International Journal of Sport Nutrition and Exercise Metabolism, 2014, 24, 177-187
http://dx.doi.org/10.1123/ijsnem.2013-0067
© 2014 Human Kinetics, Inc.

INTERNATIONAL JOURNAL OF
SPORT NUTRITION AND
EXERCISE METABOLISM
www.IJSNEM-Journal.com
ORIGINAL RESEARCH

Iron Regulation in Athletes: Exploring the Menstrual Cycle and Effects of Different Exercise Modalities on Hepcidin Production

Marc Sim, Brian Dawson, Grant Landers, Debbie Trinder, and Peter Peeling

The trace element iron plays a number of crucial physiological roles within the body. Despite its importance, iron deficiency remains a common problem among athletes. As an individual's iron stores become depleted, it can affect their well-being and athletic capacity. Recently, altered iron metabolism in athletes has been attributed to postexercise increases in the iron regulatory hormone hepcidin, which has been reported to be upregulated by exercise-induced increases in the inflammatory cytokine interleukin-6. As such, when hepcidin levels are elevated, iron absorption and recycling may be compromised. To date, however, most studies have explored the acute postexercise hepcidin response, with limited research seeking to minimize/attenuate these increases. This review summarizes the current knowledge regarding the postexercise 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 postexercise 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. Currently, the recommended daily dietary intake for males is 8 mg, increasing up to 18 mg in premenopausal 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 with males (DellaValle & Haas, 2011). In addition, in more severe cases, ID may present as iron deficiency anemia (IDA), characterized 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 et al., 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. In addition, 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. (2004b) demonstrated that hepcidin up-regulation internalizes 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

Sim, Dawson, Landers, and Peeling are with the School of Sport Science, Exercise and Health, and Trinder the School of Medicine and Pharmacology, University of Western Australia, Crawley, Australia. Address author correspondence to Marc Sim at marc.sim@uwa.edu.au.

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., 2004a); with this relationship documented in both clinical (Kemna et al., 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 $\mu g \cdot h^{-1}$, 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 with preinfusion 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 hr of LPS infusion, and that urinary hepcidin levels peaked after 6 hr (3 hr subsequent to the peak in IL-6), accompanied by a decrease in serum iron levels.

In a separate study (Nemeth et al., 2003), 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 a single turpentine injection into mice induced a sixfold increase in liver hepcidin expression and a twofold decrease in serum iron. Recent work by Hashizume et al. (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 signaling, 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 signaling molecules known as cytokines are produced. One of the cytokines commonly measured in the assessment of inflammation is IL-6 (Villarino et al., 2004), which is a key cytokine in the acute-phase response (Ostrowski et al., 2000), and is acknowledged as an important inflammatory marker (Wallberg et al., 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 postexercise IL-6 levels reported in the literature vary greatly, ranging from a twofold increase after a 10 km run at 75% of peak oxygen uptake velocity (VO_{2peak}; Peeling et al., 2009b) 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 et al., 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 1996, 1997, 1998), reporting a negative correlation between peak IL-6 concentration and run time ($r = -0.3$, $p < .05$) and a positive correlation between peak IL-6 concentration and running intensity ($r = .32$, $p < .05$). More recently, the rise in postexercise IL-6 levels has been linked to subsequent hepcidin production.

Acute Postexercise Response on Hepcidin

Within the last 6 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 hr postexercise (Peeling et al., 2009a, 2009b, 2009c; Sim et al., 2012, 2013).

Exercise induced hemolysis is typically represented by an immediate postexercise 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 altered iron metabolism through hepcidin (Peeling et al., 2009b, 2009c). This considered, Telford et al. (2003) previously had 10 well-trained male triathletes perform 1 hr of running or cycling at 75% VO_{2peak}. Immediately postexercise, 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 with cycling. It was proposed that footstrike 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 (10×1 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 hr 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. In addition, 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 postexercise 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 postexercise 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 postexercise 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 et al., 2005), even with elevated postexercise IL-6 levels (Peeling et al. 2009a). Such events raise the possibility that menstrual cycle hormones might play a role in hepcidin regulation.

