Title

Iron regulation in athletes: exploring the menstrual cycle and effects of different exercise modalities on hepcidin production.

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Abstract

The trace element iron plays a number of crucial physiological roles within the body. Despite its importance, iron deficiency remains a common problem amongst athletes. As an individual's iron stores become depleted, this can affect their well-being and athletic capacity. Recently, altered iron metabolism in athletes has been attributed to post-exercise increases in the iron regulatory hormone hepcidin; which has been reported to be up-regulated by exercise induced increases in the inflammatory cytokine interleukin-6 and hemolysis. As such, when hepcidin levels are elevated, iron absorption and recycling may be compromised. To date however, most studies have explored the acute post-exercise hepcidin response, with limited research seeking to minimise/attenuate these increases. This review summarises the current knowledge regarding the post-exercise hepcidin response under a variety of exercise scenarios, and highlights potential areas for future research such as: a) the use of hormones though the female oral contraceptive pill to manipulate the post-exercise hepcidin response, b) comparing the use of different exercise modes (e.g. cycling vs. running) on hepcidin regulation.

Keywords

Iron deficiency, estrogen, progesterone, female athletes, running, cycling

Background

Iron is an element that plays a number of critical physiological roles within the body, such as oxygen (O_2) transport and energy production (Lukaski, 2004), facilitated by the incorporation of iron into proteins and enzymes such as hemoglobin (Hb), myoglobin (Mb) and cytochrome-c (Dallman, 1986). Since iron cannot be produced within the body, adequate dietary iron is essential in maintaining healthy iron stores. To this end, the recommended daily dietary intake for males is 8 mg, increasing up to 18 mg in pre-menopausal females (Food and Nutrition Board, 2001). Such gender differences are likely associated with increased iron loss through menses (Harvey et al., 2005), possibly explaining why females are 5-7 times more likely to experience iron deficiency (ID) compared to males (DellaValle & Haas, 2011). Additionally, in more severe cases, ID may present as iron deficiency anemia (IDA), characterised by compromised iron stores that reduce Hb production. Although poor dietary intake remains the main cause of ID, exercise training can alter iron metabolism acutely (Newlin et al., 2012, Peeling et al., 2009a, 2009b, 2009c, Sim et al., 2012, 2013), potentially compromising iron status over an extended training period (McClung et al., 2009a, 2009b).

During exercise, iron loss is prevalent and occurs via several mechanisms, including hemolysis, hematuria, sweating and gastrointestinal bleeding (for reviews, see Peeling, Dawson, Goodman, Landers, & Trinder, 2008). Although acute iron losses during exercise may be minimal, the accumulation of iron losses over the course of an extended training program may affect the iron status of athletes. Additionally, the discovery of the iron regulatory hormone hepcidin and its involvement in iron metabolism may also help explain the high incidence of ID in active individuals.

Hepcidin

The initial connection between hepcidin and iron metabolism was noted by Pigeon et al., (2001) in studies investigating hepatic responses to iron overload. They found hepcidin was predominantly expressed by hepatocytes and regulated by iron and inflammatory stimuli. Since then, hepcidin has been shown to be crucial in the homeostatic regulation of iron metabolism in two main ways; a) dietary iron absorption in the intestine and b) recycling of iron by macrophages. Nemeth et al. (2004a) demonstrated that hepcidin up-regulation internalises and degrades the iron export protein ferroportin (Fpn), that is highly expressed in the intestine and macrophages; thereby limiting iron absorption and the release of iron from senescent erythrocytes by macrophages (Ganz, 2003). To date, several factors have been identified to regulate hepcidin production, including iron, hypoxia, anemia (Nicholas 2002) and inflammation (Nemeth et al., 2004a). Interestingly, these conditions are commonly associated with physical activity, with interleukin-6 (IL-6) specifically demonstrated to be the main regulator of exercise related increases in hepcidin levels (Banzet et al., 2012).

Interleukin-6 and Hepcidin

Hepcidin has been shown to be up-regulated by inflammation due to increases in IL-6 levels (Nemeth et al., 2004b); with this relationship documented in both clinical (Kemna, Pickkers, Nemeth, van der Hoeven, & Swinkels, 2005, Nemeth et al., 2004a) and exercise-based settings (Banzet et al., 2012, Newlin et al., 2012, Peeling et al. 2009a, 2009b, 2009c, Sim et al., 2012, 2013). Initially, Nemeth et al. (2004a) examined the effect of the inflammatory stimulus in healthy humans infused with recombinant human IL-6 for three hours at a rate of 30 $\mu g.h^{-1}$, which resulted in a 7.5 fold increase in urinary hepcidin concentration. Two hours after IL-6 infusion had ceased, when hepcidin excretion was at its highest, serum iron and transferrin saturation were decreased by 34 and 33% respectively, as compared to pre-

infusion levels. However, in the same investigation, when IL-6 knockout mice were injected with a turpentine solution (an inflammatory stimulus), hepcidin mRNA was suppressed. The authors suggested that the attenuation of hepcidin mRNA in the absence of IL-6 was possibly due to suppressive effects on hepcidin by other inflammatory cytokines. These findings were further substantiated by Kemna et al. (2005), where inflammatory cytokines, urinary hepcidin and serum iron levels were investigated in 10 healthy individuals after injection with lipopolysaccharide (LPS; an inflammatory stimuli). The results indicated that serum IL-6 was dramatically increased within 3 h of LPS infusion, and that urinary hepcidin levels peaked after 6 h (3 h subsequent to the peak in IL-6), accompanied by a decrease in serum iron levels.

