Received: 2 September 2018 | Revised: 8 October 2018 | Accepted: 14 October 2018

DOI: 10.1002/dta.2526

RESEARCH ARTICLE

WILEY

Worldwide distribution of blood values in elite track and field athletes: Biomarkers of altered erythropoiesis

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Abstract

For the first time, blood samples were collected in all athletes participating in a major sporting event of the International Association of Athletics Federations (IAAF) (Athletics World Championships 2011, Daegu, Korea). All variables obtained from blood analyses were incorporated into the individual blood profiles of each athlete for the so-called athlete biological passport (ABP). This unprecedented data collection highlighted differences for a few blood biomarkers commonly measured and reported for the ABP on some group of athletes. Subsequently, blood tests analyses for all athletes were repeated during the following World Championships (2013, Moscow, Russia). Both sets of blood tests were then used to set up the distribution of blood values for track and field athletes considering potential confounding factors such as gender, age, discipline, origin of the athlete (continental classification), and time of blood collection. Implementation of well-defined distribution of blood values will allow to improve the estimation of blood doping prevalence among a specific population of athletes in track and field.

KEYWORDS

athletes, blood passport, distribution, doping

1 | INTRODUCTION

Throughout the years, top-level athletes have repeatedly tried to increase their endurance capacity and performance through erythropoietin stimulating agents (ESAs) or the abuse of homologous/autologous blood transfusion. All these substances or methods were indicated in the prohibited list of the World Anti-Doping Agency (WADA) in force at the time of Track and Field World Championships in 2011 and 2013.[1] ESAs and homologous blood transfusions are currently detectable by direct anti-doping routine tests conducted either in urine and/or blood[2,3] while autologous blood transfusion cannot be detected by traditional direct methods. Indirect markers are thus used in an individual and longitudinal perspective to target and eventually sanction athletes based on significant abnormalities observed in the sequence of biomarkers of erythropoiesis.[4,5] The determinant biomarkers were the hemoglobin concentration, the reticulocyte count, the OFF-score (also called stimulation index,[6]) and the Abnormal Blood Profile Score (ABPS).[7] The longitudinal follow-up of these blood parameters over time is the basic principle of the hematological module of the so-called athlete biological passport (ABP).[7,8] The ABP focuses on abnormal individual changes of blood biomarkers using a Bayesian statistical approach.[8,9] If an athlete has never been tested in the past, then the initial limits are fixed by population-based limits. Then, each subsequent test adapts to individual limits from the initial population limits. Consequently, inter-subject variance is reduced, and the specificity of the tool is significantly increased. Initial population-based limits were set up according to the fundamental contribution of several earlier-published scientific papers.[6,10] Since then, some pre-analytical and analytical changes have occurred mainly because of the establishment of specific technical documents detailing the procedures for blood sample collection, transport, and analysis as described in the appropriate operating WADA guidelines or technical

documents.[11] The latter allowed to decrease significantly the within-subject variance due to a decrease of some components of the allowable total errors. As a consequence, the population reference values may no longer be adapted to the current ABP[12] and require a significant update. The ABP was officially validated and launched by WADA in 2008. The International Cycling Union (UCI) was the first international federation to implement the ABP with the clear intention to fight even more efficiently against blood doping. Athletes demonstrated very atypical profiles and were finally sanctioned.[13] Consequently, athletes supposedly adapted their practices since blood profiles gradually returned toward values expected from a normal population. Nevertheless, the ABP has been established as a deterrent tool in the fight against blood doping.[14,15]

One year later, the hematological passport was implemented by the International Association of Athletic Federation (IAAF) and quite a few abnormal blood data were observed as in cycling. However, some publications raised doubts on the capacity of the ABP to detect micro ESA injections and/or regular small amounts of blood transfusion.[16,17] To improve the actual ABP, there is a need to investigate whether the current distribution of blood values are still fit for purpose. Another question is whether the sensitivity of the model could be improved by replacing the universal within-subject variance by the implementation of an individual within-subject variance.[18]

The aim of the present study is to evaluate the data from two major events and to publish reference ranges in top-level track and field athletes for biomarkers of altered erythropoiesis following the prevailing ABP guidelines.[11]

2 | SUBJECTS AND METHODS

In 2011 and 2013, all athletes taking part to IAAF World Championships in Daegu (Republic of Korea) and Moscow (Russia) underwent blood withdrawal to supplement the individual hematological passport. Reference ranges in top-level track and field athletes were determined for biomarkers of altered erythropoiesis considering various heterogeneous factors such as gender, age, endurance (all disciplines with distances $\geq$800 meters) or non-endurance (all disciplines other than endurance) disciplines, detailed sport's disciplines, the origin of the athletes, and finally the time of blood sampling. Athletes were incorporated into six different groups of disciplines according to WADA's specification, namely (a) sprint (100 m to 400 m events), (b) combined events (heptathlon and decathlon), (c) jumps (long, triple, high jumps and pole vault), (d) middle distance (from 800 m to 1500 m), (e) long distance (from 3000 m to 50 km), and (f) throws (shot put, discus throw, hammer throw, and javelin throw). The origin of the athletes was determined following the IAAF countries' classification made of six different continents (North America (North America, Central America and Caribbean), South America, Africa, Europe, Asia, and Oceania). Athletes were arranged in three different age (A) groups ($A < 20$; $20 \leq A \leq 30$; $A > 30$) and finally, testing period was categorized into three separate periods (Morning $\leq$11:59; 12:00 $\leq$ Afternoon $\leq$16:59; Evening $\geq$17:00). In total, 3683 athletes from six sports disciplines (2015 male and 1668 female) were tested; they were aged between 16 and 47 years (mean age for total population was 26.0 ± 4.3 years) and originated from 173 different countries. Blood sampling took place between 07:05 and 24:00.

