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Timing is everything, but does it really matter? Impact of 8-weeks morning versus evening iron supplementation in ballet and contemporary dancers

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ABSTRACT

The effectiveness of a morning versus evening oral iron supplement strategy to increase iron stores was explored. Ballet and contemporary dancers with serum ferritin (sFer) < 50μg/L (n = 14), were supplemented daily with 105 mg elemental oral iron in either the morning (Fe_{AM}) or evening (Fe_{PM}) for 8 weeks. A control group (n = 6) with sFer >50μg/L were given no supplement over the same period. Dancers' sFer were measured at baseline and post-intervention. Assessment of daily training load, dietary intake, and menstruation were made. A significant interaction ($p < 0.001$) showed the within group sFer change over the 8-week intervention in Fe_{AM} (+25.9 ± 10.5μg/L) and $Fe_{PM,}$ (+22.3 ± 13.6μg/L) was significantly different to CON (−30.17 ± 28.7μg/L; both $p = 0.001$). This change was not different between Fe_{AM} and Fe_{PM} ($p = 0.778$). sFer levels within Fe_{AM} and Fe_{PM} significantly increased over the 8-weeks; however, they significantly decreased in the CON group (all $p < 0.05$). Post-intervention sFer levels were no longer different between the three groups ($p > 0.05$). Training load, dietary intake, and number of menstrual cycles incurred were similar between Fe_{AM} and Fe_{PM} ($p > 0.05$). Oral iron supplementation in either the morning or evening appears equally effective in increasing sFer levels in dancers with sub-optimal iron status.

Highlights

- 8 weeks of oral iron supplements increases serum ferritin levels in elite dancers.
- Dancers not consuming an iron supplement showed a decline in serum ferritin over the 8-week period.
- Consuming the iron supplement in either the morning or the evening appeared equally effective in improving serum ferritin stores.

KEYWORDS
Iron supplement; iron deficiency; nutrition; minerals

Introduction

Numerous studies to date have described the transient upregulation of the iron master regulator, hepcidin, released from the liver in the 3- to 6-h period post exercise (Barba-Moreno et al., 2022; Hennigar et al., 2021; McCormick et al., 2019; Newlin et al., 2012; Peeling et al., 2009). Furthermore, some of this literature confirms that increased post-exercise hepcidin effectively inhibits iron absorption during this time (Hennigar et al., 2021). Interestingly, hepcidin activity has been shown to follow a pattern of diurnal variation, whereby levels are lowest in the morning and reach a peak in the afternoon (Busbridge et al., 2009; Ganz et al., 2008; Kemna et al., 2007; Kroot et al., 2009; Schaap et al., 2013). Previous work has reported a 2- to 6-fold increase in serum hepcidin concentration from 0600am to 1500pm on the same day (Kemna et al., 2007). Such fluctuations across the day, and following exercise, ultimately prompt a challenge to iron absorption. Given this, a strategic approach to iron intake is warranted, with the goal to optimise absorption.

Beyond whole food dietary intervention, oral iron therapy (i.e. supplements) has been shown to be an effective method of iron replacement. Previous studies in exercising populations have highlighted the effectiveness of daily oral iron supplementation

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consisting of ~100–200 mg of elemental iron for 4–12 weeks in increasing serum ferritin (sFer) levels from baseline by 33–127% (Dawson et al., 2006; Fogelholm et al., 1992; Friedmann et al., 2001; Hinton et al., 2000; Klingshirn et al., 1992; Zhu & Haas, 1998). Given these findings, it appears that oral iron supplementation is an effective means of improving iron status in active/athlete populations. However, the optimal timing of supplement consumption remains to be fully understood.

With a focus on strategic approaches to iron supplementation, previous literature has explored the application of oral iron supplement strategies with the goal of increasing tolerability by minimising the associated negative gastrointestinal side effects, while optimising absorption (McCormick et al., 2020; McCormick et al., 2020; Stoffel et al., 2017; Stoffel et al., 2020). The potential strategies stemming from this work include supplementing on alternate days, at lower doses (i.e. 60 mg), or using controlled release tablets. However, the timing of supplementation in active/athlete populations has received less attention.