In a separate study (Nemeth et al., 2004b), urinary hepcidin was assessed in patients with anemia of inflammation due to severe infection. Here, as much as a 100-fold increase in hepcidin excretion was observed, while smaller increases were seen in patients with less severe forms of inflammatory disease. Similar observations were noted by Nicolas et al. (2002), who reported that turpentine injections into mice induced a 4-fold increase in hepcidin mRNA, and also a 2-fold decrease in serum iron. Recent work by Hashizume, Uchiyama, Horai, Tomosugi, & Mihara (2010) also demonstrated that an anti-IL-6 receptor antibody (Tocilizumab) injected into anemic monkeys (once a week over four weeks) improved iron status. These findings were attributed to a blockade of IL-6 signalling, which induced a rapid and transient decrease in hepcidin, potentially improving iron metabolism. Therefore, current literature suggests that IL-6 is the primary inflammatory mediator of the rise in hepcidin levels in a clinical setting. However, whether this model is applicable to an exercise-based scenario in humans remains unclear.

Exercise and Interleukin-6

During exercise, numerous signalling molecules known as cytokines are produced. One of the cytokines commonly measured in the assessment of inflammation is IL-6 (Villarino, Hunter, & Huang, 2004), which is a key cytokine in the acute-phase response (Ostrowski, Schjerling & Pedersen, 2000), and is acknowledged as an important inflammatory marker (Wallberg, Mattsson, Enquist & Ekblom, 2011). During exercise, IL-6 may be produced from a variety of sources (e.g. adipose tissue and leukocytes), with the greatest amount derived from exercising skeletal muscle (Keller et al., 2001). Numerous investigations have reported that exercise exponentially increases IL-6 production, with peak levels attained immediately post-exercise (Nieman et al., 1998; Ostrowski et al. 2000). Nevertheless, the post-exercise IL-6 levels reported in the literature vary greatly, ranging from a two-fold increase after a 10 km run at 75% of peak oxygen uptake velocity (vVO_{2peak}) (Peeling et al., 2009c) to a 100-fold increase after a marathon running race (42.2 km) (Ostrowski et al., 2000). These differences have been attributed to factors such as exercise duration (Ostrowski, Rohde, Zacho, Asp & Pedersen, 1998; Wallberg et al., 2011) or intensity (Ostrowski et al., 2000; Helge et al., 2003). For example, Ostrowski et al. (2000) combined data from three marathon running races (n=52, Copenhagen Marathon 1997, 1997, 1998), reporting a negative correlation between peak IL-6 concentration and run time ($r=-0.3$, $p<0.05$) and a positive correlation between peak IL-6 concentration and running intensity ($r=0.32$, $p<0.05$). To this end, the rise in post-exercise IL-6 levels has been linked to subsequent hepcidin production.

Acute Post-Exercise Response on Hepcidin

Within the last six years, numerous investigations have examined the relationship between exercise and hepcidin production. Currently, it is generally accepted that exercise-induced

increases to IL-6 and hemolysis levels are likely responsible for the subsequent peak in hepcidin levels at 3 h post-exercise (Peeling et al., 2009a, b, c, Sim et al., 2012, 2013).

Exercise induced hemolysis is typically represented by an immediate post-exercise increase in free Hb (with a corresponding decrease in serum haptoglobin [Hp]), and an increase in serum iron levels (Buchman et al., 1998) as a result of the erythrocyte destruction. Previously, the hemolytic effects of exercise have been associated with an increase in hepcidin production (Peeling et al., 2009b, c). This considered, Telford et al. (2003) previously had 10 well-trained male triathletes perform 1 h of running or cycling at 75% VO_{2peak}. Immediately post-exercise, the common markers of a hemolytic episode were altered, with free Hb being significantly higher (400%) and serum Hp significantly lower after running when compared to cycling. It was proposed that foot-strike during running was responsible for these exercise induced changes in hemolytic markers. When related specifically to iron metabolism, a reduction in hemolysis may reduce the amount of iron retained in macrophages due to changes in the hepcidin dependent regulation of Fpn. However, as previously mentioned, it is likely that any form of exercise induced hemolysis experienced commonly occurs with a corresponding increase in IL-6.