Upon their arrival at the competition sites, athletes were invited to join the designated blood-collection stations at any time of the day, at least 24 hours after their arrival in either Daegu or Moscow, to recover from their journey (hydration status, jetlag …). All blood tests were conducted before the competition following rigorously the WADA ABP operating guidelines[11] including a questionnaire specifically designed for the program. After blood collection, all tubes were deposited carefully in temperature-monitored refrigerators (for temporary storage) and transported daily under refrigerated conditions to the analytical facilities. The transport was done in an insulated cool box under refrigerated conditions and temperature was recorded by a temperature data logger as defined by the WADA standards for testing. Blood samples were all analyzed within less than 24 hours after withdrawal. All details of the blood collection and transport procedures were published elsewhere.[19] If an athlete was tested more than once during an event, only the first blood test was evaluated in this study.

Due to the high number of blood samples, each blood analytical facility was equipped with two identical hematological analyzers, the Sysmex XT-2000i (Sysmex Europe, Norderstedt, Germany). In Daegu, blood analyses were conducted by a mobile unit of the WADA-accredited laboratory of Lausanne whereas in Moscow, the analyses were conducted by the local WADA-accredited laboratory. All analyzers were carefully calibrated by the local Sysmex distributors, and internal quality controls (E-Checks, Level 1, 2, and 3) were run twice before and after each batch of samples. External quality controls provided by the Quality Control Center Switzerland (CSCQ) were also measured to assess whether instruments bias and precision were within WADA's acceptance criteria. As required by WADA's technical document and explained in detailed elsewhere,[20] each blood sample was analyzed twice and hemoglobin and reticulocyte count duplicates had to be within a very narrow range. Only the first valid test results were reported.

Samples were randomly assigned to one of the instruments at the time of their delivery to the blood analytical facilities to avoid any misinterpretation due to a possible instrument bias.

For various logistical and ethical reasons, it was not possible to record the ethnic origin of each athlete. Therefore, the indexed origin was the country which the athlete represented in the event (also called the sport's nationality). This nationality was then sorted based on IAAF appropriate continental classification.

Athletes were asked to report whether they had used hypoxia simulation devices and/or resided or trained at altitude (> 1500 meters) during the previous two weeks. Unfortunately, this information was difficult to control without knowing the exact whereabouts of each athlete. This was not systematically reported by the athletes at the time of blood sampling in the Anti-Doping Administration and Management System (ADAMS). ADAMS is a web-based database management system used by WADA to manage whereabouts information and coordinate the testing activities of the anti-doping organizations. Consequently, this information was not integrated into the evaluation.

Both blood sample collection missions were carried out under the supervision of the personnel from IAAF's Medical & Anti-Doping Department whereas blood analyses were done under the supervision of personal from the Swiss Laboratory for Doping Analyses.

3 | STATISTICAL ANALYSIS

Data analysis was conducted using the R software (R Core Team (2017)). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria), version 3.3. Before analysis, the data were cleaned, and quality controls were performed. Briefly, data were checked for consistency, and every time duplicate measurements were available, they were compared to ensure that the difference was within acceptable range (data not shown). The random distribution of samples over the two machines was assessed using both graphical and statistical tools, and showed no bias linked to gender, year of birth, continent of origin, discipline, endurance vs non-endurance, or time of the day (data not shown).

Values for the ABPS were calculated using a custom-made R package described elsewhere.[7] Briefly, the ABPS combines seven hematological markers [reticulocytes percent (RET%), hemoglobin level (HGB), hematocrit level (HCT), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC)] into a single score.

An arbitrary reference population was set up; it consisted of male European athletes, aged between 20 and 30 years old, tested in the morning and competing in sprint discipline in Daegu (40 subjects belonged to this reference population; this population was mainly selected because the prevalence of blood doping was considered low and the number of subjects was elevated). From this reference population, adjustment values were calculated for each variable using linear models. In this way, the model explains the observed result according to a series of categorical variables based on gender, age, continent of origin, discipline or "endurance", and the competition. When "endurance" is included in the model instead of the detailed disciplines, the reference value corresponds to an athlete competing in a non-endurance discipline. When necessary, the measurements were transformed as described later.

For each parameter, two main models were considered: One without any interaction between the variables, and another one where the effect of each variable was considered differently between men and women (with the inclusion of the interactions of all factors with the gender variable into the model). After adjustment of all values using the model with interactions, a normal distribution was fitted to the data, to estimate some quantiles or interest. In addition, separate models were fitted to the Daegu and Moscow data to verify that any effect found was coherent between the two sets of data.

The latter approach was deemed most relevant while other methods were still considered (robust linear models, mixed models) in our analytical approach.

For each model, results are indicated as the estimate (adjustment value) for each variable, accompanied by a 95% confidence interval (CI) and statistical information indicating whether the estimated value is significantly different from zero. P-values were not corrected for multiple testing, as they are only used to identify trends; however, most observations described below were based on a p-value threshold of 0.001 and they were made in two independent datasets (from Daegu and Moscow); in rare case where an observation was associated with a different p-value, it is indicated explicitly.

The data is represented using boxplots (using R default parameters) or histograms.

4 | RESULTS

In total, 3683 athletes were tested in Daegu (n = 1808) and/or in Moscow (n = 1875) with at least one observation per athlete for each event. The age of athletes (mean +/− SD) for Daegu was 26.0 +/− 4.2 years for males and 25.9 +/− 4.4 years for females. In Moscow, the average age for males was 25.9 +/− 4.3 years and 26.0 +/− 4.3 years for females. Table 1 summarizes the number of athletes tested for each event depending on their age group, gender, origin (continents), disciplines, endurance versus non-endurance disciplines, and finally the testing period.

