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Biological variation of inflammatory and iron metabolism markers in high-endurance recreational athletes; are these markers useful for athlete monitoring?

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Abstract

Objectives: To deliver biological variation (BV) data for serum hepcidin, soluble transferrin receptor (sTfR), erythropoietin (EPO) and interleukin 6 (IL-6) in a population of well-characterized high-endurance athletes, and to evaluate the potential influence of exercise and health-related factors on the BV.

Methods: Thirty triathletes (15 females) were sampled monthly (11 months). All samples were analyzed in duplicate and BV estimates were delivered by Bayesian and ANOVA methods. A linear mixed model was applied to study the effect of factors related to exercise, health, and sampling intervals on the BV estimates.

Results: Within-subject BV estimates (CV_I) were for hepcidin 51.9 % (95 % credibility interval 46.9–58.1), sTfR 10.3 % (8.8–12) and EPO 27.3 % (24.8–30.3). The mean concentrations were significantly different between sex, but CV_I estimates were similar and not influenced by exercise, health-related factors, or sampling intervals. The data were homogeneously distributed for EPO but not for hepcidin or sTfR. IL-6 results were mostly below the limit of detection. Factors related to exercise, health, and sampling intervals did not influence the BV estimates.

Conclusions: This study provides, for the first time, BV data for EPO, derived from a cohort of well-characterized endurance athletes and indicates that EPO is a good candidate for athlete follow-up. The application of the Bayesian method to deliver BV data illustrates that for hepcidin and sTfR, BV data are heterogeneously distributed and using a mean BV estimate may not be appropriate when using BV data for laboratory and clinical applications.

Keywords: biological variation; hepcidin; erythropoietin; soluble transferrin receptor; athletes

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Introduction

Biological variation (BV) describes the fluctuation of a measurand around its homeostatic set point in steady-state conditions, defined in an individual as the within-subject BV (CV_I). In contrast, the variation between the homeostatic set points of different individuals is denoted the between-subject BV (CV_G). The most important applications of BV include setting analytical performance specifications (APS) [1, 2], interpreting serial test results from consecutive samples from an individual by the reference change value (RCV) [3, 4] and to determine personalized reference intervals allowing an individual to have their results assessed against their own reference interval [5].

BV has been studied since the 20th century and data have been published from many healthy populations [6]. Selected data are also available from populations with stable disease settings such as cardiac or kidney disease, for which the subject can be considered in a steady state despite their pathological condition [7–11]. A high level of physical exercise maintained over time can be considered a different steady state setting, which has been shown to influence the BV of e.g. hepatic and muscle enzymes, acid-base status measurands and reticulocytes [12–14].

Higher endurance aerobic sport most likely influences biomarkers related to erythropoiesis and inflammation. Hepcidin is a marker involved in both pathways and influenced by iron stores, erythropoiesis, inflammation, and hypoxia. Anemia and hypoxia promote erythropoiesis, which decreases hepcidin activity [15]. However, hepcidin increases under inflammatory conditions because its transcription is stimulated by interleukin-6 (IL-6), facilitating a block of iron absorption [15]. Erythropoietin (EPO) also increases in response to hypoxia [16] and anemia but is inhibited by proinflammatory cytokines [17]. Resistance and endurance sports produce inflammation mediators, especially after exercise [18]. Therefore, athletes need a balance between stimulus (physical activity and hypoxia) to promote erythropoiesis and enough rest to avoid excess inflammation or increased iron loss through the gastrointestinal tract. Unlike hepcidin and ferritin, soluble transferrin receptor (sTfR) is not affected by inflammation or high-level exercise and therefore is a good marker for iron status in an athlete [19]. Considering the complexity of iron regulation in athletes, the availability of robust BV data for iron and inflammatory markers could facilitate a better understanding of iron metabolism, aid in the interpretation of results when monitoring athlete health and to assist in doping investigations. However, to our knowledge, there needs to be more high-quality studies reporting BV for athlete populations that have adhered to current recommendations for BV study design and delivery [20]. Furthermore, there needs to be more knowledge on the potential effect of high training load or other health-related factors on BV estimates in this population.

