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Oral contraception does not alter typical post-exercise interleukin-6 and hepcidin levels in females

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ABSTRACT

Objectives: The post-exercise interleukin-6 (IL-6) and hepcidin response was investigated during the hormone-deplete and hormone-replete phases of an estradiol and progestogen regulated oral contraceptive cycle (OCC).
Design: Counterbalanced, repeated measures cross-over study.
Methods: Ten active female monophasic oral contraceptive pill (OCP) users completed two 40 min treadmill running trials at 75% of their pre-determined peak oxygen uptake velocity (vVO_{2peak}). These trials were randomly performed in two specific phases of the OCC: (a) Day 2–4, representing a hormone-free withdrawal period (D – 0); (b) Day 12–14, representing the end of the first week of active hormone therapy (D + 7). Venous blood samples were drawn pre-, post- and 3 h post-exercise.
Results: In both trials, serum IL-6 was significantly elevated ($p < 0.05$) immediately post-exercise, while serum hepcidin was significantly elevated ($p < 0.05$) 3 h post-exercise, with no significant differences recorded between trials.
Conclusions: These findings suggest that exercise performed during the different phases (D – 0 vs. D + 7) of a monophasic OCP regulated cycle does not alter exercise induced IL-6 or hepcidin production. As such, future studies looking to investigate similar variables post-exercise, may not need to 'control' for different phases of the OCC, provided participants are current monophasic OCP users.

1. Introduction

Poor iron status in athletes has been attributed to factors such as inadequate dietary iron intake and exercise induced iron losses such as sweating, hematuria, gastrointestinal bleeding and hemolysis (see Ref. 1 for review). Specifically, when considering female athletes, iron losses can be exacerbated by menstruation.[2] Recently, the inflammatory response has also been shown to play a key role in affecting iron metabolism in the post-exercise recovery period, via its influence on the iron regulatory hormone hepcidin (see Ref. 3 for review).

Interleukin-6 (IL-6) is an accepted biomarker for the inflammatory state.[4] However, recent work suggests that IL-6 may also be referred to as a myokine that is involved in numerous metabolic and signalling pathways.[5] Interleukin-6 increases exponentially during exercise and peaks immediately thereafter.[6,7] To date however, most investigations have examined male participants, possibly to eliminate the need to control for hormonal fluctuations (e.g. oestrogen and progesterone) in the menstrual cycle that may affect IL-6 activity.[8,9] Of interest, IL-6 has also been shown to be one of the main regulators of the iron regulatory hormone hepcidin.[10]

Hepcidin is a peptide hormone produced by the liver which regulates body iron levels by internalising and degrading the iron exporter ferroportin (Fpn) on the surface of the macrophage and intestinal duodenum.[10,11] Previously, it has been established that peak hepcidin levels occur 3 h post-exercise.[12–14] However, to date, limited investigations have adopted female participants to determine the post-exercise hepcidin response. Recently, one investigation had 12 female athletes perform two run trials that included a 60 and 120 min run at 65% VO_{2max} on separate occasions (7–10 days after the onset of menses). The results revealed that hepcidin was 200% higher after 120 min of running as compared to 60 min.[15] Additionally, it has also been reported that a subset of females (with healthy iron status) demonstrated increases in hepcidin after a 60 min run undertaken during the menstrual phase of their cycle.[13] Based on the aforementioned investigations, the

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typical post-exercise IL-6 and hepcidin response appears similar during the aforementioned menstrual cycle phases. Nevertheless, when considering the oral contraceptive cycle (OCC) (where large amounts of extraneous ethinylestradiol and progestogen are supplemented), any effect on IL-6 and hepcidin production currently remains unclear.

Recently, hepcidin levels in fish (pond raised largemouth bass) have been reported to be down regulated by estradiol[16]; a predominant sex hormone in females that represents a sub-group of oestrogen. Furthermore, high oestrogen levels may be involved in modulating the immune response by inhibiting IL-6.[9] Therefore, hepcidin may be down-regulated by estradiol via suppression of IL-6. In support of this concept, a link between estradiol and hepcidin down-regulation has been reported in vitro in human hepatoma cells[17] and in vivo in mice.[18] Considering that progesterone has also been reported to have an inverse relationship with IL-6,[8] the combined OCP (containing estradiol and progestogen) has the potential to attenuate the post-exercise IL-6 and hepcidin response. To this end, since a large proportion of female athletes are currently OCP users, this study set out to determine if post-exercise IL-6 and hepcidin levels may be attenuated during the hormone-replete as compared to the hormone-deplete phase of an OCC.

2. Methods

Ten active females, who were OCP users participated in this study [age = 26 ± 2 year, body mass = 57.0 ± 1.7 kg, stature = 163.4 ± 1.1 cm, running peak oxygen uptake (VO_{2peak}) = 53.0 ± 1.7 ml kg^{-1} min^{-1}]. Participants were taking an OCP for a minimum of 3 months to ensure that they were accustomed to their OCP regime, thereby reducing the likelihood of non-compliance or occurrence of unexpected side effects. The sample size was determined via customised computer software (GPOWER Version 2, Department of Psychology, Bonn University, Bonn, Germany) using effect sizes attained from similar previous research.[7,12,15] A sample size of 10 was recommended to yield a power of 0.90 at a significance level of $p < 0.05$. Preceding recruitment, all participants were screened to ensure that they had a normal iron status [serum ferritin = 48.0 (±10.4) μg L^{-1}, transferrin saturation = 18.9 (±2.1)%], and were not taking any iron supplements. Prior to participation, the purpose, requirements and risks of involvement were explained, and written consent obtained. Ethical approval was granted by the Human Ethics Committee of The University of Western Australia (RA/4/1/4676).

