Ferritin status impact on hepcidin response to endurance exercise in physically active women along different phases of the menstrual cycle

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Summary

Serum ferritin has been proposed as a predictor of hepcidin concentrations in response to exercise. However, this fact has not been studied in physically-active women. Therefore, the main objective of this study was to analyse the hepcidin response at different ferritin status before and after running exercise in physically active females. Fifteen eumenorrheic women performed a 40-min running protocol at 75% of VO_{2peak} speed in different menstrual cycle phases (early-follicular phase, mid-follicular phase and luteal phase). Blood samples were collected pre-exercise, 0h post-exercise and 3h post-exercise. For statistics, participants were divided into two groups according to their pre-exercise ferritin levels (<20 and ≥20 µg/L). Through menstrual cycle, hepcidin was lower in both early follicular phase (p=0.024; 64.81±22.48 ng/ml) and mid-follicular phase (p=0.007; 64.68±23.91 ng/ml) for <20 µg/L ferritin group, in comparison with ≥20 µg/L group (81.17±27.89 and 79.54±22.72 ng/ml, respectively). Hepcidin showed no differences between both ferritin groups in either pre-exercise, 0h post-exercise and 3h post-exercise. Additionally, no association between pre-exercise ferritin and hepcidin levels 3h post-exercise (r=-0.091; p=0.554) was found. Menstrual cycle phase appears to influence hepcidin levels depending on ferritin reserves. In particular, physically-active females with depleted ferritin reserves seems to present lower hepcidin levels during the early-follicular phase and mid-follicular phase. However, no association between ferritin and hepcidin levels was found in this study. Hence, ferritin levels alone may not be a good predictor of hepcidin response to exercise in this population. Multiple factors such as sexual hormones, training loads and menstrual bleeding must be taken into account.

Impacto de las reservas de ferritina sobre la respuesta de la hepcidina al ejercicio de resistencia en las mujeres físicamente activas a lo largo de las diferentes fases del ciclo menstrual

Resumen

La ferritina sérica parece ser un predictor de la respuesta de la hepcidina al ejercicio. Sin embargo, este hecho no ha sido estudiado en mujeres físicamente activas. El objetivo fue analizar la respuesta de la hepcidina en diferentes estados de la ferritina antes y después del ejercicio. Quince mujeres eumenorreicas realizaron un protocolo de carrera de 40 minutos al 75% de la velocidad VO_{2pico} en diferentes fases del ciclo menstrual (fase folicular temprana, fase folicular media y fase lútea). Se recogieron muestras de sangre antes del ejercicio y a las 0h y 3h después del ejercicio. Las participantes se dividieron en dos grupos según sus niveles de ferritina previos al ejercicio (<20 y ≥20 µg/L). La hepcidina fue más baja tanto en la fase folicular temprana (p=0,024; 64,81±22,48 ng/ml) como en las fase folicular media (p=0,007; 64,68±23,91 ng/ml) para el grupo de ferritina <20 µg/L en comparación con el grupo de ferritina ≥20 µg/L (81,17±27,89 y 79,54±22,72 ng/ml, respectivamente). La hepcidina no mostró diferencias entre ambos grupos de ferritina para ninguno de los momentos (antes del ejercicio ejercicio, 0h y 3h después del ejercicio). No se encontró ninguna asociación entre los niveles de ferritina previos al ejercicio y los niveles de hepcidina 3h posteriores al ejercicio (r=-0,091; p=0,554). El ciclo menstrual parece influir en los niveles de hepcidina dependiendo de las reservas de ferritina. En particular, las mujeres físicamente activas con reservas de ferritina agotadas parecen presentar niveles de hepcidina más bajos durante la fase folicular temprana y la fase folicular media. Sin embargo, no se encontró ninguna asociación entre la ferritina y la hepcidina. Por lo tanto, los niveles de ferritina por sí solos pueden no ser un buen predictor de la respuesta de la hepcidina al ejercicio en esta población. Se deben tener en cuenta múltiples factores como las hormonas sexuales, las cargas de entrenamiento y el sangrado menstrual.