5 | HEMOGLOBIN OBSERVATIONS

First, box plots in Figure 1A detail the hemoglobin concentrations (HGB, g/dL) for all factors of interest. Table 2 A1 provides the mean HGB reference value with the 95% CI, as well as the necessary adjustment coefficients to apply to obtain mean HGB values for all different classes depicted in Figure 1A. For example, the mean reference HGB value for male athletes was 15.7 g/dL (15.6–15.8 95% CI). To obtain the mean value for female athletes, 1.7 g/dL must be deduced from the reference HGB value (−1.8 to −1.6 with 95% CI). Significant HGB differences with the reference population were observed. Unsurprisingly, gender had a significant impact on HGB. Time of blood collection was also an important confounding factor with a regular decrease of HGB over the day time. Mean HGB values were significantly different (p < 0.01) according to some of the origin of the athletes; for example, athletes from North America and Oceania had lower mean HGB values compared to the reference population. The impact of disciplines on HGB values was also of interest, since a significant adjustment coefficient need to be applied for all of them compared to the reference population (ie, sprint). For some disciplines, mean HGB were higher (long distance, middle distance, throws) and for jumps, mean HGB was lower. An important HGB difference was observed between both events: Mean HGB in Moscow was 0.3 g/dL lower than in Daegu. An example of how to use the reference tables is given in Table 3.

The same principle applies to Table 2 A2, but instead of considering all detailed disciplines, athletes were classified into endurance versus non-endurance classes only. The reference population was included in the non-endurance class. Figure 2 A1 shows the distribution of HGB for the reference population with only 40 observations and no adjustment. To improve the number of observations, the whole population (3683 observations) was used to set-up the distribution of HGB after adjustment (Figure 2 A2). A normal distribution was fitted

TABLE 1 Summary of the total number of athletes tested for each event depending on their age group, gender, origin (continents), disciplines, endurance versus non-endurance disciplines, and finally testing period

	Females			Males			
Age Group	Daegu	Moscow	Total F.	Daegu	Moscow	Total M.	Total
age < 20	49	34	**83**	29	40	**69**	**152**
20 ≤ age ≤ 30	670	659	**1329**	793	863	**1656**	**2985**
age > 30	129	127	**256**	138	152	**290**	**546**
Total	**848**	**820**	**1668**	**960**	**1055**	**2015**	**3683**
	Females			Males			
Continent	Daegu	Moscow	Total F.	Daegu	Moscow	Total M.	Total
Africa	106	90	**196**	150	168	**318**	**514**
Asia	128	83	**211**	156	148	**304**	**515**
Europe	393	403	**796**	374	431	**805**	**1601**
North America	151	170	**321**	189	207	**396**	**717**
Oceania	31	28	**59**	49	45	**94**	**153**
South America	39	46	**85**	42	56	**98**	**183**
Total	**848**	**820**	**1668**	**960**	**1055**	**2015**	**3683**
	Females			Males			
Discipline	Daegu	Moscow	Total F.	Daegu	Moscow	Total M.	Total
Combined Events	28	32	**60**	30	34	**64**	**124**
Jumps	130	100	**230**	125	124	**249**	**479**
Long Distance	176	205	**381**	221	292	**513**	**894**
Middle Distance	69	71	**140**	84	85	**169**	**309**
Sprint	339	303	**642**	368	400	**768**	**1410**
Throws	106	109	**215**	132	120	**252**	**467**
Total	**848**	**820**	**1668**	**960**	**1055**	**2015**	**3683**
Endurance versus non-endurance	Females			Males			
	Daegu	Moscow	Total F.	Daegu	Moscow	Total M.	Total
Non-endurance	603	544	**1147**	655	678	**1333**	**2480**
Endurance	245	276	**521**	305	377	**682**	**1203**
Total	**848**	**820**	**1668**	**960**	**1055**	**2015**	**3683**
	Females			Males			
Testing period	Daegu	Moscow	Total F.	Daegu	Moscow	Total M.	Total
7:00 > Test ≤ 11:59	322	285	**607**	332	374	**706**	**1313**
12:00 ≤ Test ≤ 16:59	285	339	**624**	341	462	**803**	**1427**
17:00 ≤ Test < 00:00	241	196	**437**	287	219	**506**	**943**
Total	**848**	**820**	**1668**	**960**	**1055**	**2015**	**3683**

to this adjusted data and corresponds to a mean of 15.5 g/dL, with standard deviation 0.9 g/dL). Some quantiles of the adjusted data and of the normal distribution fitted to this data were also calculated and are displayed on the same figure. Some extreme quantiles were not calculated for the adjusted data, as the number of observations were too low to obtain meaningful values. The other quantiles are similar between the two distributions, indicating that the normal distribution fits the data very well.

6 | RETICULOCYTES OBSERVATIONS

Based on previous publications,[21] reticulocyte percentage (%RET) measurements were transformed using a square root transformation (sqrt%RET). All sqrt%RET are given in detail in Figure 1B. The influence of all factors is also shown in detail in Table 2 B1 and Table 2 B2. Reticulocytes were significantly higher for female athletes. Except for Oceanian athletes, the reticulocytes were significantly lower in the reference population. Combined events, jumps, and long distance had lower mean reticulocytes than the reference population. Athletes from the throws' discipline had, in contrast, higher reticulocytes values.

Mean sqrt%RET was 0.03 units higher for Moscow than for Daegu. Figure 2 B1 shows the distribution of sqrt%RET of the reference population with no adjustment and Figure 2 B2 the distribution considering all observations after adjustment. The quantile of the normal distribution fitted to the adjusted data were calculated for sqrt%RET. A mean sqrt%RET of 1.00 +/− 0.18 was obtained.