This study aimed to deliver BV estimates of hepcidin, sTfR, serum EPO (sEPO) and IL-6, applying a Biological Variation Data Critical Appraisal Checklist (BIVAC) [20] compliant design in a population of well-characterized high-endurance athletes with monthly samplings, by both classical and Bayesian methods. Additionally, we aimed to evaluate the potential influence of factors related to exercise, health, and sampling intervals on the BV estimates.

Materials and methods

Study population

The study population has been described in detail previously [12, 13, 21]. Briefly, 30 subjects (15 women), median age 36 years (range 19–53) were recruited from three triathlon clubs in Madrid, Spain and fulfilled the following inclusion criteria: presumably healthy, age >18 years, >13 h of training per week, normal standard medical examination and expected results for a stress test supervised by a physician specialist in sports medicine [12]. The study was approved by the host Institutional Research Ethical Committee and all participants signed informed consent.

Sample collections

To assure a confidence interval (CI) width lower than 1/3 CV_i [22], 11 samples per subject were collected monthly (January–November 2017) on Saturday mornings (8–10 am) after 10 h fasting. In each visit, participants completed a questionnaire about fasting, vitamin supplementation and diet changes, health status and use of medication, date of last competition, details on last training (time, type and intensity) and in females, date of last menstrual period.

All aspects of the preanalytical phase were standardized, as previously reported in detail [13] and are detailed in the Supplementary File.

Sample analysis

All serum samples were analyzed in duplicate for hepcidin, sEPO and sTfR (January–August 2022) and IL-6 (January 2022–February 2023). We used the following methodology: LC-MS/MS for hepcidin [23] (limit of detection (LoD) 0.3 nmol/L); Quantikine ELISA immunoassay for IL-6 (R&D Systems Inc, Minneapolis, MN, USA) (LoD=0.7 ng/L), chemiluminescence immunoassay on Atellica analyzer (Siemens Healthineers®) for sTfR (LoD=0.5 mg/L) and chemiluminescent immunometric assay on Immulite 2000 Xpi analyzer for sEPO (LoD=1.5 IU/L). All samples from each subject were measured in the same analytical run and with the same reagent lot. All quality control results were within set limits.

Statistical analysis

Differences in biomarker mean concentrations related to sex (male/female) or the physical activity performed the previous 24 h (Yes/No) were assessed by t-tests, after a Levene's test to evaluate the data distribution.

Prior to calculating the BV components, results below the LoD were excluded. Trend analysis was performed for the overall population for all measurements. If the 95 % CI of the slope did not include 0, the trend was considered significant. In this case, trend analysis was performed for each individual by plotting each subject's results against the collection dates. Subjects with a significant trend during the study were excluded and trend analysis for the overall population was repeated. The resulting data set was considered to represent a steady-state situation and two different approaches were applied to derive BV estimates:

Classical approach: Firstly, outlier analysis was performed between replicates from the same sample and between the samples from the same subject, using the Cochran C test to assure the homoscedasticity for CV_A and CV_I [1]. Reed's criterion was applied to assessed differences between the athletes' mean values [24]. Thereafter, a CV-ANOVA was performed to deliver CV_I estimates, an ANOVA was applied to deliver CV_G estimates (Log-ANOVA), after log-transforming the data [3].

Bayesian approach: A Bayesian model, previously described in detail, was applied [25]. Previous information (priors) was included in the model for sTfR (CV_I and CV_G estimates from the general population derived from the EFLM BV database: <http://biologicalvariation.eu/>) and for hepcidin (hemodialysis patients [26]). Priors for CV_A were set based on routine analytical quality control results. For sEPO, no previous information was available. Thus, the CV_I estimate obtained from the classical approach was used to define the hyperparameters but given low weight (1/2 standard deviation). The Bayesian model provides an individual's CV_I ($CV_{P(i)}$) for each subject, used to deliver the predicted distributions ($dCV_{P(i)}$) with credibility (CrI) intervals, and the degrees of freedom for an adaptative Student t distributions for the within-subject and the analytical component. The CrI, from a Bayesian perspective, provide a range within the true parameter would lie with 95 % of probability, given observed data and prior information.

