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Biological variation of venous acid-base status measurands in athletes

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ABSTRACT

Background: Analysis of acid-base status (ABS) is requested in a wide range of clinical scenarios, including in the assessment of athletes' performance and follow up, but there is a lack of high-quality biological variation (BV) data. The aims of this study were to estimate the BV of ABS related parameters in athletes and to evaluate if variables related to exercise may influence the estimates.

Material and methods: Eleven samples from 30 triathletes were drawn, on a monthly basis. The samples were measured for pH, pCO_2, bicarbonate, base excess, TCO_2, Ca^{2+} and lactate. A CV-ANOVA was performed to calculate within-subject (CV_I) estimates and a linear mixed model was applied to analyze the effect of the following variables on the BV; health status, sampling interval, intensity and duration of the exercise.

Results: For all ABS parameters except for lactate, higher CV_I estimates were found in athletes than what have been reported for the general population. No significant effect of the exercise and sampling related variables were observed, except for Ca^{2+}.

Conclusion: This difference founds in ABS parameters between athletes and the general population could be explained by the physiological stress during exercise. Laboratories attending this population could use these BV estimates to establish quality goals.

1. Introduction

Venous acid-base analysis is performed in a wide range of clinical scenarios to provide an evaluation of the acid-base status (ABS). These include inpatients in a critical care setting analysis of lactate concentrations to evaluate the severity of sepsis [1] and in outpatients, measurement of bicarbonate/lactate to assess the progression of renal impairment reflected in metabolic acidosis [2]. ABS is often used in a monitoring situation, where current results are compared with previous results. Thus, it is essential to have knowledge on the homeostatic regulation of these biomarkers and to have a tool to distinguish physiological changes in patients from analytical noise and normal biological variation.

Biological variation (BV) describes the variation in the concentration of a certain measurand around the homeostatic set point; within an individual (within-subject BV; CV_I) or between the homeostatic points from different individuals (between-subject BV; CV_G) [3]. BV data have many important applications in laboratory medicine. These encompass setting analytical performance specifications (APS) [4], to assess the individuality of a measurand by the index of individuality (II) [5], to interpret serial results in an individual by the reference change value (RCV) [5], and to deliver personalized reference intervals [6]. However, when applying BV estimates, they must be reliable and relevant to the population to which they will be applied. The European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) has published recommendations for the design and delivery of BV studies to obtain

Abbreviations: ABS, acid-base status; BV, biological variation; CV_I, within-individual biological variation; CV_G, between-individual biological variation; RCV, reference change value; EFLM, European Federation of Clinical Chemistry and Laboratory Medicine; BIVAC, Biological Variation Data Critical Appraisal Checklist; RI, biological reference interval; $[H^+]$, hydrogen ion concentration; pCO_2, partial pressure of carbon dioxide; TCO_2, total carbon dioxide; Ca^{2+}, ionized calcium; CI, confidence interval; VO_2max, maximum oxygen intake; AT, aerobic threshold; ANT, anaerobic threshold; HR, heart rate; IQR, interquartile range; LMM, linear mixed model; CV_A, Imprecision; II, index of individuality.

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robust BV estimates [7] and the Biological Variation Data Critical Appraisal Checklist (BIVAC) [8] which is a tool for critically assessing BV studies by their quality design and methodology. A *meta*-analysis with BV estimates for many measurands is published in an EFLM database [9]. However, as of today, there are a very limited number of BV studies for ABS measurands.

In athletes, the ABS is used to derive lactate thresholds during a stress test. These thresholds are considered highly reliable to identify the workload at the transition from aerobic to anaerobic metabolism as an alternative to the ventilation thresholds [10].

Recently, lactate has also been suggested as a potential marker to detect overtraining syndrome [11], when following athletes over time, probably because an increase in lactate concentrations indicates an unbalance in the metabolism that drives an acid accumulation. In these contexts, knowledge on BV of ABS measurands in athletes could help to understand the behavior of these measurands during athlete monitoring. However, to the best of our knowledge, there are no BV data for ABS available for athletes.

Furthermore, in athletes, oxygenation and acid-basic metabolism mechanisms could be influenced by exercise, creating an imbalance between production and clearance of acids from the anaerobic metabolism [12]. The concentration of these metabolites and related routes could therefore be subject to greater variations in athletes than in sedentary subjects, and consequently, higher BV estimates could be expected in athletes than in the general population. Furthermore, the type of training, type of exercise, intensity and duration could influence the individual results.

Additionally, it may be helpful to establish specific reference intervals (RI) for athletes. The standard way to assess an individual's result is to compare it to a RI based on reference population values [13]. The RI usually represents data from the general population and is not necessarily transferable to specific conditions such as pregnancy, obesity or in this case, athletes.

The aims of this study were to deliver BV data for the ABS related parameters in athletes following a BIVAC compliant study design and to evaluate variables related to exercise that could theoretically influence these BV estimates. Furthermore, we performed a systematic review of BV studies on ABS related measurands and verified the RI published for these markers in our athlete population.

