The European Biological Variation Study (EuBIVAS)

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The European biological variation study (EuBIVAS): Biological variation data for testosterone, follicle stimulating hormone, prolactin, luteinizing hormone and dehydroepiandrosterone sulfate in men

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ABSTRACT

Background: Knowledge of biological variation (BV) of hormones is essential for interpretation of laboratory tests and for diagnostics of endocrinological and reproductive diseases. There is a lack of robust BV data for many hormones in men.
Methods: We used serum samples collected weekly over 10 weeks from the European Biological Variation Study (EuBIVAS) to determine BV of testosterone, follicle-stimulating hormone (FSH), prolactin, luteinizing hormone (LH) and dehydroepiandrosterone sulfate (DHEA-S) in 38 men. We derived within-subject (CV_I) and between-subject (CV_G) BV estimates by CV-ANOVA after trend, outlier, and homogeneity analysis and calculated reference change values, index of individuality (II), and analytical performance specifications.
Results: The CV_I estimates were 10 % for testosterone, 8 % for FSH, 13 % for prolactin, 22 % for LH, and 9 % for DHEA-S, respectively. The IIs ranged between 0.14 for FSH to 0.66 for LH, indicating high individuality.
Conclusions: In this study, we have used samples from the highly powered EuBIVAS study to derive BV estimates for testosterone, FSH, prolactin, LH and DHEA-S in men. Our data confirm previously published BV estimates of testosterone, FSH and LH. For prolactin and DHEA-S BV data for men are reported for the first time.

1. Introduction

The European Biological Variation Study (EuBIVAS) [1,2] is a highly powered biological variation (BV) study including subjects from five different European countries, which has delivered high-quality BV estimates for a large number of measurands. These data can be applied to establish analytical performance specifications (APS) [3,4], as well as reference change values (RCV) [5] to assess clinical significance of a change in laboratory results when monitoring a patient over time, index of individuality (II) and personalized reference intervals. All established

Abbreviations: APS, analytical performance specification; B_{APS}, analytical performance specification for bias; BIVAC, Biological Variation Data Critical Appraisal Checklist; BV, biological variation; CV_{APS}, analytical performance specification for imprecision; CV_I, within-subject biological variation; CV_G, between-subject biological variation; DHEA-S, dehydroepiandrosterone sulfate; EFLM, European Federation of Clinical Chemistry and Laboratory Medicine; EuBIVAS, European Biological Variation Study; FSH, follicle stimulating hormone; II, index of individuality; LH, luteinizing hormone; RCV, reference change value; QA, quality assurance.

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EuBIVAS estimates are based on standardized pre-analytical procedures, rigorous statistical analysis [6], and a representative group of healthy volunteers [7], and are derived by a methodology conforming to the Biological Variation Data Critical Appraisal Checklist (BIVAC) [8].

When deriving BV components, study subjects must be in steady state or data must be transformed prior to calculation [9]. In endocrinology, steady state has a different meaning than in many other fields of medicine, which may make the application of RCV less straight forward. This is because many hormones are governed by a daily, monthly, or other hormonal cycle. Therefore, a standardized time of phlebotomy is particularly important when studying BV of hormone markers. Hormone concentrations can also be influenced by medication, either with the purpose of stabilizing or supplementing hormone levels, or as side effects of medication. When laboratory results show pronounced changes, for example those in hormonal imbalance, application of RCV is unnecessary. However, for certain patient groups RCVs may be a valuable tool, such as when monitoring prolactin after removing a prolactinoma [10]. RCV calculated from chemically castrated prostate cancer patients can also be used for monitoring testosterone in these patients after the treatment [11].

