Introduction

VO\textsubscript{2max} is a critical factor for endurance exercise performance because it sets the upper limit for aerobic metabolism [1, 19, 20]. The primary limitation of VO\textsubscript{2max} is the rate of oxygen delivery to the working muscle [1, 24]; many physiological parameters that influence oxygen delivery during exercise have been described previously [1, 20, 23].

One parameter that influences VO\textsubscript{2max} is the total mass of hemoglobin in circulation (tHb) [35]. tHb influences VO\textsubscript{2max} both via its relationship with hemoglobin concentration ([Hb]) and arterial oxygen content (C\textsubscript{a}O\textsubscript{2}) [35], and via its relationship with total blood volume, venous return and ventricular filling, and maximal cardiac output [10, 18, 21, 35]. However, tHb and [Hb] are not the only factors that influence C\textsubscript{a}O\textsubscript{2}; arterial oxygen partial pressure and arterial oxygen saturation (S\textsubscript{a}O\textsubscript{2}) also influence C\textsubscript{a}O\textsubscript{2}. During high-intensity exercise, S\textsubscript{a}O\textsubscript{2} can drop significantly in a variety of athletes; this condition is known as exercise-induced arterial desaturation (EIAD). When EIAD was initially described in the literature, it was thought that decreased arterial oxyhemoglobin concentration during exercise led to decreased C\textsubscript{a}O\textsubscript{2}. However, high-intensity exercise increases arterial blood temperature and can lead to plasma volume shifts and hemoconcentration; in individuals that do not experience EIAD, these changes can lead to an increase in C\textsubscript{a}O\textsubscript{2} during high-intensity exercise [36], and therefore EIAD may simply prevent an increase in C\textsubscript{a}O\textsubscript{2} during high intensity [15]. Regardless, it is clear that EIAD has a detrimental effect on VO\textsubscript{2max}, because ameliorating EIAD by increasing the fraction of inspired
oxygen from 21 % to 26 % leads to an increase in VO₂max only in individuals with EIAD [15].

At sea level, EIAD is uncommon in recreationally active subjects, but ~50 % of elite endurance athletes experience some degree of EIAD [25]. As altitude increases, EIAD is exacerbated in all individuals [22]. There is also significant interindividual variability in the severity of EIAD experienced during exercise, even within groups of similarly trained athletes [12, 26]. Therefore, assuming EIAD and tHb are independent, EIAD may influence the relationship between tHb and VO₂max.

Although tHb and EIAD both influence oxygen delivery during exercise, to date there have been no studies looking at how these factors interact to influence VO₂max. Therefore, the purpose of this study was to determine whether EIAD influences the relationship between tHb and VO₂max in moderately to highly trained competitive male and female endurance athletes at moderate altitude (1,625 meters). Additionally, previous research has indicated that females may be more likely to experience EIAD than males due to anatomical differences [8, 9]. However, direct comparisons of EIAD between competitive, endurance-trained male and female athletes are lacking. Therefore, a secondary aim of this study was to compare severity of EIAD at moderate altitude in men and women after taking into account aerobic capacity.

Methods

Subjects

Seventeen female and sixteen male competitive endurance-trained cyclists and triathletes residing at moderate altitude (1,500–2,000 meters) took part in this study. Subjects were required to maintain moderate-altitude residence throughout participation in the study, and testing occurred at 1,625 meters. Endurance-trained was defined as cycling, running, and/or swimming for more than 10 h per week for men and more than 8 h per week for women over the month prior to inclusion in the study. All subjects had participated in at least one discipline-specific competitive race in the previous calendar year. At the time of the first visit, males were required to hold at a minimum a USA Cycling Category 2 or USA triathlon license; females were required to hold at a minimum a USA Cycling Category 3 or USA triathlon license. Subjects were screened to ensure that they were between the ages of 18–42 years old, free from known cardio-respiratory disease as assessed by the Physical Activity Readiness Questionnaire (PAR-Q), had not donated blood in the previous 8 weeks, and were non-smokers. Subjects were not excluded from the study if they had participated in short sojourns to sea level (< 7 days) in the 8 weeks prior to participation in the study, because hemoglobin mass has previously been shown to be stable for up to two weeks following descent to sea level in endurance-trained moderate-altitude residents [28]. Females were screened to ensure they were not pregnant or breastfeeding, and all females undertook a urine hCG test prior to participating (AccuMed, USA).