2. Materials and Methods:

2.1. Bibliographic search

A literature search for BV studies on ABS related measurands (pH as hydrogen ion concentration ($[H^+]$), partial pressure of carbon dioxide (pCO_2), bicarbonate, base excess, and total carbon dioxide (TCO_2), ionized calcium (Ca^{2+}) and lactate) was conducted with cut-date November 30th, 2020). The search was performed in Pubmed, using as key terms [the measurand] in question with each of the following combinations: "within-subject*," "between-subject*, "within-person*," "between-person *," "interindividual*," and "intraindividual *, where the asterisk denotes "biological variation," "variation," "coefficient of variation," and "CV".

2.2. Study population

The study was presented in a formal meeting and by e-mail for three triathlon clubs from Madrid (Spain) and 32 subjects were recruited using the following inclusion criteria:

Absence of any physical injury or complete recovery in the previous 4 weeks, age > 18 years, > 13 h of training per week (Including running, cycling and/or swimming) and normal medical examination and normal stress tests, including electrocardiogram, spirometry and dynamometry, based on a sports medicine physician criteria.

Thereafter, the following exclusion criteria were applied:

History of cardiovascular, hematology, metabolic, thyroid, liver or kidney disease or a blood test results that indicated a chronic or acute disease, carrier state for HBV, HCV, and HIV, hospital inpatient the previous 4 weeks, blood donation in the previous 3 months or pregnancy or breastfeeding within one year. In the first visit, preliminary analysis was performed to check the recruited subjects' health status. This included; haemogram, glucose, creatinine, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, gamma-glutamyltransferase, alanine-amino-transaminase, aspartate-amino-transaminase, total and direct bilirubin, creatinine-phosphokinase, alkaline phosphatase, C-reactive protein, total proteins, thyroid stimulating hormone. Serum HIL index (haemolysis, icterus and lipaemia index) was performed on all samples. Two subjects were excluded, a male (hereditary spherocytosis) and a female (hypothyroidism), leaving 30 subjects enrolled in the study (15 females and 15 males), aged 19 to 53 years. During the study, two individuals decided to abandon the study voluntarily, but their results (six visits) were included in the calculations.

2.3. Stress test

During the stress test (continuous ramp until exhaustion), spirometry and ergometry were performed and the ECG and blood pressure were monitored. Besides, maximum oxygen intake (VO_2max) was measured representing the maximum amount of O_2 that the body can distribute to the tissues and which is the most representative performance indicator in athletes [14]. The aerobic (AT) and anaerobic (ANT) threshold are also good markers to know the type of metabolism that is being utilized to obtain energy and to derive workload zones [15]. The ANT is the limit from which the balance between the O_2 demand and supply is disrupted, resulting in a lactate accumulation. The heart rate (HR) and power were measured at the different thresholds.

2.4. Sample collections

In accordance with the Røraas simulation [16] and to assure a confidence interval (CI) width lower than 1/3 CV_I, eleven samples per subject from 30 subjects were drawn on a monthly basis (from January to November) at 8–10 am (Saturday morning) after ten hours fasting, with participants being instructed to avoid heavy dinner and not to participate in competition for one week and not to perform high intensity exercise 24 h prior to sampling. High intensity exercise was defined as a heart rate (HR) > 82% of their maximum HR [16]. A stringent protocol was applied including standardizing of the pre-analytical phase with venous blood collection being performed by the same phlebotomist for all visits after subjects rested 15 min in the seated position.

The subjects completed at each visit a follow-up questionnaire including questions on fasting, nutritional supplements and diet changes, disease status and medication, last competition time, last training time with information on type and intensity and for women, date of last menstruation.

2.5. Sample analysis

Blood samples were drawn with a calcium balanced lithium – heparin ABS analysis Syringe (Becton-Dickinson^R) and measured within a maximum of 2 min after blood collection, in duplicate on a POCT analyzer (ABL 90 Flex. Radiometer^R). The analysed measurands were pH, (converted to [H +]), pCO_2, bicarbonate, base excess, and TCO_2, Ca^{2+} and lactate. The ABS methodology was under the Point-of-care testing (POCT) - Requirements for quality and competence (ISO 22870:2016) accreditation requirements. An overview of the analytical methods is provided in Supplemental Table 1.

2.6. Statistical analysis

The median and interquartile range (IQR) for stress test variables and ABS measurands were calculated in the subgroups of males and females. We performed a Spearman correlation test between VO_{2max} (L Kg^{-1}), as the most representative marker of aerobic performance, and concentrations of ABS related measurands (with Bonferroni correction). We also used the median test to assess whether performing the exercise the day before (within 24 h) had any effect on the ABS related measurands concentration.

A trend analysis was performed, as recommended by the BV-WG EFLM [8]: for all measurands, the results of each subject were plotted against the collection dates to observe any trends inherent to the subject, such as disease, changes in life-habits, among others. Furthermore, the total results from all subjects were also plotted to discard trends such as preanalytical or analytical error, lot to lot shifts, seasonal variations etc. If the equation had an r coefficient>0.75 and the slope 95% confidence interval did not include the 0, the trend was considered significant and the results corrected by the linear equation factors. Then, we performed outlier analysis between replicates and between samples for each person using the Cochrane C test and between mean values of different subjects applying the Reed's criterion, as recommended by Fraser [3]. To obtain the BV estimates, we performed a CV-ANOVA for CV_I and log-ANOVA for the CV_G. To calculate the mean concentration, we used the first value from the duplicate analysis.

