one representative season as a junior (A) and a senior (B) athlete. *LIT sessions with a duration < 1 h are not included in the figures.

intervals just below her target HR (85%–90% of HR_{max}) and blood lactate (2.0–3.2 $mmol \cdot L^{-1}$) zones.

The annual HIT volume (including competitions) was 36% lower as a senior compared with a junior (27 ± 1 vs. 42 ± 3 $h \cdot season^{-1}$, $P = .006$), with a correspondingly lower number of sessions performed (66 ± 2 vs. 79 ± 4 $sessions \cdot season^{-1}$, $P = .023$). As a senior athlete, most HIT sessions were performed as 6 × 4 min, 8 × 3 min, or 5 × 5 min uphill running with poles. To ensure that she

TABLE 2 Representative training weeks for the general preparation period 2 and the competition phase during junior and senior seasons.

Day/ session		General preparation period 2		Competition period	
		Junior	Senior	Junior	Senior
Mon	1.	1 h roller ski bandy + 0.6 h strength training	0.5 h shooting at rest (90 shots) 2 h LIT roller ski skating w/shooting (70 shots)	1 h LIT running + 0.6 h general strength	1.25 h LIT ski classic
	2.		1 h roller skiing classic + 0.6 h strength training 10 min dry fire training		*Travel to World Cup destination*
Tue	1.	HIT session running with poles 0.5 h LIT warm-up 0.5 h HIT (2–3–4–4–3–2–1–2–1 min) 0.5 h LIT cool-down	2 h LIT roller ski skating	1 h LIT ski skating with/shooting (50 shots)	MIT session ski skating 0.5 h LIT warm-up (20 shots) 0.8 h MIT (3 × 6 min, 20 shots) 0.5 h LIT cool-down
	2.		0.5 h shooting at rest (100 shots) 1.5 h LIT cycling 10 min dry fire training		0.5 h LIT cycling + 0.25 h strength training 10 min dry fire training
Wed	1.	1.5 h LIT roller ski classic with/ shooting (60 shots)	MIT session roller ski skating 0.5 h LIT warm-up (20 shots) 0.75 h MIT (5 × 8 min, 25 shots) 0.5 h LIT cool-down	1.25 h LIT ski classic	1.25 h LIT ski skating w/ shooting (50 shots)
	2.	1.5 h LIT running (undulating terrain)	1 h LIT roller ski classic 10 min dry fire training		10 min dry fire training
Thu	1.	0.5 h soccer + 0.6 h strength training	2 h LIT roller ski classic with shooting (70 shots)	*Travel to competition destination*	Competition 1 h LIT warm-up (20 shots) Competition (sprint) (20 min,10 shots)
	2.		0.5 h shooting at rest (100 shots) 1 h LIT cycling 10 min dry fire training		0.5 h LIT cycling 10 min dry fire training
Fri	1.	1.5 h LIT roller ski classic with shooting (80 shots)	2 h LIT roller ski skating with shooting and 8 × 15–20 s sprints (80 shots)	1.5 h LIT ski skating with 8 × 10–15 s sprints and shooting (50 shots)	1.25 h LIT ski classic with shooting (50 shots)
	2.		10 min dry fire training		10 min dry fire training
Sat	1.	HIT session roller ski skating 0.5 h LIT warm-up (30 shots) 0.4 h HIT test-race 6 km (20 shots) 0.5 h LIT cool-down	3 h LIT running/walking in the mountains	Competition 0.5 h LIT warm-up (15 shots) Competition (sprint) (20 min, 10 shots) 0.5 h LIT cool-down	Competition 1.1 h LIT warm-up (15 shots) Competition (relay) (20 min, 10 shots)
	2.				0.5 h LIT cycling 10 min dry fire training
Sun	1.	2 h LIT running/walking in the mountains	MIT session roller ski skating 0.5 h LIT warm-up (15 shots) 0.75 h MIT intervals (6 × 7 min, 35 shots) 0.5 h LIT cool-down	Competition 0.5 h LIT warm-up (15 shots) Competition (pursuit) (30 min, 20 shots) 0.5 h LIT cool-down	Competition 1.1 h LIT warm-up (15 shots) Competition (mass start) (30 min, 20 shots)
	2.		1.5 h LIT cycling 10 min dry fire training		*Travel to next World Cup destination* 10 min dry fire training
Total		Total volume (physical + shooting): 13.2 h Total volume (physical): 12.1 h LIT (hours/shots): 10 h/170 shots MIT (hours/shots): 0 h/0 shots HIT (hours/shots): 0.9 h/20 shots Strength and speed: 1.2 h Shooting at rest: 0 shots Dry fire training: 0 h	Total volume (physical + shooting): 24.5 h Total volume (physical): 21.1 h LIT (hours/shots): 18.6 h/255 shots MIT (hours/shots): 1.5 h/60 shots HIT (hours/shots): 0 h/0 shots Strength and speed: 1 h Shooting at rest: 290 shots Dry fire training: 1 h	Total volume (physical + shooting): 9.3 h Total volume (physical): 8.2 h LIT (hours/shots): 6.4 h/130 shots MIT (hours/shots): 0 h/0 shots HIT (hours/shots): 0.8 h (30 shots) Strength and speed: 1 h Shooting at rest: 0 shots Dry fire training: 0 h	Total volume (physical + shooting): 13.1 h Total volume (physical): 12.1 h LIT (hours/shots): 9.3 h/170 shots MIT (hours/shots): 0.3 h/20 shots HIT (hours/shots): 1.2 h/40 shots Strength and speed: 0.25 h Shooting at rest: 0 shots Dry fire training: 1 h

LIT, low-intensity training; MIT, moderate-intensity training; HIT, high-intensity training.

met the goal of the session, the participant rated her perceived training quality during all MIT and HIT sessions using a scale from 1 to 5. Furthermore, a simple rule of thumb was to have at least 1 day of LIT between MIT sessions and 2 days between HIT sessions. As a junior athlete, typical HIT sessions included test competitions in the skating style with shooting, or 4–8 × 2–4 min intervals, often performed as running or uphill running with poles. The participant also mentioned that she was not afraid of competing and took part in competitions both in running and orienteering in addition to XC skiing and biathlon during these years.

Strength and speed training

The annual strength training volume was 26% higher as a senior athlete compared with a junior athlete (31 ± 3 vs. 25 ± 3 h·season^{-1}, $P = .046$), with a correspondingly lower number of sessions performed (47 ± 6 vs. 32 ± 3 sessions·season^{-1}, $P = .038$). As a senior athlete, a typical strength session included 15–30 min of core/stabilization exercises followed by upper-body heavy strength training (3–4 series of 6–8 repetitions of maximal strength including 4–6 different exercises). As a junior athlete, a typical strength session included 30–45 min of various core/stabilization exercises targeting muscles involved in the force transfer during XC skiing. Speed training was unfortunately not included in the analyses, but the participant mentioned during interviews that she typically performed 6–8 × 15–20 s sprints across different terrains one to two times per week integrated as a part of LIT sessions. The content and frequency of these sessions were similar across the senior and junior athlete seasons.

Discussion

This study investigated the long-term development of performance, physiological, and training characteristics in a world-class female biathlete, with emphasis on differences between junior and senior athlete seasons. The main findings were as follows: (1) There was a long-term progression in the annual physical training volume (∼409–792 h·season^{-1}) and shots fired (∼1,163–17,328 shots·season^{-1}) from the age of 17 to 28, with a subsequent reduction in both the physical training volume (694 ± 60 h·season^{-1}) and shots fired (14,537 ± 1,109 shots·season^{-1}) during the seasons of peak performance at ages 30–33; (2) VO_{2max} in roller ski skating increased by 10% (62.9–69.2 ml·kg^{-1}·min^{-1}) from age 22 to 32; (3) comparisons of junior vs. senior seasons demonstrated 48% higher physical training volumes and 175% more shots fired as senior, mainly due to more LIT and MIT with shooting and less HIT performed in the general preparation period.

Long-term training characteristics

The participant followed a long-term progression in the annual volume of physical training (average increase of 40 h·season^{-1}) before achieving her highest training volumes at the age of 27–28. These patterns are similar to those previously described in various world-class endurance athletes (4–6, 22–24), further supporting the importance of long-term progression in training volume to reach world-class endurance performances. Interestingly, novel data from this study showed that the progression of physical training coincided with an average increase of 1,200 shots per year, reaching a peak at the age of 27–28 (17,328 shots·season^{-1}). However, a subsequent reduction to ∼13,275–15,355 annual shots during her seasons of peak performance (age of 31–33) was observed. While no previous data on the progression of shooting training is reported in the literature, the number of shots fired during the participant's seasons of peak performance is in line with the ∼12,000–15,000 annual shots previously reported in a world-class male biathlete (4). However, a substantially higher number of shots (∼22,000 shots·season^{-1}) is reported in Swedish national team biathletes (1). Possible explanations for the observed differences in the number of annual shots fired might be individual variations in the requirement for shooting-specific training, differences in the quantification of shooting training (e.g., daily registration in a training diary vs. estimation by coaches), or further developments of the sport with increased demands for shooting-specific training after the participant retired from biathlon in 2014.

An interesting aspect of the participant's long-term development process was two seasons characterized by a lack of performance development from ages 21 to 23. Although at a later stage in their career, similar periods of stagnation have previously been described in two world-class XC skiers (25, 26). Some similarities in these athletes' return from underperformance include taking a break from systematic training and competitions, changing the training stimulus, and increasing their autonomy in the planning and adjustments of training. The participant also emphasized increased shooting-specific training as necessary to achieve her international breakthrough at the age of 24. However, contrary to the world's most successful female XC skier, whose most successful seasons coincided with the highest annual training volumes (5), the participant in this study reduced her volumes of both physical and shooting training during her seasons of peak performance. In line with this, the annual volumes of ∼650–750 h·season^{-1} physical training during these seasons are within the lower range of the training volumes (∼700–900 h·season^{-1}) previously reported in world- or national-class biathletes (1, 4, 27, 28). The participant mentioned during interviews that the reductions in training volume were due to increased emphasis on improving the quality of each single training session and that this was particularly important to further develop her shooting skills. Consistent with these findings, a recent commentary highlighted the importance of training quality in endurance sports, and the quality of both the training process and the execution of each training session likely are important factors separating the highest-performing athletes from the rest (29). Although the participant's reduction in both the volume of physical and shooting training during her most successful seasons might seem contra intuitive, it likely contributed to increased load-recovery balance, training quality, and thereby better adaptations and performance development.

Furthermore, the participant's physical training volumes were ∼30% lower than the ∼900 annual training volumes reported for female world-class XC skiers (5, 14). Similar differences have previously been observed between national team XC skiers and biathletes (27, 28) and are likely explained by the additional demands for shooting-specific training in biathlon (1). Furthermore, the participant performed ∼20% higher annual training volumes in the skating style but less strength training (31 vs. ∼50–90 h·season^{-1}) than previously reported in world-class XC skiers (2, 5). These findings indicate that biathletes likely compensate for lower physical training volumes than XC skiers by performing more specific training in the skating style. The reason for the lower strength training volume compared with XC skiing can only be speculated, and most likely, it reflects the participant's own prioritizations rather than differences in sport-specific demands between biathlon and XC skiing. Taken together, the observed differences in training characteristics between biathlon and XC skiing underpin the complex and demanding nature of biathlon, which requires an adequate load-recovery balance and training quality in both physical and shooting training.

Long-term physiological characteristics

While several studies have reported VO_{2max} values in world-class endurance athletes (30), including biathletes and XC skiers during their most successful seasons (31), data on the long-term development of physiological capacities are relatively sparse. The participant increased her VO_{2max} in roller ski skating (G2 sub-technique) by 10% (~63–69 ml·kg^{-1}·min^{-1}) from the age of 22 to 27. In comparison, increases of 4%–13% in VO_{2max} over a 7-year period have been reported in elite male rowers (23, 32). However, other studies including elite to world-class athletes have reported no long-term changes in VO_{2max} (22, 33, 34). Tønnessen et al. (31) reported average VO_{2max} values in running of 66 ml·kg^{-1}·min^{-1} in female world-class biathletes, which were 10% lower than the corresponding values reported among distance XC skiers at the same performance level. Although the participant did not measure VO_{2max} during her most successful seasons, the highest VO_2 values obtained during the incremental intervals in the G3 sub-technique were ~67 ml·kg^{-1}·min^{-1}. Therefore, her VO_{2max} was likely to be at comparable values (68–70 ml·kg^{-1}·min^{-1}) with those previously reported in female world-class endurance athletes (5, 14, 22, 30, 31). Further, there was a clear reduction in HR during the incremental intervals throughout her career. These physiological changes likely had significant implications for her training and associated intensity zones, by allowing higher speeds and potentially better technical quality during LIT and MIT sessions. However, the use of different test centers, time points for testing, and lack of any direct measurements of VO_{2max} during the last seasons of her career indicate that the physiological data in this study should be interpreted with caution. By much of the same reasons, in addition to the use of different exercise modes (e.g., running vs. roller ski skating), comparisons of VO_{2max} values reported in previous studies should also be done with caution. Therefore, longitudinal data on the development of VO_{2max} and other physiological capacities are needed, both in biathletes and endurance athletes in general.

Comparison between junior and senior athlete seasons

The annual physical training volume and number of sessions were ~50% higher as a senior than junior athlete, with 175 % more shots fired. These increases in the volume of physical and shooting training from junior to senior athlete coincided with an increase in both the volume (155 to 328 h) and proportion (35% to 50%) of sport-specific training (i.e., skiing/roller skiing in the skating style). The intensity distribution of endurance training showed a transition from higher proportions of HIT as a junior athlete to higher proportions of LIT and MIT as a senior athlete. This was further confirmed by the participant during interviews, stating that the change in intensity distribution in part was due to changes in training philosophy and also a consequence of increased physiological capacities, making it possible to perform MIT sessions at higher and more competition-relevant speeds. Comparable changes in intensity distribution have previously been observed in the world's most successful female XC skier, emphasizing more HIT during the first part but more LIT and MIT during the latter part of her senior career (5, 35). However, similar intensity distributions from the age of 21 to 31 are reported in a world-class male biathlete (4). The most effective intensity distribution for endurance performance is widely debated, and while longitudinal data of endurance athletes often are characterized by increased LIT volumes, the progression and distribution of MIT and HIT are less clear (3, 36, 37). However, differences in the logging and quantification of endurance training intensity (i.e., *time in zone* vs. *session goal approach*) should be acknowledged in such interpretations (19, 36). For example, while the biathlete in the current study excluded the breaks during interval sessions, the world-class female XC skier included breaks in her logging of MIT and HIT sessions (5). Therefore, more long-term training data, following an accepted framework for quantification, would raise the possibility of comparing training characteristics across endurance athletes and sports and thus allow more valid comparisons adding considerable scientific and practical value.

The abovementioned differences in physical training were accompanied by large increases in both the volume and content of shooting training. Here, large differences between junior and senior athlete seasons were observed in the amount of dry fire training (12 vs. 29 h) and the number of shots fired at rest (1,197 vs. 5,035 shots·season^{-1}) and during LIT sessions (2,663 vs. 7,440 h·season^{-1}). As a senior athlete, shooting at rest was often performed as a session including 80–100 shots in the morning before LIT sessions, while 10 min of dry fire training typically was performed either before physical training sessions or in the evening. The participant mentioned that this training was important to fine-tune technical details connected to her shooting performance (e.g., shooting posture, triggering behavior, and rifle stability) and all movements related to reducing both the shooting and range time. This is further supported by previous studies suggesting that dry fire training is important to improve triggering behavior, rifle stability, and mental aspects of shooting (38, 39). Although the distribution of shooting-specific features as a senior athlete (36% at rest, 51% during LIT, and 24% at higher intensities) is in line with previous data in biathlon (1, 4), no comparable data for the differences in shooting training between junior and senior athletes exists. In this context, the 20-year period since our participant was a junior athlete should be acknowledged, and there is likely a need for more updated data on shooting training in both junior- and senior-level biathletes.

Substantial differences were observed in the annual training periodization between junior and senior athlete seasons. Large differences in training characteristics were found in GP1 and GP2, with almost twice the volume of physical training and five times more shots performed as a senior athlete compared with that as a junior athlete, with smaller differences observed in SP and CP. In line with previous observations (27), the senior seasons included a reduction in both the volume of physical training and shots fired at LIT and MIT, but increased HIT in sport-specific modes from GP1 to CP. In contrary, the junior seasons were characterized by an increase in both physical training volumes and shots fired, mainly caused by increased LIT, but with relatively similar amounts of HIT performed from GP1 to SP. The reasons explaining these findings are likely increased specialization and professionalism in biathlon as a senior athlete, allowing higher training volumes and more shots fired during GP1 and GP2, while more frequent traveling and competitions make it less possible to perform high training volumes in the CP. Altogether, our findings indicate that the transition from a junior to the senior elite level included a tolerance for higher sport-specific volumes of LIT and MIT (including shooting), but less HIT. In addition, an increased volume of shooting training besides the physical training (dry fire training and shooting at rest) particularly during the GP was a clear progression from a junior to a senior athlete.

Practical applications

The participant had an active childhood with relatively late specialization to biathlon at the age of 18, followed by a long-term annual progression in both the volume of physical and shooting training. The participant emphasized increased shooting training as necessary to reach her international breakthrough at the age of 24, where she also had a progressive change from emphasizing HIT to more MIT. Furthermore, the participant reduced both her volume of physical and shooting training during her most successful seasons with the intention of increasing the quality of each single training session. In comparison to XC skiers, biathletes seem to compensate for lower overall physical training volumes with higher volumes of sport-specific endurance training in the skating style. Although this study provides data on the sophisticated training characteristics of a world-class female biathlete, the limitations of a single-case approach should be considered in the interpretation of the present findings.

Conclusions

This case study provides unique insights into the long-term development of physical and shooting training from the junior to the senior elite level in a world-class female biathlete. From the age of 17, the participant had a 10-year progression in both the annual volume of physical and shooting training accompanied by development of sport-specific physiological capacities. However, a reduction in both the volume of physical and shooting training was observed during the seasons of peak performance with the intention of increasing training quality. The major differences in training characteristics between junior and senior athlete seasons were higher sport-specific volumes of LIT and MIT and less HIT particularly during the general preparation period. These differences were accompanied by more shooting-specific training, particularly at rest, and in connection with LIT. More data on the training characteristics of larger samples of biathletes at different ages and performance levels are needed to further understand the complexity of long-term training and performance development of biathletes.

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 Norwegian Center for Research Data (498575). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

GS, AF, and RT designed the study; AF and GS performed data collection; GS performed data and statistical analyses; GS, AF, and RT contributed to interpretation of the results; GS and RT wrote the draft manuscript; GS, AF, and RT contributed to the final manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Dropout and compliance to physical exercise in menopausal osteopenic women: the European "happy bones" project

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Introduction: Decline in muscle mass and bone density seem to be two of the most disabling side effects of menopause that negatively affect women's quality of life. Promoting physical activity protocols in the workplace can represent a focal point in the prevention and management of several diseases. The study aims to evaluate the compliance and drop-out of menopausal osteopenic women engaged in combined training performed inside and outside the workplace. Strength and balance were analyzed to evaluate the effect of this protocol on osteoporosis prevention and the risk of falling.

Methods: 73 menopausal women were enrolled in 5 European countries. They performed 72 lessons of a combined training proposed in the working place (IW) or sport center (SC).

Results: Out of the total 39 women enrolled in the IW, 12.8% had to leave the program, while out of the 34 women enrolled in SC, 41.2% did not complete the training. According to the compliance results, 47% of women that completed the trained IW and 85% in the SC recorded high compliance (p = 0.019). Moreover, the strength of the lower limbs ($p < 0.001$) and static balance (p = 0.001) significantly improved in the whole group.

Discussion: In conclusion, proposing well-structured training in the workplace for menopausal women seems to reduce drop-out. Strength and balance results suggest its positive impact on bone health and risk of falls, despite where it is performed.

KEYWORDS

exercise, workplace, sport center, women, compliance, health, bone

1. Introduction

Menopause is a unique event in women's life that occurs around the age of 50 and it is a stage that all women would experience (1, 2). It is characterized by the progressive decline of female hormones, estrogen, and progesterone, culminating in a total shutdown of the ovaries (3). This condition can lead to several side effects, such as an increase in fatigue, headache, and mood swing, that can negatively affect the Quality of Life (QOL) (4). Moreover, physical performance declines with aging and studies reported that this decline differs by sex, with women showing a more rapid decline than men during middle age (5, 6). This sex difference may be due to the hormonal changes that occur during the menopausal years (7, 8). For these reasons, menopausal women are particularly exposed to the risk of osteopenia and osteoporosis, and fragility fracture (9). It is known that in 2025 there will be an increase in the risk of falls, which will most likely lead to fractures in the case of osteopenia/osteoporosis (10). According to the International Osteoporosis Foundation (IOF), to avoid premature bone loss, adults should implement a nutritious diet with adequate calcium intake, maintain healthy body weight and participate in regular weight-bearing activity (8). Well-structured exercise protocols can make a series of adaptations which, in addition to having a positive effect on bone health, have a direct influence on the prevention of falls, improving the strength of the lower limbs and balance. Moreover, Physical Activity (PA) has been shown to enhance physical functions and QOL among menopausal women, improving psychosomatic well-being and physiological parameters (11, 12). It is therefore essential that menopausal women practice PA regularly, both to preserve bone health and to maintain strength and balance. Despite all this knowledge, women in general, and those in menopause in particular, do not reach the minimum amount of PA recommended (13). This highlights the urgent need to find a new strategy to implement adherence to the Moderate Vigorous PA as well as compliance with a well-tailored protocol of PA. The term adherence indicates the number of minutes engaged in Moderate-Vigorous PA in unsupervised and unstructured environments, while the term compliance indicates the frequency of participation in terms of the number of sessions attended in a supervised intervention and it is often analyzed with the addition of the drop-out rate (14). Currently, a real classification of the rate of compliance with the PA program has not yet been defined, but participation that is around 80% can be considered a satisfactory threshold (15). As it is known, the workplace represents an environment in which individuals spend a high number of hours and covers a wide age group with various risk factors, and it is often the work itself that leads individuals to high levels of a sedentary lifestyle, spending many hours sitting (16). In addition, lack of time and lack of transportation are referred to be the primary cause of physical inactivity in people (17). In this framework, the World Health Organization (WHO) Comprehensive Worker Health Action Plan (18) establishes, among other things, that the workplace should provide resources for the personal health of workers and that it should be the first intervention outpost to promote and implement a healthy lifestyle. Indeed, as it was reported in the literature, structured and supervised physical exercise interventions carried out in the workplace, with a minimum duration of four months and with at least two weekly sessions, allow a significant decrease in Body Mass Index (BMI), an increase in muscle mass (19, 20), and an improvement in systolic blood pressure (21). Moreover, in a recent review, it was shown that this type of workplace intervention leads to an increase in Metabolic Equivalent of Task (MET)-mins per week and positively impacts the cardiometabolic health of women of working age in high-income countries (22). In another recent review, it was evidenced that training performed in the workplace can modulate sedentary behavior, reducing the number of prolonged sedentary behaviour greater than 30 min during the whole day (23). According to our knowledge, no studies have so far evaluated the compliance and the effect of a well-tailored protocol proposed in the workplace on menopausal women and compared it to the same protocol performed in sport center outside the working place. Considering these findings, our study aims to evaluate the compliance and drop-out of a well-structured combined training performed inside and outside the workplace in menopausal osteopenic women. Moreover, strength, balance, and QOL will be analyzed to evaluate the effect of this protocol on physical fitness, an indicator of menopausal women's general health needed to prevent osteoporosis and risk of falling (24).

2. Materials and methods

2.1. Participants

The project was funded by the European Commission (G.A. 613137-EPP-1-2019-1-IT-SPO-SCP—ERASMUS + SPORT). Coordinated by the University of Rome "Foro Italico", the partnership consists of 5 European partners: the University of Rome "Foro Italico" (Italy), Bulgarian Sports Development Association—BSDA (Bulgaria), Alexandru Ioan Cuza University of Iași—UAIC (Romania), Gazi University—GU (Turkey) and Fundaciò Salut I Envelliment UAB–- FSIE (Spain). Before the start of the study, through the Train the Trainers methodology, the coordinator organized a specific training course for professionals of the exercise, 2 for each partner country, where it was explained the well-tailored protocol for menopausal women as well as all tests performed during the functional and psychological evaluations. Moreover, a dedicated website was created (https://www.happybones.eu/section "intellectual outputs"), where theoretical and practical manuals have been uploaded to trigger the training cascade effect and involve more operators in the sector. Once the Train the Trainers course was completed, a pilot action based on the well-tailored protocol was planned in each partner country, where at least 10 women were enrolled according to the following inclusion criteria: aged between 45 and 65 years, inactive (less than 150 min of PA per week), being in menopause and osteopenic and/or osteoporotic. The osteopenic/osteoporosis status was evaluated through a

Dual-energy x-ray absorptiometry (MOC/DEXA) report, the analysis was performed autonomously by the women no more than 12 months before the start of the training. Patients with cardiovascular disease and other complications were excluded. In Italy, Romania, and Turkey participants were enrolled in the Universities through an institutional mail sent by the Human resources office, while in Bulgaria and Spain were distributed informative leaflets in the Sport Centers. The study aimed to evaluate compliance and drop-out with the well-tailored training protocol about the place where it was proposed (workplace or sports center). Moreover, functional (strength, balance, flexibility) and psychological parameters were evaluated to analyze the effect of this protocol on women's general well-being. After signing an informative content, a total of 73 menopausal women aged between 50 and 65 were enrolled. The participants' characteristics are reported in **Table 1**. No significant differences between countries were reported at baseline. The proposed study protocol was drafted in accordance with the European Unio"s Standards of Good Clinical Practice and the current revision of the Declaration of Helsinki and was approved by the University's Ethics Committee.

From the total of 73 participants, 39 were recruited in the workplace (IW) and they performed the activity in the gym inside the working structure, which is the University involved in the European project (Italy, Romania, Turkey), while 32 women were enrolled among the general population and performed the activity outside the workplace, in Sports Centers (SC) involved in the European project (Bulgaria, Romania, Spain). A detailed flowchart of the study design is reported in **Figure 1**.

2.2. Assessments

Before the start and at the end of the well-tailored training protocol, the physical, functional, and psychological state of all participants in each country was assessed by administering evaluation tests and questionnaires described below:

1Repetition Maximum (RM): to measure the lower limb strength, and was estimated using the Brzycki formula on submaximal loadsMaximum weight = [Weight used in the test/ [1,0278—(0,0278 * number of repetitions)] (25). Participants performed three trials with increased loads on each machine used during the protocol: Leg press, Leg extension, Leg curl and Gluteus machine. The formula was carried out using the load lifted for fewer repetitions, less than 8.,

Six Minutes Walking Test: to evaluate the functional capacity. At the end of the test, the effort was indicated by the patient through the Borg scale 0–10 (26).

Handgrip Test: to measure the strength of the handheld grip using a dynamometer (Jamar Plus®, Patterson Medical Ltd.), which is representative of the general strength of the subject (27).

30-second Sit to Stand: to assess the strength of the lower limbs, balance, and risk of falls (28). The participants had to get up and sit properly from a chair and the trainer recorded the number of stands they completed in 30 s.Star Excursion Balance Test: to evaluate the dynamic balance and it is performed with a grid placed on the floor, with 8 lines extending from the center (anterolateral, anterior, anteromedial, medial, posteromedial, posterior, posterolateral, and lateral) (29). The distance reached from the center in each direction is measured in centimetres and subsequently adjusted according to the length of the subject's leg.

Single Leg Stance: to evaluate the static balance. Three trials are carried out for each leg, if 30 s are reached the trial is interrupted (30).

International Physical Activity Questionnaire (IPAQ): this questionnaire is used as a surveillance instrument of Physical Activity among adults and is self-administered. The score obtained allows to convert the activities carried out into MET-min/week (31).

Quality of life Questionnaire (QUALEFFO-41): this psychological questionnaire, developed by the IOF, measures different aspects of the subject's life, such as physical, social, and mental function, pain, and general health (32).

The compliance was analyzed through the number of attendances of each participant at the 72 supervised lessons provided for well-tailored protocol, where 100% compliance corresponds to participation in 72 lessons, while 25% compliance corresponds to participation in 18 lessons. Compliance is expressed as a percentage of the overall lessons. Moreover, the dropout of the participants and the timing of this dropout were recorded.

2.3. Well-tailored training protocol

The training protocol provides 72 lessons to perform in a period of 24 weeks; it includes home training (5 days a week) and supervised training (3 days a week), performed in the working place (IW—Italy, Romania, Turkey) or sport center (SC—Bulgaria, Romania, Spain), based on the facilities available to the partners. The supervised training includes group training,

TABLE 1 Women's characteristics.

Women's characteristics	Italy $n = 15$ (M ± SD)	Bulgaria $n = 16$ (M ± SD)	Romania $n = 12$ (M ± SD)	Turkey $n = 18$ (M ± SD)	Spain $n = 12$ (M ± SD)	Tot $n = 73$ (M ± SD)
Age (yrs)	56.1 ± 5.4	63.5 ± 2.2	54.8 ± 4	55 ± 3.7	60.5 ± 5.1	58.0 ± 4.1
Weight (Kg)	66 ± 9.9	64.3 ± 11	68.2 ± 5.8	68 ± 11.5	66.8 ± 10.8	66.9 ± 9.5
Height (cm)	164.3 ± 4.7	162.7 ± 9.1	163.5 ± 6.3	159 ± 6.6	157.2 ± 8.1	161.7 ± 6.9
BMI	21	24	20	22	26	23
Group	IW	SC	IW/SC	IW	SC	

n, number; BMI, Body Mass Index; M, mean; SD, standard deviation; Tot, total; IW, in the workplace; SC, Sport Center.

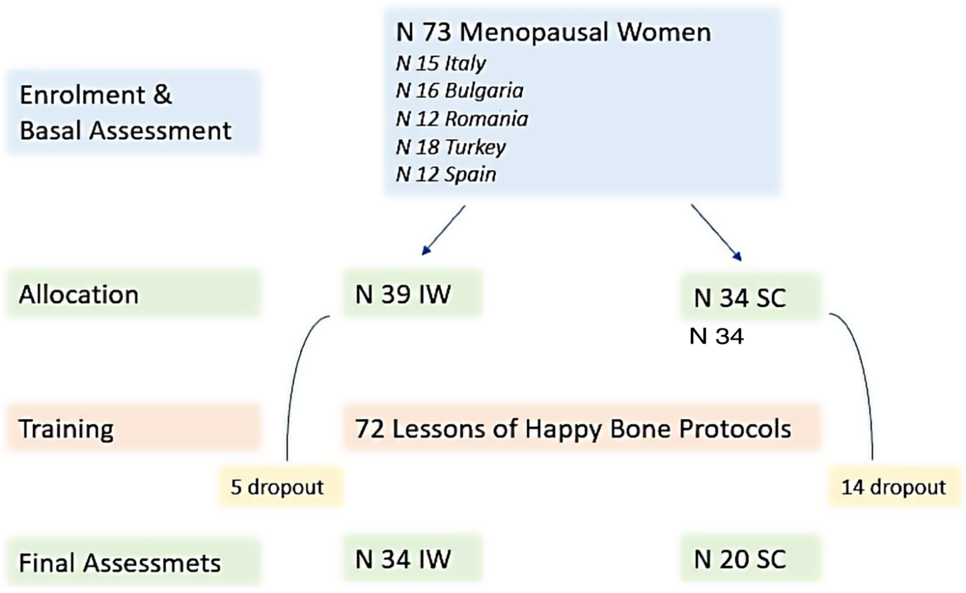

FIGURE 1
Flowchart of the study design. IW, In the working place; SC, Sport Center; N, number.

strength training, and cardiovascular training, explained below (Figure 2).

2.3.1. Home training

Participants are required to perform two exercises at home, the Single leg Standing, 1 min for each leg, and the Star-Excursion Balance, once a day for both legs. The aim of these exercises is to improve dynamic and static balance and stimulate the development of a new bone matrix.

2.3.2. Happy bones supervised training (HB)

The supervised training is organized into three different phases: (1) group training; (2) strength training; (3) cardiovascular training, starting with a warm-up and ending with a cool-down of the major muscle groups involved in the protocol. The strength and cardiovascular training were performed 3 days per week, while the group training 2; all sessions were held under the supervision of the two specialized trainers involved in the Train the Trainer course. The group training included bodyweight exercises and training with small tools to improve balance, strengthen the trunk extensor muscles, to increase flexibility, coordination, and posture. The strength training was performed using four different machines, specific for the lower limbs: the leg press machine, the leg extension machine, the leg curl machine, and the gluteus machine. The strength session started with 2 series of 10 repetitions at 50% of the 1RM and the aim was to reach, at the end of the intervention, 2–3 series of 10/15 repetitions at 70% of the 1RM. The 1RM was calculated through the Brzycki formula mentioned above. Lastly, the cardiovascular training was performed on a treadmill or elliptical machine; it started with 15 min of approximately 75% of the heart reserve rate (HRR), evaluated through the Karvonen formula (Target Heart Rate = [(max HR−resting HR) × %Intensity] + resting HR), and

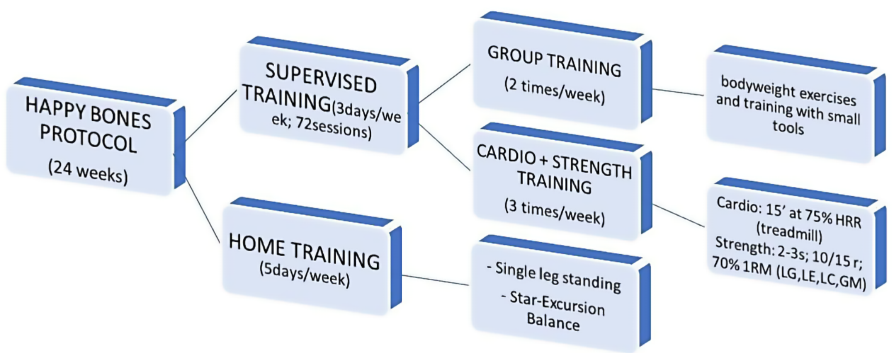

FIGURE 2
Structure of the well-tailored happy bones training protocol.

at the end of the protocol women gradually achieved 30 min of activity at 75% of their HRR.

2.4. Statistical analysis

The statistical package IBM SPSS version 19 (IBM, Chicago, IL, USA), was used for the analysis. At first, each variable was checked for normality using the Kolmogorov-Smirnov Test. For variables that showed normal distribution, separate repeated measures with ANOVA were performed to explore the effect of time, whilst data were presented as mean values and standard deviations. In addition, effect size (ES) was calculated for all variables as partial eta-squared (h2p). Partial eta-squared values below 0.01, between 0.01 and 0.06, between 0.06 and 0.14, and above 0.14 were considered to have trivial, small, medium, and large effect sizes, respectively (33). The variables with no normal distribution were tested for pre and post-intervention differences using the non-parametric Wilcoxon signed ranked test. The median and interquartile range (1st and 3rd quartile) were chosen to represent statistical dispersion for these variables. Lastly, chi-square analysis to determine differences in compliance rates between IW vs. SC was performed. The statistical significance was set at an alpha level of $p < 0.05$.

3. Results

3.1. Results of the compliance and drop-out

This is not a Randomized Control Trial, women were enrolled in the IW or in the SC group depending on the facilities available to the partners. No significant differences between countries were reported at baseline. Out of the total 39 women IW who participated in the protocol, 5 (12.8%) had to leave the program due to problems related to the Covid-19 restrictions, as highlighted in the flowchart. Out of the 34 women who carried out the program in SC,14 of them (41.2%) did not complete the training program due to issues not related to the protocol.

Italian participants carried out the protocol at the workplace, in the university gym, 5 of them registered a high participation frequency in lessons (between 75% and 100%), 7 had a moderate attendance (between 50% and 70%), 2 registered a low frequency (between 25% and 50%) and the group had only 1 drop-out not related to the training protocol.

Bulgarian participants carried out the protocol in a sports center and were recruited through an informative video; due to the pandemic, some had to take the lessons online *via* the Zoom platform. Initially, 16 women started the program but during the six months of lessons, 9 participants left the protocol for reasons not related to the proposed physical activity. The remaining 7 women concluded the protocol by attending 100% of the lessons.

Out of the 12 Romanian participants, 6 were recruited at the university (IW) and 6 externally (SC). The group carried out the protocol in the university gym. None of the participants dropped out of the study and they all completed 100% of the proposed lessons.

Turkish participants carried out the protocol at the workplace, in the university gym. Initially, 18 women were recruited, and 14 participants concluded the exercise protocol; the other 4 left the study due to problems unrelated to the proposed training. Out of the 14 participants, 5 registered a high participation frequency in the sessions (between 75% and 100%), 7 had moderate participation (between 50% and 75%), and 2 a low participation (between 25% and 50%).

Spanish participants were recruited outside the workplace and carried out the activity in the sports center. 12 women were recruited and 5 of them had to drop out of the study due to problems that were not related to the protocol. Out of the 7 women who completed the training, 4 registered a high participation frequency in the sessions (above 75%), while 3 showed moderate compliance (between 50% and 75%).

As regards participation in the proposed training activity, out of the 34 women recruited at the workplace who completed the training, 47% recorded high compliance, 41.3% moderate compliance, and 11,7% low compliance (**Figure 3**).

Out of the 20 women recruited outside the workplace, that performed the activity in an SC, and who completed the protocol, 85% showed high participation and 15% moderate participation in the proposed training sessions (**Figure 4**).

The compliance to the training protocol was statistically higher in the SC compared to the IW ($\chi 2(1) = 5.538$; $p = 0.019$).

3.2. Results of the functional parameters

The functional and psychological results will be presented as a whole group because at baseline and after the protocol was no registered differences between groups (neither between nations nor IW and SC) in the parameters analyzed. At the end of the 6

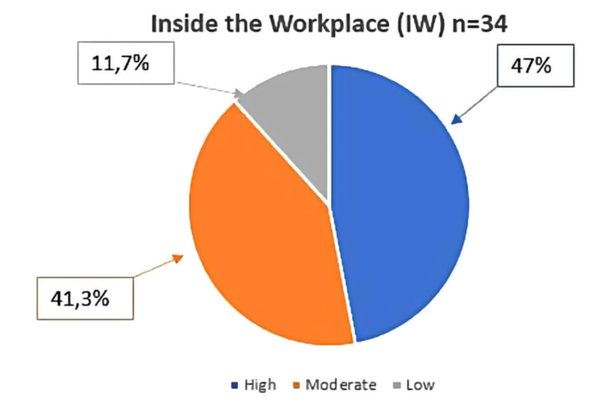

FIGURE 3
Participation rate in the protocol of women who were recruited in the working place (IW) ($n = 34$). The percentage was calculated on the total number of participants who completed the proposed activity. High = participation range between 100% and 75%; Moderate = participation range between 75% and 50%; Low = participation range between 50% and 25%.

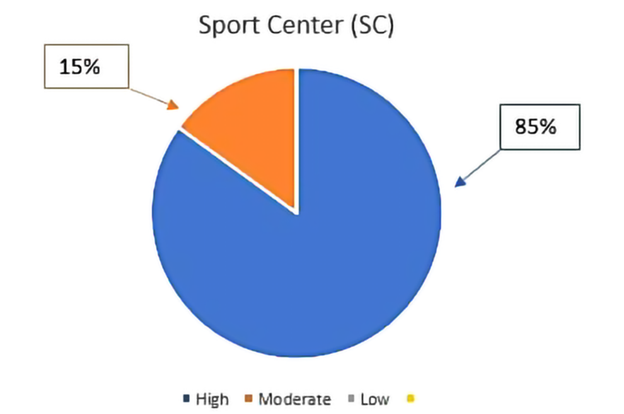

FIGURE 4
Participation rate in the protocol of women who were recruited outside the workplace ($n = 20$). The percentage was calculated on the total number of participants who completed the proposed activity. High = participation range between 100% and 75%; Moderate = participation range between 75% and 50%; Low = participation range between 50% and 25%.

months ofPA, the results of the strength and functional capacity tests showed significant improvements (Table 2). More specifically, all the evaluations made on the machines used for strength training of the lower limbs (the Leg Press, the Leg Curl, the Leg Extension, and the Gluteus Machines—$p < 0.001$) reported a statistically significant increase in this parameter. The Six minutes walking test showed a clear improvement in meters walked from about 570 before the protocol started to about 625 after its completion ($p < 0.001$). Finally, the functional test of the lower limbs (30' sit to stand) confirmed the improvement already shown in the strength tests reported above ($p = 0.005$).

All tests related to both static (Single Leg Stance—$p = 0.001$) and dynamic (Star Balance) balance showed significant improvements (Table 3). These data are very comforting as the balance is closely correlated with the risk of falls; improving this parameter can therefore prevent traumatic events that are very harmful to the general health of menopausal women who could suffer from osteopenia or osteoporosis.

The IPAQ questionnaire results showed a significant improvement in the level of activity ($p = 0.001$), which went from moderate to high, as reported by the MET which is an index linked to weekly energy expenditure due to physical activity (Table 4).

TABLE 2 Results of the total group for Pre and post functional test.

Functional Test	T0 (M ± SD)	T1 (M ± SD)	*p*-value	h2p	IC 95%
Weight (Kg)	66.9 ± 9.5	66.4 ± 9.8	n.s.	–	–
1RM LP (Kg)	69.9 ± 38.5	104.3 ± 53.4	$p < 0.001$	.581	73.86–100.41
1RM LC (Kg)	23.7 ± 14	33.5 ± 16	$p < 0.001$	.628	24.29–33.06
1RM LE (Kg)	38.4 ± 20.5	53.5 ± 20.5	$p < 0.001$	.562	40.18–51.83
1RM GM (Kg)	52.4 ± 26.3	75.1 ± 34.6	$p < 0.001$	.503	55.21–72.37
HT Right (Kg)	28.5 ± 10.7	28.6 ± 9	n. s.	–	–
HT Left (Kg)	27.8 ± 10.9	27.5 ± 8.1	n. s.	–	–
SMWT (m)	570.8 ± 103.3	625.5 ± 74	$p < 0.001$	.393	573.21–623.13
30' StS (n)	16.3 ± 5.4	18 ± 4.3	$p = 0.005$	.164	15.83–18.54

M, mean; SD, Standard Deviation; n., numbers; 1RM, one repetition maximum; 1RM LP, 1RM Leg Press; 1RM LC, 1RM Leg Curl; 1RM LE, 1RM Leg Extension; 1RM GM, 1RM Gluteus Machine; HT, Handgrip Test; SMWT, Six Minutes Walking Test; 30' StS, 30' Sit to Stand; n.s., non-significative; h2p, partial eta-squared; IC, interval confidence.

TABLE 3 Results of the total group for Pre and post-functional test.

Balance Test	T0 (M ± SD)	T1 (M ± SD)	*p*-value	h2p	IC 95%
Single Leg Stance	25.5 ± 6.6	30.4 ± 8.7	$p = 0.001$	.227	26.08–29.87
Star Balance					
Ant R	73.4 ± 11.5	79.3 ± 12.5	$p = 0.02$	.349	70.86–79.27
Ant Med R	76.5 ± 11.1	83 ± 11	$p = 0.05$	.391	74.24–82.47
Med R	75.6 ± 12	80.6 ± 12.9	$p = 0.05$	.166	72.62–80.99
Post Med R	71.9 ± 13.5	81.5 ± 13.9	$p = 0.001$	.421	71.01–79.86
Post R	70.8 ± 12.2	82 ± 13	$p = 0.001$	.623	70.80–79.43
Post Lat R	67.1 ± 12.8	76.8 ± 14.2	$p < 0.001$	.544	66.37–75.14
Lat R	60 ± 14.2	71.2 ± 14.6	$p < 0.001$	.459	60.32–68.90
Ant Lat R	68.9 ± 11.2	74.7 ± 12	$p = 0.019$	.387	66.57–74.63
Ant L	72 ± 12	80 ± 13.8	$p = 0.004$	.524	70.31–79.12
Ant Med L	74.7 ± 11.1	82.2 ± 12.5	$p = 0.003$	.406	72.91–81.27
Med L	70.4 ± 13.1	80.2 ± 15.5	$p = 0.001$	.300	69–77–78.39
Post Med L	72.1 ± 10.6	80.6 ± 14.4	$p = 0.001$	.391	70.79–79.22
Post L	69.9 ± 13.9	82.3 ± 13.9	$p < 0.001$	.615	70.33–79.42
Post Lat L	68 ± 14.2	78.3 ± 12.8	$p = 0.004$	.509	67.58–76.29
Lat L	62.5 ± 16.4	72.7 ± 14	$p = 0.001$	.391	62.05–71.09
Ant Lat L	69.1 ± 11.4	76.2 ± 10.2	$p = 0.002$	.479	67.48–75.27

M, mean; SD, Standard Deviation; h2p, partial eta-squared; IC, interval confidence; ANT, anterior; MED, medial; POST, posterior; LAT, lateral; R, Right; L, Left.

3.3. Results of the Quality of Life Questionnaire

The Quality of Life questionnaire (Qualeffo-41), administered both before and after the training protocol implementation, showed a general improvement of this parameter in the participants (Table 5). The interpretation of this questionnaire foresees that the data that decreases detect an improvement, on the contrary, those showing an increase, detect a deterioration. The total score of the test showed a decrease of 1.2%, which indicates a general improvement in the quality of life. The scores of Mental Function (−5.4%), General Health Perception (−5.5%), Leisure, Social Activity (−0.5%), and Mobility (−2.8%) also decreased. These results, despite not being significant, suggest how the physical activity protocol led to an improvement in the participants' cognitive function, the

TABLE 4 Results of the total group for Pre and post IPAQ score.