Taking this into consideration, Peeling et al. (2009b) set out to determine how training surface and intensity affected IL-6, hemolysis and hepcidin expression. These authors used an interval-based running protocol on grass (10 x 1 km interval running on grass at 90-95% vVO_{2peak} with a work-rest ratio of 2:1) and continuous running protocols on both grass and bitumen road surfaces (10 km continuous run at 70-80% vVO_{2peak}). Their results showed that irrespective of the exercise surface and intensity, hepcidin levels were significantly increased 3 h subsequent to the peak in IL-6 expression in all trials. It was concluded that any running-

based exercise resulted in an increase in hemolysis and IL-6, as well as hepcidin production. Additionally, these results showed that a greater running intensity (in the interval running trials) incurred more hemolysis and inflammation, but did not further influence the acute increases in hepcidin expression, serum iron or ferritin status. As such, the authors proposed that post-exercise increases in hepcidin levels may compromise both iron absorption and recycling, thereby negatively affecting iron metabolism in the subsequent recovery period. Although numerous studies have highlighted an association between elevated post-exercise IL-6 and hepcidin, only Banzet et al. (2012) was able to conclusively demonstrate this.

Banzet et al. (2012) demonstrated an essential role of IL-6 in hepcidin production in an exercise-based scenario. Using a rodent model of exhaustive running exercise in combination with cyclosporine A (CsA: a calcineurin inhibitor that blunts IL-6 during exercise) administration, they reported that hepcidin mRNA was significantly blunted in the CsA treated rats. Despite increases recorded in the post-exercise hepcidin response, a number of other investigations have reported that some of their female participants did not show any significant increase in hepcidin levels (Roecker, Meier-Buttermilch, Brechtel, Nemeth, & Ganz, 2005), even with elevated post-exercise IL-6 levels (Peeling et al. 2009a).

Hepcidin, Exercise and the Female Athlete

Two investigations (Peeling et al., 2009a, Roecker et al., 2005) have previously reported that a subset of their female athletes were hepcidin 'non-responders'. Initially, Roecker et al. (2005) had 14 well-trained female endurance runners perform a marathon race (42.2 km). They measured urinary hepcidin levels before the race, immediately after and then 24 and 72 h post-race. Hepcidin levels were significantly elevated 24 h post-race, but had returned to baseline by 72 h of recovery. Most importantly, they reported that only eight of the 14

participants demonstrated hepcidin increases, leading to the remaining six being classed as 'non-responders'. However, neither iron status nor inflammatory markers (e.g. IL-6) were measured to substantiate these findings. Subsequently, Peeling et al. (2009a) had 11 well-trained individuals (six male and five female) perform a 60 min run (15-min warm-up at 75–80% of peak heart rate (HR_{peak}) + 45 min at 85–90% HR_{peak}). Most importantly, serum iron and the inflammatory marker IL-6 were elevated immediately post-run, potentially explaining elevated hepcidin levels 3 h and up to 24 h post-run. Again, three female participants were found here to be hepcidin 'non-responders', but it was also evident that these athletes had low iron stores. It was postulated that the pre-existing low iron status (serum ferritin < 35 $ug.L^{-1}$) of these athletes may have prevented hepcidin up-regulation, potentially allowing increased iron absorption by the intestine and recycling by the macrophages (Nicolas et al., 2002) during a time of increased iron requirement. Although such a protective mechanism may exist for individuals with already compromised iron stores, the causes of ID amongst athletes must be further explored to determine the most appropriate methods (e.g. dietary and/or training) to prevent individuals currently with 'borderline' iron status from slipping into a state of ID.

Recently, Newlin et al. (2012) had 12 well-trained female runners perform a 60 and 120 min run at 65% of maximal oxygen uptake (VO_{2max}) on two separate occasions. To control for fluctuating hormone levels throughout the menstrual cycle, these sessions were conducted approximately four weeks apart, and occurred 7–10 days after the onset of menses (follicular phase). Here, both IL-6 and hepcidin were significantly elevated immediately and 3 h post-exercise in both run trials. Hepcidin levels were also approximately 200% higher after the 120 min as compared to the 60 min trial, leading the authors to conclude that exercise duration plays a large role in determining the post-exercise hepcidin response. As hormonal

fluctuations occur throughout the menstrual cycle, this may also play a role in regulating IL-6 and/or hepcidin production. To this end, the post-exercise hepcidin response may be different during the different phases of the menstrual cycle.

Menstrual Cycle

The average menstrual cycle for an adult female consists of 28 days, and is characterised by fluctuating levels of hormones such as estrogen and progesterone. The menstrual cycle can be divided into a number of phases, including:

1) *Menstrual phase*: typified by a discharge of menstrual fluid (~Day 1-5)
2) *Follicular phase*: increasing levels of estrogen are produced by the growing follicle until ovulation (~Day 6-14).
3) *Ovulatory Phase*: During this 24-36 h period at the end of the follicular phase, high levels of luteinising (LH) and follicle stimulating hormone (FSH) causes the oocyte to be released from the ovarian follicles into the oviduct.
4) *Luteal Phase*: Without the ovum, the remaining follicle then forms the corpus luteum that secretes high and moderate levels of progesterone and estrogen, respectively (Day 19-26). (Saladin & Miller, 2004)

Typically, peak estrogen and progesterone levels are observed towards the end of the follicular (Day 12-14) and luteal phase (Day 19-26) respectively, and are lowest during the menstrual phase (Day 1 to 5). Nevertheless, in females that are currently using a hormonal oral contraceptive pill (OCP), these responses will be altered.