Furthermore, as an indicator of the degree of heterogeneity, the estimated Harris-Brown heterogeneity ratio (HBR) was calculated by using the mean and standard deviation from $CV_{P(i)}$; $100\% (\sigma[CV_{P(i)}] / \mu[CV_{P(i)}])$. Correlations between the individual $CV_{P(i)}$ and the concentration were assessed by visual inspection. For data analysis, we used the *rstan* package (Rstudio software) and the script provided by Roraas et al. [27].

All BV components were estimated for the overall group as well as in male and female subgroups. Differences between men and women were assessed by Wilcoxon (statistically significant when $p < 0.05$). BV estimates delivered by the two models were compared based on the overlap/lack of overlap of the 95 % CIs/CrIs.

Influence of exercise and health-related factors on BV components:

We evaluated several parameters by a linear mixed model (LMM) to explore the potential influence of different factors on the BV. The BV estimates were included as random effects in the model and the following variables as fixed effects: health status, sampling interval, exercise 24 h prior to sampling, intensity and duration of exercise the week before sampling, and time from last menstruation (females). We calculated the CV_I from the model with and without the variables, as described in Supplementary Data. A difference of more than 5 % in the CV_I estimates derived from including the different variables in the LMM was considered as significant ($p < 0.05$). To calculate the index of individuality (II), the following formula was applied: $II = CV_I / CV_G$. For the calculations, we used RStudio Desktop 1.3.1093 and Microsoft Excel 2010.

Results

The mean number of training sessions recorded per week per athlete was eight, with an average of 9.2 h of exercise per week. The athletes did mainly aerobic exercise and all participants similarly performed training (types of sports,

intensity, distance and duration) indicating that the group was homogeneous concerning exercise profiles [13].

A total of 292 samples were collected corresponding to 584 results (an average of 9.7 samples per subject). All samples had the hemolysis, lipemia or icterus index below the assigned cut-off for interference. Results for sEPO, hepcidin and sTfR are depicted in Figure 1. For IL-6, BV estimates could not be calculated because about 90 % of the results were below the LoD.

Influence of sex and training on measurand concentrations

Training during the previous 24 h did not influence the mean concentration of any of the measurands (data not shown). However, the mean concentrations were significantly different ($p < 0.005$) between men and women, with women having higher results for sTfR and sEPO and lower for hepcidin (Supplementary Figure 2). Mean concentrations in men and women were 0.73 and 1.01 mg/L for sTfR, 7.7 and 11.7 IU/L for sEPO, and 2.7 and 1.0 nmol/L for hepcidin, respectively (Supplementary Figures 1 and 2).

Biological variation estimation

No trends were identified in the overall study population except for sEPO (slope 0.004, CI 95 % 0.007–0.026). Further study indicated a significant trend for sEPO in two subjects. After excluding these two, there was no longer a trend in sEPO and the remaining subjects were considered to be in a steady state.

For hepcidin and sTfR, 16.5 and 17.1 % of results were under the LoD, respectively. Out of the results over the LoD, 18.6 and 13.5 % of the data were identified as outliers for hepcidin and sTfR, respectively (Table 1). Only two results were identified as outliers for sEPO. The BV estimates derived by the classical approach, with mean concentration, included results and the number of results under the LoD and outliers are shown in Table 2. No significant differences between men and women were identified for BV estimates derived by the classical approach. Still, the distribution of the $CV_{P(i)}$ derived by the Bayesian model was different between men and women for hepcidin and sTfR (Table 2, Figures 2 and 3). No relation between the $CV_{P(i)}$ and concentrations for the different analytes was observed by visual inspection (Supplementary Figure 3).

For hepcidin, sEPO and sTfR, there was an overlap between the 95 % CI/CrI for the BV estimates obtained by Bayesian and ANOVA methods (Tables 1 and 2). Including

