AQ1 Effects of EPO on Blood Parameters and Running Performance in Kenyan Athletes

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ABSTRACT

AQ1 HAILE, D. W., J. DURUSSEL, W. MEKONEN, N. ONGARO, E. ANJILA, M. MOOSES, E. DASKALAKI, K. MOOSES, J. D. MCCLURE, S. SUTEHALL, and Y. P. PITSILADIS. Effects of EPO on Blood Parameters and Running Performance in Kenyan Athletes. *Med. Sci. Sports Exerc.*, Vol. 51, No. 2, pp. 00–00, 2019. **Introduction**: Recombinant human erythropoietin (rHuEpo) administration enhances oxygen carrying capacity and performance at sea level. It remains unknown whether similar effects would be observed in chronic altitude-adapted endurance runners. The aim of this study was to assess the effects of rHuEpo on hematological and performance parameters in chronic altitude-adapted endurance runners as compared to sea level athletes. **Methods**: Twenty well-trained Kenyan endurance runners (KEN) living and training at approximately 2150 m received rHuEpo injections of 50 IU·kg^{-1} body mass every 2 d for 4 wk and responses compared with another cohort (SCO) that underwent an identical protocol at sea level. Blood samples were obtained at baseline, during rHuEpo administration and 4 wk after the final injection. A maximal oxygen uptake ($\dot{V}O_{2max}$) test and 3000-m time trial was performed before, immediately after and 4 wk after the final rHuEpo injection. **Results**: Hematocrit (HCT) and hemoglobin concentration (HGB) were higher in KEN compared to SCO before rHuEpo but similar at the end of administration. Before rHuEpo administration, KEN had higher $\dot{V}O_{2max}$ and faster time trial performance compared to SCO. After rHuEpo administration, there was a similar increase in $\dot{V}O_{2max}$ and time trial performance in both cohorts; most effects of rHuEpo were maintained 4 wk after the final rHuEpo injection in both cohorts. **Conclusions**: Four weeks of rHuEpo increased the HGB and HCT of Kenyan endurance runners to a lesser extent than in SCO (~17% vs ~10%, respectively) and these alterations were associated with similar improvements in running performance immediately after the rHuEpo administration (~5%) and 4 wk after rHuEpo (~3%). **Key Words:** ENDURANCE PERFORMANCE, DOPING, CHRONIC ALTITUDE EXPOSURE, EAST AFRICAN

Recombinant human erythropoietin (rHuEpo) administration has been shown to increase blood oxygen carrying capacity and endurance performance in normoxic conditions. For example, it has been shown that 4 to 6 wk of rHuEpo administration increased maximal oxygen uptake ($\dot{V}O_{2max}$) by 6% to 8% (1–8), submaximal cycling capacity at 80% of $\dot{V}O_{2max}$ by ~54% using time to exhaustion (9) and submaximal running performance by approximately 6% using 3000-m time trial (4). Hence, rHuEpo is, allegedly, frequently subjected to abuse by athletes. Despite this overwhelming evidence, some authors continue to question the ergogenic potential of rHuEpo administration in elite athletes and contend that the performance-enhancing effect of rHuEpo is likely to be small or even nonexistent (10,11). In the most recent of these studies, Heuberger et al. (10) reported that rHuEpo administration enhanced $\dot{V}O_{2max}$ by 10% in well-trained cyclists but did not improve performance during submaximal exercise test or road race performance. These authors argue that time trial performance involving elite athletes is determined by muscle oxidative capacity and to a lesser extent oxygen delivery; hence the suggestion that rHuEpo enhances $\dot{V}O_{2max}$ but not submaximal road racing performance. This explanation is in stark contrast to a study that reported greater gains in performance during submaximal than maximal exercise after rHuEpo administration (9).

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In response to the extraordinary success of the Kenyan and Ethiopian middle- and long-distance runners since the 1968 Mexico City Olympics, several factors have been investigated (see (12) for review) in an attempt to explain this East African running supremacy such as the fact that most of these runners live and train at an altitude of approximately 2400 to 2700 m. For instance, the homeland of the Kalenjin tribe, which is known to produce the vast majority of the best runners in Kenya, is situated at 1830 to 2450 m (13). Specifically, it has been reported that Kenyan runners have a very high $\dot{V}O_{2max}$, high running efficiency, lower blood lactate accumulation, extremely low ammonia accumulation during exercise and higher 3-hydroxyacyl-CoA-dehydrogenase than their European counterparts (14,15).

Although chronic hypobaric and acute normobaric hypoxia can lead to very different physiological responses, it can be speculated that athletes residing at moderate altitude may benefit even more from rHuEpo administration than sea-level athletes due to the enhanced effects of rHuEpo on $\dot{V}O_{2max}$ at this altitude range (16). Robach et al. (16) compared the ergogenic effects of rHuEpo during normobaric hypoxia and normoxia in physically active subjects and found that the relative improvement in $\dot{V}O_{2max}$ induced by rHuEpo administration was greater at a simulated altitude up to 3500 m compared to normoxia, while above 3500 m, $\dot{V}O_{2max}$ remained unaltered by rHuEpo administration. Administration of rHuEpo increased the arterial oxygen content regardless of the severity of hypoxia; however, peak oxygen delivery directed to the exercising legs was only enhanced during normoxia. It remains unknown why the relative improvement in $\dot{V}O_{2max}$ was more than doubled (i.e., 17.5 vs ~8%) at a moderate altitude up to 3500 m compared to normoxia (16). Nonhematological mechanisms, such as augmented buffer capacity, mood improvement, and placebo effect, have all been proposed to account for the observed changes in exercise performance in response to rHuEpo administration (17,18).