IPAQ	T0 (Me-IQR)	T1 (Me-IQR)	*Z* score	Sign
MET	984.0 (1,478.0)	2,256.0 (2,160.0)	−5.090	0.001

IPAQ, international physical activity questionnaire; MET, metabolic equivalent of the task; Me, median; IQR, interquartile range; sign., significance.

TABLE 5 Results of the total group for the qualeffo-41 questionnaire in the different domains.

Intervention Group	T0 (Me-IQ)	T1 (Me-IQ)	*Z* score	Sign
Pain	30.0 (13.7–50.0)	30.0 (10.0–50.0)	−.650	n.s.
Activities of daily living	12.5 (6.2–53)	6.2 (6.2–59.3)	−.571	n.s.
Jobs Around the House	15.0 (0.0–47.5)	10.0 (0.0–62.5)	−.246	n.s.
Mobility	15.0 (3.1–50.0)	12.5 (3.1–53.1)	−.296	n.s.
Leisure, Social Activities	42.5 (15.4–16.3)	32.8 (15.4–63.3)	−.323	n.s.
General Health Perception	50.0 (33.3–66.6)	41.6 (25.0–66.6)	−.877	n.s.
Mental Function	38.8 (30.5–59.6)	36.1 (22.2–55.3)	−1.563	n.s.
Tot Qualeffo-41	27.6 (20.2–47.2)	22.6 (16.6–57.8)	−.876	n.s.

Me, median; IQ, interquartile; Qualeffo-41, quality of life for osteoporosis; Sign, significance; n.s., non-significative.

general perception of health, sociability, and the ability to move autonomously.

4. Discussion

According to our knowledge, this is one of the first studies that compared the compliance of a well-structured combined protocol proposed in two different settings to menopausal women.

4.1. Compliance and drop-out in HB protocol

The results show good compliance in the proposed activity with a medium-high participation rate for more than a third of the participants, both for the women enrolled inside or outside the working place. The low compliance, both in IW and SC, was affected by the covid restrictions still in place during the conduct of the protocol. This result evidence higher compliance than similar studies of PA in the workplace (34, 35). The dropout was less in the IW group concerning the SC, suggesting that a protocol proposed in the working place can reduce all those barriers that prevent a continuous practice of physical activity, i.e., lack of time or transportation. The drop-out was not related to the protocol, but due to the personal problems of the participants, regardless of the group in which they were enrolled. Throughout the study, several factors tangibly emerged that influenced, positively or negatively, the women's participation. A positive aspect was the choice to propose the protocol to employees of universities specialized in healthcare, which meant that among the participants there was greater awareness about the role of physical activity on health. Moreover, these workplaces already have well-organized facilities that can be offered to the employees. While the choice to carry out the activity during the lunch break, could represent a negative aspect, because most of the academic meetings are scheduled around this period, after the end of the lessons with students. As reported in the study of Burn et al. (36), that investigated the effectiveness of interventions of physical activity in the workplace dividing the participants into two groups "in-work" and "after-work", the "after-work" group showed better effects and above all a higher participation rate, although in both groups there was an improvement in overall health (36). Despite this is a pilot study with a small study sample and therefore cannot lead to a generalization of the effects, compared to other studies the level of compliance IW and SC was higher, and it should encourage further studies, more extensive over time, to evaluate the long-term effects on compliance, and, in addition, it may be helpful to provide sessions during and after working hours.

4.2. Functional and psychological effect of HB protocol

According to the functional results, the intervention protocol proposed improved the strength of the lower limbs, as reported by the 1 RM results, as well as functional abilities, such as a 6-minute walking test and balance. Strength and balance are two fundamental components to preventing and treating osteoporosis, which occurs during the menopausal stage (37). Increasing strength allows to intervene in the maintenance of the bone and the general health of the individuals while through the improvement of balance, it is possible to work on the prevention of falls (38). Even the 30' Sit to Stand Test result highlights this significant result, evidencing an improvement in the functional lower extremity strength, which is highly correlated with the risk of falls (39). Furthermore, questionnaires were administered to all project participants to investigate the level of physical activity and quality of life. Although self-reported measurements and population physical activity levels resulting from self-assessment should be interpreted with caution (40), this qualitative analysis through the IPAQ demonstrated an improvement in the time spent actively. In terms of quality of life, the QUALEFFO-41 questionnaire did not produce statistically significant results, although the trend shows improvement. It is important to note, however, that the QUALEFFO-41 is a questionnaire primarily designed for individuals who already suffer from osteoporosis and have fractures. But since this protocol is part of a European project, involving other countries and numerous subjects, it was necessary to use this questionnaire because it is more accessible to all and its reliability and validity have been demonstrated in multiple languages (41).

4.3. Limitations

The results from this study were positive and very encouraging, nevertheless, the study has some limitations. Primarily this is not a randomized control trial, it was performed in 5 different countries around Europe, and for this reason, the choice to be included in the working place or in the sports center group depended on the country's possibility and not from a proper randomization. Nevertheless, due to the normal distribution of the functional and psychological data we had the possibility to evaluate the group as one and compare the data from pre to post training. Moreover, we did not analyze bone health through specific equipment such as MOC or DEXA, mainly because not all partners could reach this equipment and also because, according to the literature, a 6-month protocol is not able to produce a significant result in terms of bone health (42). In addition, to make the protocol more comprehensive and allow for good performance of the Handgrip Test as well, upper limb activities and tools for objective measurements, such as pedometers or digital step-counting watches, should have been added to the training sessions. Lastly, the QUALEFFO-41 questionnaire should have been implemented with other quality-of-life questionnaires more specific to the target population. All these elements could contribute to making further studies more in-depth and capable of producing more generalizable effects.

5. Conclusions

In conclusion, the Happy Bone protocol, when it is proposed in the workplace, seems to reduce the level of dropout compared to those experienced by the women that performed the training in the sports center. Moreover, the protocol improved the general health of the participants, increasing strength and balance, regardless of where it has been proposed. Given these positive and encouraging results women's national societies should collaborate with Ministries of sport, universities of sports science, national nutrition foundations/councils, nongovernmental organizations concerned with seniors' welfare, and national sports councils to inform menopausal women, and adults in general, on their nutritional and exercise needs to maintain a healthy skeleton, avoid premature bone loss, fall and avoid malnutrition in the elderly. Moreover, it would be extremely interesting to expand and extend this project over time. In addition, it would also be important to use the model of this intervention to support the prevention and management of other types of chronic diseases and populations, making the workplace one of the first places where promote health (43).

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 University of Rome "Foro Italico" Ethics Committee: CAR 106/2021. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conceptualization, AP, MS, B-AA, R-MI, SB-A and HP; methodology, SO, I-MD, CM, AG, MB, SD, GR and ÀR; software, MB, SD; formal analysis, CC and ÀR; investigation, ET, C-EM and EM; resources, CM, C-EM and I-MD; data curation, EG, R-MI, AGS and AG; writing—original draft preparation, EG, EM and SO; writing—review and editing, ET and AD; supervision, MS, B-AA and AD; project administration, HP, SB-A and CC; funding acquisition, AP. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The effects of 10-week plyometric training program on athletic performance in youth female handball players

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Purpose: The aim of the study was to investigate the effects of a 10-week plyometric training (PT) on changes of direction, jumping ability, repeated sprint ability, and both muscular strength and power in youth female handball players.
Methods: Twenty-eight participants (age: 15.8 ± 0.2 years) were randomly divided into a plyometric group (PG; $n = 14$) or a control group (CG; $n = 14$). Significant (group × time) interaction was noted for change of direction (COD) [Modified agility *T*-test (T-half)], three jumping tests [squat jump (SJ), countermovement jump (CMJ) and standing long jump (SLJ)], repeated sprint ability (RSA), muscular strength (1-RM bench press and 1-RM half squat) and muscular power (force-velocity test for both upper and lower limb).
Results: With a group × time interaction, the PG enhanced the T-half performance [$p < 0.001$, $\Delta = 10.4$, $d = 1.95$ (large)] compared to the CG. The PG enhanced the jump performance over SJ [$p = 0.009$, $\Delta = 18.3$, $d = 0.72$ (medium)], CMJ [$p = 0.005$, $\Delta = 20.7$, $d = 0.79$ (medium)] and SLJ [$p < 0.001$, $\Delta = 24.5$, $d = 2.25$ (large)]. Three of four RSA scores increased significantly in the PG compared to the CG [$p < 0.001$, $\Delta = 2.76$, $d = 1.11$ (large); $p < 0.001$, $\Delta = 2.72$, $d = 1.23$ (large); and $p < 0.001$, $\Delta = 2.75$, $d = 1.21$ (large), in best time (RSA-BT), mean time (RSA-MT) and total time (RSA-TT), respectively]. In contrast, group × time interactions revealed no significant differences in both 1-RM bench press and 1-RM half squat performance between PG and CG. Regarding the force velocity performance, the PG enhanced 3 of 4 force velocity scores for the upper limb performance [$p < 0.001$, $d = 1$ (large); $p < 0.001$, $d = 1.13$ (large) and $p = 0.012$, $d = 0.72$ (medium) for the peak power in these two forms (W and W·kg-1), and maximal pedalling velocity, respectively], and 2 of 4 force velocity scores for the lower limb performance [$p = 0.045$, $d = 0.56$ (medium); and $p = 0.021$, $d = 0.65$ (medium) for the peak power in these two forms (W and W·kg-1), respectively].
Conclusion: It was concluded that additional PT performed two times per week during 10 weeks enhances measures related to game performance, such as COD, jump ability, RSA, and power in youth female handball players.

KEYWORDS

stretch shortening cycle, peak power, 1-RM, upper limb, lower limb

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Introduction

Handball is a team sport that requires the use of several key parameters of performance (e.g., anthropometric, physiological, psychological, and motor skill characteristics) (1–3). The physical and physiological characteristics and the on-court performances of handball players have recently been reviewed (4, 5). It is a contact sport, which includes jumping, running, changes of direction and arm throwing ball as prominent features of performance (6–9). Muscle strength and power are key components of fitness performance, required in many explosive actions (e.g., jump, change of direction, throw, sprint) and constitute an essential part of any young athlete's overall training program (10). To maintain a high level of physical performance, both strength/power training should be carefully monitored throughout the competitive season.

PT programs could be very useful to develop lower/upper limb power for both male and female handball players (11–13). PT can play a significant role in the development of young female handball players. Plyometrics involves explosive movements that aim to enhance power, speed, and agility, which are essential attributes for handball players (14). Hence, handball coaches should perform specific PT to develop these physical qualities. This type of program (i.e., PT) consists of an eccentric muscle contraction followed by a concentric muscle contraction the aim of which is to improve the stretch-shortening cycle (i.e., time between eccentric contraction and eccentric contraction) and subsequently improve physical qualities (11, 13–17). PT is based on jumping (such as hurdle jump, drop jump, etc.), skipping and hopping exercises to benefit the shortening stretch cycle (18). Incorporating this type of exercise improves the ability of the muscle-tendon unit to produce maximum force in the shortest possible time (i.e., muscular strength and muscular power) (19), and subsequently the physical qualities. Leading world fitness and health organizations guidelines, review articles, and meta-analyses (14, 18, 20, 21) indicate that PT, if correctly done, can be very beneficial for adolescents.

The ability to closely mimic powerful actions essential for success in handball makes plyometric exercises an ideal method of resistance training activity for young female handball players. In previous studies, the effects of PT on young handball athletes (11, 13) were investigated. For instance, Hammami et al. (13) found increases in sprint speed, change of direction, jump ability and repeated change of direction after 10-week upper and lower PT in young female handball players aged 15.8 ± 0.2 years. Furthermore, Chaabene et al. (11) revealed that PT (2 weekly sessions for 8 weeks) improves measures of physical fitness (i.e., linear/change of direction speed, jumping, and RSA) in young female handball players aged 15.9 years old. In fact, Hammami et al. (12), found increases in both upper limb (handgrip force, back extensor strength, and medicine ball throwing) and lower limb [sprinting, change of direction (CoD), jumping] performance after 9 weeks of combined upper and lower limb PT in U14 female handball players. Controversy, Meszler and Vaczi (22), found no significant changes in T agility test scores, balance, hamstring strength or H:Q ratio after 7 weeks of PT in female basketball players aged younger than 17 years.

Recently, the review of Ramirez-Campillo et al. (23), found that scarce information is available in the literature on PT effects in female players (23). This implies that future studies including female participants are needed to provide more in-depth knowledge for coaches and practitioners. Moreover, a recently published systematic review outlined that PT studies on the effects on physical fitness in young female athletes suffer from numerous methodological shortcomings and are limited in number. A major limitation is the lack of controls which affects the veracity of findings (21). This implies that more controlled PT trials are needed in female players.

Therefore, the aim of this study was to determine how far the substitution of a short-term plyometric program for some existing drills within a regular in-season handball training program would enhance physical performance in young female handball players. A plyometric program was introduced into the normal in-season regimen for 10 weeks for participants, without increasing their total training time. Taking into consideration the previous investigations on this topic (11, 23), we expected that 10 weeks of PT would improve change-of-direction ability, jump height, repeated sprint ability, power and strength performance.

Materials and methods

Participants

The Gpower 3.0.10 program was used to calculate the minimal sample size needed in our study, with Z1-β = 1.03 (power = 85%) and Z/2 = 1.96 (alpha = 5%). The study of Meszler and Váczi (22) showed the mean ± SD of counter movement vertical jump as 33.52 ± 3.89 (cm) in the experimental group vs. 28.72 ± 6.66 (cm) in the control group, and considering a ratio of 1 control for every case, there was a need for a minimum of 11 experimental and 11 control subjects (24). In order to explore the effects of a short-term (i.e., 10 weeks) PT program on measures of athletic performance in youth female handball players, twenty eight youth female handball players from the same club were divided by playing position, and players from each position were then randomly assigned into a plyometric group (PG) (*n* = 14; age 15.7 ± 0.2 years; body mass 63.8 ± 3.3 kg; body height 1.65 ± 0.03 m; body fat 25.4 ± 4.1%; maturity-offset 2.9 ± 0.4 years) or a control group (CG) (*n* = 14; age 15.8 ± 0.2 years; body mass 63.3 ± 4.1 kg; body height 1.67 ± 0.03 m; body fat 24.6 ± 1.8%; maturity-offset 3.0 ± 0.4 years). They were examined by the team physician, with a particular focus on conditions that might preclude elastic band training, and all were found to be in good health (the player who is not in good health, excluded from the study). All participants were classified as highly trained athletes (25). They participated in national competitions for at least 5 years and they had 3 years' experience of PT. All had already achieved a good overall physical preparation at the beginning of the season (a preliminary 6-week period of 6 training sessions per week). This preliminary phase was divided into 2 parts. The first 3 weeks included a resistance training program which aimed to improve muscular endurance by light loads (30%–50% 1 repetition maximum). The second 3-week period was devoted to improving muscular power with higher loads (40%–60% 1 repetition maximum realized at high velocity), accompanied by friendly matches each weekend. The subjects continued to participate in 5 training sessions per week during September at the championship season. The experimental intervention of biweekly PT was undertaken during the second phase of the national championships (January to March). All participants had previously engaged in five to six training sessions per week (90–120 min each session). However, for 10 weeks, the EG replaced some of their handball-specific drills with a PT program, although the overall training volume remained comparable for the two groups. Any athlete missing more than 10% of the total training sessions and/or a player who is not in good health would be excluded from the study. During the intervention, the CG followed their usual handball training (i.e., mainly technical-tactical exercises, small-sided and simulated games, or injury prevention drills).

Procedures

This current study examined whether 10-week of biweekly in-season PT would enhance certain performance-related capacities in initially well-trained youth female handball players relative to their peers who continued to follow their customary in-season training regimen [the CG followed their usual handball training (i.e., mainly technical-tactical exercises, small-sided and simulated games, or injury prevention drills)]. Two familiarization sessions were held, 2 weeks before baseline test session, to get participants acquainted with the tests. Measurements were made in a fixed order over 4 days, immediately before and 4 days after the last plyometric training session the subjects were disallowed to participate in any exhausting exercise for 24 h before testing, and to consume any food or caffeine-containing drinks 2 h before testing. The training intervention was conducted during the in-season period of the year 2018–2019. Training and measurements were made at the same time of day (5:00–7:00 PM), under approximately the same environmental conditions (temperature: 20°C–25°C) on a wooden surface at the same time of day indoors handball hall. A standardized warm-up (10–20 min of low- to moderate-intensity aerobic exercise and dynamic stretching) preceded all tests. On the first test day, participants made modified agility *T*-test (*T*-test), standing long jump (SLJ), and repeated sprint ability (RSA). The second day was devoted to upper limb force velocity test and 1-RM half squat. On the third day, anthropometric measurements were followed by jumping ability [i.e., squat jump (SJ), counter-movement jump (CMJ)]. On the fourth and last day, 1-RM bench press and lower limb force velocity test were completed. All tests were scheduled at least 48 h after the most recent training session or competition and under the same experimental conditions. Participants were instructed to use the same athletic shoes and clothes during the pre- and post-testing (**Figure 1**).

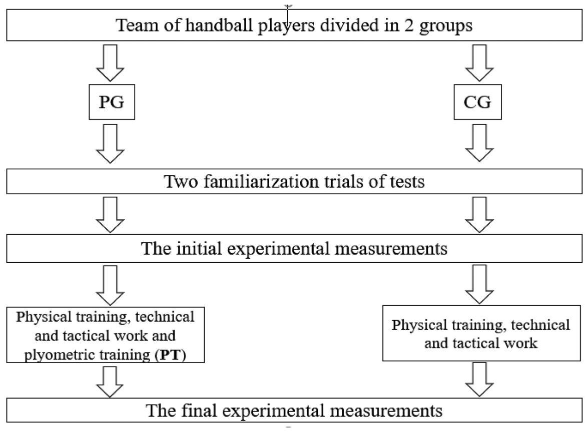

FIGURE 1
The diagram includes detailed information on the interventions received.

Testing procedures

Anthropometry

Anthropometric measurements included standing and sitting body height (stadiometer accuracy of 0.1 cm; Holtain, Crosswell, Crymych, Pembs, United Kingdom) and body mass (0.1 kg; Tanita BF683W scales, Munich, Germany). The overall percentage of body fat was estimated from the biceps, triceps, subscapular, and suprailiac skinfolds, using the equations of Durnin and Womersly (26) for children and adolescent females:

$$\%\,\text{Body fat} = (495/\text{D}) - 450$$

where $D = 1.1369–0.0598$ (Log sum of 4 skinfolds)

Maturity offset status was calculated from peak height velocity [23]:

Maturity offset = −9.38 + (0.000188 × leg length × sitting height) + (0.0022 × age × leg length) + (0.00584 × age × sitting height) + (0.0769 × weight/height ratio):

Modified agility *T*-test

As previously described (27) the modified *T*-test was performed to determine speed with directional changes such as forward sprinting, left and right shuffling, and backpedalling. Performance times were recorded to the nearest 0.01 s by paired single beam photocells (Microgate, Bolzano, Italy). Each player performed two attempts with 5 min of rest between them, and the best attempt was used for analyses.

Vertical jumps

Jump height was evaluated using an infrared photocell mat related to a digital computer (Optojump System, Microgate SARL, Bolzano, Italy). Flight times were measured with a precision of 1/1,000 s, allowing calculation of jump heights. Players started the SJ at a knee angle of 90 degrees, and made a vertical jump by pushing upwards, keeping their legs straight (28). The CMJ began from an upright position, subjects making a rapid downward movement to a knee angle of 90 degrees and simultaneously beginning to push-off. One minute of rest was permitted between the three trials of each test, the highest jump being used in subsequent analyses. Each player performed two attempts with 5 min between them, and the best attempt was used for analyses.

Standing long jump

The starting position required subjects to stand with their feet at shoulders' width behind a line marked on the ground and their arms in neutral position (28). On the command ready, set, go, participants executed a countermovement with their legs and arms and jumped at maximal effort in the horizontal direction. Participants had to land with both feet at the same time and were not allowed to fall forward or backward. The horizontal distance between the starting line and the heel of the rear foot was evaluated via tape measure to the nearest 1 cm. Each player performed two attempts with 5 min between them, and the best attempt was used for analyses.

Repeated sprint ability (RSA) test

After a standardized warm-up, the shuttle RSA test involved 6 repetitions of 2 m × 20 m shuttle sprints (approximately 7 s running time). In this test, sprints were repeated every 20 s (29–31). An active recovery was allowed through a quick walk back to the starting line. Three seconds before starting each sprint, players took an individually chosen starting position 0.5 m behind the timing gate. A digital timer started automatically when the player passed the gate. Two timing gates (Microgate Srl; Race time 2. Light Radio, Bolzano, Italy) working in opposite directions allowed subjects to start the next run from the end where they had finished the preceding sprint. Strong verbal encouragement was provided throughout, and participants were asked to perform each sprint with maximal effort, avoiding pacing. Four scores were assessed: best sprint time (RSA-BT), mean sprint time (RSA-MT), total sprint time (RSA-TT) and fatigue index (RSA-FI), the last calculated as the percentage decrement: 100—(Total time/ideal time × 100); where the ideal time = 6 × RSA best time (31).

1-RM half squat and bench press

Muscular strength of participants was evaluated by one maximum repetition (32), measured three times (just before starting the training program, at the fourth week and after 2 months). Thus, training loads (%RM) were accurately adjusted during the training program, following previous literature guidelines (24). First, the player was instructed to perform a light resistance warm-up from 10 to 12 repetitions in the assessed exercise. Then, a 1-minute rest was allowed. A warm-up load was added to allow the athlete to complete 3–5 repetitions (5%–10% for bench press and 10%–20% for leg press and back squat). A 2-minute rest time was provided. Again, a 5%–10% increase in the load was performed for bench press and 10%–20% for leg press and back squat. A 4-minute rest time was provided. The load was again increased for the athlete to attempt one maximum repetition. The load continued to be increased or decreased until the player completed one repetition with proper exercise technique. In both leg press and squat exercises, participants were asked to perform a thigh-knee 90° angle range of motion. In bench-press, also an arm-forearm 90° range of motion was defined as the final moment of the eccentric phase.

The force-velocity test

The lower limb force–velocity tests were executed on a standard cycle ergometer (model 894 E, Monark Exercise AB, Vansbro, Sweden). The corresponding maximal anaerobic power was calculated using the instantaneous peak velocity at each braking force. The maximal velocity (V0) was identified as the highest velocity attained without external loading. Peak power was defined as the power at which additional loading induced a decrease in power output. Parabolic relationships were determined only if we observed a decline of peak power over two successive braking forces.

Upper limb tests were made using an appropriately modified version of the same apparatus. Hand cranks replaced the pedals, and the saddle pillar was removed to avoid injuries. The ergometer was then mounted on a metal support that brought the crankshaft to shoulder level. The unrestrained subjects stood freely in front of the ergometer, with the exception that smaller participants were allowed to stand on a step.

The measured and calculated parameters for both tests contained Peak power of the upper (PP_{UL}) and lower (PP_{LL}) limbs, each expressed in Watts, $W \cdot kg^{-1}$ of total body mass, and the corresponding maximal forces ($F0_{UL}$ and $F0_{LL}$) and maximal velocities ($V0_{UL}$ and $V0_{LL}$). The force–velocity tests required short all-out sprints (duration about 7 s) using a suitable sequence of ergometer braking forces (33, 34). The force–velocity tests required short all-out sprints (duration about 7 s) using a suitable sequence of ergometer braking forces. After a 10-minute standardized warm-up, lower limbs tests began at a braking force equal to 2.5% of the participants' body mass (33). After a 5-minute recovery, the braking was increased to 5%, 7.5%, 8.5%, 9.5%, 10.5%, and 11.5% of body mass in randomized order. The same sequence was performed again, until an additional load induced a decrease of power at each of 2 repetitions; this value was accepted as the PP. Six to 8 all-out sprints were generally performed in a session. The upper limbs protocol was similar, beginning with a braking force equal to 1.5% of the participants' body mass. After a 10-minute warm-up, the braking was increased by 0.5% every bout, until the subject could not reach the previous peak of power in 2 successive bouts.

Plyometric training program

The PT program completed a 10-week in-season with two training sessions per-week (Tuesday and Thursday), respectively, based on the players' previous training records and research results (13, 23). PT drills were incorporated into their regular 90–120 min handball training routines, replacing some low-intensity technical-tactical handball exercises. Without counting competitive and friendly matches, the PT replacement activity represented <10% of the total training load. During the intervention, the CG followed their usual handball training (i.e., mainly technical-tactical exercises, small-sided and simulated games, and injury prevention drills).

The rating of perceived exertion RPE (35) was used to control the overall training load and ensure no differences between both groups. A standardized 8–12 min warm-up preceded each PT session, including low-intensity running, coordination exercises, dynamic movements (i.e., lunges, skips), sprints, and dynamic stretching for both upper and lower limb muscles. The intervention included push-up exercises for the upper limbs (both exercises performed at high velocity), and hurdling, lateral hurdling, and hurdle jumping (jumping with 180° rotation) exercises for the lower limbs. Exercises for the upper limbs were immediately followed by lower-limb exercises (i.e., 6–10 repetitions of dynamic push-ups + 6–8 repetitions of lower limb jumps), with no intervening rest periods (**Table 1**). The sequence of plyometric exercises for the upper and lower limbs lasted ~10 s (20, 21, 23). A time of 30 s was fixed as a recovery time between sets. All plyometrics in general (i.e., upper and lower limb exercises) were performed with maximal effort, minimizing contact time in each repetition, and no resting was allowed between jumps.

Statistical analyses

Statistical analyses were performed using the SPSS 22 program for Windows (SPSS, Inc., Armonk, NY: IBM Corp). The Kolmogorov–Smirnov test was used to verify the normality of all variables (36). Data are presented as mean (SD), and as median values for skewed variables. Initial between-group differences were analyzed using independent *t*-tests, and the effect of the intervention was determined by 2-way analyses of variance [group (PG vs. CG) x time (pre vs. post)]. To evaluate within-group pre-to-post performance changes, paired sample *t*-tests were applied. Percentage changes (delta-change) were calculated as [(post-training value—pre-training value)/pre-training value] * 100. Effect sizes were calculated by converting partial eta squared values to Cohen's d [classified as small ($0.00 \le d \le 0.49$), medium ($0.50 \le d \le 0.79$), and large ($d \ge 0.80$)] (37). Training-related effects were assessed by 2-way analyses of variance (group × time). Statistical significance was set at $p < 0.05$, whether a positive or a negative difference was seen (i.e., a 2-tailed test was adopted). The reliabilities of all dependent variables were assessed by calculating intra-class correlation coefficients (2-way mixed) and coefficients of variation.

Results

No athlete missed more than 10% of the total training sessions and/or more than two consecutive sessions, so it was not necessary to exclude any participants from the study.

Reliability of the tests

Test-retest reliabilities were generally above the accepted threshold, with intra-class correlation coefficients ranging from 0.93 to 0.98, and coefficients of variation of 2.1% to 9.2% (**Table 2**).

Between-group differences at baseline

There were no significant initial intergroup differences for any of the dependent variables.

Training-related effects

All data, collected after the 10-week intervention, showed significant increases for both PG and CG. With a group × time interaction, the PG enhanced change of direction [i.e., T-half ($p < 0.001$; $d = 1.95$)]; and jump performance [i.e., SJ ($p = 0.009$, $d = 0.72$), CMJ ($p = 0.005$, $d = 0.79$) and SLJ ($p < 0.001$, $d = 2.25$)]

TABLE 1 Plyometric training program.

	Weeks 1–2	Weeks 3–4	Weeks 5–6	Weeks 7–8	Weeks 9–10
	Set × Repetition	Set × Repetition	Set × Repetition	Set × Repetition	Set × Repetition
Upper limb					
Push-up	10 × 6	10 × 6	10 × 6	10 × 6	10 × 6
Contacts number	60	60	60	60	60
	Weeks 1–2	Weeks 3–4	Weeks 5–6	Weeks 7–8	Weeks 9–10
	H × S × R	H × S × R	H × S × R	H × S × R	H × S × R
Lower limb					
Hurdle jump	0.3 m × 2 × 6	0.3 m × 3 × 6	0.35 m × 2 × 6	0.35 m × 3 × 6	0.4 m × 2 × 6
Lateral hurdle jump	0.3 m × 2 × 6	0.3 m × 3 × 6	0.35 m × 2 × 6	0.35 m × 3 × 6	0.4 m × 2 × 6
Stretched leg jump	0.25 m × 2 × 6	0.25 m × 3 × 6	0.30 m × 2 × 6	0.30 m × 3 × 6	0.35 m × 2 × 6
Hurdle jump (jump with 180°)	0.25 m × 2 × 6	0.25 m × 3 × 6	0.30 m × 2 × 6	0.30 m × 3 × 6	0.35 m × 2 × 6
Horizontal jump	1.1 m × 2 × 6	1.1 m × 3 × 6	1.2 m × 2 × 6	1.2 m × 2 × 6	1.3 m × 2 × 6
Contacts number	60	90	60	90	60

H, height; S, sets; R, reps.

TABLE 2 Reliability and variability of change of direction and jump tests.

	ICC	95% CI	CV
T-half	0.974	0.944–0.988	2.1
SJ	0.986	0.971–0.994	8.5
CMJ	0.979	0.955–0.990	7.5
SLJ	0.932	0.852–0.968	9.2

CI, confidence intervals; CV, coefficient of variation; CMJ, counter-movement jump; ICC, intraclass correlation coefficient; SJ, squat jump; T-half, Modified agility *T*-test; SLJ, standing long jump.

compared to the controls (Table 3). Of the same, 3 of 4 repeated sprint ability scores increased significantly in the plyometric relative to the control group [$p < 0.001$, $d = 1.11$ (large); $p < 0.001$, $d = 1.23$; and $p < 0.001$, $d = 1.21$, in RSA-BT, RSA-MT and RSA-TT respectively] (Table 4). Controversially, group × time effects showed no significant difference in both 1-RM bench press and half squat performance between PG and CG (Table 3). Regarding the force velocity performance, the PG enhanced 3 of 4 force velocity scores [$p < 0.001$, $d = 1$ (large); $p < 0.001$, $d = 1.13$; and $p = 0.012$, $d = 0.72$ for PP_{UL} (W), PP_{UL} ($W.kg^{-1}$) and VO_{UL} respectively] for the upper limb performance, and 2 of 4 force velocity scores [$p = 0.045$, $d = 0.56$; and $p = 0.021$, $d = 0.65$ for PP_{LL} (W) and PP_{LL} ($W.kg^{-1}$), respectively] for the lower limb performance. However, $F0_{UL}$, $V0_{LL}$ and $F0_{LL}$ remained unchanged (Table 4).

Discussion

The current study aimed the effectiveness of a 10-week PT intervention in improving change of direction, jumping ability, repeated sprint ability, and muscular strength and power in youth female handball players. With the exception of muscular strength (1-RM bench press and 1-RM half squat), performance on these selected measures was significantly enhanced by PT in comparison with the standard regimen.

Change-of-direction capacity refers to a movement where no immediate reaction to a stimulus is required, so the direction change is preplanned (19). It has been proved among the key qualities in a handball match (38), and is affected by strength, power, and speed (19). The present finding revealed a significant improvement in COD (i.e., T-half) in the PG compared to the CG. In the literature, several studies examined the impact of plyometrics and found increases (11, 13) and decreases (22, 39) in COD performance. Discrepancy between studies may be explained by numerous factors (i.e., training level, gender, age, sport activity, or familiarity with plyometrics) and training variables (i.e., surface and type of PT, rest period between sets and training sessions, and the principle of specificity). The possible mechanisms of COD improvements could be the result of force gain and high-power output and the ability to efficiently use the stretch-shortening cycle in ballistic movements (11, 13).

TABLE 3 Change of direction, jump, repeated sprint ability, and muscular strength test performances in plyometric and control group before and after 10-week intervention.

	Control group (*n* = 14)					Plyometric group (*n* = 14)					Anova group x time interaction	
	Pre	Post	%Δ change	Paired *t* test		Pre	Post	%Δ change	Paired *t* test		*p*	Cohen's *d*
				p	Cohen's *d*				*p*	Cohen's *d*		
Change of direction												
T-half (s)	7.49 ± 0.16	7.42 ± 0.18	0.8 ± 0.8	0.001	0.43	7.47 ± 0.16	6.70 ± 0.25	10.4 ± 3.1	<0.001	3.81	<0.001	1.95 (large)
Jump												
SJ (cm)	22.7 ± 2.3	23.7 ± 1.8	4.7 ± 4.6	0.001	−0.50	22.4 ± 1.6	26.4 ± 1.8	18.3 ± 2.4	<0.001	−2.44	0.003	0.85 (large)
CMJ (cm)	23.9 ± 2.2	24.9 ± 1.9	4.1 ± 3.5	<0.001	−0.50	24.3 ± 1.4	29.3 ± 1.7	20.7 ± 3.1	<0.001	−3.33	<0.001	1.15 (large)
SLJ (m)	1.52 ± 0.15	1.69 ± 0.17	12.2 ± 13	0.003	−1.10	1.50 ± 0.13	1.86 ± 0.15	24.5 ± 13.9	<0.001	−2.66	0.033	0.60 (medium)
Repeated sprint												
RSA-BT (s)	7.54 ± 0.07	7.50 ± 0.06	0.5 ± 0.4	<0.001	0.64	7.54 ± 0.07	7.34 ± 0.08	2.6 ± 0.6	<0.001	2.76	<0.001	1.11 (large)
RSA-MT (s)	7.70 ± 0.07	7.66 ± 0.06	0.5 ± 0.5	0.001	0.64	7.71 ± 0.08	7.50 ± 0.08	2.7 ± 0.1	<0.001	2.72	<0.001	1.23 (large)
RSA-TT (s)	46.22 ± 0.42	45.97 ± 0.35	0.5 ± 0.5	0.001	0.67	46.26 ± 0.48	44.99 ± 0.48	2.7 ± 0.1	<0.001	2.75	<0.001	1.21 (large)
RSA-FI (%)	2.16 ± 0.60	2.16 ± 0.55	1.9 ± 19.5	1.000	0.00	2.31 ± 0.71	2.21 ± 0.78	4.9 ± 22.4	0.419	0.14	0.759	0.08 (small)
1-RM												
1-RM Bench press (kg)	35.3 ± 10.4	39 ± 10.7	11.2 ± 5.6	<0.001	−0.36	35.9 ± 10.7	42.9 ± 10.3	22.3 ± 17.2	<0.001	−0.71	0.561	0.16 (small)
1-RM Half squat (kg)	73.3 ± 13.4	79.5 ± 14.3	8.6 ± 2.3	<0.001	−0.46	72.2 ± 16	73.7 ± 19.1	6.1 ± 31.3	0.770	−0.09	0.580	0.15 (small)

T-half, Modified agility *T*-test; CMJ, countermovement jump; SLJ, standing long jump; RM, repetition maximal; RSA, repeated sprint ability; BT, best time; MT, mean time; TT, total time; FI, fatigue index; SJ, squat jump.

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TABLE 4 Force-velocity test performances in plyometric and control group before and after 10-week intervention.

	Control group ($n = 14$)					Plyometric group ($n = 14$)					Anova group x time interaction	
	Pre	Post	%Δ change	Paired *t* test		Pre	Post	%Δ change	Paired *t* test		*p*	Cohen's *d*
				p	Cohen's *d*				*p*	Cohen's *d*		
Upper limb												
PP (W)	146 ± 24	146.5 ± 10.7	2.7 ± 17.6	0.948	−0.03	144.8 ± 25.9	186 ± 20.5	30.8 ± 17.3	<0.001	−1.83	0.001	1.00 (large)
PP (W.kg^{-1})	1.9 ± 0.2	1.9 ± 0.2	3.8 ± 18.7	0.688	0.00	1.9 ± 0.3	2.4 ± 0.2	31.5 ± 16.6	<0.001	−2.04	<0.001	1.13 (large)
V0 (rpm)	87.3 ± 17.3	84.2 ± 8.6	0.1 ± 21.5	0.525	0.24	88.8 ± 17.1	106.5 ± 14.8	21.9 ± 14.6	<0.001	−1.15	0.012	0.72 (medium)
F0 (N)	6.5 ± 1.2	7.1 ± 0.7	11.6 ± 23.9	0.108	−0.63	6.6 ± 0.9	7.3 ± 0.7	11.1 ± 12.3	0.005	−1.42	0.816	0.06 (small)
Lower limb												
PP (W)	337.8 ± 37.9	355.4 ± 35.9	5.3 ± 3	<0.001	−0.49	345.9 ± 48.5	407.1 ± 35.9	18.5 ± 8	<0.001	−1.49	0.045	0.56 (medium)
PP (W.kg^{-1})	5.3 ± 0.5	5.4 ± 0.5	3.3 ± 3.3	0.003	−0.21	5.5 ± 0.8	6.5 ± 0.7	11.5 ± 7.1	<0.001	−1.38	0.021	0.65 (medium)
V0 (rpm)	162.9 ± 18.8	164.1 ± 22.4	0.8 ± 8.8	0.767	−0.06	164.8 ± 21.3	163.9 ± 26.6	0.6 ± 8.9	0.831	0.04	0.866	0.06 (small)
F0 (N)	7.8 ± 0.5	8.7 ± 1.1	11.1 ± 12.8	0.006	−1.09	7.8 ± 0.5	9.3 ± 1.2	20.3 ± 15.3	<0.001	−1.69	0.146	0.40 (small)

PP, peak power; V0, maximal pedaling velocity; F0, maximal braking force.

In fact, PT enhances the neuromuscular system's ability to generate and control force rapidly. This type of training stimulates the stretch-shortening cycle, which involves rapid muscle lengthening followed by a forceful contraction (18). The neuromuscular system becomes more efficient in coordinating the timing and recruitment of muscle fibers, leading to improved power production during movements involved in COD tasks (18). The change-of-direction tasks are amongst the most frequently performed activities during matches, and it is an important physical fitness attributes in handball (40). For it, coaches must include PT exercises combined with a change of direction exercise. In future studies, it is possible to include PT exercises combined with reactive agility exercise (nonplanned change of direction).

The present results demonstrated significant improvement in all jumps performances in the PG relative to CG. Some studies reported increases in vertical and horizontal jump after PT in young female athletes (11, 13). Similar to our training program, Hammami et al. (13) found increases in Squat and CMJ and horizontal jump performance in young female handball players. The PT effects on vertical jumping performance in female athletes were reported in a published meta-analysis (20), which demonstrated that less than 10 weeks of plyometrics generated small CMJ performance improvements (ES = 0.58) in female athletes (20). The efficiency of the stretch-shortening cycle, neural drive to the agonist muscles, muscle activation strategies like intermuscular and intramuscular coordination, changes to muscle size and architecture, and changes to single-fiber mechanics are just a few of the neuromuscular-related adaptations that may interact to improve jump performance (18, 20, 23). Given the substantial empirical evidence demonstrating the effectiveness of this method of training, it is not surprising that improvements in jumping performance were caused by the plyometric protocol (18, 20, 23). According to existing research, the eccentric phase of a plyometric exercise with a ground contact time of less than 250 ms demonstrates the longest stretch-shortening cycle stimulation, which maximizes performance (41). The primary neuromuscular mechanisms behind training-induced performance increases must still be investigated in more detail in new research.

Findings of this study indicated that plyometrics combined with traditional handball training induced large significant improvement in RSA scores (best, mean and total time), but no significant change in fatigue index. The lack of significant change in fatigue index could be due to the poor reproducibility of this selected measure (30). This was in accordance with previous studies that also showed trivial to moderate effect sizes for best time, total time, and fatigue index (11, 17). PT effects on the final results in progressive load tests could be explicated by reduced contact time with the surface, improved tendon and muscle rigidity, increased mechanical output caused by the muscles and tendons' elastic attributes, and better movement economy as a whole. After plyometric training, improvement in RSA scores due to higher number of recruited motor-unit and better motor-unit synchronization, increasing firing frequencies, better stretch-shortening cycle efficiency, or increased musculotendinous stiffness (18, 31).

Compared with the performance improvements seen in the change of direction, jumping and RSA tests, there were no observable enhancement in both 1-RM bench press and half-squat PT in our study. This could have occurred due to the multidimensional demands of handball training, but with no appreciable improvement in the 1-RM bench press and 1-RM half-squat performance of the intervention groups, it is unlikely that the plyometrics, as delivered in the current program, exerted any effect on performance in the 1-RM tests. That the plyometrics seemed to exert a preferential impact on SJ and CMJ is unsurprising, given the similarity of the training stimulus to the respective tests used. Likewise, the specificity of plyometric training, which does not contain exercises based on additional load (i.e., moving a load). Although, plyometric exercises primarily target the stretch-shortening cycle and focus on generating power and explosiveness. On the other hand, maximal strength exercises like the half-squat and bench press primarily aim to increase maximum force production. The specific adaptations required for each type of training may differ, and improvements in one may not directly translate to improvements in the other. For instance, Vissing et al. (42) have previously reported the sensitivity of certain physical attributes to training stimuli that share similar characteristics. They demonstrated that a greater extent increased SJ and CMJ by plyometrics than it was by conventional resistance training, thus reinforcing the principle of training specificity (43). To the authors' knowledge, only a few studies have previously focused on the effects of PT on 1-RM bench press or half squat performance in young female athletes (44, 45). The authors noted that 12-week PT can enhance strength (i.e., back squat performance) in female adolescent handball players aged 14.9 years old (45). The disagreement from present findings could be explained by methodological differences (duration of program; the type of exercise; the instrument used: dynamometer test or 1-RM test).

Power is a paramount performance determinant in handball (7). The results of our study showed moderate to large improvements for both upper and lower limb force-velocity performances (**Table 4**). To the authors' knowledge, no study has previously addressed the effects of PT on force-velocity performance in young female athletes. Using similar PT on male players, Chelly et al. (16) reported increases in upper limb force velocity scores (absolute peak power: 27.4%) and peak relative to body mass (28.7%) following an 8-week bi-weekly course of upper limb plyometric training in junior male handball players. Similarly, Chelly et al. (34) found increases of absolute Peak power and peak power relative to body mass. However, no increases of peak power per unit of muscle volume or thigh muscle volume was shown after 8-week PT in male soccer players aged 19 years. Conversely, Hammami et al. (12) failed to find any significant change in all force-velocity scores after 8-week plyometric training in male soccer players (age = 15.8 years). Regarding V0 parameter, our data revealed increases in V0 upper limb, nevertheless V0 lower limb remained unchanged. According to our findings, Chelly et al. (16) demonstrated increases in V0 upper limb performance. However, for both upper and lower limb the F0 score remained unchanged. This coincides with the results of the literature (16, 17, 34). Discrepant findings probably reflect differences in methodology (for instance, the testing of post-adolescent vs. much younger players; elite or professional players vs. regional level players; the format of the plyometric exercises, the frequency, duration and progression of training, and its timing relative to the playing season). In terms of training intensity, volume, and exercise selection we followed the principle of progressive overload, starting with lower intensities, single-joint exercises, and less complex exercise techniques, and progressing to higher intensities, multi-joint exercise, and more complex techniques. In brief, the present study outcomes showed that either plyometric training is equally effective training interventions in improving young female handball players' force-velocity performance.

This study has certain limitations that should be taken into consideration. Firstly, only physical performance was evaluated. Physiological data may provide some neuromuscular mechanisms responsible for the observed findings. Secondly, we did not assess other anthropometric measurements such as limb muscle volume, thigh muscle volume, cross-sectional area, and peak power per unit for both upper and lower limb, which would allow us to make assumptions. Thirdly, although the players were questioned whether they had a typical menstrual cycle, or if they used hormonal contraception, it was not possible to align their training according to their cycles due to the group training. As it was impossible that their cycles ran in tandem with each other, this was not taken into account.

Conclusion

This study demonstrated that a short-term, in-season PT program in place of some handball-specific drills are undoubtedly able to enhance physical fitness measures (i.e., change of direction, jumping, RSA, strength, and power) in youth female handball players. These outcomes could help coaches and practitioners to better structure their training programs concerning the types of training used. PT is a time-efficient and highly helpful method for improvement of both upper and lower limbs physical performance in youth female handball players. Supplementary studies are needed to investigate the effects of PT on muscle morphology and neural adaptations. Similarly, it will be interesting to explore the impact of maturation status as a potential moderator variable.

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 Local Ethics Committee Research Unit (UR17JS01) in conformity with principles identified in the latest version of the Declaration of Helsinki. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

MH designed the study, conducted analyses, and wrote the manuscript. MH, YC, MC, and BK assisted in acquisition, analysis and interpretation of data, and reviewed and edited the article. NG, MH and BK administered the project. RT, NG, MH, MC and BK made a substantial contribution, including conception and a critical revision of the article. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Carbohydrate intake in young female cross-country skiers is lower than recommended and affects competition performance

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Purpose: (1) To evaluate if energy availability (EA), macronutrient intake and body composition change over four training periods in young, highly trained, female cross-country skiers, and (2) to clarify if EA, macronutrient intake, body composition, and competition performance are associated with each other in this cohort.
Methods: During a one-year observational study, 25 female skiers completed 3-day food and training logs during four training periods: preparation, specific preparation, competition, and transition periods. A body composition measurement (bioimpedance analyzer) was performed at the end of the preparation, specific preparation, and competition periods. Competition performance was determined by International Ski Federation (FIS) points gathered from youth national championships.
Results: EA (36–40 kcal·kg FFM^{-1}·d^{-1}) and carbohydrate (CHO) intake (4.4–5.1 g·kg^{-1}·d^{-1}) remained similar, and at a suboptimal level, between training periods despite a decrease in exercise energy expenditure ($p = 0.002$) in the transition period. Higher EA ($r = -0.47$, $p = 0.035$) and CHO intake ($r = -0.65$, $p = 0.002$) as well as lower FM ($r = 0.60$, $p = 0.006$) and F% ($r = 0.57$, $p = 0.011$) were associated with lower (better) FIS-points. CHO intake was the best predictor of distance competition performance ($R^2 = 0.46$, $p = 0.004$).
Conclusions: Young female cross-country skiers had similar EA and CHO intake over four training periods. Both EA and CHO intake were at suboptimal levels for performance and recovery. CHO intake and body composition are important factors influencing competition performance in young female cross-country skiers.

