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RESEARCH ARTICLE

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Validation of a blood marker for plasma volume in endurance athletes during a live-high train-low altitude training camp

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Abstract

Altitude is a confounding factor within the Athlete Biological Passport (ABP) due, in part, to the plasma volume (PV) response to hypoxia. Here, a newly developed PV blood test is applied to assess the possible efficacy of reducing the influence of PV on the volumetric ABP markers; haemoglobin concentration ([Hb]) and the OFF-score. Endurance athletes (n=34) completed a 21-night simulated live-high train-low (LHTL) protocol (14 $h.d^{-1}$ at 3000 m). Bloods were collected twice pre-altitude; at days 3, 8, and 15 at altitude; and 1, 7, 21, and 42 days post-altitude. A full blood count was performed on the whole blood sample. Serum was analysed for transferrin, albumin, calcium, creatinine, total protein, and low-density lipoprotein. The PV blood test (consisting of the serum markers, [Hb] and platelets) was applied to the ABP adaptive model and new reference predictions were calculated for [Hb] and the OFF-score, thereby reducing the PV variance component. The PV correction refined the ABP reference predictions. The number of atypical passport findings (ATPFs) for [Hb] was reduced from 7 of 5 subjects to 6 of 3 subjects. The OFF-score ATPFs increased with the PV correction (from 9 to 13, 99% specificity); most likely the result of more specific reference limit predictions combined with the altitude-induced increase in red cell production. Importantly, all abnormal biomarker values were identified by a low confidence value. Although the multifaceted, individual physiological response to altitude confounded some results, the PV model appears capable of reducing the impact of PV fluctuations on [Hb].

KEYWORDS

Athlete Biological Passport, Bayesian inference, biological variation, blood doping, intravascular volumes

1 | INTRODUCTION

Rather than the direct detection of a doping substance, the Athlete Biological Passport (ABP) is focused on the physiological changes resulting from doping practices (i.e., indirect detection).[1] However, the ABP relies on a number of concentration-based markers, including haemoglobin concentration ([Hb]) and the OFF-score (OFF-score = [Hb] (g/dL) x 10 – 60 x √reticulocyte%),[2] and plasma volume (PV) fluctuations currently represent the majority of biological variation associated with these markers. Recently, PV shifts resulting from acute hyper-hydration or an increase in training load have been found to influence an ABP profile.[3,4] An alternative scenario where PV shifts occur naturally is during exposure to altitude. It is well documented that altitude promotes a natural reduction in PV in the first few days of ascent from sea level, reflected by an increase in [Hb].[5] As an athlete adapts to the low oxygen environment, red cell production is stimulated and a true increase in haemoglobin mass (HbM) is commonly observed.[6] Of note, altitude is especially problematic to the ABP, as athletes commonly live and train in hypoxic environments to naturally improve their performance, often in the weeks preceding a major competition. Nevertheless, there is currently no method in place to correct volumetric ABP markers for such naturally occurring PV shifts.

Recently, our laboratory developed a novel method to estimate absolute PV through the observed variability of a panel of common biomarkers.[7] This novel marker for PV, requiring only a blood test, was suggested as a potential tool to adjust for volume fluctuations influencing the concentration-based markers of the ABP.[8] By applying the PV correction to the adaptive model of the ABP, the specificity of the resulting reference limit calculations were refined.[8] However,

before the PV correction can be applied to anti-doping practices, validation of the model in a variety of relevant situations is necessary.

Within this study, an attempt is made to reduce the confounding effects of PV shifts at altitude with the application of the PV correction to the ABP adaptive model. It is hypothesised that altitude will stimulate an initial reduction in PV; however, with the application of the PV correction to the ABP adaptive model, this natural fluctuation of PV will be accounted for.

2 | METHODS

Thirty-four sub-elite endurance athletes (19 m, 15 f) with at least 3 years of training history completed 21 days of simulated live-high train-low (LHTL) altitude training. Mean ± standard deviation for age and VO_{2max} of males and females was 28.1 ± 9.2 years and 65.9 ± 6.6 ($ml.kg^{-1}.min^{-1}$) and 29.9 ± 6.3 years and 54.2 ± 5.6 ($ml.kg^{-1}.min^{-1}$), respectively. Athletes spent on average 14 hours per day at a simulated altitude (normobaric hypoxia) of 3000 m. All training was conducted at normoxia (~600 m, Canberra, Australia). Participants were excluded if they showed signs of iron overload (haemochromatosis, haemosiderosis), polycythaemia, iron deficiency, or recently received a blood transfusion or donated blood. Baseline haematology and HbM measures[9] were collected -14 and -1 day prior to altitude exposure (B1, B2) and then at days 3, 8,and 15 at altitude (A3, A8, A15) and +1, +7, +21 and +42 days post-altitude (P1, P7, P21, P42). Note that HbM was not measured at A3. A schematic diagram of the testing protocol is found in Figure 1. As part of a parallel investigation, subjects were split into 3 iron-supplementation groups: placebo (no supplementation), oral supplementation, or a course of intravenous (IV) injections. Details of the iron-supplementation protocol[10] and HbM protocol[7] can be found elsewhere. Venous blood was sampled following strict World Anti-Doping Agency (WADA) blood collection and analysis protocol.[11] A 3-mL whole blood K2EDTA sample (BD vacutainer, Ref. 367856, Plymouth, UK) was stored at room temperature (21 degrees) and analysed for haemoglobin concentration ([Hb]), platelets (PLT), mean corpuscular haemoglobin concentration (MCHC), and reticulocytes percentage (RET%) on a Sysmex XT 2000i (Kobe, Japan) in automatic mode within 24 hours. An 8.5-mL serum sample (BD vacutainer, Ref. 367988, Plymouth, UK) was collected, stored at room temperature