In East Africa, where champions are continuously emerging and where being a successful runner is associated with substantial socioeconomic rewards, athletes are subjected to an environment with extreme pressure to perform (13). This unique social, psychological, and economic situation may increase the likelihood of doping behavior. Therefore, the aim of this study was to assess the effects of rHuEpo administration on hematological and performance parameters in well-trained altitude-adapted endurance runners. More specifically, whether rHuEpo at moderate altitude enhances oxygen carrying capacity and indicators of performance in chronic altitude adapted endurance runners. These unique results will be compared with similar observations made in a white cohort living and training at or near sea level that underwent an identical rHuEpo administration regimen (4).

Furthermore, in the antidoping field, the Athlete Biological Passport (ABP) uses intra-individual abnormal variability over time in selected hematological parameters to indirectly detect erythropoiesis-stimulating agents, such as rHuEpo (19). In this context, knowledge of the hematological response after rHuEpo administration in athletes living and training at moderate altitude is urgently required.

METHODS

Subjects. Twenty Kenyan endurance runners from the Kalenjin tribe based at altitude (Eldoret, Kenya, ~2150 m above sea level, PIO_2: 115 mm Hg; age, 26.4 ± 4.1 yr; body mass, 56.6 ± 4.7 kg; height, 171.8 ± 6.4 cm) were recruited for this study. Eighteen subjects were long distance runners (personal best times, 5000 m: 14:22 ± 0:47 min:s; 10,000 m: 29:43 ± 1:2 min:s; half marathon, 1:03 ± 0:14 h:min; marathon, 2:12 ± 0:19 h:min) and 2 were specialized in shorter distances (800 m: 01:50 ± 0:3 min:s; 1,500 m: 3:49 ± 0:4 min:s). On average, the group had been training for 5 ± 2 yr and were training in the same group with an average running distance of 128 ± 25 $km \cdot wk^{-1}$. This cohort (KEN) was compared with a previously published cohort (SCO) of 19 white endurance trained males based at or near sea level (Glasgow, Scotland, PIO_2, 150 mm Hg; age, 26.0 ± 4.5 yr, body mass, 74.8 ± 7.9 kg; height, 179.8 ± 5.4 cm) that underwent an identical rHuEpo administration regimen, a full description of this study is available elsewhere (4).

All subjects underwent a medical assessment and provided written informed consent to participate. Subjects were requested to maintain their normal training but abstain from official sporting competition for the duration of the research study. This study was approved by the Ethics Committees of Moi University (Kenya) and University of Glasgow (Scotland) and conformed to the Declaration of Helsinki.

Experimental design. Each subject subcutaneously self-injected, under supervision, 50 $IU \cdot kg^{-1}$ body mass of rHuEpo (NeoRecormon, Roche, Welwyn Garden City, UK) every second day for 4 wk. The average rHuEpo dose was 2869 ± 46 IU per injection and 11,308 ± 986 $IU \cdot wk^{-1}$ for KEN, whereas the average dose was 3741 ± 383 per injection and 14,966 ± 1530 $IU \cdot wk^{-1}$ for SCO group, reflecting the higher body mass of the SCO cohort (74.8 ± 7.9 kg vs 56.6 ± 4.7 kg respectively). In one subject (KEN), the predetermined safety hematocrit (HCT) limit of 55% was reached on day 12 and saline injections substituted rHuEpo on five occasions (14, 16, 18, 20, and 22 d after the first injection). Thereafter, the subject resumed the standard rHuEpo regimen. Daily oral iron supplementation (~100 mg of elemental iron, ferrous sulfate tablets; Almus, Barnstaple, UK) was given during the 4 wk of rHuEpo administration. Venous blood samples were drawn from an antecubital vein into 4-mL K_2EDTA tubes (Greiner Bio-One Ltd., Stonehouse, UK) after 10 min of rest in the supine position (20). Before the analysis, blood samples were rolled at room temperature for 2 min and measured in triplicate using a Sysmex XT-2000i (Sysmex UK, Milton Keynes, UK) for HCT, hemoglobin concentration (HGB) and reticulocyte percentage (RET%). The mean

value of the triplicate was reported. OFF-score was calculated as follows (21):

$$\mathrm{HGB}\ (\mathrm{g \cdot dL^{-1}}) - 60\sqrt{\mathrm{reticulocytes}(\%)}$$

Three blood samples were taken at baseline over a period of 2 wk, 10 blood samples during rHuEpo administration and seven blood samples after rHuEpo administration over a 4-wk period as previously described (4). The third baseline measurement was used for paired comparisons to limit the potential stress-induced hemoconcentration that may occur during the first two visits (see KEN in Fig. 1A and B). At baseline, resting blood pressure and HR were assessed 3 times on both arms in the supine position before blood sampling. Unlike in SCO, total hemoglobin mass (tHb_{mass}) was not measured in KEN due to practical difficulties.

$\dot{V}O_{2max}$ and running performance. $\dot{V}O_{2max}$ was determined at baseline, 2 to 3 d and 4 wk after the final rHuEpo injection using a standard incremental test to exhaustion on a motorized treadmill (Cardionics Type 3113, Bromma, Sweden). Briefly, after 15 min of rest, running speed was set at 9 $km \cdot h^{-1}$ for 4 min with an incline of 1% and was then increased by 1 $km \cdot h^{-1} \cdot min^{-1}$ to reach 17 $km \cdot h^{-1}$. Thereafter, the speed remained constant and the incline was increased by 2.5%$\cdot min^{-1}$ until volitional exhaustion. After 30 min of rest, $\dot{V}O_{2max}$ was verified using a square-wave protocol to exhaustion at a speed and incline equivalent to the end of the incremental test. Gas exchange variables were measured breath by breath using an automated metabolic gas analysis system (K4b2; Cosmed, Rome, Italy). For each stage, an average of the last 30 s was reported and $\dot{V}O_{2max}$ was declared as the highest average oxygen uptake during a 30-s period. Additionally, attainment of $\dot{V}O_{2max}$ was confirmed if respiratory exchange ratio (R) (>1.10) and HR (±10 bpm of age-predicted maximum (i.e., 220 − subject age)). Two 3000-m running time trials separated by at least 1 d rest were performed on a 400 m outdoor athletics track (Chepkoilel Stadium, Eldoret, Kenya) at an altitude of ~2150 m 2 to 3 d before, at the end of rHuEpo administration and 4 wk after the final injection. Though the study was not blinded, there was a concerted effort to be consistent and minimize subjectivity, that is, limited verbal encouragement with standardized feedback for split time and remaining laps given in all the trials. The best performance of both trials on each occasion was used for analysis. The typical error of measurement for time trial performance calculated between the two tests on each phase was 1.1%. Borg's RPE was recorded at the completion of the time trial. Temperature, humidity and wind speed were recorded via portable