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 'nonresponders'. 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 hr postrace. Hepcidin levels were significantly elevated 24 hr postrace, but had returned to baseline by 72 hr of recovery. Most importantly, they reported that only eight of the 14 participants demonstrated hepcidin increases, leading to the remaining six being classed as *nonresponders*. 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 postrun, potentially explaining elevated hepcidin levels 3 hr and up to 24 hr postrun. Again, three female participants were found here to be hepcidin 'nonresponders', but it was also evident that these athletes had low iron stores. It was postulated that the preexisting low iron status (serum ferritin $< 35\ \mu g \cdot L^{-1}$) of these athletes may have prevented hepcidin up-regulation, potentially allowing increased iron absorption by the intestine and recycling by the macrophages 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 among 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 hr postexercise in both run trials. Hepcidin levels were also approximately 200% higher after the 120 min as compared with the 60 min trial, leading the authors to conclude that exercise duration plays a large role in determining the postexercise hepcidin response. Since fluctuations occur throughout the menstrual cycle, this may also play a role in regulating IL-6 and/or hepcidin production. To this end, the postexercise 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 characterized 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 hr 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 toward the end of the follicular (Day 12–14) and luteal phase (Day 19–26) respectively, and are lowest during the menstrual phase (Day 1–5). 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 among 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 1980s only 5–12% of female athletes were using an OCP (Prior & Vigna, 1985); however, since the late 1990s, 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 multiphasic (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 with the fixed hormone doses in MOC, the amount of progestogen or both estradiol and progestogen vary throughout the cycle for a MPOC regimen. A comprehensive summary of the commonly used OCP formulations was presented by Burrows and Peter (2007)

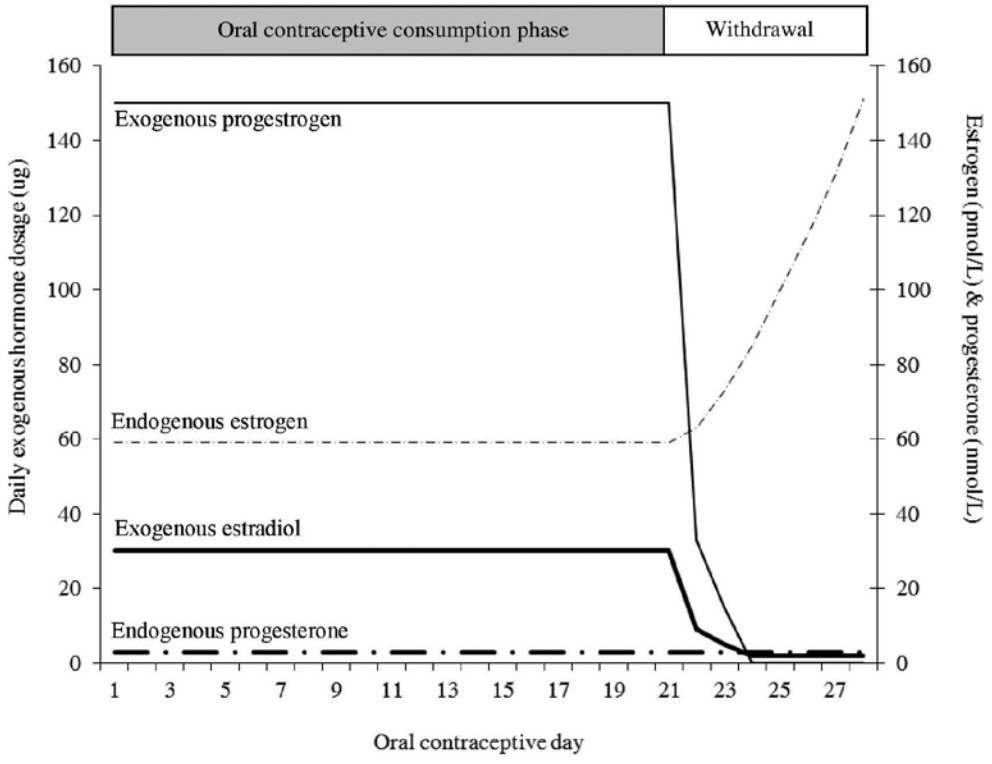

Figure 1 — Endogenous and exogenous hormone levels in a combined monophasic oral contraceptive cycle (adapted from Rechichi et al., 2009).