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Introduction

Poor iron status can affect health and athletic performance[1,2]. Daily iron needs are covered by dietary iron, iron stores as ferritin, and iron recycling by macrophages. These mechanisms are controlled by the peptide hormone hepcidin, which acts by internalizing and degrading ferroportin, the only known cellular exporter of iron. Increased expression of hepcidin reduces iron inflow to plasma, while decreased expression produces the opposite effect[3]. There are several stimuli that influence the synthesis of this hormone. Hepcidin expression is increased by intra and extra cellular iron concentrations and inflammation, mainly interleukin-6 (IL-6), while it is decreased by low iron intra and extra cellular concentrations, erythropoietic activity and hypoxic stimuli[4]. Exercise practice can modify aforementioned hepcidin regulators[5–9]. Specifically, running shows a powerful capacity to increase hepcidin expression due to IL-6 production, chiefly owing to the higher foot strike haemolysis befallen in this modality[10] and/or its production as a myokine in the muscle during aerobic exercise[11].

Moreover, gender has also influence over iron losses and stores due to menstrual menses[1,12]. Along with this, several studies have already suggested changes over hepcidin production by high concentrations of sex hormones; specifically, high oestrogen concentrations are supposed to downregulate hepcidin synthesis[13–16] and reduce ferroportin expression in cells membrane[17]. Interestingly, progesterone seems to produce an opposite effect[18]. Although these effects are still not clear, it is reasonable to hypothesize that menstrual cycle could influence the hepcidin response. For all of the above reasons, women and athletic population frequently suffer from iron deficiency (ID) and iron deficiency anaemia (IDA)[9].

Additionally, a positive correlation between ferritin and hepcidin levels has been described in the literature[19,20]. Based on this relation, some studies observed the hepcidin response to exercise depending on the previous ferritin status of the participants, obtaining that the higher the levels of ferritin were before exercise, the higher the levels of hepcidin were 3 hours post-exercise[21,22]. Only Peeling et al.[21] tested men and women´s response, but the results were presented as a whole, ignoring the possible influence of female sex hormones[13–18]. It is unknown the hepcidin response to endurance exercise of the active females in different menstrual cycle phases, depending on their pre-exercise ferritin concentrations. This information could help many active women to avoid ID and IDA due to poor training and nutrition planning. Thus, the purpose of this study was to compare the hepcidin response to exercise attending to baseline ferritin levels in physically active eumenorrheic women.

Material and method

Participants

Fifteen endurance-trained females with eumenorrheic cycles (age=35.6±4.2 years; height=163.9±5.9 cm; body mass=58.12±5.2 kg; peak oxygen consumption (VO$_{2peak}$)=50.5±3.7 ml/kg/min; body fat=24.2±7.0%; haemoglobin=13.1±0.9 g/dl; serum iron=73.9±37.1 μg/ dl; serum ferritin=37.8±22.1 μg/L) were recruited for this study. They were females between 25 and 40 years old. Inclusion criteria required participants to meet: eumenorrheic menstrual cycle during the year previous to the study; training endurance sports between 5 and 12 hours per week; presented with no IDA; not presenting thyroid problems; not consuming currently medication or dietary supplements (e.g., iron supplementation, tricyclic antidepressants, α-blockers, β-blockers, etc.); non-smokers; non-pregnant or ovariectomy. Eumenorrheic cycle is defined as normally occurring menstrual cycles, from 24 to 35 days in length[23,24]. Research Ethics Committee of the Universidad Politécnica de Madrid approved the project, which is in accordance with the ethical standards of the Helsinki Declaration. In addition, each participant signed an informed consent form.

Experimental Overview

The current study is an observational and randomized controlled study. Participants visited the laboratory to perform initial testing (body composition and VO$_{2peak}$ assessment). All the screening tests were performed during the early follicular phase (between days 2 and 5). Resting blood samples were obtained in early morning fasted state in order to verify that none of the participants showed signs of inflammation, IDA, thyroid problems or menstrual cycle dysfunction. For the last one, sexual hormones (progesterone, oestrogen, luteinizing and follicle-stimulating hormones and prolactin) were measured.

Body composition, as well as height and body mass were measured for all the participants. A stadiometer (SECA) was used for measuring height and a scale (range 0-150 kg, Beurer GmbH Germany) was used for body mass. For the body composition analysis, a Dual-Energy X-ray Absorptiometry (DEXA) scan was performed, which measured body fat mass (%), total body fat mass (kg) and free fat mass (kg), using a GE Lunar Prodigy apparatus (GE Healthcare, Madison, Wisconsin, USA).