Additionally, to explore the potential influence of different factors on the BV, we evaluated the parameters as detailed below, by a linear mixed model (LMM) [17] by including these variables as fixed effects and the BV estimates as random effects. To assess the effect, we calculated the CV_I from the model with and without the variable that was assessed for, according to the script, and considering a difference of 5%, as significant:

- Model without variable: log(measurand) ~ (1|Subject) + (1| Subject: Visit)
- Model including the variable: log(measurand) ~ Variable + (1| Subject) + (1|Subject:Visit)

Variables were defined as:

- Moderate exercise in the previous 24 h (Yes/ No).
- Health status: illness recorded in the ongoing questionnaire in the last week (Yes/ No).
- Sampling interval: time since previous visit (number of days).

Variables related to the intensity of exercise (recorded the week before sampling):

1) Average HR during the training session classified by the percentage of the maximum HR, from T1 to T5: T1 (<65%); T2 (65–80%); T3 (80–90%); T4 (95–98%); T5 (100%) [18].
2) Average of subjective perception of effort, the Borg scale: 0–4 sleep or inactive; 5–7 absence of effort; 8–9 very, very light; 10 very light; 11–12 fairly light; 13–14 somewhat hard, 15–16 hard; 17–19 very hard; 20 maximum effort. [19]

- Variable related to the duration of the exercise: number of hours of endurance activity per week (h/w).
- The CV_I and CV_G estimates were used to determine APS for imprecision (CV_A) and bias [20] and the (RCV) [3], and the II [21] were calculated as follows: $CV_A = 0{,}5 \cdot (CV_I)$
- Bias = $0.5 \cdot (CV_I^2 + CV_G^2)^{1/2}$
- II = CV_I / CV_G
- RCV = $100\% \cdot (\exp(\pm Z \cdot 2^{1/2} \cdot ((CV_{LnA}^2 + CV_{LnI}^2))^{1/2}))$;

where CVLn refers to ln-transformed data = (Ln (1 + CV2))1/2 and "Z" refers to the Z-score equal to the number of standard deviations appropriate for the selected probability. The CV_A was derived from the sample results. The bias calculation is not in accordance with the theory of measurement uncertainty, but is used by many laboratories as a practical approach.

To verify the transferability of RI, the CLSI-EP 28-A3 [22] protocol was followed. For parameters that did not fulfill the CLSI criteria, RI was calculated using a robust method [23]. For the calculations, we used RStudio Desktop 1.3.1093, Analyze-it and Microsoft Excel 2010.

3. Results

The systematic search only identified two papers for BGA, one of them included pH, (converted to [H +]), pCO_2, bicarbonate, base excess, and TCO_2 estimates [24], and the other only pCO_2, not reporting BV estimates [25]. For the specific bicarbonate search, we identified 6 publications with BV data [26-30]. For lactate, three papers were identified, two in a healthy population [31,32] and one in ICU patients [33]. For Ca^{2+}, only one publication reporting CV_I estimates was found [29].

Data for demographics and stress test parameters of the 30 athletes included in the BV study are provided in Table 1. The median values of VO_2max, an indicator of exercise performance, were close to p90 from the general population for both sexes for the 30–40 years age range (51.7 L Kg^{-1} for male and 45.3 L Kg^{-1} for females) [34]. We found significant differences for Ca^{2+}, base excess, pCO_2 and TCO_2 between genders (Table 1, Supplemental Figure 1).

No trends were identified in the data, indicating that the athletes

Table 1
Median and interquartile range for measures derived from the stress test and the ABS related measurands grouped by gender. Significant differences ($p < 0.05$) were found between genders for BE, Ca^{2+}, pCO_2, TCO_2 and bicarbonate, as indicated by *, with higher results in males.

	Men				Women			
	Median	IQR			Median	IQR		
Age (years)	41	32.5	-	42.5	34	30	-	39
VO_{2max} (L Kg^{-1})	50.0	46.7	–	55.3	43.8	40.7	–	48.2
Power at VO_{2max} (W)	383	338	–	389	268	250	–	283
Power at anaerobic threshold (W)	212	195	–	244	135	123	–	156
Relative maximum power (W Kg-1)	5.1	4.9	–	5.6	4.7	4.1	–	4.9
Minimum heart rate (bpm)	62	55	–	70	72	61	–	85
Maximum heart rate (bpm)	174	166	–	181	175	169	–	180
Ventilation in VO_{2max} (L min^{-1})	146	141	–	163	110	87	–	124
Aerobic threshold (bpm)	137	131	–	143	133	129	–	135
Anaerobic threshold (bpm)	157	150	–	161	160	152	–	163
pH	7.36	7.34	–	7.38	7.36	7.34	–	7.39
pCO_2 * (mmHg)	52.6	49.3	–	55.6	48.3	44.6	–	52.6
TCO_2 *(mmol/L)	27.6	25.9	–	29.6	25.9	24.3	–	28.1
Ca^{2+} * (mmol/L)	1.23	1.21	–	1.24	1.21	1.19	–	1.23
Bicarbonate * (mmol/L)	4.84	4.61	–	5.0	4.51	4.31	–	4.72
Lactate (mmol/L)	0.80	0.60	–	1.00	0.80	0.6	–	1.10
Gap anion	11.90	10.9	–	12.7	11.80	11.0	–	12.5
Base excess*	3.0	2.0	–	3.8	1.35	0.20	–	2.6