In this study, we have investigated the BV of testosterone, follicle-stimulating hormone (FSH), prolactin, luteinizing hormone (LH) and dehydroepiandrosterone sulfate (DHEA-S) in men. In men, analysis of testosterone is most often performed in patients who present with symptoms of low testosterone, or to monitor the effects of testosterone supplementation. FSH and LH are mostly assessed in women to evaluate menopause, polycystic ovary syndrome or fertility. In men, however, these tests are ordered primarily with the purpose of differentiating primary and secondary hypogonadism [12]. If patients with symptoms such as reduced libido, erectile dysfunction or reduced muscle strength are found to have low serum testosterone, this condition is diagnosed as hypogonadism. In primary hypogonadism (testicular failure) serum LH and FSH concentrations are elevated while they are low or normal in secondary hypogonadism (defects in the hypothalamus or anterior pituitary) [13]. Hyperprolactinemia is a common side-effect of medication, and prolactinomas have a high incidence among pituitary tumors. After medical or surgical treatment of prolactinoma, patients will be monitored to ensure normalization of prolactin levels. Adrenal DHEA-S is one of the most abundant steroids in the circulation. Following adrenarche, DHEA-S levels increase until the age of 20 years and then decreases steadily to around 20 % of peak concentration at the age of > 70 years [14,15]. In men, DHEA-S measurement is used for diagnosis of hyperandrogenism, congenital adrenal hyperplasia and androgen-producing tumors [16].

There are, for most of these markers, only a limited number of BV studies performed in male populations available. The aims of this study were therefore to derive BV estimates of these hormones in men utilizing samples from the fully BIVAC-compliant highly powered EuBIVAS and to investigate the influence of age, nationality, or BMI on hormone concentrations.

2. Materials and methods

2.1. Population and samples

This study employed 370 samples collected from 38 apparently healthy individuals, who self-reported to be men (median age 35 years, range 22–59 years), collected during 2015 in six laboratories situated in Italy, Norway, Spain, the Netherlands, and Turkey. A previous study describes the study protocol and demographic characteristics, health status, exclusion and inclusion criteria of subjects enrolled in the EuBIVAS in detail [2,7]. Fasting blood samples were collected during ten consecutive weeks into 10 mL serum tubes with clot activator (plastic, silicone coated, Becton Dickinson, USA). We did not use gel separator tubes to avoid testosterone quantification interferences [17]. Samples were centrifuged at 3000*g* for ten minutes within one hour of sampling. Serum was aliquoted and frozen at −80 °C and sent frozen on dry ice to the coordinating center (San Raffaele Hospital, Milan, Italy).

2.2. Hormone assays

The samples were thawed and mixed thoroughly prior to analysis on a Cobas® e801 analyzer (Roche Diagnostics International Ltd., Rotkreuz, Switzerland) during December 2017–January 2018. Immunoassay Electrochemiluminescence (ECLIA) reagents and calibrators used for testosterone, FHS, prolactin, LH and DHEA-S are described in Supplemental Table 1. For each subject, samples were analyzed in duplicate within the same analytical run.

The assays employed are in routine use for laboratory diagnostics. For quality management we used two level Precicontrol Universal for Roche Elecsys quality assurance (QA) samples (Roche Diagnostics). No changes in the concentrations of QA samples were detected during this study (Supplemental Table 2).

2.3. Data analysis

Data analyses have been previously described in detail [18,19]. Briefly, analytical variation (CV_A) and within-subject biological variation (CV_I) estimates were obtained by using an ANOVA method based on CV-transformation of data (CV-ANOVA) [8]. Outliers were identified and removed to obtain CV_A and CV_I homogeneity that was verified by the Bartlett test and the Cochran test, respectively. Linear regression of mean group value (n = 10) over the study period for each hormone was used to confirm steady state of the participants. If participants were not in a steady state *i.e.,* a trend was identified, the data was adjusted by applying the inverse of the regression formula ([measured concentration-(S-1) x A] where S is the week number and A is the slope) to all measurement results at each week [20]. ANOVA on the natural log-transformed data delivered between-subject biological variation (CV_G) estimates. The presence of outliers between subjects and normality was first assessed by using the Dixon q-test and the Shapiro-Wilk test, respectively. To test for differences between hormone concentrations and nationalities, and correlation between age or body mass index (BMI), we used Wilcoxon-Mann-Whitney and Spearman's correlation tests, respectively. All analyses were performed using either Microsoft Excel 2016 or Excel for Microsoft 365 and Analyse-it for Microsoft Excel 4.81.1 (Analyse-it software Ltd, http://www.analyse-it.com).