Oral Contraceptive Cycle

The use of an OCP is a prevalent practice amongst young women, especially within the athletic population. In the United States, approximately 80% of women have taken an OCP at

some point during their reproductive years, in addition to the estimated 60 million users' worldwide (Oakley, Sereika, & Bogue, 1991). Specifically, in the early 1980's only 5-12% of female athletes were using an OCP (Prior & Vigna, 1985); however, since the late 1990's, up to 47% of female team sport athletes have been reported to have adopted this practice (Brynhildsen et al., 1997). Possible explanations for such a response may be due to its ease of administration, increased awareness and most importantly, greater control in relation to the timing of menses, especially during athletic competition.

The OCP comes in a variety of formulations that contain various concentrations of synthetic ethinyl estradiol and progestogen. Currently, the OCP can be divided into two main groups: monophasic (MOC) and multi-phasic (MPOC) oral contraceptives. However, the method by which these different OCP regimes function are similar, as the exogenous hormones (progestogen and ethinyl estradiol in both MOC and MPOC) act by attenuating endogenous progesterone and estrogen production. In general, both forms of oral contraceptive consist of a 28-day regimen, where an active pill is taken for 21, 24 or 26 days, followed by placebo (sugar) pills; it is thought that by shortening the hormone free interval, this may reduce the incidence of hormone withdrawal symptoms.

The MOC are manufactured such that each active tablet contains the same dose of ethinyl estradiol and progestogen. The most common range being 30-35 µg of ethinyl estradiol, with the amount and type of progestogen (e.g. 0.1-0.25 mg of levonorgestrel or 0.25 mg norgestimate) varying based on the specific OCP formulation used. As such, the MOC ensures a constant dose of estradiol and progestogen to its users during the active pill phase (Figure 1). Further, 20 µg of ethinyl estradiol is considered a low dosage, while 50 µg is a high dose. Differences in dosages are linked to potential side effects that have been reported

at higher doses. In comparison to the fixed hormone doses in MOC, the amount of progestogen or both estradiol and progestogen vary throughout the cycle for a MPOC regime. A comprehensive summary of the commonly used OCP formulations was presented by Burrows and Peter (2007).

Oral Contraceptive Pill and Regulation of Iron Metabolism

Despite the widespread use of OCP amongst female athletes, most research has chosen to compare iron parameters and/or exercise performance in OCP users against non-users. Although numerous studies have set out to determine the effect of the OCP on exercise performance, any potential ergogenic (or ergolytic) effects of the OCP on exercise performance remain unclear. Nevertheless, the OCP has been reported to improve iron storage levels, which could be related to reduced menstrual blood loss (MBL) (Larsson, Milsom, Lindstedt, & Rybo, 1992), or possibly to the suppression of hepcidin via estradiol (Yang, Jian, Katz, Abramson, & Huang, 2012). Previously, Milman, Kirchhoff, and Jorgensen (1992) studied iron parameters in 809 Danish pre-menopausal women, of which approximately 73% were using (or had previously used) some form of hormonal contraceptive. Interestingly, this sub-group of women had significantly higher ferritin levels than those who had never used hormonal contraception. In addition, current and former pill users were found to be less likely to have low ferritin values (<15 $\mu g.L^{-1}$), with ferritin levels increasing in association to the number of years that the pill was used. Likewise, Larsson et al. (1992) examined ferritin levels in women that started taking the OCP, and the effect on MBL. After six months of OCP use, MBL had decreased by approximately 50% and ferritin levels were significantly improved in 10% of the women who had poor ferritin levels prior to starting OCP use. Similarly, Frassinelli-Gunderson, Margen, and Brown (1985) compared iron parameters in OCP users and non-users, finding that OCP users had significantly higher

serum ferritin, iron and total iron binding capacity. Furthermore, during the natural menstrual cycle, fluctuations in iron parameters have been commonly recorded. For example, transferrin saturation and serum ferritin have been reported to be significantly lower during menses, and highest in the luteal phase of the menstrual cycle (Kim, Yetley, & Calvo, 1993). As such, the increase in iron stores associated with reduced MBL in an OCP regulated cycle may prevent any transient decline in iron parameters, potentially improving iron metabolism throughout each cycle. Although the reduction of MBL has been linked to improved iron status, the mechanisms behind such findings may be linked to hormonal fluctuations observed in the oral contraceptive cycle.