KEYWORDS

endurance sport, energy availability, body composition, FIS points, female athlete, macronutrient, nutrition, periodization

1. Introduction

The annual training plan of most athletes is periodized into macrocycles (training periods), which have their own specific training and performance goals (1). The typical training year for cross-country (XC) skiers consists of a one- to three-part training period between May and November, a competition period from November to April, and a few weeks long transition period from April to May to allow for recovery before a new training year (2–5). The aim of this periodized training plan is to develop a variety of physiological, technical, and tactical capabilities that, together with competitive equipment, are needed for successful XC skiing performance (3).

Due to the variable training load and goals across training periods, nutritional needs may change. Therefore, to promote and support training goals, nutrition should also be periodized (6, 7). Periodized nutrition means that macronutrient intake is modified between training periods to support the specific training goals while maintaining adequate micronutrient intake and energy availability (EA) and avoiding negative health and performance consequences (6, 8, 9). In particular, carbohydrate (CHO) requirements may vary significantly depending on the intensity, volume and goals of the training (6). In XC skiing, most competitions and key training sessions are performed at intensities that are highly dependent on CHO based fuels (3, 10). Consequently, inadequate CHO intake may impair training intensity and competition performance, delay recovery, and increase the risk of lost training days due infection or injury (9–11).

In young athletes, the main goal of nutrition is to ensure optimal energy and nutrient availability to promote performance, recovery, training adaptations, overall health, and normal physical development (12). Physical maturation also induces changes in nutritional needs and performance capabilities, especially for young female athletes, who typically gain more body fat during adolescence between the ages of 8 and 20 years (13). As previous findings suggest that leaner body composition may confer a competitive advantage in young female XC skiers (14), more knowledge is needed, on how nutrition affects performance and body composition.

A recent study by Kettunen et al. (15) found that most young female XC skiers had suboptimal CHO intake and EA during normal training days and during an intensified 5-day training camp, despite some nutritional periodization practices. As research regarding the nutritional practices across the training year is still limited, the aim of this study was to evaluate if EA, macronutrient intake, and body composition change over four macrocycles in young female XC skiers. In addition, the study aimed to clarify how EA, macronutrient intake, body composition, and competition performance are associated with each other.

2. Materials and methods

2.1. Participants

A total of 27 female XC skiers and biathletes from a local high school sport academy (age 15–19 years) provided written informed consent to participate in this observational study. Two participants dropped out due personal reasons and thus the final number of the participants was 25. The proportion of the participants, who belonged to the youth XC or biathlon national team was 64%. Due to some missing data, the number of the participants varies between analyses, and therefore *n* values are presented in tables.

2.2. Design

Data were prospectively collected for one training year from the beginning of May to the end of April (**Figure 1**). The participants completed four 3-day food and training logs, one for each training period (macrocycle) of the training year as follows: preparation (Log_1), specific preparation (Log_2), competition (Log_3), and transition period (Log_4). Laboratory measurements were performed at the end of the preparation (M_1), specific preparation (M_2), and competition periods (M_3). M_3 was performed 12 ± 5 days after the Finnish Youth Championships in XC skiing, which was the main goal of the year for most of the participants. Total training volume (total hours) was recorded daily using an electronic training log (eLogger, eSportwise Oy, Finland). Competition performance was determined by International Ski Federation (FIS) points gathered from Finnish Youth National Championships. The study was approved by the ethical board of the University of Jyväskylä (20.3.2020) and conducted in accordance with the Declaration of Helsinki.

2.3. Anthropometric measurements (M_1, M_2, M_3)

Anthropometric measurements were completed in the morning following an overnight fast. The height of each participant was measured with a wall-mounted stadiometer. Body mass (BM), fat mass (FM), fat free mass (FFM), and fat percent (F%) were measured using bioimpedance analyzer (Inbody 720, Biospace Co., Seoul, Korea). Participants were barefoot and in their underwear during the measurement, which was performed in a private room.

2.4. Race performance

The level of XC skier performance can be evaluated by FIS points (16, 17). FIS point calculations are based on competition

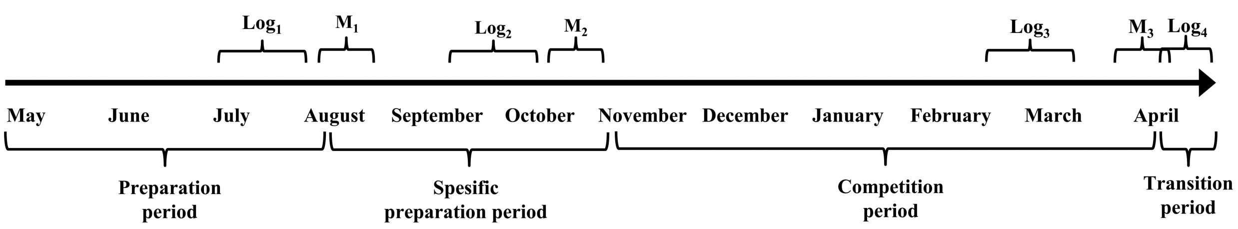

FIGURE 1
The timing of the training periods, food, and training logs (Log_1–Log_4), and measurements (M_1–M_3) during a one-year follow-up study.

performance as presented by Jones et al. (14) and lower FIS points indicate a better performance (17). As many FIS level competitions were canceled due to the COVID-19 pandemic, competition performance was evaluated by FIS points earned from Youth National Championships to which both XC skiers and biathletes took part. The best points from three distance competitions and one sprint competition were recorded separately. A total of 20 participants competed in at least one distance race and 14 participants competed in the sprint race.

2.5. Food and training logs

Three-day food and training logs were collected at four time points (Log_1–Log_4). Participants selected three subsequent days for each log from a 4-week period and were asked to select days that reflect their normal life and training as well as possible. Participants recorded the timing, type, and weight of foods and fluid consumed, quantifying their intake using kitchen scales (Idéale+, Tokmanni Oy, Mäntsälä, Finland). Verbal and written instructions were given to ensure a more accurate record keeping. Participants were instructed to take at least two photographs of the weighed portions, and whenever the scales were not available (e.g., in a restaurant) to validate what was recorded. The timing, type, and average heart rate (HR) of all exercises performed were recorded in training logs. Written and verbal instructions were given for accurate record keeping. Food logs were analyzed for energy intake (EI) and macronutrient intake using Aivodiet-software (version 2.0.2.3, Mashie, Malmö, Sweden). Although there are significant challenges in the validity of food logs, they are the best available tool for assessing dietary intake of the athletes (18).

2.6. Assessment of exercise energy expenditure and energy availability

The exercise energy expenditure (EEE) assessments were based on the individual relationships between HR, oxygen uptake (VO_2) and energy expenditure (EE). An incremental exercise test was performed by walking with ski poles on a treadmill (Telineyhtymä, Kotka, Finland) during M_1, M_2, and M_3. The test started at an inclination of 3.5° with a speed of 5.0 $km^{-1}·h^{-1}$. The inclination and/or speed of the treadmill was increased every third minute so that oxygen demand calculated using the equation by Balke & Ware (19) increased 6 kcal·kg $FFM^{-1}·d^{-1}$ every stage. The treadmill was not stopped between the stages. Breathing gases were measured continuously using Medikro 919 Ergospirometer (Medikro Oy, Kuopio, Finland). The average VO_2 and respiratory exchange ratio (RER) from the last 60 s of each stage were recorded. Heart rate (HR) was monitored continuously throughout the tests using a Polar H10 HR belt (Polar Electro Oy, Kempele, Finland), and the average HR from the last 60 s of each stage was recorded. The protocol was selected as it is commonly used at local Olympic Training Center to monitor participants' performance in a similar way as was done in the present study. Therefore, participants were familiar with the protocol.

The first five stages of the treadmill test were utilized to form an individual regression line for each participant as described by Tomten & Hostmark (20). EE during each stage was calculated as (21):

$$EE = VO2 \times (1.1 \times RER + 3.9)$$

HR was strongly linearly correlated with calculated EE at increasing workloads ($r = 0.99$ at each measurement point) (21). EEE for each training session was calculated from the duration and mean HR of the training session using the regression line. The resting EE during exercise was calculated using the Cunningham equation (22) and subtracted from EEE in line with the latest definition of EA (23). Laboratory-based measures where HR is plotted against indirect calorimetry are regarded as the best methods to assess EEE in field conditions (24). Importantly, the mean intensity of most training sessions recorded were performed at an intensity between the first and fifth stage of the treadmill test.

Daily EA was calculated as (25):

$$EA = \frac{EI - EEE}{FFM}$$

where FFM is fat free body mass obtained from the bioimpedance measurement (25).

As FFM and relationship between HR and EE may change within a year, M_1 was used to analyze Log_1, M_2 to analyze Log_2, and M_3 to analyze Log_3 and Log_4.

2.7. Statistical analyses

Statistical analyses were performed with IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY). All data were checked for normality with a Shapiro-Wilk test and nonparametric tests were used with non-normally distributed data. The changes in variables between different parts of the training year were analyzed using a one-way analysis of variance for repeated measurements followed by the Bonferroni *post-hoc* test. Wilcoxon signed rank test was used for nonparametric variables. Results are reported as means ± SD. Correlations for normally distributed data were analyzed by using Pearson's correlation coefficient (r) and nonparametric data were analyzed using Spearman's correlation coefficient (r_s). Stepwise linear regression analysis was used to determine which variables were the best predictors FIS points. The relative importance of predictors was calculated based on R Square (R^2). A p-value <0.05 was defined statistically significant.

3. Results

As presented in **Table 1**, EI, EEE, and macronutrient intake remained similar between Log_1, Log_2, and Log_3 while EI, EEE,

TABLE 1 Food and training log data between four training periods in young female XC skiers ($n = 23$).

	Preparation period (Log_1)	Specific preparation period (Log_2)	Competition period (Log_3)	Transition period (Log_4)
Energy intake (kcal·kg $FFM^{-1}·d^{-1}$)	50.8 ± 10.6	52.5 ± 9.3	53.3 ± 8.9	46.2 ± 10.7$^{bb, cc}$
Exercise energy expenditure (kcal·kg $FFM^{-1}·d^{-1}$)	14.1 ± 5.5	14.2 ± 5.3	13.3 ± 5.7	7.7 ± 6.9$^{aa, bbb, cc}$
Energy availability (kcal·kg $FFM^{-1}·d^{-1}$)	36.7 ± 11.0	37.8 ± 10.7	40.0 ± 9.6	38.5 ± 11.2
Carbohydrate intake ($g·kg^{-1}·d^{-1}$)	5.0 ± 1.0	5.0 ± 1.1	5.1 ± 1.0	4.4 ± 1.1
Protein intake ($g·kg^{-1}·d^{-1}$)	2.0 ± 0.5	2.1 ± 0.4	2.0 ± 0.4	1.6 ± 0.4$^{aaa, bbb, ccc}$
Fat intake ($g·kg^{-1}·d^{-1}$)	1.5 ± 0.5	1.5 ± 0.4	1.5 ± 0.4	1.3 ± 0.5
Training (food diaries) ($h·d^{-1}$)	2.1 ± 0.7	2.1 ± 0.7	1.9 ± 0.7	1.1 ± 0.7$^{aaa, bb, cc}$
Training (eLogger) ($h·d^{-1}$)	1.9 ± 0.3	1.8 ± 0.3	1.5 ± 0.5$^{aa, bb}$	0.5 ± 0.4$^{aaa, bbb, ccc}$

^{aa}Significantly different from preparation period (Log_1) $p < 0.01$.
$^{aaa}p < 0.001$.
^{b}Significantly different from specific preparation period (Log_2) $p < 0.05$.
$^{bb}p < 0.01$.
$^{bbb}p < 0.001$.
^{c}Significantly different from competition period (Log_3) $p < 0.05$.
$^{cc}p < 0.01$.
$^{ccc}p < 0.001$.

and protein intake were lower in Log_4 than in Log_1, Log_2, and Log_3. Nevertheless, EA remained statistically similar between the logs. Yearly training volume was 610 ± 89 h and ranged between 450 and 815 h. There were no significant differences between the daily training volume reported in the food and training logs compared to the mean training volume reported in the electronic training diaries during each training period.

As presented in Table 2, BM and body mass index (BMI) increased from M_1 to M_2 and further from M_2 to M_3. In addition, FM and F% increased from M_2 to M_3.

Mean FIS distance points for participants from the Youth National Championships were 171.6 ± 52.0 ($n = 20$, range 101.0–274.6) and mean FIS sprint points were: 186.2 ± 32.0 ($n = 14$, range 127.2–239.6).

As presented in Table 3, better success in XC distance races, indicated by lower FIS distance points, was associated with higher EA and macronutrient intake as well as with lower FM and F%. FIS sprint points were negatively associated with training volume. In addition, athletes with higher FM and F% tended to eat less CHO, protein, and fat in relation to their BM (Table 3). When stepwise linear regression was performed using the variables presented in Table 3, CHO intake explained 46% of the variance in FIS distance points ($R^2 = 0.46$, $p = 0.004$). Training volume was the best predictor for FIS sprint points ($R^2 = 0.34$, $p = 0.036$).

TABLE 2 Anthropometric variables in the end of three macrocycles in young female XC skiers.

	n	M_1 (August)	M_2 (November)	M_3 (April)
Body mass (kg)	24	61.8 ± 6.8	63.1 ± 6.8^{aaa}	64.7 ± 7.3$^{aaa, bb}$
Height	24	168.2 ± 5.2	168.5 ± 5.3	168.6 ± 5.2
BMI ($kg·m^{-2}$)	24	21.8 ± 2.2	22.2 ± 2.2^{aaa}	22.7 ± 2.7$^{aaa, bb}$
Fat free mass (kg)	23	51.0 ± 5.2	51.9 ± 5.0	51.5 ± 4.9
Fat mass (kg)	23	10.6 ± 3.6	10.9 ± 4.0	13.0 ± 4.3$^{aaa, bbb}$
Fat percent (%)	23	17.0 ± 4.8	17.1 ± 5.2	20.0 ± 5.0$^{aaa, bbb}$

^{a}Significantly different from M_1 $p < 0.05$.
$^{aa}p < 0.01$.
$^{aaa}p < 0.001$.
^{b}Significantly different from M_2 $p < 0.05$.
$^{bb}p < 0.01$.
$^{bbb}p < 0.001$.

4. Discussion

The present study assessed the changes in nutritional intake and body composition during a training year in young female XC skiers. The results showed that athletes had similar EEE, EI, EA, and macronutrient intake during preparation, specific preparation, and competition periods. However, athletes decreased EI during the transition period, where they also experienced lower EEE, thus maintaining EA. Unfortunately, in most athletes, CHO and EA were lower than recommended for performance and training adaptations (9). The second aim of the study was to assess the relationships between nutrition, body composition, and competition performance. Interestingly, better performance in XC distance competitions was associated with higher EA and macronutrient intake as well as with lower FM and F%. CHO intake was the best predictor for FIS distance points.

The EEE and training volume between preparation (Log_1), specific preparation (Log_2), and competition periods (Log_3) remained similar but decreased significantly in the transition period (Log_4). Also nutritional requirements of the training remained quite similar during the first three training periods, where athletes had similar EI, EA, and macronutrient intake. Nevertheless, during the transition period, athletes adapted to smaller energy needs by consuming less energy. Therefore, athletes seemed to periodize their EI between transition period and other training periods. Indeed, EA remained stable despite the variation in energy needs, which is in line with periodized nutrition recommendations (6). This finding is also in line with the findings of Ihalainen et al. who found that young female runners had similar EA in different parts of their training year

TABLE 3 Correlation coefficients (r_s for BM, r for others) between FIS points, EA, and macronutrient intake (the mean of Log_1, Log_2 and Log_3), anthropometrics in the end of the competition season, and yearly training volume.

	FIS_d	FIS_s	EA	CHO	Protein	Fat	BM	FFM	FM	F%	Training
FIS_d	1										
FIS_s	0.36	1									
EA	−0.47*	−0.23	1								
CHO	−0.65**	0.01	0.69***	1							
Protein	−0.51*	0.22	0.53**	0.75***	1						
Fat	−0.53*	0.31	0.82***	0.69***	0.65**	1					
BM	0.42	−0.02	−0.23	−0.28	−0.08	−0.27	1				
FFM	0.28	−0.13	−0.38	−0.16	0.11	−0.17	0.83***	1			
FM	0.60**	0.09	−0.37	−0.60**	−0.60**	−0.49*	0.77***	0.28	1		
F%	0.57*	0.17	−0.20	−0.55**	−0.64**	−0.43*	0.51*	−0.06	0.93***	1	
Training	−0.27	−0.58*	−0.31	−0.29	−0.31	−0.09	0.07	−0.02	0.13	0.11	1

FIS_d, FIS distance points; FIS_s, FIS sprint points; EA, energy availability (kcal·kg $FFM^{-1}·d^{-1}$); CHO, carbohydrate intake (g·$kg^{-1}·d^{-1}$); BM, body mass; FFM, fat free body mass (kg); FM, fat mass (kg); F%, fat percent.
*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.

(26). Unfortunately, the data of the present study does not reveal whether the lower EI during transition period was intentional or spontaneous.

The mean EA of athletes during the present one-year follow-up varied between 36 and 40 kcal·kg $FFM^{-1}·d^{-1}$, which is considered suboptimal for performance and training adaptations but adequate to maintain normal physiological functions (25). The EA detected in the present study is similar to that reported in young female XC skiers at home and training camp conditions (15) and in young female distance runners during different parts of the training year (26). Notably, the individual variation was high, and six athletes had low EA (<30 kcal·kg $FFM^{-1}·d^{-}1$) in Log_1, while four had low EA in Log_2, two in Log_3, and five in Log_4. In the long term, these individuals may be at risk to develop deleterious health and performance consequences related to low EA such as hormonal disturbances, decreased bone health, and increased risk of injury (8, 27).

When comparing the results from the first three food logs to the nutrition recommendations for endurance athletes training 1–3 h·d^{-1}, protein intake (2.0–2.1 g·$kg^{-1}·d^{-1}$) was in the upper limits (1.2–2.0 g·$kg^{-1}·d^{-1}$), fat intake (1.5 g·$kg^{-1}·d^{-1}$ i.e., −31% of total EI) was in the recommended range (20%–35% from total EI), and CHO intake (5.0–5.1 g·$kg^{-1}·d^{-1}$) was less than recommended (6–10 g·$kg^{-1}·d^{-1}$) (9). These findings are similar to those previously reported in Swedish national team XC skiers (28) and in Finnish Youth National Team XC skiers (15). Thus, it seems that consuming adequate CHO is a notable challenge for female XC skiers of different ages and nationalities.

BM and F% increased significantly over the training year and especially during the competition period, which is against the body composition periodization principles that are used to optimize performance in elite athletes (29). Importantly, an increase in BM, FM, and F% is also a normal physiological phenomenon during adolescence (13), which may explain, at least partly, why BM, FM, and F% increased as the study progressed. BM increased evenly from M_1 to M_3, while the increase from M_1 to M_2 was mostly explained by the increase in FFM while the increase from M_2 to M_3 is explained by an increase in FM, which led to a simultaneous increase in F%.

Better competition performance in distance events at the Youth National Championships was associated with higher EA and macronutrient intake as well as with lower FM and F%. CHO intake was the best predictor for FIS distance points. The present results indicate that adequate CHO intake across the training year may have an important role in optimizing competition performance. This finding is logical, as inadequate CHO intake may impair training intensity and competition performance, delay recovery and increase the risk of lost training days due infection or injury (9–11).

In addition to the findings that nutritional intake and body composition were associated with performance, we found that anthropometric and nutritional variables correlated with each other. Interestingly, higher FM and F% were associated with lower intake of all macronutrients. Because of the observational study setting, reliable causal relationships cannot be determined. One potential explanation may be that athletes with higher body fat restricted their eating to lose BM without desired results. Another explanation may be that higher body fat is an adaptation to chronic low EA as long-term low EA leads to energy conserving metabolic changes that, over the long term may become detrimental to training adaptations and sport performance (8, 27). It is also possible that athletes who have adopted proper eating practices that support their training, have succeeded in developing their performance from year to year whereby their body composition has adapted to the demands of their sport due to successful training and/or genetics. It is worth noting, that the macronutrient intake in the present study and current nutritional recommendations (9) are expressed in relation to BM. Athletes with higher FM and F% have higher amount of metabolically inactive tissue and therefore, it is possible that they do not need as much fuel in relation to their BM as their leaner counterparts.

FIS sprint points were predicted by yearly training volume but were not associated with any other variables. Similar results were reported by Jones et al. (14) who did not find any predictors for

FIS sprint points. In contrast, Carlsson et al. (30) found that lean body mass predicted XC sprint performance in elite female skiers. It is difficult to assess why training volume predicted sprint but not distance performance in present study. Nevertheless, it is important to note that only 14 participants took part in the sprint race at Youth Nationals, which increases the risk of error in FIS sprint point analyses, thus these results should be interpreted with caution.

The present study has some limitations. One of the major limitations is that the methods used to assess dietary intake and EA in field conditions are prone to errors (24, 31). To minimize these errors, we selected methods that are considered the most valid to assess EEE and dietary intake in field conditions (24). Food and training logs were completed during different parts of the training year, which gave more reliable information regarding overall practices. Notably, training logs reflected the training volume expected from each training period suggesting that training logs reflected actual training completed. Finally, FIS points were recorded only from single races instead of a yearly score. Nevertheless, we believe that this gave the best possible description of the competition performance as many races were cancelled due to the COVID-19 pandemic and the Youth National Championships were among the most important competitions for our participants. Importantly, it should be recognized that non-physiological factors such as ski selection, waxing, and environmental factors may have affected competition performance and FIS points.

4.1. Conclusions

Young female XC skiers maintained similar, but suboptimal, EA and carbohydrate intake between four training periods. EEE remained similar between preparation, specific preparation, and competition periods suggesting similar dietary requirements during these macrocycles. Nevertheless, athletes experienced lower EEE during the transition period, but maintained EA by decreasing EI. CHO intake across the training year seems to be an important predictor for XC distance competition performance in young female XC skiers. Furthermore, lower BM, FM, and F% may be beneficial for distance competition performance. Based on our results, however, restricting dietary intake may not be an optimal way to modify body composition in young female athletes. Therefore, based on the findings of present study, young female XC skiers should aim to maintain adequate EA and CHO intake throughout the training year to promote long-term development in competition performance.

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 Ethical board of the University of Jyväskylä. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

Conceptualization and methodology: OK, VL, and JI; investigation: OK; statistical analysis: OK and JM; original draft preparation: OK; Writing—review and editing: OK, RM, VL, JM, and JI; supervision: RM, VL, and JI. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Heart rate variability of elite female rowers in preparation for and during the national selection regattas: a pilot study on the relation to on water performance

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Elite athletes require a delicate balance of physiological and psychological stress and recovery—essential for achieving optimal performance. Monitoring heart rate variability (HRV) provides a non-invasive estimation of both physiological and psychological stress levels, offering potentially valuable insights into health, performance, and adaptability. Previous studies, primarily conducted on male participants, have shown an association between HRV and performance in the context of rowing training. However, given the rigorous nature of rowing training, it is crucial to investigate HRV in elite rowers, particularly during the U.S. national selection regattas (NSR).

Purpose: To comprehensively analyze elite female rowers, evaluating acute changes in HRV and subjective psychometrics during the NSR.

Methods: Five elite female rowers (26 ± 2 years, 180 ± 8 cm, 82 ± 8 kg, 19 ± 6%fat) were recruited and tracked prior to and during NSR I and II. Morning HRV measures were completed using photoplethysmography (HRV4training) along with self-reported levels of fatigue, soreness, rating of perceived exertion, mentally energy and physical condition.

Results: Significant decreases were observed in log transformed root-mean square of successive differences (LnRMSSD; $p = 0.0014$) and fatigue ($p = 0.01$) from pre-to-during NSR, while mental energy ($p = 0.01$), physical condition ($p = 0.01$), and motivation ($p = 0.006$) significantly increased. These psychometric measures returned to pre-NSR levels, at post-NSR (all $p < 0.05$), though HRV remained slightly suppressed. NSR on-water performance was not correlated to LnRMSSD or the change in LnRMSSD ($p > 0.05$).

Discussion: HRV and psychometric measures are sensitive to the stress of elite rowing competition in females. However, HRV was not associated with on-water rowing performance during an elite rowing competition.

KEYWORDS

women, cardiac autonomic activity, rowing, peformance, longitudinal, training

1. Introduction

In recent years, sport scientists and coaches have increasingly employed heart rate variability (HRV) as a non-invasive physiological marker for evaluating and enhancing athlete adaptations to training and subsequent performance (1–3). HRV serves as an index of the autonomic nervous system reflecting the interaction between the sympathetic and parasympathetic systems influence on the heart (4, 5). It is assessed by measuring the variation in R-R intervals, where a changes in the duration of R-R intervals indicates altered autonomic activity (3). It has previously been established that HRV measures are associated with health outcomes, adaptability to training regimens, and athletic performance (6–9), making HRV a good candidate for athlete monitoring. Intense training or psychological stress can suppress vagal indices of HRV, indicating reduced parasympathetic activity (7). Conversely, heightened sympathetic nervous system activity, as measured via HRV, has been linked to fatigue and overtraining (7, 8, 10). For athletes and coaches, it is important to understand and monitor the physiological and psychological stress associated with training, traveling, and competing, to optimize training and performance, but for some sports, such elite female rowers, there is a paucity of data.

Rowing is a high-intensity sport, which requires significant strength, power, anaerobic and aerobic capacity (11–14). International race distance is 2 km and which is covered in 5.5 to 7.5 min in elite rowers, depending upon boat class and gender (15) and course conditions (16). During racing, aerobic metabolism likely contributes 67%–84% of the energy requirement (15); thus, the remaining 16%–33% of energy demand is met through anaerobic energy producing pathways such as the phosphocreatine shuttle and anaerobic glycolysis (11, 17). Regarding the latter, peak rowing power over 5 or 10 stroke maximal tests has been found to be highly related to 500 m (18) and 2 km times (12). Rowing performance, both on the water and on a rowing ergometer, is dependent on several physical determinants, such as height and wing span [recently highlighted here (19)], and physiological determinants, as mentioned above, but also includes maximal oxygen consumption (VO_{2max}) and the power output associated with VO_{2max} (W_{VO2max}) (12, 20). However, quantifying on-water performance is often challenging because of the environment and the inability to assess the performance of the crew individually. Although ergometer performance tests eliminate these constraints, they do not sufficiently address the skill aspect of performance on the water (20), and can take away from technical training on the water which is also essential for performance. As such, identifying key physiological variables able to monitor and/or predict on-water performance has merit for coaches aiming to effectively periodize training workloads. Considering the rise in popularity of HRV monitoring tools for self-monitoring, in an autonomous manner, future studies should explore their real-world use.

Given the effectiveness of HRV as in indirect indicator of the body's ability to tolerate the stress of exercise training and the limited study of elite rowers, let alone female rowers, this pilot study sought to characterize a group of elite rowers (body composition, VO_{2peak}, and peak rowing power output), and assess changes in autonomously monitored HRV and subjective psychometrics leading to, during, and shortly after U.S. National Selection Regattas (NSR). For this purpose, we hypothesized that HRV, self-reported levels of mental energy, and physical condition would significantly decrease during the NSR and recover following the competition rebound post-competition. It was also hypothesized that HRV, and the perturbation in HRV in response to NSR competition, would be related to on-water rowing performance. Additionally, we hypothesized that self-reported levels fatigue, soreness, rating of perceived exertion (RPE) would significantly increase during the NSR and return to normal post-competition.

2. Methods

2.1. Participants and general procedures

Elite female rowers were recruited from the Saratoga Rowing Association Advanced Rowing Initiative of the Northeast (ARION) program. The athletes were classified as elite development as they were training for national team selection and/or national and international level regattas, with rowing experience (secondary school and/or collegiate). Participants were recruited verbally and through emails and in coordination with the head coach. In addition to being part of the ARION training group, inclusion criteria required that the participants were healthy, English-speaking, and a smart-phone user. Exclusion criteria involved any chronic disease or illness or injury that would prevent them from training or one that could alter their HRV (e.g., atrial fibrillation), which was acquired by health history form and reviewed by the study team. The athletes provided written informed consent prior to participation. This protocol was reviewed and approved by the Skidmore College Institutional Review Board (IRB#2112-1010) and conducted in accordance with the most recent revisions to the Declaration of Helsinki.

2.2. Study overview

The athletes underwent in-person baseline testing in the Human Performance Research Laboratory at Skidmore College. Upon the participants' arrival, they were screened for eligibility and written informed consent was obtained. Baseline testing included a body composition analysis, a maximal power output test and a peak oxygen consumption (VO_2peak) test (21–23). Following preliminary testing, participants tracked their HRV and psychometrics each morning leading up to and post the NSRs using the HRV4Training mobile device application. NSR 1 and NSR 2 occurred 5- and 9-weeks following baseline testing. The rowers official 2 km times during heats, semi-finals, and finals of the NSRs were used to assess the on the water performance, and the best times were used for analysis.

2.3. Baseline assessment

Height was measured using a stadiometer and body composition was obtained using air displacement plethysmography (Bod Pod, CosMed, Chicago, IL, USA) which is a known, reliable method of assessing body composition (22). To obtain the most accurate results possible, participants were asked to refrain from eating or exercise (only snack and water) at least 3 h prior to testing, use the restroom upon arrival, remove glasses and jewelry, if possible, to wear their uni-suit, and wore a provided swim cap to compress air pockets within the hair.

A maximal rowing power performance test was conducted during single trial following a participant controlled 5-min warm up (23). Starting from a rested (non-spinning) flywheel, participants were then instructed to row as hard as possible for 10 strokes completed (approximately 15 s) on a Concept2 rowing ergometer (Model D, Concept2, Morrisville, VT) a pre-determined drag factor of 10, as described previously (18, 24). The maximum, or highest, single power output in watts was recorded. A brief recovery was allotted, and then a graded VO_{2peak} test was run to characterize the aerobic fitness of the rowers (15). To measure oxygen consumption, participants were instrumented with a two-way non-rebreathe mouthpiece (8,900, HansRudolph, Shawnee, KS) attached via hose to a metabolic cart (TrueOne 2,400, Parvomedics, Sandy, UT), which has been documented to be reliable and valid assessment of VO_2 (21). The participant also wore a chest strap style heart rate monitor (H7, PolarUSA, Lake Success, NY). Participants were seated on the rowing machine until reasonable baseline relative VO_2 values were obtained (3–5 ml/kg/min) before starting the graded exercise test. The graded exercise test started at 120 watts for 3 min, increasing 30 watts per 3 min stage until volitional exhaustion and/or failure to maintain workload following a similar previously published protocol (25). Increases in work rate were achieved through increased drive force ("pressure") and/or stroke rate (strokes/minute). This was a maximal effort test. Participants' HR, ventilation, VO_2, and respiratory exchange ratio (RER) were continuously monitored throughout the test and as criteria as to whether participants achieved a near-maximal or maximal VO_2.

2.4. HRV and psychometric monitoring

Following baseline testing participants were tasked with remotely recording their heart rate variability each morning upon waking using the HRV4Training mobile device application (Amsterdam, Netherlands; see http://www.hrv4training.com/). The HRV4Training application uses photoplethysmography (PPG) to obtain R-R intervals from a continuous pulse rate reading (26, 27). Participants were given a familiarization session with the application along with an instructional document for reference; additionally, the application uses a step-by-step process to walk the user through the measurement. In the morning after voiding their bladder, participants recorded their HRV for 1 min upon waking while in a supine position while breathing at self-selected pace (28). HRV was tracked using root mean square of successive differences (RMSSD), natural log transformed RMSSD (LnRMSSD), and standard deviation of N-N intervals (SDNN). The mobile device application also tracks other factors that may influence HRV, such as sleep, training, menstrual cycle status, which the participant was asked to complete with each reading. A two-week period was used to establish a normal range for each individual, the app provided a daily assessment as to whether the athletes' HRV was within, above, or below their normal range. This data was relevant for comparing HRV and training performance.

2.5. Data analysis

During the weeks of March 22nd–25th the participants competed in NSR I and May 3rd–6th in NSR II. The rower's 2 km performance times were recorded and compared to their HRV of the corresponding day. Additionally, changes in LnRMMSD, 7-day average of LnRMMSD, RHR, SDNN, RPE, mental energy, soreness, fatigue, physical condition, and motivation pre, during, and post-NSR were assessed with a linear mixed model with the fixed effect of time (pre, during, post NSR) and the random effect of subject ID. Significant main effects were followed up by pairwise comparisons. Linear mixed models were checked for homoscedasticity via visual inspection and Q-Q plots. Finally, a linear regression was performed to assess the relationship between best on-water performance, absolute LnRMMSD during the each NSR, and the percent change in LnRMMSD from pre to during NSR. All calculations and statistical analyses were run using Microsoft Excel (Microsoft Excel, v 16.43, Redmond, United States) and open-source statistical software JAMOVI (29, 30). Estimates of effect size, using Cohen's small (0.2), medium (0.5), and large (0.8) were used in accordance with the model complement p values. The α-level was set to 0.05 and used to determine statistical significance.

3. Results

3.1. Baseline subject characteristics and performance

Participants were 26.4 ± 1.7 years, height 180 ± 2 cm, weight 81.8 ± 8.4 kg, percent body fat 19.3 ± 6.5%, and percent fat free mass 80.7 ± 6.5% (**Table 1**). Participants baseline and on water performance characteristics are presented in **Table 2**, average maximal power output was 681 ± 71 watts, peak heart rate 181 ± 17 bpm, and the relative VO_{2peak} 52.6 ± 3.4 ml/kg/min (**Table 2**). All participants VO_2 max ranked >75th percentile for age and sex specific norms. Individual daily HRV values and rolling averages are presented in **Table 3**.

3.2. Changes in estimated cardiac autonomic activity during the NSR

A significant main effect of time was observed in LnRMSSD ($F = 3.82$, $p = 0.02$, **Figure 1A**). A pairwise comparison revealed a

TABLE 1 Subject age, height, weight, % fat and % fat free mass.

Subject ID	Age	Height (cm)	Weight (kg)	% fat	% fat free Mass
1	29	184	75.0	11.1	88.9
2	25	177.5	73.0	18.8	81.2
3	27	179	93.0	26.0	74.0
4	27	179	90.8	27.2	72.8
5	24	179	77.3	13.4	86.6
Mean ± SD	26.4 ± 1.74	179.7 ± 2.23	81.8 ± 8.39	19.3 ± 6.47	80.7 ± 6.48

significant reduction in LnRMSSD occurred for pre-to-during NSRs ($p = 0.006$, $t = 2.78$, ES = 0.47). LnRMSSD was not different during-to-post NSRs ($p = 0.15$, $t = 1.87$, ES = 0.24) or pre-to-post NSRs ($p = 0.11$, $t = -1.61$, ES = 0.27). There was a significant main effect for time on RHR ($F = 5.51$, $p = 0.005$, Figure 1B). A pairwise comparison revealed a significant increase in RHR from pre-to-during NSRs ($p = 0.001$, $t = -3.32$, ES = 0.56) and from during-to-post NSRs ($p = 0.02$, $t = 2.25$, ES = 0.38). There was no significant difference in RHR from pre-to-post NSRs ($p = 0.19$, $t = -1.32$, ES = 0.22) Additionally, there was no main effect for time on SDNN ($F = 2.91$, $p = 0.05$, Figure 1C) during the NSRs. As an aspect of the current study was to utilize autonomous HRV monitoring (vs. laboratory and/or researcher based) we observed a 91% compliance rate, with the athletes completing 141 out of 154 possible measurements over the study time frame.

3.3. Changes in psychometric dimensions of performance during the NSR

A significant main effect of time was observed for mental energy ($F = 5.05$, $p = 0.008$, Figure 2C). A pairwise comparison revealed a significant increase in mental energy for pre-to-during NSRs ($p = 0.002$, $t = -3.17$, ES = 0.53) followed by a significant decrease for during-to-post NSRs ($p = 0.03$, $t = 2.20$, ES = 0.37). Pairwise comparison revealed no significant difference in mental energy between pre-to-post NSRs ($p = 0.23$, $t = -1.19$, ES = 0.20). A significant main effect of time was observed for soreness ($F = 10.4$, $p = 0.001$, Figure 2D). A pairwise comparison revealed that soreness was significantly decreased for between pre-to-during NSRs ($p = 0.001$, $t = 3.27$, ES = 0.55) and a significant increase for during-to-post NSRs ($p = 0.001$, $t = -4.52$, ES = 0.76). No significant difference was observed for pre-to-post NSRs ($p = 0.15$, $t = -1.44$, ES = 0.24). A significant main effect of time was observed for fatigue ($F = 8.03$, $p = 0.001$, Fatigue 2E). A pairwise comparison revealed a significant decrease in fatigue for pre-to-during NSRs ($p = 0.01$, $t = 3.49$, ES = 0.59), while a significant increase in fatigue was observed for during-to-post NSRs ($p = 0.001$, $t = -3.72$, ES = 0.63). There was no significant difference in fatigue for pre-to-post NSRs ($p = 0.79$, $t = -0.26$, ES = 0.04). A significant main effect for time was found for physical condition ($F = 4.84$, $p = 0.009$, Figure 2B). Additionally, a pairwise comparison found no change in physical condition for pre-to-during NSRs ($p = 0.21$, $t = -1.26$, ES = 0.21). While a significant decrease was observed for during-to-post NSRs ($p = 0.003$, $t = 2.99.56$, ES = 0.50) and between pre-to-post NSRs ($p = 0.04$, $t = 2.03$, ES = 0. 34). A significant main effect for time was observed for motivation ($F = 6.05$, $p = 0.003$, Figure 2F). A pairwise comparison found a significant increase in motivation for pre-to-during NSRs ($p = 0.001$, $t = -3.32$, ES = 0.56), while a significant decrease in motivation was observed for during-to-post NSRs ($p = 0.004$, $t = 2.92$, ES = 0.49). No significant differences in motivation were observed for pre-to-post NSRs ($p = 0.61$, $t = -0.50$, ES = 0.08). A significant main effect was observed for training RPE ($F = 13.4$, $p = 0.001$, Figure 2A). A pairwise comparison found a significant increase in training RPE for pre-to-during NSRs ($p = 0.001$, $t = -4.99$, ES = 0.91) and a significant decrease from during-to-post NSRs ($p = 0.001$, $t = 4.15$, ES = 0.76). No significant differences in training RPE for pre-to-post NSRs ($p = 0.43$, $t = -0.78$, ES = 0.14).

3.4. Estimated cardiac autonomic activity and relation to on-water rowing performance

No significant relationship was observed for LnRMSSD during the NSR 1 and on water performance ($p = 0.29$, R = 0.59, $R^2 = 0.35$, Figure 3A) or for the % change in LnRMMSD from pre to during NSR 1 and on water performance ($p = 0.95$, R = 0.03, $R^2 = 0.00$, Figure 3C). Additionally, no significant relationship was observed for LnRMMSD during NSR 2 and on water performance ($p = 0.23$, R = 0.65, $R^2 = 0.42$, Figure 3B) or for the % change in LnRMMSD from pre to during NSR 2 and on water performance ($p = 0.16$, R = 0.73, $R^2 = 0.53$, Figure 3D).

TABLE 2 Subject laboratory and on water performance characteristics.

ID	Peak power (W)	Peak HR (bpm)	Peak absolute VO_2 (L/min)	Peak relative VO_2 (ml/kg/min)	Age adjusted VO_2 ranking	Anaerobic threshold (AnT) (W)	HR at AnT (bpm)	Average NSR 1 time (s)	Average NSR 2 time (s)
1	643	184	4.22	56.3	95th %	270	173	452.64	427.01
2	571	195	4.16	57.06	95th %	240	181	461.99	425.31
3	763	190	4.64	49.89	75–80th %	240	165	445.64	N/A
4	677	188	4.49	49.44	75–90th %	240	159	469.27	435
5	750	183	3.89	50.34	75–90th %	240	168	461.00	429.71
Mean ± SD	681 ± 71	181 ± 17	4.3 ± 0.3	52.6 ± 3.4	N/A	246 ± 12	169 ± 7		

TABLE 3 Individual daily and rolling 7-day average of LnRMSSD pre, during, and post NSR 1 &2.

ID	Pre-NSR 1 daily LnRMMSD							During NSR 1 daily LnRMMSD				Post-NSR 1 daily LnRMSSD						
1	8.0	7.4	8.0	8.4	7.6	8.4	8.0	7.8	7.7	7.6	7.4	6.6	7.7	7.6	8.0	8.7	9.2	8.3
2	7.2	8.2	7.0	7.2	7.0	7.6	7.2	7.5	7.2	6.8	7.0	7.5	7.0	7.8	8.0	7.2	7.9	7.5
3	8.1	8.4	8.7	9.1	8.6	8.6	8.4	–	–	–	–	–	–	7.6	8.3	–	8.2	8.8
4	7.8	8.3	9.0	7.6	9.1	8.4	7.2	7.7	7.7	8.1	6.4	8.1	–	8.7	7.6	7.3	7.4	7.3
5	8.9	–	10.2	8.9	10.9	12.6	8.7	–	–	9.0	9.6	8.7	9.8	8.4	8.4	8.9	8.3	–
ID	Pre-NSR 2 daily LnRMMSD							During NSR 2 daily LnRMMSD				Post-NSR 2 daily LnRMSSD						
1	7.9	7.6	9.4	7.9	–	9.6	9.0	8.4	8.1	7.9	8.0	7.7	9.2	8.8	8.2	7.3	7.5	7.1
2	7.1	7.1	7.9	7.6	7.4	8.0	7.3	7.2	7.6	6.9	7.1	8.2	8.7	7.7	7.7	7.6	7.2	7.7
3	-	9.0	–	8.7	–	7.0	8.1	–	8.8	–	9.0	8.8	7.8	8.8	8.6	8.8	8.5	8.4
4	7.5	7.2	7.4	7.5	8.4	8.2	7.2	6.3	6.2	8.1	7.8	7.3	6.3	7.2	-	8.2	8.0	8.7
5	9.6	–	–	9.2	7.8	9.3	9.6	–	8.7	9.1	8.6	8.6	–	9.0	9.2	8.7	9.2	8.6
ID	Pre-NSR 1 7-day average LnRMSSD							During NSR 1 7-day average LnRMSSD				Post-NSR 1 7-day average LnRMSSD						
1	8.3	8.2	8.1	8.2	8.0	8.1	8.0	8.0	8.0	7.9	7.8	7.6	7.5	7.5	7.5	7.6	7.9	8.0
2	7.3	7.5	7.4	7.5	7.4	7.4	7.3	7.4	7.2	7.2	7.2	7.3	7.2	7.3	7.4	7.3	7.5	7.6
3	8.2	8.2	8.3	8.5	8.5	8.6	8.6	NA	NA	NA	NA	8.5	8.4	7.6	8.0	8.0	8.1	8.2
4	7.7	7.8	7.9	7.9	8.2	8.3	8.2	8.2	8.1	8.0	7.8	7.6	7.5	7.8	7.8	7.7	7.6	7.7
5	9.6	9.5	9.7	9.5	10.0	10.4	10.2	10.3	10.0	10.2	9.7	9.2	9.1	9.0	9.0	8.9	7.5	7.6
ID	Pre-NSR 2 7-day average LnRMSSD							During NSR 2 7-day average LnRMSSD				Post-NSR 2 7-day average LnRMSSD						
1	7.7	7.7	7.9	7.9	7.9	8.2	8.5	8.6	8.7	8.4	8.5	8.4	8.3	8.3	8.3	8.2	8.1	8.0
2	7.3	7.2	7.4	7.4	7.4	7.5	7.5	7.5	7.5	7.4	7.3	7.4	7.6	7.6	7.7	7.7	7.7	7.8
3	8.3	8.4	8.7	8.6	8.6	8.3	8.2	8.2	8.2	8.2	8.2	8.3	8.5	8.7	8.6	8.6	8.6	8.5
4	7.5	7.2	7.4	7.5	8.4	8.2	7.2	6.3	6.2	8.1	7.8	7.3	6.3	7.2	–	8.2	8.0	8.7
5	8.6	8.6	8.6	8.7	8.5	8.9	9.1	9.0	8.9	8.9	8.8	9.0	8.9	8.8	8.9	8.9	8.9	8.9

4. Discussion

The current pilot study was one of the first to assess the autonomously measured changes in HRV and psychometrics during high-level competition and relation to on-water performance in elite female rowers. The purpose of the current study was to characterize a group of elite rowers (body composition, peak aerobic fitness, and maximal power output), and assess acute changes in HRV, as an estimate of cardiac autonomic activity, and subjective psychometrics during the NSRs. We observed decreases in LnRMSSD during competition which rebounded 72 h post competition. However, neither HRV nor the change in LnRMSSD were related to on-water performance during the NSR. Secondly, these changes were accompanied by alterations in psychometrics, such increased mental energy, decrease in fatigue, and increased self-report of physical condition, while soreness was unaffected during the competition; these parameters returned to baseline approximately 72 h post competition. This is the first study to report autonomous HRV and perceptual dimensions of training and performance in rowers; thus, collectively we demonstrate that athletes are capable, and willing, to self-monitor (observed 91% compliance rate), which can provide an insight into their physiological and psychological status in the context of high-level competitions.