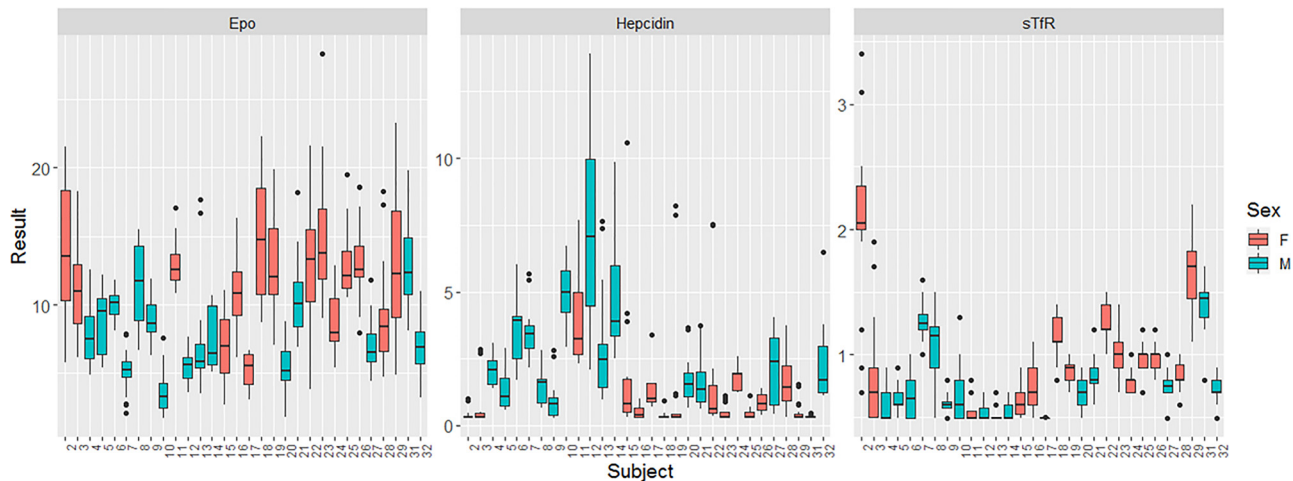


Figure 1: Distribution of results of the athletes (crude data, before outlier analysis), with red indicating females and blue males. The boxplots represent the median and interquartile range for each study participant, with the x-axis showing the participant number (two of the recruited study subjects were excluded, no. 1 and 30). EPO, erythropoietin; sTfR, soluble transferrin receptor. Units are given in Table 1.

exercise and health-related factors in the LMM did not change the CV_I for hepcidin, sEPO and sTfR when including sampling interval, perceived exertion, intensity or duration of training in the week before sampling (data not shown).

Discussion

In this study, we considered exercise as stable physiological stress maintained continuously, which may potentially influence the BV in a high-endurance athlete population. Data on physical activity confirmed that all study subjects performed constant training. Following the exclusion of two subjects for sEPO, trend analysis indicated that the study population was in a steady state setting during the study. Thus, the data are valid for such a BV study. We observed significant differences between men and women in iron-metabolism-related markers, which could indicate greater iron needs (lower hepcidin and higher sTfR concentration) and increased erythropoiesis (increased EPO) in female athletes compared to males, as also suggested in previous BV studies in the same cohort (hemoglobin, cell blood count and reticulocytes) [14] and the European Biological Variation Study (EuBIVAS) [28]. Because of hepcidin's implication in iron regulation, one could speculate that these markers could be influenced by the menstrual cycle in females. Still, we observed no significant variations for iron markers related to the menstrual cycle phases, in agreement with previous studies [29]. Furthermore, there was no correlation between the individual $CV_{p(i)}$ and the mean concentration for each study participant (Supplementary Figure 3).

The mean CV_I estimate derived by CV-ANOVA and the mean $CV_{p(i)}$ derived from the Bayesian model was similar for the assessed measurands. CV_G estimates derived by the two approaches were also similar. However, the distribution of the $CV_{p(i)}$ derived by the Bayesian method revealed differences in the individual $CV_{p(i)}$ with implications for using these markers in athlete monitoring and doping investigations.

Distribution of BV data

When applying the classical CV-ANOVA approach, the strategy is to analyze and eliminate outliers to fulfil ANOVA requirements, including normality and homogeneity between replicates and between subjects. This data management “cleans” the data, which runs a risk of results being biased, especially when the number of excluded results is high, as was the case for some of the measurands included in our study. Conversely, the Bayesian method is robust to extreme values, and outlier and homogeneity analyses are not required before analysis. When interpreting the Bayesian results, the HBR is a tool to evaluate the distribution of the individual $CV_{p(i)}$ (Figures 2 and 3). If the HBR is above a set cut-off dependent on the study population/design ($100/\sqrt{2 \cdot \text{no. sample}}$), the $CV_{p(i)}$ distribution can be considered heterogeneous. In such a case, the derived average, or median, BV estimate is unlikely to represent the studied population.