For VO$_{2peak}$ measurement, participants performed a maximal graded test consisting of 3 minutes warm up at 6 km/h followed by an incremental period in which the speed was set at 8 km/h and increased by 0.2 km/h every 12 seconds until participant´s exhaustion. 1% slope was set throughout all the test. A computerized treadmill (H/P/COSMOS 3PW 4.0, H/P/Cosmos Sports & Medical, Nussdorf-Traunstein, Germany) and a gas analyser Jaeger Oxycon Pro (Erich Jaeger, Viasys Healthcare, Germany) was used to determine their VO$_{2peak}$ and maximal aerobic speed (vVO$_{2peak}$). VO$_{2peak}$ was determined as the mean of the three highest breath-by-breath VO$_2$ measurements. vVO$_{2peak}$ was recorded as the minimum speed required to elicit VO$_{2peak}$.

After initial measurements, participants attended the laboratory on three different occasions throughout the menstrual cycle to carryout the experimental protocol. Different phases of the menstrual cycle were selected for this study in order to analyze different hormonal environments as literature suggest[25]: early follicular phase, low sex hormonal levels (days 2-5); mid-follicular phase, low progesterone but mid-high oestrogen levels (days 7-10; 8±1.09); and luteal phase, elevated progesterone and oestrogen levels (days 19-21). Nevertheless, mid-follicular phase was selected instead late follicular phase (peak oestrogen concentrations) in order to ensure testing before ovulation as no luteinizing hormone surge testing was used in this study[25]. The set value

for individuals progesterone concentration for the luteal phase was 6.0 ng.mL-1, such as literature suggest to exclude deficient luteal phases[26].

Participants were asked to complete information about the length of their last four menstrual cycles (number of days from the cycle onset to the next one) to determine their average cycle length. Day 1 of the menstrual cycle was characterized by the onset of menstrual bleeding. These data were provided to a gynecologist, who confirmed menstrual cycles were eumenorrheic and calculated the days for the different menstrual phases. In addition, blood samples were collected for determination of female steroid hormones in each menstrual phase to confirm participants were performing the tests in the correct phase.

Ferritin Group Determination

Participants were divided into two groups according to their baseline ferritin levels before each exercise protocol performed in the different menstrual cycle phases conducted in the study. The cut-off point for the division of the groups was 20 µg/L. This decision was based on two criteria: 1º) at stage two of ID, this being the state prior to the IDA[27]; 2º) the clinical depletion of ferritin reserves was found at <22 µg/L[28]. Accordingly, the sample was divided into unexpended ferritin reserves group with ≥20 µg/L (FG≥20) and depleted ferritin reserves group with <20 µg/L (FG<20). The number of participants in each ferritin group were n=8 (early follicular and luteal phase) and n=7 (mid-follicular phase) for FG<20; n=7 (early follicular and luteal phase) and n=8 (mid-follicular phase) for FG≥20.

Experimental Protocol

Participants attended the laboratory at 07 am on the test day having had breakfast two hours earlier. Composition breakfast was controlled and supervised by a nutritionist in order to avoid pro-inflammatory food and carbohydrates as it may reduce the post-exercise hepcidin response following exercise[29]. Repeat testing for each participant was conducted at the same time of day to minimize the potential effect of diurnal fluctuations.

Prior to the test, venous blood samples were drawn to measure the serum concentrations of oestrogen and progesterone to verify menstrual cycle phase.

After this, a running aerobic endurance test was performed. This test consisted of 5 minutes of warm-up at 60% of vVO_{2peak} followed by 40 minutes at 75% of vVO_{2peak} concluding with 5 minutes recovery at 30% of vVO_{2peak}. The same absolute velocity was used for each of the testing sessions. The testing protocol has been previously selected by other studies[30]. The speed that participants ran on the treadmill was 11.2±1.1 km/hour (mean±SD). Blood samples were collected before the start of exercise (Baseline), immediately post-exercise (Post-0h) and 3 h post-exercise (Post- 3h). Samples were subsequently analysed for serum hepcidin, IL-6, iron and ferritin.

Nutrition and physical activity

The participants completed a 24-hour dietary recall for the 24 hour period immediately prior to the first session, which was the replicated for the following testing sessions. Participants were asked to be adequately hydrated for each session, and to avoid caffeine and alcohol for 24 hours prior to testing. In addition, they were requested to refrain from any type of exercise for 24 hours prior to each testing session.

