Effect of altitude on second-generation blood tests to detect erythropoietin abuse by athletes

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Background and Objectives. ON- and OFF-model scores derived from blood parameters sensitive to erythropoiesis have been shown to be a useful tool to identify athletes who are currently injecting erythropoietin to enhance performance or those who have recently stopped doing so. We investigated changes in blood parameters and model scores during and after exposure to terrestrial and simulated altitudes.

Design and Methods. We retrospectively evaluated changes in hematologic data collected from 19 elite cyclists who lived and trained 2690 m above sea level for 26-31 days, from six elite Kenyan runners who lived 2100 m above sea level but descended to compete at sea level competitions, and from 39 well-trained subjects who resided at sea level but slept at a simulated altitude of 2650-3000 m for 20-23 days of either consecutive or intermittent nightly exposure.

Results. Upon ascent to a terrestrial altitude, ON- and OFF-model scores increased immediately, mainly because of an increase in hemoglobin concentration. Scores had not returned fully to baseline three weeks after return to sea level, because of the persistence of the raised hemoglobin concentration for the ON and OFF scores and a fall in reticulocyte percentage for OFF scores. Effects were smaller or negligible for simulated altitude. For Kenyan runners, ON- and OFF-model scores decreased within seven days of descent to sea level.

Interpretation and Conclusions. Our results reinforce the notion that caution should be exercised when interpreting blood results from athletes who have recently been exposed to either terrestrial or simulated altitude, and appropriate allowance should be made for the effect of altitude on blood model scores.

Key words: recombinant human erythropoietin, altitude, athletes, blood tests, doping, erythropoiesis.

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The health risks associated with the widespread practice of blood doping in elite sports, in tandem with the limitations of pharmacologic techniques to detect administration of peptide hormones such as recombinant human erythropoietin (rHuEPO), confronts elite sports with a profound challenge. One response has been to decipher hematologic information gleaned from athletes' blood samples, on the basis that current or prior use of blood doping inevitably disturbs the blood profile, and may therefore be used to highlight athletes who have undertaken this banned practice.[1-5]

The decision on which key hematologic parameters to focus upon must be based not only on their sensitivity to blood doping, but also on their stability during storage, transferability of results, and precision of measurements across different instruments. Cognisant of these issues, our group has recently published blood models that incorporate hemoglobin concentration (Hb), percent reticulocytes, and serum concentrations of erythropoietin (EPO) and transferrin receptor (sTfr).[1] These models not only enable organizations to rationalize the cost of expensive urine assays for rHuEPO by first screening blood samples for signs of recent rHuEPO use, but are also capable of detecting 20-80% of those subjects who have recently ceased administration of rHuEPO.

Our *ON* models detect current rHuEPO users on the basis of concomitant elevations of Hb, EPO and sTfr (associated with accelerated red blood cell production). Our *OFF* models detect users who have recently ceased rHuEPO injections, because these models are sensitive to an unusually high Hb in concert with suppressed EPO and percent reticulocyte values (erythropoiesis is temporarily suppressed upon cessation of rHuEPO injections). The sensitivity of our models is compromised by the inherent inter-individual variability of the component blood parameters. However this has been offset by establishing model cut-off scores based on population data, which provide a level of assurance that an unusual model score is not the result of the individual variation we have documented in an elite athlete population.[1]

In addition to inter-individual variability, factors such as ethnicity, type of sport and residence at altitude also influence blood model scores.[6] We have previously highlighted that residence at a moderate altitude (~1730-2220 m) has by far the largest influence on blood parameters. Endurance athletes incorporate various altitude training modalities to improve performance, exposing

themselves to hypoxia at terrestrial altitudes or to altitude effects simulated by hypoxic gas mixtures. There is still no consensus as to whether the changes in EPO, percent reticulocytes and sTfr documented in athletes residing at altitude are sufficient to increase total red cell mass measurably.[7] However, one universally recognized physiologic manifestation is that exposure to hypoxia precipitates a rapid increase in Hb due to hemoconcentration.[8,9] Given the primacy of Hb in our models, this change by itself will inevitably influence model scores.

The objective of our research was, therefore, to investigate the effect of common altitude training scenarios on the blood models we recommend to highlight rHuEPO use. We retrospectively analyzed data collected from elite athletes who lived and trained at terrestrial altitudes, as well as from well-trained subjects who trained at sea level but slept under various simulated altitude regimens. We also included data from a small cohort of elite runners who lived at altitude but travelled to attend competitions at sea level. We evaluated the change in model scores during and after hypoxic exposure, examining the variations we would expect to see within an elite athlete population.

Design and Methods

Subjects

We performed a retrospective evaluation of data collected from several cohorts of subjects.

The Toluca 1996 group were eight male track cyclists with VO_2 peak of 81.4±3.6 mL.kg^{-1}.min^{-1} (mean±SD) who spent 31 days in the Mexican city of Toluca, 2690 m above sea level.[10] All but one of the athletes was a current or previous gold medallist and/or world record holder at senior or junior level (the exception subsequently won a gold medal at a junior world championship). While at altitude, vitamin and iron supplements (1000 mg vitamin C, 500 IU vitamin E, 350 mg ferrous sulphate) were taken daily. Blood samples were collected prior to ascent to altitude, and on days 3, 9 and 21 after returning to sea level (no samples were collected at altitude).

The Toluca 2000 group were 11 elite-level male track cyclists who were all members of the Australian national track cycling squad eight months prior to the Sydney 2000 Olympic Games. Prior to the altitude training, all cyclists were free from illness and infection according to an assessment by a physician. While at altitude vitamin and iron supplements (1000 mg vitamin C, 500 IU vitamin E, and 350 mg ferrous sulphate equivalent to 105 mg elemental iron) were taken daily, as was a prophylactic antibiotic (norfloxacin) to prevent *E. coli* enteritis. The squad trained together and completed 2778 km in 26 days at Toluca. A resting blood sample was collected prior to ascent to altitude, on days 1, 4, 8, 11, 15, 18, 22 and 25 whilst at altitude, as well as 7 and 14 days after the cyclists' return to sea level.