Desirable APS for the analytical imprecision (CV_{APS}) and analytical bias (B_{APS}) were calculated according to

$$CV_{APS} = 0.5 \times CV_I$$

$$B_{APS} = 0.25 \sqrt{(CV_I^2 + CV_G^2)}$$

II was calculated according to

$$II = CV_I/CV_G$$

RCVs were estimated for an increase and a decrease using Z value of 1.65 for the probability level of significant change set at 95 % employing the EFLM BV database calculator (https://biologicalvariation.eu/meta_calculations) that is based on the formulas below, applying CV_A estimates based on duplicate measurement of study samples from all subjects:

$$SD(A,log)^2 = log\_e\ (CV_A^2 + 1)$$

$$SD(I,log)^2 = log\_e\ (CV_I^2 + 1)$$

$$SD(combined,log) = \sqrt{(SD(A,log)^2 + SD(I,log)^2)}$$

$$RCV\% = 100\ \% \times (exp((\pm Z \times \sqrt{2} \times SD(combined, log)))\text{-}1)$$

where Z = 1.65 for the probability level of significant change set at 95 %.

2.4. Ethics

This study was approved by the Institutional Ethical Review Board of San Raffaele Hospital, Milan, Italy in agreement with the World Medical Association Declaration of Helsinki and by the Ethical Board/Regional Ethics Committee as relevant for each involved center. All participants signed informed consent.

3. Results

The number of subjects and results included in the estimation of BV estimates after exclusion of outliers are presented in Supplemental Table 3. The fraction of outliers for each hormone varied between 0.6 % – 2.3 %. We found an increasing trend for DHEA-S over the study period of ten weeks according to an equation Y = 7.286 (95 % CI 7.22–7.35) + 0.033 (95 % CI 0.022–0.043)X, where Y is the mean DHEA-S (μmol/L) of all individuals per sampling and X is the sampling week number (Supplemental Fig. 1). To counteract the effect of the trend, the data was corrected at each time point before calculating the BV measures.

In Fig. 1, box plots of serum hormone concentrations in each study participant are displayed. The individual median concentrations of testosterone, FSH, prolactin, LH and DHEA-S varied between 9.7 and 25.7 nmol/L, 1.6 – 10.7 IU/L, 105.2 – 473.7 mIU/L, 2.2 – 9.9 IU/L and 2.9 – 14.5 μmol/L, respectively. The mean testosterone concentration was 18.2 nmol/L (95 % CI 17.9 – 18.5 nmol/L). In one study subject (29 years, BMI 29.4), the concentration was 9.7 nmol/L (Fig. 1A). All hormones and other laboratory tests (glucose, creatinine, cholesterol, triglycerides, gamma-glutamyltransferase, alanine aminotransferase, creatine kinase, C-reactive protein) were within the respective reference range. For FSH, the mean concentration was 4.5 IU/L (95 % CI 4.7 – 5.0 IU/L). However, in three men (54, 29 and 26 years) the mean FSH concentrations were 9.3 IU/L, 10.2 IU/L and 10.6 IU/L, respectively (Fig. 1B). These men had BMIs of 29.4, 18.1 and 19.5, respectively. Likewise, mean LH concentration in all men was 4.8 IU/L (95 % CI 4.7 – 5.0 IU/L). In two men (32 and 28 years) the mean LH concentrations were 8.9 IU/L and 10.4 IU/L, respectively (Fig. 1D), which are above the upper reference limit of 8.6 IU/L. The latter was a smoker and had an elevated CK concentration at sampling week #5. There was no clinical information that could explain these minor deviations in hormone concentrations. No significant differences in hormone concentrations