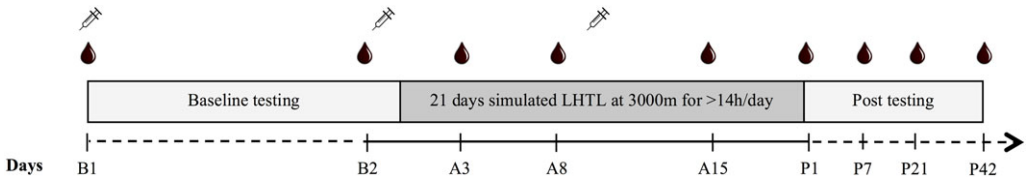

FIGURE 1 **Study design.** Baseline HbM calculations (determined by the CO-rebreathing test) and blood collection were performed on day -14 and -1 day prior to altitude (B1, B2) (represented by the droplet). Subsequent measures occurred at days 3, 8, and 15 at altitude (A3, A8, A15) and +1, +7, +21 and +42 days post altitude (P1, P7, P21, P42). HbM was not collected at A3. Subjects received an oral supplement (iron or placebo glucose tablets) daily from day -14 to day 21 at altitude and 3 IV injections (iron or placebo), prescribed at day -14, day -1 prior to altitude and day 10 at altitude (represented by the needle) [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Observed change values from baseline in haemoglobin concentration and plasma volume

Study Day Males	[Hb] (g/L) Mean	%Δ (min, max)	PV (mL) Mean	%Δ (min, max)
B2	148 ± 7	0	4336.0 ± 707.9	0
A3	152 ± 8	3.3 (-6, 13.7)	4139.7 ± 760.3	-4.5 (-19.4, 8.7)
A8	157 ± 8	6.7 (-1.3, 20.6)	3919.1 ± 682.9	-9.6 (-26.8, 1.7)
A15	157 ± 8	6.7 (0, 19.2)	3999.5 ± 687.9	-7.7 (-22.7, 5.9)
P1	156 ± 9	5.7 (-1.3, 17.1)	4106.0 ± 725.5	-5.3 (-20.9, 4.2)
P7	154 ± 8	4.2 (-2.5, 13.9)	4205.8 ± 747.4	-4.9 (-18.9, 6.6)
P21	152 ± 6	2.2 (-3.4, 8.7)	4205.4 ± 706.5	-2.5 (-11.5, 4.5)
P42	150 ± 7	1.3 (-3.8, 12.3)	4171.2 ± 748.1	-1.9 (-15.3, 9.0)
Females	**Mean**	**%Δ (min, max)**	**Mean**	**%Δ (min, max)**
B2	136 ± 9	0	3162.8 ± 344.9	0
A3	140 ± 10	3.5 (-6.8, 15.1)	3007.3 ± 479.7	-5.1 (-19.7, 10.6)
A8	142 ± 7	4.5 (-4.2, 10.1)	3002.1 ± 377.7	-5.1 (-13.5, 8.5)
A15	144 ± 11	6.3 (-7.6, 18.5)	2993.1 ± 400.7	-5.3 (-18.0, 15.9)
P1	144 ± 8	5.9 (-2.1, 15.3)	3024.2 ± 455.6	-4.7 (-13.0, 6.2)
P7	141 ± 7	2.6 (-1.4, 8.5)	3144.7 ± 305.1	-2.4 (-11.1, 7.2)
P21	138 ± 7	0.4 (-6.1, 8.7)	3199.7 ± 388.5	0.3 (-9.6, 14.4)
P42	138 ± 7	1.9 (-3.5, 12.2)	3102.5 ± 414.8	-1.4 (-13.0, 11.9)

All placebo subjects (n = 9; 5 M, 4 F) have been removed to reduce the impact of differences in the erythropoietic response to altitude influencing [Hb] and thus PV values.

for 15 minutes before centrifugation at 4°C and 1500 rpm for 10 minutes. Serum was aliquoted and stored at -80°C and batch analysed in random order with a COBAS Integra 400 (Roche Diagnostics, Switzerland) for the following 6 chemistry variables: Transferrin (Tfn), Albumin (ALB), Calcium (Ca), Creatinine (CRE), Total Protein (TP), and Low-Density Lipoprotein (LDL). The Australian Institute of Sport human ethics committee approved the study and all subjects provided written consent before participating.

