






# Is the acid-base status at rest related to endurance performance in 10-km runners?

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## ABSTRACT

This study aimed to compare acid-base parameters between elite (ER) and amateur (AR) runners at rest and to explore potential correlations with 10-km running. Each participant completed a 10-km time trial on a 400-meter track, underwent an incremental exercise test in laboratory conditions, and provided a resting blood sample for analysis. Capillary blood sample were collected from the fingertip at rest. Measurements included pH, partial pressure of dioxide carbon ( $p\text{CO}_2$ ), haematocrit (Hct), haemoglobin (Hb) and lactate ( $\text{Lac}^-$ ), sodium ( $\text{Na}^+$ ), potassium ( $\text{K}^+$ ), chloride ( $\text{Cl}^-$ ) and bicarbonate ( $\text{HCO}_3^-$ ) ions. Base excess (BE) and strong ions difference (SID) was calculated. No significant differences were observed between ER and AR for Hb,  $\text{K}^+$ ,  $\text{Lac}^-$ , and pH ( $p > .05$ ). ER exhibited significantly higher values for  $\text{HCO}_3^-$  (ER =  $28.5 \pm 1.8$ ; AR =  $25.7 \pm 1.7 \text{ mmol}\cdot\text{l}^{-1}$ ),  $\text{Cl}^-$  (ER =  $104.4 \pm 3.83$ ; AR =  $100.1 \pm 3.89 \text{ mmol}\cdot\text{l}^{-1}$ ), BE (ER =  $5.6 \pm 1.6$ ; AR =  $3.21 \pm 1.43 \text{ mmol}\cdot\text{l}^{-1}$ ) and  $p\text{CO}_2$  (ER =  $36.9 \pm 3.7$ ; AR =  $33.9 \pm 2.9 \text{ mmHg}$ ;  $p < .05$ ). SID (ER =  $49.0 \pm 5.70$ ; AR =  $41.3 \pm 5.23 \text{ mmol}\cdot\text{l}^{-1}$ ;  $p < .05$ ) and  $\text{Na}^+$  (ER =  $140.0 \pm 4.1$ ; AR =  $143.5 \pm 3.3 \text{ mmol}\cdot\text{l}^{-1}$ ;  $p < .05$ ) were significantly lower in ER. Strong correlations were found between  $\text{HCO}_3^-$ , SID, ventilatory threshold parameters and 10-km performance ( $p < .05$ ). These findings suggest that resting acid-base status can be a useful indicator of 10-km performance and can assist in monitoring training-induced adaptations.

**Keywords:** Sport medicine, Acid-base profile, Running, Athletes.

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## INTRODUCTION

Endurance performance is closely associated with maximal aerobic capacity (i.e., maximal oxygen uptake -  $VO_{2max}$ ), anaerobic capacity, and running economy (Joyner et al., 2008). Recently, the concept of critical power/speed (CS) has emerged as an additional determinant of endurance performance. This concept facilitates the prediction of exercise tolerance by identifying the critical power (CP) and the work or distance ( $W'$  or  $D'$ ) achievable above CP. The CS model characterizes the transition between heavy and severe exercise intensity domains, distinguishing between sustainable and unsustainable speeds over a given distance while maintaining in a physiological steady state (i.e. stable pulmonary  $VO_2$ , blood lactate levels, muscle [phosphocreatine] and pH, muscle  $O_2$  saturation) (Jones et al., 2019; Poole et al., 2016).

Under laboratory conditions, one commonly used method to identify this domain transition involves analysing expired gases and the identification of the second ventilatory, also known as threshold or respiratory compensation point (RCP). The RCP has been associated with the total extra- and intra-muscular buffering capacity (Bhambhani et al., 2007; Wasserman et al., 2011) indicating that intensities above RCP lead to a failure to maintain pH levels, resulting in rapid acidosis. Our previous research (Lourenço et al., 2019a) demonstrated a strong correlation between 10-km performance and the running speed at the RCP (sRCP) ( $r = 0.96$ ). Moreover, we observed that both amateur and elite runners select a running pace very close to sRCP during 10-km race.

Blood bicarbonate ( $HCO_3^-$ ) is a crucial chemical buffer, playing a pivotal role in maintaining blood pH during exercise. An increase in resting  $HCO_3^-$  concentration has been shown to enhance buffering capacity. Acute and chronic sodium bicarbonate supplementation has been associated with elevated resting  $HCO_3^-$  levels, prolonging time to exhaustion and delaying the onset of acidosis during high-intensity tasks (Carr et al., 2011; Hadzic et al., 2019). However, no studies have specifically compared endogenous blood  $HCO_3^-$  levels at rest among runners and their correlation with competitive 10-km performance.