Experimental design

Because duplicate measurements reduce the typical error of a measurement by √2 [17], measurements of all primary outcomes (tHb, VO₂max, and S₉O₂) were performed twice. On the first of four visits to the lab, written informed consent was obtained. In order to confirm that there were no changes in tHb throughout the study, visits one and four consisted of identical measurements of tHb, whereas visits two and three consisted of identical graded exercise tests (GXT) to measure maximal oxygen uptake and S₉O₂ during exercise. All visits were separated by at least one day, and for each subject, GXTs were performed at the same time of day, plus or minus one hour. This study was conducted in accordance with the ethical standards of the International Journal of Sports Medicine [16].

Total hemoglobin mass

Total hemoglobin mass was measured via the optimized carbon monoxide rebreathing procedure [27, 34] as described previously [32, 33]. Subjects were instructed to refrain from exercise for two hours preceding these visits due to the possible interactions between exercise and carboxyhemoglobin kinetics. For this study, the coefficient of variability was 2.7 % (95 % confidence interval: 2.2 %–3.7 %).

Graded exercise test

On visits two and three, subjects performed a maximal GXT on a cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). For these visits, subjects were instructed to arrive at the lab two hours postprandial, and were instructed to not consume alcohol or perform vigorous activity for 24 h prior to either GXT. Prior to the GXT, body mass was measured on a digital scale (COMBICS 1, Sartorius Weighing Technology, Göttingen, Germany) in cycling clothes and without shoes. Oxygen consumption and other metabolic parameters were measured via computerized open-circuit indirect calorimetry, which was calibrated according to the manufacturer’s specifications (TrueOne 2400, Parvo Medics, Sandy, UT, USA). Heart rate was measured using a heart rate monitor (Polar Electro, Kempele, Finland), and peripheral oxygen saturation was measured continuously via forehead pulse oximetry (Nellcor N-595, Medtronic, Minneapolis, MN, USA). Forehead pulse oximetry was chosen due to its ability to determine S₉O₂ with relatively low bias and high precision compared to other non-invasive measurement options [37]. After five minutes of rest on the ergometer, baseline S₉O₂ was measured for one minute. Each subject was then allowed a 10-min warm-up period during which the subject rode at a self-selected power not exceeding the power output of the first stage of the GXT. Following the warm-up period, the subject put on a nose clip and began breathing through the open-circuit spirometry system. The GXT began at an individualized power output of 4 W·kg⁻¹ for males and 3 W·kg⁻¹ for females, rounded down to the nearest 20 W increment, and increased 20 W every minute until subjects reached volitional exhaustion or until cadence could not be maintained above 60 RPM. This sex-specific protocol was used in order to elicit volitional exhaustion in this subject population in about 10–12 min, which has been reported to be an optimal duration for determining VO₂max [4]. During each GXT, all subjects reached an RPE > 17 and a HRmax within 10 % of age-predicted HRmax. As previously described, VO₂max was calculated as the highest 30-s average oxygen consumption; S₉O₂ at VO₂max was calculated as the average S₉O₂ during the same 30 s used to determine.
VO₂ max [3, 32]. Peak power output was calculated as described previously using the following equation [3, 32]:

\[
\text{Peak power output} = \text{penultimate stage power output} + (\text{power increase between stages} \times (\text{seconds into the final stage}/60 \text{ seconds}))
\]

Mild, moderate, and severe EIAD were classified as a Sₐ O₂ at VO₂ max between 93–95 %, 88–93 %, and < 88 %, respectively [7]. The coefficients of variation for VO₂ max and Sₐ O₂ at VO₂ max using these duplicate measurements were 3.5 % (95 % confidence interval: 2.8–4.7 %) and 1.4 % (95 % confidence interval: 1.2–1.8 %), respectively.

Saturation-adjusted tHb
To determine how saturation influences the relationship between tHb and VO₂ max, as well as the relationship between saturation-adjusted tHb and VO₂ max, simple linear regressions were performed. Linear regression models were compared using Williams t-test. To assess differences in slopes, multiple linear regression was performed with sex as an independent variable and Sₐ O₂ at VO₂ max as the dependent variable. For all regression analyses, bivariate normality was assessed using Q-Q plots and Shapiro-Wilk tests on the residuals, homoscedasticity and linearity were assessed using scatter plots of normalized residuals, and autocorrelation was assessed using the Box-Pierce test. Unstandardized regression coefficients from linear models are reported using the symbol β. All analyses were performed in R, version 3.3.2 (R Core Team, Vienna, Austria), and alpha was set to 0.05. Trends were noted if 0.05 < p < 0.1. Results are represented as the mean ± SD.

