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APPENDIX: Development of reference ranges in elite athletes for markers of altered erythropoiesis

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Appendix to Methods

Blood collection and analysis

Erythrocyte and reticulocyte parameters were analysed using the ADVIA 120 Hematology Analyzer (Bayer Diagnostics, Tarrytown NY) which performs flow cytometric measurements . Where possible, analysis was completed within 8 hr of collection. A total of 13 ADVIAs located in 11 countries (two each in France and Italy) were used during the study. However in two locations no ADVIA analyser was available, so samples from Kenya (to South Africa) and Chinese Hong Kong (to Australia) were air-freighted to a nearby ADVIA and analysed within 24 hr of collection. Because samples from Kenya and Chinese Hong Kong were analysed in a different country, this led to a total of 15 Country-ADVIA groups. For simplicity, in what follows, the term ADVIA is used to distinguish between these 15 groups of athletes in order to allow for possible differences between ADVIAs as well as differences between countries.

Statistical analysis

(a) Estimating reference ranges for haematological parameters and model scores. For each of the haematological parameters and model scores, means, standard deviations and 95% reference ranges were estimated using the mixed modeling procedure (Proc Mixed) in the Statistical Analysis System (SAS Version 8.1, SAS Institute, Cary NC). The fixed Effects were Ethnicity, Age, Sport, Altitude, Visit, Time since last exercise, and Time of day of the visit. Levels of some of these effects were chosen in an iterative process of analysis to identify those for which there was a substantial difference and for which there was a reasonable number of athletes on each level. The levels for each of the fixed effects are shown in the tables. While a small number of athletes in other locations recorded their residence during the study as being at moderate altitude, the majority resided in Kenya, Mexico, South Africa and the USA, and the effect of Altitude was evaluated as a comparison between these four nations (Altitude) and all of the others (non-Altitude). For Sport the only comparison we have considered is that between Endurance (groups 4 and 7 in Table 1) and non-Endurance. The first visit of the sub-group of athletes with the following characteristics was nominated as the reference group: Caucasian, non-Endurance athletes of age 19-24 years, who resided less than 610 m above sea level, and whose blood sample was collected between 07:30AM and 4:00PM at least 12 hours after exercise. The levels chosen for the reference group were those with the largest number of athletes. To avoid possible confusion with reference ranges, this group is subsequently referred to as the modal group. Findings for other levels of the fixed effects are expressed as departures from this modal group. The use of a different instrument to analyse the blood of athletes in each country and the analysis of three blood samples from each athlete permitted estimation of between- and within-instrument variation as well as between- and within-athlete variation. The random effects corresponding to these variations were respectively: ADVIA identity, the interaction of ADVIA identity with the identity of the day on

which the analysis was performed, athlete identity, and residual error. The Between-instrument variation represents differences between individual ADVIAs, including any systematic difference in calibration between instruments. This component includes any differences between laboratories caused by different storing, handling and processing of samples. The Within-instrument variation refers to differences that may result from day to day changes in operating conditions, including effects of machine re-calibration. The Between-athlete variation refers to inter-individual differences free of any measurement error or within-athlete variation. Within-athlete variation refers to differences due to biological influences that may alter haematological parameters, including but not limited to diurnal variation, the time since exercise and the intensity of the exercise. Although the variation attributable to some of these factors has already been incorporated into the 95% reference ranges estimated in this study, these are average overall effects, and any individual differences in the way these factors affect athletes is included in the Within-athlete variation. We generated reference ranges for each ADVIA measure by assuming that the probability distribution of the value of a randomly chosen athlete would be at distribution. The mean of the distribution was the mean for athletes on the given level of the Fixed Effect, and on the level of the modal group for all other fixed effects. The variance of the distribution was obtained by summing the variances of all the Random Effects with the variance of the estimate of the mean. The degrees of freedom of the distribution were estimated using the Satterthwaite approximation.[16] Estimates and confidence limits for differences between means of the levels of the fixed effects were provided directly by Proc Mixed. A similar approach was adopted for estimating 95% reference ranges and Effects for EPO and sTfr. All assays were performed in the one laboratory, so there was no Between-laboratory variation to estimate. Standard deviations for the modal group reported in the Tables are the values appropriate for predicting future values and include an allowance for the precision in estimating the mean, while the 95% reference ranges are obtained as the mean plus or minus a t-value (usually about 2.1) times the standard deviation.

