The effect of the competitive season in professional basketball on inflammation and iron metabolism

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ABSTRACT: Following acute physical activity, blood hepcidin concentration appears to increase in response to exercise-induced inflammation, but the long-term impact of exercise on hepcidin remains unclear. Here we investigated changes in hepcidin and the inflammation marker interleukin-6 to evaluate professional basketball players’ response to a season of training and games. The analysis also included vitamin D (25(OH)D³) assessment, owing to its anti-inflammatory effects. Blood samples were collected for 14 players and 10 control non-athletes prior to and after the 8-month competitive season. Athletes’ performance was assessed with the NBA efficiency score. At the baseline hepcidin correlated with blood ferritin (r=0.61; 90% CL ±0.31), but at the end of the season this correlation was absent. Compared with the control subjects, athletes experienced clear large increases in hepcidin (50%; 90% CI 15-96%) and interleukin-6 (77%; 90% CI 35-131%) and a clear small decrease in vitamin D (-12%; 90% CI -20 to -3%) at the season completion. Correlations between change scores of these variables were unclear (r = -0.21 to 0.24, 90% CL ±0.5), but their uncertainty generally excluded strong relationships. Athletes were hence concluded to have experienced acute inflammation at the beginning but chronic inflammation at the end of the competitive season. At the same time, the moderate correlation between changes in vitamin D and players’ performance (r=0.43) was suggestive of its beneficial influence. Maintaining the appropriative concentration of vitamin D is thus necessary for basketball players’ performance and efficiency. The assessment of hepcidin has proven to be useful in diagnosing inflammation in response to chronic exercise.

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INTRODUCTION

Effective energy metabolism and transport of oxygen both need to engage several iron-containing proteins, such as cytochromes in the mitochondria, myoglobin and haemoglobin. Thus, proper iron homeostasis is essential, particularly during long lasting athletic competitions. In another way, iron overload can stimulate formation of reactive oxygen species (ROS) [1]. Most of the iron stored in ferritin, yet it does not stimulate ROS formation. Still, certain stressors such as UV light, TNF-α or hydrogen peroxide can trigger iron release from ferritin, hence stimulating iron-dependent ROS formation [2]. At the same time, a systemic inflammatory response may be triggered by excessive exercise, common in professional sport [3]. The pro-inflammatory response has the potential to disturb the intracellular iron homeostasis. In consequence, the inflammation acts as a signal activating regeneration processes including proliferation of satellite cells and the molecular mechanism of protein biosynthesis. Hence, inflammation may contribute to the improvement of adaptation to exercise [4].

Nevertheless, the mechanism responsible for this anti-inflammatory response in training and regular exercise needs to be recognized. Zembron-Lacny and co-workers reported significant shifts in markers of ROS activity – thiol redox status and inflammatory mediators (IL-6, TNFα) – following professional basketball training and found the changes in thiol redox and cytokine response to be correlated [5]. In our previous paper we concluded that the pro-inflammatory cytokine IL-1β, most likely induced by an extensive workload during the tournament season, was responsible for the low level of ferritin in young, professional tennis players [6]. Moreover, the study revealed that increased levels of the pro-inflammatory cytokines were associated with an elevated level of hepcidin – a liver-produced hormone regulating iron metabolism in the gut and macrophages [7, 8]. These...
data are in agreement with the paper by Nemeth, who stated that the inflammatory cytokine interleukin-6 (IL-6) is the primary mediator responsible for regulating hepcidin activity [9]. Hepcidin is also expressed in response to hypoxia, elevated iron levels and exercise [10, 11]. Clinical research demonstrated the peak hepcidin level to occur 3 h following the IL-6 peak rise [12]. The relationship between hepcidin and IL-6 was confirmed, investigating the response to a single dose of exercise: endurance [13] as well as interval training [14]. In addition, Roecker and co-workers observed a considerable increase in hepcidin level 24 h after a 42.2-km marathon run in female athletes [15]. Data determining the elevated resting serum hepcidin values in response to long-lasting training and competition in professional athletes are, however, limited. Ma and co-workers showed that hepcidin was not chronically elevated with sustained training in competitive collegiate runners [16]. Still, 85% of those runners were supplemented with iron. A significant elevation in hepcidin was also observed in specific groups with anaemia, associated with inflammation and/or chronic diseases [17].

These data confirm that inflammation plays a significant role in hepcidin synthesis. In physical training, especially when combined with competition stress, it is essential to maintain the appropriate balance between the pro- and anti-inflammatory response [3]. We assumed that changes in iron status and hepcidin response may be one of the mechanisms which helps in achieving this goal.