The Kenyan group were six elite runners who were born and lived in Kenya at an altitude ~2100 m. This group of runners had collectively won three Olympic medals, five World Championship medals and held numerous World Records. Although they travelled to compete at various international meetings during the competitive season, all athletes had resided at altitude continuously since the end of the previous track season (at least six months prior to the sample collected at altitude) except for one athlete who spent a month at sea level three months before the first collection at altitude. The first blood sample was collected during a training camp at ~2100 m, and sea level samples were collected 7 and 14 days after arriving at sea level to compete at various competitions.

The Sim Alt 3000 group were six male endurance athletes (VO_2 peak 70.2±2.9 mL.kg^{-1}.min^{-1}) who slept for 23 nights at a simulated altitude of 3000 m but trained outdoors at approximately 600 m during the study.[11] Although all subjects had normal serum ferritin values (> 20 μg.L^{-1}), iron supplementation (350 mg.week-1 ferrous sulphate) was provided to ensure adequate iron stores. In addition, each subject took vitamin and mineral supplements (500 mg vitamin C, 500 IU vitamin E, and multi-vitamin/mineral pills with 100% of the recommended daily intake). Subjects spent 8-10 hours per night in a room with enriched nitrogen simulating an altitude of 3000 m in normobaric hypoxia (O_2 15.55%). Blood samples were collected prior to and on days 3, 5, 14 and 21 of simulated altitude exposure, as well as 1 and 7 days after leaving the nitrogen house.

The two remaining cohorts exposed to a simulated high altitude were a subgroup of 33 male endurance-trained athletes (nine triathletes and 24 cyclists). Subjects were divided into three groups (Consecutive, Intermittent and Control) matched for initial maximal oxygen consumption. The Consecutive 2650 group (n=12, VO_{2max} 64.8±7.9 mL.kg^{-1}.min-1) spent 8–10 h per day for 20 consecutive nights in a room enriched with nitrogen, simulating an altitude of 2,650 m in normobaric hypoxia (16.3% inspired O_2, 710 Torr ambient barometric pressure). The Intermittent 2650 group (n = 10, VO_2max 64.8±4.1 mL.kg^{-1}.min^{-1}) also spent a total of 20 nights sleeping in hypoxia at a simulated altitude of 2,650 m, comprising four *blocks* of five nights in hypoxia, with each block interspersed by two nights of sleep in normoxia at a natural altitude of 600 m. The Control 2650 group (n= 11, VO_2max 67.0±4.3 mL.kg^{-1}.min^{-1}) slept in dormitory-style accommodation under ambient conditions (600 m above sea level) during the entire experimental protocol. Day-

time hours for all subjects were spent in ambient conditions.[12] Subjects maintained their own training during the study and kept a daily log of duration, mode, and frequency of training beginning one week before and continuing throughout the experimental period.

Sample analysis

All blood samples were collected by trained phlebotomists and were sampled from an antecubital vein after a brief (less than 10 minutes) period of supine rest which was kept constant within each specific study to minimize variation due to postural effects.

The instrument used for hematologic and biochemical assays for each cohort was dictated by availability. Unless noted otherwise, Hb and percent reticulocytes were measured using a locally based ADVIA 120 Hematology Analyzer (Bayer Diagnostics, Tarrytown, NY, USA) in Canberra (Consecutive 2650, Intermittent 2650, and Control 2650 groups), Mexico City (Toluca 2000 group), or London (altitude samples were shipped from Kenya and analysed within 24 hours, whilst sea level samples were collected in London). For the Toluca 1996 group, a Coulter Counter (Model JT, Hialeah, FL, USA) was used to measure Hb and a Becton Dickinson FACScan flow cytometer (San José, CA, USA) to measure reticulocytes. The Sim Alt 3000 study measured Hb and reticulocytes using a Technicon H*3 analyzer (Bayer Diagnostics, Tarrytown, USA) which is the predecessor to the ADVIA 120.

Serum EPO and sTfr concentrations were determined using an automated solid-phase chemiluminescent immunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA) and an automated immunonephelometric assay (Dade Behring, Germany), respectively. The exceptions to this were: Toluca 1996 group in whom EPO was measured using a Boehringer Mannheim Elisa kit read on a Bio-Rad Microplate reader (model 3550, Hercules, CA, USA) and no sTfr measures were taken, and the Sim Alt 3000 study which utilized an R&D Human EPO Quantikine IVD enzyme-linked immunosorbant assay kit to measure EPO.

Model evaluation

The models we chose for evaluation were as follows:[1]

ON-he = Hb + 9.74ln(EPO);
ON-hes = Hb + 6.62ln(EPO) + 19.4ln(sTfr);
OFF-hr = Hb - 60$\sqrt{}$(Ret);
OFF-hre = Hb - 50$\sqrt{}$(Ret) - 7ln(EPO).

Abbreviations (and units): ln, natural logarithm; Hb, hemoglobin concentration ($g.L^{-1}$); Ret, percent reticulocytes; EPO, erythropoietin concentration ($mU.mL^{-1}$); sTfr, serum transferrin receptor ($mg.L^{-1}$).

Since there were (approximately) ten subjects in each of the cohorts, it was appropriate to compare the model scores for these cohorts with the 1 in 10 false positive cut-off thresholds we have published for each of the models (one subject in 10 would be expected to surpass this threshold).