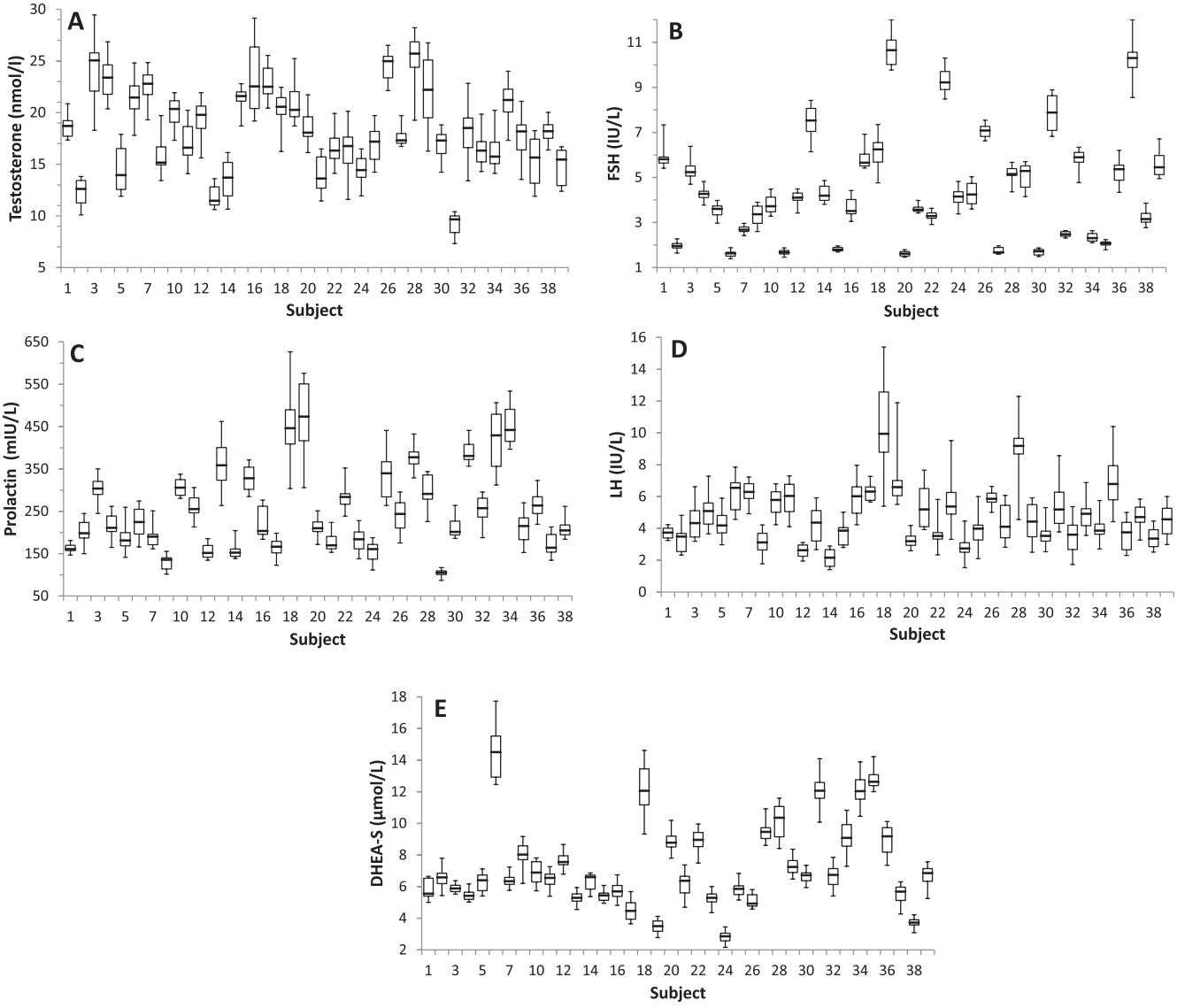

Fig. 1. Hormone concentrations in each study subject. Box plot of serum testosterone (A), follicle-stimulating hormone (FSH) (B), prolactin (C), luteinizing hormone (LH) (D), and dehydroepiandrosterone sulfate (DHEA-S) (E) concentrations in each study subject. The box denotes the interquartile range (IQR), and the bar denotes median for each subject, while the whiskers extend to the furthest observations within +/- 1.5 x IQR of the 1st or 3rd quartile.

between the nationalities were found ($p > 0.07$). There was no significant correlation between hormone concentrations and age ($p > 0.05$) or BMI ($p > 0.18$). For testosterone the correlation between concentration and age was close to significance (Spearman's rs −0.319, 95 % CI −0.586 – 0.010) with a p-value of 0.0506.