were in a stable state over the study period. Results for lactate for all 11 samplings for the 30 athletes are displayed in Fig. 1. We found a negative correlation between VO_2max (L Kg^{-1}) and lactate concentration (r = -0.19, p = 0.006) and a positive correlation between VO_2max (L Kg^{-1}) and bicarbonate concentration (r = 0.12, p = 0.049). No significant correlations were observed (p > 0.05) for the other ABS parameters (data not shown).

There were no differences in ABS parameters from blood collections where athletes had indicated having performed moderate exercise the previous 24 h compared to collections where they indicated having rested (Supplemental Table 2 and Supplemental Figure 2).

CV_I results were similar between genders, except for base excess where the 95 %CI did not overlap (Table 2, Supplemental Table 3). All the ABS parameters were associated with a high II (>0.6) (Table 3). Regarding variables that potentially may affect the CV_I estimate, no differences were found except for Ca^{2+} (3%), where the CV_I decreased from 1.87 (1.69–2.07) to 1.81 (1.64–2.01) (p < 0.05), after including the variable "duration" (number of hours of endurance activity performed during the previous week to the blood collection) in the LMM. Suggested athlete specific RI are presented in Table 4.

4. Discussion

Little data on BV for ABS have been published, with current data for BGA parameters being derived from one only publication [24]. There, Harding and Fraser published BV estimates for pH, reported as $[H^+]$, partial (pCO_2), bicarbonate, base excess, and TCO_2 measured in capillary specimens of whole blood from a cohort of 14 healthy subjects. This study is graded as a BIVAC grade C in the EFLM Biological Variation Database [9]. Through the bibliographic search, we also found BV data for bicarbonate, lactate and Ca^{2+}, but all these publications were graded C as well [25-33]. So the BV data published for these measurands in the literature are very limited and not of high-quality. Moreover, no data for BV in athletes or other groups with a physiological state that can influence the BV estimates are available. For athletes, it is important to assess whether exercise-related factors may influence the BV estimates.

4.1. Acid-base status parameters in athletes

The athletes, probably due to the adaptation to "their physiological condition" i.e. a high-exercise regime, showed different concentrations of metabolites than those found in the general, more sedate, population. They could also have a higher clearance rate to compensate for the high production rate of metabolic products to maintain the homeostatic balance. As we can see in Table 4, our results indicate that ABS parameters are higher than those of the general population, indicating that athlete-specific RI may be relevant to consider.

Correlation analysis between factors that potentially may influence the concentrations of venous ABS parameters identified a negative correlation between lactate and VO_2max and a positive correlation between VO_2max and bicarbonate. This finding could be explained by the greater VO_2max in athletes, caused by a more efficient distribution of the oxygen in the body leading to a lower level of anaerobic metabolite accumulation and a greater buffer capacity for lactate.

We observed significantly higher values for Ca^{2+}, EB, TCO_2, and bicarbonate in males than females. Therefore, it seems that although men have higher concentrations of metabolic products, they maintain the pH within the RI.

4.2. Biological variation in athletes

BV estimates for ABS parameters are particularly of interest in high-endurance athletes where oxygenation and acid-base metabolism mechanisms could be influenced by the type of training, exercise type, intensity and duration of exercise. Consequently, this could lead to different BV estimates from those observed in the general population.

Compared to BV estimates published by Fraser, where capillary blood was used [24], we observed higher CV_I and CV_G estimates for pCO_2, TCO_2 and pH in a ratio scale (H^+) derived from the venous blood gas analysis, (Table 2). This finding could be explained by the increased production and clearance of metabolism products due to the exercise practice in athletes [35]. However, it should be taken into account that differences between sample material (venous and capillary blood) or study design may explain this finding.

No differences in CV_I estimates were observed between genders, except in the case of base excess. The CV_I for male athletes (46.5%) was much lower than the CV_I for female athletes (90.1%), which was closer to the CV_I estimate published for the general population (76.4%). However, the CV_G in athletes (both genders) was similar to the general population (Table 2).