2.1 | Statistics

A validation of the previously describe PV model is performed here. Detailed descriptions of the statistical development of the PV model[7] and its application to the adaptive model of the ABP paradigm can be found elsewhere.[8] Within the PV model, a confidence level is associated with the calculated PV Z-score returned by the model. The confidence level is equal to the exponential sum of the weighting function (calculated as a normality probability distribution of residuals in the variations of the markers) and is normalised between 0 and 1, so that values close to 0 have low confidence while values close to 1 have high confidence. The confidence level weights the variance that is used to calculate the Z-score associated to PV shifts. Confidence in the PV model prediction is, therefore, related to the uniformity of the PV marker Z-scores.

By applying an adaptive Bayesian model, and the principles of the ABP, a longitudinal profile for [Hb] and OFF-score calculations for each individual subject was produced (specificities were set at both 99% and 99.9%). The individual [Hb] and OFF-score values were entered into the Bayesian model, which produced individualised reference limits. The individual estimations of PV, derived from the panel of 8 PV markers ([Hb], Tfn, CRE, Ca, PLT, LDL, ALB, and TP) applied to an adaptive model, were then used to correct the individual ABP reference calculations for [Hb] and the OFF-score.

3 | RESULTS

Table 1 quantifies the mean change from baseline levels (using the B2 measure only) in [Hb] and PV. The largest PV mean change from baseline occurred at A8 in males (-9.6%) and A15 in females (-5.3%), calculated from the individual change values from baseline. The largest %change from baseline in PV occurred concurrently with the largest %change value in [Hb]. The individual PV response to altitude varied, and females presented with more variable change values (ranging from -19.7% at A3 to 15.9% at A15) in comparison to males who tended to present with PV contractions from baseline levels (ranging from -26.8% at A8 to 9.0% at P42).

From a total of 283 observations from 34 subjects the number of Atypical Passport Findings (ATPFs) for [Hb] was reduced when the PV model was applied. Without the correction, 7 ATPFs of 5 subjects (99% set specificity) and 3 of 3 subjects (99.9% set specificity) were recorded. This was reduced to 6 of 3 subjects (99% specificity) and 1 (99.9% specificity) ATPF with the PV correction (Table 2, the original [Hb] ATPF data are presented elsewhere).[10] However the OFF-score recorded an increased number of ATPFs with the inclusion of the PV correction (at a set specificity of 99%). The ATPFs were increased from 9 of

TABLE 2 Atypical passport findings for haemoglobin concentration with and without the plasma volume correction

Subject	Test	ATPF (upper or lower limit)	Confidence (%)
Without Correction			
S6	A15	Lower	
S8	A3	Upper	
S8	A8	Upper *	
S8	A15	Upper	
S17	A3	Upper *	
S21	A8	Upper	
S30	A15	Upper *	
With Correction			
S8	A3	Upper	0
S8	A8	Upper *	0
S8	A15	Upper	0
S1	*A15*	*Upper*	*2*
S1	*P1*	*Upper*	*0*
S25	*P1*	*Upper*	*0*

(*) Reference limits set at 99.9% specificity were also flagged. Italics indicate subjects who recorded an ATPF only when the PV correction was applied.

TABLE 3 Atypical passport findings for the OFF-score with and without the plasma volume correction

Subject	Test	ATPF (upper or lower limit)	Confidence (%)
Without Correction			
S1	P7	Upper	
S6	B2	Lower *	
S6	P42	Upper	
S8	A15	Upper	
S13	A15	Upper	
S15	B2	Upper *	
S17	A3	Upper *	
S23	P42	Upper *	
S30	A15	Upper	
With Correction			
S1	P7	Upper *	29
S6	B2	Lower *	40
S8	A3	Upper	0
S8	A15	Upper	0
S23	P42	Upper *	30
S5	*P1*	*Upper*	*5*
S13	*P7*	*Upper*	*79*
S20	*P7*	*Upper*	*85*
S20	*P21*	*Upper*	*100*
S25	*P1*	*Upper*	*0*
S28	*P21*	*Upper*	*40*
S30	*P1*	*Upper*	*2*
S35	*B2*	*Lower*	*45*

(*) Reference limits set at 99.9% specificity were also flagged. Italics indicate subjects who recorded an ATPF only when the PV correction was applied.