Therefore, the first aim of our study was to evaluate hepcidin level among professional basketball players at baseline and in response to long-lasting physical workload, as well as training and a game. Owing to the well-documented anti-inflammatory effect of vitamin D (25(OH)D3) [18], we assumed that lower hepcidin would be observed among athletes with higher levels of vitamin D.

MATERIALS AND METHODS

Data collection. To monitor the influence of training and game workload on iron status, blood analysis was conducted in a professional basketball team. The same blood parameters were assessed in the control group as well. Body composition was also measured. These examinations were performed at the onset of the season (September, baseline) and two days directly after the last game of the first round of the games (March). This 8-month period in-between included all indoor games scheduled by the premier basketball league. Throughout this period physical training and game workload as well as each game efficiency were recorded. All the subjects were informed of the aim of the study and gave their written consent for participation in the project. The protocol of the study was approved by the local ethics committee, in accordance with the Declaration of Helsinki.

Subjects

Two groups took part in the investigation: basketball players and non-athletes (as a control group). On average, the players had 20 ± 5.0 years of training and competition experience. Non-athletes were instructed not to change their lifestyle in the period between examinations.

Body composition assessment

Body mass (BM) and body composition were estimated using a multi-frequency impedance plethysmograph body composition analyzer (InBody 720, Biospace, Korea). Using a diverse range of frequencies from 1 kHz to 1 MHz, InBody 720 accurately measured the amount of body water as well as body composition, including fat mass, free fat mass and skeletal muscle mass. Precision of the repeated measurements was expressed as the coefficient of variation, which on average was 0.6% for the fat mass percentage [19, 20]. The measurements were taken one hour before breakfast. The participants had emptied their bladders and bowels prior to the assessment. During the measurement, the participants were only briefly and remained barefoot [21].

Training and game workload

The training workload during this period was recorded by coaches. The team analyst recorded the NBA efficiency score for each player in each game as a measure of players’ performance. The formula created by John Hollinger and modified by the statistician Martin Manley is officially used by NBA and Basketball-Reference.com. The change in the score for each player over the season was derived by regressing the score against the game number (28 games) and deriving the difference between predicted scores in the first and last games (the slope times 27).

Blood sampling and cytokine analysis

Blood samples were taken from an antecubital vein into single-use containers with an anticoagulant (EDTA-K$_2$). After collection, the samples were immediately stored at a temperature of 4°C. Within 10 minutes, they were centrifuged at 3000 g and 4°C for 10 min. Aliquots of the plasma were stored at -80°C.

Blood was collected at rest, at the same time of the day (8.00 a.m.). Red blood cell counts [10$^6$ µL$^{-1}$ (RBC), haematocrit [% (Hct) and blood haemoglobin concentration [g·dL$^{-1}$] (Hb)] were determined from the venous blood samples by conventional methods using a COULTER LH 750 Hematology Analyser (Beckman-Coulter, USA). Plasma interleukin-6 (IL-6) level was determined via the enzyme immunoassay method using commercial kits (R&D Systems, USA). The detection limit for IL-6 was 0.039 pg·ml$^{-1}$, with the average intra-assay CV below 8.0%.

Serum levels of 25(OH)D3 were determined by electrochemiluminescence (ECLIA) using the Elecsys system (Roche, Switzerland). The intra assay and inter assay coefficients of variation (CVs) were 5.6% and 8.0%, respectively, and the limit of detection was 4 ng·mL$^{-1}$.

Serum hepcidin was determined using the DRG Elisa kit (DRG Instruments, GmbH, Germany) according to the manufacturer’s protocol. This assay detects the 25 amino-acid form of hepcidin – the
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biologically active form of the hormone. The dynamic range of the assay was 0.9-140 ng mL⁻¹. The average CVs precisions of intra-assay was 4.41% and 9.70%, respectively. The normal 5-95% range in healthy adults described by the manufacturer corresponds to 13.3-54.4 ng mL⁻¹. Analytical sensitivity of the hepcidin kit was calculated by subtracting two standard deviations from the mean of 20 replicate analyses of the zero standard. Similar hepcidin measurements (Elisa kit) were used in a previous study [22] – at the moment it seems to be the most time- and cost-effective method.