Blood samples analysis

All blood samples were obtained in a rested state by venipuncture using clot activator vacutainer tubes. Following inversion and clotting, the whole blood was centrifuged (Biosan LMC-3000 version V.5AD) for ten minutes at 3000 rpm and transferred into Eppendorf tubes and stored frozen at -80ºC until further analysis. Total 17β-estradiol and progesterone were measured via ADVIA Centaur® solid-phase competitive chemiluminescent enzymatic immunoassay (Siemens Healthineers, Germany). Coefficients of variation reported by the laboratory for sexual hormones were: 7.48% for estradiol, 14.11% for progesterone, 7.74% for FSH, 10.77% for LH, 5.65% for prolactin. And for iron parameters: 4.15% for iron, 4.92% for ferritin, 1.4% for transferrin, 5.9% for IL-6 and 4.55% for hepcidin.

Serum samples were analysed in a AU400 clinical analyser (Beckman Coulter Ltd) for Iron (OSR6186), Ferritin (OSR61203), using Beckman re-actives; and in a Immulite 1000 (Siemens Healthineers) for Interleukin-6 (IL-6 Ref 6604071). Hepcidin was analysed using a microplate ELISA Kit (Elabscience Human Hepc25 Elisa kit. Catalog Nº. E-EL-H5497). Samples were assayed as duplicates. A Thermofisher Multiskan Ascent microplate reader connected to a computer was used to read ELISA microplate absorbance. The ascent software used controls microplate readings and ELISA microplate calibration data to calculate the final concentration of hepcidin in each sample.

Statistical analysis

All statistical analysis was conducted using the software package SPSS version 22 (IBM; Armonk, NY, USA) and the alpha level of significance was set at $p<0.05$. Data are expressed as means ± SD. All data were initially tested for normality with the Shapiro-Wilk test was used to determine data´s normal distribution. For data with a normal distribution (iron post_0h and iron post_3h) Independent t-test was used, while for data that were not normally distributed (the rest of them) the non-parametric Mann-Whitney test was performed. For the data with a normal distribution the Levene´s test was used to assess the equality of variances. Additionally, Pearson's correlation coefficients were calculated to assess any association between serum ferritin and hepcidin.

The effect size was used to examine the magnitude of change between ferritin groups. Their interpretation was based on the following criteria: <0.2 = trivial, 0.2 to 0.6 = small effect, >0.6 to 1.2 = moderate effect, >1.2 to 2.0 = large effect, and >2.0 = very large[31].

Results

Hormone levels (mean±SD) presented in each phase of the cycle fulfilled the expected fluctuations for a typical menstrual cycle. Oestrogen showed the lowest levels in the early follicular phase (39.4±18.4 pg/ml), increasing during the mid-follicular phase (82.7±51.3 pg/ml) and presenting its highest levels in the luteal phase (110.7±33.6 pg/ml).

Progesterone exhibited its highest concentrations in the luteal phase (10.43±5.58 ng/ml) in comparison with the low levels found in early follicular (0.91±0.79 ng/ml) and mid-follicular phases (0.53±0.31 ng/ml). The mean±SD for the testing days of the different phases were the following ones: day 3±0.85 for the early follicular, day 8±1.09 for the mid-follicular and day 21±1.87 for the luteal (Table 1).

The mean hepcidin levels across the menstrual cycle for each ferritin group are presented in Figure 1A, while baseline and post-exercise values are shown in Figure 1B. FG<20 presented lower hepcidin concentrations than FG≥20 during the early follicular (p=0.024, d=0.04) and mid-follicular phases (p=0.007, d=0.24).

IL-6 and iron concentrations in the different phases of the menstrual cycle are shown in Table 2 for each ferritin group. FG<20 plasma iron was lower in comparison with FG≥20 throughout all the menstrual cycle (early follicular, p<0.001, d=1.17; mid-follicular, p<0.001, d=0.75; luteal, p=0.002, d=1.79). Furthermore, baseline and post-exercise values are presented in Table 3. FG<20 showed lower iron concentrations than FG≥20 at Baseline (p<0.001, d=0.90), Post-0h (p<0.001, d=0.95) and Post-3h (p<0.001, d=0.85). IL-6 reported no significant differences between

Table 2. Mean±SD serum IL-6 and iron of each FG<20 and FG≥20 during different menstrual cycle phases.