Monophasic OCP preparations used by participants during this investigation included; Levlen ($n = 7$), Yasmin ($n = 2$) (both manufactured by Bayer Australia Limited, New South Wales, Australia), and Estelle (Douglas Pharmaceuticals Australia Ltd., New South Wales, Australia) ($n = 1$). During the hormonal-deplete phase, the mean daily doses of both estradiol and progestogen were 0 μg. During the hormone-replete phase, the mean daily dose of ethinylestradiol was 30 ($n = 9$) or 35 μg ($n = 1$), and for progestogen either 150 μg of levonorgestrel ($n = 7$), 3000 μg of drosperinone ($n = 2$) or 2000 μg of cyproterone ($n = 1$).

Participants attended three laboratory-based testing sessions within a 28 day period. No manual labour or structured exercise was performed during the 24 h prior to each testing session. Session one was classed as a familiarisation session, performed on a motorised treadmill, and included a graded exercise test (GXT) (as previously described[7]) to determine each individual's running peak oxygen uptake (VO_{2peak}), and the corresponding velocity (vVO_{2peak}). The two subsequent experimental sessions occurred in a randomised counter-balanced order during specific phases of each participant's OCC, and included:

(a) Day 2–4 of the OCC, representing a hormone-free withdrawal period (D – 0).
(b) Day 12–14 of the OCC, representing the end of the first week of active hormone therapy (D + 7).

Day 1 of each participant's cycle was determined as the first day that the placebo pill was taken. Therefore, the different OCP formulations (Yaz vs. Levlen: 5 vs. 7 placebo pills) determined when the subsequent experimental session occurred. For each of the experimental sessions, participants arrived at 0700 and performed a 40 min run at 75% of their pre-determined vVO_{2peak}. The run trials were performed under comfortable laboratory conditions (22.0 ± 0.3 °C and 46.0 ± 1.4% relative humidity), with 300 ml of water consumed ad libitum, to minimise any potential hemoconcentration effects. Venous blood was collected on three separate occasions; (a) on arrival (baseline); (b) immediately after each exercise trial (post-exercise); and (c) at 3 h post-exercise. Serum iron studies were analysed at all three time points to assess iron metabolism, while IL-6 was measured at baseline and immediately post-exercise. Serum hepcidin was analysed at baseline and 3 h post-exercise. These specific time points were selected based on previous research demonstrating when peak IL-6 and hepcidin levels occur.[6,7,15] During the 3 h post-exercise recovery period, participants rested at the laboratory, and after 1 h were fed a standardised low iron containing meal [300 g of boiled pasta (Balducci, Spaghetti No. 3) in 150 g of a tomato puree (Siena, Tomato Puree), containing a total of ~3281 kJ that consisted of ~193 g of carbohydrates, 6 g of fat and 31 g of protein]. A low iron content meal was selected to minimise any potential effects on hepcidin, while replicating a high carbohydrate post-exercise meal. Lastly, participants completed a food diary for the 24 h before the run trial, and were instructed to replicate their eating habits prior to each experimental session. Heart rate was measured using a polar HR monitor (Polar RS 400, Finland), and RPE using Borg's 6–20 scale (6 = no exertion to 20 = maximal exertion).[19]

Venous blood was collected via venepuncture of an antecubital forearm vein (previously described[7]). Serum iron and ferritin measurements were performed at the Royal Perth Hospital Pathology Laboratory (Pathwest, Perth, Western Australia, Australia). Serum iron was measured using the Architect analyser (c1600210), and determined using an Iron Reagent (Sentinel Diagnostics, Milano, Italy). Coefficient of variation (CV) for iron determination at 12.01 and 43.35 μmol L^{-1} was 1.73 and 0.61%, respectively. Serum ferritin levels were determined using an Architect analyser (1SR06055) and a Ferritin Reagent (Abbott Diagnostics, Illinois, USA). The CV for ferritin determination at 28.62, 223.05 and 497.85 μg L^{-1} was 4.58, 4.46 and 4.36%, respectively. Serum IL-6 was measured at the School of Medicine and Pharmacology at The University of Western Australia (Fremantle, Western Australia, Australia) using a commercially available ELISA (Quantikine HS IL-6, R&D Systems, Minneapolis, USA), with CV values at 0.49 and 2.78 pg ml^{-1} of 9.6% and 7.2%, respectively. Serum hepcidin measurements were performed (Hepcidinanalysis.com, Nijmegen, The Netherlands) by a combination of weak cation exchange chromatography and time-of-flight mass spectrometry (WCX-TOF MS).[20] The lower limit of detection of this method was 0.5 nM; average coefficients of variation were 2.7% (intra-run) and 6.5% (inter-run). The median reference level of serum hepcidin-25 is 2.0 nM for pre-menopausal women, with a range of 0.5–12.3 nM.[21]

Results are expressed as mean and standard error of the mean (±SEM). A two-way repeated measures ANOVA analysed time, trial and time*trial effects of the different OCC phases on IL-6, hepcidin and iron parameters in the post-exercise recovery period. Post hoc LSD pairwise comparisons determined where specific trial differences existed. Mean HR and RPE were compared using students