The CV_A of all assays was ≤ 3.4 %. The mean concentrations, BV estimates with 95 % CIs, II, CV_{APS}, B_{APS} and RCV% are presented in Table 1. The CV_I was the lowest for FSH (8.1 %, 95 % CI 7.5–8.7 %), for DHEA-S it was 8.6 % (95 % CI 7.9–9.3 %), for testosterone 10.2 % (95 % CI 9.5–11.1 %), for prolactin 13.3 % (95 % CI 12.3–14.4 %), and the highest for LH (21.9 %, 95 % CI 20.2–23.6 %). The RCV depends on CV_I and CV_A. Thus, the limits for significant differences between repeated analyses of these hormones increased accordingly from −17.5 % and 21.2 % (decrease and increase) for FSH to −39.8 % and 66.2 % for LH, respectively. CV_G was the lowest for testosterone (20.4 %, 95 % CI 16.8–26.9 %) and the highest for FSH (58.9 %, 95 % CI 46.4–79.8 %). The II for all hormones was low (<0.66) indicating high individuality (Table 1).

4. Discussion

In laboratory diagnostics, there is a need for well characterized BV data that can be applied meaningfully to the target populations. Differences between data sets to produce BV estimates may be due to physiology, pathology, and methodology. They all should be understood in terms of impact on efficacy of the clinical applications of BV data. Previous studies of hormone BV in men differ in the number and age of included study subjects, sampling intervals, study duration, pre-analytical phase and statistical approaches. Apart from the study of Collier *et al.* [21] (n = 87) most previous studies included a lower number of participants (n = 13–20) than our study. Andersson *et al.* [22] collected samples monthly during a 17-month period, while Collier *et al.* [21] collected samples twice, four weeks apart. In the study of Collier *et al.* the individuals were > 50 years of age [21] whereas in the study of Andersson *et al.* they were 20–40 years [22]. Only the study of Maes *et al.* [23] and Orentreich *et al.* [15] report using samples after overnight fasting. In this study, we report robust CV_I and CV_G estimates for testosterone, FSH, LH, and for the first time, for prolactin and DHEA-S in men, based on a CV-ANOVA approach and fasting serum samples collected from thirty-eight 22–59-year-old individuals over a ten-week period.

When considering BV, testosterone is the most studied sexual hormone in men [21–26], as expected considering its clinical utility. We found that the CV_I and CV_G estimates for testosterone were 10.2 % (95 % CI 9.5–11.1 %) and 20.4 % (95 % CI 16.8–26.9 %), respectively, based on the immunoassay results of the men included in the EuBIVAS. This is lower than most other previous studies, which have reported CV_I's, ranging from 10.9 to 17.6 % and CV_G's from 17 − 40.8 % for men (n = 15–27) [21–24]. In our study, we collected weekly fasting morning samples, whereas most other studies have applied different collection protocols. Based on samples drawn at noon from 15 men aged 21–63 years, at five different time points four weeks apart, van der Veen *et al.* reported a CV_I of 13.5 % (95 % CI 11.4–15.6 %) and CV_G 15.1 % [25]. A recent study by Røys *et al.* used different approaches to calculate CV_I's and CV_G's for sex hormones and adrenal steroids in non-fasting samples collected from 14 men aged 18–75 years [26]. They reported higher CV_I's of 15 % (95 % CI 13–18 %) and 14 % (95 % CI 12–16 %) for testosterone by ANOVA and Bayesian approaches, respectively, than those in our study. In addition, they estimated CV_I by the indirect method based on result ratios derived from laboratory databases as 17 % (95 % CI not reported). For the two first approaches *i.e.,* the direct methods, our studies share the same sample intervals (ten consecutive weeks) but differ by the fact that Røys *et al.* used in-house mass spectrometric assay and samples from non-fasting individuals. It is known that for testosterone there is a postprandial effect [27], which may affect both the CV_I and CV_G and thus, BV for testosterone is best estimated

Table 1
Mean values, analytical variation (CV_A), within-subject (CV_I) and between-subject (CV_G) biological variation estimates with 95% CIs, index of individuality, analytical performance specifications (APS) for imprecision (CV_{APS}) and bias (B_{APS}), and reference change values (RCV) for serum testosterone, follicle-stimulating hormone (FSH), prolactin, luteinizing hormone (LH) and dehydroepiandrosterone sulfate (DHEA-S) in men.