Levels of  $HCO_3^-$  are influenced by the partial pressure of carbon dioxide ( $CO_2$ ) and haemoglobin (Hb) (Geers et al., 2000), both of which are affected by mitochondrial activity and red blood cells concentration - key adaptations induced by endurance training (Egan et al., 2013; Montero et al., 2017; Uciá et al., 2000). Therefore, resting  $HCO_3^-$  levels may reveal significant training-induced adaptations.

In addition to  $HCO_3^-$ , several other factors such as non-bicarbonate buffers, base excess (BE) and strong ions difference (SID) contribute to quantifying metabolic components related to blood pH control (Kellum, 2005). BE represents the amount of acid or base required to restore pH to 7.40, whilst SID is calculated as the difference between concentrations of strong cations and strong anions ( $SID = [Na^+ + K^+] - [Cl^- + Lactate^-]$ ) affecting pH based on the principles of electroneutrality and mass conservation (Stewart, 1983).

Although BE has been shown to decrease during incremental exercise in marathon runners (Zoladz et al., 1993), it is currently unclear how BE is related to running performance. Considering the relevance of acid-base balance to endurance performance and the significant contribution of BE and SID to blood pH regulation, we hypothesized that resting BE and SID are associated with endurance running performance. This study aims to compare the acid-base profiles of elite and amateur runners at rest and explore their relationships with 10-km running performance.

## MATERIALS AND METHODS

### **Experimental design**

Initially, both professional and amateur 10-km runners individually performed a 10-km time trial on a 400-m outdoor track, with their time to complete the distance being recorded. Two days later, they visit the laboratory to collect blood samples at rest and underwent a maximal incremental running test to determine  $VO_{2max}$  and the running speed at the gas exchange thresholds.

### **Participants**

Twenty-six amateur runners (AR; age:  $35 \pm 6$  years, body mass:  $69.0 \pm 10.1$  kg, stature:  $174 \pm 0.1$  cm) and nineteen elite runners (ER; age:  $26 \pm 6$  years, body mass:  $67.9 \pm 8.7$  kg, stature:  $174 \pm 0.1$  cm) participated in the study. The ER were ranked among the top ten national ranking of 5-km and 10-km and were actively competing in national and international events at the time of the study. All athletes refrained from exercise for at least 48h before the tests and were instructed to maintain their usual diets for three days prior to the study. Informed consent was obtained from all participants in accordance with the guidelines of the Ethical Committee of University Research (n° 523/2010).

### **Procedures**

#### *Test 1: 10-km running trial*

A 10-15-minute warm-up period preceded the test, which commenced at 9 A.M.. Participants were allowed to hydrate *ad libitum* during the trial. Each subject was verbally encouraged to exert maximal effort and was not permitted to use any time devices. The run took place on an official 400-m track, with lap times recorded to calculate average running speed and total time test, thereby determining the average speed over the 10-km distance ( $s_{10km}$ ).

#### *Test 2. Blood analysis at rest*

After fifteen minutes rest time (seated) capillary blood samples were collected from the fingertip using disposable lancets (Accu-Chek SoftClix®, Roche®) and heparinized glass micro-haematocrit capillary tubes (Clinitubes®, Radiometer Copenhagen®). Blood pH, carbonic dioxide partial pressure ( $pCO_2$ ), haematocrit (Hct), Hb and blood lactate (Lac-) were immediately analysed using the Stat Profile®-pHOx®PlusL blood gas analyser (Nova Biomedical®, MA, USA). Equipment calibration was performed immediately before and at regular intervals during the experiment as per the manufacturer's instructions. Coefficients of analytical variation (CVA) are showed in Table 2.

Sodium ( $Na^+$ ), potassium ( $K^+$ ) and chloride ( $Cl^-$ ) ions concentrations were measured using the same equipment with ion-sensors.  $HCO_3^-$  plasma concentration was derived from  $pCO_2$  using Handerson-Hasselbach equation and BE was calculated from Hb,  $HCO_3^-$  and pH values by Van Skyle equation (Lang et al., 2002).

The SID was calculated according to following equation (Stewart, 1983).

$$\text{Equation 1: } [SID] = ([Na^+] + [K^+]) - ([Cl^-] + [Lac^-])$$

#### *Test 3. Maximal incremental test*

Seventy-two hours following the time trial, all athletes performed a maximal incremental test on a treadmill set at a 1% grade, with speed increments of  $0.3 \text{ km} \cdot \text{h}^{-1}$  every 25 seconds until volitional exhaustion (Lourengo et al., 2011).