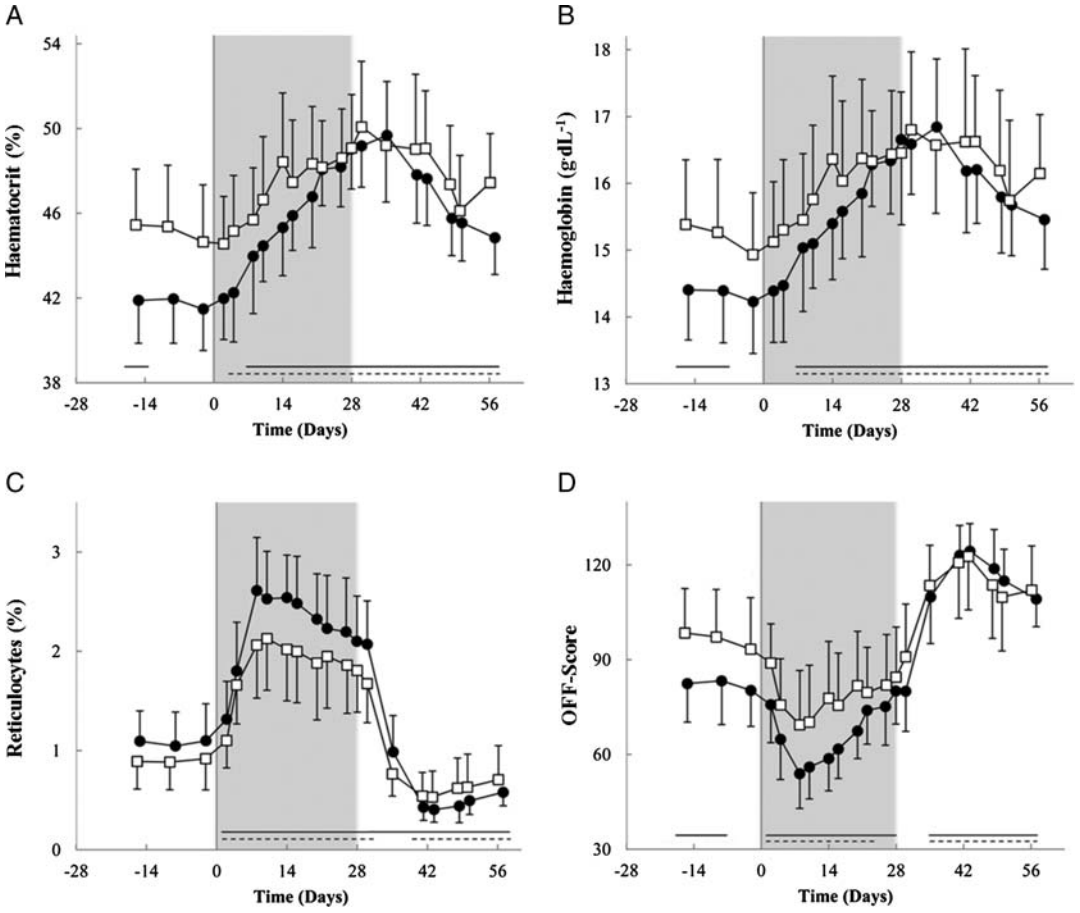

FIGURE 1—Mean ± SD of changes in HCT (A), hemoglobin concentration (B), reticulocyte percent (C) and OFF score (D) in KEN (*white squares*) and SCO (*black circles*). The gray zone represents the 4 wk of rHuEpo administration. Significant differences compared to the third baseline values in KEN (n = 20) and SCO (n = 19) are indicated by *solid and dashed lines* $P < 0.05$, respectively.

TABLE 1. Ventilatory parameters at maximal exertion in both cohorts at different phases of the study.

	KEN			SCO		
	Baseline	End of rHuEpo	End of the Study	Baseline	End of rHuEpo	End of the Study
$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})	66.2^{a} (4.9)	70.7^{ab} (7.3)	68.2^{a} (5.3)	57.7 (5.4)	62.1^{b} (5.0)	60.3 (4.8)
$\dot{V}O_2$ (L·min^{-1})	3.60^{a} (0.32)	3.80^{a} (0.48)	3.65^{a} (0.41)	4.24 (0.41)	4.54^{b} (0.46)	4.43 (0.49)
$\dot{V}CO_2$ (L·min^{-1})	3.74^{a} (0.31)	4.14^{ab} (0.55)	4.25^{a,b} (0.37)	4.57 (0.42)	4.95^{b} (0.64)	4.68 (0.46)
$\dot{V}_E/\dot{V}O_2$	42.1^{a} (3.6)	40.5^{a} (6.3)	43.0^{a} (4.8)	34.8 (3.3)	33.2 (4.4)	32.8 (3.3)
$\dot{V}_E/\dot{V}CO_2$	40.2^{a} (3.2)	34.8^{ab} (2.7)	37.2^{a,b} (3.8)	32.3 (3.4)	30.2^{b} (2.6)	30.9 (2.9)
RR (bpm)	65 (5)	62 (5)	63 (9)	61 (8)	59 (7)	58 (9)
$PETO_2$ (mm Hg)	84^{a} (2)	83^{a} (4)	85^{a} (2)	115 (3)	113 (5)	113 (4)
$PETCO_2$ (mm Hg)	31^{a} (3)	34^{ab} (2)	33^{a} (3)	36 (4)	39^{b} (4)	38 (3)
R	1.04 (0.03)	1.10 (0.12)	1.18^{b} (0.12)	1.07 (0.04)	1.08 (0.08)	1.06 (0.07)
$\dot{V}_E$ (L·min^{-1})	145.0 (21.1)	146.8 (18.5)	154.8 (13.3)	150.3 (15.6)	150.8 (17.5)	148.5 (13.7)
HR (bpm)	185 (7)	188 (6)	189^{a} (6)	184 (9)	183 (6)	181 (10)