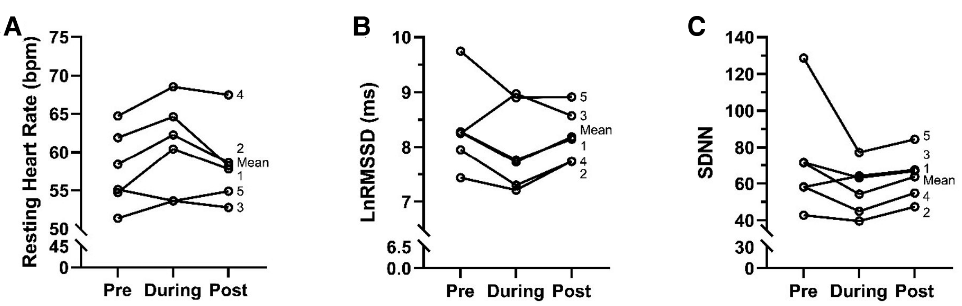

FIGURE 1
Estimated cardiac autonomic assessments pre, during, and post NSRs in elite female rowers (n = 5). (A) Resting heart rate, RHR (beats/min), (B) natural log transformed root mean square of successive differences, LnRMMSD, (C) standard deviation of N-N intervals, SDNN.

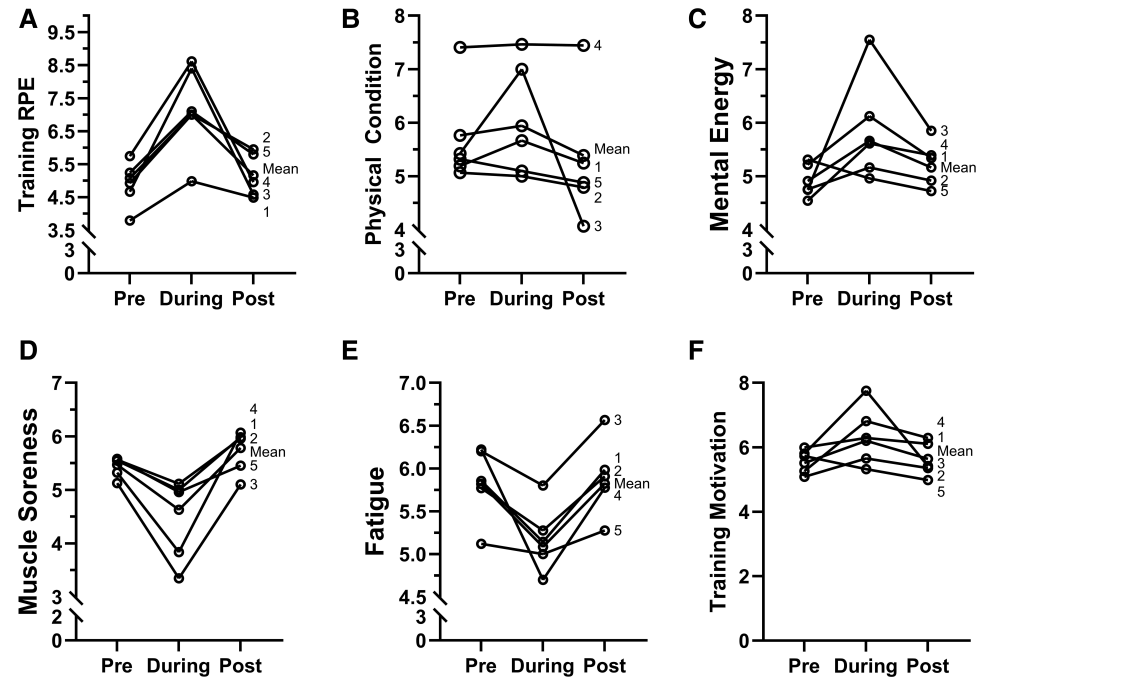

FIGURE 2
Performance related psychometrics for pre, during, and post NSRs in elite female rowers (n = 5). (A) Training rating of perceived exertion (training RPE), (B) physical condition (C) mental energy, (D) muscle soreness, (E) fatigue, (F) training motivation.

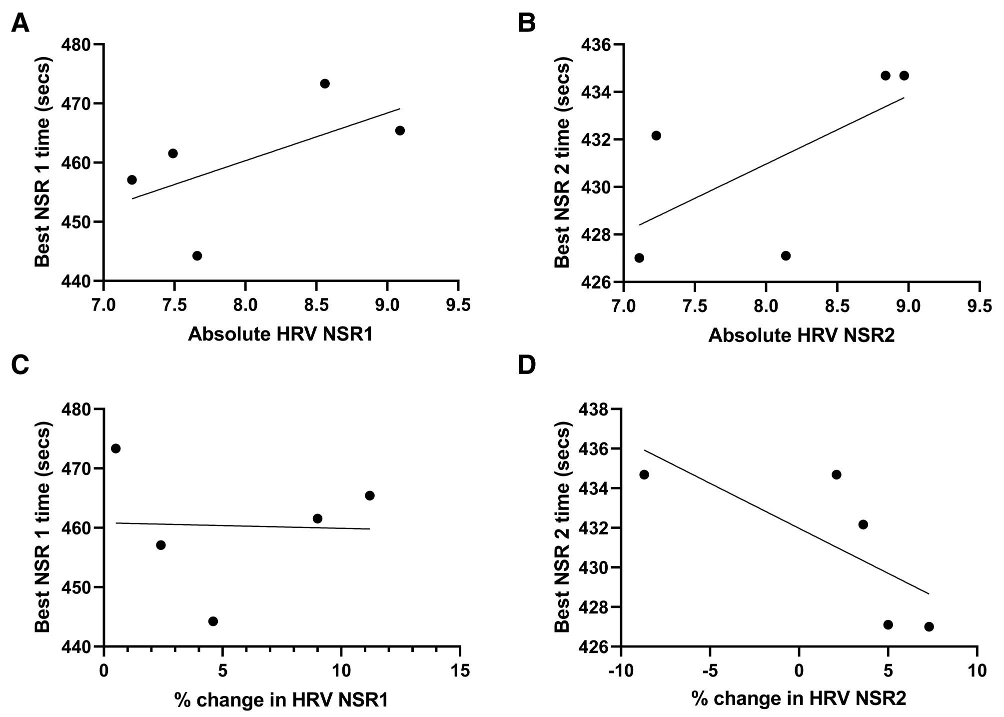

FIGURE 3
The relationship between on-water performance and average absolute LnRMMSD and % change in LnRMMSD during NSR 1 & 2 in elite female rowers (n = 5). (A) Absolute HRV (LnRMSSD) and best NSR 1 performance, (B) absolute HRV (LnRMSSD) and best NSR 2 performance, (C) % change in HRV (LnRMSSD) and best NSR 1 performance, (D) % change in HRV (LnRMSSD) and best NSR 2 performance.

4.1. Changes in estimated cardiac autonomic activity during the NSRs

We observed significant acute decreases in LnRMSSD from pre to during the NSRs with a rebound occurring 72 h post NSR (**Figure 1**). An oversimplification or lack of context to this response could be a cause for concern since decreases in HRV during a training cycle are often interpreted as indicative of a lack of adaptability to training (31), and when depressed chronically, LnRMMSD is related to all-cause mortality (32, 33). The observed acute decrease in HRV can be attributed to pre-performance anxiety or stress response as previous research has demonstrated an anticipatory response to stressful tasks, meaningful competitions and high-intensity training sessions decreases HRV (34–36). The performance impact of HRV reductions are equivocal, and could be attributed to the level of athlete and/or the importance of the forthcoming performance bout (37–39). As such, these acute changes can be seen as expected or beneficial, as increases in sympathetic activity facilitate increases in norepinephrine, epinephrine (36), bioenergetic pathways (40, 41), and neurological processes (i.e., reaction time) (42). Thus, this short-term suppression of HRV estimated cardiac autonomic activity may indicate a "readiness to perform", as indicated in previous literature (43). Our observation that parasympathetic reactivation did not occur within 72 h partially contradicts previous literature where an observed rebound occurred within 48 h (39, 44). This difference might be attributed to the effects of a multiday event delaying full autonomic recovery, and/or the potential for travel to suppress HRV (45). Therefore, athletes, coaches and or sport scientists using HRV monitoring strategies should consider adopting an individualized return to training plan.

4.2. Changes in psychometric dimensions of performance during the NSRs

This study found that psychometric profiles improved during NSRs and returned to pre-NSR values following the competition (**Figure 2**). Specifically, we found improvements in mental energy, physical condition, motivation, in addition to the reductions in fatigue occurred during competition as LnRMSSD decreased. Flatt et al., (2017b) observed that both psychological (stress, soreness, fatigue, and mood) and LnRMMSD profiles improved (i.e., LnRMMSD increased and perceived fatigue decreased) as Division I swimmers tapered for a national competition. The improvements in psychological and physiological status when reducing training volume likely contribute to the ergogenic effects associated with tapering (46). Similarly, our group has demonstrated significant reductions in fatigue and improvements in motivation in recreationally active volunteers participating in HRV-modulated training prescribed with lower training volumes (47). Reductions in LnRMSSD have previously been associated with reductions in fatigue and soreness (48); however, these findings are not universal and may be subject to interindividual variability associated with HRV outcomes (49). While reductions in LnRMSSD have been associated with increased training stress and incomplete recovery (50) the changes in LnRMSSD are interpreted as a positive response due to the increases in psychological status, which other investigators have suggested are key to interpreting HRV outcomes (51). Thus, the inclusion of perceptual or psychometric assessments is valuable to coaches, athletes, and sports scientists monitoring athlete workload.

4.3. Estimated cardiac autonomic activity and on-water performance during the NSRs

This study found that neither absolute LnRMMSD or the % change in LnRMMSD during competition was related to on-water performance. This finding supports the previous research by DeBlauw et al. (2022), that found no difference in 40-min cycling time trial performance when HRV (LnRMMSD) was within or outside an individual's smallest worthwhile change window. There is substantial evidence that daily HRV monitoring is a useful tool for monitoring and adapting training cycles (52–54) as well as identifying when negative adaptations or overtraining may be present (8). However, HRV's relation to performance may be more nuanced than initially thought, in that parasympathetic (LnRMSSD) HRV metrics suggest that an inverse relation may exist insofar as greater HRV may actually be associated with slower on water racing times ($R = 0.6–0.7$, **Figure 3**), and the percent change in the same metric is unrelated to on water performance. Thus, rowers and/or coaches may consider hiding or masking HRV values during competition to avoid further anxiety around their values and interpretation.

4.4. Experimental considerations

The sample size used in the current study was small but was representative of female rowers from an elite team. No sample size estimation or power analysis was conducted because we aimed to recruit all of the rowers on the team who were currently at the training center. Due to intensive training camp logistics we were only able to recruit 5 athletes for this study, similar to other investigations ranging from 2 to 6 elite athletes (Edmonds et al., 2014; Plews et al, 2012). Due to the remote nature of the HRV data collection we were unable to enforce a pre-reading stabilization period or seated position for HRV readings which may have an effect on resting HRV values (27, 55). Additionally, the statistical analysis used in our previous investigations demonstrated that changes in HRV is an appropriate method in similar small populations (56). Finally, we did not control for the menstrual cycle phase in this study. It is important to note that (57) has demonstrated that in female rowers large perturbations in autonomic activity can occur during the menses phase of the ovarian cycle, which may overlap with competition, and this did occur within our one of our specific athletes. This may have influenced their respective LnRMMSD values. However, these athletes will still be expected by coaches and themselves to perform at high-level regardless of menses. Despite this, our approach represents an ecologically valid perspective given that these women likely train and compete during all phases of the menstrual cycle. Further research may be necessary to determine more significant relationships and findings, but the current study provides effect sizes for subsequent studies and meta-analyses.

4.5. Future directions

There has been limited previous literature on how HRV may affect water performance for rowers, especially female populations. The importance of assessing HRV in an underrepresented population may provide extremely useful feedback for coaches when designing an effective exercise program. Further applications on HRV could provide a stronger or more significant relationships of HRV and on water performance. Further assessment of an individual's HRV within and outside of their normal window (above, below and normal ranges) and the effect on rowing performance.

5. Conclusion

The current pilot study is one of the first to use autonomous monitoring of HRV and psychometrics in elite female rowers leading up to, during, and following U.S. National Selection Regattas, and characterized their performance related parameters (body composition, peak aerobic fitness, and maximal power output). We observed decreases in LnRMSSD during competition, which was mirrored by increased mental energy, decrease in fatigue, and increased self-report of physical condition, which reversed 72 h post competition. However, neither HRV nor the change in LnRMSSD were related to on-water performance during the NSR, perhaps the reductions in HRV are reflecting a readiness to perform and not an acute maladaptive response. Collectively, this novel pilot study highlights that athletes are capable of autonomous monitoring HRV and perceptual dimensions of training and performance; which can provide an insight into their physiological and psychological state but the temporal relations amongst these variables may be complex and context dependent.

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 humans were approved by Skidmore College Institutional Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

JD, JS, and SI: prepared the original manuscript, figures, and tables and analyzed the data. JD, JS, RH, and SI: interpreted the results and edited the manuscript. JD, CB, MH, SM, IE, and SI: performed the testing and collection of study data. JD, RE, and SI: conceived and designed the experiment. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Free-living competitive racewalkers and runners with energy availability estimates of <35 kcal·kg fat-free mass^{-1}·day^{-1} exhibit peak serum progesterone concentrations indicative of ovulatory disturbances: a pilot study

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Introduction: The release of luteinising hormone (LH) before ovulation is disrupted during a state of low energy availability (EA). However, it remains unknown whether a threshold EA exists in athletic populations to trigger ovulatory disturbances (anovulation and luteal phase deficiency) as indicated by peak/mid-luteal serum progesterone concentration (Pk-PRG) during the menstrual cycle.
Methods: We assessed EA and Pk-PRG in 15 menstrual cycles to investigate the relationship between EA and Pk-PRG in free-living, competitive (trained-elite) Guatemalan racewalkers ($n = 8$) and runners ($n = 7$) [aged: 20 (14–41) years; post-menarche: 5 (2–26) years; height: 1.53 ± 0.09 m; mass: 49 ± 6 kg (41 ± 5 kg fat-free mass "FFM")]. EA was estimated over 7 consecutive days within the follicular phase using food, training, and physical activity diaries. A fasted blood sample was collected during the Pk-PRG period, 6–8 days after the LH peak, but before the final 2 days of each cycle. Serum progesterone concentration was quantified using electrochemiluminescence immunoassay.
Results: Participants that reported an EA of <35 kcal·kg FFM^{-1}·day^{-1} ($n = 7$) exhibited ovulatory disturbances (Pk-PRG ≤9.40 ng·mL^{-1}). Athletes with EA ≥36 kcal·kg FFM^{-1}·day^{-1} ($n = 8$) recorded "normal"/"potentially fertile" cycles (Pk-PRG >9.40 ng·mL^{-1}), except for a single racewalker with the lowest reported protein intake (1.1 g·kg body mass^{-1}·day^{-1}). EA was positively associated with Pk-PRG [$r(9) = 0.79$, 95% confidence interval (CI): 0.37–0.94; $p = 0.003$; $1 - \beta = 0.99$] after excluding participants ($n = 4$) that likely under-reported/reduced their dietary intake.
Conclusions: The result from the linear regression analysis suggests that an EA ≥ 36 kcal·kg FFM^{-1}·day^{-1} is required to achieve "normal ovulation." The threshold EA associated with ovulatory disturbances in athletes and non-invasive means of monitoring the ovulatory status warrant further research.

KEYWORDS

anovulation, luteal phase deficiency, short luteal phase, female athletes, endurance sports, menstrual cycle, exercise, energy availability

1. Introduction

Energy availability (EA) is a concept in sports nutrition developed by Loucks et al. (1) to represent dietary energy intake (EI) available to support all physiological processes and human health. Accordingly, EA is calculated by subtracting the total energy cost of exercise in surplus of non-exercise waking activity [exercise energy expenditure (EEE)] from EI and then normalising to individual fat-free mass (FFM). Hence, the unit of expression for EA is kcal·kg FFM^{-1}·day^{-1}. Healthy females typically achieve energy balance at ≈45 kcal·kg FFM^{-1}·day^{-1} (2). However, restricting EA to 30 kcal·kg FFM^{-1}·day^{-1} results in a decline in biomarkers of bone formation (3) and hormonal changes, specifically a decrease in the concentration of insulin, triiodothyronine, and leptin and an increase in the concentration of cortisol (4).

The pulsatile release of luteinising hormone (LH) is disrupted at a threshold EA of <30 kcal·kg FFM^{-1}·day^{-1} (4). Moreover, the concentration of follicle-stimulating hormone is increased as EA declines to 10 kcal·kg FFM^{-1}·day^{-1}, although this trend is reported only if EA restriction is caused by EEE (1). Furthermore, bone resorption is increased at an EA of 10 kcal·kg FFM^{-1}·day^{-1} (3). These physiological changes were documented in young women after 4–5 days of EA restriction under controlled laboratory conditions (1, 3, 4). Since estimates of self-reported EA are prone to error, research conducted on free-living athletes has failed to determine thresholds or associations between EA and disruptions to metabolic hormones (5) or ovulatory disturbances (6), i.e., anovulation and luteal (post-ovulatory) phase deficiency. Hence, carefully designed studies are warranted to fill this gap in knowledge.

The surge in LH concentration stimulates ovulation (7), and the ovarian follicle responsible for releasing the ovum develops into a transient gland that mainly produces progesterone (8). "Ovulation" is assumed with a serum progesterone concentration of ≥3.0 ng·mL^{-1} [≥9.54 nmol·L^{-1} (9)] or a peak concentration of >6.0 ng·mL^{-1} (10). Nevertheless, ovulatory cycles may exhibit "luteal phase deficiency or defect," defined as a serum progesterone concentration of <5.0 ng·mL^{-1} assessed at any timepoint during the luteal phase (11). The luteal phase is typically ∼14 days in duration, regardless of the length of the menstrual cycle (7). Luteal phase deficiency exhibited as "late ovulation" or "short luteal phase" (<10 days as of the second day after the LH peak) is associated with a peak serum progesterone concentration of <10.0 ng·mL^{-1} (12). In contrast, a single mid-luteal serum progesterone concentration of >9.4 ng·mL^{-1} indicates a "potentially fertile" cycle (13).

Ovulatory menstrual cycles have lower bone resorption rates during their luteal phase compared with anovulatory cycles (10), but optimum peak progesterone concentrations for bone health remain to be fully elucidated. When the oestradiol status is maintained, luteal phase defects cause no apparent change in bone health after 3 months (14). However, during a 1-year follow-up, ≥2 cycles with a "short luteal phase" [<10 days by basal body temperature (BBT) quantitative interpretation] are associated with a decline in bone mineral density, with women exhibiting anovulation more prone to greater spinal bone loss (15). This association between frequent ovulatory disturbances and negative changes in bone mass has been confirmed in several prospective studies (16). Moreover, bone health, menstrual function, and EA constitute a triad (17) within a host of health issues characterised by the syndrome of Relative Energy Deficiency in Sport or "RED-S" (18), as observed in athletes that chronically fail to meet energy demands. Athletes in a state of low energy availability (LEA), defined as <30 kcal·kg FFM^{-1}·day^{-1}, that experience menstrual disturbances, i.e., oligomenorrhoea or amenorrhoea, often exhibit a lower resting metabolic rate (RMR) than eumenorrheic athletes who report adequate EA, i.e., ≥45 kcal·kg FFM^{-1}·day^{-1} (19). Interestingly, the frequency of injury is greater in athletes with menstrual disturbances (20), while female endurance athletes with symptoms of LEA are at higher risk of developing bone stress injury due to exhibiting poor bone health (21). Accordingly, with regard to long-term health and performance in female athletes, energy restriction should not trigger anovulation or ≥2 "short luteal phases" per year (15). Nonetheless, whether a threshold exists for the association of EA with ovulatory disturbances remains unknown.

By design, female runners (22–24) and racewalkers (22) are frequently in a state of LEA and exhibit ovulatory disturbances (14, 15, 25), especially when failing to increase EI with training overload (26). However, to our knowledge, no study has investigated the association between EA and peak progesterone concentration or identified the threshold of EA that compromises fertility [mid-luteal serum progesterone ≤9.4 ng·mL^{-1} (13)]. Therefore, the primary aim of this study is to estimate EA during free-living conditions using a field-based methodology and explore the relationship between EA and subsequent peak progesterone concentration in competitive racewalkers and runners that were not using hormonal contraception.

2. Materials and methods

This study received ethical approval for invasive research in human participants from the NHS, Invasive or Clinical Research (NICR) Committee at the University of Stirling (1 June 2017, NICR 16/17—Paper No. 58) and local endorsements from three sports institutions in Guatemala (refer to **Ethics statement**).

2.1. Eligibility and recruitment

The Low Energy Availability in Females Questionnaire (LEAF-Q) was used to determine study eligibility: score <8 points, "not at risk of LEA" (27). The criteria included participants that self-reported being non-smokers, not pregnant or lactating, not taking medications associated with any chronic disease, ≥2 years post-menarche, without signs or symptoms of perimenopause, not using hormonal contraception during the preceding 6 months, and "naturally menstruating" (11) in three previous menstrual cycles. A total of 34 eligible athletes were informed regarding this study through the cooperation of coaches and staff members from the National Athletics Federation. In total, 28 Guatemalan racewalkers and runners voluntarily agreed to participate in this research, and provided informed consent prior to their involvement. However, only 26 athletes started the study.

2.2. Study design and data collection

Figure 1 summarises the study protocol and illustrates the timing of assessments during each menstrual cycle. Researchers explained all data collection procedures and monitored athletes in person and via chat apps or phone calls. Prospective observational data were collected under free-living conditions.

2.3. Basal body temperature

Athletes conducted daily measurements of sublingual BBT using a digital thermometer (Omron MC−343F) with an accuracy of 0.1°C. The BBT chart was tracked throughout the menstrual cycle (Figure 1). A female is assumed to have ovulated after observing 3 consecutive days of elevated BBT measurements (28) that are higher than those of the previous 6 days (Sensiplan® rules for quantitative interpretation of BBT requires further verification of algorithms to confirm ovulation). This shift from lower to higher BBT provided a benchmark to schedule progesterone assessment if the day of LH peak was missed, i.e., day 1 of elevated BBT reflected day 1 of the luteal or "high-BBT" phase.

2.4. Luteinising hormone detection or "ovulation testing"

Participants used the hLH Cassette 002L040 (UltiMed™, Germany) to self-detect LH peak concentrations in urine following manufacturer instructions. This rapid-chromatographic-immunoassay test detects LH only and not LH metabolites. First-morning urine was not assayed because it could miss the LH peak (29). Moreover, given that most athletes are under the time pressure of training or school in the morning, volunteers conducted this test during the expected peak of LH (Figure 1) once a day at 8 p.m. or 10 a.m. if forgotten the night before. The first author notified the participants individually when to start testing, e.g., day 11 if expecting a 28-day cycle or

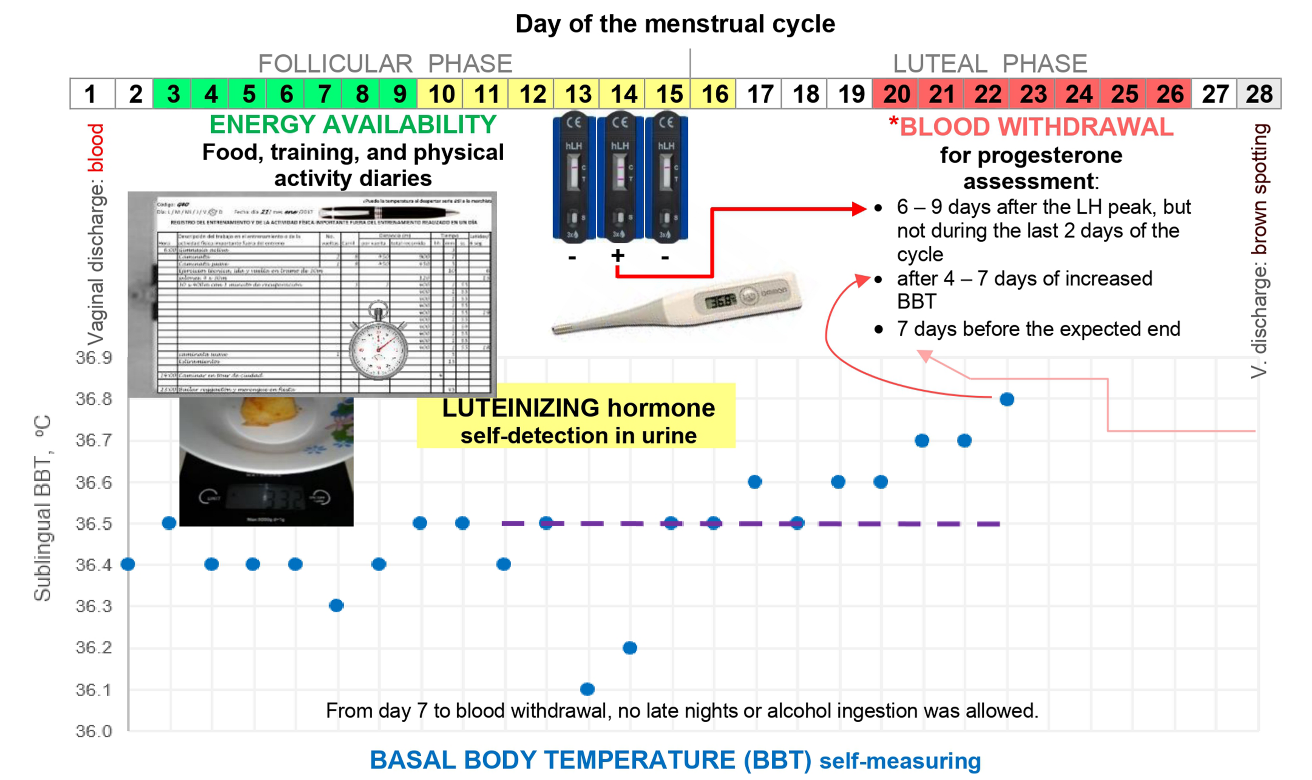

FIGURE 1
Overview of the study protocol during a 28-day menstrual cycle. *Points of reference are stated in order of priority. The shift from low to high BBT, from day 15 to 16, is doubtful at the beginning because BBT was back to the previous lower BBT level on day 17. BBT was measured immediately upon awakening after a night-time sleep, while still in bed. BBT log included remarks about vaginal discharge to define cycle length and factors that could alter BBT such as alcohol ingestion, signs and symptoms of infections, duration and quality of sleep, use of medications, and unusual environment or stress (28).

earlier/later for shorter/longer cycles. Qualitative detection of LH [≥30 mIU·ml^{-1}] continued until the day after a positive result.

Given that surges in LH concentration vary in amplitude, duration, and peak configuration (single, double, multiple, or plateau), the day of ovulation as determined by ultrasound may occur at the onset or end, during, or after the LH peak (30). However, ovulation occurs on the day (15%), the day after (76%), or ≥2 days after the first detection (9%) and not before urinary detection of LH >30 IU·L^{-1} (31). During cycles with long LH surges, higher BBT measurements begin the day after ovulation [Supplemental Figure 2B in Direito et al. (30)]. Therefore, we define the "day of LH peak" as the last consecutive day with a positive detection of LH and the presumed ovulation day as the final day of the follicular phase. We adhere to the definition of Schliep et al. (12) for the "presumed ovulation day" as the day of LH peak plus 1 day. LH was positive for two consecutive days in three menstrual cycles. In two of these cycles, the luteal phase length was the same as the high-BBT phase length.

2.5. Progesterone quantification

The participants involved in the study resided in four different cities. Two accredited laboratories (*Centro Médico* and *TecniScan*) determined serum progesterone concentrations using electrochemiluminescence immunoassay with an automated Cobas e601 analyser (Roche Diagnostics). Identical results were obtained for extremely low progesterone concentration, although differences of 1.01–1.10 ng·mL^{-1} were observed between laboratories for duplicate analysis of samples with intermediate and high concentrations. The average peak progesterone concentration of duplicates was used in the data analysis. To minimise participant burden, we planned blood withdrawal once within the expected progesterone peak period of each cycle. The athletes were encouraged to be euhydrated for blood sampling that was scheduled at 7 a.m. in an overnight fasted state. Blood vacutainers were centrifuged to separate the serum and were refrigerated until further analysis within 48 h.

"Peak progesterone" refers to a concentration quantified 6–9 days after the day of LH peak, but before the final 2 days of the cycle (10). In line with this definition, we documented progesterone concentration 6–8 days after the day of LH peak for six participants. Despite missing the LH peak ($n = 6$), progesterone concentration was quantified during days 5–8 of the high-BBT phase, within the mid-luteal period defined by BBT interpretation (13) and without statistical differences in the timing of assessment between groups of cycles by ovulatory status (**Table 1**). For cases with no LH surge during expected peak and progesterone concentrations indicative of anovulation, we verified that quantification occurred within the period before the end of the cycle with expected high concentrations (13). The peak, mid-luteal, and progesterone concentrations quantified during the expected peak are all indicative of the ovulatory status of a menstrual cycle. Therefore, we classified ovulatory status with progesterone concentrations as follows: "anovulatory" if ≤6.00 ng·mL^{-1} (10), "luteal phase defect" if 6.01–9.40 ng·mL^{-1}, and "potentially fertile" or "normal ovulatory" if >9.40 ng·mL^{-1} (13). Three cycles had peak ($n = 2$) or mid-luteal ($n = 1$) progesterone concentrations that approached the critical cut-off point between "ovulatory disturbed" and "potentially fertile" (9.00–10.00 ng·mL^{-1}). We highlight that the Quantitative Basal Temperature (QBT) method (32) and Sensiplan® rules were both consistent with the progesterone concentrations in classifying these cycles as "luteal phase defect" ($n = 2$) or "normal ovulatory" ($n = 1$).

2.6. Energy availability

Prior to expected ovulation and within days 3–12 of the menstrual cycle, the food diaries, training diaries, and physical activity questionnaires were completed over 7 consecutive days to estimate EA based on the 7-day average of both EI and EEE (refer to **Supplementary Table S1** for data collection details). A 7-day period represents the repetitive lifestyle pattern and a complete training micro-cycle, including all types of workouts and 1 day of rest over the weekend whereas a longer period was considered too onerous (33). The estimation period for EA occurred prior to the day of the LH peak or the end of the follicular phase as shown by the quantitative interpretation of BBT (**Table 1**). If an athlete reported any difference between the first and second weeks of the studied cycle in terms of (i) dietary pattern due to travelling or festivities likely changing EI, (ii) amount of regular food consumed, or (iii) training volume (intensified or tapered), the estimated EA was deemed non-representative of the follicular phase, and the cycle was discarded from the analysis.

2.6.1. Dietary assessment

The participants recorded a weighed and photographed food diary that included the data on family meal recipes with the consumed proportion. All athletes weighed their food, except for two runners who reported portion sizes using measuring cups and spoons. If unable to weigh food (unplanned eating), the photograph and description of a meal or snack were used to estimate the portion size and weight. To ensure valid and accurate data, an accredited Sports Dietitian (first author) interviewed the athletes daily and within 7 days following the dietary register week and checked all food items for their code and weight to confirm the agreement with the portion reported or photographed prior to conducting dietary analysis with NutrINCAP® (version 2.1) software. NutrINCAP® uses the Food Composition Tables of Central America, with the possibility to incorporate data for additional products. Data were verified by double-checking the records of days with low or high energy or nutrient intakes. The Diet Quality Index International (DQI-I) score (34) was estimated.

2.6.2. Exercise "training and physical activity" energy expenditure

The first author and assistant researchers documented the training in printed form by observation or, otherwise, it was self-

TABLE 1 Details and timing of assessments during menstrual cycles.

	n	Timing of assessment		
The 7-day period of energy availability estimation ended before expected ovulation, specifically on:				
	3	Day of the anovulatory cycles		10 ± 2, 9–12
	6	Days before the "day of LH peak"		6 ± 3, 2–9
	6	Days before the "last day of the follicular phase" by BBT		9 ± 3, 6–14
LH				
Not detected	2	Progesterone during the expected peak period by cycle length indicative of anovulation		
False positive[a]	1			
Detection, LH+	6	On 1 day only ($n = 3$), for 2 consecutive days ($n = 3$)		
Missed detection[b]	6	Progesterone during the peak period by BBT indicative of ovulation		
Day of LH peak	6	Day of the cycle		16 ± 3, 12–21
Serum progesterone quantification[c]	6	By *Centro Médico* (CM)		
	8	By *TecniScan* (TS)		
	1	By both laboratories		
	15	Day of the cycle		24 ± 3, 19–32
	6	Days after the "day of LH peak"		8 (6–8)
	6	Day of the luteal (post-ovulatory) phase by LH peak		7 (5–7)
	6	Day of the high-BBT phase[d] if LH detection was missed		6 ± 1, 5–8
	15	Days before the last day of the cycle		5 (4–10)
Timing of serum progesterone (PRG) quantification[e]				
	Ovulatory status			
	Anovulatory	Luteal phase defect	Potentially fertile	High PRG
Progesterone concentration cut-offs, ng·mL^{-1}	<6.00	6.00–9.40	9.41–16.00	>16.00
N	3	5	4	3
Day of the cycle	23 ± 3	24 ± 2	24 ± 1	26 ± 7
	21–27	21–27	23–25	19–32
Days after the "day of LH peak"	7	8 (6–8)	8	6
	(false LH+)	$n = 4$	$n = 1$	$n = 1$
Day of the luteal phase, defined by LH peak		7 (5–7)	7	5
		$n = 4$	$n = 1$	$n = 1$
Day of the high-BBT phase (n = missed LH detection)		5[f]	6 ± 1, 5–8[f]	6[f]
		$n = 1$	$n = 3$	$n = 2$
Days before the last day of the cycle	5 (5–8)[f]	6 (4–10)	7 (4–9)	5 (5–9)

LH, Luteinizing hormone. Day of LH peak: last consecutive day with positive urinary detection of LH (LH+). BBT, basal body temperature. Last day of the follicular phase: day of LH peak + 1 day (12) or the last day of the "low-BBT phase" by BBT quantitative interpretation.
[a]Progesterone of 2.89 ng·mL^{-1} was quantified 5 days before the last day of the cycle on day 7 after LH+, thus false LH+ for ovulation.
[b]A participant forgot to test on the day that could have been LH+, the others ($n = 5$) had shorter or longer cycles than expected.
[c]CM quantified 0.62, 11.13, and 22.82, while TS quantified, respectively, 0.62, 10.03, and 23.83 ng·mL^{-1}.
[d]High-BBT phase length was defined by Sensiplan® rules ($n = 5$) and the Quantitative Basal Temperature "QBT" method ($n = 1$).
[e]There was no significant difference in progesterone quantification timing between groups of cycles classified by their ovulatory status.
[f]Within the period of expected high progesterone concentrations and within the mid-luteal period for the ovulatory (13).

reported by the athlete. The participants also detailed their physical activity outside of training to the nearest minute. The first author assigned metabolic equivalent of tasks (METs) value for each physical activity after interviewing the athlete to verify the accuracy of self-reported information or observations made by assistant researchers. If METs data were unavailable for adolescents, i.e., running >12.9 km·h^{-1}, the adult value was used (**Table 2**). After data tabulation, EEE was estimated using Excel® 365. EEE was calculated as the total energy cost of training and physical activity minus the energy cost of being awake but not exercising over the same period (see non-exercise energy cost below).

2.6.2.1. Resting metabolic rate

Due to a lack of validated equations for our specific athletic population, we chose the Harris and Benedict (35) equation to estimate RMR in adult participants, as it predicts RMR in female athletes (40) including sports that predispose a low body mass type (41). The Schofield (36) equation that predicts RMR from body mass was used in our adolescent participants with a 5% correction, as suggested by the Institute of Nutrition of Central America and Panama "INCAP" (42).

2.6.2.2. Non-exercise energy cost

The non-exercise energy cost was defined as 1.3 × RMR per minute, multiplied by the time engaged in training and physical activity (**Table 2**). This conversion factor is based on the estimated energy cost of a 10-h rest plus a 14-h very light activity in adults (43). This value was substituted in adolescents for 1.33, which was estimated with data from Torún et al. (42) using the same rest-activity ratio and accounting for growth energy estimates in females aged 14–17.9 years.

TABLE 2 Equations to estimate energy availability.

Abbreviations	Equation or definition	Units
FFM: fat-free mass	FFM = body mass − body fat mass[a]	kg
RMR: resting metabolic rate		
RMR, female adult aged ≥18 years:	Harris and Benedict (35)	$\frac{kcal}{day}$
RMR, female adolescent aged 14–17.9 years:	[b]Schofield (36) − 5%	
RMR per minute	$\frac{RMR}{min} = RMR \cdot \frac{1\ day}{1440\ min}$	$\frac{kcal}{min}$
METs: metabolic equivalent of tasks	Databases. Adult: Ainsworth et al. (37). Adolescent: Butte et al. (38).	
TPA: training + physical activity	activity ≥4 METs + 3.5–3.9 METs if completed for ≥10 $min \cdot day^{-1}$	
[c]TECsA: total energy cost of a specific activity (sA)	$TECsA = (MET\ sA)\frac{RMR}{min}(min\ sA)$	kcal
TECE: total energy cost of exercise "TPA"	$TECE = \Sigma TECsA$	kcal
[d]ECBANE: energy cost of being awake but not exercising, during the time engaged in TPA	$ECBANE = 1.3 \cdot \frac{RMR}{min} \cdot \frac{min\ TPA}{day}$	$\frac{kcal}{day}$
EEE: exercise "TPA" energy expenditure	$EEE = TECE - ECBANE$	$\frac{kcal}{day}$
EA: energy availability EI: dietary energy intake	$EA = \frac{EI - EEE}{FFM}$	$\frac{kcal}{kg\ FFM \cdot day}$

[a]Body fat percentage, to estimate body fat mass, as per the equation in Yuhasz (39) that requires 6 skinfolds.
[b]Predicts RMR from body mass.
[c]As per Butte et al. (38).
[d]Factor 1.3 for adults, but 1.33 for adolescents.

2.6.2.3. Total energy cost of exercise "training and physical activity"

The total energy cost of exercise was estimated from the data analysis of training diaries, excluding passive stretching, and physical activity questionnaires. Physical activity was defined as efforts ≥3.5 METs. The data regarding non-training activities ≥4 METs (i.e., dancing, physical-household chores such as wood piling, biking, or walking for transportation, carrying a backpack or child) were included in the estimation of EEE (44). Moderate household chores equivalent to 3.5–3.9 METs (i.e., floor or bathroom cleaning) were computed if completed for ≥10 min per day. METs were used as a multiple of individual RMR (**Table 2**). Regular walking, running, and corrected running METs were used to estimate the total energy cost of racewalking according to speed (**Table 3**).

TABLE 3 Metabolic equivalents of tasks (METs) used to estimate the total cost of exercise.

Racewalking		
speed, $km \cdot h^{-1}$	METs	Assumed due to:
<8.0	Regular walking	Hagber and Coyle (45)
8.0–9.7	Running	
> 9.7	Running + 1.6	"1.6 METs": oxygen consumption data of highly-trained male and female racewalkers in Mora-Rodriguez et al. (46) comparing running and racewalking at 10.9 $km \cdot h^{-1}$
Typical training activities[a]		
Running or racewalking	0.2[b]	Flat surface with ∼1 kg around each wrist
	0.3[b]	Uphill
	0.4[b]	Uphill (very steep)
	0.5[b]	Flat surface wearing a vest of at least 5 kg
	4.0	Continuously active gymnastics or callisthenics Isometric exercises, e.g., maintaining tough yoga positions
	5.0	Technique drills or multiple jump exercises Resistance exercises, moderate effort
	6.0	Tough resistance exercises with own body mass (e.g., pull-ups, push-ups), high intensity
	8.0	Gym circuit, severe effort, and minimal rest

[a]We used heart rate values upon task completion to assign METs for specific activities not included in databases, e.g., heart rates immediately after "technique drills" and "resistance exercises–moderate effort" were similar.
[b]Amounts added to the specific METs: determined from the adult database (37); e.g., the difference between walking or running at a specific speed on "inclined" and "flat" surfaces.

2.6.3. Body composition

FFM was estimated during the previous or studied menstrual cycle using a two-compartment body composition model with anthropometry [equation proposed by Yuhasz (39)]. All measurements were conducted before the first training session using the International Society for the Advancement of Kinanthropometry (ISAK) methodology by the same qualified anthropometry practitioner (**Supplementary Table S1**). The assessment was not undertaken before or during menstruation (vaginal discharge of the inner lining of the uterus) when self-reported scores for fluid retention or bloating (puffiness + oedema + nocturia) are typically highest (47). The technical error of measurement of skinfolds used to estimate body fat percentage was ≤3.6%.

2.7. Ovulatory status prior to study

The participants were not required to be "eumenorrheic" (11). Two athletes recorded their BBT during the menstrual cycle prior to participation in the study. Several participants voluntarily repeated the study during the following cycle as data generated in the first attempt were discarded from the analysis as explained in **Supplementary Table S2**. Therefore, we documented the ovulatory status of the menstrual cycle prior to this study in six participants as per progesterone ($n = 3$), BBT ($n = 2$), and LH ($n = 1$).

2.8. Statistical analysis

EI relative to the measured RMR (EI:mRMR) <1.35 has been recognised as incompatible with long-term survival (48) and is typically used as an indicator of presumed EI underreporting (44). Although RMR was not measured, EI was the average of only 7 days, and ovulatory disturbances were expected (14, 15, 25), particularly if associated with reports of low EI. Hence, we chose to present data for all volunteers but conduct correlation analysis excluding four participants with EI relative to estimated RMR (EI:eRMR) <1.35. The relationship between EA and peak/mid-luteal progesterone was analysed using Pearson's correlation coefficient, $r(n-2)$, with estimated 95% confidence interval (CI). We used a regression analysis to determine the EA required by our participants to achieve fertile progesterone concentrations.

Descriptive statistics are presented as mean ± SD (including range if the data set is skewed and if minimum and maximum values are critical) or median (range) if not normally distributed as per Shapiro–Wilk test (IBM©-SPSS®). We compared three "anovulatory" cycles with those exhibiting the three highest progesterone concentrations, introducing an additional ovulatory status group: "high progesterone" (>16.0 ng·mL^{-1}). One-way analysis of variance (ANOVA) with Tukey's HSD *post-hoc* test was conducted to investigate the differences in EA between menstrual cycles with "anovulatory," "luteal phase defect," "potentially fertile," and "high" peak/mid-luteal progesterone and also to explore the differences in variables among groups of participants classified by ovulatory status. The data of a variable were presented and analysed non-parametrically if it was not normally distributed in one or more of these groups. Kruskal–Wallis with Dunn's *post-hoc* test was used as the non-parametric alternative. Significance level for all tests was set at an $\alpha = 0.05$. The *post-hoc* power ($1 - \beta$) was estimated using G*Power 3.1.9.4 (49).

3. Results

Two of the 26 volunteers who started this study dropped out. We failed to quantify the progesterone concentration within the peak period for five athletes. The ovulatory status of 19 participants was recorded based on their progesterone concentration, but four cases were excluded. The lost cases and final exclusions are described in **Supplementary Table S2**.

3.1. Participants

The final data set consisted of competitive racewalkers ($n = 8$) and runners ($n = 7$), 5 (2–26) years post-menarche, with training and performance classification (50) from trained (tier 2) to elite/international level (tier 4). The descriptive characteristics of the participants are shown in **Table 4**. A total of 15 menstrual cycles were examined, with each participant contributing one cycle for analysis (**Table 5**).

The ovulatory status of nine athletes prior to this study was unknown. In six participants, the menstrual cycle prior to this study exhibited normal ovulation based on BBT interpretation ($n = 1$) and peak progesterone concentration ($n = 1$) and ovulatory disturbances based on mid-luteal progesterone ($n = 2$), BBT ($n = 1$), and LH ($n = 1$) measurements. The ovulatory status remained constant during the study in four athletes, whereas a marginal change was reported in two participants, i.e., luteal phase defect into either anovulatory or potentially fertile.

3.2. Ovulatory status

Eight menstrual cycles that were considered normal in length displayed "ovulatory disturbances": "anovulation" ($n = 3$), "short luteal phase" (12) ($n = 1$), and LH peak at expected timing during the cycle but with progesterone concentration indicative of "luteal phase deficiency" ($n = 4$). The cycles with progesterone indicative of anovulation were also deemed "anovulatory" by BBT quantitative interpretation (no high-BBT phase). However, only seven cycles were "potentially fertile," including a longer-than-normal or "oligomenorrheic" (11) 37-day cycle. Consistent with the findings of Direito et al. (30), a large variation (22–44 days) in ovulatory cycle length was reported (27–37 days).

3.3. Diet

All participants reported an omnivorous diet with DQI-I scores of 67 ± 9 points [0–100 points (34)] with food records including two or more protein sources, and seven or more grain portions daily. Four registers included all food groups each day with three displaying frequent intake of nuts and/or seeds. Eight records exhibited low intake of fruits and/or vegetables, some detailing a monotonous pattern of intake. Although the DQI-I score was not associated with ovulatory status, scores >70 points were only achieved by athletes with "normal ovulatory" cycles, while those with ovulatory disturbances obtained all ≤3 of 6 points in the "empty-calorie" DQI-I component. Macronutrient and fibre intake are described in **Table 5**.

3.4. Energy availability and progesterone

Estimates of EA and progesterone concentrations indicative of the ovulatory status of the studied menstrual cycles ranged from 28 to 46 kcal·kg FFM^{-1}·day^{-1} and 0.41–22.51 ng·mL^{-1}, respectively. EA in our participants that exhibited ovulatory disturbances (32 ± 3 kcal·kg FFM^{-1}·day^{-1}) appeared to be greater than in runners with more severe menstrual abnormalities, i.e., amenorrhoea [18 ± 7 kcal·kg lean body mass^{-1}·day^{-1} (24)]. The ovulatory peak/mid-luteal progesterone concentrations of our participants (7.63–22.51 ng·mL^{-1}) are within the range [5.39–78.5 nmol·L^{-1} (1.69–24.69 ng·mL^{-1})] quantified using similar

TABLE 4 Characteristics, body composition, training, and physical activity of participants.