		Early	Mid	Luteal
IL-6	FG<20	3.95±3.73	2.81±1.28	6.21±7.52
pg/ml	FG≥20	2.67±1.09	3.28±1.61	4.00±4.34
Iron	FG<20	39.58±19.67	54.75±25.33	57.91±29.10
µg/dl	FG≥20	80.40±33.92*	101.07±32.17*	103.43±46.49*

Early: Early follicular phase; Mid: Mid-follicular phase; Luteal: Luteal phase; IL-6: Interleukin-6; FG<20: Depleted ferritin reserves group; FG≥20: Unexpended ferritin reserves group. *Significantly different from FG<20.

Table 3. Mean±SD serum IL-6 and iron of the FG<20 and FG≥20 pre-exercise, post-exercise and 3 hours post-exercise.

		Baseline	Post-0h	Post-3h
IlL-6	FG<20	2.92±2.66	4.39±3.96	5.86±7.36
pg/ml	FG≥20	2.10±0.33	3.33±1.29	4.51±4.24
Iron	FG<20	47.46±25.16	49.51±24.46	53.75±28.24
µg/dl	FG≥20	89.85±41.25*	99.06±41.16*	96.82±34.19*

Post-0h: 0 hours post-exercise; Post-3h: 3 hours post-exercise; IL-6: Interleukin-6; FG<20: Depleted ferritin reserves group; FG≥20: Unexpended ferritin reserves group. *Significantly different from FG<20.

ferritin groups for menstrual cycle phases (early follicular, mid-follicular and luteal; p>0.05) or time (Baseline, Post-0h and Post-3h; p>0.05).

Correlation analysis did not reveal any association between baseline ferritin and hepcidin levels. We did not find any other association in each menstrual cycle phase for these two variables or between baseline ferritin and hepcidin at the different times of measurement (p>0.05). Figure 2 shows the association between Post-3h hepcidin response and Baseline serum ferritin levels in each menstrual cycle phase (r=-0.091; p=0.554).

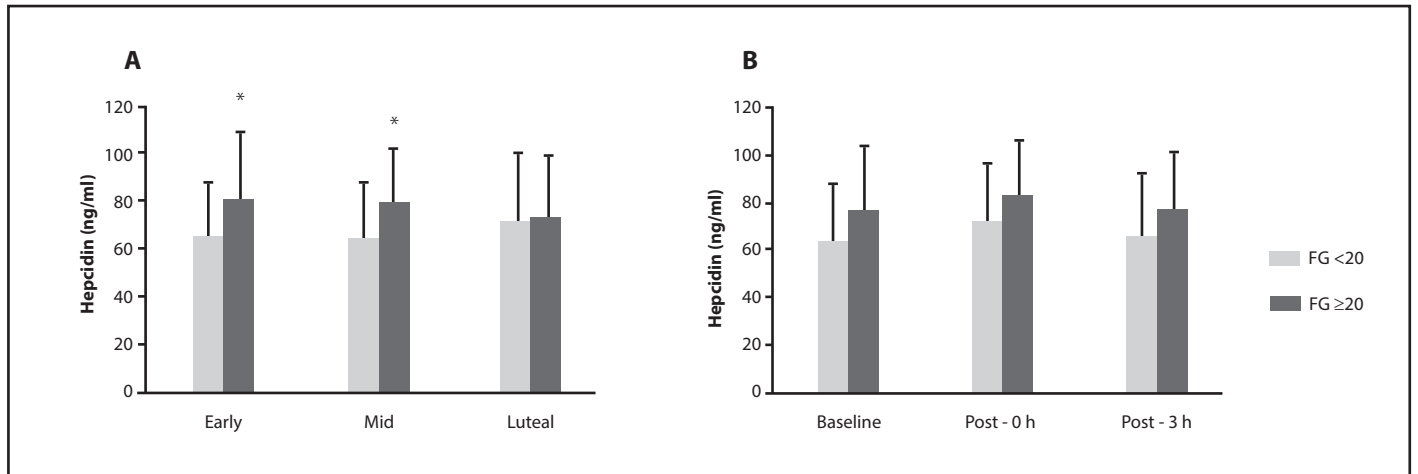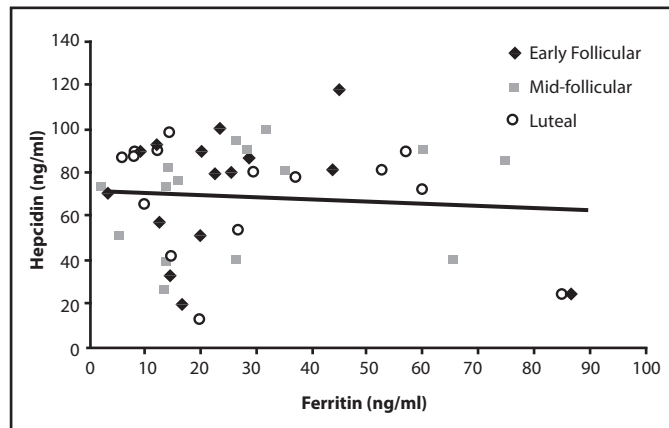**Table 1. Ferritin, 17β-estradiol and progesterone of the participants in each menstrual cycle phase (Mean±SD).**