Values are mean ± SD. Significant differences in KEN compared with SCO are indicated by $^{a}P < 0.05$. Significant differences compared to baseline values in both groups are indicated by $^{b}P < 0.05$. KEN, $n = 12$ unless indicated otherwise; SCO, $n = 14$ unless indicated otherwise; $\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1}), $\dot{V}O_2$ (L·min^{-1}, KEN = 9), and $\dot{V}CO_2$ (KEN = 9); $\dot{V}_E/\dot{V}O_2$, ventilatory equivalent for oxygen (KEN = 11), $\dot{V}_E/\dot{V}CO_2$, ventilatory equivalent for carbon dioxide; RR, respiratory rate; $PETO_2$, end tidal partial pressure for O_2; R, respiratory quotient (KEN = 9); $\dot{V}_E$, respiratory minute ventilation (KEN = 11) and HR (SCO = 5).

hygrometer and anemometer. During data collection, the laboratory in East Africa was flooded resulting in the damage of our metabolic gas analyzer and loss of some data as reflected in the numbers presented in Table 1. T1

Statistical analysis. Time trial performance, $\dot{V}O_{2max}$ and blood parameters data were analyzed using a two-way ANOVA for repeated measures over time (two groups: SCO vs KEN and three measurements: baseline, end of rHuEpo and 4 wk after the final injection). When *F*-statistic was significant in the ANOVA, planned pairwise-specific comparisons were carried out using Student's paired, two-sample *t* test, Mann–Whitney or Wilcoxon Signed Rank test where appropriate. A one-way, repeated-measures ANOVA over time for each group was also performed. Relationships between changes in HGB, time trial performance and $\dot{V}O_{2max}$ were assessed using the Pearson's product moment correlation coefficient. Statistical significance was declared at $P < 0.05$. Data are described as mean ± SD. The results of KEN precede SCO when values of the two groups are compared.

RESULTS

Hematological parameters. Hematocrit and HGB were significantly higher in KEN compared with SCO at baseline (HCT, 44.74% ± 2.71% vs 41.54% ± 2.0%; $P < 0.001$ and HGB, 14.9 ± 0.9 g·dL^{-1} vs 14.2 ± 0.8 g·dL^{-1}; $P = 0.017$, respectively, Fig. 1A and B). A main effect analysis showed that, although HCT and HGB significantly increased in both groups after rHuEpo administration ($P < 0.01$, Fig. 1A and B, Table 2), these blood parameters reached similar maximum values approximately 3 wk after the rHuEpo administration T2 period (50.1% ± 3.1% vs 49.2% ± 1.9%, $P = 0.96$ and 16.8 ± 1.2 g·dL^{-1} vs 16.6 ± 0.8 g·dL^{-1}, $P = 0.551$, respectively). Between group differences in both HCT and HGB values reappeared approximately 4 wk after the cessation of rHuEpo administration (47.0% ± 2.1% vs 45.1% ± 1.7%, $P < 0.05$ and 16.1 ± 0.8 g·dL^{-1} vs 15.6 ± 0.7 g·dL^{-1}, $P < 0.05$, respectively) (Fig. 1A and B). RET% followed a similar pattern in both groups (Fig. 1C). From similar baseline values, RET% increased rapidly and significantly after the first week of administration but attained lower values in KEN compared to SCO during the rHuEpo administration (2.06% ± 0.53% vs 2.61% ± 0.54%, $P = 0.004$) (Fig. 1C). Nadir was reached approximately 2 wk after the last rHuEpo injection in KEN and SCO (0.53% ± 0.26% vs 0.41% ± 0.13%, $P = 0.169$), which were significantly lower compared to baseline values in both groups ($P < 0.001$) (Fig. 1C). When both groups were taken together, there was a significant interaction (HCT: $F_{2,114} = 6.98$, $P < 0.001$; HGB: $F_{2,114} = 3.97$, $P < 0.05$). Regarding RET%, there were no interactions ($F_{2,114} = 0.03$, $P = 0.96$), and therefore, we analyzed the main effects of rHuEpo ($F_{2,114} = 142.32$, $P < 0.001$) and altitude ($F_{2,114} = 2.38$, $P = 0.13$). OFF-score in KEN was significantly elevated at baseline and until the third week of the rHuEpo administration compared with SCO (Fig. 1D). OFF-score significantly decreased during the first 10 d of rHuEpo administration and increased thereafter to reach maximum values 2 wk after the last injection in both groups (Fig. 1D). One subject in KEN had a distinctive hematological response to rHuEpo administration. His baseline HCT (47.1%) and HGB (16.4 g·dL^{-1}) remained relatively stable throughout the study and the response in RET% was blunted.

TABLE 2. Relative changes in running performance and blood parameters in KEN and SCO.