One recent study (McCormick et al., 2019) assessed the impact of morning versus afternoon exercise on iron absorption in endurance runners, finding that iron was best absorbed in the morning, consumed immediately (within 30 min) following an exercise bout. In this this study, hepcidin levels were higher in the afternoon, both following and in the absence of exercise, subsequently reducing (compared to a morning exercise trial) the amount of iron absorbed. Here, the increased afternoon hepcidin levels were likely a combination of the diurnal effect of hepcidin and the effect of exercise-induced inflammation (McCormick et al., 2019). Regardless, the outcomes of this short-term study by McCormick and colleagues only provides insight into the potential *acute effect* of the diurnal nature of hepcidin in an active/athletic population, and therefore, there is a need for longer-term studies that investigate the outcomes of daily oral iron supplement timing. Moreover, consideration of the impact of diurnal variation will help to further refine current supplement strategies to optimise iron absorption in active/athletic populations that are specific to their unique training environment and schedule. Therefore, the aim of this study was to evaluate the effectiveness of a morning versus afternoon oral iron supplementation regime (over 8 weeks) as a means of increasing iron levels in an athletic population with identified sub-optimal iron stores. We hypothesised that; (a) 8-weeks of oral iron supplementation will increase iron stores (sFer) regardless of supplement protocol; and (b) total iron absorption will be greater when iron is supplemented in the morning as compared to the evening, which will be reflected by a greater increase in sFer levels in the morning supplemented group.

Methods

Participants

Twenty classical ballet and contemporary dancers (1 male, 19 females) aged 16–30 years were recruited from a pre-professional undergraduate dance institution, local dance schools, and a professional dance company. Dancers were recruited for this investigation as a unique athlete population who are at heightened risk of iron deficiency due to the increased iron demands of their heavy physical workloads, paired with the potential for dietary (and therefore mineral) restriction due to the artistic and aesthetic demands of the discipline, which traditionally favour a lean body composition (Koutedakis & Jamurtas, 2004). Inclusion criteria required participants not to consume iron supplements within two weeks of the study commencement. Written informed consent was obtained from all participants. Ethics approval to conduct this study was obtained from the host institution Human Research Ethics Committee (2021/ ET000881).

Experimental overview

This study consisted of an 8-week intervention, utilising a parallel-group study design. Prior to random group allocation, interested participants were required to have a venous blood sample collected. This sample served a dual purpose; to determine (a) supplement group allocation eligibility; and (b) pre-intervention sFer levels. Participants meeting the criteria of sub-optimal iron status (sFer levels <50µg/L; n = 14) (McCormick et al., 2020) were then randomly allocated to one of two groups that received a daily oral iron supplement (Ferro Grad C, Abbott Laboratories, Botany Bay, Australia), containing 105 mg of elemental iron, for an 8-week period, to be consumed at a predetermined time as either: (1) In the morning between 0600–0800 am (Fe_{AM}; n = 7); or (2) in the evening between 1700–1900 pm (Fe_{PM}; n = 7). A control group (CON; n = 6) of those volunteers with sFer >50µg/L were given no supplement over the same period. Although the sample size in each group appears small, the sample is justified by a power analysis (G*Power version 3.1.9.7, Universitat Kiel, Germany) conducted to explore the typical change in sFer expected from daily oral iron supplementation (105mg/day) over an 8-week period (~24.0 ± 16.4 µg/L, $d = 1.54$; McCormick et al., 2020), congruent with the methods implemented

in the present study. The a-priori power analysis (critical-t = 2.57; power = 0.85; alpha = 0.05) suggested a sample of n = 6 per group to confidently see any effect of the supplement period on sFer levels. The effectiveness of supplementation protocols was assessed via comparison of post-intervention sFer levels to baseline, whilst differences between conditions were also quantified. Daily exercise duration and intensity, in addition to the occurrence of menstruation was recorded over the 8-week period using daily online diaries. Additionally, participants completed a 3-day food record during week 4 of the intervention period.