		Early	Mid	Luteal
Ferritin	FG<20	13.4±5.73	11.09±5.26	18.19±17.36
µg/L	FG≥20	39.11±22.73	43.44±19.89	49.54±20.5
17β-estradiol	FG<20	35.49±13.81	61.17±33.16	123.46±30.64
pg/ml	FG≥20	43.87±22.94	101.61±58.79	96.2±32.84
Progesterone	FG<20	0.66±0.21	0.71±0.25	9.19±5.76
ng/ml	FG≥20	1.2±1.11	0.36±0.28	11.85±5.49

Early: Early follicular phase; Mid: Mid-follicular phase; Luteal: Luteal phase; FG<20: Depleted ferritin reserves group; FG≥20: Unexpended ferritin reserves group.

Figure 1. Serum hepcidin levels of each FG<20 and FG≥20 during eumenorrheic cycle group phases and moments.

Early follicular phase (Early); Mid-follicular phase (Mid); Luteal phase (Luteal); 0 hours post-exercise (Post-0h); 3 hours post-exercise (Post-3h) Depleted ferritin reserves group (FG<20); Unexpended ferritin reserves group (FG≥20). *Significantly different from FG<20. *Significantly different from FG<20. Early follicular phase (p=0.024); Mid-follicular phase (p=0.007).

Figure 2. Scatter plot and linear trend line of each individual's Post-3h hepcidin response in association with their baseline serum ferritin levels in each menstrual cycle phase (r=-0.091; *p*=0.554).

Discussion

The main finding of this study is that hepcidin concentrations showed differences between ferritin groups in the early follicular phase and in the mid-follicular phase. However, this fact did not occur in the luteal phase.

During early follicular and mid-follicular phases, participants with low ferritin levels showed lower hepcidin levels. This is supported by other studies, finding lower hepcidin concentrations when ferritin levels are low in men and women, disregarding menstrual cycle phase effect[21,22]. Nevertheless, this fact did not occur during the luteal phase where both groups showed similar hepcidin levels. Noting that previous literature did not take into account the influence of the menstrual cycle, a potential justification for the similarity between the two ferritin groups is the influence of sex hormones. Some authors[18] recently found that progesterone presence in animals could lead to an increase in hepcidin expression, as well as elevated hepcidin levels in women who received progesterone supplementation due to a fertility treatment. Therefore, progesterone concentrations may also influence on iron metabolism. Moreover, although our study did not statistically compare menstrual cycle phases, a higher IL-6 was observed at luteal phase.

So in this phase, the lower hepcidin levels theoretically expected in the FG<20 could be counteracted or affected by the presence of noticeably higher IL-6 and progesterone concentrations[18]. Conversely, it is unknown why there was no proportional increase in the FG≥20 group. Maybe the aforementioned mechanisms act by "blocking" the reduced hepcidin expression in an iron-deficient state (FG<20), but this is only a hypothesis that need to be explored in depth with further research.

Contrary to the results found throughout the menstrual cycle, ferritin groups showed no differences at any time, whereas other studies reported changes in hepcidin levels for different ferritin status[21,22]. Peeling *et al.*[22] tested elite male athletes in a 25-km running protocol at 75% of vVO$_{2peak}$. The mean duration of this protocol was approximately 120 minutes and produced 9.4±4.6 pg/ml of post-exercise IL-6. Our protocol was 40 minutes long, also at 75% of vVO$_{2peak}$. This produced lower levels

of IL-6 (about 3.5-4.0 pg/ml), which could reduce hepcidin response and therefore mask the differences between ferritin groups. However, Peeling *et al.*[21] obtained similar IL-6 increments as our results at post-exercise with comparable protocols in duration and intensity, noting significant differences between ferritin groups in hepcidin levels at baseline and 3 hours post-exercise. Thus, IL-6 post-exercise response magnitude seems unlikely to mask hepcidin differences between ferritin groups.