We also evaluated the 1 in 100 (ON models) and 1 in 1000 (OFF models) cut-offs we have recommended as being useful in an antidoping setting.[1] We used the cut-off scores for worst case endurance athletes (as defined previously),[1] and made allowance for altitude during the periods athletes were exposed to either terrestrial or simulated altitude, but used sea level cut-offs when the athletes had returned to sea level or ceased their hypoxic training regimens.

Results

Changes in hematologic parameters

The values for hematologic variables included in our models are shown in Table 1. A notable disparity between subjects exposed to either terrestrial or simulated high altitude was the change in Hb. A marked increase in Hb was evident in the Toluca 2000 group and was still present two weeks after descent to sea level, and a similar post-altitude increase was noted in the Toluca 1996 group, although this had largely dissipated after nine days. In contrast, the mean Hb of the three groups exposed to a simulated high altitude did not change in the same manner. The only exception to this was samples taken after 21 nights of sleeping at a simulated altitude of 3000 m: the mean Hb of blood samples taken next morning (Alt 2) was noticeably higher than baseline, however the value taken the morning after a further two nights of hypoxia (Post 1) was midway between the baseline and peak values, implying there was a degree of noise in these measurements.

The mean serum erythropoietin concentration in the first sample collected from subjects after exposure to either terrestrial or simulated altitude was higher than baseline in all cohorts; however the increase in athletes training at terrestrial altitudes was noticeably higher (97%) than that of subjects exposed to a comparable level of simulated altitude (46%, 64% and 30% for Sim Alt 3000, Consecutive 2650 and Intermittent 2650 groups, respectively). For both terrestrial and simulated altitude, there was an increase in the mean sTfr value at some stage during exposure to hypoxia. Upon descent to sea level, the mean sTfr for both the Toluca 2000 and Kenyan cohorts was lower than that of the baseline sample. This pattern was not apparent in any of the groups exposed to a simulated altitude when a blood sample was drawn on the first morning after completing hypoxic exposure.

Table 1. Hematologic parameters (mean±SD) for subjects exposed to terrestrial (Toluca 1996, Toluca 2000, Kenya) or simulated (Sim Alt 3000, Consecutive 2650, Intermittent 2650) altitude.

	Pre	*Alt 1*	*Alt 2*	*Post 1*	*Post 2*	*Post 3*
Toluca 1996 (n=7)						
Hb	146±6			151±8	148±7	147±5
Retic	*2.7±0.6			*1.7±0.6	*2.2±0.4	*2.2±0.6
EPO	18.2±2.1			17.6±3.8	17.8±4.3	15.5±3.4
sTfr	n/a			n/a	n/a	n/a
Toluca 2000 (n=11)						
Hb	145±7	160±6	157±5	159±7	155±9	
Retic	1.3±0.3	1.4±0.3	1.0±0.3	0.9±0.2	1.1±0.3	
EPO	10.4±2.7	20.4±4.9	12.1±2.8	10.1±4.3	8.7±2.5	
sTfr	1.40±0.35	1.51±0.39	1.50±0.49	1.43±0.40	1.30±0.24	
Kenya (n=6)						
Hb	158±14				141±14	142±16
Retic	1.5±0.4				1.1±0.3	1.1±0.2
EPO	6.5±2.3				5.9±2.7	5.9±1.1
sTfr	1.22±0.42				1.05±0.29	1.00±0.05
Sim Alt 3000 (n=6)						
Hb	145±7	150±5	156±10	150±8	144±11	
Retic	1.0±0.3	1.0±0.2	0.8±0.2	0.9±0.2	0.8±0.3	
EPO	8.6±3.2	12.6±5.8	11.2±4.7	5.9±2.4	9.0±3.4	
sTfr	1.13±0.21	1.25±0.27	1.46±0.37	1.38±0.24	1.37±0.38	
Consecutive 2650 (n=9)						
Hb	150±6	150±5	149±4	149±6		
Retic	1.5±0.4	1.4±0.3	1.5±0.4	1.5±0.6		
EPO	11.4±3.5	18.8±6.3	13.0±3.3	9.7±3.2		
sTfr	1.42±0.26	1.48±0.32	1.54±0.30	1.58±0.35		
Intermittent 2650 (n=9)						
Hb	153±12	153±10	154±10	155±13		
Retic	1.6±0.3	1.6±0.3	1.7±0.4	1.4±0.2		
EPO	13.7±3.1	17.8±8.8	14.9±4.2	11.0±3.1		
sTfr	1.39±0.30	1.47±0.33	1.56±0.29	1.56±0.31		
Control 2650 (n=9)						
Hb	150±14	150±7	148±10	151±9		
Retic	1.3±0.3	1.3±0.2	1.6±0.3	1.3±0.1		
EPO	12.2±5.2	19.5±10.2	16.4±7.9	14.0±4.0		
sTfr	1.41±0.15	1.37±0.16	1.39±0.17	1.50±0.13		

*Values were collected as follows: Pre – before altitude/hypoxia, Alt 1 – first sample collection at altitude/hypoxia, Alt 2 – final sample collection at altitude/hypoxia, Post 1, 2, 3 –samples collected at sea level after returning from altitude/hypoxia. When no sample was collected, the cell is left blank (n/a depicts sample collected but this parameter not measured). *Reticulocytes measured with a Becton Dickinson FACScan. Abbreviations (and units): Hb, hemoglobin concentration (g/L); Retic, percent reticulocytes; EPO, erythropoietin concentration (mU/mL); sTfr, serum transferrin receptor (mg/L).*

ON model scores (ON-he and ON-hes)

Compared with values collected at sea level, ON model scores for the Toluca 2000 group were elevated upon arrival at an altitude of 2690 m. As indicated by a comparison between the graphs for terrestrial and simulated altitude environments (Figure 1), the increase in ON model scores for subjects who slept at a simulated altitude of 3000 m was more gradual, and there was little if any change in ON model scores for the two groups who slept at 2650 m using either a consecutive or intermittent protocol. It was also notable that the amelioration in ON model scores of the Toluca 2000 athletes during their stay at terrestrial altitude (there were no blood samples collected at altitude for the Toluca 1996 study) was opposite in direction to the gradual increase in mean values shown by the Sim Alt 3000 group who slept in a simulated altitude environment. Upon descent to sea level, there was a sharp decline in the mean ON model scores of the Kenyan runners, which was still evident after two weeks at sea level.