Statistical analysis

Statistical analysis was performed in two ways. Comparison of body composition and its changes was performed using STATISTICA 9.0 (StatSoft, USA). Results are expressed as the mean and standard deviation (X±SD). The normality of data was tested using the Shapiro-Wilk W-test. The level of significance was set at 0.05 for all analyses. Additionally, to determine the differential significance at baseline and after an 8-month period the t-test was used. Means related to haematological parameters were analysed with a spreadsheet for analysis of parallel-group controlled trials, and effects were interpreted using magnitude-based inference [23,24]. All data were log-transformed to reduce bias arising from the error non-uniformity. Means of the score changes in both groups, standard deviations of the score changes and the obtained effects (differences in the changes of the means and their certainty limits) were back-transformed to percentage units. Mean changes and effects were adjusted to the overall mean baseline value for both groups by including the baseline value as a covariate in the analysis. Magnitudes of the effects were evaluated with the log-transformed data by standardising with the standard deviation of the baseline values of the control group. Threshold values for assessing magnitudes of standardised effects were 0.20, 0.60, 1.2 and 2.0 for small, moderate, large and very large respectively. Uncertainty in each effect was expressed as the 90% confidence limit as well as the probability that the true effect was substantially positive (an increase) or negative (a decrease). These probabilities were used to make a qualitative, probabilistic, non-clinical inference about the true effect: if the probability of the effect being a substantial increase or a substantial decrease was >5% in both cases (equivalent of 90% confidence interval overlapping thresholds for a substantial increase and decrease), the effect was reported as unclear; otherwise, it was considered clear and assigned the relevant magnitude value, with the qualitative probability that the true effect was a substantial increase, substantial decrease, or a trivial difference (whichever outcome had the largest probability). The following scale was used for interpreting the probabilities: 25-75%, possible; 75-95%, likely; 95-99.5%, very likely; >99.5%, most likely. This study involved assessment of substantial changes in five measures. To maintain an overall error rate of <5% for declaring one or more changes to have opposite magnitudes (a substantial decrease instead of an increase, and vice versa), the effects were also evaluated as clear or unclear with a threshold of 5%/5 (1%), equivalent to consideration of overlap of substantial values with a 98% confidence interval.

The spreadsheet [23] also provided an estimate of a standard deviation representing individual responses to the season of training in the athletes (the variation in the change scores in the athlete group additional to that in the control group). The standard deviation was given by the square root of the difference in the variance of the change scores, and confidence limits were provided by assuming asymptotic normality of the variance. When the difference in the variance or a confidence limit was negative, the sign was changed before taking the square root, and the standard deviation was shown as a negative value. Individual responses were interpreted with magnitude-based inference as above, but the standardised magnitude thresholds were halved to effectively assess the magnitude of twice the standard deviation. The usual scale for correlation coefficients (0.1, 0.3, 0.5, 0.7 and 0.9 for low, moderate, high, very high and nearly perfect, respectively) was used for interpretation of relationships between selected variables and players’ performance Confidence limits (90% CL) for coefficients of correlation were calculated using dedicated spreadsheet.

RESULTS

All basketball players completed the tournament round with no adverse events or injury (serious, excluded from the games) being reported. Athletes’ body compositions are outlined in Table 1, which shows only significant changes in water status (intracellular and extracellular water amount) that took place during the investigated period. Changes in body composition for non-athletes were not significant. In agreement with our expectations, non-athletes differed in comparison to basketball players (data for non-athletes are not presented).

Baseline values of basic morphological measures from the athlete and control groups are presented in Table 2. In order to evaluate the influence of the tournament season on changes in blood parameters the same analyses were repeated. Obtained data are also presented in Table 2, including changes in basic measures in both groups throughout the season and a comparison of the observed changes.

| Variable     | Baseline | After season | P value |
|--------------|----------|--------------|---------|
| Height (cm)  | 196.7±8.7| 196.7±8.7    | ns      |
| Weight (kg)  | 94.4±6.0 | 92.1±6.4     | ns      |
| FFM (kg)     | 84.7±6.7 | 83.4±7.0     | ns      |
| SMM (kg)     | 49.1±5.2 | 48.2±4.2     | ns      |
| Fat (kg)     | 9.3±3.7  | 8.7±2.5      | ns      |
| Fat (%)      | 9.8±4.0  | 9.5±3.0      | ns      |
| VFA (cm²)    | 57.8±25.4| 62.9±15.5    | ns      |
| ECW (kg)     | 23.2±2.5 | 22.9±2.5     | 0.002   |
| ICFW (kg)    | 39.1±4.2 | 39.0±4.1     | ns      |