	KEN		SCO	
	End of rHuEpo	End of the Study	End of rHuEpo	End of the Study
Hematocrit	9.7 ± 4.7%^{a,b}	4.1 ± 5.0%^{a,b}	17.6 ± 4.9%^{b}	7.8 ± 4.5%^{b}
Hemoglobin concentration	10.1 ± 5.5%^{a,b}	5.9 ± 5.8%^{b}	16.2 ± 5.2%^{b}	8.2 ± 5.1%^{b}
3000 m performance	−4.6 ± 2.4%^{b}	−3.3 ± 2.6%^{b}	−5.7 ± 2.5%^{b}	−3.4 ± 2.6%^{b}
Maximal oxygen uptake	5.8 ± 8.0%^{b}	2.6 ± 8.6%	8.1 ± 6.8%^{b}	4.4 ± 5.6%^{b}

Values are mean ± SD (95% confidence intervals). Significant differences in KEN compared to SCO are indicated by $^{a}P < 0.05$. Significant differences compared to baseline values in both groups are indicated by $^{b}P < 0.05$. 15 KEN and 19 SCO subjects were included in the analysis of time trial performance, 12 KEN and 14 SCO subjects were included in the analysis of $\dot{V}O_{2max}$ and 20 KEN and 19 SCO subjects were included for analysis of the other variables.

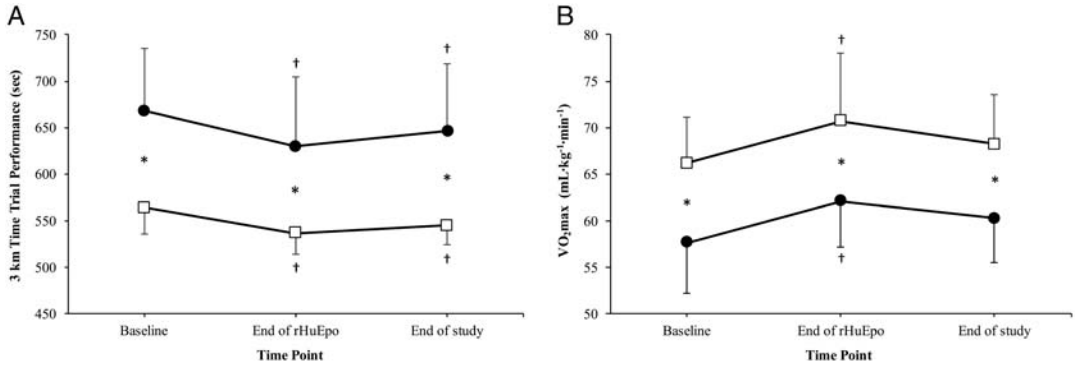

FIGURE 2—Mean ± SD changes in 3,000 m running time trial performance (A), and $\dot{V}O_{2max}$ (B) at baseline, immediately after the rHuEpo administration (i.e., end of rHuEpo) and 4 wk after the administration (i.e., end of the study) in KEN (*white squares*) and SCO (*black circles*). 15 KEN and 19 SCO subjects were included in the analysis of time trial performance and 12 KEN and 14 SCO subjects were included in the analysis of $\dot{V}O_{2max}$ Significant differences in KEN compared with SCO are indicated by *$P < 0.05$. Significant differences compared to baseline values in both groups are indicated by †$P < 0.05$.

$\dot{V}O_{2max}$ and running performance. Both time trial performance and relative $\dot{V}O_{2max}$ were significantly better in KEN compared with SCO at baseline (9:24 ± 0:28 min:s vs 11:08 ± 1:15 min:s, $P < 0.001$ and 66.2 ± 4.9 mL·kg^{-1}·min^{-1} vs 57.7 ± 5.4 mL·kg^{-1}·min^{-1}, $P < 0.001$) after rHuEpo administration (8:57 ± 0:23 min:s vs 10:30 ± 1:07 min:s, $P < 0.001$ and 70.7 ± 7.3 mL·kg^{-1}·min^{-1} vs 62.1 ± 5.0 mL·kg^{-1}·min^{-1}, $P < 0.001$) and 4 wk postadministration (9:05 ± 0:21 min:s vs 10:46 ± 1:13 min:s, $P < 0.001$ and 68.2 ± 5.3 mL·kg^{-1}·min^{-1} vs 60.3 ± 4.8 mL·kg^{-1}·min^{-1}, $P < 0.001$, Fig. 2, Table 3). However, the relative improvements were not significantly different between the two groups for time trial performance as well as for $\dot{V}O_{2max}$ immediately postadministration and 4 wk after rHuEpo (Table 2). Across group analysis revealed that there were no interaction effects of $\dot{V}O_{2max}$ ($F_{2,114} = 0.038$, $P = 0.89$) and time trial ($F_{2,114} = 0.16$, $P = 0.87$, Table 2). The subject who had a blunted hematological response did not improve upon his baseline time trial performance immediately after rHuEpo administration or 4 wk after the final rHuEpo injection (~9:29 min:s at each phase). Unlike SCO whose RPE posttime trial remained relatively constant throughout the study, RPE in KEN increased at the end of rHuEpo compared to baseline (Table 3). Time trial performance was significantly correlated with $\dot{V}O_{2max}$ throughout the study when KEN was grouped with SCO ($r > -0.78$, $P < 0.001$) but not when KEN alone was analyzed ($r < -0.26$, $P > 0.337$). A significant correlation in individual responses compared to baseline was observed for time trial performance and $\dot{V}O_{2max}$ at the end of rHuEpo when KEN was analyzed alone and when grouped with SCO ($r = -0.86$, $P < 0.001$, $r = -0.62$, $P < 0.001$, respectively). The relative improvement was higher in the third lap as compared to the second (Table 3). However, there was no significant correlation between HGB and either time trial performance or $\dot{V}O_{2max}$.