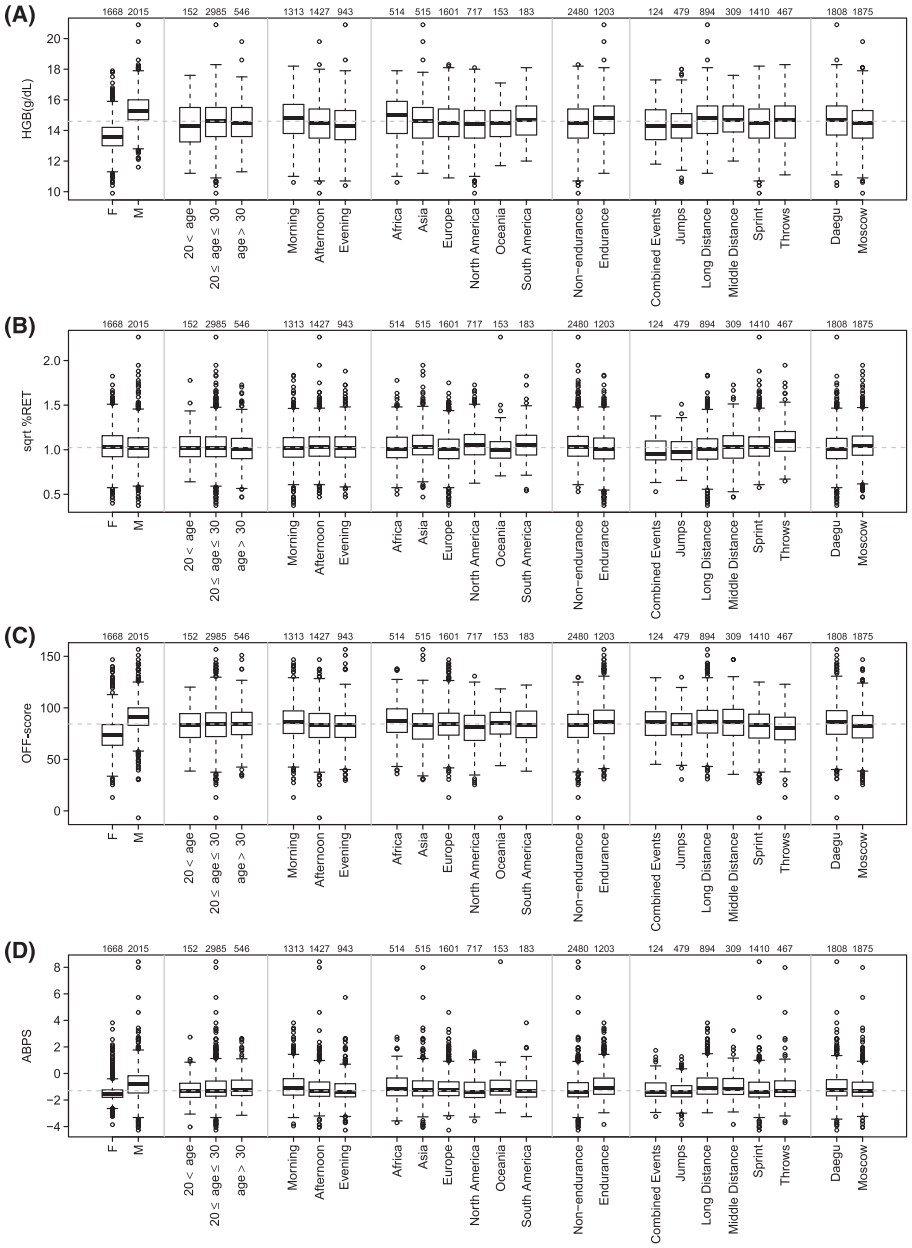

FIGURE 1 Boxplots showing the distributions of hemoglobin levels. A,HGB, g/dL; B, sqrt%RET values (%); C, OFF-scores; D, ABPS values in each subgroups taking into account various confounding factors such as gender, age, time of blood collection, origin of athlete, endurance or non-endurance disciplines, disciplines, and competitions. The number of athletes in each subgroup is indicated above the graph. The boxes of the boxplots represent scores between 25% and 75%, with the median indicated with a bold line in the box; outliers are scores >1.5 times the interquartile range (75%–25%, the length of the box) from the box and are indicated by dots; whiskers extend to the highest or lowest not considered to be an outlier

7 | OFF-SCORE OBSERVATIONS

The OFF-score is calculated as HGB [g/L] – 60*√*Ret*[%].[6,21] Box plots show the influence of the different factors on OFF-score values (Figure 1C). Table 2 C1 and Table 2 C2 give the relevant adjustment coefficients to apply for each factor of interest. Mean OFF-score values were significantly different ($p < 0.001$) to the reference population for gender, time of sample collection, athletes coming from Asia, North America, and Oceania ($p < 0.01$ for this last continent). For some disciplines, the mean OFF-score values were also significantly different in particular for long distance, throws, and to some extent middle distance. Furthermore, the event has a significant impact on OFF-score values (higher in Daegu compared to Moscow). OFF-score distribution with and without adjustment is given in Figure 2 C1 and Figure 2 C2, respectively. The quantile of the normal distribution fitted to the adjusted data were calculated for OFF-score. A mean OFF-score of 95.16 +/− 14.17 was obtained. Both figures confirm that OFF-score is normally distributed; in particular, the quantiles obtained for the adjusted data and the normal distribution are very similar.

TABLE 2 1) List of adjustment coefficients for the different subgroups and different markers, as identified using a linear model, accompanied by their 95% confidence interval. The "reference" row corresponds to the baseline value for the reference population, as described in the text (male, between 20 and 30 years old, blood collected in the morning, from Europe, competing in sprint in Daegu); other rows indicate the coefficient, along with its significance (***: p-value below 0.001; **: p-value below 0.01; * p-value below 0.05; p-values were not corrected for multiple testing). Values that were rounded to 0 are indicated with a dash. A1: HGB concentration (g/dL); B1: sqrt%RET levels (%); C1: OFF-score values; D1: ABPS values. 2) Similar adjustments as mentioned above except that the "reference" row corresponds to the baseline value for the reference population, the male, between 20 and 30 years old, blood collected in the morning, from Europe, competing in non-endurance discipline in Daegu). A2: HGB concentration (g/dL); B2: sqrt%RET levels (%); C2: OFF-score values; D2: ABPS values.