Oral Contraceptive Pill and Regulation of Iron Metabolism

Despite the widespread use of OCP among female athletes, most research has chosen to compare iron parameters and/or exercise performance in OCP users against nonusers. 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 et al., 1992), or possibly to the suppression of hepcidin via estradiol (Yang, Jian, Katz, Abramson, & Huang, 2012). Previously, Milman et al. (1992) studied iron parameters in 809 Danish premenopausal women, of which approximately 73% were using (or had previously used) some form of hormonal contraceptive. Interestingly, this subgroup 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 ($<15\ \mu g \cdot L^{-1}$), 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 before starting OCP use. Similarly, Frassinelli-Gunderson et al. (1985) compared iron parameters in OCP users and nonusers, 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 et al., 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 et al., 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 et al., 1994, 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 et al. (1997) reported that in healthy premenopausal 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 et al. (2001), had women take an ethinyl estradiol (20–40 μg) and progestogen (six structurally different formulations ranging from 0.05 to 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 hr, 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 postexercise 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 randomized cross-over design, with blood samples collected pretrial, after 30 min and again at 1, 2, 4, 12, and 24 hr post trial. As anticipated, iron parameters (serum iron and ferritin, transferrin) were significantly elevated immediately postexercise, 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 postexercise recovery period. It was proposed that these differences may be related to the reduced degree of eccentric muscle contractions of cycling compared with 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 nonweight 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 nonweight 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 postexercise and 3 hr postexercise respectively, within each trial. Therefore, regardless of exercise mode or intensity, postexercise increases in IL-6 may be expected, likely influencing a subsequent elevation in hepcidin. Finally, although the postexercise 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 postexercise hemolytic response in 32 swimmers (9 college collegiate and 23 master swimmers) after completing an endurance swimming event (1.5–10 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 such as oxidative stress and/or muscular compressions on the vasculature may be present (as seen in Selby & Eichner, 1986). In relation to the acute phase response, postexercise increases in IL-6 have also been reported in swimmers. Previously, Peeling et al. (2012) had eight elite swimmers complete 20 × 200 m efforts (mean HR~172 bpm), showing that IL-6 was significantly elevated up to 30 min postexercise as compared with baseline. Since hemolysis and inflammation may be present after swimming, increases in IL-6 may cause a rise in hepcidin activity, which could subsequently compromise iron recycling by the macrophage. However, such results should be interpreted with caution, since the exercising population, exercise modality, intensity and/or duration could alter the postexercise IL-6 response, thereby affecting subsequent hepcidin production (as shown by Troadec et al., 2009).

For example, studies examining how a 2 hr rowing session completed at ~82% of heart rate max (HR_{max}) might affect the acute phase response in 15 elite female rowers revealed that no significant increase in IL-6 was observed immediately postexercise (despite reporting ~37% increase; Henson et al., 2000). On the contrary, Ramson et al. (2008) reported a fivefold increase in IL-6 after completing a 2-hr endurance rowing session at ~87% of HR_{max} in eight trained male rowers. Potentially, such differences between studies (irrespective of their comparable intensity), may be linked to the aforementioned hormonal fluctuations in the menstrual cycle that may alter cytokine levels. In addition, irrespective of rowing being a weight supported activity, it requires a large proportion of upper and lower body muscle mass recruitment; thereby explaining the previously reported rise in hemolysis after a rowing session (Eichner, 1989). Since IL-6 has been linked to hepcidin increases, while hemolysis might exacerbate iron loss, more work needs to be undertaken to determine if these exercise modalities (rowing and swimming) are likely to influence the typical postexercise hepcidin response. To this end, future research could also explore the use of a multimodality 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. (2009b) 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 formalized daily physical training sessions taking place 4–6 d/week, comprising of 1–1.5 hr 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 et al. (1986), who previously investigated the effect of 6 weeks of aerobic training on premenopausal 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 mobilization 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., 2009b). 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. (2009a) 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 sulfate) 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 serum ferritin reduced, and the sTfr levels elevated after training as compared to baseline. In contrast, iron supplementation limited any decline in iron status, with posttraining 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 vigor 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 $\mu g \cdot L^{-1}$ with Hb <115 $g \cdot L^{-1}$ 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.