For hepcidin, an HBR below 24.9 would in our study indicate a homogenous distribution. However, the HBR was 31.9 for all subjects, and for the female subgroup even higher (HBR=58.5; cut-off <26.7) than for men (HBR=30.7; cut-off <23.4).

Table 1: Number of included subjects with total number of results (crude data), results under the limit of detection and number of included/excluded results following outlier and homogeneity analysis.

Measurand, units	Sex	No. results (crude data)	No. results <LoD, %	No. outliers, % ^a	No. results included	No. subjects included	Mean (CI 95 %)	CV _I (CI 95 %)	CV _G (CI 95 %)	CV _A (CI 95 %)	Index of individuality
Hepcidin, nmol/L	All	584	99 (17.1)	90 (18.6)	397	25	2.20 (2.16–2.23)	51.9 (46.9–58.1)	92.9 (66.7–154.3)	4.6 (4.2–5.1)	0.6
	F	292	82 (28.1)	68 (32.4)	142	11	1.22 (1.17–1.26) ^b	52.8 (44.8–64.3)	73.1 (45.0–176.4)	5.1 (4.4–6.2)	0.7
	M	292	17 (5.8)	21 (7.6)	254	14	2.91 (2.85–2.98) ^b	51.4 (45.4–59.1)	77.6 (51.8–154.3)	4.35 (3.9–5)	0.7
sTfR, nmol/L	All	584	96 (16.5)	66 (13.5)	422	27	10.42 (10.37–10.48)	10.3 (8.8–12.0)	28.9 (22.3–40.9)	9.6 (8.8–10.6)	0.4
	F	292	34 (11.6)	58 (22.4)	200	12	11.77 (11.63–11.89) ^b	11.1 (9.0–13.6)	25.3 (17.2–46.5)	9.3 (8.1–10.8)	0.4
	M	292	68 (23.2)	2 (0.8)	222	15	9.45 (9.37–9.55) ^b	9.5 (7.3–11.9)	28.2 (20.2–46.2)	10.0 (88.8–11.5)	0.3
EPO, IU/L	All	584	0	2 (0.3)	538	28	8.93 (8.88–8.99)	27.3 (24.8–30.3)	37.9 (28.8–54.5)	9.8 (8.8–11.2)	0.7
	F	292		2 (0.6)	246	13 ^c	10.4 (10.30–10.55) ^b	29.1 (25.8–33.3)	29.1 (19.4–51.9)	8.8 (7.7–10.3)	1.0
	M	292		0 (0)	292	15	7.85 (7.76–7.94) ^b	26.0 (22.7–30.3)	36.1 (24.9–62.7)	9.8 (8.7–11.2)	0.7

Mean concentrations, estimates analytical imprecision (CV_A), within-subject (CV_I) and between-subject (CV_G) BV estimates are presented with 95 % confidence intervals Hepcidin, sTfR, soluble transferrin receptor; EPO, erythropoietin. ^aPercentage of outliers=No of outliers/(No of results (crude data) – No of results<LoD) × 100. ^bSignificant differences between mean concentrations of male and female subgroups (p<0.05). ^cTwo subjects were excluded after the trend analysis.

Table 2: Number of included results, estimates of the within-subject ((CV_I(i))) and between-subject (CV_G) BV with their 95 % credibility intervals (CrI), the median of the predicted distributions d(CV_P(i))₅₀ with their 20th and 80th, percentiles and analytical variation (CV_A) for the whole study population and in subgroups defined by sex derived from the Bayesian model, Harris-Brown heterogeneity ratio and the cutoff (100/√2 · no. sample) to consider it non-homogeneous.