Oxygen uptake ( $\text{VO}_2$ ), carbon dioxide production ( $\text{VCO}_2$ ), breathing frequency (Bf) and tidal volume ( $\text{Vt}$ ) were continuously measured in a breath-by-breath system (CPX/D Med Graphics, St. Paul, MN). Data was smoothed by averaging each 25-second interval as recommended (Robergs et al., 2010). The analyser was calibrated before each test using a known gas mixture (12%  $\text{O}_2$  and 5%  $\text{CO}_2$ ), and the volume sensor was calibrated using a 3-L syringe. Laboratory conditions were set at  $21 \pm 1^\circ\text{C}$  with relative humidity between 45-50%.

#### *VT and RCP determinations*

The ventilatory threshold running speed (sVT) and sRCP were determined using the V-Slope method (Beaver et al., 1986) through visual inspection by three independent and experienced researchers.  $\text{VO}_{2\text{max}}$  and the speed of  $\text{VO}_{2\text{max}}$  (s $\text{VO}_{2\text{max}}$ ) were identified as the values corresponding to the last stage completed with respiratory exchange ratio (RER) greater than 1.10 (D C Poole et al., 2008).

#### **Analysis**

Data are presented as mean  $\pm$  SEM. Differences between AR and ER groups were assessed using unpaired t-tests. Pearson's correlation coefficient was calculated to determine association between blood variables and running test parameters. Correlations magnitudes were interpreted using the following scale:  $<0.1$ , trivial;  $0.1-0.29$ , small;  $0.3-0.49$ , moderate;  $0.5-0.69$ , strong;  $0.7-0.9$ , very strong;  $>0.9$ , nearly perfect (Hopkins et al., 2009). Statistical significance was set at 5% ( $p < .05$ ).

## **RESULTS**

#### ***Ventilatory parameters and 10-km time trial***

Elite runners (ER) exhibited significantly higher s10km compared to amateur runners (AR) ( $p < .05$ ;  $t = -11.22$ ), as well as higher sVT ( $p < .05$ ;  $t = -12.10$ ); sRCP ( $p < .05$ ;  $t = -11.98$ ) and s $\text{VO}_{2\text{max}}$  ( $p < .05$ ;  $t = -8.91$ ). sVT was significantly lower and s $\text{VO}_{2\text{max}}$  significantly higher than the s10km in both groups ( $p < .05$ ;  $t = -13.78$ ), while no differences were observed between s10-km and sRCP ( $p = .65$ ;  $F = -1.39$ ).

Table 1. Respiratory parameters related to maximal incremental test and 10-km running performance of amateur and elite runners. Data are available in mean  $\pm$  standard deviation.

	AR (n = 26)	ER (n = 20)
Mean 10 km running speed ( $\text{km}\cdot\text{h}^{-1}$ )	$13.4 \pm 1.4^f$	$18.4 \pm 1.6^*$
sVT ( $\text{km}\cdot\text{h}^{-1}$ )	$11.5 \pm 1.1$	$15.9 \pm 1.0^*$
sRCP ( $\text{km}\cdot\text{h}^{-1}$ )	$13.2 \pm 1.3^f$	$18.4 \pm 1.2^*$
s $\text{VO}_{2\text{max}}$ ( $\text{km}\cdot\text{h}^{-1}$ )	$16.7 \pm 1.2^{\#}$	$21.4 \pm 1.7^{\#*}$
$\text{VO}_{2\text{max}}$ ( $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ )	$57.5 \pm 9.6$	$75.8 \pm 5.4^*$

Note. \*- $p < .05$  related to AR; f- $p < .05$  related to sVT; #- $p < .05$  related to sRCP; sVT-Running speed related to ventilatory threshold; sRCP-Running speed related to respiratory compensation point; s $\text{VO}_{2\text{max}}$ -Running speed related to maximal oxygen consumption.

#### ***Blood parameters***

No significant differences between elite and amateur runners for resting pH,  $\text{Lac}^-$ , Hb and Hct ( $p < .05$ ). However, BE,  $\text{pCO}_2$  and  $\text{Cl}^-$  and  $\text{HCO}_3^-$  concentrations were significantly higher in ER ( $p < .05$ ; Table 2) and  $\text{Na}^+$  concentration and SID were significantly higher in AR ( $p < .05$ ).