Recently, Auersperger et al. (2012) also investigated the effect of an extended exercise training program on hepcidin production and iron status in athletes. These authors had 18 female runners randomly assigned into either a continuous (CONT) or interval (INT) based 8 week training program. This comprised two 3-week overload periods each separated by a week of recovery, and was concluded with a 10 km or 21 km competitive run. Participants in the INT group had four training sessions per week, consisting of two interval runs (one at 88–95% HR_{max} and the second up to 100% HR_{max}), and two distance runs (at 70–87% HR_{max}) of 6–8 km and 12–18 km. The CONT group had three training sessions per week consisting of one interval training (*fartlek,* or speed play, at 80–90% HR_{max}) and two distance runs (at 70–87% HR_{max}) similar to those in the INT group. The main finding of this study was that serum hepcidin had decreased over time, while sTfR levels were elevated after the 8 week training period in both groups.

Although the aim of Auersperger et al. (2012) was to investigate how different running intensities/programs might affect iron regulation over the course of an extended training program, some methodological issues may affect the interpretation of these results. Firstly, blood samples were only obtained at the end of each training block and recovery week to measure serum hepcidin levels. Previously, it has been shown that hepcidin levels are highest 3 hr postexercise subsequent to the peak in IL-6 immediately postexercise (Newlin et al., 2012, Peeling et al., 2009a, 2009b, 2009c, Sim et al., 2012, 2013), before returning to baseline levels by 24 hr of recovery (Sim et al., 2012). Therefore, any variations in hepcidin levels reported by Auersperger and colleagues may not have been a direct reflection of any exercise-induced changes. Such an explanation may also account for the findings of Ma et al. (2013), where basal hepcidin levels were not different between females undertaking a high (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 $\mu g \cdot L^{-1}$, Hb > 115 $g \cdot L^{-1}$, transferrin saturation >16%) before 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 pretraining 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 $\mu g \cdot L^{-1}$). However, at the conclusion of the program, the percentage of participants with serum ferritin <20 $\mu g \cdot L^{-1}$ 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 minimize 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, 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 multimodality training program might have on IL-6, hemolysis and hepcidin production, and its impact on iron metabolism.

Conclusion

In summary, acute exercise-induced increases in IL-6 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 hr 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 minimize/attenuate any postexercise 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 postexercise 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 nonweight bearing exercise such as cycling, rowing or swimming (as compared with 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.

Acknowledgments

Debbie Trinder is the recipient of a senior research fellowship from the National Health and Medical Research Council of Australia (APP1020437).

References

Angstwurm, M.W.A., Gärtner, R., & Ziegler-Heitbrock, H. (1997). Cyclic plasma IL-6 levels during normal menstrual cycle. *Cytokine, 9*(5), 370–374. PubMed doi:10.1006/cyto.1996.0178

Auersperger, I., Knap, B., Jerin, A., Blagus, R., Lainscak, M., Skitek, M., & Skof, B. (2012). The effects of 8 weeks of endurance running on hepcidin concentrations, inflammatory parameters, and iron status in female runners. *International Journal of Sport Nutrition and Exercise Metabolism, 22*(1), 55–63. PubMed

Auersperger, I., Skof, B., Leskosek, B., Knap, B., Jerin, A., & Lainscak, M. (2013). Exercise-induced changes in iron status and hepcidin response in female runners. *PLoS ONE, 8*(3), e58090. PubMed doi:10.1371/journal.pone.0058090

Banzet, S., Sanchez, H., Chapot, R., Bigard, X., Vaulont, S., & Koulmann, N. (2012). Interleukin-6 contributes to hepcidin mRNA increase in response to exercise. *Cytokine, 58*(2), 158–161. PubMed doi:10.1016/j.cyto.2012.01.006

Blum, S.M., Sherman, A.R., & Boileau, R.A. (1986). The effects of fitness-type exercise on iron status in adult women. *The American Journal of Clinical Nutrition, 43*(3), 456–463. PubMed

Brynhildsen, J., Lennartsson, H., Klemetz, M., Dahlquist, P., Hedin, B., & Hammar, M. (1997). Oral contraceptive use among female elite athletes and age-matched controls and its relation to low back pain. *Acta Obstetricia et Gynecologica Scandinavica, 76*(9), 873-878.