Note: Values are means ± SD; FFM – fat-free mass, SMM – skeletal muscle mass, Fat – fat mass, Fat% – percentage of body fat, VFA – visceral fat area, ns – no statistically significant differences between measurements.
The main purpose of this study was to evaluate whether the whole tournament season impaired iron metabolism and if these changes were related to hepcidin and IL-6 concentrations. This hypothesis was based on previous observations from professional athletes, both basketball and tennis players, who exhibited low ferritin levels (<25 µg·L\(^{-1}\)). Ferritin levels in blood should reflect the intracellular ferritin concentration. Cellular ferritin synthesis also changes in parallel to cellular iron levels. Hence, low blood ferritin is considered to be the best indicator of iron deficiency. Nonetheless, contrarily to our assumptions the average values of parameters characterising the iron status in the athlete group were in their reference ranges at the baseline as well at the game round completion. Table 3 shows baseline values of the measures related to iron status in the athlete and control groups.
The value of average slope of efficiency was -0.1 ± 3.3. A likely 7.5 with between-athletes SD = 4.5, and within-athletes SD = 5.6. Unclear. The average players’ NBA efficiency of all the 28 games was while those for vitamin D (2%; 90% CI-12 to 13%) were trivial and respectively large and moderate observed values but were unclear, (20%; 90% CI -5 to 30%) and IL-6 (16%; 90% CI -29 to 49%) had (90% CI 95 to 168%; very large), while that for vitamin D was lower (-21%; 90% CI -35 to -5%; moderate). Differences for IL-6 (18%; 90% CI -9 to 53%), ferritin (18%; 90% CI -22 to 79%) and iron (-11%; 90% CI -28 to 11%) were small but unclear. The changes in the measures across an 8-month period in each group and the comparison of the changes adjusted to the overall mean at baseline are also shown in Table 3. There were large, clear increases in hepcidin and IL-6, and a small increase in ferritin. Iron and vitamin D exhibited small clear reductions. The effects for hepcidin, IL-6 and vitamin D were still clear at the 98% level. Moreover, a strong correlation between the changes in blood ferritin and hepcidin and iron in the athlete and control groups for one of the measures, hepcidin, is illustrated in Figure 1. It shows how the mean changes and their differences were adjusted to the mean baseline hepcidin concentration: the adjusted changes in each group are the changes predicted for the mean baseline value using the linear regression.

The baseline concentration of hepcidin in the athlete group was over twice that of the control subjects (a difference of 129%; 90% CI 14 to 43%; large) and for ferritin (32%; 90% CI -6 to 49%; moderate). Individual responses expressed as coefficients of variation were all up-regulated by NF-κB (29). Consequently, iron is described as a molecule capable of activating transcriptional nuclear factor-κB (NF-κB) (29). It might lead to both enhanced gene expression and protein synthesis [30]. Among others, pro-inflammatory mediators such as IL-1β, IL-6 and TNF-α are all up-regulated by NF-κB. Reduced iron absor-
tion due to rising blood hepcidin is one way which allows the inflammatory process to self-limit. It is important to note that the observed changes in iron metabolism seem to have been a very sensitive marker of inflammation in athletes, who probably experienced overreaching from the game season.

One reason why training and game workload induced inflammation could be a low concentration of vitamin D. The anti-inflammatory effects of vitamin D are well-documented [31, 32] the deficiency is often observed in indoor athletes [33]. Moreover, a paper by Kope et al. showed that even in outdoor sport the recommended levels of 25(OH)D3 were recorded in 50% of the players after the summer period but only in 16.7% of the players after the winter time [34]. Additionally, hypovitaminosis D was also recorded among Tunisian athletes despite living in a sun-rich environment [35]. In our study, the first assessment was performed directly after summer (a period of high exposure to UVB), but the values of vitamin D were low in both groups. The vitamin D deficiency was found to have progressed throughout the season, which could be one of the causes of inflammation. Data confirmed that athletes characterized by a smaller reduction in vitamin D were able to maintain their best performance efficiency during the whole game season. The literature reports that 25(OH)D3 concentration can positively affect muscle function [36] as well as muscle tissue [37]. Agergaard et al. revealed that 12 weeks of resistance training supported by supplementation of vitamin D does not influence the whole muscle hypertrophy or muscle strength, but improved muscle quality in the elderly and fibre type morphology in the young, indicating an effect of vitamin D on skeletal muscle remodelling [38]. Although we did not measure muscle strength, we can speculate based on the obtained correlation that the players characterized by elevated vitamin D exhibited higher exercise tolerance and underwent the recovery process faster, which helped them maintain better performance during the whole investigation period.

In our observations, we noted a parallel increase of the exercise-induced inflammatory markers hepcidin and IL-6. Both markers could be considered as indicators of the inflammatory response in professional athletes. It appears particularly interesting to uncouple the relationship between ferritin and hepcidin at the end of the season, which could also be considered a good marker of inflammation.

CONCLUSIONS

In summary, our current data show that overreaching, which is an effect of the basketball tournament season, leads to an increase in blood hepcidin. The strong correlation observed between blood ferritin and hepcidin faded at the end of the season, indicating that it could be a good and sensitive marker of inflammation and overreaching among athletes. Regular assessment of vitamin D should also be included due to its likely role in ameliorating exercise-induced inflammation and the possible benefit to athletes’ performance.

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