Measurand	Number of individuals	Total number of results	Mean number of samples/ individual	Mean number of replicates/ sample	Mean value (95% CI)	CV_A % (95% CI)[1]	CV_I % (95% CI)	CV_G% (95% CI)	Index of individuality [2]	CV_{APS}%[3]	B_{APS}%[4]	RCV% (decrease/ increase)[5]
Testosterone	38	714	9.39	2.00	18.25 nmol/L (17.91–18.60)	2.01 (1.9–2.2)	10.20 (9.5–11.1)	20.37 (16.8–26.9)	0.50	8.05	42,705	−21.4/27.2
FSH	38	731	9.66	1.98	4.46 IU/L (4.26–4.66)	2.20 (2.1–2.4)	8.05 (7.5–8.7)	58.88 (46.4–79.8)	0.14	4.03	29.71	−17.5/21.2
Prolactin	37	685	9.41	1.94	258.72 mIU/L (249.8–267.9)	1.20 (1.1–1.3)	13.34 (12.3–14.4)	39.37 (30.77–51.33)	0.34	6.67	20.78	−26.5/36.1
LH	38	713	9.47	1.96	4.84 IU/L (4.67–5.01)	3.35 (3.1–3.6)	21.86 (20.2–23.6)	33.15 (26.67–44.55)	0.66	10.93	19.85	−39.8/66.2
DHEA-S	38	728	9.61	1.99	7.32 μmol/L (7.09–7.55)	3.38 (3.2–3.6)	8.55 (7.9–9.3)	37.64 (29.99–49.37)	0.23	4.28	19.30	−19.3/23.9

[1] Analytical variation (CV_A) estimates were based on CV-ANOVA of duplicate analysis of all study samples.
[2] Index of individuality = CV_I/CV_G.
[3] $CV_{APS} = 0.50\ CV_I$.
[4] $B_{APS} = 0.25\ (CV_I^2 + CV_G^2)^{0.5}$.
[5] RCV were calculated delivering asymmetric values for rise and fall at the probability level of 95% for significant unidirectional change, applying CV_A estimates based on duplicate measurement of all study samples.

under standardized, fasting sampling conditions. The CV_I and CV_G of our study are similar to those of Valero-Politi and Fuentes-Arderiu [24] who studied samples from 20 men aged 26–47 years during 12 months and are among the lowest of those published.

In our study, the CV_I and CV_G estimates derived for men FSH were 8.1 % (95 % CI 7.5–8.7 %) and 59 % (95 % CI 46–80 %), respectively. These are in line with the CV_I's and CV_G's in the above mentioned study of Røys *et al.* by the ANOVA and Bayesian approaches [26] Similarly, for LH we obtained CV_I and CV_G estimates of 22 % (95 % CI 20–24 %) and 33 % (95 % CI 27–45 %), respectively. These too, are close to those published by Røys *et al.* by the two direct methods, except that the CV_G was clearly lower (4.9 %) by the Bayesian method [26]. Two other publications [22,24] with younger study populations (20–40 years and 26–47 years) report similar estimates for FSH and LH based on analysis of samples collected monthly during 12–17 months compared to samples collected during ten consecutive weeks in our study and in that of Røys *et al.* [26].

No prior research has specifically addressed the BV of prolactin in male populations. While one study included men in their investigation of prolactin BV, it did not conduct a subgroup analysis [23]. The reported estimates included the entire study population comprising 13 men and 13 women, reporting a CV_I 39 % and CV_G 65 %. In contrast, our study yielded lower CV_I and CV_G estimates *i.e.,* 13 % and 39 %, respectively. Another study focused on BV of prolactin in women (n = 21) [28] reporting CV_I and CV_G estimates of 20 % and 49 %. Given the established knowledge that prolactin concentrations are higher and the reference interval is wider in women than in men, and the menstrual cycle's influence, where prolactin peaks in the luteal phase compared to the follicular phase [29], may explain the higher BV estimates for women or combined genders than for men alone. Regarding DHEA-S in men, there is a lack of high-quality published data on BV. One longitudinal study examining weekly samples from four men (36–59 years) disclosed individual variability of 19 % for DHEA-S [15]. Based on our data, the calculated CV_I is markedly lower at 8.6 % (95 % CI 7.9–9.3 %).