Experimental procedures

Iron supplementation

During the 8-week supplementation period, participants consumed their daily 105 mg of elemental iron in the form of ferrous sulfate (each tablet containing 325 mg of ferrous sulfate and 500 mg of ascorbic acid). The Fe_{AM} group consumed the tablet upon waking (between 0600–0800am), and the Fe_{PM} group between 1700–1900pm. Participants received a daily reminder text message at the start of the 2-h window to consume the iron tablet. The time of supplement consumption was documented in a daily journal along with any side effects experienced after taking the supplement each day. Of note, participants were asked to avoid the consumption of vitamin C, dairy, coffee, and/or tea with their supplement intake, and for the ensuing 60 min, as a result of the known influence that co-consumption of these nutrients can have on intestinal iron absorption (i.e. vitamin C enhances iron uptake, whereas tannins, polyphenols, and calcium are known inhibitors; (Collings et al., 2013)).

Blood collection

Upon volunteering for the study, a resting venous blood sample (4 ml) was collected from a forearm vein and analysed to determine (a) supplement group allocation eligibility; and (b) pre-intervention sFer levels. This process was conducted by a commercial pathology laboratory (Clinipath Pathology, Western Australia). Participants were required to follow the pre-blood test recommendations for iron screening of Sim et al. (2019), entailing participants be in a well-rested/hydrated state. Accordingly, participants were rested at for least 12-h post training, with adequate levels of hydration encouraged, and blood samples collected in the morning following an overnight fast (Sim et al., 2019). Following the 8-week supplementation period, a second venous blood sample was collected (under the same conditions) to determine any change in sFer levels.

Exercise, dietary and menstrual monitoring

Participants were required to document their daily exercise, noting the activity, time of day performed, duration (min) and level of exertion using the Borg CR-10 scale (where 0 = no exertion, and 10 = maximal exertion; (Borg, 1998)). These data were used to calculate a daily training impulse (TRIMP) using the Bannister TRIMP method (Foster et al., 1995). Additionally, participants completed a 3-day food record during week 4 using the mobile application *Research Food Diary* (Xyris Software, version 6.0.0202). This information was analysed for energy intake, macronutrient and micronutrient content using the nutritional analysis software, *Foodworks* (Xyris Software, version 10.0.4266, AusBrands 2019 and AusFoods 2019 databases). Finally, female participants documented the onset of menstruation on each occasion incurred over the intervention period using a dedicated section of the daily diary provided.

Statistical analysis

Repeated measures ANCOVA using Fe_{AM} and $Fe_{PM,}$ and CON as a grouping variable to account for independent samples, and total elemental iron intake from the supplement as a covariate was used to establish time, group, and interaction effects in sFer. In the event of a main effect, paired samples t-test was used to further explore the outcome. Additionally, one-way ANOVA was used to assess any between group differences in diary entries for training load, nutrient intake, and number of menstrual cycles in the Fe_{AM} and Fe_{PM} groups only. Important to note here is that all participants successfully provided both the pre- and post-intervention blood samples for sFer analysis; however, although participants in the Fe_{AM} and Fe_{PM} groups provided consistent and complete diaries over the 8-week intervention, only four of the six CON group participants provided consistent and reliable diary entries over this time. As a result, the CON data has only been included in the analysis of sFer, with the mean ± SD diary entry data not incorporated into the analysis, and is instead, only provided as descriptive indicative outcomes from n = 4 CON participants.

Results

Supplement compliance

The Fe_{AM} group consumed 50 ± 11 iron supplements (~89% compliance: 5190 ± 1232 mg of elemental iron across the 8-week intervention) and the Fe_{PM} group consumed 49 ± 6 iron supplements (~88% compliance: 5070 ± 538 mg of elemental iron across the 8-week intervention). Total number of supplements and the total

amount of elemental iron consumed over the intervention period was not different between groups (both $p = 0.817$).