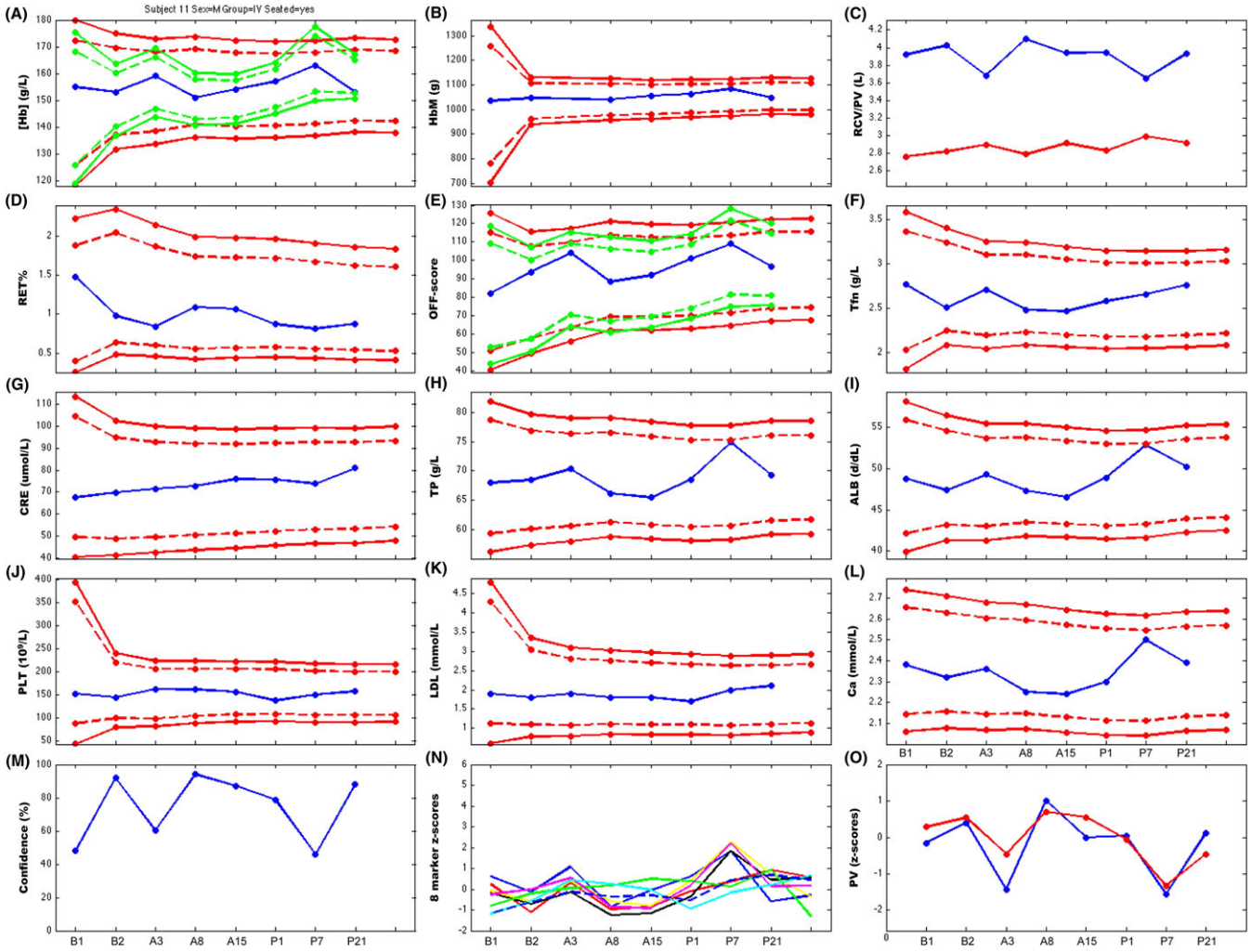

FIGURE 2 **Subject 11.** Red lines indicate the ABP adaptive model reference limits set at 99% (dashed lines) and 99.9% (solid lines) specificity (panels A, B, D–L). The green reference limits for [Hb] (A) and the OFF-score (E) represent the ABP reference limit calculations with the inclusion of the PV correction. Panel C shows the red blood cell (RBC) (red line) and plasma volume (blue line) values calculated with the CO-rebreathing method. Panel M represents the confidence calculation in the PV estimation. Panel N shows the z-scores for markers [Hb] (blue), Tfn (red), ALB (yellow), Ca (black), CRE (green), TP (pink), PLT (cyan), LDL (dashed blue). Panel O represents the plasma volume z-score calculations using the CO-rebreathing method (blue line) and PV model (red line) [Colour figure can be viewed at wileyonlinelibrary.com]

8 subjects (99% set specificity) without the correction, to 13 of 11 subjects with the correction (Table 3). At a 99.9% set specificity, the OFF-score ATPFs were reduced from 4 of 4 subjects to 3 of 3 subjects. Two values were removed due to high abnormal chemistry values resulting from sampling errors (A15 from Subject 24 and P7 from Subject 29).

Figure 2 (Subject 11) gives an example of the PV models function and describes the expected PV contraction at A3, resulting in an increased [Hb]. Subsequent changes in PV were also inversely related to [Hb] and the OFF-score. With the inclusion of the PV correction, the specificity of the predicted reference ranges for [Hb] and the OFF-score were improved (Figure 2A,E, respectively). For example, for Subject 11, at P7 the calculated Z-scores for PLT, LDL, CRE, and Tfn were lower than the remaining 4 biomarkers (Figure 2N), and therefore, the confidence calculation dropped to ~45% for this particular test (Figure 2M).

Subject 8 (Figure 3) presented with uncharacteristic results whereby [Hb] was the only PV biomarker to respond to a PV contraction observed between B2 and A8. From B2, A3, and A8 [Hb] increased from 146 to 166 and 176 g/L, respectively. The calculated PV decrease (using the CO-rebreathing method) at A3 and A8 was -19.4% and -26.8%, respectively. This volume shift was not reflected in any of the PV biomarkers. Importantly, the confidence in the PV estimation for A3 and A8 was 0%.