At maximal exertion, $\dot{V}O_2$ relative to body mass was higher in KEN than in SCO; however, owing to their lower body mass, KEN had a lower absolute $\dot{V}O_2$ at maximal exertion at all time points (Table 1). At maximal exercise, $\dot{V}O_2$ in relative (mL·kg^{-1}·min^{-1}) terms, carbon dioxide production ($\dot{V}CO_2$), ventilatory equivalent for CO_2 ($\dot{V}_E/\dot{V}CO_2$) and end tidal partial pressure for CO_2 ($PETCO_2$) were significantly higher at the end of rHuEpo administration in both groups. While in absolute terms, $\dot{V}O_2$ (L·min^{-1}) increased in both KEN and SCO, it was only significant in SCO (3.60 ± 0.32 L·min^{-1} vs 3.80 ± 0.48 L·min^{-1}, $P = 0.147$ and 4.24 ± 0.41 L·min^{-1} vs 4.54 ± 0.46 L·min^{-1}, $P < 0.05$ respectively) (Table 1). At the end of the study, $\dot{V}CO_2$ was significantly higher and $\dot{V}_E/\dot{V}CO_2$ was significantly lower at maximal exercise in KEN compared to baseline, whereas there were no significant changes compared with baseline in SCO (Table 1). There was a trend for $PETCO_2$ to be higher in

TABLE 3. Running 3000 m time trial performance and $\dot{V}O_{2max}$ in KEN.

		KEN		
		Baseline	**End of rHuEpo**	**End of the Study**
3000 m time trial	3000 m total time (min:s)	9:24 ± 0:28	8:57 ± 0:23[a]	9:05 ± 0:21[a]
	First 1 km split time (min:s)	3:03 ± 0:08	2:57 ± 0:08[a]	2:58 ± 0:08[a]
	Second 1 km split time (min:s)	3:12 ± 0:11	3:03 ± 0:09[a]	3:04 ± 0:08[a]
	Third 1 km split time (min:s)	3:09 ± 0:11	2:59 ± 0:09[a]	3:03 ± 0:09[a]
	RPE scale (6–20)	14.6 ± 1.5	16.1 ± 1.1[a]	15.3 ± 1.2
	Temperature (°C)	24.0 ± 2.5	25.4 ± 2.4	23.3 ± 3.9
	Humidity (%)	55.4 ± 16.1	50.4 ± 9.2	58.5 ± 15.9
	Wind speed (m·s^{-1})	1.6 ± 0.8	1.9 ± 0.8	2.8 ± 0.7[a]
Max test	$\dot{V}O_{2max}$ (L·min^{-1})	3.60 ± 0.32	3.80 ± 0.80	3.65 ± 0.41
	$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})	66.2 ± 4.9	70.7 ± 7.3[a]	68.2 ± 5.3

Values are mean ± SD. Significant differences compared to baseline values are indicated by [a]$P < 0.05$. A total of 15, 9, and 12 KEN subjects were included in the analysis of the time trial, $\dot{V}O_{2max}$ in absolute (L·min^{-1}) and in relative (mL·kg^{-1}·min^{-1}) terms, respectively: Kenyan runners living and training at moderate altitude. RPE: Borg's RPE.

KEN at the end of the study; however, this failed to reach statistical significance ($P = 0.054$). KEN had a lower resting systolic blood pressure (122 ± 6 vs 128 ± 9, $P = 0.005$) and HR (57 ± 8 vs 62 ± 8, $P = 0.024$) compared with SCO but similar diastolic blood pressure (67 ± 5 vs 70 ± 8, $P = 0.12$). No significant changes were observed after rHuEpo administration in resting systolic and diastolic blood pressure or resting HR; however, at maximal exercise, HR was significantly higher in KEN compared with SCO at the end of the study period (189 ± 6 bpm vs 181 ± 10 bpm, respectively; $P < 0.05$).

DISCUSSION

This study is the first to report the effects of rHuEpo administration on blood parameters, $\dot{V}O_{2max}$ and running time trial performance in Kenyan endurance runners living and training at moderate altitude (~2150 m) compared with data reported in white trained males living and training at or near sea level (4). Although baseline values were markedly different between the groups and blood parameters did not change as much in KEN as in SCO, the relative improvements in running performance immediately after rHuEpo administration (~5%) and 4 wk after the final injection (~3%) were relatively similar in both groups. Although oxygen carrying capacity of the blood was not directly measured in the present study, it is tempting to conclude that not all the potential ergogenic benefit provided by the increase in HGB was used given the improvement in performance was less than the increase in HGB; the reason(s) for this remains to be determined.

The mean HGB and HCT level reported in our study were a little lower but generally in accordance with the values previously reported in the literature (21,22). This small difference may be due to the posture adopted before blood sampling, which was not reported in the other studies and may affect hematological parameters (20). Regardless of these minor discrepancies, baseline HCT and HGB values were, as expected, higher in KEN than SCO (Table 2 and Fig. 1). There was also a significant increase in RET% after rHuEpo administration (Fig. 1C). HCT and HGB continued to rise significantly in both groups reaching a maximum of approximately 50% and 17 $g \cdot dL^{-1}$ 1 wk after the cessation of rHuEpo administration respectively. Although increasing HGB enhances the blood oxygen carrying capacity, it is also associated with elevated blood viscosity, which in turn can limit capillary flow, increase cardiac work and impair exercise performance (22,23). However, the moderate altitude (2000–2500 m) at which Kenyan endurance athletes typically live and train may represent an optimal altitude to increase endogenous erythropoietin production and consequently, augmented HCT and performance. Although not universally accepted, some authors reported that the optimal HCT for human exercise performance should be within the broad range of 43% to 55% (23,24). Others have speculated that it should be close to the measurements reported in a Finnish cross-country skier and three times Olympic gold medalist with an autosomal dominant mutation in erythropoietin receptor resulting in a higher than normal HCT and HGB above 65% and 20 $g \cdot dL^{-1}$, respectively (22,25). Interestingly, a study comprising of 217 male and 200 female elite speed skaters failed to find an association between HGB and skating performance (26). The suggestion of an elevated blood viscosity after rHuEpo administration in the present study, illustrated by the increase in HCT and HGB which peaked at similar times and levels in both groups, did not appear to hinder significant improvements in running performance either in KEN or in SCO. This finding is in agreement with a study by Calbet et al (27) where after 9 wk at 5260 m above sea level, HGB increased by 36% but this increased blood viscosity did not impair maximal cardiac output during maximal exercise at altitude nor did subsequently decreasing the HGB by 24% via hemodilution. Consequently, the effect of the small increase in blood viscosity assumed by the modest increase in HCT in the present study is too small to have any real impact on peak cardiac output.