(b) Transformations. The reference ranges are based on the assumption that the different values of the parameter for different athletes are normally distributed. The distributions of the raw values of several parameters were highly skewed, so these parameters were transformed appropriately. Square-root transformations were used for RetHct, reticulocyte count and %Retics, while log transformations were used for EPO, and sTfr. The transformation of macrocyte counts was log(count + 0.1), to accommodate observations with zero counts. For those parameters that were square-root transformed, all means, standard deviations and effect sizes are reported on the square-root scale. For those that were log transformed, the mean for the modal group has been back-transformed to the original scale; the standard deviations and effect sizes are reported as multiplicative effects, so that all values must be >1.0, and values close to 1.0 indicate little variation.

(c) Changes in model scores over time. Changes in the ON- and OFF-model scores were analysed for the subgroup of 995 athletes (366 females and 629 males) who had at least two visits. For tractability of the analyses, the dependent variable was the change in model score between each of the three pairs of tests, and we assumed each change score came from a different athlete. The fixed effects were Ethnicity, Age, Sport, Altitude, the Pair of visits, Change in time of visit since last exercise, Change in time of day of the visit, and the Time between the visits. The levels of these fixed effects are shown in the tables. Because ÔCHANGEÕ scores remove between-athlete and between-ADVIA variation, only within-athlete and within-ADVIA random effects were required. By use of the Repeated step in Proc Mixed we specified a different within-athlete effect for the three groups of CHANGE scores defined by the time between visits (to allow for any increase in Within-athlete variation with increasing time between visits).

Appendix to Results

Interpretation of reference ranges

Reference ranges in Tables 2 and 3 for groups other than the modal group are appropriate for individuals that differ only by a single category from the modal group, for example if they are a different Ethnic group, or play a different Sport, or reside at Altitude. Were an athlete exposed to multiple Effects, the appropriate reference range can be estimated using the approach illustrated below. For all parameters except those that were log/square-root transformed, (approximate) 95% reference ranges can be obtained by adding/subtracting 2.1 times the standard deviation from the mean. As an illustration of this procedure, for females in our modal group, mean Hct was 39.6 and the standard deviation was 2.6 (Table 4), therefore the 95%

reference range is given by 39.6 ± (2.1 x 2.6) = 34.1 to 45.1. Note the rounding errors associated with this approach give a reference range slightly different to the range derived directly from our data (34.3 to 45.0, Table 2). To estimate the reference range for athletes who differ from the modal group, the mean value must first be combined with the Effect size (listed in Tables 4 and 5) before it is modified by the standard deviation component. Therefore for African females, where the Effect is -0.7, the modified mean Hct score is 39.6 - 0.7 = 38.9, which is then treated identically to the mean score for the modal group, namely the reference range is given by 38.9 ± (2.1 x 2.6) = 33.4 to 44.4. Finally, for females that differ from the modal group by multiple factors (for example they are African, compete in an Endurance sport and live/train at Altitude), the preliminary mean score is calculated as (39.6 - 0.7 + 1.1 + 1.8) = 41.8. Then 41.8 ± (2.1 x 2.6) gives a reference range of 36.3 to 47.3. For those parameters (RetHct, reticulocyte count and %Retics) that were square-root transformed, the values generated by these calculations must be back-transformed before they are in the original units. For example, males in our modal group possessed a mean RetHct value of 0.81±0.12 (Table 4). On the transformed scale, the reference range is given by 0.81 ± (2.1 x 0.12) = 0.56 to 1.06. However 0.56 and 1.06 must be back-transformed by squaring each value to give the 95% reference range of 0.31 to 1.12. The same principle outlined above for estimating scores for athletes that differ by one or more factors is followed when using transformed parameters. Therefore for males who reside at Altitude (Effect is 0.10), are Oceanian (Effect is 0.04) and less than 19 years of Age (Effect is Δ0.02), the mean score on the transformed scale is (0.81 + 0.10 + 0.04 - 0.02) = 0.93. Then 0.93 ± (2.1 x 0.12) gives the values 0.68 and 1.18, which when back-transformed by squaring give a 95% reference range of 0.46 to 1.39. For those parameters where data were log transformed (%macro(+ 0.1), EPO and sTfr), the log transform implies that Effects are multiplicative and reference ranges have been expressed as the median multiplied/divided by the appropriate factor, rather than as the mean±standard deviation. To illustrate, our male modal group have a median EPO score of 9.2 x/ü1.43 (Table 4). Instead of multiplying the standard deviation of 1.43 by 2.1, it must be raised to the power 2.1 before applying to the median score: $1.43^{2.1}$ = 2.12, and 9.2 x/ü 2.12 gives a 95% reference range of 4.3 to 19.5 (note the discrepancy with the reference ranges derived directly from our data in Table 2, which is caused by rounding errors). To compensate for one or more Effects, the mean score is first altered by multiplying by the Effect size listed in Table 4, then multiplying/dividing this score by the relevant factor raised to the power 2.1. For example, the mean score for males who are Endurance athletes (Effect 1.12) and reside at Altitude (Effect is 1.07) is given by (9.2 x 1.12 x 1.07) = 11.02. When this value is multiplied/divided by $1.43^{2.1}$ (11.02 ü 2.12 and 11.02 x 2.12), the resulting 95% reference range is 5.2 to 23.4.