Subject 1 (female, IV group) recorded an increase in HbM towards the end of the altitude sojourn (Figure 4B), presenting with increases in HbM greater than the female sample mean (between 33% and 67%). The increase in HbM was reflected with an increase in [Hb] at A15 and P1, which, as expected, was not reflected in any other PV biomarker. This increase in [Hb], due to the HbM increase (rather than a PV shift), resulted in a decline the confidence to near 0% of the PV calculation.

4 | DISCUSSION

Exposure to hypoxia results in a number of inherent physiological adaptations, which alter an athlete's haematological profile.[12] The volumetric response to altitude is of interest here, and specifically, the influence of the initial haemoconcentration on the ABP markers, [Hb]

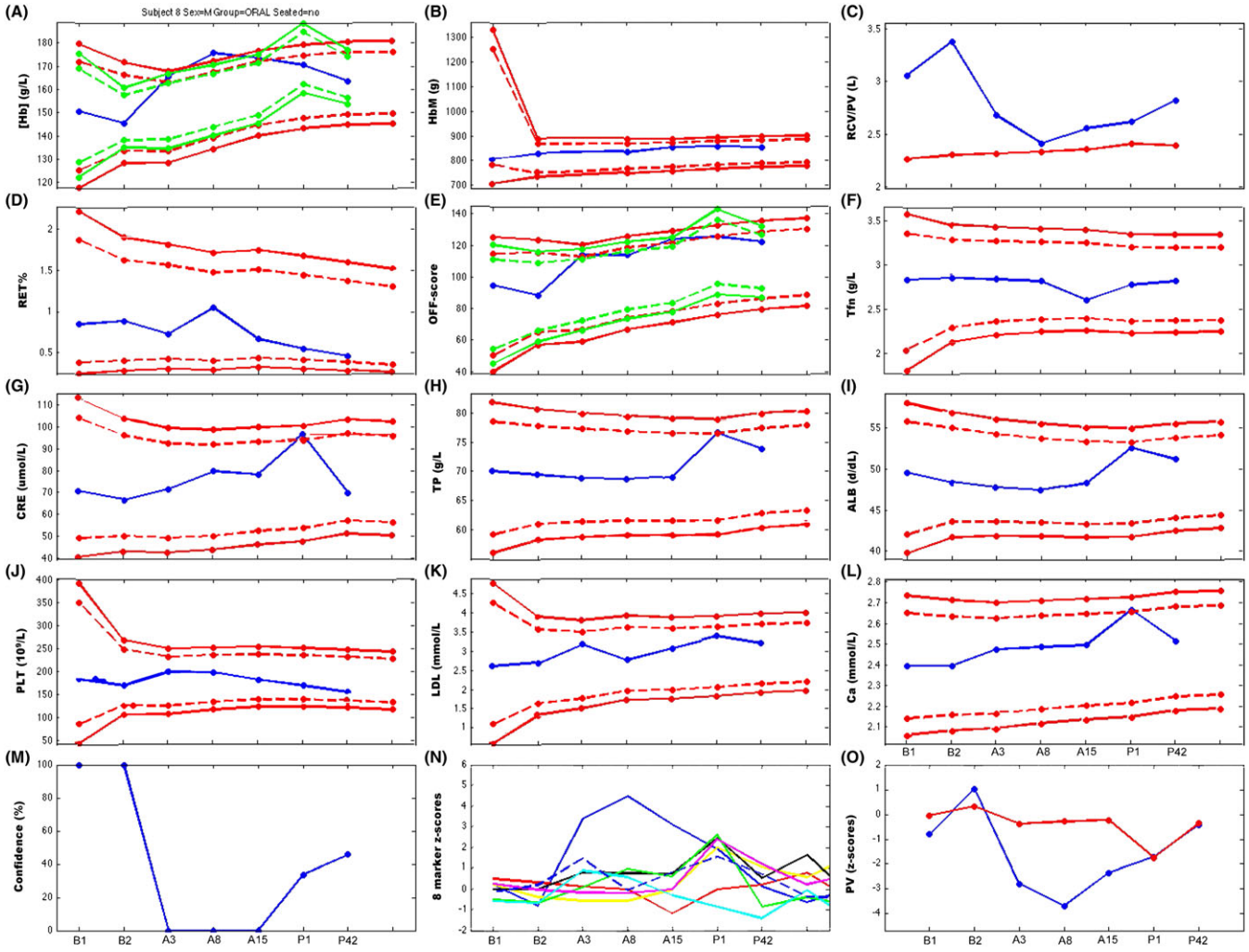

FIGURE 3 **Subject 8.** Refer to Figure 2 for panel descriptions. Note tests P7 and P21 are missing [Colour figure can be viewed at wileyonlinelibrary.com]

and the OFF-score. A maximum mean decrease in PV was observed concurrently with the maximum mean increase in [Hb] for both males and females, confirming the initial primary confounder of [Hb] fluctuations to be the PV contraction. A few ATPFs were observed for [Hb] and the OFF-score values during and post-altitude, most likely the result of the initial PV shift, as well as the expected increase in red cell production. Importantly, when the PV correction was applied to the adaptive model, the reference limits were refined and the number of ATPFs for [Hb] was reduced. However, the number of ATPFs recorded for the OFF-score increased with the inclusion of the PV correction (99% set specificity). It has to be highlighted that the study setting in this investigation presents a "worst case scenario" to test the functionality of the PV model: Indeed, PV is influenced by altitude, but at the same time, HbM will be impacted by the hypoxic exposure and the various iron supplementation protocols. While this is a possible scenario in certain athletes, the strongest (and most common) modulator of PV in a normal setting is exercise alone.[13]