	Ovulatory status				
	Anovulatory	Luteal phase defect	Potentially fertile	High PRG	Total
Serum progesterone (PRG) cut-offs, ng·mL^{-1}	<6.00	6.00–9.40	9.41–16.00	>16.00	
N	3	5	4	3	15
Characteristics					
Rw: racewalkers, *n*	Rw = 2	Rw = 2	Rw = 3	Rw = 1	Rw = 8
R: runners, *n*	R = 1	R = 3	R = 1	R = 2	R = 7
Training and performance calibre,[a] *n* per tier	2: *n* = 1	2: *n* = 4	2: *n* = 1	2: *n* = 1	2: *n* = 7
	3: *n* = 2	3: *n* = 1	3: *n* = 3	3: *n* = 1	3: *n* = 7
				4: *n* = 1	4: *n* = 1
Chronological age, years	18	18	24	20	20
	(16–20)	(14–21)	(17–41)	(19–22)	(14.8–41.1)
Gynaecological age, years post-menarche	3	4	12	6	5
	(1.7–5.0)	(2–8)	(4–26)	(4–9)	(1.7–26.1)
LEAF-Q score,[b] points	6	4	5	6	5
	(3–7)	(0–5)	(1–9)	(2–8)	(0–9)
Anthropometry and body composition					
Body mass, kg	48.2 ± 0.6	49.2 ± 5.3	50.3 ± 8.1	46.3 ± 7.6	49 ± 6 40–61
Height, m	1.53 ± 0.01	1.49 ± 0.04	1.54 ± 0.14	1.55 ± 0.15	1.53 ± 0.09 1.39–1.74
Body mass index, kg·m^{-2}	20.5	20.6	21.0	19.1	20.5
	(20.5–21.1)	(19.4–25.9)	(20.2–21.9)	(18.0–20.4)	(18.0–25.9)
Sum of 8 skinfolds, mm		* ’	’	*	
	106	110	82	83	96
	(90–114)	(96–162)	(80–101)	(71–87)	(71–162)
Body fat, % as per Yuhasz (39)		* ’	’	*	
	15	16	13	14	15
	(15–17)	(15–22)	(13–16)	(12–14)	(12–22)
Training and physical activity "TPA" ≥ 3.5 metabolic equivalent of tasks (METs)					
Volume, km per week	62	23	62	44	44
	(40–103)	(12–90)	(18–92)	(26–60)	(12–103)
Volume, h per week	6	5	9	5	6
	(5–23)	(3–12)	(5–14)	(4–8)	(3–23)
EEE: exercise "TPA" energy expenditure, kcal per day	493	195	443	388	385
	(318–690)	(150–572)	(204–696)	(189–394)	(150–696)

Significant differences between groups: * and ’ $p < 0.05$.
[a]As per McKay et al. (50); tier 2: local level representation; tier 3: competitive athletes at both the National (Guatemala) and Central American levels.
[b]LEAF-Q: Low Energy Availability (LEA) in Females Questionnaire, score ≥8 points = risk for LEA (27). Two athletes with 8 and 9 points were accepted as scores reflected respectively 3-week absence of training (posterior tibial syndrome) and postpartum amenorrhoea.

methodology during the intermediate luteal phase defined by Anckaert et al. (51). Eleven participants reported "reduced" EA (30–45 kcal·kg FFM^{-1}·day^{-1}), whereas three athletes and one runner reported LEA and adequate EA, respectively. A positive correlation was observed between EA and progesterone concentration [statistics of significant correlation using our entire sample ($n = 15$) not shown]. After excluding four cases for possible underreporting or reduced EI while recording diet (EI: eRMR <1.35), a moderate correlation was observed between EA and progesterone [$r(9) = 0.79$, 95% CI: 0.37–0.94; $p = 0.003$; $1 - \beta = 0.99$], with EA explaining 63% ($r^2 = 0.63$) of progesterone variance (**Figure 2**). Athletes with a reported EA <35 kcal·kg FFM^{-1}·day^{-1} ($n = 7$) had all ovulatory disturbed cycles. In contrast, seven of eight participants with EA ≥36 kcal·kg FFM^{-1}·day^{-1} exhibited progesterone indicative of "normal ovulation." Interestingly, the exception was the racewalker that reported the lowest protein intake (1.1 g·kg body mass^{-1}·day^{-1}) and the highest percentage of EI derived from refined sugars (14%) in this subgroup. Linear regression analysis with EA as a predictor of peak/mid-luteal progesterone [serum progesterone concentration = 1.13 (EA) − 30.77] indicates that EA ≥36 kcal·kg FFM^{-1}·day^{-1} is required to achieve normal ovulation. EA estimated during cycles with "high" progesterone was greater [$F(3, 11) = 7.45$, $p = 0.005$; $1 - \beta = 0.78$] than EA during cycles with concentrations indicative of "anovulation" ($p = 0.010$) and "luteal phase deficiency" ($p = 0.014$) [mean ± SD (95% CI)]: 42 ± 4 (31–52) vs. 31 ± 2 (26–36) and 33 ± 4 (28–37) kcal·kg FFM^{-1}·day^{-1} (**Figure 3**).

TABLE 5 Diet and menstrual cycles of participants.

	Ovulatory status				
	Anovulatory	Luteal phase defect	Potentially fertile	High PRG	Total
Serum progesterone (PRG) cut-offs, ng·mL^{-1}	<6.00	6.00–9.40	9.41–16.00	>16.00	
N	3	5	4	3	15
Reported dietary intake, 7-day average					
Energy (EI), kcal		*	*		
	1,756 ± 265	1,583 ± 202	2,096 ± 282	1,982 ± 174	1,834 ± 302 1,329–2,349
Energy, kcal per kg body mass		* ’	*	’	
	36 ± 6	32 ± 3	42 ± 4	43 ± 3	38 ± 6; 28–46
EI:$_e$RMR		**	**		
	1.36 ± 0.21	1.23 ± 0.14	1.62 ± 0.16	1.54 ± 0.02	1.42 ± 0.22; 1.06–1.81
EI:$_e$RMR < 1.35, *n*	1	3	0	0	4
Protein, g per kg body mass		* **	**	*	
	1.1 (0.9–1.3)	1.0 (0.8–1.1)	1.4 (1.4–1.7)	1.3 (1.2–1.7)	1.2 (0.8–1.7)
Carbohydrate, g per kg body mass	5.4 ± 1.5	4.9 ± 1.0	6.4 ± 1.1	6.6 ± 1.1	5.7 ± 1.3; 4.0–7.7
Carbohydrate, g per kg fat-free mass	5.6 (5.3–8.5)	5.4 (4.7–7.4)	7.3 (5.9–9.1)	7.8 (6.3–8.7)	6.7 (4.7–9.1)
Energy from refined sugars,[a] % kcal	* **	’	** ’	*	
	14.4 ± 4.2	10.7 ± 2.8	4.6 ± 1.3	6.8 ± 3.2	9.0 ± 4.5; 3.1–19.2
Fibre, grams	*	**	* **		
	12 (12–14)	11 (10–15)	25 (17–28)	16 (9–17)	14 (9–28)
Diet quality index international “DQI-I,” points	64 ± 5	62 ± 8	73 ± 7	69 ± 13	67 ± 9; 50–82
Menstrual cycle					
Length, days	29 ± 3	31 ± 2	31 ± 3	32 ± 5	31 ± 3
	27–32	27–33	28–34	28–37	27–37
Luteal phase length by LH detection in urine (≥30 mIU·mL^{-1}), days		12 ± 2			13 ± 2
		9–13	15	14	9–15
		$n = 4$[b]	$n = 1$	$n = 1$	$n = 6$
High-BBT phase length by Sensiplan® rules, days (*n* = missed LH detection)			11 ± 2		12 ± 2
		15	10–13	11	10–15
		$n = 1$[b]	$n = 3$	$n = 2$	$n = 6$
Serum progesterone, ng·mL^{-1}	* **	* “	**	** “	
	2.96 ± 2.58	8.64 ± 0.69	10.90 ± 1.38	19.83 ± 3.34	10.34 ± 5.93
	0.41–5.57	7.63–9.36	9.58–12.84	16.09–22.51	

eRMR, estimated resting metabolic rate.
Significant differences between groups: * and ’ $p < 0.05$ or ** and ” $p < 0.01$.
[a]Reported dietary energy from added table sugar and refined sugars within commercial food, drinks, and candy.
[b]High BBT phase length was indicated by the Quantitative Basal Temperature “QBT” method; PRG was quantified 10 days before the last day; Sensiplan® rules and QBT method indicated both a “luteal phase deficiency.”

4. Discussion

This observational study explored the relationship between EA (estimated with field-based methodology) and the subsequent peak/mid-luteal serum progesterone concentration, which is indicative of the ovulatory status of a menstrual cycle. Our data in free-living Guatemalan competitive racewalkers and runners who prospectively recorded ≥3 cycles of normal length before this study showed a positive correlation between EA and the subsequent peak/mid-luteal progesterone concentration (**Figure 2**). Ovulatory disturbances (peak/mid-luteal progesterone ≤9.40 ng·mL^{-1}) were observed with EA <35 kcal·kg FFM^{-1}·day^{-1}, and “normal ovulation” was associated with EA ≥36 kcal·kg FFM^{-1}·day^{-1}. Our estimates of EA (**Figure 3**) successfully distinguished between ovulation with “high” progesterone (>16.00 ng·mL^{-1}) and both “anovulation” (≤6.00 ng·mL^{-1}) and “luteal phase deficiency” (6.01–9.40 ng·mL^{-1}).

Five days of LEA during the follicular phase of the menstrual cycle has been shown to disrupt the pulsatile release of LH (4). We provide further evidence of anovulation ($n = 2$) and luteal phase deficiency ($n = 1$) with progesterone concentrations during the expected peak or peak period in trained-to-highly-trained athletes who reported LEA (28–<30 kcal·kg FFM^{-1}·day^{-1}) during 7 consecutive days within the follicular phase of the same cycle. Insulin is critical in the control of reproduction, i.e., hyper and hypo-insulinemia are associated with disturbed gonadotropin-releasing hormone and LH pulse and release patterns (52). However, insulin declines linearly with acute EA restriction (4). Moreover, daily energy deficits (caloric restriction + exercise) of 470–813 kcal generate an incidence of ovulatory disturbances

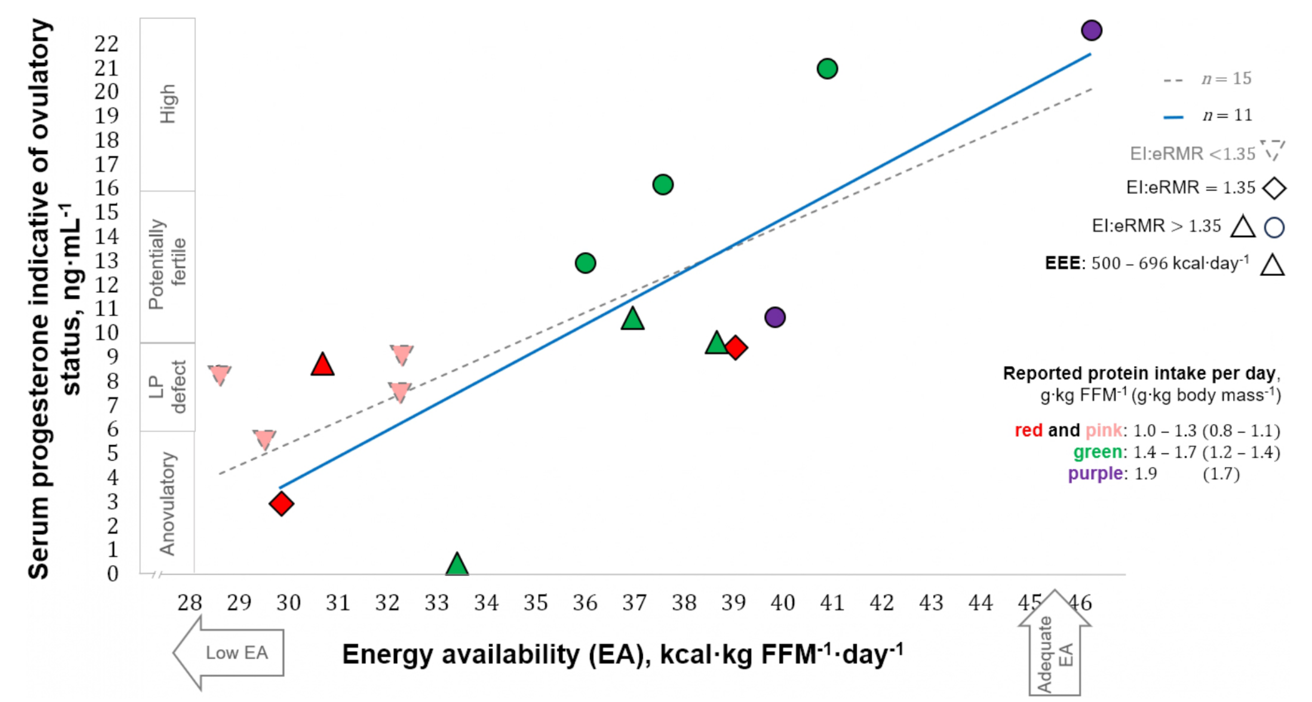

FIGURE 2
Correlation between energy availability and progesterone concentration. LP, luteal phase; FFM, fat-free mass; EI, reported energy intake; eRMR, estimated resting metabolic rate; EEE, reported exercise “training and physical activity” energy expenditure. Shapes of data points indicate EI:eRMR with triangles also showing the highest reported EEE. Colours in data points indicate reported protein intake.

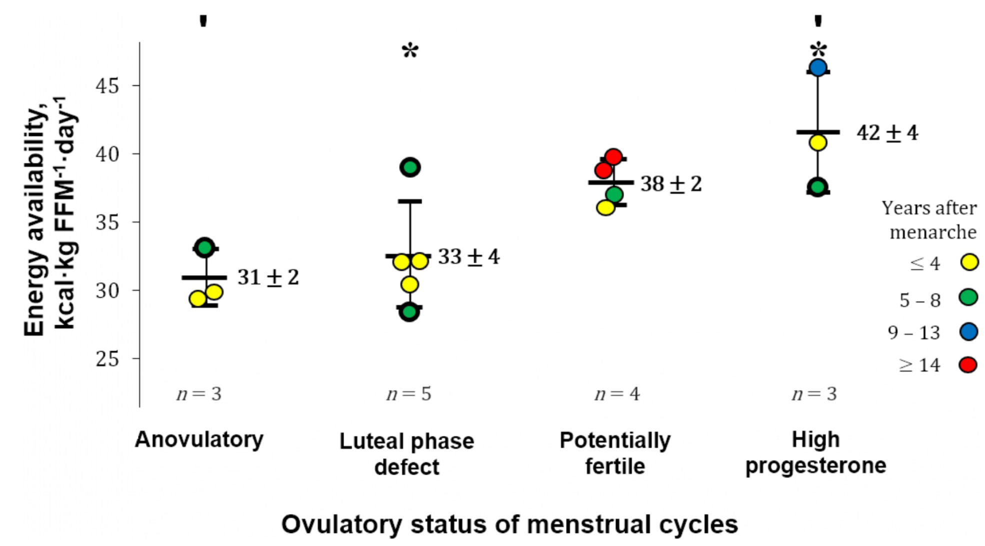

FIGURE 3
Energy availability by ovulatory status. FFM, fat-free mass; Error bars, reported mean energy availability (EA) ± *SD*. Significant differences between groups: ′*p* = 0.010 and **p* = 0.014. The gynaechological age of participants is depicted in the colours of the data points. CASE HISTORIES (thick-border data points). ANOVULATORY. (1) Reported daily protein intake of 1.3 g·kg body mass^{-1} and exercise “training and physical activity” energy expenditure (EEE) of 690 kcal·day^{-1}. LUTEAL PHASE DEFECT. (2) Lowest EA: The following 2 cycles were deemed ovulatory disturbed and anovulatory by quantitative interpretation of basal body temperature. (3) Highest EA: reported daily protein intake of 1.1 g·kg body mass^{-1}; percentage energy intake derived from refined sugars of 14%. HIGH PROGESTERONE. (4) This elite athlete [tier 4 (50)] reported EA during the competitive season (not her highest training volume).

during the first menstrual cycle of 38%–42% (including 13% anovulation within the highest deficit), with incidence rates increasing and luteal phase length decreasing over three menstrual cycles (53). According to Lieberman et al. (54), the likelihood of ovulatory disturbances and oligomenorrhoea increases with a decrease in EA, with >50% probability of experiencing these disruptions with LEA, but not supporting the notion that a specific threshold of EA exists. Nevertheless, in this study (54), EEE was calculated as "total energy cost of exercise minus RMR," while Loucks et al. (1) deducted for "non-exercise waking activity." Furthermore, it is not clear whether the oligomenorrheic cycles reported were ovulatory disturbed which is prudent since several studies have documented extended ovulatory cycles of 36–44 days (30, 55). Intuitively, individual factors influence the onset and degree of ovulatory or menstrual disturbance during energy deficit (53). Despite these observations, it appears that acute EA restriction before (from follicular recruitment, i.e., late luteal phase of previous cycle, to LH peak) or after ovulation (from LH peak to menses) has a similar impact on the luteal phase of the actual menstrual cycle. Hence, irrespective of phase of the ovarian cycle in which an abrupt onset of short-term exercise occurred, the luteal phase was disturbed in women who did not increase their EI (56). Notably, these previous studies (4, 53, 54, 56) were conducted in habitually sedentary eumenorrheic women rather than in athletic populations. Hence, a strength of the present study is the specialised group of competitive endurance athletes recruited, although we did not control for the ovulatory status of the previous cycle. Therefore, our current investigation should be considered a pilot study to extend understanding regarding the effect of EA on the ovulatory status of free-living athletes. Future intervention studies are warranted to understand the effect of EA restriction on normal ovulation in eumenorrheic athletes and should be long-term (2–3 cycles) in design with a follow-up included (**Supplementary Table S3**). Given that research into how psychological stress might impact menstrual function is inconclusive (57), future studies should focus on monitoring heart rate variability (HRV) as an indicator of autonomic nervous function while being cognisant that HRV changes in response to progesterone fluctuations during normal menstrual cycles (58).

Free-living estimates of EA in highly trained and elite athletes have been considered "snapshots," and thus not necessarily an accurate representation of long-term EA status (19). Our 7-day estimates of EA are self-reported as representative of the follicular phase, i.e., without changes in diet and exercise in the first 14 days of the studied menstrual cycle. We verified that the EA estimation period ended before the expected ovulation (**Table 1**) and showed that estimates of EA were related to the ovulatory status of the same menstrual cycle. This relationship is consistent with the findings of Lieberman et al. (54) that reported EA as a predictor of menstrual disturbances within the same but not the subsequent cycle. Moreover, Schliep et al. (12) showed an association between hormonal deficiencies in the follicular phase and hormonal deficiencies in the luteal phase of the same cycle. Accordingly, our observations represent an acute rather than a chronic state of EA and can only be interpreted as the relationship between EA status before ovulation and its impact on the ovulatory status within the same menstrual cycle.

Estimates of EA under free-living conditions have previously been shown to discriminate between amenorrhoea and eumenorrhea [31 ± 2 vs. 37 ± 2 kcal·kg lean body mass^{-1}·day^{-1} ± SEM, respectively (6)]. However, no distinction was observed for subclinical ovulatory status as diagnosed with urinary metabolites of oestradiol and progesterone, i.e., no evidence of a statistical difference was reported regarding estimates of EA in physically active females who consistently exhibited ovulatory, disturbed, or anovulatory cycles during one to three menstrual cycles. Several methodological differences existed between our estimates of EA and those of Reed et al. (6), particularly regarding assessments of EEE, body composition, and RMR. Reed et al. (6) assessed EI over a 3-day period that was not standardised to a cycle phase, while we estimated EI and EEE simultaneously during the follicular phase with a similar timing of EA restriction in previous studies (1, 4). Given that ovulatory disturbances were expected (14, 15, 25), and since EI and expenditure are increased after ovulation (59), concomitant with slightly higher RMR during the luteal phase (60), we avoided the unfair comparison of EI between participants that would be influenced by ovulatory status. There is a lack of a single protocol for the assessment of EA in free-living situations (5). Hence, the design of our protocol did not include self-reporting a difference in diet or exercise during the first 2 weeks of the studied menstrual cycle. However, during the first two cycles studied, we recognised the importance of exhibiting EA estimates representative of the follicular phase. Our free-living estimates of EA were correlated with peak/mid-luteal progesterone concentration and distinguished between ovulatory status. To our knowledge, this is the first report of a significant association between EA and peak/mid-luteal progesterone concentration in free-living competitive endurance athletes.

Monitoring the menstrual cycle of athletes in a free-living situation is challenging. In the context of "normal ovulatory" or "fertile" menstrual cycles, progesterone remains elevated from day 10 to day 5 prior to menstruation (13). Progesterone was assessed 4 days before the last day in two cycles, near the midpoint of their post-ovulatory phases. One of these cycles exhibited a 9-day (short) luteal phase with progesterone concentration quantified 6 days after the day of LH peak. However, if the assessment was planned 9 days after the LH peak [within the peak period in "normal" cycles (10)], we would have failed to document peak progesterone with a quantification scheduled 1 day before the last of this cycle. Thus, practitioners or researchers that use our protocol to control for cycle phase or document progesterone concentration during the peak period in athletes with expected ovulatory disturbances should consider that assessments are based on "normal" cycles. In the present study, as ovulatory disturbances became severe, it was more difficult to quantify progesterone within the peak period, which in a normal cycle is from about 2 days before until 2 days after the middle of the luteal phase.

Prior evidence suggests that normal-length menstrual cycles may mask ovulatory disturbances (15, 25). The prevalence rate of ovulatory disturbances in our cohort of athletes was 53% which falls within the range (29%–79%) that was previously reported in active females (14, 15, 25). Our data also suggest that monitoring menstrual cycle length effectively detects disturbances until deemed clinically evident with substantial delay or absence of menstruation. However, recent evidence indicates that menstrual cycle monitoring is not widespread, with only 54% of elite and highly trained athletes tracking their cycles (61). Hence, rather than waiting until the period is absent, practitioners are encouraged to take a proactive approach to detecting LEA at an early stage as consequences of RED-S such as anaemia, decreased muscle strength and glycogen stores, vulnerability to illness, and stress fractures can be detrimental to health and performance (18). Thus, in practical terms, practitioners supporting athletes vulnerable to RED-S may attempt to monitor the ovulatory status of those individuals not using hormonal contraception because hormonal contraceptives will often prevent ovulation. Based on our LH detection analysis, it was possible to record short luteal phases [positive urinary LH detection ≤10 days before the last day of the cycle, defining a luteal length of <10 days (12)], although this activity required continued searching from the expected timing of LH peak until almost the end of the menstrual cycle. Whereas Park et al. (55) documented anovulation in 4.7% of cycles with LH surges, we observed three false LH "positives for ovulation" with a level of urinary detection of ≥30 $mIU·mL^{-1}$ (**Table 1** and **Supplementary Table S2**). Furthermore, we documented normal-length luteal phases with peak progesterone indicative of "luteal phase deficiency." Therefore, monitoring the timing of "ovulation" is not sufficient in athletic populations, where the assessment of hormonal adequacy in the luteal phase becomes important.

Establishing comprehensive guidelines for EA restriction to achieve body composition goals in female athletes warrants further investigation, alongside practical means of monitoring ovulatory status. In female athletes, a decline in body mass of 0.7% per week appears commensurate with resistance training goals (62). However, recommendations for EA restriction without disturbing the ovulatory status to the extent of impairing long-term health and performance remain undetermined. While fertility might not be a concern for many athletes, ovulatory cycles also have a role in bone health (10, 15). Indeed, a lower than expected bone mineral density (z-score < 0) has been associated with LEA (63). In addition, athletes should be aware that LEA impairs muscle protein synthesis (64) and that failure of progesterone to rise adequately during the menstrual cycle is not only associated with LEA but also with impaired athletic performance (65), i.e., when athletes fail to increase EI with increased training loads, ovulatory disturbances are induced and performance is impaired (26). Hence, the practitioner may monitor progesterone during the peak period as a tool to confirm an EA status that is sufficient to maintain normal ovulatory function in naturally menstruating athletes, instead of the time-consuming estimation of EA. However, there could be exceptions in terms of ovulatory or menstrual disturbances without obvious indications of chronic energy deficiency, such as in polycystic ovary syndrome (66). Moreover, the quantitative interpretation of carefully documented BBT during menstrual cycles may be considered equally promising as a non-invasive and low-cost screening tool to assess ovulatory status. Our BBT data support the use of BBT quantitative interpretation to indicate a peak, mid-luteal, or progesterone concentration at the expected peak (Castellanos-Mendoza et al., unpublished observations), although further validation in the context of preventing LEA is needed. Given that some female athletes use hormonal contraception methods that may mask LEA (67) as users may interpret withdrawal bleeding as a "period" when they may otherwise have menstrual disturbances, it remains relevant to establish the minimum EA required to maintain normal ovulation to guide the prescription of energy restriction. Since access to gold standard methods is limited, a parallel estimation of EA with accessible methodology and an indication of the equivalent energy deficit is useful for practitioners. In **Supplementary Tables S2, S3**, we provide our feasibility analysis and suggestions for future research.

The challenges of estimating EA in a field setting are well recognised (5, 67); hence, care was taken in the present study to minimise the chance of possible errors, with special emphasis on the estimation of EI and the total energy cost of training and physical activity (**Methods** and **Supplementary Table S1**). While adding food images to food diaries may reduce the margin of error (68), participant motivation and "attitude to food" likely influence the validity of self-reported EI (69). In terms of energy balance (EB) expressed as percentage of total energy expenditure (TEE), the reported mean error of EI by weighed diet records without food images was −34% to −1% in female athletes with estimated TEE by doubly labelled water (DLW) (33, 69). The highest difference was observed in runners that were required to self-report dietary intake over 3 weeks, and light-weight rowers. In contrast, the smallest difference was documented when dietitians weighed food portions during meals, with snacks and sports drinks considered as the source of individual error [−18.4% to +19.2% (70)]. Nevertheless, most previous studies included a shorter EI assessment period than estimation of TEE, and no study controlled EEE or derived "EEE + NEAT (non-exercise activity thermogenesis)" from TEE and RMR as in Silva et al. (71). Moreover, the degree of error explained by undereating, underreporting, and/or dietary analysis drawbacks remains unknown (69). Although the limitations of using METs to estimate individual energy cost of exercise are recognised (37, 38), 11 of 25 studies reviewed by Burke et al. (5) used METs as an independent metric of self-reported EEE estimation. To our knowledge, no validation study has been conducted to address the error in using METs to estimate EEE from training and physical activity diaries in female athletes. TEE determined by DLW ranged from 2,350 to 3,735 $kcal·day^{-1}$ in free-living female runners (72, 73) that on average were older, taller, of greater body mass, and with higher training volume than the participants of this study. Interestingly, the estimated TEE [eRMR·1.3 (1.33 for adolescents) + EEE] with our data was 1,821–2,453 $kcal·day^{-1}$, with EB remarkably similar to previous reports (−11% ± 12%; −30% to +17%).

To our knowledge, this study is novel in suggesting that a threshold for EA is associated with ovulatory disturbances and in highlighting the impact of EA on the serum peak/mid-luteal progesterone concentration. A graphical summary of the short-term effects of LEA on hormones [Figure 3 in Areta et al. (74)] shows the lack of evidence in terms of peak progesterone concentration. Linear regression analysis suggests that EA =

35 kcal·kg FFM^{-1}·day^{-1} reflects ovulatory disturbances, but this EA value was not reported. Due to our study limitations, including lack of precision in field-based methods, lack of control of variables known to alter the menstrual cycle in a free-living setting, a small sample size, and use of two laboratories to quantify progesterone, we report the threshold EA between ovulatory disturbances and "normal ovulation" with a gap representing our observations without statistical analysis. These EA thresholds for ovulatory disturbances (EA <35 kcal·kg FFM^{-1}·day^{-1}) and "normal ovulation" (EA ≥36 kcal·kg FFM^{-1}·day^{-1}) are based on the participants studied with our methodology. Highlighting the pilot nature of this study, we speculate that other methods to estimate EA and participant restriction by training and performance classification, or years after menarche (y.a.m.) [2–4 y.a.m. "adolescents" vs. > 14 y.a.m. "mature women" (75)] likely impact the threshold EA for ovulatory disturbances. While our analysis is limited to carbohydrate intake per FFM (**Table 5**) as we did not assess the intensity or metabolic effect of EEE, it remains unknown if carbohydrate availability [intake minus oxidation during exercise (76)] has a greater impact than EA on the ovulatory status.

Two additional limitations are associated with this study. First, recreationally active (tier 1) and world-class (tier 5) athletes were not represented, while elite (tier 4) athletes (50) were underrepresented and most participants (87%) were between 2 and 9 years post-menarche. Consequently, our findings can only reliably be extrapolated to trained and highly trained (tier 2–3) female athletes aged 14–23 years given that most participants were within this age range except for two aged 27 and 41 years. Second, we did not conduct interviews or questionnaires to investigate eating behaviour to objectively verify "restrictive eaters" and discern whether the low EI was due to "consciously eating less while keeping a detailed register of food intake" rather than "underreporting." Nevertheless, we excluded athletes with EI:eRMR <1.35 to formulate our conclusion.

5. Conclusions

We conclude that EA during the follicular phase of the menstrual cycle impacts the ovulatory status of the same cycle in competitive racewalkers and runners. Our free-living estimates of EA <35 and ≥36 kcal·kg FFM^{-1}·day^{-1} are associated with subsequent progesterone concentrations indicative of ovulatory disturbances and normal ovulation, respectively. Further research is warranted to elucidate the threshold EA associated with ovulatory disturbances in athletes and develop non-invasive means of monitoring ovulatory status in trained individuals.

Data availability statement

Due to participant confidentiality and privacy, an unidentifiable data set supporting the conclusions of this article is only available upon request to be directed to the corresponding author.

Ethics statement

This project was approved by the NHS, Invasive or Clinical Research (NICR) Committee at the University of Stirling (1 June 2017, NICR 16/17—Paper No. 58) and Sports Confederation (31 May 2017), National Olympic Committee (24 May 2017), and Athletics Federation (8 May 2017) of Guatemala. All participants provided their written informed consent to participate in this study with parental approval for the athletes of <18 years of age.

Author contributions

MC: Conceptualisation, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Visualisation, Writing – Original draft, Writing – Review & editing. SG: Conceptualisation, Funding acquisition, Methodology, Writing – Review & editing, Formal analysis, Supervision. OW: Conceptualisation, Formal analysis, Supervision, Writing – Review & editing, Methodology.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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Short-term effects and long-term changes of FUEL—a digital sports nutrition intervention on REDs related symptoms in female athletes

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Female endurance athletes are at high risk for developing Relative Energy Deficiency in Sport (REDs), resulting in symptoms such as menstrual dysfunction and gastrointestinal (GI) problems. The primary aim of this study was to investigate effects of the FUEL (Food and nUtrition for Endurance athletes—a Learning program) intervention consisting of weekly online lectures combined with individual athlete-centered nutrition counseling every other week for sixteen weeks on REDs related symptoms in female endurance athletes at risk of low energy availability [Low Energy Availability in Females Questionnaire (LEAF-Q) score ≥8]. Female endurance athletes from Norway (n = 60), Sweden (n = 84), Ireland (n = 17), and Germany (n = 47) were recruited. Fifty athletes with risk of REDs (LEAF-Q score ≥8) and with low risk of eating disorders [Eating Disorder Examination Questionnaire (EDE-Q) global score <2.5], with no use of hormonal contraceptives and no chronic diseases, were allocated to either the FUEL intervention (n = 32) (FUEL) or a sixteen-week control period (n = 18) (CON). All but one completed FUEL and n = 15 completed CON. While no evidence for difference in change in LEAF-Q total or subscale scores between groups was detected post-intervention ($BF_{incl} < 1$), the 6- and 12-months follow-up revealed strong evidence for improved LEAF-Q total ($BF_{incl} = 123$) and menstrual score ($BF_{incl} = 840$) and weak evidence for improved GI-score ($BF_{incl} = 2.3$) among FUEL athletes. In addition, differences in change between groups was found for EDE-Q global score post-intervention ($BF_{incl} = 1.9$). The reduction in EDE-Q score remained at 6- and 12- months follow-up among FUEL athletes. Therefore, the FUEL intervention may improve REDs related symptoms in female endurance athletes.

Clinical Trial Registration: www.clinicaltrials.gov (NCT04959565).

KEYWORDS

sports injuries, menstrual disturbances, low energy availability, endurance exercise, women's health

1. Introduction

Sustainable and low-cost management of symptoms related to the syndrome Relative Energy Deficiency in Sport (REDs) is of key interest in female endurance athletes (1–3) due to the high reported prevalence of symptoms, ranging from 31% to 80% (4–8). The frequent occurrence of negative health and performance related consequences, including menstrual dysfunction with associated low bone mineral density and overuse injuries (3) calls for action. Insufficient energy intake relative to exercise energy expenditure, often denoted low energy availability (LEA), is the underlying etiological factor for REDs (3). The recommended treatment is therefore to increase energy intake, reduce exercise energy expenditure or a combination of both (9, 10). However, the evidence of intervention efficacy for the managements of REDs symptoms is limited and primarily based on case studies (11–13) and interventions without a control group (14, 15), or in non-competitive females (16). Successful nutrition interventions have suggested that future studies implementing strategies to provide more personalized dietary interventions accounting for food preferences, dietary patterns across the day, timing of food intake and macronutrient composition may have the potential to be more effective (16).

The menstrual cycle is an energy demanding process, involving hormonal synthesis and follicular development, and eumenorrhea is recognized as an important health indicator for female athletes (17). Therefore, menstrual function is a frequently used marker when screening for LEA and REDs and assessing the safety of female athletes' sports participation (4, 10, 18). In fact, it has been suggested that assessing self-reported symptoms of LEA, including menstrual function, provides a better assessment of the overall health status of an athlete compared to a snapshot of current energy availability, where assessment of dietary intake and exercise energy expenditure is susceptible to several sources of errors (6, 19, 20). The Low Energy Availability in Females Questionnaire (LEAF-Q) (4), where the main emphasis is laid on menstrual function, is one of the most frequently used screening tools for detecting female athletes at risk of LEA and REDs (20). The LEAF-Q is validated in endurance athletes (4) and this is also where one of the highest prevalence of menstrual dysfunction is reported in sports ranging from 0% to 20% for primary amenorrhea (late menarche), 0%–56% for secondary amenorrhea (no bleeding for minimum of three consecutive menstrual cycles), and 0%–39% for oligomenorrhea (<9 menstrual bleedings per year), depending on the diagnostic method used (21). Although the infertility associated with menstrual dysfunction in athletes may be transient (22), prolonged or severe LEA and the multiple metabolic and endocrine alteration associated with menstrual disturbances e.g., elevated cortisol and lowered estradiol, insulin and T3 levels can have serious negative impact on bone health via an estrogen-dependent and estrogen-independent pathway, which may be irreversible (23, 24). Low bone mineral density constitutes an increased risk for bone stress injuries, resulting in long absences from sport participation (25, 26), emphasizing the need for prevention at all levels (27).

Negative gastrointestinal tract function has also been associated with LEA and REDs (3). More specifically, persistent LEA can result in mucosal atrophy characterized by diminished intestinal function and morphological changes including decreased villous height, crypt depth, surface area, and epithelial cell numbers (28). In addition, LEA and REDs have been associated with an excessive dietary fiber intake among female endurance athletes (29). The gastrointestinal problems may appear as delayed gastric emptying, bloating, constipation, and increased intestinal transit time (3). Gastrointestinal problems, commonly reported by endurance athletes, may not only be detrimental to health and quality of life, but also to athletic performance (30, 31).

As formulated by Ackerman et al. (32) "*It is time for a drastic paradigm change in women's sport, coupled with education at all levels to improve the long-term health and athletic achievement of female athletes*" [(32), p.1 line 15–19]. One of the proposed steps in the management of REDs is to raise awareness of the negative effects of LEA so athletes can make wise decisions for their own long-term health (32). In essence, inadequate knowledge of optimal sports nutrition and the negative health and performance consequences of LEA, coupled with a normalization of REDs symptoms, e.g., menstrual dysfunction, appears to be frequent underlying causes of LEA (33, 34). Though, adequate nutrition knowledge is necessary for optimal nutrition habits, it may not be a sufficient factor for ensuring a true change in nutritional behavior in athletes (35). Furthermore, motivation, enabling, and supporting athletes have been identified as additional components necessary for changes in nutritional behavior (33). We have previously reported strong evidence for improved sports nutrition knowledge along with weaker evidence for increased energy intake in female endurance athletes with risk of REDs after a 16-week sports nutrition intervention, consisting of online sports nutrition lectures combined with individual athlete-centered nutrition counseling (the FUEL study) (36).

Parallel to measure physiological symptoms that may be affected by a nutrition intervention, it is important to monitor psychological symptoms associated with LEA. This includes eating disorder symptoms. Nutrition education and counseling have been reported to increase eating disorder symptoms in young ballet dancers (37, 38). Hence, although LEA and REDs occurs frequently without disordered eating behavior or eating disorders (39–41), one may fear that a nutrition intervention aiming at increasing energy intake may pose a risk for the development of eating disorders in an already high-risk group (42). Furthermore, there is a reported association between symptoms of eating disorders and exercise addiction (43) and REDs may therefore be associated with exercise addiction in endurance athletes (39, 44).

Therefore, the primary aim of the present analysis was to investigate immediate effects (pre-post intervention) and long-term changes (6- and 12-months follow-up) of the FUEL intervention study, on common symptoms associated with REDs; menstrual and gastrointestinal function and injuries. Secondary, the aim was to investigate any symptoms related to the risk of eating disorders and exercise addiction. Specifically, the goal of this analysis was to investigate whether the LEAF-Q, Eating Disorder Examination (EDE-Q), and Exercise Addiction Inventory (EAI) scores change differently from the pre- to postintervention in the intervention group compared to the control group, and to investigate how the symptoms develop up to 12-months follow-up in the intervention group.

2. Methods

The study design, recruitment process, and intervention content have been described in detail elsewhere (36). The study was approved by the regional ethics committee in Norway (31,640), Sweden (2019-04809), and by the Norwegian Centre for Research Data (968,634) and registered at www.clinicaltrials.gov (NCT04959565). Originally, the study was planned and approved to include a wide range of REDs related clinical biomarker measurements and a control group prior to initiation of the intervention. Due to the COVID-19 pandemic the first round of recruitment had to be cancelled. Further, since all physical contact with the participants was prohibited, the final design and measures are strongly influenced by the pandemic restrictions. Consequently, all medical procedures were excluded in the final research plan, thus, the study did not need an additional ethical approval at the other study sites (Germany and Ireland).

2.1. Study design

This was a multicenter study recruiting female endurance athletes from Norway, Sweden, Ireland, and Germany. Athletes were seasonally allocated to the FUEL intervention (FUEL) or a control condition (CON). The intervention group received weekly online lectures in sports nutrition combined with individual athlete-centered nutrition counseling with an experienced sports nutritionist for sixteen weeks. The control group received no lectures or counseling. The study was initiated with a screening phase, where athletes completed an online survey via the data collection tool Nettskjema that was connected to the safe Services for Sensitive Data (TSD) platform (University of Oslo). In this part of the study, athletes provided background information and completed the LEAF-Q (4), EDE-Q (45), and the Exercise Addiction Inventory (46). Athletes with risk of LEA, defined as a LEAF-Q score ≥8 (4), and low risk of disordered eating behavior, defined as an EDE-Q global score <2.5 (47), were invited to participate in the study. Athletes completed the same questionnaires after the 16-week FUEL/CON condition. In addition, the FUEL intervention group completed a 6- and 12-months follow-up answering the LEAF-Q, the EDE-Q, and the EAI. Other assessments included in the study, including diet and training log, has previously been described and analyzed (36).

2.2. Eligibility criteria

Eligibility criteria for the study were (1) competitive female endurance athlete, (2) 18–35 years of age, (3) training ≥5 times/week, (4) no use of hormonal contraceptives for at least six weeks prior to the study, (5) no chronic disease (e.g., Crohn's disease or hypothyroidism) or diagnosed menstrual dysfunctions not related to LEA (e.g., polycystic ovarian syndrome or endometriosis), (6) non-smoker, (7) not pregnant or planning a pregnancy, (7) speaking/understanding Norwegian, Swedish, English, or German.

2.3. Recruitment

Athletes were recruited from November 2020 to September 2021 via Norwegian, Swedish, Irish, and German competitive endurance sports clubs, coaches in endurance sports at the Olympic sports center in Norway and via social media with a link to the project website. The recruitment targeted summer endurance disciplines (runners, orienteers, cyclists, and triathletes) during November/December with the initiation to the intervention in January, while the recruitment targeted winter endurance disciplines (biathletes and cross-country skiers) in May with the initiation to the intervention in June.

In total, 208 participants signed up for the study. Of these, 141 were excluded: $n = 2$ male athletes; $n = 2 < 18$ years; $n = 1 > 35$ years.; $n = 1$ badminton player; $n = 3$ with chronic diseases ($n = 1$: Crohn's disease, $n = 1$: Hashimoto's thyroiditis, $n = 1$: hypothyroidism); $n = 55$ hormonal contraceptive users; $n = 23$ with a EDE-Q global score ≥2.5; $n = 51$ with a LEAF-Q score <8, and $n = 3$ for not providing any contact information. The LEAF-Q responses of $n = 67$ athletes were analyzed in more detail, and some were contacted to clarify their answers. This resulted in $n = 7$ athletes being excluded due to a suspected false positive identification of the risk of LEA. Further, $n = 4$ athletes were unavailable, $n = 3$ responded too late in relation to intervention start-up and allocation to sports nutritionists, and $n = 3$ athletes declared severe illness ahead of the baseline measurements (i.e., abdominal surgery and COVID-19). In total, $n = 18$ athletes, who had signed up during their competition season, were allocated to a 16-week waiting control condition (CON) of which $n = 15$ athletes completed ($n = 1$ wanted to start using hormonal contraceptives, while we were unable to contact $n = 2$). In total, $n = 32$ athletes were directly allocated to the FUEL intervention, while $n = 1$ terminated participation in the project in week 13 due to experiencing too much work related to the project. Consequently, $n = 31$ (97%) completed the FUEL intervention and $n = 15$ (83%) completed the CON condition.

2.3.1. Final inclusion of participants in the analyses

One athlete in FUEL missed the postintervention survey relevant for this paper (but completed the other measurements), while all participants in CON completed the survey with the LEAF-Q, EDE-Q, and EAI pre- and postintervention. Consequently, $n = 30$ and $n = 15$ athletes were included in the analyses comparing pre- and post-measurements for FUEL and CON, respectively. Twenty-six of the 30 FUEL athletes completed the 6-months follow-up. In terms of the LEAF-Q analysis, $n = 3$ had started using hormonal contraceptives, $n = 1$ reported pregnancy/breastfeeding and was therefore excluded from the 6-months follow-up. Twenty-three FUEL athletes completed the 12-months follow-up. Additional $n = 2$ had started using hormonal contraceptives and $n = 1$ reported pregnancy/breastfeeding and was therefore excluded in the 12-months follow-up for the LEAF-Q analyses.

2.4. Nutrition intervention

The 16-week intervention consisted of weekly online lectures in sports nutrition targeting female endurance athletes with risk of REDs, combined with individual athlete-centered nutrition counseling every other week.

The sixteen sports nutrition lectures integrated evidence-based sports nutrition information and recommendations. They were developed by four researchers and practicing sports nutritionists, initially in Norway and Sweden, including a comprehensive manuscript for each session, and subsequently translated into English and German. All sixteen lectures were comprehensively reviewed and finally approved by all four researchers/sports nutritionists. The recorded lectures had a mean duration time of 25.0 ± 8.4 min. Key topics were information about REDs, the importance of the menstrual cycle for health and performance, macronutrient recommendations for endurance athletes, and nutritional periodization. Every week during the intervention, participants received an e-mail with a link and password to the lecture of the week located on a closed online platform. Participants had the opportunity to watch the lectures when suitable during their everyday lives and to watch them repeatedly if they wanted.

The nutrition counseling was administrated via the teleconferencing platform Zoom, Zoom Video Communication, Inc. (California, USA). The first consultation was scheduled to run for 1.5 h (actual duration: 73 ± 15 min), while the following seven consultations were scheduled to run for approximately 1 h (actual duration: 55 ± 6 min). The team of counsellors, consisted of three Norwegian, four Swedish, two Irish, and one German highly experienced sports nutritionists, who work with Elite athletes on a daily basis. Self-determination theory was chosen as a core foundation for the FUEL counseling, since this approach has been found to be effective in promoting behavior change (48). An athlete-centered, empathic communication approach, inspired by core skills in motivational interviewing (49) was utilized.