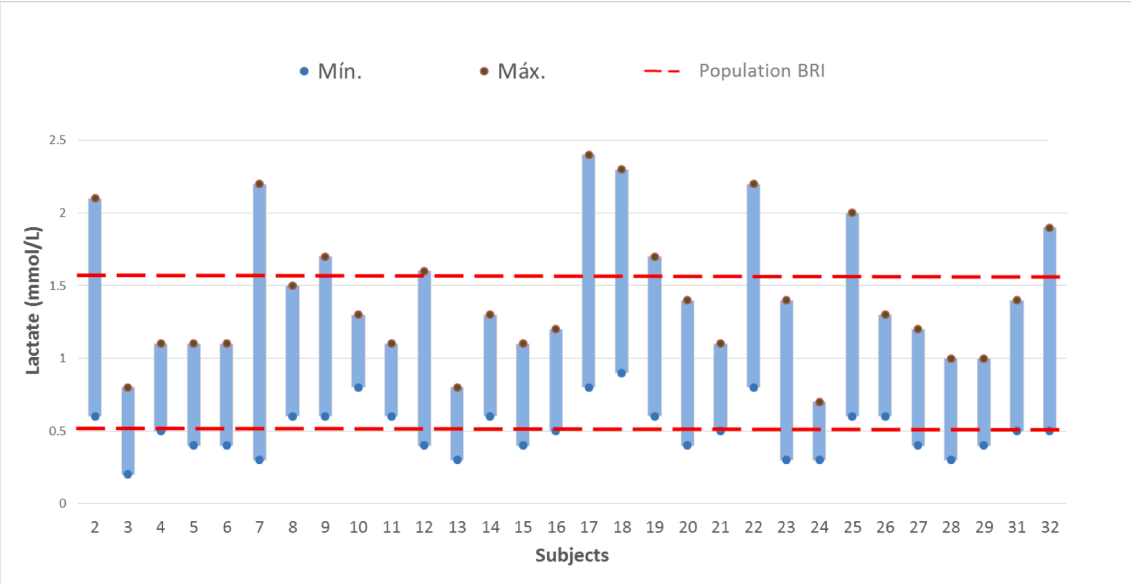

Fig. 1. Range of lactate concentration for the 30 athletes included in the study and the reference interval reported by Radiometer (reagent provider), for the general population, indicated by the red lines. Participants number 1 and 30 were excluded. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Number of subjects and included results, percentage of excluded outliers, mean and standard deviation (SD), analytical imprecision (CV_A), within-individual (CV_I) and between-individual (CV_G) BV estimates and their 95% confidence intervals for the ABS related measurands. BV estimates from health population (8).

Measurand	N. of subjects	N. included results	Outliers (%)	Mean (SD)	Gender	CV_A (%)	CV_I	CV_G	Healthy population	Healthy population
							CV_I (%) (95% CI)	CV_G (%) (95% CI)	CV_I (%)	CV_G (%)
Bicarbonate (mmol/L)	30	574	1.7	4.62 (0.3)	♂/♀	0.6	4.6 (4.2–5.0)	6.7 (67.9–8.9)	3.4–7.2	5.3–17.4
Ca^{2+} (mmol/L)	30	573	1.9	1.2 (0.0)	♂/♀	0.5	1.8 (1.6–2.0)	2.0 (1.5–2.7)	2.4	3.6
***pCO_2* (mmHg)**	30	578	1.0	50.0 (5.2)	♂/♀	0.8	7.8 (7.2–8.6)	10.4 (8.0–13.9)	4.8	5.3
TCO_2 (mmol/L)	30	567	3.2	27.0 (3.0)	♂/♀	0.6	9.3 (8.5–10.1)	6.3 (4.6–8.7)	4	4
$[H^+]$ nmol/L	30	571	2.2	42.7 (3.0)	♂/♀	0.9	4.8 (4.4–5.3)	5.1 (3.9–6.8)	3.5	2*
Lactate (mmol/L)	24	452	22.6	0.9 (0.3)	♂/♀	8.1	30.5 (27.6–34.1)	19.3 (13.2–28.4)	30.1	29
Gap anion	30	564	3.4	11.3 (1.0)	♂/♀	7.5	12.4 (11.0–13.9)	5.5 (3.2–8.3)	NA	NA
Base excess	30	565	3.3	+2.8 (1.4)	♂	7.2	46.5 (40.7–54.0)	41.0 (26.6–68.59	76.4	43.2
				+1.3 (1.7)	♀	13.8	90.1 (75.5–111.0)	46.6 (24.6–87.4)		

Table 3
Index of Individuality (II), Reference Change Values (RCV) and analytical performance specifications based on BV of ABS parameters from athletes. RCV_{pos} indicates an increase and RCV_{neg} a decrease. APS-CV_A indicates desirable APS for imprecision and APS- Bias, for bias.*The bias calculation is not in accordance with the theory of measurement uncertainty.

Measurand	Gender	II (CVI / CVG)	RCV neg (%)	RCV pos (%)	APS -CV_A (%)	APS-Bias (%)*
pCO_2	♂/♀	**0.76**	**−16.6**	**20.0**	**2.3**	**2.0**
TCO_2	♂/♀	**1.48**	**−19.5**	**24.2**	**4.7**	**2.8**
Lactate	♂/♀	**1.58**	**−51.3**	**105.2**	**15.3**	**9.0**
$[H^+]$	♂/♀	**0.95**	**−10.8**	**12.1**	**2.4**	**1.7**
Ca^{2+}	♂/♀	**0.89**	**−4.3**	**4.4**	**0.9**	**0.7**
Bicarbonate	♂/♀	**0.69**	**−10.2**	**11.4**	**2.3**	**2.0**
Base excess	♂	**1.13**	**−64.8**	**183.7**	**23.5**	**15.5**
	♀	**1.93**	**−83.8**	**517.9**	**45.1**	**25.4**

For bicarbonate, BV estimates were slightly higher in athletes than those published by Fraser et al [24]. However, if we assess the range of published estimates including data from both whole blood and serum [26-30], no clear difference is seen, as the athlete-based estimates are within the ranges reported from the healthy population (Table 2).