Buchman, A.L., Keen, C., Commisso, J., Killip, D., Ou, C-N., Rognerud, C.L., ... Dunn, J.K. (1998). The effect of a marathon run on plasma and urine mineral and metal concentrations. *Journal of the American College of Nutrition*, *17*(2), 124–127.

Burrows, M., & Peters, C. E. (2007). The influence of oral contraceptives on athletic performance in female athletes. *Sports Medicine (Auckland, N.Z.), 37*(7), 557–574.

Dallman, P.R. (1986). Biochemical basis for the manifestations of iron deficiency. *Annual Review of Nutrition, 6*(1), 13–40. PubMed

DellaValle, D.M., & Haas, J.D. (2011). Impact of iron depletion without anemia on performance in trained endurance athletes at the beginning of a training season: a study of female

collegiate rowers. *International Journal of Sport Nutrition and Exercise Metabolism, 21*(6), 501–506. PubMed

Eichner, E.R., Strauss, R., Sherman, W., Dernbach, A., & Lamb, D. (1989). Intravascular haemolysis in elite college rowers. *Medicine and Science in Sports and Exercise, 21*(2), S78.

Food and Nutrition Board. (2001). *Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc* (pp. 290–393). Washington, D.C: National Academy Press.

Frassinelli-Gunderson, E.P., Margen, S., & Brown, J.R. (1985). Iron stores in users of oral contraceptive agents. *The American Journal of Clinical Nutrition, 41*(4), 703–712. PubMed

Ganz, T. (2003). Hepcidin, a key regulator of iron metabolism and mediator of anemia of inflammation. *Blood, 102*(3), 783–788. PubMed doi:10.1182/blood-2003-03-0672

Harvey, L.J., Armah, C.N., Dainty, J.R., Foxall, R.J., John Lewis, D., Langford, N.J., . . . Srinivasan, K. (2005). Impact of menstrual blood loss and diet on iron deficiency among women in the UK. *The British Journal of Nutrition, 94*(4), 557–564. PubMed doi:10.1079/BJN20051493

Hashizume, M., Uchiyama, Y., Horai, N., Tomosugi, N., & Mihara, M. (2010). Tocilizumab, a humanized anti-interleukin-6 receptor antibody, improved anemia in monkey arthritis by suppressing IL-6-induced hepcidin production. *Rheumatology International, 30*(7), 917–923.

Helge, J. W., Stallknecht, B., Pedersen, B. K., Galbo, H., Kiens, B., & Richter, E. A. (2003). The effect of graded exercise on IL-6 release and glucose uptake in human skeletal muscle. *The Journal of Physiology, 546*(1), 299–305.

Henson, D.A., Nieman, D.C., Nehlsen-Cannarella, S.L., Fagoaga, O.R., Shannon, M., Bolton, M.R., . . . Austin, M.D. (2000). Influence of carbohydrate on cytokine and phagocytic responses to 2 h of rowing. *Medicine and Science in Sports and Exercise, 32*(8), 1384–1389. PubMed doi:10.1097/00005768-200008000-00005

Hou, Y., Zhang, S., Wang, L., Li, J., Qu, G., He, J., . . . Liu, S. (2012). Estrogen regulates iron homeostasis through governing hepatic hepcidin expression via an estrogen response element. *Gene, 511*(2), 398–403. PubMed doi:10.1016/j.gene.2012.09.060

Jilma, B., Dirnberger, E., Löscher, I., Rumplmayr, A., Hildebrandt, J., Eichler, H.G., . . . Wagner, O.F. (1997). Menstrual cycle-associated changes in blood levels of interleukin-6, 1a acid glycoprotein, and C-reactive protein. *The Journal of Laboratory and Clinical Medicine, 130*(1), 69–75. PubMed