Hormonal Influence on Intereukin-6 and Hepcidin

Estrogen

As previously mentioned, estrogen plays a vital role in the regulation of the menstrual cycle. However, when related specifically to the oral contraceptive cycle, endogenous estrogen levels are attenuated by exogenous ethinyl estradiol supplementation. The first study to demonstrate a relationship between estradiol and hepcidin was reported in fish (Robertson, Iwanowicz, & Marranca, 2009). In this investigation, pond-raised largemouth bass were injected with 17-β estradiol or with corn oil (control), resulting in the estradiol treated group showing significantly reduced hepcidin levels. Although IL-6 was not measured, it was suggested that hepcidin down regulation may be linked to IL-6, since estrogen and bisphenol-A (an estrogen mimic) can attenuate IL-6 production (Sugita-Konishi et al., 2003; Pottratz Bellido, Mocharla, Crabb, & Manolagas, 1993), and as previously discussed, IL-6 is a key regulator of hepcidin synthesis. Also it has been reported that estradiol treatment suppressed hepcidin transcription directly by binding to an estrogen responsive element in the hepcidin gene promoter (Hou et al. 2012; Yang et al, 2012). The authors suggested that hepcidin

inhibition by estradiol may serve as a protective mechanism to increase iron uptake to compensate for iron losses that occur during menses. To this end, recent work would suggest a link between estradiol and hepcidin production that may possibly involve IL-6.

Progesterone

Similar to how estraiol attenuates estrogen production in the oral contraceptive cycle, progestogen performs the same action on progesterone. Angstwurm, Gartner, and Ziegler-Heitbrock (1997) reported that in healthy pre-menopausal women that were not taking oral contraceptive, the low progesterone levels recorded during the follicular phase were accompanied by high IL-6 levels. However, after ovulation when progesterone levels had increased (by 1000%), they observed a significant reduction in IL-6 levels. Conversely, Jilma et al. (1997) reported that IL-6 levels remained unchanged during the three different phases of the menstrual cycle. Similarities may also be observed in the oral contraceptive cycle; however this may be complicated by the cocktail of estradiol and progestogen found in an OCP, making it hard to attribute the effects of each specific hormone on IL-6. Salkeld, MacAulay, Ball, & Cannon (2001), had women take an ethinyl estradiol (20-40 µg) and progestogen (six structurally different formulations ranging from 0.05-1.0 mg) containing OCP on days 1 through 21, which constituted the quasi-luteal (QL) phase; after which, they then took either placebos or no pills for days 22–28, which constituted the quasi-follicular phase (QF). Blood samples were obtained between 0700-1100 h, once at the end of the QF phase (between days 26 and 28), and once during mid-QL phase (between days 11 and 14). Results revealed that IL-6 levels were not significantly different between QF and QL. Although these studies report that progestogen and estradiol had no effect on basal IL-6 levels, it is possible that the human body may possess an inherent ‘lower-limit’ of IL-6 levels, and the effects of exogenous estradiol and/or progestogen (from the OCP) may only alter IL-

6 production during times of abnormal cytokine production, such as inflammation or exercise. Future studies should explore the interaction between the post-exercise hepcidin response during different phases in both the oral contraceptive (placebo vs. active pill) and menstrual cycle (menstrual vs. follicular vs. luteal phase) to determine its impact on iron metabolism. Such findings will help determine if extraneous hormones in an oral contraceptive cycle may benefit iron metabolism.

Exercise Modality and Intensity on Hepcidin Production

A recent review by Peeling (2010) compared investigations that explored the interaction of exercise and hepcidin production. However, to date only two investigations have examined the use of a cycling exercise task on hepcidin production. Previously, Troadec et al. (2009) had 14 untrained healthy males (18-40 y) perform two trials comprising; a) 45 min of submaximal cycle exercise at 60% of heart rate reserve (HRR), b) 45 min of seated rest. These sessions were conducted in a randomised cross-over design, with blood samples collected pre-trial, after 30 min and again at 1, 2, 4, 12 and 24 h post-trial. As anticipated, iron parameters (serum iron and ferritin, transferrin) were significantly elevated immediately post-exercise, but contrary to previous running-based studies (Peeling et al., 2009a, 2009b, 2009c, Sim et al., 2012), IL-6 and hepcidin levels remained unchanged in the post-exercise recovery period. It was proposed that these differences may be related to the reduced degree of eccentric muscle contractions of cycling compared to running, which may have failed to increase IL-6 production and the subsequent up-regulation of hepcidin.

This hypothesis may be subject to criticism as it has been proposed that exercise (whether largely eccentric or concentric in nature) will elevate IL-6 levels, with exercise intensity (Ostrowski et al., 2000; Helge et al., 2003) and/or duration (Ostrowski et al., 1998; Wallberg

et al., 2011) playing a greater role in determining the response. In addition, alternate explanations for such findings may be attributed to: a) the low intensity of 60% of HRR and/or duration that the cycle trial was performed at; b) the non-weight bearing nature of cycling that may have reduced the demand (strain) placed on the exercising skeletal muscle (which has been shown to be the main source of IL-6 production during exercise); and c) the non-weight bearing nature of the cycle trial that may have reduced the degree of exercise induced hemolysis. To examine this in greater detail, Sim et al. (2013) had 10 well-trained male triathletes perform four exercise trials; (a) 40 min low intensity continuous run at 65% vVO_{2peak} (L-R); (b) 40 min high intensity interval run session at 85% vVO_{2peak} (H-R); (c) 40 min low intensity continuous cycle at 65% of peak oxygen uptake power (pVO_{2peak}) (L-C); (d) 40 min high intensity interval cycle session at 85% pVO_{2peak} (H-C). Results revealed that regardless of exercise mode or intensity, IL-6 and hepcidin levels were significantly elevated post-exercise and 3 h post-exercise respectively, within each trial. Therefore, regardless of exercise mode or intensity, post-exercise increases in IL-6 may be expected, likely influencing a subsequent elevation in hepcidin. Finally, although the post-exercise hepcidin response has been investigated in running and cycling exercise, the use of other modalities such as rowing and swimming currently remains unknown.