Measurand	Sex	No. results	Mean concentration	Mean [CV _{P(0)}] (95 % CrI)	Predicted d (CV _{P(0)}) ₅₀ (20–80 %) ^S	CV _G (95 % CrI)	CV _A %	Harris-Brown ratio	Harris-Brown ratio cutoff
Hepcidin, nmol/L	All	485	2.2	55.3 (44.7–66.3)	58.1 (39.9–78.6)	90.1 (66.2–123.6)	8.6	31.9	<24.9
	F	210	1.36	49.8 (29.2–67.5)	63.1 (32.7–99.8)	68 (44.3–103.2)	8.8	58.5	<26.7
	M	275	2.84	46.6 (34–57.2)	49.2 (34.6–63.3)	79.9 (53.8–118.3)	5.9	30.7	<23.4
sTfR, nmol/L	All	482	11.51	10.2 (7.9–12.6)	12.3 (6.8–17.8)	28.3 (23.4–33.9)	12.7	43.1	<24.9
	F	258	12.8	8.1 (5.1–10.8)	11.6 (4.7–20.5)	26.3 (20.1–33.1)	13.2	43.1	<24.1
	M	224	9.99	8.2 (5.9–10.3)	9 (6.3–11.9)	24.6 (19.1–30.9)	10.6	34.4	<25.9
EPO, IU/L	All	582	9.79	29.0 (25.0–33.2)	29.6 (26.6–32.7)	39.5 (29.9–52.9)	10.2	11.8	<22.7
	F	290	11.82	30.7 (24.2–37.5)	31.7 (26.4–37.3)	29.7 (19.1–44.7)	8.9	18.0	<22.7
	M	292	7.77	27.4 (22.4–32.6)	27.9 (24.7–31.4)	37.7 (25.6–57.1)	11.1	12.5	<22.7

sTfR, soluble transferrin receptor; EPO, erythropoietin.

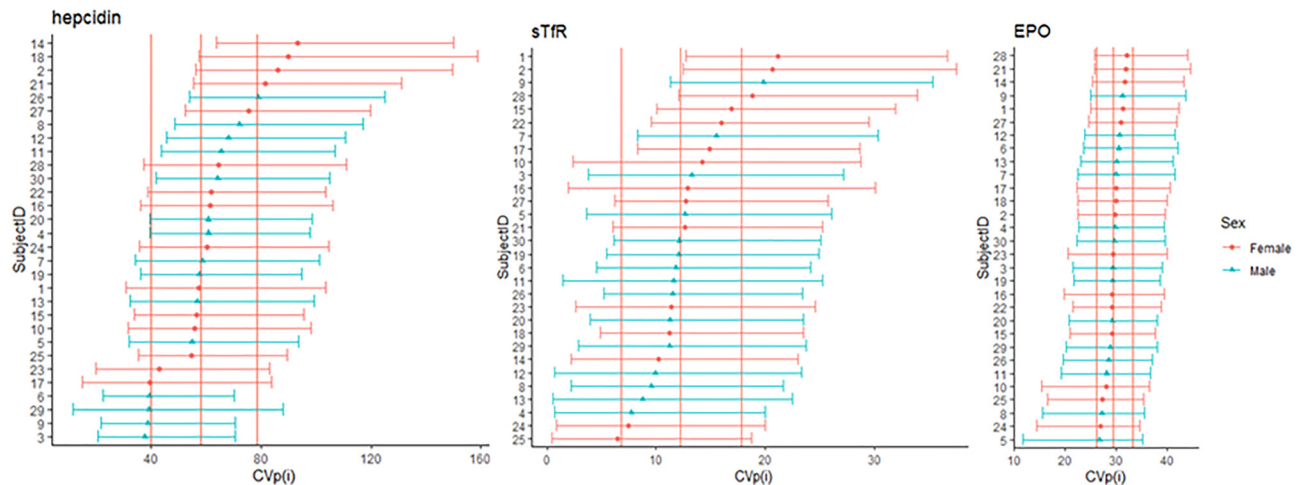


Figure 2: $CVp(i)$ of the athletes for hepcidin, sTfR, soluble transferrin receptor; EPO, erythropoietin. Circles indicate males and triangles females.