Serum ferritin

Figure 1 depicts the mean ± SD pre- and post-intervention sFer concentrations for the Fe_{AM}, Fe_{PM} and CON groups. A significant time*group interaction ($p < 0.0001$) showed the *within* group sFer change over the 8-week intervention in Fe_{AM} (+25.9 ± 10.5µg/L) and $Fe_{PM,}$ (+22.3 ± 13.6µg/L) were significantly different to CON (−30.2 ± 28.7µg/L; both $p = 0.001$); however, this change over time was not different *between* Fe_{AM} and Fe_{PM} ($p = 0.778$). Of note, the sFer levels within both Fe_{AM} and Fe_{PM} were significantly increased over the 8-week intervention, whereas the sFer levels within the CON group were significantly decreased (all $p < 0.05$). Post intervention, the sFer levels between all three groups were no longer different ($p > 0.05$).

Training load, dietary intake, and menstrual cycle

Table 1 presents the mean ± SD weekly training volume and intensity (captured as a weekly training impulse score [TRIMP]), typical daily energy, carbohydrate, protein, fat, and iron intake from the 3-day food record provided in week 4 of the intervention, and the number of menstrual cycles incurred over the 8-week period for the Fe_{AM}, Fe_{PM}, and CON groups. No differences were evident in the average 8-week training load between Fe_{AM} and Fe_{PM} ($p = 0.514$); further, no differences were evident in any variable of the typical daily dietary intakes between Fe_{AM} and Fe_{PM} (all $p > 0.05$); finally, no differences were evident in the number of menstrual cycles incurred between Fe_{AM} and Fe_{PM} ($p = 0.826$).

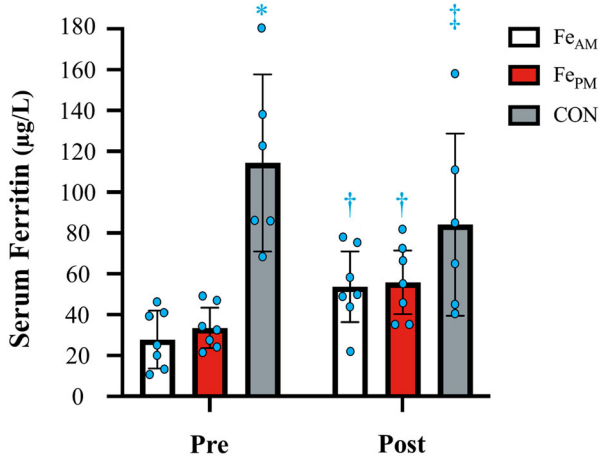

Figure 1. Mean ± SD Serum Ferritin (sFer) of the morning supplement (FeAM), evening supplement (FePM), and no supplement (CON) groups at pre- and post- 8-week intervention. *CON group significantly greater than FeAM and FePM at pre-intervention †Significant within group increase to sFer (pre- to post-intervention) ‡Significant within group decrease to sFer (pre- to post-intervention).

Discussion

In support of our first hypothesis, we showed an increase in sFer over an 8-week iron supplement intervention independent of the time of day that the supplement was consumed. Both the Fe_{AM} and Fe_{PM} groups saw a 1.7-2.1-fold increase in sFer levels from baseline, which corroborates findings from previous studies in athletic populations that describe a 33–127% increase in sFer following 6–12 weeks of supplementation with ~100–200 mg of elemental iron administered daily (Fogelholm et al., 1992; Friedmann et al., 2001; Hinton et al., 2000; Klingshirn et al., 1992; Zhu & Haas, 1998). Accordingly, we provide further evidence to show the efficacy of daily oral iron supplementation in athlete populations with sub-optimal iron levels (defined as sFer <50 µg/L) (McCormick et al., 2020). Interestingly, participants in the CON trial of this investigation reported a significant decline in sFer levels over time, likely reflecting the negative impact of consistent high physical training demands over time on the iron stores of elite dancers. Accordingly, our results provide strong rationale for consistent monitoring of iron stores in 'at risk' athlete populations, as per the guidelines recommended by Sim et al. (2019).