A1			A2		
	Factor	95% CI		Factor	95% CI
Reference	15.7	15.6–15.8	Reference	15.6	15.6–15.7
Female	−1.7 (***)	−1.8 – −1.6	Female	−1.7 (***)	−1.8 – −1.6
Age < 20	—	−0.1 – 0.2	Age < 20	—	−0.1 – 0.2
Age > 30	—	−0.1 – 0.1	Age > 30	—	−0.1 – 0.1
Afternoon	−0.3 (***)	−0.4 – −0.2	Afternoon	−0.3 (***)	−0.4 – −0.2
Evening	−0.4 (***)	−0.5 – −0.3	Evening	−0.4 (***)	−0.5 – −0.3
Africa	0.1	0–0.2	Africa	0.1 (*)	0–0.2
Asia	−0.1	−0.2 – 0	Asia	−0.1	−0.2 – 0
North America	−0.1 (**)	−0.2 – 0	North America	−0.1 (**)	−0.2 – 0
Oceania	−0.2 (**)	−0.4 – −0.1	Oceania	−0.2 (**)	−0.4 – −0.1
South America	0.1	−0.1 – 0.2	South America	0.1	-0.1 – 0.2
Combined Events	—	−0.2 – 0.2	Endurance	0.2 (***)	0.2–0.3
Jumps	−0.2 (**)	−0.3 – −0.1	Moscow	−0.3 (***)	−0.4 – −0.3
Long distance	0.2 (***)	0.1–0.3			
Middle Distance	0.3 (***)	0.2–0.4			
Throws	0.1 (*)	0–0.2			
Moscow	-0.3 (***)	−0.4 – −0.3			
B1			**B2**		
	Factor	95% CI		Factor	95% CI
Reference	1	0.98–1.01	Reference	1	0.98–1.01
Female	0.02 (**)	0.01–0.03	Female	0.02 (**)	0.01–0.03
Age < 20	−0.01	−0.04 – 0.02	Age < 20	−0.01	−0.04 – 0.02
Age > 30	−0.01	−0.03 – 0	Age > 30	−0.01	−0.03 – 0.01
Afternoon	—	−0.02 – 0.01	Afternoon	—	−0.02 – 0.01
Evening	—	−0.02 – 0.01	Evening	—	−0.02 – 0.01
Africa	0.03 (**)	0.01–0.05	Africa	0.03 (**)	0.01–0.05
Asia	0.05 (***)	0.04–0.07	Asia	0.05 (***)	0.03–0.07
North America	0.05 (***)	0.04–0.07	North America	0.05 (***)	0.03–0.07
Oceania	0.01	−0.02 – 0.04	Oceania	0.01	−0.02 – 0.04
South America	0.05 (***)	0.02–0.07	South America	0.05 (***)	0.02–0.07
Combined Events	−0.05 (**)	−0.08 – −0.02	Endurance	−0.03 (***)	−0.04 – −0.02
Jumps	−0.05 (***)	−0.07 – −0.04	Moscow	0.03 (***)	0.02–0.05
Long distance	-0.04 (***)	−0.05 – −0.02			
Middle Distance	—	-0.02 – 0.02			
Throws	0.07 (***)	0.05–0.09			
Moscow	0.03 (***)	0.02–0.05			
C1			**C2**		
	Factor	95% CI		Factor	95% CI
Reference	96.76	95.45–98.07	Reference	96.64	95.46–97.82
Female	−18.08 (***)	−19.01 – −17.15	Female	−18.06 (***)	−19 – −17.12
Age < 20	0.75	−1.61 – 3.12	Age < 20	0.82	−1.55 – 3.18
Age > 30	0.71	−0.63 – 2.05	Age > 30	0.47	−0.85 – 1.8
Afternoon	−2.83 (***)	−3.92 – −1.73	Afternoon	−2.85 (***)	−3.94 – −1.75
Evening	−3.8 (***)	−5 – −2.6	Evening	−3.85 (***)	−5.06 – −2.65

(Continues)

TABLE 2 (Continued)

A1			A2		
	Factor	95% CI		Factor	95% CI
Africa	−0.76	−2.26 – 0.73	Africa	−0.73	−2.21 – 0.76
Asia	−4.06 (***)	−5.51 – −2.6	Asia	−4.03 (***)	−5.49 – −2.58
North America	−4.38 (***)	−5.67 – −3.09	North America	−4.25 (***)	−5.53 – −2.97
Oceania	−3.17 (**)	−5.58 – −0.76	Oceania	−3.26 (**)	−5.67 – −0.85
South America	−1.88	−4.07 – 0.31	South America	−1.86	−4.06 – 0.33
Combined Events	2.8 (*)	0.17–5.44	Endurance	4.14 (***)	3.11–5.17
Jumps	1.62 (*)	0.12–3.11	Moscow	−5.2 (***)	−6.13 – −4.27
Long distance	4.36 (***)	3.11–5.6			
Middle Distance	2.98 (**)	1.2–4.75			
Throws	−3.01 (***)	−4.54 – −1.48			
Moscow	−5.21 (***)	−6.14 – −4.28			
D1			D2		
	Factor	95% CI		Factor	95% CI
Reference	−0.61	−0.69 – −0.53	Reference	−0.62	−0.69 – −0.55
Female	−0.7 (***)	−0.76 – −0.65	Female	−0.71 (***)	−0.76 – −0.65
Age < 20	—	−0.15 – 0.14	Age < 20	−0.01	−0.15 – 0.14
Age > 30	0.03	−0.05 – 0.11	Age > 30	0.05	−0.03 – 0.13
Afternoon	−0.18 (***)	−0.25 – −0.12	Afternoon	−0.18 (***)	−0.25 – −0.11
Evening	−0.28 (***)	−0.35 – −0.21	Evening	−0.28 (***)	−0.35 – −0.21
Africa	−0.11 (*)	−0.21 – −0.02	Africa	−0.12 (*)	−0.21 – −0.03
Asia	−0.04	−0.13 – 0.04	Asia	−0.04	−0.13 – 0.05
North America	−0.17 (***)	−0.24 – −0.09	North America	−0.17 (***)	−0.25 – −0.09
Oceania	-0.09	−0.23 – 0.06	Oceania	−0.08	−0.22 – 0.07
South America	−0.04	−0.18 – 0.09	South America	−0.04	−0.17 – 0.09
Combined Events	—	−0.16 – 0.16	Endurance	0.33 (***)	0.27–0.39
Jumps	−0.12 (*)	−0.21 – −0.03	Moscow	−0.16 (***)	−0.22 – −0.1
Long distance	0.35 (***)	0.27–0.42			
Middle Distance	0.26 (***)	0.15–0.37			
Throws	0.08	−0.01 – 0.18			
Moscow	−0.16 (***)	−0.22 – −0.1			