Results
Subject characteristics and measures of reliability between duplicate measures are given in Table 1. Of the 33 subjects who participated, 32 completed all experimental procedures; one female subject was withdrawn due to illness after completing the first two visits, so her results represent only a single measurement of each parameter.

There were no significant differences between tests for the following measures: tHb, body mass, Sₐ O₂ at rest, Sₐ O₂ at VO₂ max, desaturation from rest, maximum heart rate, maximum RER, peak power output, and maximum RPE (all p = N.S.). VO₂ max was significantly higher for the second GXT, both when expressed as L · min⁻¹ (delta = 0.064 L · min⁻¹, p = 0.048) and when expressed as mL · min⁻¹ · kg⁻¹ (delta = 1.21 mL · min⁻¹ · kg⁻¹, p = 0.02). The change in VO₂ max between trials was not different between males and females (p = N.S.). Although there was no significant difference in peak power output between tests, the difference in VO₂ max between tests was related to the difference in peak power output (p < 0.001, r² = 0.44), which indicates that there may have been a learning effect that took place between GXTs. The magnitude of the difference in VO₂ max between tests was small (< 2 %). To examine whether this difference influenced our conclusions, all fur-

<table>
<thead>
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<th>Table 1 Subject Characteristics and Measurement Variability.</th>
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<tr>
<td><strong>Male n = 16</strong></td>
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<td>Age</td>
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<td>Weekly Training Duration (hours)</td>
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<td>Body Mass (kg)</td>
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<td>Absolute tHb (g)</td>
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<td>Normalized tHb (g · kg⁻¹)</td>
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Values are mean ± SD. VO₂ max: Maximal oxygen uptake during graded exercise test; RER: Respiratory exchange ratio; HR max: maximum heart rate during graded exercise test; Peak Power Output: peak power output during graded exercise test; Sₐ O₂: arterial oxyhemoglobin saturation; tHb: total hemoglobin mass. Weekly training duration was calculated from self-reported hours of endurance training per week over the month preceding inclusion in study. tHb parameters are the average of two measurements, whereas all graded exercise test parameters (VO₂ max, RER at VO₂ max, HR max, Peak Power Output, Sₐ O₂ at VO₂ max, and Desaturation from Rest) are taken from the 2nd graded exercise test, because VO₂ max was significantly higher for this test.
Other analyses were performed twice, once using results from the average of both GXTs, and once using results from only the second GXT. There were no differences in any conclusion regardless of which variables were used, and therefore all results are presented from the second GXT, including VO\(_2\)\(_{\text{max}}\), \(S_{\text{a}O_2}\) at VO\(_2\)\(_{\text{max}}\), peak RER, and peak power output.

**Exercise-induced arterial desaturation**

At VO\(_2\)\(_{\text{max}}\), the average desaturation from rest was 9.1 ± 3.5 % for males and 6.9 ± 2.6 % for females. In males, \(S_{\text{a}O_2}\) at VO\(_2\)\(_{\text{max}}\) ranged from 81.7 % to 94.0 %; in females \(S_{\text{a}O_2}\) at VO\(_2\)\(_{\text{max}}\) ranged from 85.7 % to 95 %. Overall, 94 % of subjects experienced greater than 4 % desaturation from rest (95 % confidence interval: 80 %–99 %), with no statistical difference between men and women (▶Table 2). \(S_{\text{a}O_2}\) at VO\(_2\)\(_{\text{max}}\) was negatively related to VO\(_2\)\(_{\text{max}}\), both when expressed as an absolute (\(r = –0.58\), \(p < 0.001\)) and when normalized to body mass (\(r = –0.55\), \(p < 0.001\)). When split by sex, this relationship was observed only in the female cohort (for females, absolute: \(r = –0.59\), \(p < 0.05\); normalized: \(r = –0.62\), \(p < 0.05\)). There was no significant difference in the severity of EIAD between males and females after accounting for VO\(_2\)\(_{\text{max}}\) (\(p = \text{N.S.}\)).