In the present study, an average improvement of 27 s (corresponding to a ~5% improvement) during the 3000 m run at ~2150 m above sea level after rHuEpo administration was found. While the hematological changes after rHuEpo were not as pronounced in KEN as in SCO, reflected by an increase in HGB and HCT of ~10% in KEN compared with ~17% in SCO, the relative improvements in running performance and $\dot{V}O_{2max}$ were comparable (~5 vs ~7%). Similar percent changes in all the phases between groups justified the changes in performance variables were mostly related to rHuEpo. Despite some methodological differences in the frequency of injections and in the dosage used, rHuEpo administration for 4 to 6 wk has been shown to increase $\dot{V}O_{2max}$ by approximately 6% to 8% in normoxia (1–8).

Despite the overwhelming scientific evidence to support the ergogenic effects of rHuEpo, some question the application of these primarily laboratory findings to real-life competitive conditions or races involving elite athletes (10,11). For example, Heuberger et al (10) reported that while performance was enhanced in laboratory-based high-intensity tests, endurance and road race performances were similar between rHuEpo and placebo groups questioning a multitude of findings which state otherwise (1–8). The difference in subject characteristics, mode, and intensity of the performance tests may account for some of the lack of agreement between their findings and the current study. Furthermore, the current study was not double-blind, and therefore, the extent of the placebo-effect cannot be quantified.

It has previously been reported that the relative improvement of $\dot{V}O_{2max}$ induced by rHuEpo administration was more than doubled (i.e., 17.5 vs ~8%) at a simulated moderate altitude up to 3500 m compared with normoxia (16). Despite the smaller increase in the hematological parameters in KEN after rHuEpo administration, improvements in $\dot{V}O_{2max}$ and time trial performance was similar between KEN compared with SCO (Table 2). Absolute $\dot{V}O_{2max}$ in

KEN increased but not significantly ($P = 0.147$) after the end of rHuEpo administration, this however may be due to a lack of statistical power after the loss of some data after a severe flood in the laboratory. The Kenyan athletes recruited in the present study were chronically adapted to moderate-altitude and were of a higher training status than their counterparts in SCO. It is therefore likely that KEN athletes had a larger cardiac output and may have subsequently desaturated to a greater extent when exercising at $\dot{V}O_{2max}$ after rHuEpo administration. The mode of testing could be another factor because desaturation is more common during running than during bicycling (28). The present data revealed a decrease in $\dot{V}_E/\dot{V}CO_2$ despite an increase in $PETCO_2$, potentially reflecting a ventilatory limitation in these athletes. As described by Foster et al (29), a ventilatory limitation is most likely primarily due to mechanical stress in the lung. Using data presented by Foster et al (29), equation by Severinghaus (30) and the $PETO_2$ data in KEN in the present study, arterial oxygen saturation (SaO_2) during maximal exercise can be estimated. Before rHuEpo administration and at an altitude of approximately 2150 m, the KEN athletes had an estimated SaO_2 of 91% at $\dot{V}O_{2max}$. This level of SaO_2 is similar to that reported at maximal exercise when endurance-trained athletes were exposed acutely to an altitude of 2500 m (i.e., albeit normobaric hypoxia): 91.3% ± 1.9% at $\dot{V}O_{2max}$ (31). This similar SaO_2 at maximal exercise in the chronically adapted to altitude KEN athletes in the present study and sea-level endurance athletes exposed to a similar degree of hypoxia (31) suggests similar limitations in pulmonary gas exchange during maximal exercise. Specifically, the large cardiac output of endurance-trained athletes at maximal exercise and consequently, the reduced transit time of red blood cells across the alveoli, would be expected to decrease SaO_2. This limitation in pulmonary gas exchange during maximal exercise could explain the lack of significant difference in absolute $\dot{V}O_{2max}$ when O_2 carrying capacity was increased after rHuEpo in the present study (Table 1).