TABLE 3 Example of how to use the reference Table to correct the HGB concentration (data extracted from Table 2 A1) of a female athlete, tested in Daegu, in the evening, coming from Oceania, and competing in throws, the reference HGB level

15.7	Baseline for the reference population
- 1.7	Adjustment for female athletes
- 0.4	Adjustment for testing in the evening
- 0.2	Adjustment for an athlete coming from Oceania
+ 0.1	Adjustment for an athlete competing in throws
13.5	HGB baseline for the athlete

8 | ABPS OBSERVATIONS

ABPS box plots are given in Figure 1D considering the various factors of interest. Table 2 D1 and Table 2 D2 detail the adjustment coefficient to apply to the mean ABPS value of the reference population for each factor. ABPS distribution with and without adjustment is given in Figure 2 D1 and Figure 2 D2, respectively, and quantile of the normal distribution fitted to the adjusted data were calculated for ABPS. A mean ABPS of −0.67 +/− 0.86 was obtained; again, the figures show that the normal distribution fit the data well.

9 | OTHER VARIABLES COMMONLY REPORTED IN A COMPLETE HEMOGRAM

All other blood parameters (RBC, HCT, MCV, MCH, and MCHC) used for the calculation of ABPS are presented in detail in the Supporting Information. Additional blood parameters (platelet count: Plt; red blood cell distribution width: RDW.SD; absolute reticulocyte count: #RET; immature reticulocyte count: IRF commonly) reported in a usual hemogram together with serum EPO and ferritin concentrations are also in the Supporting Information.