2.5. Measures and instruments

2.5.1. Low energy availability in females questionnaire

The validated screening tool LEAF-Q (4) was used to assess self-reported symptoms of LEA; injury frequency the past year, current gastrointestinal function, and current and past reproductive function. The LEAF-Q is validated in female endurance athletes and has a total of 9–25 questions depending on the respondent's answer, including those related to hormonal contraceptive use. A total score ≥8 was considered at risk of LEA (4). Athletes completed the LEAF-Q at pre- and postintervention/control period, and also at 6- and 12-months follow-up for the FUEL group. Because a LEAF-Q score ≥8 was used as an inclusion criterion, all athletes in the present study had a LEAF-Q score ≥8 at pretest. Since the LEAF-Q assesses injuries the past year, the injury score was considered less important at postintervention measurement. Similarly, it is not possible to change the answer to some of the questions related to menstrual function during a 16-week period ["*How old were when you had your first period?*" and "*Did your first menstruation come naturally (by itself)?*"]. Therefore, it was of interest to look at possible changes on single questions related to menstrual function, namely: "*Do you have normal menstruation?*" and "*Do you experience that your menstruation changes when you increase your exercise intensity, frequency or duration?*" Minor clarifications from the original LEAF-Q were added and has been described previously (39).

2.5.2. Eating disorder examination questionnaire

The EDE-Q was used to measure behavioral and cognitive symptoms of eating disorders the past 28 days (45). It has been validated in an athletic population (50) and is a frequently used screening tool for disordered eating and LEA/REDs (20). The EDE-Q consists of 28 items which can be divided into four subscales (restraint, eating concern, shape concern, and weight concern) and a global score averaging the subscales, used as cut-off for eating disorder pathology. In the present study, a global EDE-Q score ≥2.5 was used to classify athletes with disordered eating behavior (39, 47, 51). Because an EDE-Q global score <2.5 was used as an inclusion criterion, all included athletes had an EDE-Q global score <2.5 at pretest.

2.5.3. Exercise addiction inventory

The EAI was used to assess symptoms of exercise addiction with a score ≥24 considering participants at risk of exercise addiction (46, 52). The EAI consists of six general components describing the degree of addiction rated on a five-point Likert scale: salience (exercise is the most important thing in life), conflicts (e.g., interpersonal conflicts due to the exercise behavior), mood modification (a coping strategy to regulate emotions), tolerance (increasing amounts of exercise is needed to achieve effect), withdrawal symptoms (e.g., irritability when an exercise session is missed), and relapse (reversions to earlier patterns). Originally, the EAI was validated in recreational exercisers but has later been validated in elite athletes (53).

2.6. Statistics

Data analyses were conducted using JASP (version 0.17.1.0). All analyses were conducted within the Bayesian statistical framework (54, 55). Descriptive statistics were expressed as frequencies with percentage for binary and categorical data and as means ± standard deviation (SD) for continuous data. Group comparisons for baseline characteristics were conducted using Bayesian Independent Samples *t*-test for normally distributed data and Mann–Whitney test for non-normally distributed data. Bayesian contingency table tests were used to compare groups for categorical data. Within-group differences from pre- to postintervention were investigated using Bayesian Paired Samples *t*-test for normally distributed data and with Wilcoxon Signed-Rank test for non-normally distributed data. Group comparisons from pre- to postintervention were conducted using a Bayesian repeated measures analysis of variance (ANOVA) with default priors and compared to the null model. Non-normally distributed data were transformed using SPSS [version 28.0.1.1 (14)] but did not change the interpretations of the results compared to analyzing the non-transformed data. A group × time interaction effect was hypothesized, i.e., that the FUEL and CON group's LEAF-Q scores would change differently over time (alternative hypothesis). To calculate the Bayes Factor (BF) for the interaction effect only inclusion probabilities for matched models were considered (55). BFs between 1 and 3 were considered to indicate weak evidence for the alternative hypothesis, BFs between 3 and 10 were considered moderate evidence for the alternative hypothesis, while BFs greater than 10 were considered as strong evidence for the alternative hypothesis (56). Menstrual function for individual questions in the LEAF-Q was analyzed in a descriptive manner due to insufficient number of participants. Within FUEL group comparisons for LEAF-Q, EDE-Q, and EAI scores for the four measurement time points (pre-, postintervention, 6- and 12-months follow-up), were conducted using a Bayesian repeated measures ANOVA. Menstrual function for individual questions in the LEAF-Q was analyzed in a descriptive manner.

3. Results

Endurance athletes from Norway ($n = 11$), Sweden ($n = 17$), Ireland ($n = 5$), and Germany ($n = 12$) were included from the following endurance disciplines: running ($n = 14$), orienteering ($n = 7$), triathlon ($n = 12$), cycling ($n = 5$), cross country skiing ($n = 1$), and biathlon ($n = 6$). Participant characteristics are presented in **Table 1**. There was no evidence of statistical differences when comparing the two groups' baseline characteristics (BFs < 1).

TABLE 1 Participant characteristics divided by intervention (FUEL) and control (CON) groups.

	FUEL ($n = 30$)	CON ($n = 15$)
Age (years)	25.2 ± 4.09	24.1 ± 4.7
Height (cm)	169.5 ± 6.3	171.2 ± 7.1
Body weight (kg)	59.6 ± 7.1	59.3 ± 5.0
Body Mass Index (kg/m^2)	20.7 ± 2.1	20.3 ± 1.7
Training volume (h/month)	45.2 ± 16.5	47.0 ± 18.9
Full-time athlete (%)	16.7	20.0
Level of competition		
Club (%)	63.3	86.7
National team (%)	20.0	6.7
Professional (%)	10.0	6.7
Others (%)[a]	6.7	0.0

FUEL, the FUEL intervention group; CON, the control group.
Continuous data are presented as mean ± SD and categorical data as percentage.
[a]Athletes who did not identify themselves as competing at club-, national team-, or professional level, e.g., competing in one of the endurance disciplines but not affiliating within a club.

3.1. Symptoms of low energy availability

3.1.1. Comparing pre- and postintervention group differences

The FUEL athletes reduced the LEAF-Q total score from 12.0 ± 2.8 to 9.8 ± 4.3 ($BF_{10} = 20.92$) compared to CON athletes reducing the LEAF-Q total score from 11.0 ± 3.0 to 10.3 ± 2.5 ($BF_{10} = 0.79$) with no evidence for difference in change between groups (**Table 2**). Nor did any of the changes in the LEAF-Q subscale scores differ between groups as indicated by the lack of an interaction effect ($BF_{incl} < 1$). At posttest, total LEAF-Q score was <8 for $n = 11$ (37%) of the FUEL athletes and $n = 2$ (13%) of the CON athletes ($BF_{10} = 1.267$).

The number of participants that reported eumenorrhea increased among FUEL athletes from 30% ($n = 9$ athletes) at pretest to 67% ($n = 20$ athletes) at posttest and decreased among CON athletes from 73% ($n = 11$) to 53% ($n = 8$) (**Figure 1**). Five of the 14 (36%) FUEL athletes, who reported menstrual dysfunction at pretest, reported eumenorrhea at posttest. Of the FUEL athletes who reported menstrual dysfunction at pretest and eumenorrhea at posttest, all reported their latest menstruation within the last 0–3 month at pretest. Three FUEL athletes and one CON athlete reported secondary amenorrhea at pretest. None of them improved their menstrual function form pre to posttest.

Seven (23%) FUEL athletes and three (20%) CON athletes were unaware whether they had normal menstruation at pretest. All FUEL athletes were able to define whether they had normal menstruation or not at posttest, while the number was unchanged among CON athletes. The number of athletes who reported reduced or absence of menstrual bleedings with increased training load decreased from $n = 21$ (70%) to $n = 14$ (47%) among FUEL athletes while the number was unchanged among CON athletes ($n = 14/73\%$) (**Figure 2**). Twelve (40%) FUEL athletes and four (27%) CON athletes reported late menarche a (menarche after 15 years of age).

3.1.2. Six- and 12-months follow-up

Six- and 12-months follow-up revealed strong evidence for improvement in LEAF-Q total score for FUEL athletes comparing the three ($BF_{incl} = 441$) and four ($BF_{incl} = 123$) measurement points, respectively (**Figure 3A**). This was explained by improvements in the menstrual score (6-months: $BF_{incl} = 4{,}486$, 12-months: $BF_{incl} = 840$) (**Figure 3B**) and the gastrointestinal score (6-months: $BF_{incl} = 9.5$, 12-months: $BF_{incl} = 2.3$) (**Figure 3C**). We found weak evidence for an improvement in the gastrointestinal score from 6- to 12-months follow-up ($BF_{10} = 1.2$) while no evidence for improvement in LEAF-Q total score, menstrual score or injury score when comparing 6- and 12-months follow-up ($BF_{10} < 1$) (**Figure 3D**).

At 6-months follow-up, 45% of FUEL athletes had a total LEAF-Q score <8, and 21% at 12-months follow-up. The two FUEL athletes with secondary amenorrhea at pretest, who was eligible for long-term follow-up, still had not improved menstrual function at 6 months follow-up, but one reported eumenorrhea at 12-months follow-up.

TABLE 2 Low energy availability in females questionnaire scores pre- and postintervention.

	FUEL ($n = 30$)			CON ($n = 15$)			Difference in change between groups, BF_{incl}
	Pre	Post	Difference pre-post	Pre	Post	Difference pre-post	
LEAF-Q total score	12.0 ± 2.8	9.8 ± 4.3	−2.2 ± 3.6	11.0 ± 3.0	10.3 ± 2.5	−0.7 ± 1.7	0.850
Injury score	3.2 ± 2.3	2.8 ± 2.3	−0.3 ± 2.0	3.7 ± 2.0	3.5 ± 2.1	−0.3 ± 1.4	0.320
Gastro-intestinal score	2.3 ± 2.1	1.7 ± 1.5	−0.6 ± 1.6	2.3 ± 1.7	2.1 ± 1.5	−0.1 ± 1.6	0.544
Menstrual score	6.6 ± 2.5	5.3 ± 3.0	−1.2 ± 1.8	5.1 ± 2.7	4.7 ± 2.3	−0.4 ± 2.2	0.729

Data are presented as mean ± SD.
BF_{incl}, bayes factor for inclusion of group × time interaction; CON, control group; FUEL, the FUEL intervention group, LEAF-Q, low energy availability in females questionnaire.

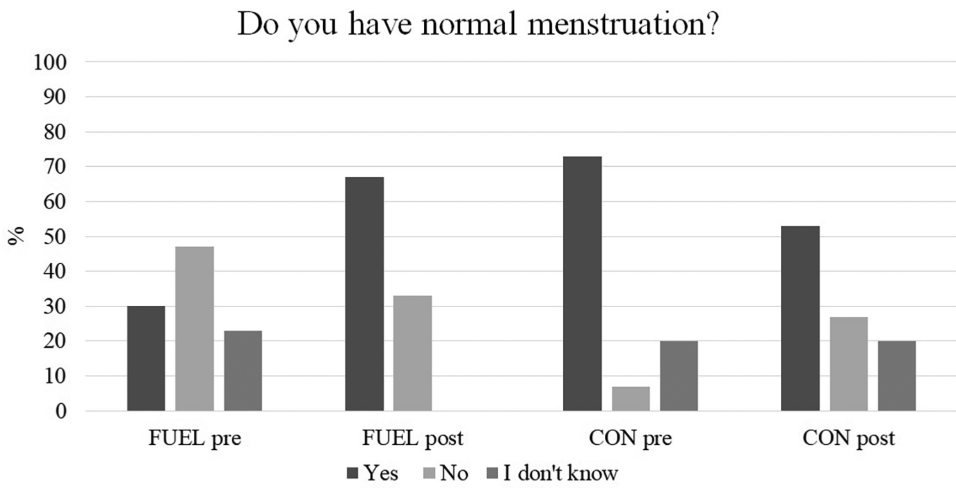

FIGURE 1
Self-reported eumenorrhea from the low energy availability questionnaire pre-and postintervention. Data are presented as percentages. CON, the control group; FUEL, the FUEL intervention group.

3.2. Symptoms of disordered eating behavior

3.2.1. Comparing pre- and postintervention group differences

The EDE-Q global score decreased from 1.03 ± 0.73 to 0.72 ± 0.69 ($BF_{10} = 11.84$) among FUEL athletes and was unchanged among CON athletes (0.80 ± 0.74 at pretest and 0.96 ± 0.85 at posttest, $BF_{10} = 0.41$) with weak evidence for a difference in change between groups as indicated by the interaction effect of $BF_{incl =}$ 1.858 (Table 3). The largest within-group difference among FUEL athletes for the EDE-Q subscales was detected for the restraint subscale score (BF_{10} = 14.87). In contrast, weak evidence for an increase in the EDE-Q subscale weight concern was found among controls ($BF_{10} = 1.68$).

The EDE-Q global score increased above the 2.5 threshold post-intervention for two (7%) FUEL athletes (pre-intervention EDE-Q global scores of 0.4 and 2.4, respectively) and one (7%) CON athlete (pre-intervention EDE-Q global score 2.4) to EDE-Q global scores 2.8, 3.0, and 2.5, respectively.

3.2.2. Six- and 12-months follow-up

Long-term follow-up revealed moderate evidence ($BF_{incl} = 5.18$) for reduced EDE-Q global score for FUEL athletes comparing all four measuring points (Figure 4). The largest reduction in the EDE-Q subscale scores was seen in the restraint subscale ($BF_{incl} = 16.45$).

The two FUEL athletes with EDE-Q global scores ≥2.5 at postintervention, had EDE-Q global scores of 0.0 and 0.3, respectively, at 6-months follow-up and 0.3 and 0.8, respectively, at 12-months follow-up.

3.3. Exercise addiction inventory

3.3.1. Comparing pre- and postintervention group differences

Within group analyses revealed no evidence for changes from pre- to posttest in EAI total or the six item scores among FUEL nor CON athletes ($BF_{10} < 1$). Nor did we find evidence for difference in change between groups for the EAI total score (FUEL pre: 20.7 ± 3.0, FUEL post: 20.8 ± 2.7 vs. CON pre: 20.6 ± 3.0, CON post: 21.1 ± 2.9) or any of the six item scores ($BF_{incl} < 1$).

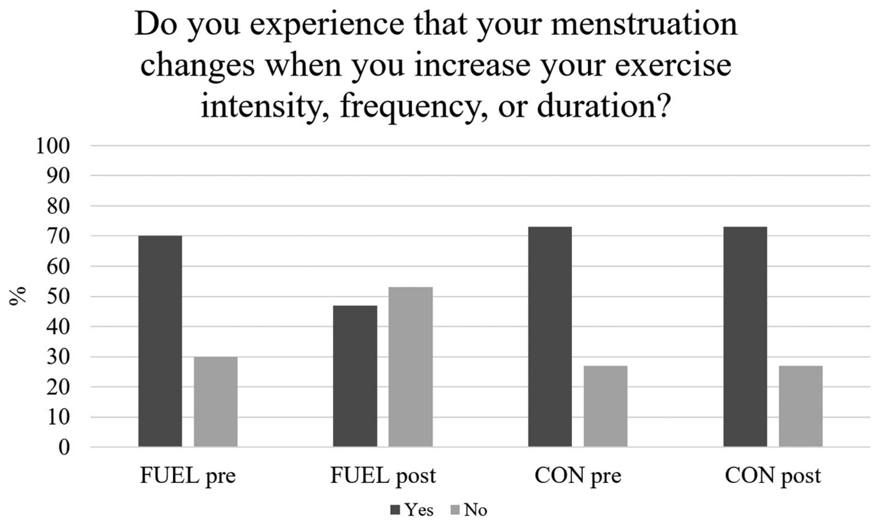

FIGURE 2
Reduced, or absence of, menstrual bleedings with increased training load from the low energy availability questionnaire pre-and postintervention. Data are presented as percentages. CON, the control group; FUEL, the FUEL intervention group.

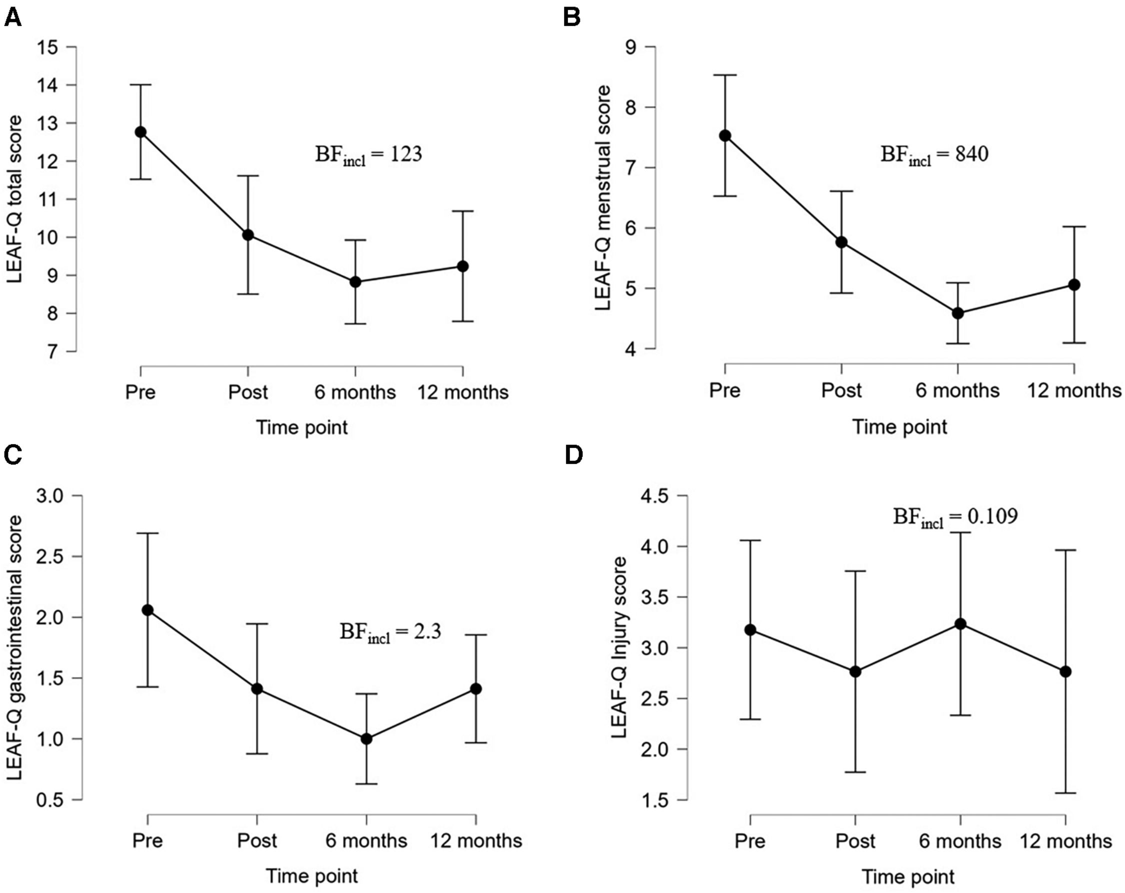

FIGURE 3
Changes in LEAF-Q (A) total score, (B) menstrual score, (C) gastrointestinal score, and (D) injury score for the FUEL athletes at pre- and postintervention, and at 6- and 12-months follow-up. Data are presented as mean and 95% credible intervals. BF_{incl}, bayes factor for inclusion of time interaction, LEAF-Q, low energy availability in females questionnaire.

TABLE 3 Eating disorder examination questionnaire scores pre- and postintervention among FUEL (n = 30) and CON athletes (n = 15).

	FUEL (n = 30)			CON (n = 15)			Difference in change between groups, BF_{incl}
	Pre	Post	Difference pre-post	Pre	Post	Difference pre-post	
EDE-Q global	1.03 ± 0.73	0.72 ± 0.69	−0.31 ± 0.76	0.80 ± 0.74	0.96 ± 0.85	0.16 ± 0.56	1.858
EDE-Q restraint	0.79 ± 0.94	0.38 ± 0.62	−0.41 ± 0.98	0.96 ± 1.21	0.83 ± 1.08	−0.16 ± 0.51	0.498
EDE-Q eating concern	0.72 ± 0.75	0.46 ± 0.62	−0.25 ± 0.64	0.39 ± 0.39	0.51 ± 0.45	0.12 ± 0.30	2.006
EDE-Q weight concern	1.18 ± 0.85	0.97 ± 0.90	−0.21 ± 0.96	0.92 ± 1.08	1.4 ± 1.3	0.46 ± 1.08	1.768
EDE-Q shape concern	1.42 ± 0.94	1.05 ± 0.95	−0.37 ± 1.02	0.98 ± 0.65	1.16 ± 1.11	0.18 ± 0.83	1.230

Data are presented as mean ± SD.
BF_{incl}, bayes factor for inclusion of group × time interaction; CON, the control group; FUEL, the FUEL intervention group; EDE-Q, eating disorder examination questionnaire.

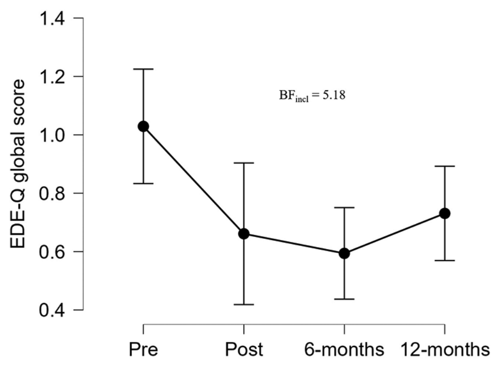

FIGURE 4
Eating disorder examination questionnaire global score for the FUEL athletes at pre- and postintervention, and at 6- and 12-months follow-up. Data are presented as mean and 95% credible intervals. BF_{incl}, bayes factor for inclusion of time interaction; EDE-Q, eating disorder examination questionnaire.

3.3.2. Six- and 12-months follow-up

Six- and 12-months follow-up revealed strong evidence (BF_{incl} = 31.50) for reduced EAI total score for FUEL athletes comparing the four measuring points (**Figure 5**).

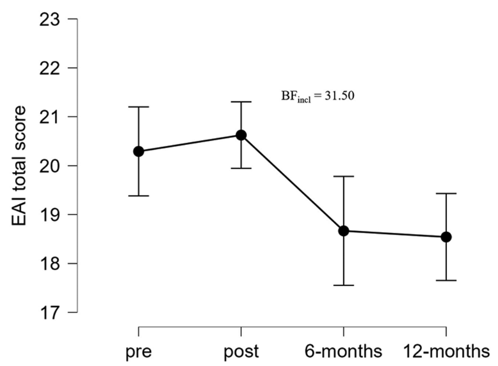

FIGURE 5
Exercise addiction inventory total score for the FUEL athletes at pre- and postintervention, and at 6- and 12-months follow-up. Data are presented as mean and 95% credible intervals. BF_{incl}, bayes factor for inclusion of time interaction; EAI, exercise addiction inventory.

4. Discussion

To our knowledge, this is the first study to explore changes on several REDs related symptoms in female endurance athletes with risk of REDs after a nutrition intervention using validated screening tools, comparison with a control group, and inclusion of long-term follow-up. More specifically the current study explored changes in menstrual and gastrointestinal function, injuries, eating disorder and exercise addiction symptoms. The FUEL study was an international multicenter study with weekly online sports nutrition lectures combined with individual consultations every other week. The lectures were specifically designed for female endurance athletes with risk of REDs. Although no evidence for difference in change between FUEL and CON athletes pre- to postintervention were found in LEAF-Q scores, long-term follow-up revealed strong evidence for reduced LEAF-Q total and menstrual scores among FUEL athletes. Importantly, the nutrition intervention did not result in negative effects related to eating disorder or exercise addiction symptoms. Rather, there was evidence for improved EDE-Q scores after the FUEL intervention. The reduction in eating disorder symptoms for FUEL athletes remained at 6- and 12-month follow-up.

In this study, athletes were categorized with risk of REDs using the LEAF-Q (4). The LEAF-Q has been validated in female endurance athletes, 18–39 years of age, training ≥5 times/week with Cronbach's Alpha 0.61–0.79, and an acceptable sensitivity (78%) and specificity (90%) (4), making it a good alternative to assess symptoms of LEA in this group of athletes. The LEAF-Q has subsequently been validated in a mixed sport-cohort (n = 75, 18–32 years), which demonstrated high sensitivity for the detection of low bone mineral density and menstrual dysfunction, suggesting that injury and menstrual function cutoff score also may be appropriate in mixed-sport cohort (57). The researchers concluded that LEAF-Q total score <8 can be used to determine females at low risk of LEA related conditions given the high negative predictive values identified in this study (57). In the present study we examined all LEAF-Q responses in detail and excluded athletes who had been diagnosed with menstrual dysfunction not related to LEA and others where false positive identification of problematic LEA was expected (e.g., athletes who had been involved in a bicycle crash and menstrual dysfunction in the past resulting in a LEAF-Q total score ≥8). Nevertheless, using screening tools as inclusion and exclusion criteria contains a risk of including false positive cases (e.g., high LEAF-Q total score due to acute injuries), including false negative cases (e.g., athletes reporting eumenorrhea while undetected subclinical menstrual dysfunction (23, 58), as well as excluding false negative cases (e.g., high menstrual function score due to polycystic ovarian syndrome while coexisting symptoms of LEA).

In our study, the decline in LEAF-Q total score was 18% among FUEL and 6% among CON athletes. Although there was a lower risk rate of LEA among FUEL (64%) compared to CON (87%) athletes at posttest, we did not detect between group difference from pre- to post intervention when comparing LEAF-Q total score. The 16-week intervention period may have been too short to detect differences in the measured symptoms. Especially regarding the LEAF-Q injury score, which is related to the previous year and associated with low bone mineral density (4, 57), where an improvement cannot be expected within the time frame of the study period (59). But the absence of intervention effect may also be attributed to the time required to restore normal menstrual function and the complexity of changing eating habits.

All FUEL athletes who reported menstrual dysfunction at pretest but eumenorrhea at posttest, had reported a recent bleeding at pretest, while none of the three FUEL athletes reporting long-term absence of bleeding at pretest, had improved menstrual function at posttest. The two FUEL athletes with long-term absence of bleeding, who were eligible for long-term follow-up, still had not improved menstrual function at 6-months follow-up, but one reported eumenorrhea at 12-months follow-up, suggesting that recovery time from more severe menstrual disorders may be longer. Previous studies have reported mean time to restoration of menstruation to be as high as 16 ± 3 months among college athletes with nonpharmacologic therapies (60) and researchers have suggested that the time required to resume menstruation depends to a large extend on the starting point, including the duration of the menstrual dysfunction (14, 15). Unfortunately, the maximum duration of menstrual dysfunction assessed in the present study was ≥6 months (corresponding to the response option when answering "*no*" to "*do you have normal menstruation*"). In the present study six athletes, all with menstrual dysfunction, reported bone stress injuries during the last year at pretest, indicating long-term exposure of LEA.

Indeed, habits may take more than sixteen weeks to change (61) and we have previously emphasized the complexity of improving eating habits in this group of athletes (36). Since the increase in energy intake was modest among FUEL athletes (138 ± 453 kcal/day, corresponding to an increase of only 5%) (36), it may have been insufficient for improving REDs related symptoms in some of the athletes. The five FUEL athletes who reported menstrual dysfunction at pretest and eumenorrhea at posttest had a slightly higher increase in energy intake compared to the nine FUEL athletes who reported menstrual dysfunction both at pre- and posttest (7% vs. 1%). Previous studies with athletes and active females have reported increase in energy intake of 17% (14) and 18% (15, 16) after nutrition interventions of 6, 9, and 12 months, respectively. In these studies, 88% (14) and 23% (15) of the athletes restored regular menstruation after the intervention, while De Souza et al. reported improved menstrual function in 64% in a group of active females (16). Although we recognized the complexity of habitual changes in the study planning phase and implemented individual athlete-centered nutrition counseling, sixteen weeks may be too short for changing eating habits that can result in improvement of REDs symptoms. Since energy availability is energy intake relative to exercise energy expenditure, changes in LEAF-Q score could also be attributed to changes in training volume. We have previously reported decreased training volume among FUEL and CON athletes from pre to posttest with no difference in change between groups (36). Hence, training load was reduced independent of group and athletic season. Although training adjustment was not a part of the FUEL intervention, it is possible that some athletes deliberately have reduced their training volume to improve REDs symptoms. While the relative increase in energy intake may be crucial, it has been suggested that increase in body fat mass is an important predictor of restoration of menstrual function in athletes and active females (15, 16). Unfortunately, body composition was not possible to measure in the present study due to the COVID-19 pandemic (39).

At pretest ∼20% of the participants in the present study were unable to define their menstrual status while all FUEL athletes could define their menstrual status at posttest, with unchanged results for CON athletes, suggesting that the FUEL intervention succeeded in increasing the awareness of the menstrual cycle. Being aware of one's menstrual cycle, and the importance of having a regular menstrual cycle, seems like an obvious first step in the prevention of problematic LEA and REDs for female athletes. Especially since menstrual dysfunction is associated with low bone mineral density reported in 17%–45% of female endurance athletes (6, 40, 62–64) and an increased risk for bone stress injuries, resulting in long absences from sport participation (25, 26).

Among FUEL athletes the 6- and 12-months follow-up revealed strong evidence for improvement of LEAF-Q total score explained by improvements in the gastrointestinal score and in particular the menstrual score. Although positive changes in LEAF-Q total and subscale scores were observed, menstrual-, injury-, and total scores were all above the suggested cut-offs [≥2 for injuries, ≥2 for gastrointestinal symptoms, ≥4 for menstrual function, and ≥8 for total score (4)] at all four measuring points. At 6-months follow-up, 45% had a LEAF-Q score <8, while only 21% at 12-months follow-up. These findings may indicate that these female athletes need continuous nutritional support (e.g., individual follow-up sessions).

Importantly, no adverse effects on eating disorder or exercise addiction symptoms were found after participating in the FUEL intervention. Rather, we found evidence for a difference in change between groups for the EDE-Q global score, while long-term follow-up for FUEL athletes suggested persistent reduction in EDE-Q global and a reduction in EAI score. A recent systematic review of eating psychopathology interventions delivered to athletes (65, 66) found that less than half of the included studies reported sustained reductions in eating psychopathology, while two studies on ballet dancers reported an increase in eating psychopathology symptoms following the interventions. Importantly, our study differentiates from the studies in the systematic review by excluding athletes with risk of eating disorders, since these athletes are recommended an interdisciplinary treatment including psychiatric treatment (66). Interestingly, the authors of the review conclude that future interventions should investigate other modes of delivery beyond face-to-face group sessions, including digital approaches, which makes intervention retention more flexible for the participants but also serve to overcome stigma (65). This may in part explain the positive development in EDE-Q scores among FUEL athletes in the present study, but the explanation may also be found in the principles of the FUEL intervention reflected in the teaching videos and the individual consultations: Focus away from body weight and more towards food as fuel and that there are no "good" or "bad" foods. Two FUEL athletes had increased EDE-Q global score above the 2.5 cut-off from pre- to posttest which reduced well-below the 2.5 at long-term follow-up indicating that some athletes may have transient changes in eating disorder symptoms during the athletic seasons, and that regular screening and follow up assessments are needed.

Among FUEL athletes, post-hoc tests found weak to moderate evidence for change comparing the preintervention EAI total score with 6- and 12-months follow-up, respectively. Although no difference in changes in EAI scores between FUEL and CON athletes were detected postintervention, it is interesting that FUEL athletes reduced their score at long-term follow-up. This should be seen in light of the reduced LEA and eating disorder symptoms at 6- and 12-months follow-up, symptoms that have been reported to be associated with symptoms of exercise addiction (39, 43, 44). However, both changes in athletic season (67) and the COVID-19 pandemic (68) may also be explanatory factors to the changes in exercise addiction symptoms. Further, FUEL athletes with risk of primary exercise addiction preintervention, reduced and increased LEAF-Q total score, respectively, suggesting a complex symptom picture and the potential interaction between exercise addiction and risk of REDs.

4.1. Strengths and limitations

A strength of the present study is the combined intervention design, including both online lectures and individual consultations, which were athlete-centered and aimed at inducing long-term behavioral change by enabling female athletes to actively formulate nutritional and behavioral goals to support their own long-term health, as researchers have requested (32). As previously recommended (16), this type of intervention opens for a more individual-centered approach compared to previous studies aiming at improving REDs related symptoms in females (16). The knowledge and tools acquired by the athletes presumably enables a longer-lasting behavior change compared to studies where the participants are given nutritional supplements only (14). Other strengths of the present study are the use of validated screening tools, long-term follow-up, and inclusion of a control group, which have been lacking in previous studies (14, 15). In addition, hormonal contraceptive users were excluded, in order to get the true picture of menstrual function. This, however, complicates the recruitment of the participants since the prevalence of hormonal contraceptive users among endurance athletes have been reported to be as high as 68% (69). Although the participant information material described that hormonal contraception users could not participate, we had to exclude 28% of the athletes who had signed up for the study due to the use of hormonal contraceptives. Unfortunately, the exclusion of hormonal contraceptive users may have prevented potential REDs cases to participate, e.g., since hormonal contraceptives may mask underlying menstrual dysfunctions.

A limitation of the present study is the lack of long-term follow-up in the CON athletes. Consequently, the long-term effects of the FUEL intervention can only be speculative. By having long-term follow-up of the CON condition, athletes would have to wait an additional year before being offered the FUEL intervention, thereby increasing the risk of a higher drop-out rate in this group. There may also be ethical considerations, since these athletes all have REDs related symptoms, early intervention is important. While prioritizing the intervention in athletes' off-season, it is a limitation that data assessment was conducted at different phases of the athletic season for the intervention group and the control group, which may reduce comparability between the two groups.

The low number of participants in the CON group is also a limitation. Based on an initial analysis during the recruitment phase with an expected improvement in LEAF-Q score of 3 and type I and Type II error of 5% and 20% respectively, the power calculation suggested 28 subjects in each group, suggesting an insufficient number of CON athletes. In addition, the expected improvement in LEAF-Q score of 3 is theoretically founded based on a project group discussion, without any previous studies to lean on.

Although it would have been interesting to collect data via physical laboratory tests, e.g., including body composition and female sex hormones to verify menstrual status, this study and its measurement methods more closely reflect what is practically possible for most athlete-based centers where time and resources are a critical constraint. The intervention and methods used may therefore more easily be implemented in real life settings.

Despite that this study included a combination of online lectures and individual consultations using behavior change theories and approaches, intervening athletes alone may be insufficient for behavior change and thus changes in REDs related symptoms. As previously addressed (36), cultural revolutions and changes in social norms are needed, which involves inclusion of coaches, health professionals, entire teams/clubs, and relatives of the athletes. Hence, future research should aim for also including the athletes' entourage.

5. Conclusion

In this group of endurance athletes, participating in the FUEL intervention implies long-term improvement of REDs related symptoms, including menstrual function. In addition, short and long-term follow-up suggest no adverse effects on eating disorder symptoms. The lack of long-term follow-up for the CON condition indicates, however, that the results should be interpreted with caution. Nevertheless, the FUEL intervention seems promising as a part of management of REDs related symptoms in female endurance athletes.

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 study was approved by the regional ethics committee in Norway (31,640), Sweden (2019-04809), and by the Norwegian Centre for Research Data (968,634) and registered at www.clinicaltrials.gov (NCT04959565).

Author contributions

IF: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. AM: Conceptualization, Investigation, Methodology, Supervision, Writing – review & editing. IG: Conceptualization, Methodology, Supervision, Writing – review & editing. PW: Investigation, Writing – review & editing, Data curation, Methodology, Resources. AI: Formal Analysis, Methodology, Supervision, Writing – review & editing. S-MH-S: Methodology, Resources, Writing – review & editing, Conceptualization, Supervision. KK: Methodology, Resources, Writing – review & editing, Investigation. DL: Investigation, Resources, Writing – review & editing. SM: Investigation, Resources, Writing – review & editing. MG: Investigation, Writing – review & editing. MT: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Menstruation-related symptoms are associated with physical activity and midpoint of sleep: a pilot study

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Introduction: Menstruation-related symptoms (MRSs) significantly impact women's health and contribute to economic burdens worldwide. Current interventions, primarily pharmacological ones, have limitations and side effects that underscore the need for alternative management strategies. This study explores the association between MRSs and lifestyle factors, specifically physical activity and sleep timing across menstrual cycle phases, to inform non-pharmacological intervention development.

Methods: Fourteen female students from Ritsumeikan University, Japan, with regular menstrual cycles (25–38 days), not on hormonal treatment or engaged in shift work, participated in this observational study. Using a Fitbit Inspire 2, total daily energy expenditure (TDEE) and sleep timing were monitored over a complete cycle. Menstrual cycle phases were defined based on ovulation day, predicted using home luteinizing hormone tests. Participants completed daily electronic questionnaires rating MRSs using a modified menstrual distress questionnaire. Data were analyzed using a generalized linear mixed model with a gamma distribution and logarithmic link function, examining the relationship of TDEE and the midpoint of sleep time (MS time) with MRS severity.

Results and discussion: The following observations were noted: first, MRS severity, except for behavioral change symptoms, significantly increased during the menstrual and luteal phases compared to the follicular phase. Second, delayed MS time was associated with reduced pain, concentration symptoms, water retention, and negative affect during the menstrual phase and reduced negative affect during the luteal phase. Finally, an increase in TDEE was associated with reduced concentration symptoms, autonomic reaction symptoms, and negative affect during the menstrual and luteal phases and reduced water retention only during the luteal phase. This study provides insights into the relationship between MRSs and TDEE/MS time, suggesting potential non-therapeutic approaches for symptom management, though further research is needed to substantiate these findings for practical applications.

KEYWORDS
menstruation-related symptoms, physical activity, sleep, menstrual cycle, menstrual cycle phase, generalized linear mixed model, wearable device

1. Introduction

Hormonal fluctuations associated with the menstrual cycle affect women's health. Menstruation-related symptoms (MRSs) include heavy menstrual bleeding, dysmenorrhea with lower abdominal pain associated with menstrual bleeding, and premenstrual syndrome, in which physical and mental discomfort persists for 3–10 days before menstrual bleeding. Sleep quality subjectively deteriorates during the menstrual and luteal phases in women with menstrual pain and premenstrual syndrome (1, 2). Objectively measured sleep quality has been found to be impaired in women with premenstrual syndrome, particularly premenstrual dysphoric disorder (PMDD) (3). MRSs are significant, leading to reduced academic performance and an increased economic burden due to decreased labor productivity (4, 5). Therefore, addressing MRSs transcends individual health, bearing significant implications for societal productivity and economic burden.

A survey conducted by Japan's Ministry of Health, Labor and Welfare of 3,000 women revealed that enduring symptoms and taking over-the-counter medications are common ways of coping with MRSs, and that the rate of visits to a medical institution is only around 10% (6). Painkillers and hormonal therapy are effective in alleviating MRSs, while nonsteroidal anti-inflammatory drugs relieve primary dysmenorrhea (7). Meanwhile, some individuals express concerns regarding the drug regimen and its associated side effects. Therefore, there is a need to develop non-drug methods to alleviate MRSs.

Recent studies have suggested exercise and light therapy approaches for alleviating MRSs. Exercise effectively improves physical symptoms, such as pain, constipation, and breast sensitivity, and mental symptoms, such as anxiety and anger (8, 9). Aerobic exercise has been reported to relieve dysmenorrhea (10). This effect is thought to be due to the fact that exercise stimulates estradiol secretion in the body and serotonin secretion in the brain (11, 12). Since an acrophase of circadian rhythms for patients with PMDD are advanced, light therapy was effective in significantly reducing their depressive symptoms (13). On the other hand, inappropriate sleep timing (social jet lag and shift work) may exacerbate these circadian rhythm disturbances and worsen MRSs (14).

While there has been significant research into the relationship between MRSs and physical activity/sleep timing, several methodological limitations persist in existing literature. Notably, many studies have relied on retrospective self-reports of symptoms, which are susceptible to recall bias and may not accurately capture the nuances of daily menstrual experiences. Furthermore, few have provided an integrated approach that considers physical activity, sleep timing, and MRSs within the context of the menstrual cycle phase (MCP) using daily data.

Our objective was to investigate the relationship between the severity of MRSs and total daily energy expenditure (TDEE) as physical activity and the midpoint of sleep time (MS time) according to the MCP. Our hypothesis was that increased TDEE, and delayed MS time during the menstrual and luteal phases are related to reduced MRSs. Establishing this relationship can pave the way for personalized exercise and sleep timing recommendations tailored to individual MCPs, thereby alleviating MRSs.

2. Methods

2.1. Participants

This study included 14 female undergraduate and graduate students at Ritsumeikan University, Japan. Participants were eligible for the study if they self-reported that they were not currently receiving treatment for sleeping disorders, had a menstrual cycle of 25–38 days (definition of a normal menstrual cycle by the Japanese Society of Obstetrics and Gynecology), were not taking hormonal medication that could affect their menstrual cycle, and were not shift workers.

2.2. Experimental protocol

Applicants were briefed about the experiment, and oral and written informed consent were obtained before the experiment. Participants completed questionnaires regarding basic information, such as age and height, and received instructions on the use of the experimental apparatus and procedures.

The participants wore a Fitbit Inspire 2 (Google, Mountain View, CA) wristwatch-type wearable device for 24 h during one menstrual cycle, except during bathing, under free-living conditions. Their TDEE, bedtime, and wake-up time were measured. Ovulation day prediction tests using ClearBlue (OMRON, Tokyo, Japan) were started 17 days before the expected start of menstruation to obtain reference data. The participants completed an electronic questionnaire every night before bedtime, reporting menstrual bleeding, ovulation-day predictor test results, and MRS scores. As the data were obtained under free-living conditions, there were no interventions regarding bedtime, wake-up time, caffeine or alcohol consumptioan, or exercise restrictions.

All study procedures were conducted in accordance with the principles of the Declaration of Helsinki. This study was approved by the Ethical Review Committee of Ritsumeikan University (approval number: BKC-LSMH-2021-063).

2.3. Survey items and data collection

2.3.1. Basic information

Age, height, weight, and menstrual start/end dates were obtained from participants' self-reports, and body mass index was calculated based on height and weight. The MCP for each participant was determined based on these data: the menstrual phase was defined as the period of bleeding (excluding irregular bleeding) in the first half of the menstrual cycle; the follicular phase was defined as the period from the day after the last day of the menstrual phase to the day of ovulation day predictive test positivity; and the luteal phase was defined as the period from the day after ovulation day predictive test positivity to the day before the next menstrual period start date (15). Because the length of the menstrual period varies among individuals, the self-reported menstrual period was used (16). The reference data for the day of ovulation were the days after the first positive result in the ovulation day prediction test. Home luteinizing hormone tests are widely used to detect ovulation and determine fertility (17).

2.3.2. Physical activity parameter: total daily energy expenditure

TDEE as a physical activity parameter was measured by Fitbit Inspire 2. The device is suitable for measuring TDEE (18). This device combines basal metabolic rate, activity data, and heart rate data to estimate TDEE (19). Data were obtained using the Fitbit Web API.

2.3.3. Sleep timing parameter: midpoint of sleep time

MS time as a sleep timing parameter was measured by Fitbit Inspire 2. MS time was calculated by identifying the time halfway between bedtime and waketime. For example, the MS time is 4:00 am if sleep onset is at 00:00, and the wake-up time is 8:00 am (20). MS time has been shown to be positively correlated with the circadian rhythm phase and is often used as a surrogate for the circadian rhythm phase (21). This device estimates sleep stages by analyzing patterns of movement and heart rate, determining sleep when there is no movement detected for approximately one hour (22). Prior research on this device has indicated it to have high sensitivity (93.9%) and accuracy (76%), and its use as an objective tool for measuring sleep in daily life is considered appropriate (23).

2.3.4. MRS parameters: MRS questionnaire score

The modified menstrual distress questionnaire (mMDQ), a 35-item Japanese version of the questionnaire, was used to collect MRS data. The questionnaire consists of six subscales: pain (six items), concentration symptoms (eight items), behavioral change symptoms (five items), autonomic reaction symptoms (four items), water retention (four items), and negative affect (eight items). Responses were scored on a 6-point Likert scale ranging from 1 (no response at all) to 6 (acute or partially disabling), with higher total scores indicating more severe symptoms. The original version of the MDQ was developed to assess MRS scores during menstruation, one week before menstruation, and the rest of the menstrual cycle (4, 24). In Japan, the two domains of arousal and control are often examined in six parts because there are few complaints in these domains (25). Therefore, 35 items from six environments were used in this study. In general, retrospective methods have limited reliability because the incidence and severity of symptoms are substantial and are often related to social conventions and attitudes (26). Therefore, we employed a direct method in which respondents answered the day's symptoms every day upon entering bed. The total score on each subscale of the mMDQ was used as a parameter for the MRSs.

2.4. Statistical analysis

The following two criteria were set for the data to be analyzed: a daily rate of at least 80% for obtaining bedtime/wake-up time information via Fitbit and a response rate of at least 90% for the electronic survey at bedtime. These criteria were used to ensure the reliability and accuracy of the data.

All statistical analyses were performed using R (R Foundation, Vienna, Austria). The significance level was set at $p < 0.05$. The generalized linear mixed model (GLMM) examined the relationship between MRSs and TDEE/MS time according to the MCP during one menstrual cycle. The objective variables for each model are as follows: Model 1, pain; Model 2, concentration symptoms; Model 3, behavioral change symptoms; Model 4, autonomic reaction symptoms; Model 5, water retention; Model 6, negative affect. The fixed effects were the same for all models and are as follows: main effects: TDEE, MS time, menstrual and luteal phases; reciprocal action: TDEE*Menstrual phase, TDEE*Luteal phase, MS time*Menstrual phase, MS time*Luteal phase. To avoid multicollinearity, the follicular phase was used as the criterion. Because of the significant individual differences in these effects, we employed a GLMM, which can be estimated more appropriately based on the random effects of human variation (27). To evaluate whether MCP acts causally, we established an interaction effect. **Table 1** lists the targets and main effects of each GLMM. In this study, the Bonferroni correction was applied to adjusted *P*-values to prevent inflation of type I errors.