Previously, endurance swimming has been shown to increase hemolysis (Selby & Eichner, 1986). This study examined the post-exercise hemolytic response in 32 swimmers (9 college collegiate and 23 masters swimmers) after completing an endurance swimming event (1.5 to 10 km). Immediately post-swim, the fastest swimmers in the longest events displayed the greatest decrease in serum Hp, indicating a hemolytic episode. Typically, the greatest amount of hemolysis during exercise is associated with foot-strike during running (Telford et al., 2003). However, in the absence of such ground reaction forces, other sources of hemolysis

401 such as oxidative stress and/or muscular compressions on the vasculature may be present (as
402 seen here). In relation to the acute phase response, post-exercise increases in IL-6 have also
403 been reported in swimmers. Previously, Peeling, Fulton, Sim and White (2012) had eight elite
404 swimmers complete 20 x 200 m efforts (mean HR~ 172 bpm), showing that IL-6 was
405 significantly elevated up to 30 min post-exercise as compared to baseline. As both hemolysis
406 and inflammation may be present after swimming, this provides the typical stimulus for a
407 subsequent rise in hepcidin activity. However, such results should be interpreted with
408 caution, since the exercising population, exercise modality, intensity and/or duration could
409 alter the post-exercise IL-6 and hemolytic response (as shown by Troadec et al., 2009),
410 thereby affecting subsequent hepcidin production.
411
412 For example, studies examining how a 2 h rowing session completed at ~82% of heart rate
413 max (HR_{max}) might affect the acute phase response in 15 elite female rowers revealed that no
414 significant increase in IL-6 was observed immediately post-exercise (despite reporting ~ 37%
415 increase) (Henson et al., 2000). On the contrary, Ramson, Jurimae, Jurimae, and Maestu
416 (2008) reported a five-fold increase in IL-6 after completing a 2 h endurance rowing session
417 at ~87% of HR_{max} in eight trained male rowers. Potentially, such differences between studies
418 (irrespective of their comparable intensity), may be linked to the aforementioned hormonal
419 fluctuations in the menstrual cycle that may alter cytokine levels. Additionally, irrespective of
420 rowing being a weight supported activity, it requires a large proportion of upper and lower
421 body muscle mass recruitment; thereby explaining the previously reported rise in hemolysis
422 after a rowing session (Eichner, 1989). As both IL-6 and hemolysis can affect hepcidin
423 production, more work needs to be undertaken to determine if these exercise modalities
424 (rowing and swimming) are likely to influence the typical post-exercise hepcidin response.
425 To this end, future research could also explore the use of a multi-modality cross training

program (e.g. running with swimming recovery) to determine its impact on hepcidin production and subsequent iron metabolism.

Accumulated Effects of Exercise on Iron Status

To date, there have been a number of investigations that have explored how exercise performed over an extended training period might influence iron status in active individuals. McClung et al. (2009a) investigated how a 9 week basic military combat training (BCT) program affected iron status in female soldiers (n=94). The BCT program included both aerobic and muscle strength training, with formalised daily physical training sessions taking place 4–6 d/week, comprising of 1–1.5 h of cardiorespiratory (road marching, distance running and sprinting) and muscle strength (callisthenic exercises, sit-ups and push-ups) training. The authors suggested that this equated to approximately 16,000 steps/d; the equivalent of nearly 12 km.

To assess iron status, blood markers such as Hb concentration, erythrocyte width, serum ferritin, transferrin saturation and soluble transferrin receptor (sTfR) were used. All markers (except for Hb) had significantly diminished after completing the BCT program, demonstrating that the increase in activity levels had significantly reduced iron status in the female soldiers. However, although serum ferritin had decreased, Hb levels increased by approximately 10%, similar to the results of Blum, Sherman, and Boileau (1986), who previously investigated the effect of 6 weeks of aerobic training on pre-menopausal women. To explain these findings, Blum et al. (1986) proposed that increased Hb levels coupled with diminished serum ferritin levels indicated a shift in iron from storage to functional O_2 delivery. In addition, exercise may have stimulated erythrocyte production, resulting in increased Hb levels and mobilisation of iron from ferritin. Interestingly, 3.2 km running time

trial (TT) performance was slower despite the observed increase in Hb levels (McClung et al., 2009a). Together, these findings suggest that iron status may be compromised by an extended block of physical activity, which may be associated with decrements in exercise performance. Fortunately, any decline in iron status during a block of intense physical exercise can be counteracted by an appropriate iron supplementation (Karl et al., 2010, McClung et al., 2009b).