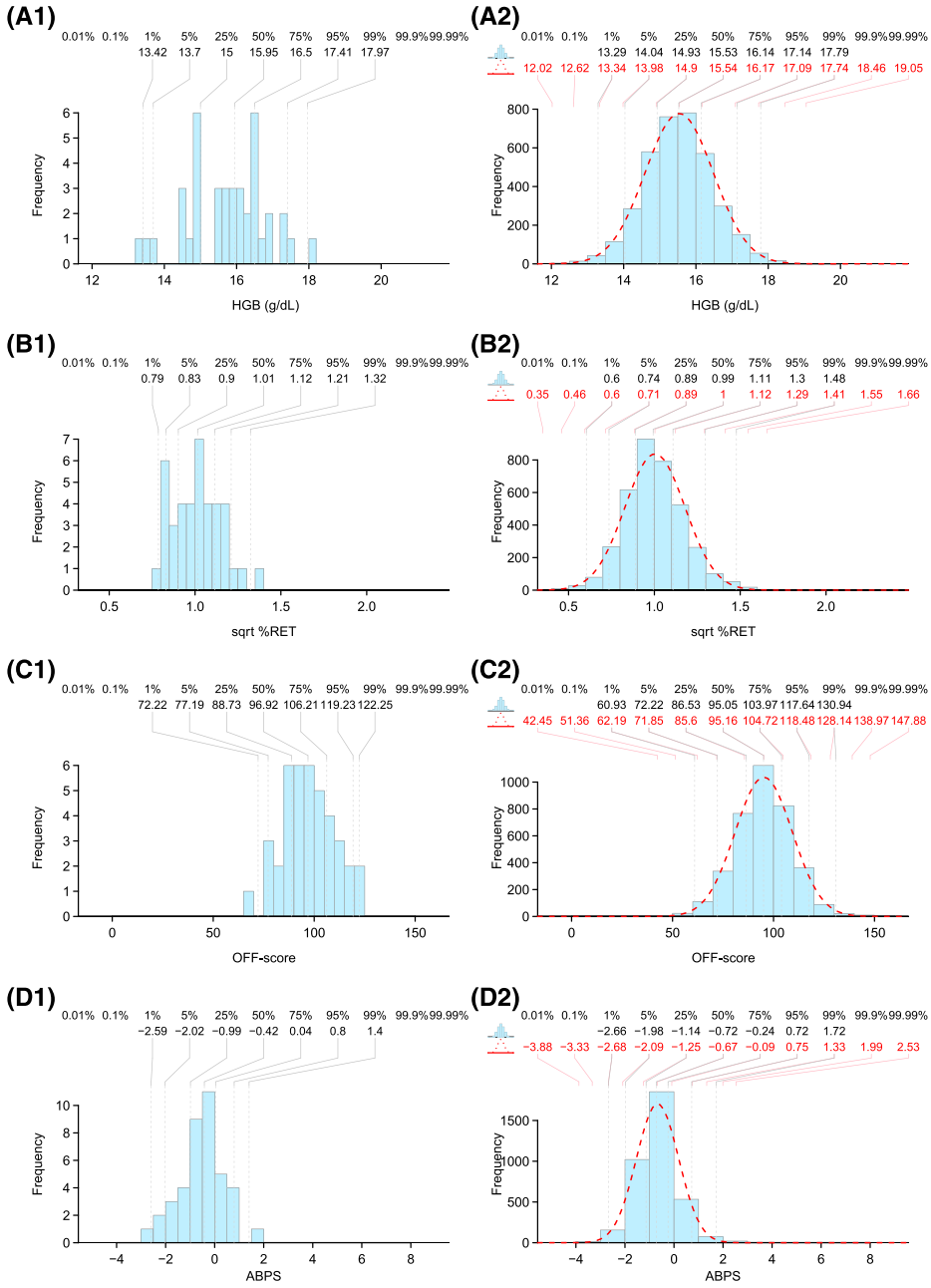

FIGURE 2 A, Histograms showing the distribution of the various markers (in our reference population (40 athletes). The values of some quantiles of interest are indicated above the graph. A1, HGB concentration (g/dL); B1, sqrt%RET levels (%); C1, OFF-score values; D1, ABPS values.B, Histogram showing the distribution of the various markers in our complete population (3683 athletes), after adjustment of the levels according to factors described in the text. The normal distribution fitting the data is plotted in red dashed line over the histogram. Estimated quantiles are indicated above the plot for both the histogram and the fitted normal distribution. A2, HGB concentration (g/dL); B2, sqrt%RET levels (%); C2, OFF-score values; D2, ABPS values.

10 | DISCUSSION

All data presented in this article were obtained in an anti-doping context and not in a medical context. Therefore, the observations presented here, include also data collected on athletes with various pathologies such as iron deficiency, malaria, and other possible diseases nevertheless compatible with sport at the highest level of competition. It cannot be excluded that some athletes doped prior to the blood testing; this could have an impact on the blood distribution of blood values.

It is important to mention that when the Daegu results were obtained, the effects of some of the confounding factors we considered on the variability of the blood parameters such as gender, origin of the athletes, time of collection, and disciplines were more important than initially expected. This was one of the reasons to conduct once again such a massive blood testing during a major event. The results

obtained on the second occasion finally confirmed those obtained during the first blood testing.

11 | GENERAL CONSIDERATIONS

Hemoglobin is recognized to be a robust variable and easily measured worldwide, especially when following the same pre-analytical and analytical protocols and using the same hematological analyzer, which was the case in the present study. A recent study demonstrated that through over 13 000 samples "the current estimate of within-subject variance of HGB used by the ABP remains credible."[18] A seasonable effect has already been observed in several studies with a significant decrease of HGB during warm months within athletes[18,22] and non-athlete populations.[23] It was notably found out that the decrease of HGB during the daytime was higher in Daegu than in Moscow, most likely due to plasma volume changes (data not shown) which might be explained by the differences in weather conditions, and in accordance with the pre-cited literature.[18,22,23] As previously described, the reticulocyte percentage has been transformed in square root values. This transformation has been published elsewhere by Sharpe et al.[6] It is an elegant way to get symmetrical values that are close to a normal distribution and makes the interpretation of values easier to observe. On average, during the event in Moscow, the sqrt%RET was slightly higher (0.03 $p < 0.001$), but the difference may originate from multiple sources. Most likely, the difference was due to variations in calibration of the instruments. Although these differences were statistically significant, they were analytically not important and within the acceptance criteria of the normal function. Indeed, having two analytical sites reporting similar data two years apart is highly improbable. The setting of the instruments and the standard operating procedures prevent as much as possible any drift, but the precision of sqrt%RET to that level still suffers some limitations. On the other hand, the number of low and very low sqt%RET observed in Daegu have nearly disappeared for Moscow. For the first time and on such a large number of athletes, the sqrt%RET distribution curve is given. This distribution is symmetrical and does not show two separate populations. The mean sqrt%RET is close to 1.00 (corresponding to 1.00%RET) with very low or very high values which can be attributed to pathologies or most probably to doping, as most of the pathologies are incompatible with sport at the highest level, except maybe a bleeding ulcer which may influence the blood profile and for which the prevalence is quite high, especially among endurance athletes.[24]

12 | AGE AND GENDER

The mean HGB difference between male and female athletes was equal to 1.7 g/dL, in line with a previous study which found a difference of 1.6 g/dL between genders (1376 males and 1073 females[18]). While the relation between hemoglobin concentration and aging is well-known, with a decrease of 0.06 g/dL per year,[25] in this population of relatively young athletes (mean age of 26 ± 4 years), age difference had no significant impact on HGB for male and female athletes. Studies of this relation are usually made on elder populations and our results are in line with another study[18] where the fixed effect of age was not significant either in a similar population (ie, elite athletes, 22.3 ± 4.6 years).

The sqrt%RET were significantly higher ($p < 0.01$) for women compared to men. Significant differences between male and female athletes were reported elsewhere[26,27] and are similar to our results and at the same magnitude (ie, higher for women). It has been hypothesized that these differences between genders could be induced by the estrogen-dependent production of erythropoietin by the female reproductive system.[28] Thus, it has been suggested that the response to erythropoietin production and expression of its receptor could be more pronounced in female athletes.[26] Nevertheless, further studies are needed to better understand the physiological and especially hematological adaptations of female athletes.

13 | ORIGIN OF THE ATHLETES

African and South American male athletes had on average a mean HGB higher than the reference population whereas the Oceanian athletes had lower HGB values. These observations were not the same for female athletes, because for both events, mean HGB values were higher for European athletes than for any other female athlete population. Knowing that altitude information could not be taken into account and considering that male and female athletes most probably follow the same kind of training camps, it seems that there was a difference in mean HGB concentration between male athletes from different continents and furthermore, these differences were not the same for male and female athletes. Therefore, the origin of the athletes does not have a similar impact on male and female athletes (data not shown).