3. Results

3.1. Characteristics of the analysis subjects

Nine of the 14 women were included in the analysis, two of whom may have been anovulatory based on the results of ovulation day predictor tests. Three other women were excluded from the analysis because their Fitbit bedtime/waketime data acquisition rate was less than 80%. The average and standard deviations of the menstrual cycles of the analyzed subjects were 30.6 ± 3.7 days, all within the normal range. They were not pregnant. **Table 2** summarizes the mean TDEE, MS time, and mMDQ subscale scores for each MCP in all participants. The mean TDEE was 1,757.2 ± 246.9 kcal for the menstrual phase, 1,717.0 ± 221.9 kcal for the luteal phase, and 1,698.9 ± 215.5 kcal

TABLE 1 Target and main effects for each GLMM.

Model no.	Target	Fixed factors
Model 1	Total pain score	TDEE, MS time, Menstrual phase, Luteal phase, TDEE*Menstrual phase, TDEE*Luteal phase, MS time*Menstrual phase, MS time*Luteal phase
Model 2	Total concentration score	
Model 3	Total behavioral change score	
Model 4	Total autonomic reactions score	
Model 5	Total water retention score	
Model 6	Total negative affect score	

GLMM, generalized linear mixed model; TDEE, total daily energy expenditure; MS time, midpoint of sleep time.

TABLE 2 Average and standard deviation of TDEE, MS time, and mMDQ scores per MCP for all participants.

MCP		Menstrual phase	Follicular phase	Luteal phase
TDEE [kcal]		1,757.2 ± 246.9	1,698.9 ± 215.5	1,717.0 ± 221.9
MS time [h]		5.35 ± 1.70	5.29 ± 1.52	5.49 ± 1.31
The score of the mMDQ [point]	Pain	10.2 ± 3.0	8.2 ± 2.0	8.5 ± 2.3
	Concentration	12.1 ± 5.4	10.7 ± 3.8	11.1 ± 4.8
	Behavioral change	8.8 ± 3.8	8.3 ± 3.7	8.6 ± 3.2
	Autonomic reactions	5.4 ± 1.4	4.5 ± 0.7	4.8 ± 0.9
	Water retention	6.5 ± 2.0	5.4 ± 1.4	6.4 ± 1.7
	Negative affect	11.8 ± 4.5	8.8 ± 1.1	10.2 ± 3.5

TDEE, total daily energy expenditure; MS time, midpoint of sleep time; mMDQ, modified menstrual distress questionnaire; MCP, menstrual cycle phase.

for the follicular phase. The mean MS time was 5.49 ± 1.31 h for the luteal phase, 5.35 ± 1.70 h for the menstrual phase, and 5.29 ± 1.52 h for the follicular phase. The menstrual phase group had the highest, and the follicular phase group had the lowest total mMDQ scores on all subscales.

3.2. Relationship evaluation using GLMM

The following were the results of the relationship between MRSs and TDEE/MS time according to the MCP using GLMM. The probability distribution was gamma, and the link function

TABLE 3 Fixed coefficients of models 1–4.

Target	Fixed factors	Estimate	SE	*t* value	Adjusted *p*-value
Model 1 Pain	Intercept	1.9**	0.54	3.6	0.0024
	TDEE	0.000080	0.000130	0.62	1.0
	MS time	−0.0069	0.042	−0.16	1.0
	Menstrual phase	0.75***	0.19	3.9	<0.001
	Luteal phase	0.49**	0.14	3.5	0.0036
	TDEE*Menstrual phase	−0.000017	0.000058	−0.29	1.0
	TDEE*Luteal phase	−0.00011	0.000042	−2.6	0.081
	MS time*Menstrual phase	−0.10*	0.029	−3.5	0.0047
	MS time*Luteal phase	−0.049	0.020	−2.5	0.11
Model 2 Concentration	Intercept	1.9***	0.22	8.4	<0.001
	TDEE	0.00016	0.00007	2.3	0.20
	MS time	0.032	0.023	1.4	1.0
	Menstrual phase	1.1***	0.14	7.8	<0.001
	Luteal phase	0.84***	0.11	7.9	<0.001
	TDEE*Menstrual phase	−0.00035***	0.000043	−8.2	<0.001
	TDEE*Luteal phase	−0.00039***	0.000032	−12	<0.001
	MS time*Menstrual phase	−0.076***	0.019	−3.9	<0.001
	MS time*Luteal phase	−0.035	0.016	−2.2	0.24
Model 3 Behavioral change	Intercept	2.9***	0.31	9.5	<0.001
	TDEE	−0.00061***	0.00013	−4.5	<0.001
	MS time	0.024	0.035	0.67	1.0
	Menstrual phase	−0.42	0.21	−2.0	0.43
	Luteal phase	0.12	0.16	0.76	1.0
	TDEE*Menstrual phase	0.00041***	0.000072	5.7	<0.001
	TDEE*Luteal phase	0.000030	0.000051	0.60	1.0
	MS time*Menstrual phase	−0.038	0.026	−1.4	1.0
	MS time*Luteal phase	−0.023	0.021	−1.1	1.0
Model 4 Autonomic reactions	Intercept	1.40***	0.26	5.4	<0.001
	TDEE	0.000027	0.000076	0.36	1.0
	MS time	0.008	0.074	0.11	1.0
	Menstrual phase	0.72***	0.16	4.4	<0.001
	Luteal phase	0.69***	0.12	5.8	<0.001
	TDEE*Menstrual phase	−0.00019**	0.000052	−3.7	0.0021
	TDEE*Luteal phase	−0.00032***	0.000037	−8.7	<0.001
	MS time*Menstrual phase	−0.053	0.022	−2.4	0.15
	MS time*Luteal phase	−0.019	0.017	−1.1	1.0

SE, standard error; TDEE, total daily energy expenditure; MS time, midpoint of sleep time.

*Significant at the $p < 0.05$ level.

**Significant at the $p < 0.01$ level.

***Significant at the $p < 0.001$ level.

TABLE 4 Fixed coefficients of models 5–6.

Target	Fixed factors	Estimate	SE	*t* value	Adjusted *p*-value
Model 5 Water retention	Intercept	1.4***	0.23	6.1	<0.001
	TDEE	0.000077	0.000094	0.82	1.0
	MS time	0.026	0.064	0.40	1.0
	Menstrual phase	0.68**	0.18	3.7	0.0023
	Luteal phase	0.94***	0.14	6.8	<0.001
	TDEE*Menstrual phase	−0.00011	0.000063	−1.7	0.78
	TDEE*Luteal phase	−0.00033***	0.000045	−7.3	<0.001
	MS time*Menstrual phase	−0.067*	0.023	−2.9	0.036
	MS time*Luteal phase	−0.042	0.019	−2.2	0.27
Model 6 Negative affect	Intercept	1.9***	0.18	10	<0.001
	TDEE	0.00013	0.000060	2.2	0.23
	MS time	0.019	0.021	0.93	1.0
	Menstrual phase	1.4***	0.20	7.1	<0.001
	Luteal phase	1.0***	0.15	6.8	<0.001
	TDEE*Menstrual phase	−0.00038***	0.000066	−5.7	<0.001
	TDEE*Luteal phase	−0.00033***	0.000046	−7.1	<0.001
	MS time*Menstrual phase	−0.10**	0.026	−3.7	0.0016
	MS time*Luteal phase	−0.064*	0.021	−3.1	0.017

SE, standard error; TDEE, total daily energy expenditure; MS time, midpoint of sleep time.
*Significant at the $p < 0.05$ level.
**Significant at the $p < 0.01$ level.
***Significant at the $p < 0.001$ level.

was logarithmic. Tables 3, 4 show the results for the GLMM fixed coefficients.

3.2.1. Relationship between pain and TDEE/MS time according to the MCP

In Model 1, where the target variable was pain, we observed a significant increase in pain during the menstrual and luteal phases compared to the follicular phase (Menstrual phase: Estimate = 0.75, standard error [SE] = 0.19, adjusted $p < 0.001$; Luteal phase: Estimate = 0.49, SE = 0.14, adjusted $p = 0.0036$). Additionally, there was a notable interaction effect between MS time and the menstrual phase, with a decrease in pain associated with a delayed MS time during the menstrual phase (Estimate = −0.10, SE = 0.029, adjusted $p = 0.0047$). Figure 1 shows the relationship between pain and MS time.

3.2.2. Relationship between concentration symptoms and TDEE/MS time according to the MCP

In Model 2, where the target variable was concentration symptoms, we observed a significant increase in concentration symptoms during the menstrual and luteal phases compared to the follicular phase (Menstrual phase: Estimate = 1.1, SE = 0.14, adjusted $p < 0.001$; Luteal phase: Estimate = 0.84, SE = 0.11, adjusted $p <$ 0.001). Furthermore, an increase in TDEE during these phases was associated with a decrease in concentration symptoms (TDEE*

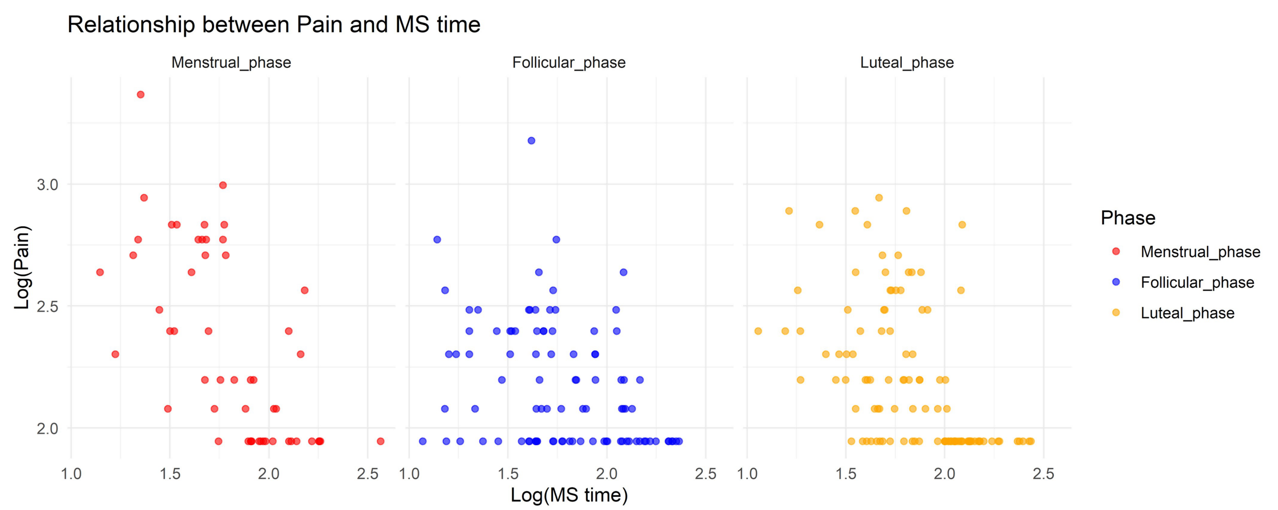

FIGURE 1
Relationship between pain and midpoint of sleep time.

Menstrual phase: Estimate = −0.00035, SE = 0.000043, adjusted $p < 0.001$; TDEE*Luteal phase: Estimate = −0.00039, SE = 0.000032, adjusted $p < 0.001$). Additionally, a regression in MS time during the menstrual phase was related to a reduction in concentration symptoms (MS time* Menstrual phase: Estimate = −0.076, SE = 0.019, adjusted $p < 0.001$). **Figure 2** shows the relationship between concentration symptoms and TDEE/MS time.

3.2.3. Relationship between behavioral change symptoms and TDEE/MS time according to the MCP

In Model 3, where the target variable was behavioral change symptoms, we observed a significant association with TDEE, where an increase in TDEE was linked with a decrease in behavioral change symptoms (Estimate = −0.00061, SE = 0.00013, adjusted $p < 0.001$). Moreover, the interaction term between TDEE and the menstrual phase was significant, indicating that an increase in TDEE during the menstrual phase is associated with an increase in behavioral change symptoms (Estimate = 0.00041, SE = 0.000072, adjusted $p < 0.001$). No significant effects were found for the menstrual and luteal phases on their own, nor for their interactions with TDEE. **Figure 3** shows the relationship between behavioral change symptoms and TDEE.

3.2.4. Relationship between autonomic reaction symptoms and TDEE/MS time according to the MCP

In Model 4, where the target variable was autonomic reaction symptoms, we observed a significant increase in autonomic reaction symptoms during the menstrual and luteal phases compared to the follicular phase (Menstrual phase: Estimate = 0.72, SE = 0.16, adjusted $p < 0.001$; Luteal phase: Estimate = 0.69, SE = 0.12, adjusted $p < 0.001$). Furthermore, an increase in TDEE during these phases was associated with a decrease in autonomic reaction symptoms (TDEE* Menstrual phase: Estimate = −0.00019, SE = 0.000052, adjusted $p = 0.0021$; TDEE*Luteal phase: Estimate = −0.00032, SE = 0.000037, adjusted $p < 0.001$). **Figure 4** shows the relationship between autonomic reaction symptoms and TDEE.

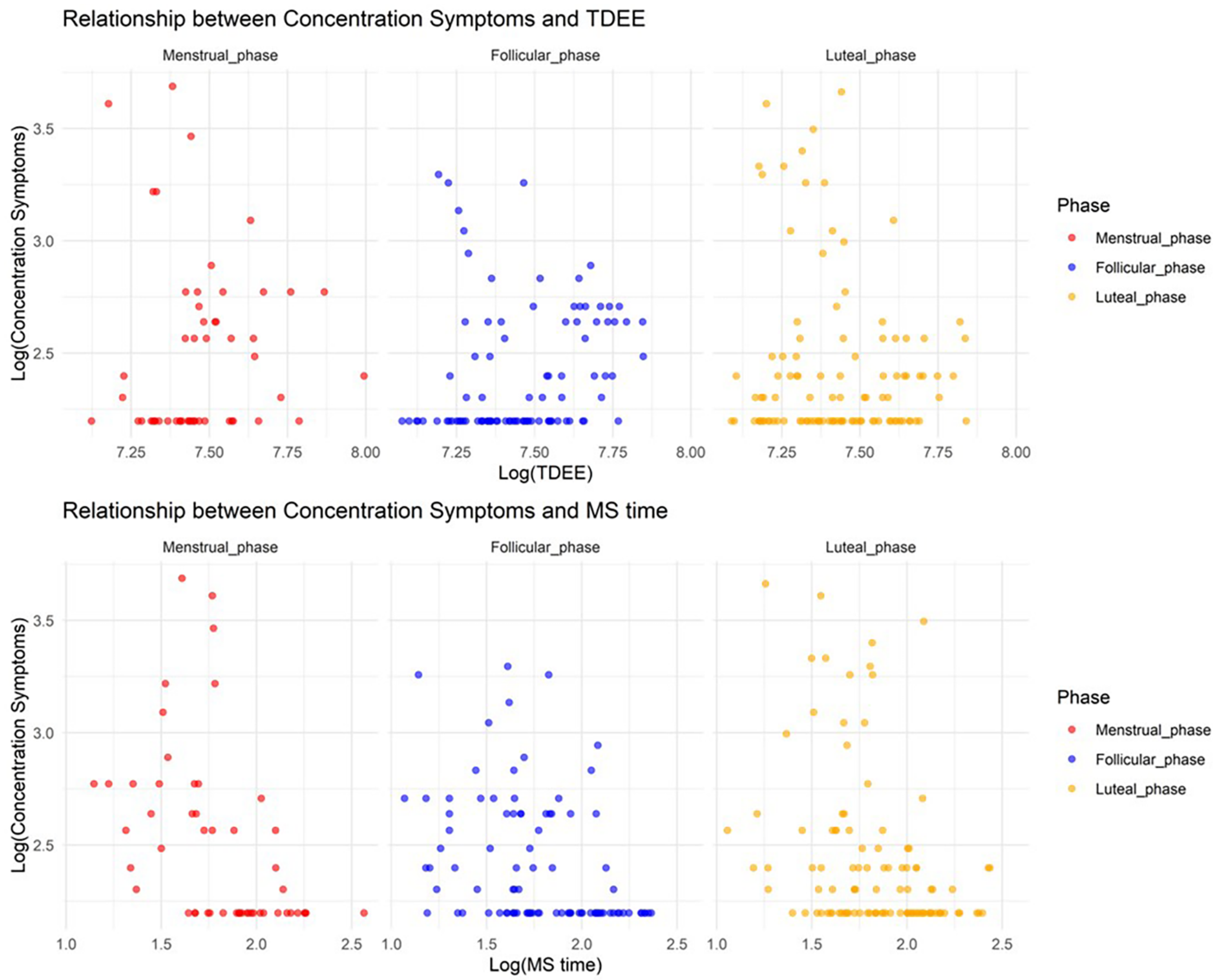

FIGURE 2
Relationship between concentration symptoms and total daily energy expenditure/midpoint of sleep time.

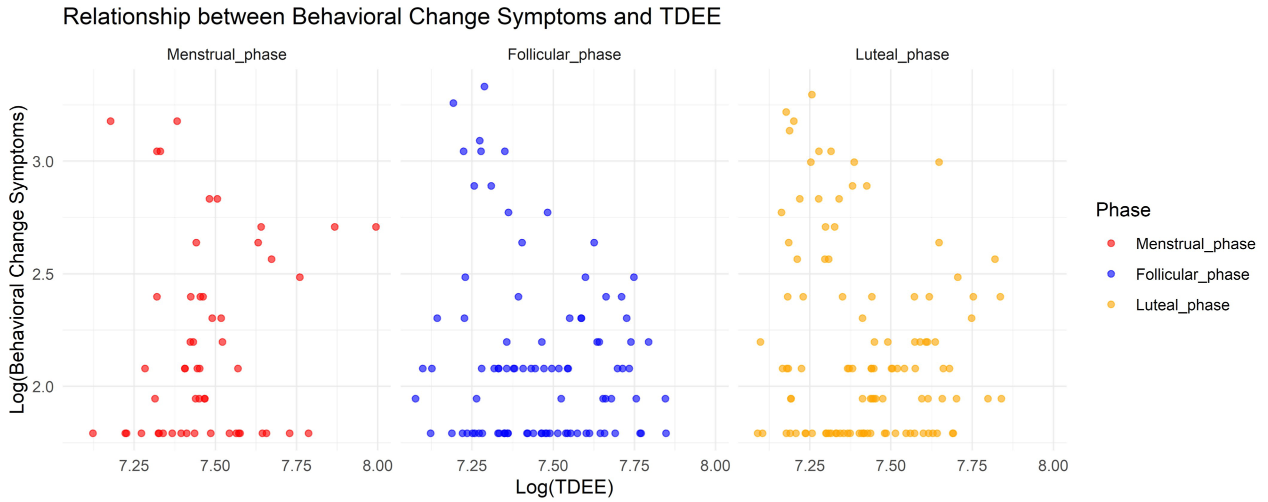

FIGURE 3
Relationship between behavioral change symptoms and total daily energy expenditure.

3.2.5. Relationship between water retention and TDEE/MS time according to the MCP

In Model 5, where the target variable was water retention, we observed a significant increase in water retention during the menstrual and luteal phases compared to the follicular phase (Menstrual phase: Estimate = 0.68, SE = 0.18, adjusted $p = 0.0023$; Luteal phase: Estimate = 0.94, SE = 0.14, adjusted $p < 0.001$). Furthermore, an increase in TDEE during the luteal phase was associated with a decrease in water retention (TDEE*Luteal phase: Estimate = −0.00033, SE = 0.000045, adjusted $p < 0.001$). Additionally, a regression in MS time during the menstrual phase was related to a reduction in water retention (MS time* Menstrual phase: Estimate = −0.067, SE = 0.023, adjusted $p = 0.036$). Figure 5 shows the relationship between water retention and TDEE/MS time.

3.2.6. Relationship between negative affect and TDEE/MS time according to the MCP

In Model 6, where the target variable was negative affect, we observed a significant increase in negative affect during the menstrual and luteal phases compared to the follicular phase (Menstrual phase: Estimate = 1.4, SE = 0.20, adjusted $p < 0.001$; Luteal phase: Estimate = 1.0, SE = 0.15, adjusted $p < 0.001$). Furthermore, an increase in TDEE during these phases was associated with a decrease in negative affect (TDEE* Menstrual phase: Estimate = −0.00038, SE = 0.000066, adjusted $p < 0.001$; TDEE*Luteal phase: Estimate = −0.00033, SE = 0.000046, adjusted $p < 0.001$). Additionally, a regression in MS time during these phases was related to a reduction in negative affect (MS time* Menstrual phase: Estimate = −0.10, SE = 0.026, adjusted

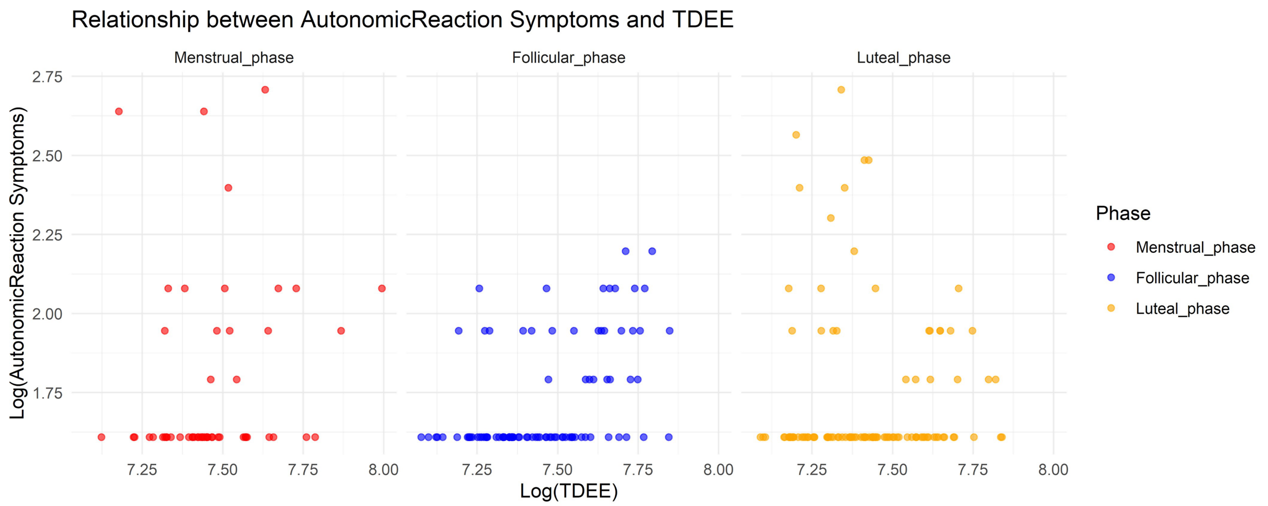

FIGURE 4
Relationship between autonomic reaction symptoms and total daily energy expenditure.

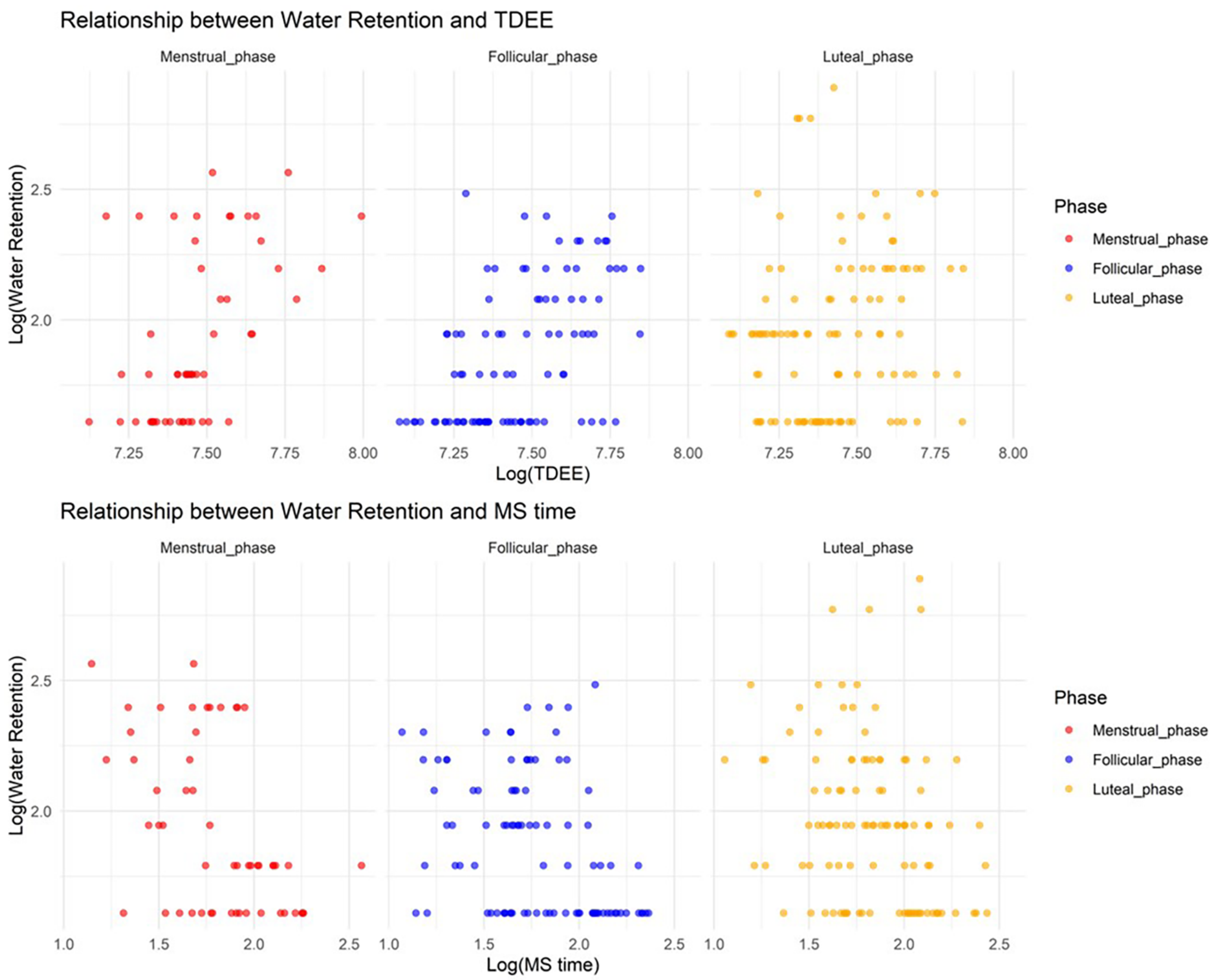

FIGURE 5
Relationship between water retention and total daily energy expenditure/midpoint of sleep time.

$p = 0.0016$; MS time*Luteal phase: Estimate = −0.064, SE = 0.021, adjusted $p = 0.017$). **Figure 6** shows the relationship between negative affect and TDEE/MS time.

3.3. Summary of results

This study evaluated the relationship between MRSs (pain, concentration symptoms, behavioral change symptoms, autonomic reaction symptoms, water retention, and negative affect) and TDEE, as well as MS time according to the MCP. Firstly, all MRSs, except behavioral change symptoms, were significantly more pronounced during the menstrual and luteal phases compared to the follicular phase. Secondly, delayed MS time was associated with reduced pain, concentration symptoms, water retention, and negative affect during the menstrual phase, and reduced negative affect during the luteal phase. Finally, an increase in TDEE was associated with reduced concentration symptoms, autonomic reaction symptoms, and negative affect during the menstrual and luteal phases and with reduced water retention only during the luteal phase.

4. Discussion

This study investigated the relationship between MRSs and TDEE as well as MS time according to the MCP. The findings support the hypothesis that MRS severity is associated with variation in TDEE and MS time according to the MCP.

Consistent with hormonal fluctuation theories, our results indicate that MRSs (except behavioral change symptoms) are significantly more severe during the menstrual and luteal phases. The elevated presence of MRSs (pain, concentration symptoms, autonomic reaction symptoms, water retention, and negative affect) during these phases could be attributed to the hormonal changes in the fluctuations in estrogen and progesterone levels. The results of the present study are consistent with those of previous studies showing increased scores of mMDQ subscales in the menstrual phase group compared with those in the follicular phase group. Previous studies have shown that women generally experience unpleasant MRSs, and the results of the present study confirmed this (24). Furthermore, in a survey of 200 female

FIGURE 6
Relationship between negative affect and total daily energy expenditure/midpoint of sleep time.

college students, many reported premenstrual syndrome appearing during the luteal phase, although the degree of severity varied (28). The lack of significant variance in behavioral change symptoms across the MCP may suggest the predominance of external factors such as daily stressors over hormonal influences, a hypothesis that warrants further investigation.

A noteworthy finding is that delayed MS time was associated with reduced pain, concentration symptoms, water retention, and negative affect during the menstrual phase, and reduced negative affect during the luteal phase. This suggests that sleep timing may play a critical role in the exacerbation or alleviation of MRSs. Recent reports have indicated that women with dysmenorrhea experience higher levels of oxidative stress than healthy individuals (29). Melatonin has antioxidant and anti-inflammatory effects (30). Oral administration of melatonin at bedtime during menstruation has been suggested to alleviate the pain associated with dysmenorrhea and improve sleep quality (31, 32). Water retention during the menstrual phase is thought to be due to changes in the estrogen and progesterone levels. The delayed MS time may have positively affected sleep quality and reduced concentration symptoms and water retention. Therefore, regression of the melatonin secretion rhythm and prolonging the secretion time due to late awakening may be involved in alleviating the symptoms of pain and water retention during menstruation. A previous study reported that women with PMDD tended to have advanced circadian rhythm acrophase (13). Our study showed that there was a relationship between decreased negative affect and delayed MS time in both the luteal and menstrual phases. On the other hand, previous studies have indicated an association between the magnitude of social jet lag, circadian rhythm disturbances, and MRSs (14). Therefore, it is considered important to maintain a daily rhythm according to the MCP, to delay MS time during periods when MRSs are severe, and to advance the MS time when they are not. There was no relationship between autonomic reactions and MS time during the menstrual phase because this symptom has large temporal variability and requires more precise assessment. More objective methods for assessing this relationship, such as electroencephalography, would provide a more accurate understanding.

Our study revealed that an increase in TDEE was associated with reduced concentration symptoms, autonomic reaction symptoms, and negative affect during the menstrual and luteal phases and reduced water retention during the only luteal phase. This was considered to be because exercise stimulates estradiol secretion in the body and serotonin secretion in the brain (11, 12). On the other hand, it may be the result of sex hormones affecting TDEE. In an online survey, approximately 40% of respondents reported reduced activity levels during the menstrual phase (33). In the luteal phase, a significant increase in average core body temperature and energy expenditure were observed (34).

In this study, physical activity and sleep timing were monitored using a wearable device during one menstrual cycle, allowing for more objective data to be collected. In a previous study, physical activity measured using wearable devices was found to be more reliable than self-reported physical activity (35). Furthermore, we provided integrated models that consider the relationship between MRSs and physical activity/sleep timing according to the MCP during daily life, allowing the relationship to be evaluated under real-life conditions.

This study has some limitations. Firstly, the relatively small sample size of our study may limit the robustness and generalizability of our findings. A larger and more diverse cohort would enhance the statistical power and enable more definitive conclusions about the complex interactions we have explored. Secondly, our analysis did not include chronotype measures. Among university students, the "eveningness" chronotype is associated with MRSs (36). The absence of this variable may have restricted our understanding of the nuances in the relationship between MRSs and sleep timing. Another limitation lies in the scope of our study, which was conducted over a single menstrual cycle. The cyclical nature of menstrual symptoms and their modulators may be better understood through longitudinal studies spanning multiple cycles, providing a richer, more detailed analysis of these patterns over time. Future research should aim to include a more diverse demographic and extend its scope to encompass other influential lifestyle factors such as diet, stress, and hormonal fluctuations, which could play a significant role in MRSs.

In conclusion, this study clarified the relationship between MRSs and physical activity/sleep timing according to the MCP. This study evaluated the relationship between MRSs and TDEE as well as MS time according to the MCP. Delayed MS time was associated with reduced pain, concentration symptoms, water retention, and negative affect during the menstrual phase and reduced negative affect during the luteal phase. Increased TDEE was associated with reduced concentration symptoms, autonomic reaction symptoms, and negative affect during the menstrual and luteal phases and reduced water retention only during the luteal phase. In Japan, 74.0% of women experience MRSs, and the annual socioeconomic loss due to MRSs is estimated to be approximately 682.8 billion yen (4). Future research must be done to clarify the guidelines for proper physical activity and sleep timing according to the MCP, thereby improving women's health management. Furthermore, the possibility of a tailor-made health management approach utilizing wearable devices has been suggested. The findings of this study can be used to develop applications, services, and workstyles in the future. Many countries have begun to adopt teleworking and staggered working hours after coronavirus disease 2019 (37). One specific application is the provision of applications that support the optimization of sleep and activity according to an individual's MCP. Combining this with wearable devices that estimate ovulation periods would also be adequate (38). This would enable women to better understand their physical condition, physical activity, and sleep timing and take appropriate non-therapy measures to alleviate MRSs.

Data availability statement

The datasets presented in this article are not readily available because the ethical review of our study permits the use of the data only for this research and does not permit the release of the data. If you wish to obtain data as in this study, please contact us through collaborative research. Requests to access the datasets should be directed to HM, rr0095ie@ed.ritsumei.ac.jp.

Ethics statement

The studies involving humans were approved by Ethical Review Committee of Ritsumeikan University. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

HM: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Visualization, Writing – original draft, Validation. SO: Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Sex differences in the determination of prescribed load in ballistic bench press

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Introduction: The objectives of the present study were twofold: first, to identify the specific relative load at which the concentric motion transforms into a purely propulsive action among women, and second, to compare the load-velocity relationships between men and women during the bench press throw.

Methods: Fourteen men and fourteen women participated in a test where they progressively increased the load until reaching their one-repetition maximum (1RM) in the bench press exercise. Linear regression models were employed to elucidate the relationships between load and velocity, as well as load and the propulsive phase (% of total concentric time). Additionally, ANCOVA was utilized to compare the linear regression models between men and women.

Results: The results revealed strong and linear associations between load and mean propulsive velocity (MPV) for both men and women, as well as between load and the propulsive phase. Notably, there were significant differences in MPV and the propulsive phase concerning load between men and women. Women transitioned into a fully propulsive concentric phase at approximately 80% of their 1RM, while men achieved this entirely propulsive phase at around 85% of their 1RM. Furthermore, women exhibited reduced velocities when handling lighter relative loads compared to men. Conversely, women demonstrated higher velocities when dealing with loads exceeding 85% of their 1RM in contrast to their male counterparts.

Discussion: These findings hold notable implications for prescribing bench press throw loads for women, which should differ from those recommended for men. Further studies are necessary to validate the efficacy of the proposed load recommendations.

KEYWORDS

training load, propulsive phase, ballistic exercise, movement velocity, resistance training

Introduction

Since athletes need to improve their performance through exercise, strength and conditioning coaches have focused on various techniques and training programs that can enhance athletic performance. One of the most common exercises is the bench press, which in turn helps athletes develop neuromuscular qualities and subsequently improve skills-related athletic performance (Sanchez-Medina et al., 2009). In this context, bench press throw is widely used to improve power-related capacities among athletes (Suchomel et al., 2018). Specifically, subjects need to accelerate the load throughout the movement from the beginning until the end of projection. Hence, since a variety of motions in athletic movements are required explosive motor actions, ballistic bench press exercise can assist athletes by maintaining velocity while improving strength, which in turn can enhance power-related capacities (Loturco et al., 2019).

Previous studies (Taber et al., 2016; Suchomel et al., 2018; Carvalho et al., 2022) demonstrated that prescribed training loads affect different aspects of muscle performance, in which very heavy weights (90%–95% 1RM) are employed to boost maximal strength (Suchomel et al., 2018), high loads (approximately 80% 1RM) are associated with muscle strength and hypertrophy improvements (Carvalho et al., 2022), and lighter loads (40%–60% 1RM) are used to enhance characteristics such as the rate of force development (RFD) and power-related qualities (Taber et al., 2016). Whereas ballistic bench press exercise can help improve athletic performance, there is still a concern related to prescribed loads. That is, an increase in load can lead to a decrease in velocity, such that if athletes tend to train in the presence of a high load (e.g., 80% 1RM), they cannot maintain propulsive acceleration throughout the concentric activation of exercise, which means movement velocity is too low. It is, therefore, impractical to project a loaded barbell into the air. In this vein, a recent study (Loturco et al., 2020) demonstrated that mean propulsive acceleration is close to zero during bench press throw after 80% 1RM, which suggests athletes cannot project a loaded barbell into the air. It was, therefore, proposed that strength and conditioning coaches prescribe any load below 80% 1RM if they tend to maintain the characteristics of ballistic exercise.

Given the substantial body of literature addressing sex differences in neuromuscular characteristics, including strength (Mata et al., 2016) and fatigability (Hicks et al., 2001; Davies et al., 2018), it is expected women demonstrate different responses compared to men in relation to ballistic bench press exercise. In particular, previous studies demonstrated that men with comparable training backgrounds exhibit a steeper slope in the load-velocity relationship compared to women (Torrejón et al., 2019; García-Ramos et al., 2021; Nieto-Acevedo et al., 2023). Thus, general equations that were formerly published to detect the propulsive phase of the concentric contraction might not be well-suited for women (Pareja-Blanco et al., 2020). Conducting research is essential to determining the appropriate load to maintain velocity in female athletes during a bench press throw. To the best of our knowledge, no study has been carried out to ascertain the specific relative loads at which women reach the point where the concentric action transforms into a purely propulsive phase during a bench press throw, meaning athletes are unable to project the load into the air. Therefore, the primary objective of the current study was to identify the specific relative load, represented as a percentage of 1RM, at which the concentric motion shifts into a purely propulsive action among women during the bench press. Additionally, to gain a deeper understanding of the differences between sexes in terms of the recommended load, we aimed to compare load-velocity relationships between men and women during the bench press throw. We hypothesized that female athletes would exhibit a different threshold load in the achievement of the pure propulsive phase during concentric action when compared to their male counterparts. It is also hypothesized that the change in velocity for a specific change in %1RM would be greater among men.

Methods

Subjects

While previous power analyses (Sreckovic et al., 2015) suggested that differences in mechanical variables (velocity, force, and power) could be detected with sample sizes as low as 3 to 9 participants, we conservatively enrolled 14 male collegiate athletes (age = 28.78 ± 3.19 years; body mass = 76 ± 6.23 kg; body height = 177.78 ± 4.99 cm) and 14 female collegiate athletes (age = 27.71 ± 2.33 years; body mass = 51.21 ± 8.91 kg; body height = 166.92 ± 3.33 cm) in the current study. The athletes possessed a range of 2–6 years of experience in weight training and were actively engaged in training, with 3 sessions per week, during the time of measurement for the study. These athletes had no history of musculoskeletal injuries in the past 6 months and any physical limitations that could affect the result of the study. Subjects were informed about the type of test and how to perform the bench press throw; however, were not informed regarding the outcomes of any their evaluations. Participants signed the informed consent before performing the test, and the present study was approved by the Institutional Review Board at the University of x.

Procedures

Participants started to perform 1 RM test after a 10-min standardized warm-up, which consisted of jogging on a treadmill, stretching and upper-body joint mobilization exercises, and 1 set of 5 repetitions of bench press with a load of 8 kg (the weight of the Smith machine barbell). To perform the 1 RM test, the initial load was set at 8 kg for both male and female athletes. The external load was progressively increased by 10 and 5 kg for male and female athletes, respectively, until the achieved mean propulsive velocity (MPV) was less than 0.5 m s^{-1}. Then, the load was increased by 5–1 kg to attain the precise estimation of 1RM bench press, such that 1RM was determined when an athlete could lift the heaviest load with the full extension of his/her elbow. Three, two, and one repetitions were executed for the lighter (MPV >1 m s^{-1}), medium (0.65 m s^{-1} ≤ MPV ≤1 m s^{-1}), and heaviest loads (MPV <0.65 m s^{-1}), respectively. The rest period was 3 min for lighter and medium loads, while it was 5 min for the heaviest loads. The rest period between the repetitions executed with the same load was also 10 s (Sanchez-Medina et al., 2009; Torrejón et al., 2019).

Subjects performed the bench press throw in accordance with the method, which was extensively described in previous studies (Sanchez-Medina et al., 2009; Davies et al., 2018). The participants were first asked to execute the eccentric phase with control, holding a static position for at least one second at the end of this phase, ensuring that the bar lightly touched the chest. This was done to reduce the influence of the rebound effect and enhance measurement consistency. Following this, they were instructed to perform the concentric action with maximal effort. To provide safety for participants and give them feedback to keep their

TABLE 1 Sex differences in mean propulsive velocity (in meters per second) corresponding to various loads (as a percentage of 1RM) and the proportion of the propulsive phase's contribution to the overall concentric duration.

Load (%1RM)	Men (*n* = 14)		Women (*n* = 14)	
	Propulsive phase (%)	MPV ($m.s^{-1}$)	Propulsive phase (%)	MPV ($m.s^{-1}$)
20	69	1.42	87	1.06
25	71	1.34	88	1.02
30	74	1.27	89	1
35	76	1.20	90	0.93
40	79	1.12	91	0.88
45	81	1.04	92	0.84
50	83	0.97	93	0.80
55	86	0.90	94	0.75
60	88	0.82	95	0.70
65	90	0.74	96	0.66
70	93	0.67	97	0.61
75	95	0.60	99	0.57
80	97	0.52	100	0.52
85	100	0.44	100	0.48
90	100	0.37	100	0.43
95	100	0.30	100	0.39
100	100	0.22	100	0.34

MPV, mean propulsive velocity.

maximum velocity, two trained spotters were present on both sides of the barbell.

A Smith machine (JK Fitness Equipment) along with a dynamic measurement system (i.e., a linear velocity transducer that was sampled at a frequency of 1,000 Hz (T-Force System, Ergotech, Murcia, Spain)) was used to measure the MPV of the barbell during bench press throw. MPV was assessed throughout the concentric phase of the BP; in particular, the propulsive phase was determined as the portion of the concentric phase in which the acceleration of the movement was greater than the acceleration caused by gravity (i.e., g = 9.81 m s^{-2}). The validity and reliability of the T-Force system were reported in previous studies (Sanchez-Medina et al., 2009; Perez-Castilla et al., 2019).

Statistical analysis

Data were assessed for normality and homogeneity of variance using the Shapiro-Wilks test and Levene's test, respectively. Linear regression models were employed to elucidate the association between load (%1RM) and MPV, as well as load (%1RM) and the propulsive phase (% of total concentric time). ANCOVA was utilized, with load as a covariate, to assess the sex-related differences in linear regression models. To better comprehend the distinctions between sexes in relation to dependent variables, we examined Cohen's effect size (ES) along with its 95% confidence interval. This analysis was conducted across 5% increments, ranging from 20% 1RM to 100% 1RM. The criteria for interpreting the ES magnitude encompassed the following categories: trivial (2.0), small (0.2–0.6), moderate (0.6–1.2), large (1.2–2.0), and extremely large (>2.0) (Hopkins et al., 2009). Independent t-tests were also used to compare 1RM strength with respect to sex. Analyses were conducted using SPSS software version 26, and the level of significance was set at $p < 05$.

Results

The 1RM strength was significantly different between men and women ($p < 0.001$; ES = 4.47; men = 88.71 ± 14.12 kg; women = 40.57 ± 5.63 kg).

Table 1 displays the breakdown of concentric time into propulsive and braking phases at various percentages (from 20% to 100%) of 1RM for both men and women. The results from our linear regression models reveal a strong correlation between the load (1RM%) and the relative contribution of the propulsive phase to the total duration of the concentric lift for men ($R^2 = 0.817$, $p < 0.001$) and women ($R^2 = 0.644$, $p < 0.001$), as illustrated in Figure 1. A. The results of the ANCOVA analysis reveal significant differences between men and women concerning the relative contribution of the propulsive phase to the total duration of the concentric bench press throw (F = 43.431, $p < 0.001$). Specifically, at 20% 1RM, men accounted for 69% of the concentric time in the propulsive phase, whereas women demonstrated an 87% contribution to the propulsive phase at the same relative load.

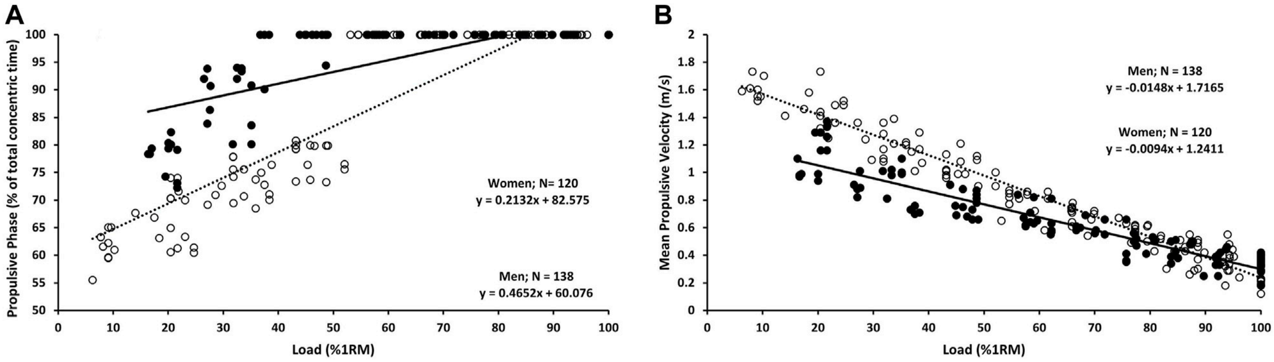

FIGURE 1
(A) Relationships between relative load (% 1RM) and Propulsive Phase for men (open dots and dashed line) and women (filled dots and solid line); **(B)** Relationships between relative load (% 1RM) and MPV for men (open dots and dashed line) and women (filled dots and solid line).