In addition to confirming the efficacy of oral iron supplements in highly trained athlete populations, our data show that the time of day a daily iron supplement is consumed *does not* appear to affect the change in sFer. This outcome contradicts our second hypothesis, that total iron absorption, as reflected by an increase in sFer from pre- to post-intervention, would be greater in Fe_{AM} than Fe_{PM}. Our hypothesis was based upon research characterising a 2–6-fold increase in serum hepcidin concentrations between 0600 am to 1500 pm on the same day (Kemna et al., 2007), a response supported by numerous other studies in the body of literature (Busbridge et al., 2009; Ganz et al., 2008; Kroot et al., 2009; Schaap et al., 2013). Accordingly, it was expected that iron absorption from afternoon supplementation would be inhibited because of hepcidin-related decreases in gastrointestinal iron uptake. However, we saw that chronic iron supplementation, indiscriminate of time of day consumed, brought about comparable increases in sFer levels.

One possible explanation for this outcome may relate to the homeostatic mechanisms driving iron regulation,

Table 1. Mean ± SD weekly training volume and intensity (captured as a weekly training impulse score [TRIMP]), typical daily energy, carbohydrate, protein, fat, and iron intake (from a 3-day food record provided in week 4 of the intervention), and number of menstrual cycles over the 8-week intervention period for the morning supplement (Fe_{AM}), evening supplement (Fe_{PM}), and no supplement (CON) groups.

			Dietary Analysis (Daily Intake)					
		Weekly Training (Av. Weekly TRIMP; AU)	Energy Intake (kJ)	Carbohydrate (g)	Protein (g)	Fat (g)	Dietary Iron (mg)	Number of Menstrual Cycles
FeAM	*mean*	4978	7566.0	209.2	69.3	71.4	8.9	1.8
	±SD	3575	1931.0	50.8	25.8	24.1	1.9	0.8
FePM	*mean*	3774	6798.8	176.8	68.6	67.1	8.5	1.7
	±SD	3116	2147.8	54.3	19.8	29.0	3.5	0.5
**CON	*mean*	4734	5591.3	147.3	57.9	53.0	10.9	1.3
	±SD	3227	1925.0	70.6	7.0	13.6	3.6	0.5

****Note:** Only four of the six CON group participants provided consistent and reliable diary entries over the 8-week period. As a result, the CON data has only been provided as descriptive indicative outcomes from n = 4 CON participants, and was not included in the analysis.

since it is well established that hepcidin release is dictated by iron levels in the body in order to maintain appropriate iron availability (Nicolas et al., 2002). Accordingly, in response to low iron stores, or when iron demand is higher (due to anaemia or hypoxia), hepcidin release is supressed to promote an increase in iron absorption, and therefore, maintain an adequate iron supply for essential physiological processes (Galetti et al., 2021; Nicolas et al., 2002). Recent findings suggest that when sFer is <50 µg/L, hepcidin levels become supressed in order for the body to increase iron absorption to meet the iron demand (Galetti et al., 2021). Accordingly, it is possible that the magnitude of the diurnal increase of hepcidin in Fe_{AM} and Fe_{PM} groups of the current study may have been reduced given the pre-intervention iron status categorising our dancers in the 'sub-optimal/iron deplete' range. However, diurnal hepcidin levels were not measured here (a result of both logistical and financial limitations), and therefore, further work is required to confirm this mechanism of effect on the efficacy of iron supplementation at different times of the day in iron compromised individuals.