(Figure 4). Thus, a common $CVp(i)$ based on a central value (mean) would not represent all the included subjects for hepcidin. This also fits very well with the results of the CV-ANOVA analysis where 18.6 % of the results were excluded as outliers (Table 1), indicating that to obtain data homogeneity, required for CV-ANOVA analysis, was difficult. For sTfR, the HBR was also higher (43.1 and 34.4 for women and men, respectively) than the respective cut-offs (Table 2), indicating non-homogenous distribution for both subgroups. For sEPO, on the other hand, the $CVp(i)$ distribution was homogeneous for both subgroups. The HBR was, nevertheless, higher in women than in men (18 and 12.5, respectively), indicating a wider distribution, as illustrated in Figure 3. The heterogeneity observed for the individual $CVp(i)$ indicates that it may not be appropriate to use a mean $CVp(i)$ for hepcidin and sTfR for the different BV applications such as APS or RCV. Instead, using a higher or lower percentile of the $CVp(i)$ may be more appropriate, depending on the clinical situation.

Soluble transferrin receptor

sTfR is especially useful in the work-up of anemia in athletes to differentiate between iron deficiency and anemia of chronic disease. Inflammatory processes linked to exercise have previously been shown to inhibit iron absorption [18]. The BV estimates for sTfR were significantly higher in athletes (10.3 % (95 % CI; 8.8–12.0)) than those published for a healthy, general population (6.95 % (6.5–7.3)) [30]. These results could be explained by a higher iron demand in endurance athletes [18, 31]. However, these two studies are not directly comparable, as they had different study designs; weekly samplings over 10 weeks [30] and monthly samplings over one year in our study. Therefore, further study may be necessary to clarify differences between general and athlete populations for sTfR. Furthermore, previous studies in healthy individuals applied classical methods and there needs to be knowledge on whether the heterogeneous BV

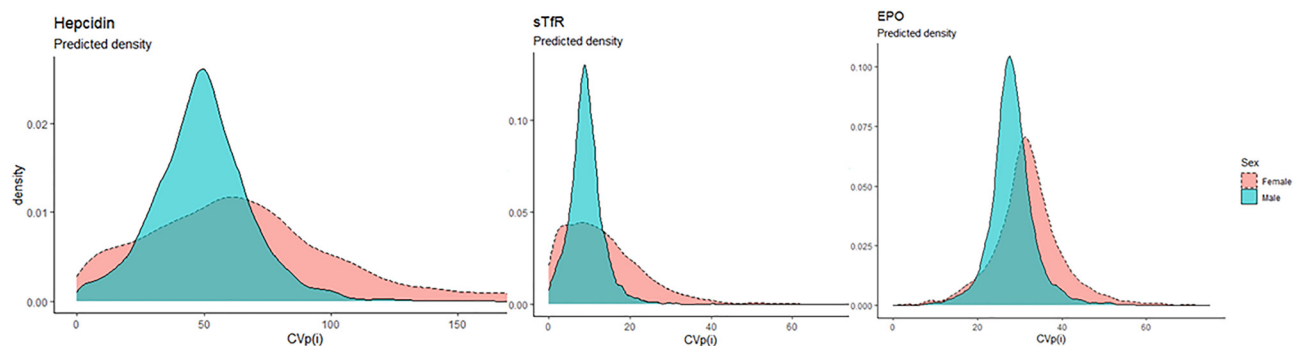


Figure 3: Distribution of the $CVp(i)$ of the athletes for hepcidin, sTfR, soluble transferrin receptor; EPO, erythropoietin. Dashed lines represent -females and continuous lines -males.

data distribution of sTfR is specific for athlete populations or also a feature also in the general population.

Hepcidin

There is generally little published BV data for hepcidin, and only one previous BV study, based on within-day samplings, performed in a healthy population could be identified [32]. The CV_I of 30.3 % reported by Krook et al. in this study [32], derived by ANOVA, was lower than the estimate observed in our study (51.9 %). However, the different sampling intervals make direct comparisons difficult. Two publications have reported BV of hepcidin in hemodialysis patients [26, 33]. In these studies the study duration was two and six weeks, respectively [26, 33] and the CV_I estimate reported were much lower (23 % [17–28]) than that obtained for the athletes in this study. Previously, we have assessed BV in this cohort for other iron markers by classical methods (ANOVA). However, for serum ferritin, data were so heterogeneously distributed that we did not estimate the BV [13]. When applying a Bayesian model on serum ferritin, the BV in women for ferritin was very heterogeneously distributed, with a HBR of 51.8, greater than that for men (37.1) (Supplementary Figure 4). Similarly, we also found a high predicted $CV_{p(t)}$ (63.1 % for females and 49.2 % for males) for ferritin suggesting that both markers may not be under homeostatic control or at least highly influenced by inflammatory mechanisms in athletes [15]. Hepcidin, in combination with other iron markers (iron, transferrin, ferritin and erythroferrone) [34], has been suggested used to detect autologous transfusions – used for doping purposes because its concentration clearly peaks up one day after blood transfusion [35]. However, as demonstrated by our study, hepcidin results show great variability. Therefore, serial results for these measurands (ferritin and hepcidin) would be difficult to interpret in endurance athletes.