In our investigation, we collected samples spanning a ten-week period from week 13 to 25 [7]. Consequently, any potential circannual rhythms, if present, would minimally impact the presented BV estimates. Notably, we observed an increasing trend in DHEA-S concentrations within our study population over the study period. While cyclic seasonal variation in DHEA-S concentration has been documented in women (n = 21) [28,30], a prospective study involving 1421 women or 1540 men found no discernible monthly, seasonal or annual rhythmicity [31]. The underlying reason for the observed trend in our study remains unclear. Annual rhythmic variations have been reported for testosterone [32–34], LH [32,34], and FSH [33]. A retrospective, large-scale data analysis involving results from 7491 men analyzed over nine years suggested circannual rhythms for testosterone and LH, but not for FSH [34]. While we collected our samples in the springtime, Røys *et al.* collected the samples in autumn [26]. An eventual circannual rhythm of testosterone could therefore explain the differences in the estimates observed. Conversely, other studies reported no evidence of circannual rhythms for testosterone [23], LH [22,33], prolactin [23] or FSH [22,34]. Contradictory findings on circannual rhythm in testosterone, FSH and LH concentrations in early studies have also been noted by Valero-Politi and Fuentes-Arderiu [33]. In summary, the seasonal variation of these hormones remains a subject requiring further elucidation, including its potential impact on the presented BV estimates.

All the hormones examined in this study exhibited low II values, with testosterone at 0.50, FSH at 0.14, prolactin at 0.34, LH at 0.66, and DHEA-S at 0.23. This underscores the importance of considering BV and the resulting RCVs alongside population-based reference intervals when interpreting laboratory test results over time for patient monitoring. Notably, only LH showed an II slightly above 0.6. An II between 0.6 and 1.6 indicates that population-based reference intervals may be of some diagnostical utility. Our study therefore emphasizes the potential for clinical utility of RCV and potentially personalized reference intervals as valuable tools for assessment of the significance of changes in serial laboratory test results for an individual. However, it is important to note, that the presented BV data may not be applicable to women due to the influence of the menstrual cycle, particularly on LH, FSH and PRL concentrations. In addition, hormone concentrations in women and children differ from those in men, *e.g.* testosterone concentrations are significantly higher in men. Thus, further research on BV of hormones in women and children is needed.

Based on the results from our study, desirable APS for the analytical imprecision was the lowest for FSH (4,0 %), followed by DHEA-S (4.3 %) and testosterone (5.1 %). The presented CV_{APS}'s are based on duplicate measurements of each sample. A previous study relied on CV_I and CV_G data published earlier, and derived CV_{APS} of 5.3 % for testosterone measurements [35]. Clinical laboratories measure hormones using a variety of technologies ranging from commercially available automated immunoassays to in-house mass spectrometric assays. Based on published reports it seems a challenge for some but not all assays to reach the required the BV derived CV_{APS} criteria. A comparison of five automated testosterone immunoassays revealed the highest total CV as 9 % [36], and for six automated FSH, LH and testosterone assays it varied between 2 and 25 % assessed using five sample pools [37].

In our study, well-defined inclusion and exclusion criteria were uniformly implemented across all six participating laboratories in five different countries to define our study population, and samples were collected under standardized fasting sampling conditions [7]. The occurrence of sample outliers for each hormone was low with a percentage not exceeding 2.3 %. Apart from DHEA-S, participants were in a steady-state. Adequate data transformation was applied to DHEA-S data before calculating the BV estimates. We conclude that samples collected in our study are highly appropriate for establishing the BV of hormones.

5. Conclusions

In this study, we present robust BV estimates for testosterone, FSH, prolactin, LH and DHEA-S, derived from high-quality data obtained through overnight fasting samples collected from 38 men representing five different countries in the EuBIVAS project. Our analysis revealed no significant variations in hormone concentrations based on nationality, age, or BMI. The presented data demonstrates BV estimates that align with or are lower than those in prior studies for testosterone, FSH and LH. Notably, our study introduces, for the first time, reliable BV estimates for prolactin and DHEA-S in men.

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CRediT authorship contribution statement

Outi Itkonen: . **Niels Jonker:** Writing – review & editing, Data curation. **Aasne K. Aarsand:** . **Sverre Sandberg:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Conceptualization. **Jorge Diaz-Garzon:** Writing – review & editing, Validation, Conceptualization. **Pilar Fernandez–Calle:** Writing – review & editing, Conceptualization. **Abdurrahman Coskun:** Writing – review & editing, Conceptualization. **William A. Bartlett:** Writing – review & editing. **Massimo Locatelli:** Writing – review & editing. **Anna Carobene:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2024.117806.

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