The ergogenic effects of rHuEpo administration appear to be explained primarily by an increase in oxygen carrying capacity as no measurable nonhematopoietic ergogenic effect of rHuEpo on exercise performance in normoxic conditions was observed in two separate studies conducted by the same research group (32,33). The oxygen supply to exercising muscle depends on the HGB and cardiac output. However, HGB is only weakly related to $\dot{V}O_{2max,}$ whereas the relationship between tHb_{mass} and $\dot{V}O_{2max}$ is much stronger (34). Unlike HGB, tHb_{mass} is not affected by changes in plasma volume and is therefore a key complementary parameter to evaluate blood oxygen carrying capacity (34). Living and training at moderate altitude has been shown to increase blood volume and tHb_{mass} in endurance athletes (35), and therefore, it would have been useful to have also measured tHb_{mass} in addition to the other blood-related parameters to better account for the observed changes in $\dot{V}O_{2max}$. A direct comparison of the effect of rHuEpo in the two cohorts cannot be performed without information on, at least, SaO_2 as elite athletes desaturate more at altitude than nonelite athletes and this effect is proportional to that of $\dot{V}O_{2max}$ (36). The present study was designed primarily to identify differentially expressed gene transcripts and therefore, SaO_2 and tHb_{mass} were not measured for practical reasons nor was the study blinded or include a control group. Therefore, the novel data generated within the study limitations, although useful for comparison with the literature, require further replication. The altitude and sea-level comparison is secondary and given the limitations outlined should be interpreted with caution. Factors unrelated to rHuEpo such as altered motivation (e.g., placebo, order effect) may partly explain the reported performance effects and reflected in the small but significant rise in RPE after rHuEpo in KEN. Proponents of "PlacEpo" claimed that the true contribution of doping is overestimated as the claimed 3%–6% improvement in $\dot{V}O_{2max}$ by rHuEpo doping is similar to the day-to-day variation (37). Studies have shown that placebo can improve 3000 m race time by 1.2% (38) and $\dot{V}O_2$max by 0% to 1.5% (1,9) and therefore, much less than observed in the present study (~5% vs 5%–7%, respectively). Nevertheless, it is tempting to speculate that the similar relative improvements in running performance and $\dot{V}O_{2max}$, despite the blunted hematological response in KEN may be due to mechanisms unrelated to blood oxygen carrying capacity, such as a greater recruitment of motor units (39) and mood alterations (18) that can influence training and performance due to the substantial socioeconomic rewards that are perceived to come with performance success (13).

In the present study, the subjects achieved a higher perception of effort at the end of the rHuEpo trial (Table 3). Several studies (18,32,40) describe the presence of erythropoietin receptors in heart, brain, and striated muscle tissues, and therefore, a potential central mechanism of action of rHuEpo in addition to the well-established enhanced oxygen carrying capacity. Recently, the understanding of the way in which rHuEpo exerts its effects have advanced with our recent gene expression studies (41), identifying differentially expressed gene transcripts relevant to the transport of oxygen, carbon dioxide and blood pH regulation (e.g., hemoglobin delta, 2,3-bisphosphoglycerate mutase, carbonic anhydrase I and solute carrier family 4, anion exchanger-member 1).

Based on the blunted response in the hematological parameters and exercise performance, one Kenyan subject was classified as a "nonresponder" to rHuEpo administration. It is well known that there is marked variability in the sensitivity to rHuEpo in anaemic patients as well as in rHuEpo dose requirement (42). In addition, the sensitivity to hypoxia exposure during altitude training in healthy volunteers or athletes is also variable as reflected by significant but not clear-cut differences in plasma erythropoietin concentrations (43,44). Athletes who responded to altitude training demonstrated higher erythropoietin levels which in turn seemed to trigger a sufficient increase in tHb_{mass} and associated improvements in exercise performance (43,44). The particular subject in KEN who demonstrated a blunted hematological

and exercise performance response after rHuEpo may therefore be representative of the 10% of the treated patients or of the "nonresponders" to altitude training. In stark contrast, another subject in the present study was required to prematurely discontinue rHuEpo injections due to his pronounced response in HCT. This subject achieved the predetermined health limit of 55% after 2 wk of rHuEpo use and reported moderate-severe headaches after training.

The use of rHuEpo is prohibited by the World Anti-Doping Agency. The direct urinary test to detect rHuEpo was developed and published by Lasne et al in 2000 (45). However, although the effects of rHuEpo administration on hematological parameters and exercise performance can last for several weeks, as shown in the present study, the test has a rather limited detection window, that is, a few hours up to a few days depending on the dose used. This limitation in detection has prompted the development of indirect tests to detect rHuEpo using blood markers of altered erythropoiesis (3). The ABP method was introduced as a new tool to indirectly detect blood doping as well as to intelligently target athletes for additional testing (19). The ABP approach relies on identifying intra-individual abnormal variability over time of selected hematological parameters including HGB, HCT, RET% and OFF-score (46). In this context, factors such as residence at altitude and ethnicity have been shown to significantly influence these hematological parameters (47) and confirmed in the present study as the magnitude of change in HGB, RET%, and OFF-score used in the ABP was reduced in KEN as compared with SCO in response to the same rHuEpo administration regimen. As a result, it may be more difficult to detect rHuEpo doping at altitude compared to sea level using the current antidoping methods due to the blunted hematological response. There are indeed concerns about the risk of the misuse of altitude exposure by some athletes to mask blood doping practices, such as the administration of rHuEpo.

In conclusion, this is the first study to demonstrate that 4 wk of rHuEpo administration significantly increased the already relatively high basal HGB and HCT values of Kenyan endurance runners living and training at moderate altitude by ~10%. Although the rHuEpo-induced increase in key hematological parameters in these altitude-adapted Kenyan endurance runners was blunted compared with white athletes living and training at or near sea level, the relative improvements of ~5% and ~3% in running performance immediately after the rHuEpo administration and 4 wk after the last injection, respectively, were similar in both groups. This finding is also in line with the overwhelming scientific literature. Although caution is required when extrapolating findings from subelite to truly elite athletes, the significant improvements in running performance we report would almost certainly translate into a worthwhile enhancement in elite performance. Moreover, in professional cycling stage races, such as the Tour de France, the mountain stages, which peak at altitudes ranging from approximately 1500 m to above 2500 m, often separates winners from contenders. If the ergogenic effects of rHuEpo are indeed enhanced at moderate altitude or can at least significantly reduce the hypoxia-induced impairment in exercise performance, rHuEpo administration has the potential to improve further the athlete's chance of winning the key stages and the overall classification. However, further research with larger sample size, complete data set, measurement of tHb_{mass}, arterial blood gases, SaO_2 and improved experimental design (e.g., randomized, placebo-controlled crossover study design) is required to confirm these findings. The results from this study also have important implications for current antidoping practices.

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All authors declare no conflict of interest.

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