Erythropoietin

No study reporting BV data for EPO has been published in the general population or athletes. Thus, this is the first study that reports BV estimates for sEPO. EPO is well known to stimulate the production of red blood cells and due to its performance-enhancing effect, the use of recombinant human EPO (rHuEPO) is prohibited by the World Anti-Doping Agency (WADA). Direct and indirect methods have been developed to detect EPO misuse, especially when rHuEPO microdoses are administered [36]. Among the available methods are different methodologies that attempt to measure EPO directly. However, to detect

rHuEPO microdoses administration, one of the suggested methods is the Athlete Biological Passport (ABP). The ABP is a tool used by WADA to monitor selected biomarkers (hemoglobin and reticulocytes among others) over time that flags variations not compatible with a normal physiological condition, bringing out a possible drug misuse. Clark et al. reported changes in performance, physiology, and blood markers included in the hematology ABP module, especially after high dosing followed by microdosed of rHuEPO [37]. However, the ABP utility for this purpose still needs to be demonstrated. Previous publications have reported that sEPO is affected by confounding factors [38]. However, our results indicate that the CV_I of sEPO was apparently not affected by physical activity, duration or intensity. Furthermore, the $CV_{p(t)}$ estimates were homogeneously distributed and the II relatively low (0.7), which suggest that sEPO could be a potential candidate to be included in the ABP strategy to detect rHuEPO misuse. The immunoassay used in this study (Immulite 2000 Xpi analyzer) showed an acceptable performance ($CV_A=10.2\%$), fulfilling the APS for imprecision of $0.5 \times CV_I$ of about 15 %. Measured by chemiluminescence, circulating EPO levels determination is relatively inexpensive compared with other methods currently in use to detect EPO in urine (isoelectric focusing or sodium dodecylsulfate polyacrylamide gel electrophoresis) [39], and the assay procedure is fully automated. Thus, this marker could be a candidate to add to WADA strategies for detecting rHuEPO misuse and may be considered included in the ABP module as a complementary method to urine EPO detection for increasing the sensitivity. However, further study to evaluate its performance for this purpose must be performed prior to implementation.

Interleukin-6

BV data for IL-6 could not be estimated, as the assay used to measure IL-6 was not sensitive enough to quantify the low concentrations of the athletes included in our study, indicating that any inflammatory process related to exercise is not pronounced by the increase of this measurand. We, however, observed that one of the subjects had detectable values in all the collected samples (range 14.8–37.3 ng/L). Further investigation revealed that this subject had been diagnosed with a mild psoriasis, not mentioned in the recruitment questionnaire, which could likely explain the results. Results for this participant for sTfR, hepcidin and EPO were within the reference intervals.

Study limitations

For the Bayesian model, no prior information on BV components could be identified for EPO in the literature.

Therefore, we used the results from the CV-ANOVA and ANOVA analysis derived from the same cohort to set the hyperparameters, however, giving them low credibility. The inclusion of a control group into our study, consisting of non-athletes using the same sampling period, would have been useful to better assess differences in BV in iron and inflammatory markers between athletes and the general population.

Conclusions

In this study, assessing BV in a cohort of well-characterized endurance athletes, we provide BV data for EPO, which were previously unavailable. These results indicate that EPO is not apparently affected by physical activity, duration or intensity. The application of the Bayesian method to deliver BV data illustrate that for hepcidin and sTfR, BV data are heterogeneously distributed and the application of a mean BV estimate may not be appropriate. Furthermore, our data indicate that hepcidin in athletes may not be under strict homeostatic control in athletes, which must be taken into account when interpreting serial test results.

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