Reference ranges for hematologic parameters

The span of the 95% reference range for Hct and Hb was of similar magnitude for females and males, whilst the mean value for males exceeded the mean female value by approximately 5 points and 16 $g.L^{-1}$ for Hct and Hb respectively. The largest Effect on Hb and Hct values, consistent across gender, was due to altitude. Residing at a moderate altitude of ~1730Δ2220 m was associated with an average increase of 1.8-2.3 points and 12-15 $g.L^{-1}$ for Hct and Hb respectively. Ethnicity was shown to have a subtle influence on Hb (when delineated from the effect of Altitude), with both the 95% reference range and mean scores for Hb being 5-6 $g.L^{-1}$ lower in males and females within the African group. This difference was not easily discernible in Hct units. The mean Hb values for male and females categorised as endurance athletes were on average 2-4 $g.L^{-1}$ higher than non-endurance athletes, although this difference was less apparent in Hct scores. The 95% reference range for reticulocytes (both absolute and percentage) was similar in both sexes. Although the 95% reference range for the MCV of reticulocytes was also similar across gender, the reference range for erythrocytes with an MCV > 120 fL (macrocytes) was nearly twice as wide for females as for males (Table 2), although this difference was less apparent in terms of the mean score (Table 4), which was only slightly higher for females than males. Serum EPO levels were comparable between females and males (Table 4). The Effect of the various factors, including Time of day, Altitude or Sport, was neither substantial nor consistent between sexes. The highest value recorded in a female was 83.3 $mU.ml^{-1}$ from an iron-deficient athlete (serum ferritin 4.4 $ng.ml^{-1}$, Hct 0.30). The two highest serum EPO values recorded in males were 81.9 and 75.6 $mU.ml^{-1}$. The Effect of Ethnicity on sTfr levels was modest with appreciable differences between all four ethnic groups and both sexes (Table 2). Although the span of the 95%

reference range was similar in all groups, the upper range for African and Asian athletes was higher than in Caucasians (for both males and females). The small sample size in our Oceanian athlete group makes extrapolation from our data tenuous for this group. Although not included in the detection models, ferritin concentration was measured to confirm iron deficiency anaemia in cases where Hct and/or Hb was low. Nineteen female and 13 male athletes had serum ferritin values under 10 and 20 $ng.ml^{-1}$, respectively. The highest ferritin values were 694 and 1154 $ng.ml^{-1}$; the lower score was for a male athlete who had a previously diagnosed disorder of hereditary spherocytosis. The Effect of the various factors on serum ferritin levels, including Time of day, Altitude or Sport, was neither substantial nor consistent between sexes.

Reference ranges for the ON-model

The width of the 95% reference ranges for both males and females was comparable, whilst mean scores were higher for males than females (Table 2). The Effects of Ethnicity, Age, Sport, Time since exercise or Time of day were neither marked nor consistent between sexes (Table 4). The largest single Effect was due to Altitude, which increased ON-model scores in males by an average of 0.14. The influence of this Effect was not evident in female athletes. Both male and female athletes who were categorised as Endurance athletes demonstrated ON-model scores that were 0.05-0.06 higher than non-Endurance athletes. The 95% reference range for males was 1.78-2.51. There were nine males who recorded an ON-model score greater than 2.80, and a further three who recorded ON-model scores of 3.00 or more (3.01, 3.06 and 3.32). The (Central/Southern African) athlete who achieved the highest score presented with low leukocyte ($2.2 \times 10^9.L^{-1}$) and platelet ($100 \times 10^9.L^{-1}$) counts, suggesting the presence of a significant abnormality. Unfortunately our attempts to follow-up this result failed due to our inability to locate the athlete. By comparison, all 26 male recreational athletes injected with rHuEPO in trials held in Sydney and Beijing reached ON-model scores of greater than 2.88 by the end of the 4-week period of rHuEPO administration, and 72% exceeded a score of 3.00 at some time during the four weeks.[2] For females the 95% reference range was 1.63-2.42, with a maximum score of 2.76 recorded in one athlete. By comparison, in the Sydney and Beijing rHuEPO administration trials, all 15 females injected with rHuEPO attained an ON-model score of more than 2.54 by the end of the 4-week period of rHuEPO administration, and 80% exceeded a score of 2.76 at some time during the four weeks.[2]