A measurable increase in HbM has been reported after just 10 days at altitude,[14] but typically does not present until a sufficient hypoxic dose[15] has been achieved. Therefore, the initial PV reduction upon arrival at altitude is most likely the primary factor influencing [Hb] fluctuations within the initial stages of altitude exposure.[16] In our investigation, the initial haemoconcentration was not observed in all athletes, and there was substantial variability in the individual response to altitude (Table 1). Beidleman et al. also described variability in the individual response, where the reported standard error for [Hb] is large for the impact of the time spent at altitude (Table 3 of Beidleman et al[5]), suggesting considerable inter-individual variability at altitude. Additionally, the intermittent nature of the LHTL protocol,[12] differences in training load,[17] and the non-standardisation of blood collection times may have impacted the variable volumetric response reported here. Due to scheduling restrictions in the current study, the timing of the blood withdrawals and training programmes were not standardised. Nevertheless, testing schedules can be random in an anti-doping setting, thus reflecting the practical application of the current results.

4.1 | Frequency of atypical passport findings

When the PV correction was applied to the ABP adaptive model, the predicted reference limits were narrowed and the number of ATPFs for [Hb] was reduced, which supports the model. With the PV correction, 4 subjects no longer recorded ATPFs, however; Subject 1 and Subject 25 recorded ATPFs only when the PV correction was applied