Karl, J.P., Lieberman, H.R., Cable, S.J., Williams, K.W., Young, A.J., & McClung, J.P. (2010). Randomized, double-blind, placebo-controlled trial of an iron-fortified food product in female soldiers during military training: relations between iron status, serum hepcidin, and inflammation. *The American Journal of Clinical Nutrition, 92*(1), 93–100. PubMed doi:10.3945/ajcn.2010.29185

Keller, C., Steensberg, A., Pilegaard, H., Osada, T., Saltin, B., Pedersen, B.K., & Neufer, P.D. (2001). Transcriptional activation of the IL-6 gene in human contracting skeletal muscle: influence of muscle glycogen content. *FASEB Journal, 15*(14), 2748–2750. PubMed

Kemna, E., Pickkers, P., Nemeth, E., van der Hoeven, H., & Swinkels, D. (2005). Time-course analysis of hepcidin, serum iron, and plasma cytokine levels in humans injected with LPS. *Blood, 106*(5), 1864–1866. PubMed doi:10.1182/blood-2005-03-1159

Kim, I., Yetley, E.A., & Calvo, M.S. (1993). Variations in iron-status measures during the menstrual cycle. *The American Journal of Clinical Nutrition, 58*(5), 705–709. PubMed

Larsson, G., Milsom, L., Lindstedt, G., & Rybo, G. (1992). The influence of a low-dose combined oral contraceptive on menstrual blood loss and iron status. *Contraception, 46*(4), 327–334. PubMed doi:10.1016/0010-7824(92)90095-B

Lukaski, H.C. (2004). Vitamin and mineral status: effects on physical performance. *Nutrition (Burbank, Los Angeles County, Calif.), 20*(7-8), 632–644. PubMed doi:10.1016/j.nut.2004.04.001

Ma, X., Patterson, K.J., Gieschen, K.M., & Bodary, P.F. (2013). Are serum hepcidin levels chronically elevated in collegiate female distance runners? *International Journal of Sport Nutrition and Exercise Metabolism, 23*(5), 513–521..

McClung, J.P., Karl, J.P., Cable, S.J., Williams, K.W., Nindl, B.C., Young, A.J., & Lieberman, H.R. (2009a). Randomized, double-blind, placebo-controlled trial of iron supplementation in female soldiers during military training: effects on iron status, physical performance, and mood. *The American Journal of Clinical Nutrition, 90*(1), 124–131. PubMed doi:10.3945/ajcn.2009.27774

McClung, J.P., Karl, J.P., Cable, S.J., Williams, K.W., Young, A.J., & Lieberman, H.R. (2009b). Longitudinal decrements in iron status during military training in female soldiers. *The British Journal of Nutrition, 102*(4), 605–609. PubMed doi:10.1017/S0007114509220873

Milman, N., Kirchhoff, M., & Jorgensen, T. (1992). Iron status markers, serum ferritin and hemoglobin in 1359 Danish women in relation to menstruation, hormonal contraception, parity, and postmenopausal hormone treatment. *Annals of Hematology, 65*(2), 96–102. PubMed doi:10.1007/BF01698138

Nemeth, E., Rivera, S., Gabayan, V., Keller, C., Taudorf, S., Pedersen, B.K., & Ganz, T. (2004a). IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. *The Journal of Clinical Investigation, 113*(9), 1271–1276. PubMed doi:10.1172/JCI200420945

Nemeth, E., Tuttle, M.S., Powelson, J., Vaughn, M.B., Donovan, A., Ward, D.M., . . . Kaplan, J. (2004b). Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science, 306*(5704), 2090–2093. PubMed doi:10.1126/science.1104742

Newlin, M.K., Williams, S., McNamara, T., Tjalsma, H., Swinkels, D.W., & Haymes, E.M. (2012). The effects of acute exercise bouts on hepcidin in women. *International Journal of Sport Nutrition and Exercise Metabolism, 22*(2), 79–88. PubMed

Nicolas, G., Chauvet, C., Viatte, L., Danan, J. L., Bigard, X., Devaux, I., … Vaulont, S. (2002). The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation. *Journal of Clinical Investigation*, *110*(7), 1037–1044.