Although lactate concentrations were greater in athletes than in the general population (Fig. 1 and Table 4), published CV_I estimates for the healthy population [31,32] (CV_I = 31.0% and 27.2%) were similar to the CV_I found in this study (30.5%). The CV_G estimate, on the other hand, was higher in the general population than the athletes (Table 2).

It is expected that the concentrations of the products of the aerobic and anaerobic metabolisms may be more elevated in athletes than in the general population. However, Cadegiani and Kater have found lactate concentrations in athletes even lower than sedentary controls [11]. The authors explain this circumstance by a possible adaptation of enzymatic metabolism, increasing their performance to achieve a better clearance of these products. We do not have data from a control group to compare with, but if assessing population RI we can see that the RI derived from the general population are not directly transferable to the athlete group. Our population showed higher and wider RI than the general population for all the ABS magnitudes except pH. Highly remarkable is the gap anion RI, which is right-skewed, probably due to the organic acids accumulation in athletes.

We compared our data derived from our athlete population with other studies. The inclusion of a control group in our study, consisting of non-athletes, would have provided better grounds for assessing differences between athletes and the general population.

In our study, all subjects were quite homogeneous with regard to type, duration and intensity of training, as most of the training consisted of running, cycling and swimming at similar intensities and durations (Supplemental Table 3). Moderate exercise 24 h before sample collection, appears not to influence the results of the ABS magnitudes (Supplemental Figure 2). We did not identify any factor that had a significant effect on the CV_I estimates, except for the case of Ca^{2+}. Thus, the differences in estimates observed between the general population and our athlete population could not be explained by the different factors related

Table 4
Overview of reference intervals (RI) delivered by the reagent provider (Radiometer) for acid-basis status measurands in a healthy population and suggested RI for the athlete population where the RI were not transferable according to the CLSI EP-28 A-3, with the corresponding statistical significance (p).

Measurand	Units	Gender	General population RI	p-value	Verified by CLSI EP28-A3C protocol	Athletes RI
pH	–	♂/♀	7.33–7.43	0.66	Yes	–
pCO_2	mmHg	♂/♀	38–50	<0.05	No	37–61
TCO_2	mmol/L	♂/♀	22–26	<0.05	No	21–33
Ca^{2+}	mmol/L	♂/♀	1.00–1.20	<0.05	No	1.15–1.28
Bicarbonate	mmol/L	♂/♀	3.77–4.43	<0.05	No	3.77–5.24
Lactate	mg/dL	♂/♀	0.5–1.6	<0.05	No	0.4–2.0
Base Excess	–	♀	−3.4–1.4	<0.05	No	−0.2–5.2
		♂	−2.7–2.5			−1.6–4.6
Anion Gap	–	♂/♀	0.8–16	<0.05	No	7.8–14.1

to intensity and duration of exercise, such as HR, subjective perception of effort, duration, health complaints the week before, or the time between visits. Considering these findings, the differences in CV_I estimates may be explained by the adaptation to the endurance training routine in athletes. However, there could be other factors related to the physical exercise not recorded during this study that could have an influence on the BV. Additionally, robust data on BV for ABS parameters in healthy individuals are lacking and for direct comparison, both study populations should be included in the same study.

Another limitation is that for some measurand such as lactate, a high percentage of results (22.3 % as outliers) had to be eliminated to achieve the homoscedasticity criteria required for the nested ANOVA. Thus, these estimates need to be applied with caution, as they may not be representative and not all the ABS parameters may fully fit the BV model in athletes. A Bayesian approach to calculate BV may have been more appropriate, as it does not depend on the variance homogeneity of the data [36].

Furthermore, the CV_G estimates are based on data from 30 subjects so these estimates and the derived RI are not fully representative. The RI were calculated with a robust, non-parametric method, but the CLSI recommends a greater sample size to achieve a narrow CI 90% in the upper and lower limit of the RI, to be representative of the selected population.

4.3. Analytical performance specifications

One of the most important applications of BV data is the possibility to establish APS to assure the accuracy of patients' results. In the case of ABS measurands, the current BV estimates are based on very few publications derived from the general population, all of which have been appraised as BIVAC grade C [8]. BV estimates derived from an athlete population in this study is higher than those previously published for the general population, except for Ca^{2+}. The most remarkable differences, if focusing on desirable BV based APS for imprecision calculated as $0.5*CV_I$, were for pCO_2 (3.9 vs 2.4%) and anion gap (6.2 vs 4.8%), respectively.

For general laboratories which have established their APS based on BV data derived from the general population, these APS will have relevance also for the athletes.