In contrast to our study, the above mentioned studies[21,22] split the participants according to their ferritin levels with higher cut-off points than ours. "Being the lowest points 35 µg/L[21] and bottom 50th percentile of the participants, presenting a mean serum ferritin of 58±7.8 µg/L[22]. Our cut-off point was 20 µg/L and it is justified in *"Ferritin Group Determination"* methodology section, but with the current knowledge it is not possible to accurately establish the cut-off point where ferritin status is not blocking or reducing hepcidin response post-exercise. Due to the aforementioned, 20 µg/L of ferritin could be an inadequate point in order to confirm, in physically active women, the influence of ferritin status on post-exercise hepcidin levels[21,22]. In spite of this, endurance-trained females usually have low ferritin levels, as they are the population group most affected by iron deficiency[9].

In addition to this issue, Peeling *et al.*[21,22] found no differences in serum iron concentrations between ferritin groups. However, our results markedly showed lower serum iron in the FG<20 compared with the FG≥20. This result can be explained due to the differences in ferritin reserves between studies. Literature has previously suggested decreased serum iron levels in individuals with ferritin <30 µg/L[32], as it is the case of the FG<20 in our study. Therefore, in the case of physically active females, our results suggest a slightly lower cut-off point to evidence significant decrements in serum iron.

Curiously, no correlations were found between ferritin and hepcidin levels at baseline. Once again contradicting previous studies, which found positive correlations between ferritin and hepcidin in general population at baseline conditions[19,20]. As mentioned previously, endurance-trained females seem to have a poorer iron status[9], and ferritin levels are commonly low or deficient. This particular scenario for physically active women could differ from healthy women since ferritin means and ranges are wider in these studies (46, 18-140 µg/L[19] and 81.6, 8.7-368.6 µg/L[20]) compared with our study (37.8, 7.5-79.8 µg/L). Similarly, we did not obtain association between baseline ferritin and pre- and post-exercise hepcidin, although a positive association have been shown in studies with male participants[21,22]. Consequently, it is difficult to compare the results since they had higher and wider ferritin values and did not consider the possible influence of the menstrual cycle phase on this association.

Hence, considering menstrual cycle phases, physically active female could have a different ferritin/hepcidin association compared to physically active men and general population. This is not surprising knowing that endurance-trained eumenorrheic females are the population group most affected by different factors when regulating hepcidin, as they have been mentioned previously (Sangkhae and Nemeth 2017; Latunde-Dada 2013; Roecker *et al.* 2005; Sim *et al.* 2014; Peeling *et al.* 2008; Peeling *et al.* 2009; McClung 2012; Goldstein 2016; Pedersen and Febbraio 2008; Constantini, Dubnov, and Lebrun 2005; Lehtihet *et al.* 2016; Yang *et al.* 2012; Hou *et al.* 2012; Qian *et al.* 2015; Li *et al.* 2016; Bajbouj *et al.* 2018).

The main limitation of the present study was the small sample size recruited. Research with a larger sample could reinforce the new results found in our study. No ovulation test was carried out despite the verification of hormone levels in each phase, so it is not possible to guarantee the ovulation of participants. Furthermore, protocols with greater intensity, duration, or both could allow us to observe additional differences in blood parameters. These results suggest that further research is needed in order to clarify the ferritin/hepcidin relation in endurance-trained females due to their special conditions regarding iron metabolism.

Ferritin levels do not necessarily have to be a good predictor of hepcidin response to exercise in endurance-trained females. However, during the early follicular and mid-follicular phases the hepcidin response of those women with lower ferritin reserves was lower. Therefore, these phases appear to be favourable for assuming iron intake either by diet or by supplementation, being a good strategy to maximize iron absorption in individuals with low ferritin levels.

Menstrual cycle phase appears to influence hepcidin levels depending on ferritin reserves. In particular, physically active females with depleted ferritin reserves seems to present lower hepcidin levels during the early follicular and mid-follicular phases. Lastly, menstrual cycle and ferritin status should be considered together in future research since our results found different hepcidin levels in different hormonal environments.

Acknowledgments

Conflict of interest

The authors do not declare a conflict of interest.

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