In a follow up study, McClung et al. (2009b) examined 219 female soldiers during an 8 week BCT program. Here, the soldiers were randomly assigned into two groups; one of which received an iron supplement daily (100 mg ferrous sulphate) throughout BCT, while the other received a placebo. Similar to their previous investigation, individuals who had received the placebo displayed signs of compromised iron status; with the erythrocyte distribution width and serum ferritin reduced, and the sTfr levels elevated after training as compared to baseline. In contrast, iron supplementation attenuated any decline in iron status, with post-training serum ferritin levels maintained in the iron supplemented group. Most importantly, individuals who were diagnosed with IDA pre-BCT, and who were placed in the iron supplemented group, reported improved vigour scores on the Profile of Mood States and improved running time in the 3.2 km running TT post-BCT. These results suggest that during periods of heavy training, iron supplementation may be beneficial for individuals who have poor iron status (a combination of serum ferritin <35 $\mu g.L^{-1}$ with Hb <115 $g.L^{-1}$ or transferrin saturation <16%). Similar results were reported by Karl et al. (2010), who found that serum hepcidin levels were unchanged after a comparable 9 week BCT program; however, hepcidin concentrations were lower in IDA soldiers than in those with normal iron status. These responses may be linked to the body's inherent 'protective mechanism' to increase iron absorption and recycling in iron compromised individuals.

476 Recently, Auersperger et al. (2012) also investigated the effect of an extended exercise
477 training program on hepcidin production and iron status in athletes. These authors had 18
478 female runners randomly assigned into either a continuous (CONT) or interval (INT) based 8
479 week training program. This comprised two 3-week overload periods each separated by a
480 week of recovery, and was concluded with a 10 km or 21 km competitive run. Participants in
481 the INT group had four training sessions per week, consisting of two interval runs (one at 88–
482 95% HR_{max} and the second up to 100% HR_{max}), and two distance runs (at 70–87% HR_{max}) of
483 6–8 km and 12–18 km. The CONT group had three training sessions per week consisting of
484 one interval training (*fartlek*, or speed play, at 80–90% HR_{max}) and two distance runs (at 70–
485 87% HR_{max}) similar to those in the INT group. The main finding of this study was that serum
486 hepcidin had decreased, while serum soluble transferrin receptor (sTfR) levels were elevated
487 after the 8 week training period in both groups.
488
489 Although the aim of Auersperger et al. (2012) was to investigate how different running
490 intensities/programs might affect iron regulation over the course of an extended training
491 program, some methodological issues may affect the interpretation of these results. Firstly,
492 blood samples were only obtained at the end of each training block and recovery week to
493 measure serum hepcidin levels. Previously, it has been shown that hepcidin levels are highest
494 3 h post-exercise subsequent to the peak in IL-6 immediately post-exercise (Newlin et al.,
495 2012, Peeling et al., 2009a, 2009b, 2009c, Sim et al., 2012, 2013), before returning to
496 baseline levels by 24 h of recovery (Sim et al., 2012). Therefore, any variations in hepcidin
497 levels reported by Auersperger and colleagues may not have been a direct reflection of any
498 exercise-induced changes. Such an explanation may also account for the findings of Ma et al.
499 (2013), where basal hepcidin levels were not different between females undertaking a high
500 (441.8 min/week) vs. low (51.5 min/week) volume of running exercise. Finally, serum

ferritin in the CONT group at the start of the investigation was only 18.86 ug.L^{-1} (vs 41.67 ug.L^{-1} in the INT group), which suggests that, according to previous criteria (Peeling et al., 2008), these individuals were Stage 1 Iron Deficient (serum ferritin < 35 ug.L^{-1}, Hb>115 g.L^{-1}, transferrin saturation >16%) prior to starting the training program. As reported previously (Peeling et al., 2009a), poor existing iron status may be linked to an attenuated hepcidin response. Therefore, the results of Auersperger et al. (2012) might have been compromised by the contrasting pre-training iron status between the CONT and INT groups.

Subsequently, Auersperger et al. (2013) reported that basal hepcidin levels and iron stores were reduced after an 8-week running program in 14 female runners. These runners were divided into two equal groups based on their existing iron status (SF <20 vs. >20 ug.L^{-1}). However, at the conclusion of the program, the percentage of participants with serum ferritin <20 ug.L^{-1} increased from 50 to 71%. Reductions in basal hepcidin levels suggest that in a small active population of iron compromised females, the body may possess an inherent mechanism that suppresses hepcidin production to minimise the effects of altered iron metabolism. However, such a protective mechanism may only be present once individuals are ID, and it is possible that the initial cause of iron depletion may be a combination of exercise induced losses and excessive hepcidin accumulation over time.

Currently, the literature investigating the effect of exercise performed under a variety of conditions (e.g. different phases of the menstrual cycle, exercise modalities and intensities), and its impact on subsequent hepcidin production still requires investigation. Most importantly, future work should explore the cumulative effect of acute disruptions to iron metabolism (caused by exercise-induced hemolysis and increased hepcidin) on an individual's iron status. This can be achieved by adopting a protocol comprising cumulative

bouts of running and cycling training protocols, in order to determine how this might affect subsequent hepcidin production acutely, and any chronic effect on iron regulation. This may provide greater insight into the effect an extended multi-modality training program might have on IL-6, hemolysis and hepcidin production, and its impact on iron metabolism.