Laboratories dedicated specifically to athletes' healthcare, performance monitoring and point of care testing during a stress test, could apply the APS derived from our athlete population (Table 3).

4.4. Results interpretation: Reference change value vs biological reference intervals

In the case of athlete monitoring the goal will most often be to detect a metabolite accumulation (organic acids) and the RCV could be calculated by the one-sided approach (alpha = 5% and Z = 1.65) (Table 3). In this context this means significant increases in the case of lactate, pCO2, gap anion and bicarbonate or decreases in pH and base excess. All the ABS magnitudes had II above 0.6 (Table 4) [37] indicating that RI can be recommended for monitoring by serial test results. pCO_2 and bicarbonate, however, had borderline results (II: 0.76 and 0.69, respectively) and for these, RCV may be helpful when monitoring of results. Our data emphasizes the need for reliable and transferable RI for ABS measurands derived from a sufficiently high number of athletes.

5. Conclusions

BV estimates for the ABS measurands are higher in athletes than those previously published for the general population. This difference does not seem to be directly explained by factors such as the intensity and duration of the exercise or moderate exercise in the 24 before sampling. However, it could be explained by the "chronic" physiological stress produced by exercise derived from the accumulation and increase of clearance rate of aerobic and anaerobic metabolites. Laboratories attending specifically to an athlete population could use these BV estimates to derive APS. Our data indicates that the best approach to interpret the ABS measurands in this population may be the use of specific biological RI instead of the RCV, due to the lack of individuality of most of these measurands. Our study provides suggested RI for an athlete population, but these should be applied cautiously as the low number of included subjects does not guarantee transferability and robustness of these intervals.

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CRediT authorship contribution statement

Jorge Diaz–Garzon: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Pilar Fernandez-Calle:** Conceptualization, Methodology, Writing – review & editing, Supervision. **Aasne K. Aarsand:** Writing – review & editing, Visualization, Supervision. **Sverre Sandberg:** Writing – review & editing, Visualization, Supervision. **Antonio Buno:** Writing - review & editing, Funding acquisition, Supervision, Project administration.

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.

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Appendix A. Supplementary material

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References

[1] M. Singer, C.S. Deutschman, C.W. Seymour, M. Shankar-Hari, D. Annane, M. Bauer, et al., The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3), JAMA. 315 (2016) 801–810.
[2] KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Chapter 3. Management of progression and complications of CKD. Kidney International Supplements. 2013;3:73–90.
[3] G.G. Fraser, E.K. Harris, Generation and application of data on biological variation in clinical chemistry, Crit. Rev. Clin. Lab. Sci. 27 (5) (1989) 409–437.
[4] M. Panteghini, F. Ceriotti, G. Jones, W. Oosterhuis, M. Plebani, S. Sandberg, Strategies to define performance specifications in laboratory medicine: 3 years on from the Milan Strategic Conference, Clin. Chem. Lab. Med. 55 (2017) 1849–1856.
[5] C.G. Fraser, Reference change values, Clin. Chem. Lab. Med. 50 (2011) 807–812.
[6] A. Coşkun, S. Sandberg, I. Unsal, C. Cavusoglu, M. Serteser, M. Kilercik, A. K. Aarsand, Personalized Reference Intervals in Laboratory Medicine: A New Model Based on Within-Subject Biological Variation, Clin. Chem. 67 (2021) 374–384.
[7] Bartlett WA, Braga F, Carobene A, Coşkun A, Prusa R, Fernandez-Calle P et al. A checklist for critical appraisal of studies of biological variation. Clin. Chem. Lab Med. 2015;53:879–85.
[8] A.K. Aarsand, T. Røraas, P. Fernandez-Calle, C. Ricos, J. Díaz-Garzón, N. Jonker, et al., The Biological Variation Data Critical Appraisal Checklist: A Standard for Evaluating Studies on Biological Variation, Clin. Chem. 64 (2018) 501–514.