14 | SPORTS DISCIPLINE

In comparison with the reference population (ie, sprint discipline), mean HGB was higher for long, middle distances, and (to a lesser extent) throws, and was lower for jumps. Indeed, considering the type of effort produced by the athletes in the jumps category, ie, very explosive impulse with only a weak aerobic component, it is logical to obtain higher HGB concentration in the sprint discipline compared to jumps. In addition, it is important to keep in mind that the sprint discipline included distances up to 400 m, during which respectively 29 and 43% of the exercise comes from the aerobic capacity.[29] Moreover, in middle and long distances, the aerobic energetic cost rises to 66 and 84% and obviously goes further when the distance is longer (3000 m, 5000 m, and 10000 m). There were various possible explanations for that; logically, athletes competing in these distance categories would most likely train at altitude to perform better and therefore obtain higher HGB values in comparison to the reference. Objectively, blood doping (ESAs or blood transfusion) could not be excluded for some athletes. HGB distribution was normal and was similar to more recently published data.[18] On average, and considering the lack of standardization of pre-analytical and analytical conditions, the data reported by Lobigs et al are quite similar to our observations. On the other hand, HGB patterns vary between different disciplines. The lack of information regarding testing period during the day time

and the origin of athletes could eventually explain some of these differences.

15 | OFF-SCORE AND ABPS

Considering that OFF-score and ABPS are mathematically calculated scores based on all parameters previously discussed, their respective fluctuations are directly induced by the latter. Therefore, a significantly lower OFF-score has been found for women compared to men induced by a combination of higher sqrt%RET and lower HGB. ABPS is based on seven hematological variables among which HGB is also included. Therefore, the behavior of ABPS for each factor is very like HGB. Under our experimental conditions, both parameters were highly correlated (data not shown).

16 | FURTHER CONSIDERATIONS AND LIMITATIONS

One of the most important conclusions of this study is certainly that in the current ABP, the population limits defined for all athletes must be removed from the system. This population limit is in fact not "fitting for all," because of the significant effects of confounding factors described in this study. The interpretation of the individual ABP cannot be appropriate when the initial population limits do not consider the confounding factors the individual may be submitted to. This makes the work of ABP experts less accurate.

Moreover, it must be assumed that the intra-individual variability is not negligible. Therefore, it should be considered to remove the universal within-subject variance and replace it by an individual within-subject variance which takes better into account important factors in order to improve the sensitivity of the ABP.

It is also important to keep in mind that the proposed reference values are adapted to the selected population of this study only, ie, elite track and field athletes.

17 | ALTITUDE

As mentioned earlier, all athletes had to fill in the usual WADA questionnaire documenting altitude residency, training and competition (altitude higher than 1500 m) as well as the eventual use of hypoxia simulation devices (normobaric or hypobaric simulation, intensity and duration of the exposure to hypoxia) within the two weeks prior to the blood collection. The level of understanding of some athletes was limited (questionnaire in English only and not in the mother tongue of the athlete, lack of education of some athletes) and some questions were too vague resulting in a poor quality of answers. On top of that, when double checking the information in the whereabouts section of ADAMS, it appeared that the information reported in the questionnaires was not always in agreement with the information reported in ADAMS. Currently, the information provided by the WADA questionnaire generates more background than useful and reliable information. It cannot be used to finetune the distribution of blood values among athletes and cannot be used to better understand atypical blood profiles observed among certain athletes.

18 | LIMITATIONS OF THIS STUDY

There are a few limitations to this study. Although some publications show that plasma volume changes are not that important during the daytime, this study shows on a large cohort of athletes, that these changes are significant and should not be ignored. Another limitation is the quality of the calibration of the instruments; a slight bias could have a major impact on the distribution of some blood values. It is also important to add that the blood distributions are valid for track and field athletes who have been tested following the appropriate operating WADA guidelines or technical documents. Any deviation could have a significant impact on blood data.

19 | CONCLUSION

With newer guidelines for blood collection and analyses, our study gathered blood samples of all athletes competing in two major events in 2011 and 2013 to define solid reference values. New reference values for elite track and field athletes are thus proposed here with considerations of the actual ABP guidelines and several factors altering ABP data. Our findings reveal that selected factors (ie, gender, origin, time of collection, discipline) have a significant influence on several hematological parameters involved in the ABP. In addition, significant differences were observed between both events which may highlight a climatic influence (eg, ambient temperature, humidity) on hematological parameters. This new reference dataset can be used to better select reference populations and set individual references for the current ABP. Furthermore, these data will be used in the future to determine more precisely which sub-population of athletes (classification done by age group, sport, discipline, sport's nationality, event) were likely doping (blood doping with ESA and/or transfusion).[30]

ACKNOWLEDGEMENTS

The authors wish to acknowledge WADA's Science Department for the financial support of this study and to the IAAF medical and anti-doping office in Monaco, especially to Thomas Capdevielle and Pierre-Yves Garnier for the medical and logistic organization of the study.

Special recognition is also due to the personal of the Swiss Laboratory for Doping Analyses (LAD) for their technical support.

A special thanks to Dr. Tiia Kuuranne, current Director of LAD, for her help in the critical review of the paper.

CONFLICT OF INTEREST

When finalizing the drafting and submission of the article, Neil Robinson was working for the ITA (International Testing Agency). At the moment of data acquisition, Neil Robinson and Martial Saugy were working at the Swiss Laboratory for Doping analyses (LAD, Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne, Switzerland).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Robinson N, Saugy J, Schütz F, et al. Worldwide distribution of blood values in elite track and field athletes: Biomarkers of altered erythropoiesis. *Drug Test Anal*. 2018;1–11. https://doi.org/10.1002/dta.2526