Conclusion

In summary, exercise-induced increases in IL-6 and hemolysis can result in elevated hepcidin levels. These changes may prevent both the release of iron from macrophages as well as a reduction in the absorption of dietary iron in the intestine. Since elevations in hepcidin levels peak approximately 3 h post-exercise, concerns have been raised with regard to how these elevated levels may impose a challenge to and/or negatively affect an athlete's iron stores. As such, future work should explore ways to minimise/attenuate any post-exercise increases in hepcidin production. For example, the interaction between hormones involved in regulating the menstrual cycle and its effect on hepcidin production still remains unclear. Specifically, the use of exogenous estradiol and progestogens via the OCP may attenuate any post-exercise increases in IL-6 and hepcidin production, potentially improving iron status in its users. Lastly, elite athletes who engage in multiple, prolonged training sessions in a single day may be exposed to increased hemolysis, with subsequent elevations in hepcidin levels compromising their iron status over time. Therefore, any acute reductions in hemolysis associated with non-weight bearing exercise such as cycling, rowing or swimming (as compared to running) might be beneficial to the individual only after an extended training period. This may be particularly beneficial to individuals with iron levels that are only slightly above normal levels at the start of a training program, as they might become iron-deficient during their training program if a running-based protocol was adopted. Ultimately, this could result in substantial performance decrements even if optimal training and

nutritional programs are implemented. To this end, potential methods aiming to limit or attenuate exercise induced increases in hepcidin levels should be explored to assist individuals with poor iron status.

Acknowledgements

Debbie Trinder is the recipient of a Senior Research Fellowship from the National Health and Medical Research Council of Australia (APP1020437).

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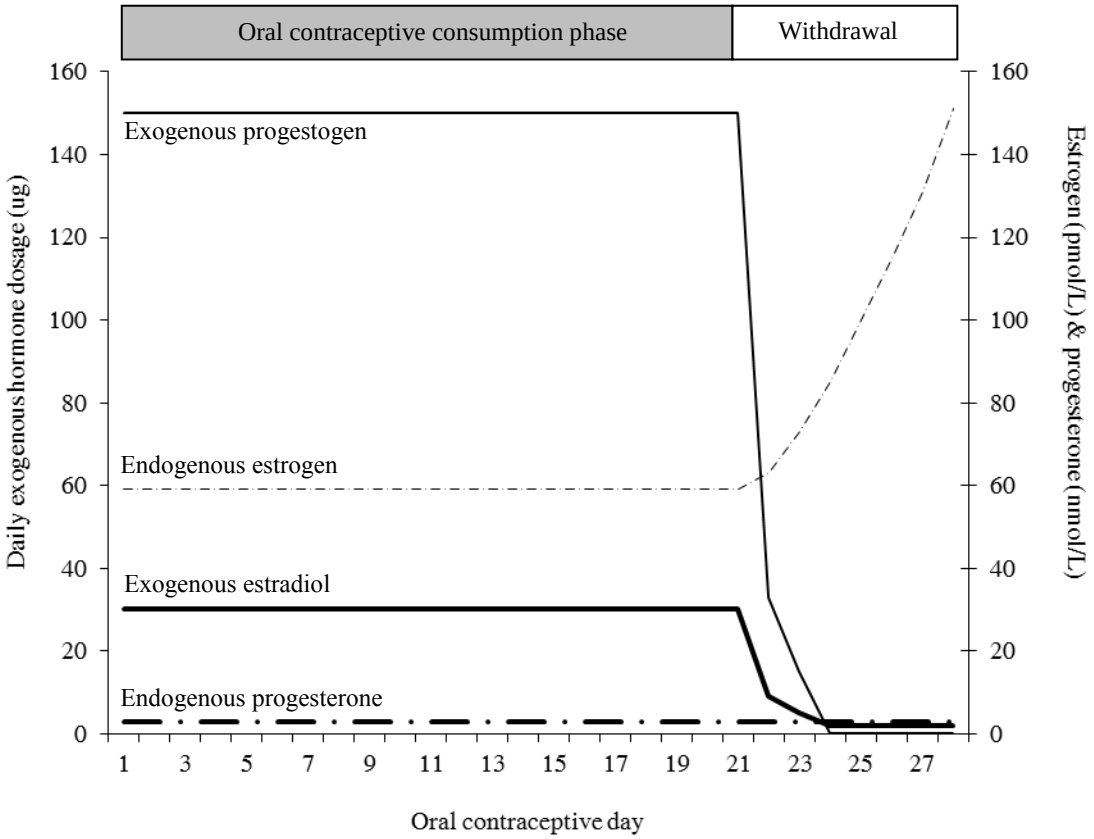

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Figure 1. Endogenous and exogenous hormones levels in a combined monophasic

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oral contraceptive cycle (Adapted from Rechichi, Dawson, & Goodman, 2009).

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