As indicated by the range bars in Figure 1, none of the elite cyclists or runners exceeded the 1 in 10 threshold score for either of the ON models whilst at altitude. However upon return to sea level, two cyclists in the Toluca 2000 group exceeded the ON-he cut-off (Hb 165 $g.L^{-1}$ with EPO 17.7 $mU.mL^{-1}$; Hb 171 $g.L^{-1}$ with EPO 8.3 $mU.mL^{-1}$ one and two weeks after returning to sea level respectively). Three athletes who slept intermittently at 2650 m exceeded the 1 in 10 threshold for the ON-he model; one athlete exceeded the threshold before (but not after) hypoxic exposure (Hb 172 $g.L^{-1}$ with EPO 17.0 $mU.mL^{-1}$ and sTfr 2.05 $mg.L^{-1}$), and another two athletes exceeded the threshold on the morning after the hypoxic regimen had been complete (Hb 168 $g.L^{-1}$ with EPO 17.8 $mU.mL^{-1}$ and sTfr 1.67 $mg.L^{-1}$; Hb 177 $g.L^{-1}$ with EPO 22.6 $mU.mL^{-1}$ sTfr 1.77 $mg.L^{-1}$, respectively). These three athletes also exceeded the 1 in 10 cut-off for the ON-hes models at the same time points that they exceeded the ON-he cut-off, moreover the athlete who exceeded the ON-he threshold before hypoxic exposure exceeded the ON-hes threshold before as well as after sleeping intermittently at 2650 m for 20 nights (post Hb 163 $g.L^{-1}$ with EPO 15.5 $mU.mL^{-1}$ and sTfr 1.94 $mg.L^{-1}$).

OFF model scores (OFF-hr and OFF-hre)

Although the mean OFF model scores for the Toluca 1996 group were considerably lower than those of both the Toluca 2000 group and the 95% reference range for endurance athletes (Figure 2), it should be noted that reticulocyte percentages for Toluca 1996 samples were the only ones derived using a FACScan analyzer. Subsequent comparison between the FACScan and ADVIA 120 supported the notion that the FACScan tends to give higher reticulocyte values than the ADVIA 120 analyzer (*unpublished observations*). However, we made no adjustment for this tendency. A higher reticulocyte percentage would result in lower OFF model scores, and this is the most likely explanation for the lower OFF model scores in the Toluca 1996 cohort. Nevertheless, the direction and magnitude of changes pre- to post-altitude in the Toluca 1996 group were consistent with the gradual increase (and persistence) in OFF model scores exhibited by the Toluca 2000 group. The mean OFF model scores for the Kenyan runners were lower than their val-