Reference ranges for the OFF-model

The width of the 95% reference ranges for both males and females was comparable, with mean scores higher for males than females. The influence of Effects, including Ethnicity, Age, Sport, Altitude, Time of day or Time since exercise, was not consistent across sexes. The largest Effect was due to Ethnicity, with female African athletes demonstrating OFF-scores on average 0.14 lower than the modal group. This effect was not evident in male African athletes. An effect attributable to Endurance sport was evident in female athletes (scores 0.07 higher) but not their male counterparts. Both male and female athletes recorded OFF-model scores during their third and final visit that were on average 0.07-0.08 lower than previous visits. For males the 95% reference range was 1.24-2.24, with a maximum score of 2.55 recorded in one athlete. In the Sydney and Beijing administration trials, 92% of the males who were injected with rHuEPO reached OFF-model scores greater than 2.24 at some stage after their last injection. The 95% reference range for females was 1.01-2.01, with a maximum score of 2.12 recorded in one athlete. Every female injected with rHuEPO during the Sydney and Beijing trials exceeded an OFF-model score of 2.01 at some stage after rHuEPO administration ceased.

Changes in the ON- and OFF-model scores over time

Of particular interest is the effect of the time between visits on the change in model scores. For both males and females the magnitude of the change in ON-model scores, as indicated by the width in the reference ranges, was larger with a longer time between visits whereas the effect on changes in OFF-model scores was relatively small (Table 6). The largest change in ON-model scores over this time period was Δ0.43 for males and Δ0.52 for females (the negative sign indicates that the change was a decrease over time). The largest change in OFF-model score was 1.21 for males and Δ0.93 for females.

Components of variation over time

The components of and total variation of the blood parameters and model scores are given in Table 7 as standard deviations. These total standard deviations, increased slightly to allow for the precision with which the means for the modal group have been estimated, are the standard deviations given for the modal group in Table 4. In all cases the increase required was trivial in magnitude.

Appendix to Discussion

Variation in the haematological parameters and models over time

An important issue for the derivation and use of reference ranges is the variation in measured variables, and its causes. There are two direct applications of this in the context of using blood tests to identify rHuEPO users. First, biological variation will impinge on the sensitivity of a test relying on disturbances in these parameters. Second, if athletes are tested in different cities using different analysers, it is important to understand the associated additional variation. As illustrated in Table 7, the greatest source of variation in both males and females, for every parameter and for both model scores, was between athletes. This source of variation is inherent and unavoidable in a value for a single test, but it disappears in CHANGE scores, as the athlete is used as their own control. With the sole exception of macrocyte count, the next largest source of variation arose from within the athletes. For most parameters, within-athlete variation for females was greater than that for males, possibly because of the effects of menstruation. Within-athlete variation for ON- and OFF-model change scores was greater than that of the corresponding single scores by the expected factor of Ã2, as was the within-ADVIA variation. Nevertheless, the resulting total variation in the change scores was about three-quarters that of the single scores, because of the absence of between-subject and between-ADVIA variation in the change scores. The smaller variation in change scores relative to single scores is the reason change scores have potential for better sensitivity and specificity in detection of rHuEPO abuse. In total, 13 ADVIAs were each used on multiple days in this study, allowing us to estimate two sources of variation that are directly relevant for athletes who may be tested in different countries or cities: Between- and Within-ADVIA variation. The overall contribution to total variation of these two components was not substantial, and was exceeded by the Within- and Between-athlete variation in all cases except the macrocyte count for both males and females, where the Within-subject variation was slightly less than the variation attributed to both Between- and Within-ADVIAs. It is worth noting that the quality controls recommended by the manufacturer of the ADVIA reflect their intended use as an aid in disease diagnosis. For the detection of rHuEPO use by athletes, more accurate measures would be desirable. One way to assess the gains that could be attained with more precision is to estimate the reduction in total variation that would be achieved if the Between- and Within-instrument, or Between-assay, variation could be eliminated. This reduced variation can be estimated as the square-root of the sum of squares of the Between- and Within-athlete standard deviations. Reductions achieved varied between 0.2% and 25%. The smallest reductions were obtained for EPO and sTfr, in part because there was no between laboratory variation to omit, while the largest reductions were for Hct (15% for females, 11% for males) and RetHct (11% for females, 25% for males). For the ON-model, the reductions were 12% for females and 10% for males while for the OFF-model the reductions were 7% for both females and males. Reductions in the variation of model scores of this magnitude would permit notable reductions to be made in cut-off scores, which in turn would substantially improve the sensitivity of our models to detect rHuEPO users. Both Hct and RetHct, the parameters with the greatest instrument variation, depend on cell volume and this cellular characteristic is known to be sensitive to the duration and nature of storage conditions. In contrast, Hb and %Retic, which depend on cell numbers rather than volume, are less affected by instrument variation. We are evaluating models that use these parameters in place of Hct and RetHct.