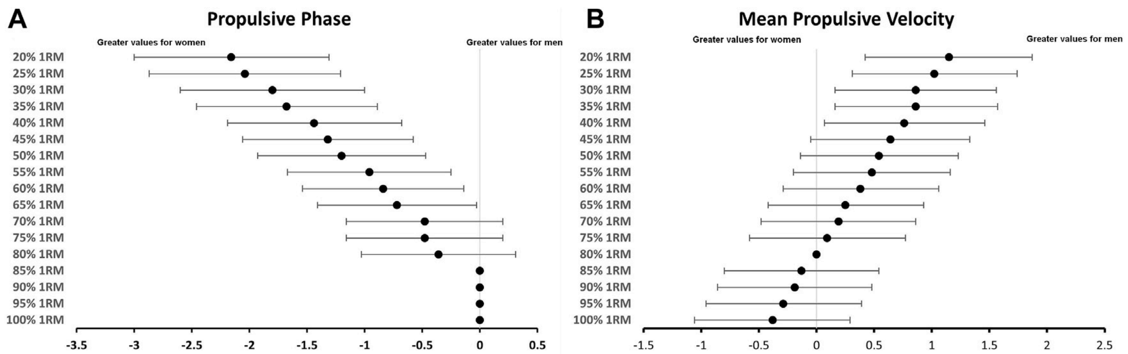

FIGURE 2
Effect size and its 95% confidence interval, represented as horizontal lines, are depicted in **(A)** for the Propulsive Phase and in **(B)** for the Mean Propulsive Velocity. Positive numbers indicate great values for men.

Notably, men reached a full propulsive phase at 85% 1RM, while women achieved the total propulsive phase at 80% 1RM, as detailed in Table 1. The effect size, along with its 95% confidence intervals, for the relative contribution of the propulsive phase to the total duration, is visually represented in Figure 2A.

The data analysis revealed a robust relationship between load (1RM%) and MPV in both men ($R^2 = 0.939$, $p < 0.001$) and women ($R^2 = 0.855$, $p < 0.001$), as depicted in Figure 1B. Furthermore, the results from the ANCOVA indicated significant differences between men and women regarding MPV ($F = 19.745$, $p < 0.001$). A comprehensive representation of MPV across various loads (ranging from 20% to 100% 1RM) can be found in Table 1. Additionally, Figure 2B visually illustrates the effect size of MPV, along with its corresponding 95% confidence intervals.

Discussion

The aim of this study was to investigate the threshold loads at which women exhibit a solely propulsive phase during the bench press throw and to compare the load-velocity relationship between men and women. Our results revealed that, at approximately 80% of their 1RM, women transitioned into a completely propulsive concentric phase. In contrast, men exhibited this purely propulsive phase at around 85% of their 1RM. Furthermore, we observed a significant difference in the load-velocity relationship between men and women. Specifically, women displayed lower velocities when handling lighter relative loads compared to men. Conversely, women exhibited higher velocities when dealing with loads exceeding 85% of their 1RM, in comparison to their male counterparts.

In theory, ballistic exercises such as the bench press throw, which involve high-velocity movements, have the potential to enhance athletes' power-related attributes, thereby elevating their performance in sports and competitive events (Loturco et al., 2020). However, it is important to note that the effectiveness of ballistic exercises in improving athletes' performance can be influenced by the prescribed load, which alters their kinematics and kinetics. Specifically, heavier loads result in reduced velocity, ultimately negating the benefits of these exercises by preventing the effective projection of the load into the air. In this vein, prior studies (Sanchez-Medina et al., 2009; Moir et al., 2018; Loturco et al., 2020) have shown that when male athletes lift loads surpassing approximately 75%–80%

of their 1RM, they are unable to harness the benefits of the ballistic bench press. This is because, at these specified loads, the concentric phase of the exercise primarily becomes propulsive, preventing athletes from effectively launching the loaded barbell into the air. In other words, the concentric contraction comprises both propulsive and braking phases. When lifting lighter loads (resulting in higher velocity), there is a prolonged braking phase during which acceleration exceeds that of gravity. However, when handling heavier loads (resulting in lower velocities), the braking phase diminishes, and acceleration falls below that of gravity (Sanchez-Medina et al., 2009). Consequently, athletes are unable to propel the barbell into the air. According to our data, women displayed a distinct propulsive phase at 80% of their 1RM, whereas men exhibited this phase at 85% of their 1RM. To delve deeper, when lifting at 20% 1RM, women demonstrated a significantly larger propulsive phase compared to men (87% for women and 69% for men). Interestingly, women exhibited an 87% propulsive phase even at just 20% of their 1RM, and from 35% to 75% of their 1RM, the propulsive phase ranged from 90% to 99%. This suggests that propelling the barbell into the air during this phase becomes particularly challenging. In line with prior research (Torrejón et al., 2019; García-Ramos et al., 2021; Nieto-Acevedo et al., 2023) advocating for the use of a specific equation to predict load-velocity relationships in women, our findings confirmed that women exhibited a higher value for the propulsive phase of concentric contraction than men.

In our current study, we observed a significant difference in the load-velocity relationship between men and women. This finding aligns with previous research (Torrejón et al., 2019; García-Ramos et al., 2021), which has consistently shown that women tend to exhibit lower velocities at lower relative loads compared to men. However, at higher relative loads (~80% 1RM), women demonstrate higher velocities compared to men. While a limited number of studies have investigated load-velocity differences based on sex, these studies have utilized mean velocity and mean propulsive velocity as key variables in developing their models (Nieto-Acevedo et al., 2023). It is worth noting that while there is some variability among individuals, a clear pattern emerges when the movement becomes purely propulsive: both mean mechanical and mean propulsive variables converge, becoming indistinguishable. However, during phases that are not entirely propulsive, mean propulsive variables surpass mean mechanical variables in magnitude (Sanchez-Medina et al., 2009). Our data revealed that men demonstrated lower velocities compared to women when the bench press throw became entirely propulsive. The difference between the sexes may result from variations in muscle fiber types between men and women (Torrejón et al., 2019). Specifically, the higher prevalence of slow muscle fibers in women compared to men might contribute to their reduced speed when handling lighter relative loads (Mata et al., 2016). It can also stem from range of motion (ROM) (Nieto-Acevedo et al., 2023). In this vein, since men are taller and have longer limbs compared to women, prior studies (MOOKERJEE & RATAMESS, 1999; Martínez-Cava et al., 2019) have demonstrated that variations in ROM can affect RFD, activation, and synchronization of motor units. However, further research is needed to identify the mechanisms underlying these sex differences and to determine whether they are attributed to muscle fiber types and ROM.

The findings of the present study must be interpreted in light of certain limitations. Firstly, the athletes in our study were not engaged in supervised weight training at the time of data collection. Additionally, due to the inherent constraints associated with cross-sectional studies, it is imperative that the results of our study are validated through future research. Lastly, as our study exclusively involved athletes with prior resistance training experience, it is important to note that the findings may not apply to different athletic groups. Therefore, it is recommended to investigate the relative loads at which a concentric contraction shifts entirely to a propulsive phase among women participating in diverse sports disciplines.

Practical applications

In the process of executing a proper barbell throw, athletes are required to maintain a persistent net positive force over an extended portion of the lift, thereby creating a more pronounced acceleration path throughout the upward phase of the motion. Furthermore, athletes need to decelerate the barbell to bring it to a complete stop during the concentric phase. The absence of a braking phase in this context renders it impossible to project the barbell into the air, as is typically the case in the traditional bench press (Loturco et al., 2020). As soon as the acceleration phase becomes entirely propulsive, it becomes unfeasible to project the barbell into the air. This point can be regarded as the 1RM for the bench press throw (Loturco et al., 2020). Therefore, coaches are advised to consider 80% of bench press-1RM for women and 85% of bench press-1RM for men as bench press throw-1RM when prescribing loads to athletes. In other words, 85% of bench press-1RM is equivalent to bench press throw-1RM. This approach aims to maintain the mechanical characteristics of ballistic exercises and optimize their performance.

Conclusion

Our research revealed that women transitioned into a fully propulsive concentric phase at roughly 80% of their 1RM, while men achieved this entirely propulsive phase at approximately 85% of their 1RM. Additionally, a significant disparity emerged in the load-velocity relationship between men and women. To elaborate, women exhibited reduced velocities when handling lighter relative loads in contrast to men. Conversely, women demonstrated higher velocities when dealing with loads exceeding 85% of their 1RM, as compared to their male counterparts. These findings hold notable implications for prescribing bench press throw loads for women, which should differ from those recommended for men. Further studies are necessary to validate the efficacy of the proposed load recommendations.

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 humans were approved by the Comitato di Bioetica at the University of Palermo. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

MI: Conceptualization, Data curation, Writing–original draft, Writing–review and editing. GP: Formal Analysis, Investigation, Methodology, Writing–review and editing. ET: Conceptualization, Data curation, Validation, Writing–review and editing. GB: Visualization, Writing–review and editing. AB: Formal Analysis, Methodology, Writing–review and editing. MB: Supervision, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The oral contraceptive cycle and its influences on maximal and submaximal endurance parameters in elite handball players

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The usage of the oral contraceptive pill is widespread among athletes of various levels. However, there is limited knowledge on how the intake of the pill alters the submaximal and maximal endurance parameters between the oral contraceptive phases. Therefore, the aim of this study was to examine potential differences between the pill intake and withdrawal phase on endurance-related parameters in first-division handball players. In total, 15 female team handball players performed two graded exercise tests until volitional exhaustion on a motorized treadmill. Tests were performed during the pill intake (days 16–17) and withdrawal phase (day 2–3). Throughout the test, respiratory gases were measured breath-by-breath, and the heart rate was measured continuously. Before and after the graded exercise test, blood samples were obtained in order to assess the blood lactate concentration. Before each test, venous blood samples were taken to determine endogenous sex hormone levels. Ventilatory parameters ($\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}E$, and respiratory equivalents for $\dot{V}O_2$ and $\dot{V}CO_2$) were measured, and the oxidation of fat and carbohydrates was calculated. A paired-sample *t*-test was used to assess differences between the two time points, and the significance was accepted as $p < 0.050$. Significant differences with lower values during the consumption phase were found for absolute (mean difference ± SD: 88 ± 131 mL·min^{-1}; $p = 0.021$) and relative $\dot{V}O_{2peak}$ (mean difference ± SD: 1 ± 2 mL·min^{-1}·kg^{-1}; $p = 0.012$). Higher values during the consumption phase were found for submaximal respiratory equivalents for $\dot{V}O_2$ (mean difference ± SD: −1.1 ± 1.7; $p = 0.028$) and $\dot{V}CO_2$ (mean difference ± SD: −0.9 ± 1.5; $p = 0.032$). No differences were found for all other parameters, including differences for endogenous sex hormones ($p > 0.050$).

Abbreviations: [BLa$^-$], blood lactate; CONS, consumption phase; CHO, carbohydrate; CO_2, carbon dioxide; E2, estradiol; GXT, graded exercise test; HR, heart rate; MAS, maximum aerobic speed; OCC, oral contraceptive cycle; OCP, oral contraceptive pill; O_2, oxygen; prog, progesterone; SD, standard deviation; SEM, standard error of the mean; testo, testosterone; TTL, total energy expenditure; $\dot{V}E$, minute ventilation; VT, ventilatory threshold; WITH, withdrawal phase.

The results of the current study suggest only marginal and physiologically insignificant differences in endurance-related parameters between oral contraceptive phases.

KEYWORDS

withdrawal bleeding, athletes, hormonal contraceptive, female endurance performance, endurance, hormonal cycle, monophasic oral contraceptive pill

Introduction

The length of the eumenorrheic menstrual cycle and, consequently, the day of onset of bleeding are, in some athletes, more consistent than in others. According to a recent survey, about half of adolescent athletes reported having irregular menstrual cycles (Rosen et al., 2020), and especially in endurance sports, menstrual irregularities are widely spread across athletes (Rosen et al., 2020). The chance of having a regular menstrual cycle and controlling its timing is a desire of numerous women, particularly competitive athletes (Elliott-Sale et al., 2020).

An often-used oral contraceptive pill (OCP) type is the combined monophasic OCP, which includes exogenous estrogen and exogenous progesterone (also progestogen). It is used for 21 days of consumption (CONS), followed by 7 days of withdrawal (WITH) or the intake of placebo pills. The intake of the exogenous hormones results in endogenous hormones like estradiol (E2), progesterone (prog), and testosterone (testo) remaining at low or stable concentrations or might increase in WITH when bleeding occurs (McNulty et al., 2020). The hormone profile across an oral contraceptive cycle (OCC) is not similar to the hormone profile of regular menstruation (McNulty et al., 2020).

The OCPs are not only used for contraceptive reasons but also for controlling the timing and duration of bleeding as well as to skip bleeding. Furthermore, the intake of OCP reduces symptoms of dysmenorrhea (e.g., cramps, pain, and headache); moreover, it diminishes menorrhagia (McNulty et al., 2020) and facilitates training planning as well as competition scheduling. Therefore, it is not surprising that the usage of OCPs in elite sports is widely spread. Recent data show that approximately a third of adolescent athletes (Rosen et al., 2020) and half of female adult athletes are using OCPs (Martin et al., 2018). In this context, 70% of female elite athletes reported already using hormonal contraceptives (Martin et al., 2018).

It is not fully understood whether the initiation of OCP intake might have a negative influence on physical performance (McNulty et al., 2020), and even variations within the OCC have been discussed lately. In terms of strength performance, most studies found no differences within the OCC, with constant and stable strength performances (Elliott et al., 2005; Ekenros et al., 2013; Elliott-Sale et al., 2020; McNulty et al., 2020; Reif et al., 2021). When it comes to endurance performance, recent results are controversial.

On the one hand, no differences through OCC phases for endurance parameters have been detected (Grucza et al., 1993; Vaiksaar et al., 2011b; Jurimae et al., 2011; Joyce et al., 2013; Gordon et al., 2018). On the other hand, previous literature found increased minute ventilation ($\dot{V}E$) during OCP intake compared to WITH (Barba-Moreno et al., 2019). During OCP intake, decreased submaximal oxygen uptake ($\dot{V}O_2$) and enhanced running economy have been found, suggesting a positive influence of OCP intake on physiological and biomechanical factors in running (Giacomoni and Falgairette, 2000). In another study, [blood lactate] (BLa^-) and rating of perceived exertion, as well as breathing frequency, were higher during OCP intake; however, it is concluded that there is no need to be concerned about or manipulate OCC to optimize endurance performance for competitions (Rechichi et al., 2009). This is in accordance with another study reporting differences in ventilation and interpreting them as not relevant for cycling competition (Vaiksaar et al., 2011a).

A number of studies have been conducted on maximal and submaximal endurance-related parameters, but the results are contradictory. The inconsistency of previous research might be explained by the heterogeneous training status of the study participants and also by different types of sports and disciplines. The present study aims to minimize the lack of data on consistently well-trained team sport athletes in order to facilitate their transfer into the practice of (elite) sports. According to a recent survey on elite athletes, more than a third of female elite athletes use OCPs (Martin et al., 2018), and the monophasic combined pill is the most commonly used OCP in Europe, according to our experience. It is, however, of great interest to analyze the effects of the intake on submaximal and maximal endurance performance parameters. It is hypothesized that endurance performance-related parameters are enhanced in WITH due to a higher level of endogenous E2.

Materials and methods

Participants

The sample consisted of 15 female team sport athletes playing in the first Austrian handball league and training at least three times per week (age: 22.9 ± 3.2 years, body mass: 66.9 ± 8.6 kg, and body stature: 1.69 ± 0.08 m). Furthermore, inclusion criteria were a training history of at least 3 years and the use of a commercially available low- to middle-dose monophasic OCP (0.020–0.035 mg ethinylestradiol combined with 0.10–2.00 mg gestodene). Participants used OCPs, which were characterized by 21 days of OCP intake and an additional 7 days of WITH when no-pill or placebo pills were taken (introducing withdrawal bleeding). Moreover, participants were included in the study only when they used OCP for more than half a year and according to the instructions. An additional calculation of subgroups has been performed, including only participants with lower hormone levels in CONS than in WITH.

Study design

The present cross-sectional study evaluates endurance-related parameters [i.e., $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}E$, RE, substrate metabolism, (BLa^-), and heart rate] across a single OCC of female team sport athletes. Rechichi et al. (2009) reported that the intake of OCPs is associated with a more stable concentration of sex hormones. Consequently, the evaluation of a single OCC seemed feasible for this research since endogenous hormones stay low during chronic OCP intake and the dosage of exogenous hormone intake and the length of an OCC are predictable. Participants were reported to the laboratory twice to analyze aerobic endurance performance in two different phases of the OC. First, in the WITH phase, which lasts for 7 days and includes menses (testing days on days 2 or 3 of WITH), WITH has been defined as the reference state for comparisons. Second, during the CONS phase, which lasts for 21 days, athletes were tested on days 16 or 17 of the OCP cycle, when OCP consumption had been re-initiated for 9 or 10 days. To avoid a potential bias caused by the order of testing days, visits in WITH and CONS were in different orders. During both visits, participants performed a graded exercise test (GXT) until volitional exhaustion to evaluate potential differences in aerobic endurance performance. Athletes were in a fed and hydrated state when they arrived at the laboratory and were instructed to replicate their food intake the day prior, from the first to the second test. Subjects were required to avoid intense exercise and alcohol for 24 h prior to testing. In addition, caffeine and sports drink intake were not allowed in the last 3 h prior to the tests. The tests were performed in a humidity- and air-condition-controlled laboratory at 20°C–22°C and between 45% and 55% humidity. To avoid potential effects of the diurnal rhythm, tests were performed at the same time of the day (±1 h) for each person. Participants were fully informed of all testing procedures and risks of this study and provided written informed consent. The University of Vienna´s Ethics Committee (#00435) approved all procedures of the study, which conformed to the principles of the World Medical Association's Declaration of Helsinki (2013).

Measures

Pre-test measures and warm-up

Using a commercially available stadiometer and scale prior to each test, body stature and body mass were measured; the latter was to examine potential differences in time points across OCC phases. Subsequently, participants executed a standardized warm-up program with a predefined workload of 0.75 W/kg body mass for 10 min on a stationary ergometer (Racer 9, Kettler Freizeit GmbH, Ense-Parsit, Germany).

Graded exercise test

To obtain relevant endurance-related parameters, a GXT on a motorized treadmill (Saturn, h/p/cosmos, Traunstein, Germany) was performed during WITH and CONS. After baseline walking at 1.39 m·s^{-1} for 3 min (which was used for baseline $\dot{V}O_2$ measures), athletes commenced running at 1.67 m·s^{-1} with an increment of 0.14 m·s^{-1} every minute until volitional exhaustion, despite strong verbal encouragement by the investigators. The treadmill incline was set to 1%. During the test, respiratory gases were measured breath-by-breath using a mobile gas analyzer (MetaMax3B-R2, Cortex Biophysik GmbH, Leipzig, Germany). Before each test flow, volume was calibrated using a 3-L syringe, and the gas analyzer was calibrated according to the recommendations of the manufacturer using known gases (15% O_2 and 5% CO_2, Cortex Medical GmbH).

Submaximal measures and determination of thresholds

Submaximal $\dot{V}O_2$, respiratory exchange ratio (i.e., the ratio of $\dot{V}CO_2$ and $\dot{V}O_2$; RER), $\dot{V}E$, as well as ventilatory equivalents for O_2 and carbon dioxide (CO_2) (i.e., $\dot{V}E/\dot{V}O_2$ and $\dot{V}E/\dot{V}CO_2$), and submaximal heart rate (HR) at 2.50 m·s^{-1} work stage were analyzed during the last 30 s and 5 s, respectively. Submaximal measurement for $\dot{V}O_2$, running economy, and respiratory exchange ratio had to be excluded for one participant as the respiratory exchange ratio was higher than 1.00 during the final 30 s of the 2.50 m·s^{-1} stage, indicating non-steady-state conditions. Running economy was calculated as oxygen consumption for 1 km at 2.50 m·s^{-1}, normalized to body mass, and expressed as mL·kg^{-1}·km^{-1}.

Ventilatory threshold (VT) 1 was determined using the following criteria: 1) an increase in $\dot{V}E/\dot{V}O_2$ without a simultaneous increase in $\dot{V}E/\dot{V}CO_2$, 2) the first loss of linearity in minute ventilation ($\dot{V}E$), and 3) a non-linear increase in $\dot{V}CO_2$ (Beaver et al., 1986). VT2 was determined using the following criteria: 1) a secondary increase in $\dot{V}E/\dot{V}O_2$ and $\dot{V}E/\dot{V}CO_2$, 2) changes in the end-tidal respiratory pressure (increase in $PetO_2$ and decrease in $PetCO_2$), and 3) a secondary increase in $\dot{V}E$ (Wasserman, 1984).

Maximal measures

The highest 30-s rolling average measured was accepted as $\dot{V}O_{2peak}$, and the highest speed obtained was considered the maximal aerobic speed (MAS). If the last work rate could not be fully completed, MAS was calculated using Eq. 1 proposed by Kuipers et al. (1985):

$$MAS = sL + t/60 \times 0.14, \tag{1}$$

where MAS is the maximal aerobic speed, sL is the speed of the last fully completed work stage (m·s^{-1}), and t is the time of the not fully completed work stage (s). Maximal values were also obtained for $\dot{V}E$, as well as $\dot{V}E/\dot{V}O_2$ and $\dot{V}E/\dot{V}CO_2$.

Carbohydrate and fat oxidation and total energy expenditure

The oxidation of carbohydrate (CHO) and fat was estimated using measures of $\dot{V}O_2$ and $\dot{V}CO_2$. To calculate substrate oxidation under steady-state conditions, the data of the last 30 s of the 2.50 m·s^{-1} stage were used (n = 14). The following equations, according to Jeukendrup and Wallis (2005), for moderate-to-high-intensity exercise were used in order to calculate CHO and fat oxidation; Eqs 2, 3 were modified in order to convert g·min^{-1} into J·s^{-1}:

$$\text{CHO oxidation } (J \cdot s^{-1}) = \left(4.210 \text{ x } \dot{V}CO_2 - 2.962 \text{ x } \dot{V}O_2\right) \text{ x } 284.0, \tag{2}$$

$$\text{Fat oxidation } (J \cdot s^{-1}) = \left(1.695 \text{ x } \dot{V}O_2 - 1.701 \text{ x } \dot{V}CO_2\right) \text{ x } 680.3 \tag{3}$$

TABLE 1 Overview of comparison between withdrawal and consumption of OCP (WITH−CONS).

	N	WITH (mean ± SD)	CONS (mean ± SD)	Mean differences ± SEM	*p*-value
Endurance parameter					
Baseline $\dot{V}O_2$ (mL·min^{-1})	15	969 ± 115	936 ± 93	−33 ± 19	0.104
$\dot{V}O_2$ at 2.50 m·s^{-1} (mL·min^{-1})	14	2,347 ± 334	2,310 ± 310	−37 ± 22	0.113
RE at 2.50 m·s^{-1} (mL·kg^{-1}·km^{-1})	14	232 ± 17	228 ± 16	−4 ± 2	0.128
RER at 2.50 m·s^{-1}	15	0.90 ± 0.04	0.90 ± 0.04	0.00 ± 0.01	0.760
Relative $\dot{V}O_{2peak}$ (mL·min^{-1}·kg^{-1})	15	44 ± 3	43 ± 4	−1 ± 1	0.012*
Absolute $\dot{V}O_{2peak}$ (mL·min^{-1})	15	2,950 ± 422	2,862 ± 462	−88 ± 34	0.021*
MAS (m·s^{-1})	15	3.51 ± 0.25	3.46 ± 0.30	−0.05 ± 0.03	0.144
VT1 (m·s^{-1})	15	2.10 ± 0.21	2.13 ± 0.24	0.03 ± 0.03	0.374
VT2 (m·s^{-1})	15	2.95 ± 0.25	2.96 ± 0.28	0.02 ± 0.04	0.665
$\dot{V}E_{max}$	15	110 ± 13	106 ± 15	−3 ± 1.5	0.071
$\dot{V}E$ at 2.50 m·s^{-1}	15	65 ± 10	67 ± 10	2 ± 1	0.119
$\dot{V}E/\dot{V}O_2$ at exhaustion	15	34 ± 2	34 ± 1	0 ± 0	0.798
$\dot{V}E/\dot{V}O2$ at 2.50 m·s^{-1}	15	25 ± 1	26 ± 2	1 ± 0	0.028*
$\dot{V}E/\dot{V}CO_2$ at exhaustion	15	31 ± 2	32 ± 2	0 ± 0	0.305
$\dot{V}E/\dot{V}CO_2$ at 2.50 m·s^{-1}	15	28 ± 1	29 ± 1	1 ± 0	0.032*
Energy metabolism					
Fat oxidation (J·s^{-1})	15	263. ± 116	249 ± 119	−14 ± 22	0.533
CHO oxidation (J·s^{-1})	15	541 ± 135	547 ± 157	7 ± 24	0.785
TTL (J·s^{-1})	15	804 ± 112	796 ± 111	−7 ± 9	0.439
Metabolic system and heart rate					
Baseline [BLa$^-$] (mmol·L^{-1})	15	1.1 ± 0.3	1.0 ± 0.3	−0.1 ± 0.1	0.228
[BLa$^-$]$_{max}$ (mmol·L^{-1})	15	8.5 ± 2.8	8.1 ± 2.4	−0.4 ± 0.4	0.274
HR at 2.50 m·s^{-1} (bpm)	14	167 ± 12	166 ± 14	−1 ± 2	0.521
HR_{max} (bpm)	14	189 ± 9	188 ± 10	−1 ± 1	0.559
Hormones					
E2 (pg·mL^{-1})	15	22.5 ± 20.3	15.1 ± 17.8	−7.4 ± 7.4	0.335
Prog (ng·mL^{-1})	15	0.38 ± 0.33	0.34 ± 0.28	−0.04 ± 0.07	0.556
testo (ng·mL^{-1})	15	0.32 ± 0.19	0.28 ± 0.14	−0.04 ± 0.02	0.059
Anthropometry					
Body mass (kg)	15	66.9 ± 8.6	67.1 ± 8.8	0.2 ± 0.2	0.433

WITH, withdrawal phase; CONS, consumption phase; SD, standard deviation; SEM, standard error of the mean; $\dot{V}O_2$, oxygen uptake; RER, respiratory exchange ratio; [BLa$^-$], blood lactate concentration; MAS, maximal aerobic speed; VT, ventilatory threshold; HR, heart rate; CHO, carbohydrate; TTL, total energy expenditure; E2, estradiol; prog, progesterone; testo, testosterone.

Total energy expenditure was estimated as the sum of CHO and fat oxidation, whereas the contribution of protein oxidation was neglected (Jeukendrup and Wallis, 2005).

Heart rate

Throughout the GXT, HR was measured continuously using a chest-strapped attached HR sensor (H7, Polar Electro Oy, Kempele, Finland), which was connected via Bluetooth™ to the gas analyzer. HR_{max} was taken as the highest value throughout the GXT. Due to the malfunction of the HR sensor in a single participant, one dataset for HR is missing (n = 14).

Blood lactate concentration and venous blood samples

Blood lactate concentration was analyzed at baseline, immediately, and 3 min after terminating the GXT. Blood samples

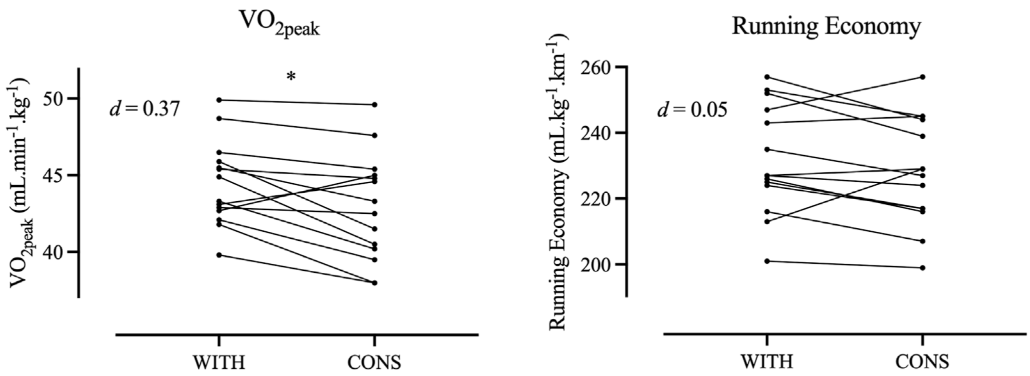

FIGURE 1
Individual responses for maximal oxygen uptake and running economy during withdrawal phase (WITH) and consumption phase (CONS).

(20 µL) were taken from a hyperemic earlobe and diluted immediately in 1,000 µL of glucose solution. Samples were analyzed using an automated lactate analyzer (Biosen S_Line; EKF-diagnostic GmbH, Barleben, Germany). Furthermore, on both test days, venous blood samples were taken to analyze for endogenous hormone levels of prog, testo, and E2. The blood samples were collected in a serum gel tube, and after a 30-min rest, they were centrifuged for 10 min at a relative centrifugal force of 3,500 × g (Rotina 420R, Hettich, Vienna, Austria). Samples were collected and frozen at −40°C until all samples were analyzed in a certified laboratory using Beckman Coulter Access Immunoassays (Beckman Coulter Inc., Brea, CA, United States).

Statistical analyses

All data are reported as means ± standard deviations. The normal distribution of data was checked using the procedures of Shapiro–Wilk and an additional visual inspection of boxplots. A paired-sample *t*-test was used to assess the differences between OCC phases (WITH vs. CONS). In cases of violation of normal distribution and for analysis of subgroups, a non-parametric Wilcoxon signed-rank test has been used, and descriptive data are reported as medians and interquartile ranges. The standard error of the mean was calculated as the ratio of the standard deviation of the mean differences and the square root of the number of participants. Statistical analyses were conducted using SPSS software package version 27 (IBM SPSS Statistics, SPSS Inc., Chicago, United States), and graphs were made using GraphPad Prism (v.10.0.1 for Mac, GraphPad Software, Boston, Massachusetts, United States, "www.graphpad.com"). Significance was accepted at an alpha level of $p < 0.050$.

Results

The mean body mass of the participants (N = 15) was 66.9 ± 8.6 kg, body stature was 1.69 ± 0.08 m, and $\dot{V}O_{2peak}$ was 2,950 ± 422 mL·min^{-1}·in WITH. The results of the treadmill tests are displayed in Table 1. Individual responses between differences in time points are depicted in Figures 1, 2. Significant differences between WITH and CONS were only revealed for absolute and relative $\dot{V}O_{2peak}$ ($p = 0.021$ and $p = 0.012$, respectively) and submaximal ventilatory equivalents for O_2 and CO_2 ($p = 0.028$ and $p = 0.032$, respectively). However, the effect sizes were *trivial* to *small*. All other parameters were not significantly different between WITH and CONS ($0.104 < p < 0.785$).

Subgroup analysis

Between OCC phases, a significant difference and *large* effect sizes in E2 ($p = 0.005$), prog ($p = 0.027$), and testo ($p = 0.005$) have been found for the respective subgroups.

In the subgroup that had significantly higher prog levels in WITH than in CONS ($n = 6$), no significant differences were reported in all evaluated parameters ($0.075 < p < 0.893$) except for relative $\dot{V}O_{2peak}$ [WITH: 42.5 mL·min^{-1}·kg^{-1} (41.2, 46.6); CONS: 40.5 mL·min^{-1}·kg^{-1} (38.8, 43.8); $p = 0.028$], baseline [BLa$^-$] [WITH: 1.2 mmol·L^{-1} (0.9, 1.4); CONS: 0.9 mmol·L^{-1} (0.5, 1.2); $p = 0.044$], and [BLa$^-$]$_{max}$ [WITH: 7.7 mmol·L^{-1} (5.8, 12.6); CONS: 6.2 mmol·L^{-1} (5.8, 11.7); $p = 0.046$].

In the subgroup with significantly lower levels of E2 in CONS than in WITH ($n = 10$), no significant differences were found in all assessed parameters ($0.050 < p < 0.953$).

The analysis of a subgroup with a significant decrease in testo ($n = 10$) from WITH to CONS showed no significant differences in all the assessed parameters ($0.066 < p < 0.862$).

Discussion

The present study aimed to investigate the differences between OCC phases in endurance-related performance parameters obtained from an incremental running test. The results of the present work suggest no meaningful influence of OCC phases on submaximal systemic responses (e.g., RE, substrate oxidation, and HR). However, maximal oxygen consumption was significantly lower during CONS without an effect on MAS or maximal blood lactate concentration. This might be due to the lower efficiency of the aerobic system during CONS. This is represented by a significantly lower oxygen uptake, which, however, was not translated into a lower maximal running speed. Therefore, the hypothesis that maximal and submaximal endurance performance are reduced during CONS due to a lower level of endogenous E2 was not confirmed.

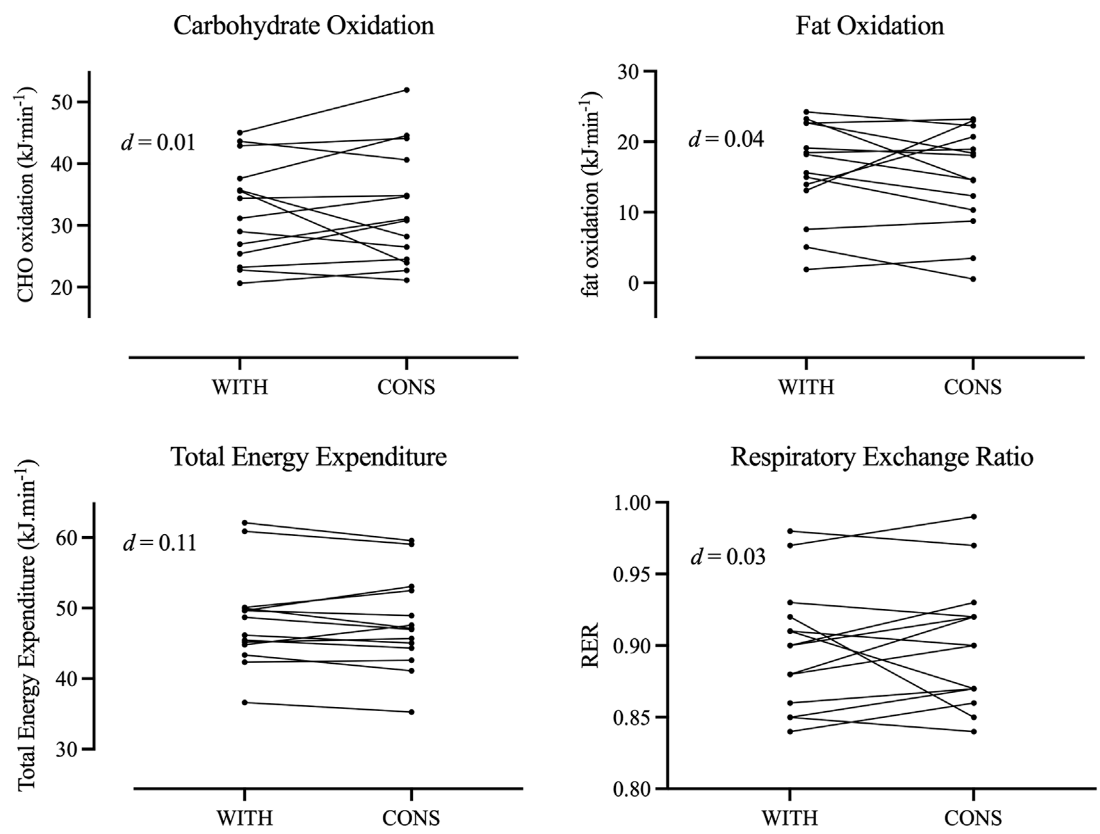

FIGURE 2
Individual responses for oxidation of carbohydrates and fats as well as for total energy expenditure and respiratory exchange ratio during withdrawal phase (WITH) and consumption phase (CONS).

Endurance-related parameters

Previous literature is controversial, as some studies did not find significant differences in endurance performance through OCC phases during chronic or short term use of OCP (Grucza et al., 1993; Bryner et al., 1996; Casazza et al., 2002; Vaiksaar et al., 2011b; Jurimae et al., 2011; Joyce et al., 2013; Gordon et al., 2018; Nakamura and Nose-Ogura, 2021), while others found significant differences (e.g., Giacomoni and Falgairette, 2000; Mattu et al., 2020). Across all exercise intensities (i.e., moderate, heavy, and severe exercise intensity domains), lower $\dot{V}O_2$ values were found, similar to previous work (Giacomoni and Falgairette, 2000). Furthermore, the present work found significantly lower absolute and relative $\dot{V}O_{2peak}$ values in CONS than in WITH.

It has been previously shown that the chronic use of OCP reduces $\dot{V}O_{2peak}$ (Giacomoni and Falgairette, 2000; Casazza et al., 2002; Suh et al., 2003). Therefore, it might be suggested that even a short-term absence could potentially lead to an elevation of exogenous estrogen E2 levels and consequently the detrimental effects on $\dot{V}O_{2peak}$ decrease. Significantly lower absolute and relative $\dot{V}O_{2peak}$ values in CONS in the present work are suggested to be attributed to notably lower (−30% in CONS) E2 levels. However, as E2 levels are not significantly different and the potential effect of still remaining exogenous hormones from OCP intake is unclear, this interpretation has to be done with caution. Anyway, a possible reason for a lower $\dot{V}O_{2peak}$ in CONS might be a negative influence of E2 on the efficiency of the mitochondrion and, therefore, a reduced use of oxygen in the aerobic metabolism.

Interestingly, no changes between OCC phases have been found for submaximal $\dot{V}O_2$ and running economy under steady-state conditions. A higher maximal response in $\dot{V}O_2$ is suggested with an enhanced efficiency of the mitochondrion. However, this was not the case in the present work for submaximal $\dot{V}O_2$. Moreover, present results suggest that OCC phases do not seem to influence other factors determining running economy, like muscle stiffness, neuromuscular control, or other biomechanical factors. No changes in the speed associated with VT1 and VT2 were found, and therefore, it is suggested that the OCC phase does not influence threshold speeds. Consequently, the intensities that, on the one hand, elicit changes in [BLa^-] and blood pH and, on the other hand, demarcate the boundary between steady-state and non-steady-state conditions are not influenced by OCP use.

In accordance with the previous literature, significantly higher values in CONS have been found in $\dot{V}E/\dot{V}O_2$ (Rechichi et al., 2008; Barba-Moreno et al., 2019) and $\dot{V}E/\dot{V}CO_2$ (Barba-Moreno et al., 2019) during submaximal exercise. However, the differences in $\dot{V}E$ (Rechichi et al., 2008; Barba-Moreno et al., 2019) have not been reproduced in the present study. Nevertheless, our findings confirm the notion of previous work (Rechichi et al., 2008; Barba-Moreno et al., 2019) that ventilatory inefficiency and a lower aerobic capacity during CONS are evident. Consequently, during the intake phase, an increase in respiratory drive is obvious. This is also reflected by a lower $\dot{V}O_{2peak}$ during CONS.

Hormones

No significant differences between OCC phases have been found for hormonal analyses. This is in accordance with previous studies (Vaiksaar et al., 2011b; Jurimae et al., 2011), which might be explained by the suppressing effect of OCPs on endogenous hormones. On the other hand, previous research has shown lower E2 levels at the end of CONS compared to WITH. There was even a difference between early and late WITH, which leads to the suggestion that testing on days 2 or 3 in the present study might have been too early to detect a difference in E2 and, consequently, an effect on the mitochondrion or the aerobic system in general. The results of the present study show a very low level of E2; in some participants, it has even been below the detection limit of the analysis. This was probably due to the suppression of OCP intake and might also be explained by the timing of testing, which was in the early phase of WITH.

Energy metabolism

In accordance with the total energy expenditure, no shift in substrate metabolism at 2.5 $m \cdot s^{-1}$ was found. However, Mattu et al. (2020) and Giacomoni and Falgairette (2000) found significant differences in RER, with the latter also observing differences in CHO and fat oxidation and total energy expenditure, which were not observed in this study. The results have shown that different E2 levels are usually linked to changes in fat and CHO oxidation, with enhanced fat oxidation at high levels of E2 and consequently a similar change in the respiratory exchange ratio (Giacomoni and Falgairette, 2000). However, our results did not demonstrate these shifts and are in accordance with Vaiksaar et al. (2011b). These authors maintained that the disaccording findings of previous works might be due to differences in exercise modes (e.g., cycling vs. running vs. rowing), the duration and intensity of the exercise, and aerobic endurance level of the subjects. Present results suggest that at lower intensities in the heavy exercise intensity domain, no effects of OCP intake concerning energy expenditure are evident. The reduced ventilatory efficiency and potentially lower aerobic capacity do not affect energy expenditure. Furthermore, a ~6% decrease in fat oxidation was found, which might be explained by a possible lower E2 concentration in CONS. This would be in accordance with recent works suggesting that a lower concentration reduces the oxidation of lipids (Giacomoni and Falgairette, 2000); however, these differences did not reach statistical significance. Based on the current results, we suggest that a greater difference in E2 between OCC phases might result in significant differences. Interestingly, Mattu et al. (2020) found no significant differences between OCC phases in time to exhaustion during a cycle-ergometer test, which suggests no influence of OCP intake on fatigue resistance.

Cardiometabolic system

Previous literature found significantly higher [BLa^-] during CONS than during WITH (Rechichi et al., 2008). However, Rechichi et al. (2008) stated as an explanation that the reported statistically significant differences of approximately 1 $mmol.L^{-1}$ in [BLa^-] are within the typical error of the measurement and might be caused by this error. In line with this statement, the present study found neither baseline nor maximal [BLa^-] differences between OCC phases. Similar to [BLa^-], no notable differences were demonstrated between OCC phases for submaximal and maximal HR. This suggests no differences in glycolytic metabolism and no influence on athletic performance concerning the cardiac system due to the intake of OCPs–when HR is suggested as a surrogate for the cardiac system.

Anthropometry

Body mass did not differ significantly between OCC phases, which is in accordance with previous results for moderately trained (Giacomoni et al., 2000; Vaiksaar et al., 2011a; 2011b; Joyce et al., 2013) and endurance-trained athletes (Rechichi et al., 2008; Barba-Moreno et al., 2019). It is, therefore, suggested that neither the exogenous hormone intake nor fluctuations in the endogenous hormone profile had a notable influence on body mass.

Subgroup analyses

A recent review by Elliott-Sale et al. (2020) suggested that the reasons for controversial findings might be explained by different concentrations of endogenous sex hormones, which vary individually through OCC phases and especially increase in the WITH phase. Moreover, physiologically meaningful differences in endogenous sex hormones have the potential to influence various physiological systems (e.g., Elliott-Sale et al., 2020; McNulty et al., 2020). Consequently, we analyzed the individual responses of E2, prog, and testo. Subsequently, we divided the total sample into subgroups where the inclusion criteria were lower hormone levels in CONS compared to WITH.

These further analyses showed influences on performance parameters when prog was increased (i.e., WITH) since [BLa^-] at rest and maximal [BLa^-] were significantly higher, represented by a *large* effect size and a significantly higher $\dot{V}O_{2peak}$ similar to the total sample. The higher [BLa^-] might be associated with a slightly affected mitochondrial function, as previously demonstrated in patients with multiple sclerosis (Paling et al., 2011) or cancer (Vyas et al., 2016). However, this is in contrast to the decrease in $\dot{V}O_{2max}$ in the present work during CONS, which is related to decreased mitochondrial function and thus lower aerobic capabilities. It might be suggested that elevated body temperatures caused by higher prog levels have an influence on anaerobic glycolysis, but this remains unclear and warrants further investigation. In subgroups with increased E2 and testo, no statistically significant effects on performance parameters have been found. Although hormone levels of E2, prog, and testo were significantly higher in WITH of the subgroup, hormone fluctuation might not have been intense enough to influence performance and reach statistical significance.

Limitations

A note of caution is due here since the fluctuation of endogenous blood hormones during daytime was not considered. Elliott-Sale et al. (2020) indicated that exogenous blood values might peak within 1 h after intake, which was not considered in the blood sample analysis. In future study designs, the measurement of hormonal-binding proteins (SHBG) should be considered to allow further interpretation. A potential cross reactivity between E2, prog, and testo assays and the components of the OCP might have influenced the analyses of hormone levels in venous blood samples. These cross reactions have been found to influence the analysis in previous animal studies, but the extrapolation of human data is unclear (Krasowski et al., 2014). In order not to influence participants' daily routine and not to counteract the instructions for OCP intake, participants were advised to keep their usual OCP intake timing. A limitation of this study is that a detailed analysis of exogenous hormonal blood values is missing. It might have been of further interest to find out whether in WITH, exogenous hormones still "exist" in athletes' bodies. Another limitation might be that submaximal parameters were obtained from a 1-min GXT protocol, which could affect the results derived from $\dot{V}O_2$ and $\dot{V}CO_2$ data due to different $\dot{V}O_2$ on-kinetics.

Conclusion

Only small changes in some endurance parameters have been found. Interpretation has to be done with caution, as no differences in hormone levels have been found, but further analyses suggest that an increase in prog might be responsible for a lower efficient metabolism in the WITH phase. Although it seems that the effects might be negligible for most participants, in elite sports, even small physiological changes can differentiate between winning and losing. Therefore, individual analysis of hormonal influences seems to be justified in elite athletes. Hormonal contraception containing only synthetic prog (and no estrogen) might have fewer suppressing effects on hormonal levels. Consequently, endurance performance might be influenced differently, but research and data concerning this topic are lacking. As a future perspective, it might be useful to study participants using OCPs containing only progestogen, as well as the use of hormonal IUDs (similarly containing only synthetic prog).

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 humans were approved by the Ethics Committee of the University of Vienna. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

AM: conceptualization, data curation, formal analysis, investigation, methodology, and writing–original draft. BW: conceptualization, data curation, formal analysis, and writing–review and editing. PH: investigation and writing–review and editing. HT: conceptualization, resources, and writing–review and editing. CT: conceptualization, data curation, formal analysis, methodology, supervision, and writing–original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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