Correlation analyses revealed nearly perfect positive associations between s10-km and ventilatory parameters, with the strongest correlation being between sRCP ( $0.96$ ;  $p < .05$ ) and s10-km followed by sVT ( $0.95$ ;  $p < .05$ ) and s $\text{VO}_{2\text{max}}$  ( $0.94$ ;  $p < .05$ ) (Table 3). Strong relationships were also observed certain acid-

base parameters and 10-km. Resting HCO<sub>3</sub><sup>-</sup> concentrations were strongly correlated with 10-km performance ( $r = 0.74$ ;  $p < .05$ ) and with all cardiorespiratory parameters (sVT, sRCP and sVO<sub>2max</sub>). pCO<sub>2</sub> exhibited a strong relationship with 10-km performance ( $r = 0.68$ ;  $p < .05$ ) and sRCP ( $r = 0.68$ ;  $p < .05$ ), and a very large relationship with sVT ( $r = 0.71$ ;  $p < .05$ ) and sVO<sub>2max</sub> ( $r = 0.76$ ). SID showed a very strong negative relationship with all performance parameters except for sRCP ( $r = -0.67$ ;  $p < .05$ ).

Table 2. Rest acid-base blood profile and coefficients of analytical variation (CVA) of amateur (AR) and elite (ER). Data are available in mean ± standard deviation.

Parameter	AR	ER	CVA (%)
Hb (g/dL)	15.2 ± 0.9	14.8 ± 0.8	1.4
BE (mmol/L)	3.21 ± 1.43	5.6 ± 1.6*	-
HCO <sub>3</sub> <sup>-</sup> (mmol/L)	25.7 ± 1.65	28.3 ± 2.0*	-
Hct (%)	45.7 ± 2.9	44.4 ± 2.7	3.2
K <sup>+</sup> (mEq/L)	5.27 ± 0.72	5.18 ± 1.0	0.4
Lac <sup>-</sup> (mmol/L)	2.86 ± 0.86	2.59 ± 0.9	4.9
Na <sup>+</sup> (mEq/L)	143.5 ± 3.3	140.0 ± 4.1*	0.7
Cl <sup>-</sup> (mmol/L)	100.1 ± 3.89	104.4 ± 3.83*	0.2
pCO <sub>2</sub> (mmHg)	33.9 ± 2.9	36.9 ± 3.7*	7.2
pH	7.47 ± 0.02	7.48 ± 0.03	0.9
SID	49.0 ± 5.70	41.3 ± 5.23*	-

Note. Hb–haemoglobin; BE–base excess; HCO<sub>3</sub><sup>-</sup>–bicarbonate ion; Hct–haematocrit; K<sup>+</sup>–potassium ion; Lac–lactate ion; Na<sup>+</sup>–sodium ion; Cl<sup>-</sup>–chloride ion; pCO<sub>2</sub>–CO<sub>2</sub> partial pressure; SID–strong ion difference; \*–significant difference in relation to AR.

Table 3. Coefficient of correlation (r) among the blood acid-base parameters, 10-km running performance and running speeds related to the VT, RCP and VO<sub>2max</sub>.

	s10km	sVT	sRCP	sVO <sub>2max</sub>	BE	Hb	HCO <sub>3</sub> <sup>-</sup>	pCO <sub>2</sub>	pH	SID
s10 km	-									
sVT	.95*	-								
sRCP	.96*	.96*	-							
sVO <sub>2max</sub>	.94*	.94*	.95*	-						
BE	.54*	.54*	.51*	.48*	-					
Hb	.40*	.40*	.44*	.52*	.03	-				
HCO <sub>3</sub> <sup>-</sup>	.74*	.78*	.75*	.82*	.61	.58*	-			
pCO <sub>2</sub>	.68*	.71*	.68*	.76*	.51	.52	.94*	-		
pH	.57*	.62*	.61*	.71*	.15	.75*	.85*	.77	-	
SID	-.70*	-.72*	-.67*	-.71*	-.64	.81*	-.69	-.65	-.02	-

Note. 10-km–10-km running speed; Lac–Lactate ion; SID–Strong ion difference; pH–blood pH; pCO<sub>2</sub>–Carbonic dioxide partial pressure; Hct–Haematocrit; Hb–Haemoglobin; HCO<sub>3</sub><sup>-</sup>–Bicarbonate ion; BE–Base excess; sVT–Running speed related to ventilatory threshold; sRCP–Running speed related to respiratory compensation point; sVO<sub>2max</sub>–Running speed related to maximal oxygen consumption. \*  $p < .05$ .

## DISCUSSION

We hypothesized that indicator of the blood buffering capacity at rest (e.g., HCO<sub>3</sub><sup>-</sup> and/or BE) could be positively correlated with endurance performance. Our primary finding is that SID and HCO<sub>3</sub><sup>-</sup> concentrations were strongly related with 10-km running speed. To our knowledge, these results are the first to describe these relationships and compare acid-base status at rest between amateur and elite athletes.