Recently, McCormick and colleagues established an acute window of greater iron absorption, whereby the uptake of a stable iron isotope was greatest in the morning (30-min post exercise), as compared to iron consumed in the afternoon (McCormick et al., 2019). These findings prompted us to explore the impact of a long-term comparison of the timing of iron administration, specifically in the form of oral supplements provided in the morning and afternoon. Accordingly, our findings, considered in conjunction with those of McCormick and colleagues, suggest that regular iron supplementation consumed over the long-term, regardless of the time of day consumed, will assist in improving iron levels, even if the critical daily window of increased iron absorption is missed. Again, this may reflect the potential for supressed hepcidin levels at any time of the day in the iron compromised group; however, these outcomes may also aid in practitioners' recommendations to athletes, allowing some confidence in the fact that if trying to correct an iron deficiency, simply getting the iron into the system is of prime concern, and timing of intake can likely come down to 'when it is convenient' for the athlete. Nonetheless, the outcomes of McCormick and colleagues do present a potential window of greater iron absorption (i.e. 30 min post-morning exercise), which was not specifically targeted here, and therefore, there may be an argument for absorption optimisation that might *maximise* sFer increases if a periodised and specifically timed supplement approach was taken. As such, further research is required to specifically target such a periodised supplementation approach.

Beyond the impact of morning versus evening supplementation protocols on sFer; we also considered the overall nutrient intake of the dancers studied here. Interestingly, the average daily energy intake of our entire cohort, as determined from the 3-day food diary, was 6785 ± 2043 kJ/day, with an estimated dietary iron intake of 9.2 ± 3.0 mg/day (50% of the female RDA). As it was not the aim of this study to quantify energy balance or energy availability, we cannot specifically determine if the energy intake values were adequate for our dancer cohort; however, these average daily energy intake values are slightly lower than the typical daily energy intake reported in prior studies of elite dancers, which corresponded with a state of negative energy balance (Brown et al., 2017; Kim et al., 2019) or low energy availability (Civil et al., 2019). This suggests that the energy intake of our cohort was potentially insufficient to support activity demands and/or promote optimal physiological functioning. Of note, low energy intake increases the risk of inadequate iron intake (Young et al., 2018), of which, the negative consequences are likely reflected in the CON group where we report a significant decline in sFer levels in the absence of a supplement to mitigate the risk of an associated low iron intake.

Of course, the present study is not without limitation. Firstly, we were limited (financially and logistically) in our ability to assess a variety of inflammatory and iron status markers (i.e. cytokines, hepcidin, transferrin, Hb, etc.) on a more regular basis; such data may have provided additional validation of our results. Further, sFer is an acute phase reactant, which in athletic populations, can be elevated in the presence of inflammatory stimuli such as training. However, as mentioned in our methods, we attempted to standardise our blood collection protocols according to established frameworks for athlete blood screening of iron (Sim et al., 2019), and therefore, followed best practice guidelines to minimise any spurious fluctuations in sFer. With respect to training volume, energy intake, and dietary iron content, we are bound by the limitations of self-reported training load metrics (Saw et al., 2017) and 3-day food diaries (Larson-Meyer et al., 2018); therefore, the potential implications of high training loads, low energy intake, and low dietary iron availability cannot be fully elucidated. Finally, we were only able to ask our female dancers a binary question in their daily diary regarding if they had menstruated or not. Accordingly, we are not able to fully elucidate the duration or severity of menstrual blood loss from our current approach, rendering our ability to interpret the impact here of the menstrual cycle ineffective. With this in mind, research that examines the implications of the menstrual cycle on iron metabolism is clearly an area of need in future work.

Conclusion

The current study indicates that oral iron supplementation in either the morning or evening appears equally effective in increasing sFer levels in dancers with suboptimal iron status over an 8-week period. From an optimisation perspective, current literature would suggest supplementing in the morning, soon after exercise cessation, appears most effective in promoting acute dietary iron absorption (McCormick et al., 2019). However, in the event that routine morning supplementation is not achievable, the practical benefit to the current work shows that supplementing whenever possible can make up for this missed window of opportunity over the longer term. Finally, given the negative impact on iron stores reported in the CON group of our dancer cohort, we recommend that elite dancers work with a trained sports dietician to develop an individualised approach to optimise their iron intake, and that regular screening (i.e. 2–3 times annually) occur when training volumes are high.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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