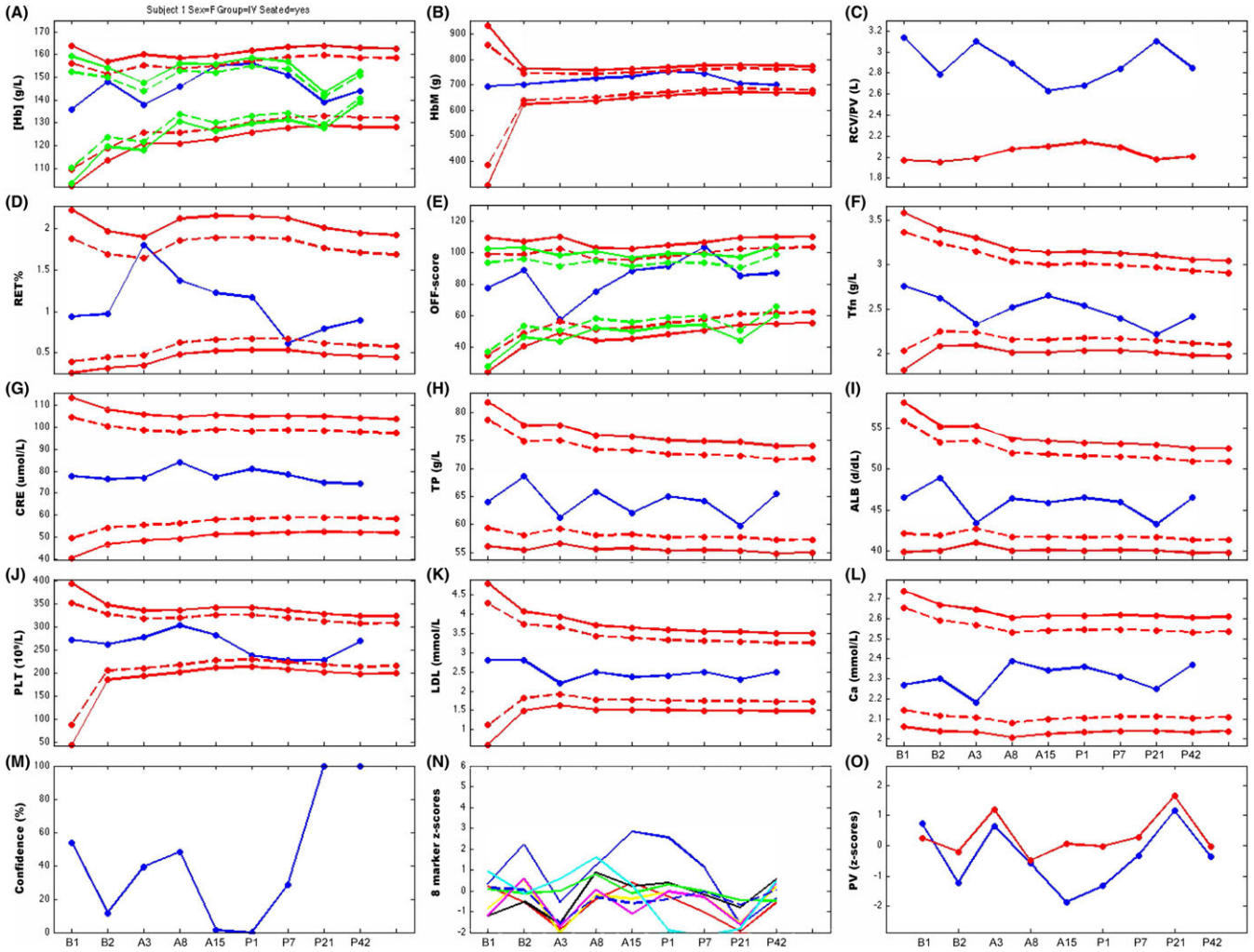

FIGURE 4 **Subject 1.** Refer to Figure 2 for panel descriptions [Colour figure can be viewed at wileyonlinelibrary.com]

(Table 2). This may be a reflection of the additional confounding effects of altitude on the ABP markers, namely an increased rate of erythropoiesis, which is reflected in the augmentation of reticulocytes.[18-20] Subject 1 (Figure 4) and 25 recorded increases in RET% (which is not affected by PV) at A3 and A8, respectively, indicating an increased red cell production influencing the [Hb] increase.

On the other hand, the OFF-score recorded an increased rate of ATPFs with the PV correction (99% set specificity). The OFF-score is probably more sensitive to the increased rates of erythropoiesis at altitude due to the inclusion of RET% in the OFF-score algorithm. Therefore, when the PV correction is applied in an altitude scenario, the resulting reference limits become too narrow, causing in an increased rate of false positives. However, with 99.9% specificity the OFF-score ATPFs were reduced by one when the PV correction was applied (Table 3). The time between sample collections must also be considered. Blood collections acquired in quick succession, such as a few days apart, are at risk of the influence of sample autocorrelation and the time between measures was brief within this investigation.[10]

Overall, applying the PV correction to both [Hb] and the OFF-score was viable in a number of individuals who did not present with ATPFs with the inclusion of the PV correction (only 2% and 5% of the 283 observations were atypical for [Hb] and the OFF-score, respectively). However, because of some false positives, further research into the PV correction and its use within altitude scenarios is required.

4.2 | The weighing function of the PV model

The components of biomarker variance were higher than expected in some subjects, which resulted in a low confidence in the PV estimation (calculated with the weighting function within the PV model algorithm). If the PV correction is to be considered for the ABP it is vital that the number of false positive ATPFs is kept to an absolute minimum and not increased. An important observation from this investigation was the association between the low confidence value and uncharacteristic chemistry values. In a hypothetical anti-doping scenario where one or more of the PV biomarkers fluctuate abnormally, resulting in a low confidence value, it is recommended that the ABP adaptive model excludes the PV correction for this particular observation.

A few subjects within this investigation recorded PV estimations associated with a low confidence value due to non-uniform PV biomarker Z-score calculations. Subject 8 (Figure 3) presented with a significant decrease in PV between B2 and A8, resulting in an increase in [Hb]. However, no other PV biomarker responded in the same manner to this volume shift. Additionally, Subject 8 presented with 3 of the 7 [Hb] ATPFs both with and without the addition of the PV correction

(Table 2). Subject 8 presented with a uniform PV biomarker variance (represented as Z-score calculations) at time points B1 and B2, resulting in a confidence calculation of 100%. However, for tests A3, A8, and A15 [Hb] increased significantly, due to a PV shift (calculated from the CO-rebreathing technique). This PV shift was not reflected in any other PV biomarker, resulting in a decrease in the confidence calculation to 0%. For P1 and P42 (Tests P7 and P21 were not performed due to subject availability at the required time of testing), the biomarker variance was more uniform, and consequently, the confidence in the PV estimation increased. As a result, there was disparity between the calculated PV Z-scores using the CO-rebreathing method and PV marker method (Figure 3O). In this particular case, the components of the [Hb] fluctuation are realistic of a PV shift. The absence of a response from the remaining 7 volumetric biomarkers of the PV model is disconcerting. However, encouragingly, the weighting function within the model identified this disconnect in the biomarker variances and is reflected by the low confidence estimation.

4.3 | Limitations

Although the present study was performed in accordance to current WADA protocol and every effort was made to adhere strictly to the sample collection, handling, and analysis protocols, it is important to recognise that the data presented here were collected under a research setting. For the PV marker to be introduced into anti-doping practices, stringent protocols must be developed for the sample collection, handling, analysis, and storage of the proposed chemistry markers, similar to those which are in place for the established ABP markers such as [Hb] or RET%. Additional factors that were not standardised within this investigation include diet, training, and the timing of blood withdrawals. Further, the testing was performed during the Canberra winter, and a few subjects presented with upper respiratory tract infections. Finally, it must be mentioned that the likelihood of any of the subjects doping within this investigation is extremely low, yet it cannot be excluded entirely. Of relevance however, each subject signed a statutory declaration prior to commencing the investigation, stating they have not and will not participate in any doping practices.