[9] Aarsand AK, Fernandez-Calle P, Webster C, Coskun A, Gonzales-Lao E, Diaz-Garzon J, et al. The EFLM Biological Variation Database. https://biologicalvariation.eu/ (Accessed Jun 2021).
[10] J.G. Pallarés, R. Morán-Navarro, J.F. Ortega, V.E. Fernández-Elías, R. Mora-Rodriguez, Ø. Sandbakk, Validity and Reliability of Ventilatory and Blood Lactate Thresholds in Well-Trained Cyclists, PLoS One. 11 (9) (2016) e0163389.
[11] F. Cadegiani, C. Kater, Basal Hormones and Biochemical Markers as Predictors of Overtraining Syndrome in Male Athletes: The EROS-BASAL Study, J. Athl. Train. 54 (2019) 906–914.
[12] J.A.A.C. Heuberger, P. Gal, F.E. Stuurman, W.A.S. de Muinck Keizer, Y. Mejia Miranda, A.F. Cohen, L. Mourot, Repeatability and predictive value of lactate threshold concepts in endurance sports, PLoS One. 13 (11) (2018) e0206846.
[13] M.L. Parker, K. Adeli, Pediatric and adult reference interval harmonization in Canada: an update, Clin. Chem. Lab Med. 57 (2018) 57–60.
[14] N.M. Beltz, A.L. Gibson, J.M. Janot, L. Kravitz, C.M. Mermier, L.C. Dalleck, Graded exercise testing protocols for the determination of VO2max: Historical perspectives, progress, and future considerations, J. Sports Med. 2016 (2016) 1–12.
[15] M.J. Joyner, E.F. Coyle, Endurance exercise performance: The physiology of champions, J. Physiol. 586 (2008) 35–44.
[16] Røraas T, Petersen PH, Sandberg S. Confidence intervals and power calculations for within-person biological variation: effect of analytical imprecision, number of replicates, number of samples, and number of individuals. Clin. Chem. 2012;58 (9): 1306-.
[17] Bates D, Machler M, Bolker BM and Walker SC. Fitting Linear Mixed-Effects Models Using lme4. https://cran.r-project.org/web/packages/lme4/vignettes/lmer.pdf (Accessed Jun 2021).
[18] J. Pallarés, R. Morán-Navarro, Methodological approach to the Cardiorespiratory Endurance Training, J. Sport Health Res. 4 (2012) 119–136.
[19] G.A. Borg, Psychophysical bases of perceived exertion, Med. Sci. Sports Exercise. 14 (1982) 377–378.
[20] T. Røraas, B. Støve, P.H. Petersen, S. Sandberg, Biological variation: the effect of different distributions on estimated within-person variation and reference change values, Clin. Chem. 62 (2016) 725–726.
[21] Fraser CG, Sandberg S. Biological variation. In: N Rifai, AR Horvath, CT, Wittwer editors. Tietz textbook of clinical chemistry and molecular biology. 6th Ed. St. Louis (MO): Elsevier Saunders; 2018. p. 157–70.
[22] CLSI. Defining, establishing, and verifying reference intervals in the clinical laboratory: Approved guideline. 3rd Edition. CLSI document C28-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
[23] P.S. Horn, A.J. Pesce, Reference Intervals. A User's Guide, AACC Press, Washington, DC, 2005.
[24] Harding PJ, Fraser CG. Biological Variation of Blood Acid-Base Status: Consequences for Analytical Goal-Setting and Interpretation of Results. 1987; 33: 1416–18.
[25] A. Crosby, P.A. Robbins, Variability in end-tidal pCO_2 and blood gas values in humans, Exp. Physiol. 88 (2003) 603–610.
[26] C.G. Fraser, P. Williams, Short-term biological variation of plasma analytes in renal disease, Clin. Chem. 29 (1983) 508–510.
[27] E.K. Harris, P. Kanofsky, G. Shakarji, E. Cotlove, Biological and analytic components of variation in long-term studies of serum constituents in normal subjects, Clin. Chem. 16 (1970) 1022–1027.
[28] D. Bailey, V. Bevilacqua, D.A. Colantonio, M.D. Pasic, N. Perumal, M.K. Chan, K. Adeli, Pediatric within-day biological variation and quality specifications for 38 biochemical markers in the CALIPER cohort, Clin. Chem. 60 (2014) 518–529.
[29] D.A. Lacher, J.P. Hughes, M.D. Carroll, Estimate of biological variation of laboratory analytes based on the third national health and nutrition examination survey, Clin. Chem. 51 (2005) 450–452.
[30] T.P. Loh, M.P. Metz, Indirect estimation of pediatric between-individual biological variation data from 22 common serum biochemistries, Am. J. Clin. Pathol. 143 (2015) 683–693.
[31] M. Panteghini, F. Pagani, Biological Variation of lactate and Piruvate in blood, Clin. Chem. 39 (1993) 908.
[32] A. Widjaja, R.J. Morris, J.C. Levy, K.N. Frayn, S.E. Manley, R.C. Turner, Within- and between-subject variation in commonly measured anthropometric and biochemical variables, Clin Chem. 45 (1999) 561–566.
[33] Tighter precision target required for lactate testing in patients with lactic acidosis, Versluys KA, Redel S, Kunst AN, Rimkus M, Chin D, Tran D, Holmes D and Cembrowski GS. Clin. Chem. Lab Med. 2014;52:809-13.
[34] B. Vainshelboim, R. Arena, L.A. Kaminsky, J. Myers, Reference Standards for Ventilatory Threshold Measured With Cardiopulmonary Exercise Testing: The Fitness Registry and the Importance of Exercise: A National Database, Chest. 157 (6) (2020) 1531–1537.
[35] R.A. Robergs, F. Ghiasvand, D. Parker, Biochemistry of exercise-induced metabolic acidosis, Am. J. Physiol. Regul. Integr. Comp. Physiol. 287 (3) (2004) R502–R516.
[36] T. Røraas, S. Sandberg, A.K. Aarsand, B. Støve, A Bayesian Approach to Biological Variation Analysis, Clin. Chem. 65 (2019) 995–1005.
[37] C.G. Fraser, Inherent biological variation and reference values, Clin. Chem. Lab. Med. 42 (2004) 758–764.