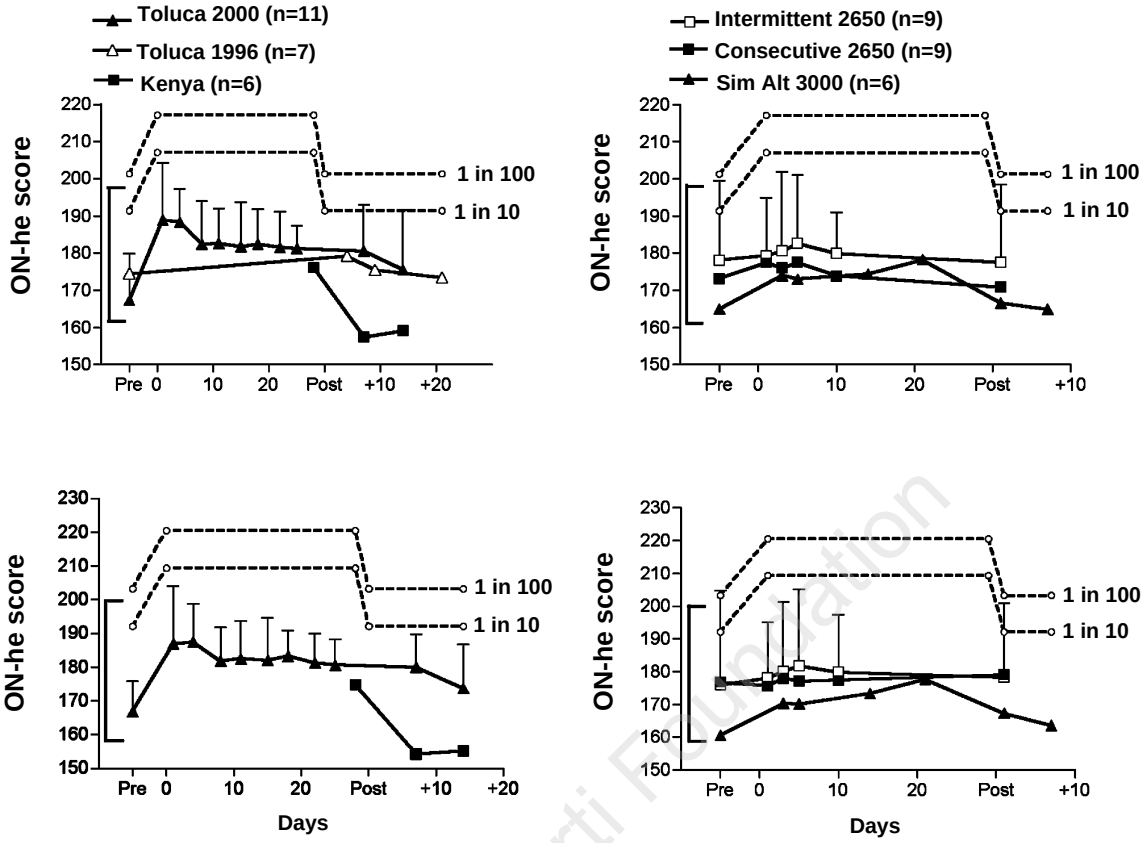

Figure 1. Mean ON model scores over time (error bars depict maximum score recorded in any group) for different groups exposed to terrestrial altitude (left hand column) or simulated altitude (right hand column). The x-axis represents the time spent at altitude, or days after returning to sea level (after *Post*). The solid vertical line between the y-axis and Pre value depicts the upper and lower limits of the 95% reference range for *worst-case* (those with characteristics producing the highest model scores as derived in a previous publication[1]) elite endurance athletes measured at sea level. The 1 in 10 and 1 in 100 cut-off scores correspond to selected false-positive rates for worst-case endurance athletes; cut-offs are higher when samples are collected at altitude since residence at altitude increases model scores. No values are reported for ON-hes of the Toluca 1996 group since no sTfr measurements were conducted.

ues when the athletes were resident at altitude. There was no discernible change in the mean OFF model scores for athletes who slept at a simulated altitude of 2650 m, although for the athletes who slept at 3000 m the mean OFF model scores were elevated during the hypoxic exposure and immediately after completing the hypoxic regimen.

When OFF-hr model scores were evaluated against the appropriate thresholds, two of the 11 cyclists in the Toluca 2000 group exceeded the 1 in 10 threshold whilst at altitude. One athlete exceeded the cut-off on each of the last three measurements at altitude (Hb 169 g.L^{-1} with 0.7% reticulocytes and EPO 9.1 mU.mL^{-1}; Hb 170 g.L^{-1} with 0.7% reticulocytes and EPO 8.1 mU.mL^{-1}; Hb 165 g.L^{-1} with 0.6% reticulocytes and EPO 9.8 mU.mL^{-1} respectively) whilst the other athlete exceeded the threshold only on the last measurement taken (Hb 165 g.L^{-1} with 0.7% reticulocytes). The individual who had exceeded the threshold three times whilst at altitude also exceeded the sea level cut-off one week after descent from altitude (Hb 161 g.L^{-1} with 0.6% reticulocytes and EPO 8.6 mU.mL^{-1}). One of the well-trained subjects who slept at 3000 m simulated altitude exceeded the altitude cut-off after 21 nights spent in hypoxia (Hb 167 g.L^{-1} with 0.7% reticulocytes and EPO 6.9 mU.mL^{-1}) and both this subject and another of his cohort exceeded the sea level cut-off on the first morning after ceasing their hypoxic regimen (Hb 153 g.L^{-1} with 0.6% reticulocytes and EPO 3.5 mU.mL^{-1}; Hb 159 g.L^{-1} with 0.8% reticulocytes and EPO 4.8 mU.mL^{-1}, respectively). For the OFF-hre model, the same athlete in the Toluca 2000 group who exceeded the 1 in 10 OFF-hr threshold for the last three measures taken at altitude also exceed-