Tables

Table 1.

Ethnicity and sport group of international athletes.

Ethnicity		Country of residence during study	Male (n)	Female (n)
Caucasian		Australia, France, Italy, Mexico, New Zealand, Norway, South Africa, USA	378	242
Asian		China, Malaysia, Singapore	221	126
African		Kenya, South Africa	87	27
Oceanian		Australia, New Zealand	19	7
Other			34	11
Totals			739	413
Group	**Sporting Category**	**Example**	**Male (n)**	**Female (n)**
1	Aesthetic Sports	Gymnastics, Synchronised swimming, Diving	11	19
2	Athletics	Track and Field	65	27
3	Combat Sports	Boxing, Wrestling, Judo, Karate, Fencing, Taekwondo	149	58
4	Endurance Sports	Marathon running; Cycling, Orienteering; Triathlon, Nordic Ski	84	41
5	Multiple	Modern pentathlon, Decathlon, Heptathlon, Biathlon	6	1
6	Power Sports	Weightlifting, Athletic field events such as hammer, discus	36	13
7	Power/ Endurance	Rowing, Canoeing, Swimming, Track cycling	133	84
8	Racquet Sports	Tennis, Badminton, Squash	33	20
9	Skill Sports	Table tennis, Shooting, Archery	16	14
10	Team Ball Games	Basketball, Netball, Volleyball, Hockey, Soccer	201	136
11	Not stated		5	0

Athletes self-identified to which ethnic group they belonged. Caucasian includes European, North African, South West Asian, Arabian, Persian and Indians; Asian include Chinese, Korean, Japanese, Siberian, American Indian and Eskimos; African includes both Central and South Africans; Oceanian refers to Australian Aboriginals, Melanesians and Polynesians; Other includes those who did not state their ethnic background or athletes of mixed ethnic background.

Table 2.

95% reference ranges for the five component haematological and serum parameters, as well as ON- and OFF-model scores. Modal group is Caucasian non-endurance athletes of age 19-24 y, measured <610 m above sea level on their first laboratory visit between 07:30 and 16:00 at least 12 h after exercise. Reference ranges for other groups are shown only when they are substantially different from those of the modal group.

FEMALES	Haematocrit (%)	Reticulocyte haematocrit (%)	Macrocytes (%)	EPO ($mU.ml^{-1}$)	sTfr ($mg.L^{-1}$)	ON-model score	OFF-model score
Modal group	34.3–45.0	0.30–1.08	0.0–1.9	4.4–23.5	0.76–2.02	1.63–2.42	1.01–2.01
Ethnicity							
Asian					0.83–2.21		
African	33.4–44.5	0.34–1.20			0.93–2.53		0.85–1.88
Oceanian			0.0–1.1	3.6–21.0	0.65–1.84	1.47–2.30	
Age							
<19 y			0.0–1.6	4.0–21.5		1.59–2.38	
≥25 y							
Sport							
Endurance	35.3–46.1		0.0–2.5			1.68–2.47	1.08–2.08
Altitude							
1730 to 2220 m	35.9–46.8	0.36–1.20	0.0–1.1		0.80–2.14		
Visit							
Second							
Third	33.5–44.2						0.92–1.92
Time since exercise							
≥4 to 12 h							
≥1.5 to 4 h			0.0–2.3				
<1.5 h							
Time of day							
<07:30	34.8–45.5					1.67–2.46	
≥16:00				4.9–26.2			

MALES	Haematocrit (%)	Reticulocyte haematocrit (%)	Macrocytes (%)	EPO ($mU.ml^{-1}$)	sTfr ($mg.L^{-1}$)	ON-model score	OFF-model score
Modal group	38.8–49.6	0.31–1.12	0.0–1.1	4.6–18.6	0.81–1.87	1.78–2.51	1.24–2.24
Ethnicity							
Asian	39.4–50.3				0.85–1.95	1.83–2.56	
African					0.87–2.03		
Oceanian		0.35–1.21			0.89–2.09		
Age							
<19 y		0.29–1.08	0.0–1.0			1.75–2.48	
≥25 y							
Sport							
Endurance			0.0–1.4	5.1–20.8	0.84–1.94	1.84–2.56	
Altitude							
1730 to 2220 m	41.0–52.0	0.42–1.34	0.0–0.8	4.9–20.0	0.89–2.05	1.91–2.65	
Visit							
Second							1.19–2.19
Third		0.34–1.18					1.18–2.17
Time since exercise							
≥4 to 12 h							
≥1.5 to 4 h							
<1.5 h							
Time of day							
<07:30							
≥16:00				4.9–20.0			

Table 3.