Nieman, D.C., Nehlsen-Cannarella, S.L., Fagoaga, O.R., Henson, D.A., Utter, A., Davis, J.M., . . . Butterworth, D.E. (1998). Influence of mode and carbohydrate on the cytokine response to heavy exertion. *Medicine and Science in Sports and Exercise, 30*(5), 671–678. PubMed doi:10.1097/00005768-199805000-00005

Oakley, D., Sereika, S., & Bogue, E.L. (1991). Oral contraceptive pill use after an initial visit to a family planning clinic. *Family Planning Perspectives,23*(4), 150–154. PubMed doi:10.2307/2135737

Ostrowski, K., Rohde, T., Zacho, M., Asp, S., & Pedersen, B. (1998). Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running. *The Journal of Physiology, 508*(Pt 3), 949–953. PubMed doi:10.1111/j.1469-7793.1998.949bp.x

Ostrowski, K., Schjerling, P., & Pedersen, B.K. (2000). Physical activity and plasma interleukin-6 in humans – effect of intensity of exercise. *European Journal of Applied Physiology, 83*(6), 512–515. PubMed doi:10.1007/s004210000312

Peeling, P. (2010). Exercise as a mediator of hepcidin activity in athletes. *European Journal of Applied Physiology, 110*(5), 877–883. PubMed doi:10.1007/s00421-010-1594-4

Peeling, P., Dawson, B., Goodman, C., Landers, G., & Trinder, D. (2008). Athletic induced iron deficiency: new insights into the role of inflammation, cytokines and hormones. *European Journal of Applied Physiology, 103*(4), 381–391. PubMed doi:10.1007/s00421-008-0726-6

Peeling, P., Dawson, B., Goodman, C., Landers, G., Wiegerinck, E., Swinkels, D., & Trinder, D. (2009a). Effects of exercise on hepcidin response and iron metabolism during recovery. *International Journal of Sport Nutrition and Exercise Metabolism, 19*(6), 583–597. PubMed

Peeling, P., Dawson, B., Goodman, C., Landers, G., Wiegerinck, E., Swinkels, D., & Trinder, D. (2009b). Training surface and intensity: inflammation, hemolysis, and hepcidin expression. *Medicine and Science in Sports and Exercise, 41*(5), 1138–1145. PubMed doi:10.1249/MSS.0b013e318192ce58

Peeling, P., Dawson, B., Goodman, C., Landers, G., Wiegerinck, E., Swinkels, D., & Trinder, D. (2009c). Cumulative effects of consecutive running sessions on hemolysis, inflammation and hepcidin activity. *European Journal of Applied Physiology, 106*(1), 51–59. PubMed doi:10.1007/s00421-009-0988-7

Peeling, P., Fulton, S., Sim, M., & White, J. (2012). Recovery effects of hyperoxic gas inhalation or contrast water immersion on the post-exercise cytokine response, perceptual recovery, and next day exercise performance. *Journal of Strength and Conditioning Research, 26*(4), 968–975. PubMed doi:10.1519/JSC.0b013e31822dcc5b

Pigeon, C., Ilyin, G., Courselaud, B., Leroyer, P., Turlin, B., Brissot, P., & Loreal, O. (2001). A new mouse liver-specific gene, encoding a protein homologous to human antimicrobial peptide hepcidin, is overexpressed during iron overload. *The Journal of Biological Chemistry, 276*(11), 7811–7819. PubMed doi:10.1074/jbc.M008923200

Pottratz, S.T., Bellido, T., Mocharla, H., Crabb, D., & Manolagas, S.C. (1994). 17-β Estradiol inhibits expression of human interleukin-6 promoter-reporter constructs by a receptor-dependent mechanism. *The Journal of Clinical Investigation, 93*(3), 944–950. PubMed doi:10.1172/JCI117100

Prior, J.C, & Vigna, Y. (1985). Gonadal steroids in athletic women contraception, complications and performance. *Sports Medicine (Auckland, N.Z.), 2*(4), 287–295. PubMed doi:10.2165/00007256-198502040-00006

Ramson, R., Jurimae, J., Jurimae, T., & Maestu, J. (2008). The influence of increased training volume on cytokines and ghrelin concentration in college level male rowers. *European Journal of Applied Physiology, 104*(5), 839–846. PubMed doi:10.1007/s00421-008-0839-y