4.4 | Conclusion and practical recommendations

The results presented here demonstrate the potential of the PV correction to refine the ABP reference limit calculations by removing the influence of PV on volumetric markers. It appears that the model is able to reduce the number of ATPFs on [Hb] in a reliable manner in most subjects. However, the multifaceted, individual physiological response to altitude confounded the results for some individuals. Nevertheless, the weighting function allowed an assessment of the outcome confidence, and could be included in any possible evaluation of an ABP. As such, it is recommended to include the confidence calculation when implementing the model, because it allows one to appropriately weight the evidence added by the PV correction. This investigation also highlights the importance of further research and development into a standardised protocol for the collection, handling, analysis, and storage of the novel PV biomarkers.

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REFERENCES

1. Sottas PE, Robinson N, Saugy M. The athlete's biological passport and indirect markers of blood doping. *Handb Exp Pharmacol*. 2010;195:305-326.
2. Gore CJ, Parisotto R, Ashenden M, et al. Second-generation blood tests to detect erythropoietin abuse by athletes. *Haematologica*. 2003;88:333-344.
3. Bejder J, Andersen AB, Goetze JP, Aachmann-Andersen NJ, Nordsborg NB. Plasma volume reduction and hematological fluctuations in high-level athletes after an increased training load. *Scand J Med Sci Sports*. 2017;27:1-11.
4. Bejder J, Hoffmann MF, Ashenden M, Nordsborg NB, Karstoft K, Mørkeberg J. Acute hyperhydration reduces athlete biological passport OFF-hr score. *Scand J Med Sci Sports*. 2015;26:338-347.
5. Beidleman BA, Staab JE, Muza SR, Sawka MN. Quantitative model of hematologic and plasma volume responses after ascent and acclimation to moderate to high altitudes. *Am J Physiol Regul Intergr Comp Physiol*. 2016;312:R265-R272.
6. Gore CJ, Sharpe K, Garvican-Lewis L, et al. Altitude training and haemoglobin mass from the optimised carbon monoxide rebreathing method determined by a meta-analysis. *Br J Sports Med*. 2013;47:i31-i39.
7. Lobigs LM, Sottas PE, Bourdon PC, et al. The use of biomarkers to describe plasma-, red cell- and blood volume from a simple blood test. *Am J Hematol*. 2017;92:62-67.
8. Lobigs LM, Sottas PE, Bourdon PC, et al. A step towards removing plasma volume variance from the Athlete's Biological Passport: The use of biomarkers to describe vascular volumes from a simple blood test. *Drug Test Anal*. 2018;10:294-300.
9. Schmidt W, Prommer N. The optimised CO-rebreathing method: a new tool to determine total Hb mass routinely. *Eur J Appl Physiol*. 2005;95:486-495.
10. Garvican-Lewis LA, Vuong VL, Govus AD. Influence of combined iron supplementation and simulated hypoxia on the haematological module of the Athlete Biological Passport. *Drug Test Anal*. 2017. https://doi.org/10.1002/dta.2303
11. World Anti-Doping Agency (WADA). Athlete Biological Passport Operating Guidelines V. 5.0. 2014.
12. Lobigs LM, Sharpe K, Garvican-Lewis LA, et al. The athlete's hematological response to hypoxia: A meta-analysis on the influence of altitude exposure on key biomarkers of erythropoiesis. *Am J Hematol*. 2018;93:74-83.
13. Sawka MN, Convertino V, Eichner ER, Schnieder SM, Young AJ. Blood volume: importance and adaptations to exercise training, environmental stresses, and trauma/sickness. *Med Sci Sports Exerc*. 2000;32:332-348.
14. Garvican L, Martin D, Quod M, Stephens B, Sassi A, Gore C. Time course of the hemoglobin mass response to natural altitude training in elite endurance cyclists. *Scand J Med Sci Sports*. 2012;22:95-103.
15. Garvican-Lewis LA, Sharpe K, Gore CJ. Time for a new metric for hypoxic dose? *J Appl Physiol*. 2016;121:352-355.

16. Wachsmuth NB, Kley M, Spielvogel H, et al. Changes in blood gas transport of altitude native soccer players near sea-level and sea-level native soccer players at altitude (ISA3600). *Br J Sports Med*. 2013;47:i93-i99.
17. Voss SC, Alsayrafi M, Alsowaidi N, et al. The influence of exercise and circadian rhythm of haemoglobin concentration associated changes in plasma volume on the biological passport. *Br J Sports Med*. 2011;45:A7-A7.
18. Schumacher YO, Christian R, Lobigs L, Gore CJ, Garvican LA. High altitude, heavy exercise and the athlete's biological passport. *Drug Test Anal*. 2015;7:48-55.
19. Bonne TC, Lundby C, Lundby AK, Sander M, Bejder J, Nordsborg NB. Altitude training causes haematological fluctuations with relevance for the Athlete Biological Passport. *Drug Test Anal*. 2015;7:655-662.
20. Sanchis-Gomar F, Pareja-Galeano H, Brioche T, Martinez-Bello V, Lippi G. Altitude exposure in sports: the Athlete Biological Passport standpoint. *Drug Test Anal*. 2014;6:190-193.

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