95% reference ranges for blood parameters of athletes measured on an ADVIA. Modal group is Caucasian non-endurance athletes of age 19-24 y, measured <610 m above sea level on their first laboratory visit between 07:30 and 16:00 at least 12 h after exercise. Reference ranges for other groups are shown only when they are substantially different from those of the modal group.

FEMALES	Haemoglobin concentration ($g.L^{-1}$)	Red cell count ($10^{12}.L^{-1}$)	Mean cell volume (fL)	Reticulocyte count ($10^{9}.L^{-1}$)	Reticulocyte percentage (%)	Mean cell volume of reticulocytes (fL)
Modal group	114–151	3.85–5.19	78.0–96.7	29–105	0.65–2.27	94–114
Ethnicity						
Asian						
African	107--146		76.0–95.4	35–118	0.76–2.52	
Oceanian			75.6–95.3			91–112
Age						
<19 y		3.92–5.26	76.7–95.5			
≥25 y						
Sport						
Endurance	118–155		79.4–98.1			96–116
Altitude						
1730 to 2220 m	126–163	4.25–5.60	74.6–93.5	35–117		
Visit						
Second						
Third	112–149	3.79–5.12			0.72–2.38	95–115
Time since exercise						
≥4 to 12 h						
≥1.5 to 4 h						
<1.5 h						
Time of day						
<07:30						
≥16:00						

MALES	Haemoglobin concentration ($g.L^{-1}$)	Red cell count ($10^{12}.L^{-1}$)	Mean cell volume (fL)	Reticulocyte count ($10^{9}.L^{-1}$)	Reticulocyte percentage (%)	Mean cell volume of reticulocytes (fL)
Modal group	131–167	4.41–5.85	78.3–94.0	30–108	0.62–2.08	94–113
Ethnicity						
Asian		4.49–5.93				
African	126–163					
Oceanian	133–170			34–118		
Age						
<19 y	129–166			28–104	0.57–2.00	
≥25 y						
Sport						
Endurance	133–169					
Altitude						
1730 to 2220 m	146–183	4.83–6.27	76.1–92.0	42-130	0.75–2.33	
Visit						
Second	129–166	4.35–5.78				
Third		4.36–5.79		33–114	0.69–2.21	
Time since exercise						
≥4 to 12 h						
≥1.5 to 4 h						
<1.5 h						
Time of day						
<07:30						
≥16:00						

Table 4.

Statistics for the five component haematological and serum parameters, as well as ON- and OFF-model scores: means and standard deviations for the modal group, and substantial mean values (with 95% confidence limits) of a given level of an effect minus the modal group level. Modal group is Caucasian non-endurance athletes of age 19-24 y, measured <610 m above sea level on their first laboratory visit between 07:30 and 16:00 at least 12 h after exercise.

		Haematocrit (%)	Reticulocyte haematocrit[a] (√%)	Macrocytes[b] (%)	EPO[a] (mU.ml^{-1})	sTfr[a] (mg.L^{-1})	ON-model score	OFF-model score
Modal group	F	39.6 ± 2.6	0.79 ± 0.12	0.29 x/÷ 2.24	10.1 x/÷ 1.53	1.24 x/÷ 1.28	2.03 ± 0.19	1.51 ± 0.25
	M	44.2 ± 2.7	0.81 ± 0.12	0.21 x/÷ 1.97	9.2 x/÷ 1.43	1.23 x/÷ 1.24	2.14 ± 0.18	1.74 ± 0.25
Ethnicity (vs Caucasians)								
Asian	F					x1.09 (x/÷1.07)		
	M	0.7 (±0.8)				x1.04 (x/÷1.05)		
African	F	-0.7 (±1.4)	0.05 (±0.06)			x1.23 (x/÷1.12)	0.05 (±0.06)	-0.14 (±0.12)
	M					x1.08 (x/÷1.06)		
Oceanian	F			x0.52 (x/÷1.79)	0.86 (x/÷1.33)	x0.88 (x/÷1.21)		
	M		0.04 (±0.05)			x1.11 (x/÷1.10)	-0.14 (±0.13)	
Age (vs 19 to <25 y)								
<19 y	F			x0.83 (x/÷1.21)	x0.91 (x/÷1.10)		-0.04 (±0.04)	
	M		-0.02 (±0.02)	x0.89 (x/÷1.14)				
≥25 y	F							
	M							
Sport (vs Non-endurance)								
Endurance	F	1.1 (±0.6)		x1.27 (x/÷1.22)			0.05 (±0.05)	0.07 (±0.06)
	M			x1.19 (x/÷1.15)	x1.12 (x/÷1.06)	x1.04 (x/÷1.04)	0.06 (±0.04)	
Altitude (vs <610 m)								
1730 to 2220 m	F	1.8 (±1.7)	0.05 (±0.05)	x0.59 (x/÷1.52)		x1.06 (x/÷1.08)		
	M	2.3 (±1.4)	0.10 (±0.07)	x0.73 (x/÷1.41)	x1.07 (x/÷1.09)	x1.10 (x/÷1.06)	0.14 (±0.09)	
Visit (vs First)								
Second	F							
	M							-0.05 (±0.03)
Third	F	-0.7 (±0.4)						-0.08 (±0.04)
	M		0.03 (±0.02)					-0.07 (±0.03)
Time since exercise (vs ≥12 h)								
≥4 to 12 h	F							
	M							
≥1.5 to 4 h	F			x1.17 (x/÷1.12)				
	M							
<1.5 h	F							
Time of day (vs ≥07:30 to 16:00)								
<07:30	F	0.5 (±0.5)						
	M							
≥16:00	F							
	M							