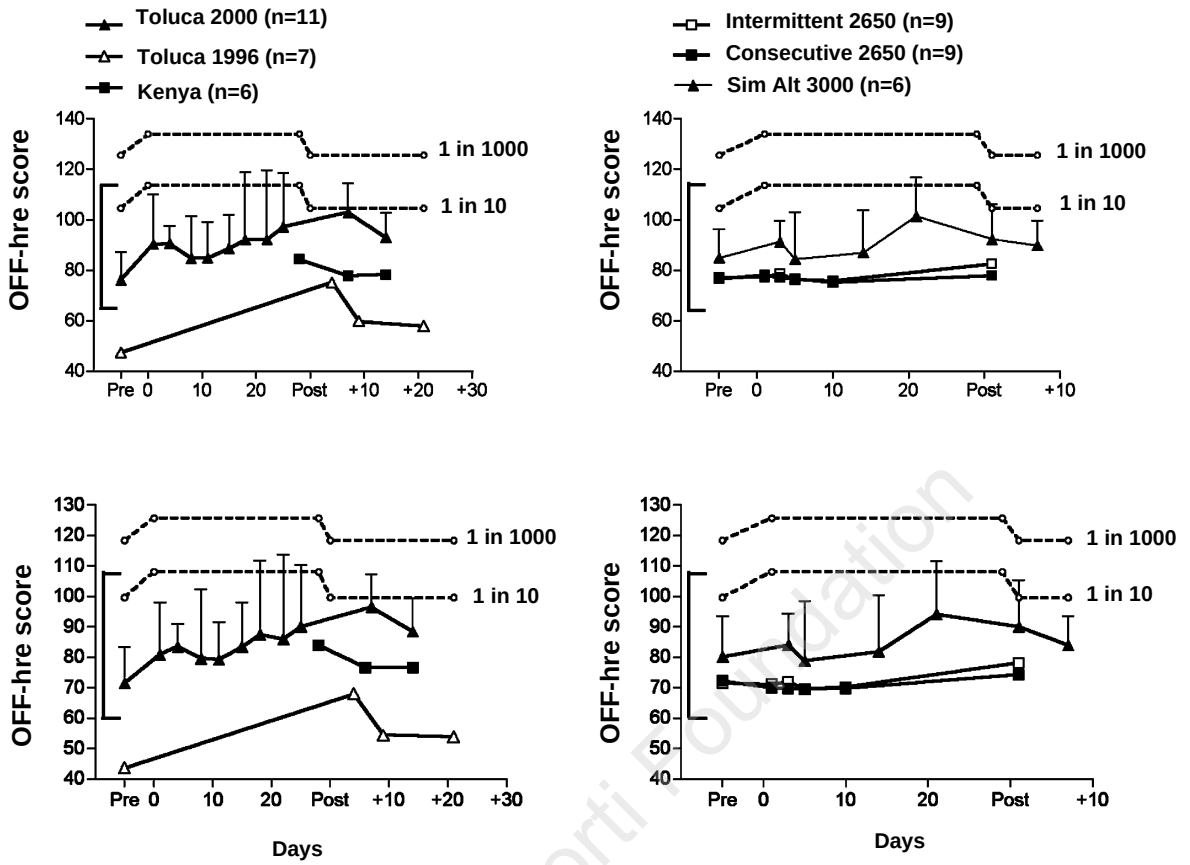

Figure 2. Mean OFF model scores over time (error bars depict maximum score recorded in any group) for different groups exposed to terrestrial altitude (left hand column) or simulated altitude (right hand column). The x-axis is representative of time spent at altitude, or days after returning to sea level (after *Post*). The solid vertical line between the y-axis and Pre value depicts the upper and lower limits of the 95% reference range for *worst-case* (those with characteristics producing the highest model scores as derived in a previous publication)[1] elite endurance athletes measured at sea level. The 1 in 10 and 1 in 1000 cut-off scores correspond to selected false-positive rates for worst-case endurance athletes; cut-offs are higher when samples are collected at altitude since residence at altitude increases model scores.

ed the OFF-hre threshold during these three visits. Furthermore this athlete, plus four others of his group, also exceeded the 1 in 10 sea level threshold for the OFF-hre model one week after descending from an altitude of 2650 m (Hb 159 g.L^{-1} with 0.7% reticulocytes and EPO 7.3 mU.mL-1; Hb 164 g.L^{-1} with 1.0% reticulocytes and EPO 6.7 mU.mL^{-1}; Hb 168 g.L-1 with 1.0% reticulocytes and 7.6 mU.mL^{-1}; Hb 167 g.L^{-1} with 0.9% reticulocytes and EPO 6.7 mU.mL^{-1} for the four colleagues). Within the Sim Alt 3000 cohort of athletes sleeping at 3000 m, the two athletes who exceeded the OFF-hr thresholds also exceeded the corresponding cut-off for the OFF-hre model, at the same time points that they exceeded the OFF-hr threshold.

Influence of hemoglobin concentration in elite athletes training at altitude

Figure 3 (ON-he) and Figure 4 (OFF-hr) illustrate the influence of Hb on our blood model scores for elite athletes training at a terrestrial altitude of 2,690 m (Toluca 2000). For both ON-he and OFF-hr, the initial hemoconcentration upon ascent to altitude was associated with a marked elevation in model scores (the same Hb data were used in both graphs).

The upper panel of Figure 3 demonstrates that the ON-he model score tended to track the amelioration of Hb (and EPO) over time, resulting in a gradual decrease in model score throughout the altitude block. Despite EPO levels falling below baseline values upon descent to sea level, the

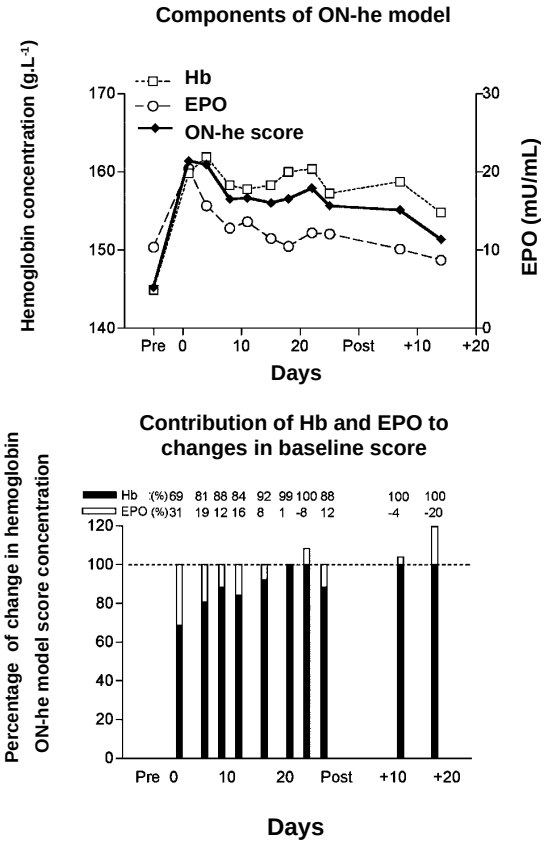

Figure 3. Hematologic and ON-he model score changes in 11 elite cyclists living and training 2690 m above sea level (Toluca 2000 group). The x-axis is representative of time spent at altitude, or days after returning to sea level (after *Post*). The upper panel depicts mean changes in Hb and EPO, whilst variations in the mean ON-he model score over time have been overlain (solid line, but does not correspond to either y-axis). The lower panel represents the percentage contribution of Hb and EPO (actual percentages shown next to legends) to the change in ON-he model scores compared with the pre-altitude value (*Pre*). The segments above the dotted line depict when EPO was lower than baseline and therefore had a negative impact on model scores.