Rechichi, C., Dawson, B., & Goodman, C. (2009). Athletic performance and the oral contraceptive. *International Journal of Sports Physiology and Performance, 4,* 151–162. PubMed

Robertson, L.S., Iwanowicz, L.R., & Marranca, J.M. (2009). Identification of centrarchid hepcidins and evidence that 17beta-estradiol disrupts constitutive expression of hepcidin-1 and inducible expression of hepcidin-2 in largemouth bass (Micropterus salmoides). *Fish & Shellfish Immunology, 26*(6), 898–907. PubMed doi:10.1016/j.fsi.2009.03.023

Roecker, L., Meier-Buttermilch, R., Brechtel, L., Nemeth, E., & Ganz, T. (2005). Iron-regulatory protein hepcidin is increased in female athletes after a marathon. *European Journal of Applied Physiology, 95*(5–6), 569–571. PubMed doi:10.1007/s00421-005-0055-y

Saladin, K.S., & Miller, L. (2004). *Anatomy and Physiology: The Unity of Form and Function* (3rd ed.). New York: The McGraw-Hill Companies Inc.

Salkeld, B.D., MacAulay, J.C., Ball, R.W., & Cannon, J.G. (2001). Modulation of body temperature, interleukin-6 and leptin by oral contraceptive use. *Neuroimmunomodulation, 9*(6), 319–325. PubMed doi:10.1159/000059389

Selby, G.B., & Eichner, E.R. (1986). Endurance swimming, intravascular hemolysis, anemia, and iron depletion. New perspective on athlete's anemia. *The American Journal of Medicine, 81*(5), 791–794. PubMed doi:10.1016/0002-9343(86)90347-5

Sim, M., Dawson, B., Landers, G., Wiegerinck, E.T., Swinkels, D.W., Townsend, M.A., . . . Peeling, P. (2012). The effects of carbohydrate ingestion during endurance running on post-exercise inflammation and hepcidin levels. *European Journal of Applied Physiology, 112,* 1889–1898. PubMed doi:10.1007/s00421-011-2156-0

Sim, M., Dawson, B., Landers, G., Swinkels, D.W., Tjalsma, H., Trinder, D., & Peeling, P. (2013). Effect of exercise modality and intensity on post-exercise interleukin-6 and hepcidin levels. *International Journal of Sport Nutrition and Exercise Metabolism, 23,* 178–186. PubMed

Sugita-Konishi, Y., Shimura, S., Nishikawa, T., Sunaga, F., Naito, H., & Suzuki, Y. (2003). Effect of Bisphenol A on non-specific immunodefenses against non-pathogenic Escherichia coli. *Toxicology Letters, 136*(3), 217–227. PubMed doi:10.1016/S0378-4274(02)00388-0

Telford, R.D., Sly, G.J., Hahn, A.G., Cunningham, R.B., Bryant, C., & Smith, J.A. (2003). Footstrike is the major cause of hemolysis during running. *Journal of Applied Physiology, 94*(1), 38–42. PubMed

Troadec, M-B., Laine, F., Daniel, V., Rochcongar, P., Ropert, M., Cabillic, F., . . . Brissot, P. (2009). Daily regulation of serum and urinary hepcidin is not influenced by submaximal cycling exercise in humans with normal iron metabolism. *European Journal of Applied Physiology, 106*(3), 435–443. PubMed doi:10.1007/s00421-009-1031-8

Villarino, A.V., Huang, E., & Hunter, C.A. (2004). Understanding the pro- and anti-inflammatory properties of IL-27. *Journal of Immunology (Baltimore, MD.: 1950), 173*(2), 715–720. PubMed

Wallberg, L., Mikael Mattsson, C., Enqvist, J.K., & Ekblom, B. (2011). Plasma IL-6 concentration during ultra-endurance exercise. *European Journal of Applied Physiology, 111*(6), 1081–1088. PubMed doi:10.1007/s00421-010-1737-7

Yang, Q., Jian, J., Katz, S., Abramson, S.B., & Huang, X. (2012). 17β-estradiol inhibits iron Hormone hepcidin through an estrogen responsive element half-site. *Endocrinology, 153*(7), 3170–3178. PubMed doi:10.1210/en.2011-2045