F, female; M, male.

[a]Data shown are for the square root of the value.

[b]Mean macrocyte percent shown is the back-transformed mean of the log-transformed value; standard deviation, effects, and confidence limits of the effects are multiplicative factors that are applied to this mean.

Table 5.

Statistics for blood parameters measured on ADVIA blood analysers: means and standard deviations for the modal group, and substantial mean values (with 95% confidence limits) of a given level of an effect minus the reference level. Modal group is Caucasian non-endurance athletes of age 19-24 y, measured <610 m above sea level on their first laboratory visit between 07:30 and 16:00 at least 12 h after exercise.

		Haemoglobin concentration ($g.L^{-1}$)	Red cell count ($10^{12}.L^{-1}$)	Mean cell volume (fL)	Reticulocyte count[a] ($\sqrt{10^9.L^{-1}}$)	Reticulocyte percentage ($\sqrt{\%}$)[a]	Mean cell volume of reticulocytes (fL)
Modal group	F	133 ± 9	4.52 ± 0.33	87.4 ± 4.6	7.8 ± 1.2	1.16 ± 0.17	103.9 ± 4.9
	M	149 ± 9	5.13 ± 0.36	86.2 ± 3.9	8.0 ± 1.2	1.11 ± 0.16	103.2 ± 4.8
Ethnicity (vs Caucasians)							
Asian	F						
	M		0.08 (±0.12)				
African	F	-6 (±5)		-1.7 (± 2.6)	0.6 (±0.6)	0.07 (±0.08)	
	M	-5 (±3)					
Oceanian	F			-2.0 (± 3.3)			-2.6 (±3.0)
	M	2 (±4)			0.4 (±0.5)		
Age (vs 19 to <25 y)							
<19 y	F		0.07 (±0.08)	-1.3 (± 1.1)			
	M	-2 (±2)			-0.2 (±0.2)	-0.03 (±0.03)	
≥25 y	F						
	M						
Sport (vs Non-endurance)							
Endurance	F	4 (±2)		1.3 (± 1.1)			2.0 (±1.1)
	M	2 (±2)					
Altitude (vs <610 m)							
1730 to 2220 m	F	12 (±5)	0.41 (±0.17)	-3.3 (±2.6)	0.6 (±0.5)		
	M	15 (±4)	0.42 (±0.17)	-2.1 (± 2.0)	1.0 (±0.6)	0.08 (±0.08)	
Visit (vs First)							
Second	F						
	M	-2 (±1)	-0.07 (±0.03)				
Third	F	-3 (±1)	-0.06 (±0.05)			0.04 (±0.03)	1.0 (±1.1)
	M		-0.06 (±0.04)		0.3 (±0.2)	0.04 (±0.02)	
Time since exercise (vs ≥12 h)							
≥4 to 12 h	F						
	M						
≥1.5 to 4 h	F						
	M						
<1.5 h	F						
	M						
Time of day (vs ≥07:30 to 16:00)							
<07:30	F						
	M						
≥16:00	F						
	M						

F, female; M, male.

[a]Data shown are for the square root of the value provided by the ADVIA.

Table 6.