Exercise training is known to stimulate mitochondrial biogenesis in skeletal muscle (Freyssen et al., 1996). Furthermore, acute bicarbonate supplementation has been associated with increased blood buffering capacity and improved high-intensity exercise performance in exercises lasting to 1-7 minutes (Grgic et al., 2021; Hadzic et al., 2019). However, no studies have specifically investigated the relationship between resting  $\text{HCO}_3^-$  concentration and endurance performance or training status.

Jones (2008) reported that endurance training leads to a reduction in  $\text{CO}_2$  output ( $\text{VCO}_2$ ), a modest increase in arterial  $\text{pCO}_2$  ( $\text{PaCO}_2$ ) and elevated  $\text{HCO}_3^-$  during submaximal exercise. Previous studies (Tas et al., 2019; Zoll et al., 2006) also reported that carbonic anhydrase (CA) activity can increase by 50% after 6 weeks of continuous or interval training. Our data align with these findings, as elite runners showed higher  $\text{pCO}_2$  and  $\text{HCO}_3^-$  values at rest.

Within erythrocytes,  $\text{CO}_2$  is hydrated by CA to form  $\text{HCO}_3^-$  and  $\text{H}^+$ .  $\text{HCO}_3^-$  ions are transported out via AE1 transporter while haemoglobin buffers  $\text{H}^+$  (Geers et al., 2000). Studies have shown that endurance training not only increases CA transcription (Ponsot et al., 2006), but also enhances AE1 (Juel et al., 2003) expression, facilitating  $\text{HCO}_3^-$  transport to plasma to further support its buffering function in blood (Putman et al., 2003). This may also explain the strong relationship between resting  $\text{HCO}_3^-$  concentration and sRCP observed in this study. Since runners maintain speeds close to sRCP for nearly the entire race time (30 to 45 minutes) (Lourenço et al., 2019b) with blood pH stable, the maintenance of  $\text{HCO}_3^-$  concentration is crucial. These findings suggest that monitoring resting blood bicarbonate concentrations could serve as a predictor of performance or training-induced adaptations. BE, representing a non-respiratory (metabolic) component of acid-base status, indicates that endurance training also contributes to buffering agents beyond  $\text{HCO}_3^-$ , such as plasma proteins and haemoglobin (Zander et al., 2004).

SID, as an independent variable in the physicochemical approach, represents the sum of strong acid anions and strong base cations (Greenbaum et al., 2005). Both groups, amateur and elite runners, showed higher SID values compared to clinical reference values for healthy people ( $39 \pm 1$  mmol/L) (Kellum, 2005). Higher SID values increase blood pH (alkalosis), suggesting that endurance training induces adaptations in this system. Lower SID values in elite runners particularly due to lower  $\text{Na}^+$  concentrations, were observed.

On the other hand, it is well established that  $\text{Cl}^-$  plays essential roles in cell physiology, varying distributions across plasma membranes. Cells actively manage  $\text{Cl}^-$  levels, with some extruding and others actively accumulating it. The  $\text{Cl}^-$  concentration is influenced by anion transporting proteins such as AE1, which mediates  $\text{Cl}^-$  and  $\text{HCO}_3^-$  exchange in rate of 1  $\text{Cl}^-$ :2  $\text{HCO}_3^-$  in red blood cell (Geers et al., 2000). This may also explain the difference in SID values found in this study, once to favour  $\text{HCO}_3^-$  output from red blood cells, will be necessary higher  $\text{Cl}^-$  concentration in blood. However, further studies are needed to clarify the importance of SID on this issue. We also suggest studies that investigate these parameters with resting blood sample collected immediately before both, 10-km running and incremental exercise. A potential limitation of the study was the timing of the resting blood sample collection, which occurred two days after the 10-km test.

## CONCLUSION

In conclusion, resting blood  $\text{HCO}_3^-$  concentration, along with other acid-base monitoring tools such as base excess and strong ion difference, is related to endurance performance. Endurance training induces adaptations that may enhance plasma buffering capacity, supporting higher exercise intensities during training and competition. Monitoring these adaptations at rest could provide valuable insights into training effectiveness and performance potential.

## AUTHOR CONTRIBUTIONS

All authors contributed to all stages of the project including the writing of this manuscript. All authors have read and agreed to the published version of the manuscript. Thiago Fernando Loureço conceived, and designed the study, collected the data, analysed, and interpreted the data, wrote the paper. Lazaro Alessandro Soares Nunes collected the data, interpreted the data, wrote the paper while Guilherme G. Artioli, Luiz E. B. Martins and Denise Vaz de Macedo interpreted the data and wrote the paper.

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## DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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