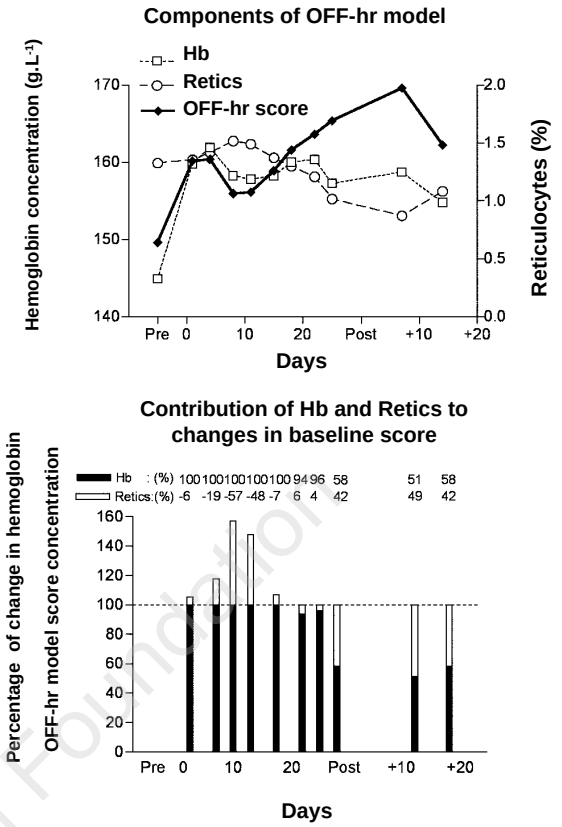

Figure 4. Hematologic and OFF-hr model score changes in 11 elite cyclists living and training 2690 m above sea level (Toluca 2000 group). The x-axis is representative of time spent at altitude, or days after returning to sea level (after *Post*). The upper panel depicts mean changes in Hb and percent reticulocytes (Retics), whilst variations in the mean OFF-hr model score over time have been overlain (solid line, but does not correspond to either y-axis). The lower panel represents the percentage contribution of Hb and reticulocytes (actual percentages shown next to legends) to the change in OFF-hr model scores compared with the pre-altitude value (*Pre*). The segments above the dotted line depict when the reticulocytes were higher than baseline and therefore had a negative impact on model scores.

increase in Hb was still sufficient to elevate ON model scores compared with baseline. The lower panel demonstrates the respective contribution of Hb and EPO to changes in the ON-he model scores relative to baseline values; after one day at altitude the transient spike in EPO was responsible for 31% of the increase in ON-he score, but the relative influence of EPO subsequently decreased throughout the sojourn at altitude. Seven and fourteen days after descent to sea level, the segments above the dotted line depict how the fall in EPO below baseline values had a negative impact on the ON-he model scores (i.e. reducing them) demonstrating that Hb was solely responsible for the heightened ON-he model scores found in these athletes after a sojourn at altitude.

In contrast, following a transient dip on days 8 and 11 (when Hb also dipped below the initial peak), the mean OFF-hr score increased throughout the remainder of the sojourn at altitude (Figure 4, upper panel). The mean score was higher after one week at sea level than at the last measurement taken at altitude. These changes in OFF model scores after day 3 inversely track the per-

centage of reticulocytes in the circulation, which increased initially, then fell below baseline for the remainder of the study period. The transient increase in reticulocytes counterbalanced the tendency for Hb to increase OFF-hr model scores, as shown in the lower panel of Figure 4. The relative contribution of reticulocytes during the first 15 days at altitude was negative. However the subsequent decrease of reticulocytes during the latter period at altitude, which continued after descent to sea level, served to increase OFF-hr model scores. This modest suppression of reticulocytosis in elite athletes after they descended from an altitude training camp was responsible for approximately half of the increase in OFF-hr model score above pre-altitude values.

Discussion

This study retrospectively evaluated changes in blood parameters that we recorded during various studies involving hypoxic exposure. The three data sets associated with terrestrial altitude were each collected on elite athletes – either cyclists or runners who had competed internationally and/or won medals at World Championship or Olympic competitions. Each data set was collected either during a *bona fide* altitude training camp (cyclists) or in the lead-up to sea level competitions (runners) and should, therefore, be representative of the changes that might be encountered when evaluating blood profiles of elite athletes in a competition setting. In contrast, our three data sets involving simulated altitude were collected in a pure research setting. Although the subjects were well-trained, appropriate caution should be exercised when extrapolating these findings to those that might be encountered when testing elite athletes during a competitive season.

We have argued previously that the confounding effect of plasma volume shifts needs to be taken into account for blood testing conducted at altitude, or after a training block conducted at altitude.[6] We, therefore, made allowance for the effect of moderate altitude (~1730-2220 m) when establishing appropriate cut-off scores for each of our models (by estimating a value for this fixed effect from analyzing model scores derived from 1152 elite athletes).[1] The difference in cut-off scores for *worst-case* athletes at either sea level or altitude was shown to be of sufficient magnitude to compensate for the changes we found in the 11 cyclists (Toluca 2000) immediately after their ascent to 2690 m. Although ON model scores decreased during the altitude sojourn, as the initial spikes in both Hb and EPO ameliorated over time (such changes are well documented during continuous altitude exposure), the opposite was found for the OFF models.

During the cyclists' 26-day stay at 2690 m, OFF model scores continued to increase over and above the immediate spike recorded upon arrival at altitude. The overall increase was approximately two-fold higher than the allowance we had previously made for the fixed effect of altitude, and this increase in model score increases the chance of exceeding the 1 in 1000 cut-off to approximately 1 in 100. The continued elevation of OFF model scores whilst at altitude can be attributed to the concomitant decrease of reticulocytes and EPO throughout the sojourn which, in concert with the elevated Hb, led to markedly higher OFF model scores. During 26 days of exposure to 2690 m, there appeared to be a biphasic response of our blood model scores, with a dramatic increase in scores of all models immediately upon arrival at high altitude, followed by a gradual decline in ON model scores and a corresponding increase in OFF model scores, which both appear to be attributable to the physiologic compensations made at altitude.