Statistics (means, standard deviations) and 95% reference ranges for changes in ON- and OFF-model scores between pairs of visits. Modal group is Caucasian non-endurance athletes of age 19-24 y, measured <610 m above sea level on their first two laboratory visits (second - first) within 2 h of the same time of day, with an unknown change in time since exercise, and with 7-8 d between visits. Reference ranges for other groups and within-subject changes are shown only when they are substantially different from those of the modal group.

		ON-model change		OFF-model change	
		Mean±SD	95% Reference range	Mean±SD	95% Reference range
Modal group	F	0.02 ± 0.14	-0.26 – 0.29	-0.05 ± 0.22	-0.48 – 0.39
	M	0.00 ± 0.12	-0.25 – 0.24	-0.10 ± 0.18	-0.45 – 0.26
Ethnicity (vs Caucasians)					
Asian	F				
	M				
African	F				
	M				
Oceanian	F				
	M	0.04 (±0.04)	-0.21 – 0.29		
Age (vs 19 to <25 y)					
<19 y	F				
	M				
≥25 y	F				
	M				
Sport (vs Non-endurance)					
Endurance	F				
	M				
Altitude (vs <610 m)					
1730 to 2220 m	F				
	M			0.08 (±0.05)	-0.38 – 0.33
Visit difference (vs Second - First)					
Third - First	F	-0.06 (±0.08)	-0.33 – 0.24	-0.09 (±0.12)	-0.58 – 0.31
	M	-0.06 (±0.05)	-0.32 – 0.18	-0.04 (±0.07)	-0.49 – 0.22
Third - Second	F				
	M			0.04 (±0.04)	-0.41 – 0.30
Time between visits (vs 6 to 8 d)					
<6 d	F	-0.03 (±0.04)	-0.29 – 0.27		
	M	-0.03 (±0.03)	-0.28 – 0.20	0.05 (±0.04)	-0.40 – 0.30
≥9 d	F	0.04 (±0.07)	-0.24 – 0.35		
	M	0.08 (±0.05)	-0.20 – 0.34		
Change in time since exercise (vs unknown)					
>4 h earlier	F			-0.05 (±0.04)	-0.53 – 0.34
	M				
within ±4 h	F			-0.04 (±0.04)	-0.52 – 0.35
	M				
≥4 h later	F				
	M	0.03 (±0.03)	-0.22 – 0.27		
Change in time of day (vs within ±2 h)					
>2 h earlier	F			0.05 (±0.05)	-0.44 – 0.44
	M				
≥2 h later	F				
	M			-0.06 (±0.04)	-0.51 – 0.20

F, female; M, male.

Table 7.

Components of and total variation of the blood parameters as well as model scores. For reticulocyte haematocrit and both reticulocyte counts, the standard deviation is the square root of the variable. For macrocytes, EPO and sTfr the standard deviations are multiplicative (x/÷).

		Analytical error			Biological variation		Total
		Between ADVIAs	Within ADVIAs	Between assays	Within athletes	Between athletes	
Haematocrit	F	1.1	0.7	-	1.3	1.8	2.6
(%)	M	1.0	0.8	-	1.3	2.0	2.7
Haemoglobin concentration	F	2.8	2.5	-	4.3	7.0	9.0
($g.L^{-1}$)	M	2.1	2.1	-	4.0	7.5	9.0
Reticulocyte haematocrit	F	0.02	0.04	-	0.07	0.08	0.12
($\sqrt{\%}$)	M	0.04	0.04	-	0.06	0.09	0.12
Reticulocyte percentage	F	0.02	0.06	-	0.10	0.12	0.17
($\sqrt{\%}$)	M	0.05	0.04	-	0.08	0.12	0.16
Reticulocyte count	F	0.15	0.37	-	0.69	0.84	1.15
($\sqrt{10^9 L^{-1}}$)	M	0.41	0.33	-	0.58	0.91	1.21
Macrocyte count	F	1.25	1.25	-	1.24	2.01	2.22
(%)	M	1.24	1.23	-	1.22	1.77	1.96
EPO	F			1.09	1.31	1.36	1.52
($mU.ml^{-1}$)	M			1.05	1.24	1.32	1.42
sTfr	F			1.03	1.08	1.26	1.28
($mg.L^{-1}$)	M			1.04	1.06	1.22	1.24
ON-model score	F	0.07	0.06	-	0.08	0.15	0.19
	M	0.06	0.05	-	0.08	0.14	0.18
Change in ON-model	F		0.09		0.11		0.14
	M		0.06		0.11		0.12
OFF-model score	F	0.04	0.08	-	0.13	0.19	0.25
	M	0.06	0.07	-	0.12	0.20	0.25
Change in OFF-model	F		0.12		0.18		0.22
	M		0.09		0.15		0.18