Although no blood samples were collected from the first group of cyclists during their 31-day stay at 2690 m (Toluca 1996), the difference noted between their pre-altitude model scores and those collected during the first week after descent to sea level virtually mimic the changes noted for the Toluca 2000 cohort. This observation supports the contention that immediately after returning from several weeks spent living and training at 2690 m, the OFF model scores for elite cyclists will be elevated to a greater degree than we had previously attributed to altitude exposure. For both groups, a second blood sample collected within several days of the first post-altitude measurement revealed that model scores had ameliorated substantially, so that the elevation above sea level values was at that stage commensurate with the value we had previously ascribed to altitude. It is noteworthy that the fixed effect of altitude was tailored on athletes resident at altitude, and the underestimation of elevated OFF model scores after athletes acutely exposed to altitude have descended to sea level is a novel finding with important implications for blood testing.

Another unusual, although not unprecedented finding,[11,13,14] was the absence of any substantial alteration in hematologic parameters associated with sleeping at a simulated altitude of 2650-3000 m. The most notable difference in the response to terrestrial or simulated altitude environments was the absence of marked hemoconcentration at the beginning of hypoxic exposure. There was no concomitant elevation of model scores for both groups sleeping at 2650 m and values were essentially unchanged both during and immediately following simulated altitude exposure. In contrast, in well-

trained subjects who slept for 23 nights at a simulated altitude of 3000 m, there was a modest increase in Hb and sTfr and a decrease in EPO and reticulocytes that resulted in elevated ON and OFF model scores. The change was most apparent in the sample collected on the morning after the 21st night, and all models were elevated by approximately the amount we had attributed to the fixed effect of altitude. The change was halved when the next measurement was taken after a further two nights of simulated altitude, and had disappeared altogether after seven nights of sleeping at near sea level. Further studies are required to elucidate whether the changes we observed in recreational athletes are also found in elite-level athletes sleeping at simulated altitude, and whether the duration or extent of hypoxic exposure has a reliable influence on the variation in model scores.

In contrast to the persistence of the altitude-induced increase in Hb in elite cyclists returning from altitude, we found an immediate drop in Hb in a small cohort of elite-level Kenyan runners upon their descent to sea level after a continuous stay at an altitude of ~2100 m. Within seven days of descent to sea level to compete in the European summer track season, this group of elite runners were found to have a dramatic reduction in Hb, almost equal but opposite in direction, to the increase we found in cyclists upon their ascent to moderate altitude. This fall in Hb was responsible for a marked drop in blood model scores. Our data suggest that the time course of achieving pre-altitude Hb levels upon descent to sea level varies, perhaps as a consequence of sport but more likely due to different responses to chronic versus acute altitude.

Despite the plethora of scientific studies concerning the hematologic response to terrestrial altitude, and the acknowledgment that ascent to moderate altitude will result in immediate hemoconcentration, existing literature is equivocal concerning the persistence of elevated Hb levels post-altitude. Although several articles report that the reversal of elevated Hb levels is ostensibly complete within several days,[9,15] and previous papers have shown a return to baseline levels within two weeks of altitude exposure,[16-18] our results indicate that this pattern may not always be present. Until such time as further data have been collected to support or refute our findings, it seems prudent for antidoping agencies to make allowance for the influence of hypoxic exposure on our blood model scores — not only while the athlete is at altitude but also for an as yet unspecified period post-hypoxia.

According to the findings of our study, an athlete who has recently returned from an acute sojourn at altitude is more at risk of exceeding a nominal cut-off threshold for both the ON and OFF models. The largest increase in either ON or OFF model scores associated with altitude, and therefore the increased likelihood of exceeding a nominal cut-off, is roughly equivalent to the disparate risk faced by athletes with Hb levels at either end of the normal range (the athlete with a higher Hb has an increased risk of exceeding the threshold). Although there would be no consequence for an elevated ON model score (since this evidence must be ratified by the presence of rHuEPO in the urine before a positive doping sanction can be applied), it would seem judicious for antidoping agencies to seek information concerning recent altitude exposure before instigating any consequences for an athlete with elevated OFF model scores. Further research is warranted to obtain a global understanding of the influence of altitude on blood model scores. Particular attention should be focused on documenting the persistence of elevated Hb levels in elite athletes after training camps at terrestrial altitude, and whether this increase is influenced by the elevation and duration at altitude.

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Pre-publication Report & Outcomes of Peer Review

Contributions

MJA contributed to the conception and design of the study, analysis and interpretation of the data, drafting and final approval. CJG contributed to conception and design, revising and final approval of the paper. RP contributed to conception and design, revising and final approval. KS contributed to analysis and interpretation, drafting and revising, and final approval. WGH contributed to analysis and interpretation, drafting and revising, and final approval. AGH contributed to conception and design, revising and final approval.

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In the following paragraphs, Professor Brugnara summarizes the peer-review process and its outcomes.

What is already known on this topic

A method combining hematologic and biochemical parameters has shown promise in detecting the abnormally accelerated erythropoiesis induced by recent abuse of recombinant human erythropoietin (r–HuEPO) in competitive athletes (ON model). The abnormally low erythropoietic rate which follows r–HuEPO abuse can also be detected by hematologic and biochemical parameters (OFF model).

What this study adds

Since altitude training is widely used by competitive athletes in a variety of endurance sports, the effect of this training method was assessed on the parameters of the ON and OFF models. Changes in these scores with altitude training were minor for the ON model, but were significant for the OFF model, to a level that would produce a significant number of falsely positive scores for r-HuEPO abuse.

Caveats

This study demonstrates the difficulties in separating, with a high level of confidence, the changes in erythropoietic rates induced by r-HuEPO abuse with those induced by altitude training. It will be important to develop individual hematologic profiles for competitive athletes to help in the proper identification of r-HuEPO abuse or any other illicit method aimed at expanding red cell mass.