**Relationship between tHb and VO\(_2\)\(_{\text{max}}\)**

tHb was positively related to VO\(_2\)\(_{\text{max}}\) when both parameters were expressed as absolute values (\(\beta_{\text{tHb}} = 5.07\); \(r^2 = 0.88\), \(p < 0.001\); ▶Fig. 1a) and when both parameters were normalized to body mass (\(\beta_{\text{tHb}} = 4.70\); \(r^2 = 0.73\), \(p < 0.001\); ▶Fig. 2a). When split by sex, the magnitude of the correlation decreased, but similar relationships were observed in both males (absolute: \(r^2 = 0.44\), \(p < 0.01\); normalized: \(r^2 = 0.32\), \(p < 0.02\)) and in females (absolute: \(r^2 = 0.67\), \(p < 0.01\); normalized: \(r^2 = 0.42\), \(p < 0.01\)).

**Saturation-adjusted tHb**

When tHb was adjusted by \(S_{\text{a}O_2}\) at VO\(_2\)\(_{\text{max}}\), this parameter was positively related to VO\(_2\)\(_{\text{max}}\), both when expressed as absolute values (\(\beta = 6.05\); \(r^2 = 0.87\), \(p < 0.001\); ▶Fig. 1b) and when normalized to body mass (\(\beta = 5.65\); \(r^2 = 0.68\), \(p < 0.01\); ▶Fig. 2b). When compared to the model between T HB and VO\(_2\)\(_{\text{max}}\), there was no significant difference between the amounts of explained variability, either for the absolute (\(p = \text{N.S.}\); ▶Fig. 1) or for the body mass normalized models (\(p = \text{N.S.}\); ▶Fig. 2).

**tHb and \(S_{\text{a}O_2}\) at VO\(_2\)\(_{\text{max}}\)**

Across all subjects, when tHb was normalized by body mass, it was negatively related to \(S_{\text{a}O_2}\) at maximal exercise (\(r^2 = 0.32\), \(p < 0.001\); ▶Fig. 3). When split by sex, there was a trend for this relationship to show up in females (\(r^2 = 0.22\), \(p = 0.06\)) but not males (\(r^2 = 0.06\), \(p = \text{N.S.}\)). After diagnostic testing of this model, this analysis was re-run after removing one male subject who exerted a high degree of influence on the original model. In the reduced data set, there was still a significant relationship between tHb and \(S_{\text{a}O_2}\) at VO\(_2\)\(_{\text{max}}\) (\(r^2 = 0.202\), \(p = 0.01\)). A secondary analysis of this data revealed that when only subjects who experienced an \(S_{\text{a}O_2}\) at VO\(_2\)\(_{\text{max}}\) less than 91 % were analyzed (which included 15 subjects), \(r^2\) increased from 0.32 to 0.48 (\(p < 0.01\) for this model).

**Discussion**

The primary findings from this study are that 1) adjusting tHb by \(S_{\text{a}O_2}\) at VO\(_2\)\(_{\text{max}}\) did not improve the amount of explained variability in VO\(_2\)\(_{\text{max}}\), and 2) after correcting for aerobic capacity, there was no difference in severity of EIAD between males and females.
Additionally, we found that tHb was negatively related to $S_a O_2$ at VO$_{2 \text{max}}$.

The finding that $S_a O_2$ at VO$_{2 \text{max}}$ did not explain additional variability in the relationship between tHb and VO$_{2 \text{max}}$ was contrary to our hypothesis. If tHb and $S_a O_2$ at VO$_{2 \text{max}}$ were both independent predictors of VO$_{2 \text{max}}$, it would be expected that the relationship between tHb and VO$_{2 \text{max}}$ would improve after taking into account $S_a O_2$ at VO$_{2 \text{max}}$. However, accounting for $S_a O_2$ at VO$_{2 \text{max}}$ resulted in no significant changes in the relationship between these variables, which indicates that tHb and EIAD may not be independent. This concept is supported by our finding that $S_a O_2$ at VO$_{2 \text{max}}$ was negatively correlated to tHb (▶ Fig. 3). Although we do not have direct evidence to explain these results, there are two possible physiological explanations. One possibility is that individuals with high tHb may experience more severe desaturation during exercise due to the presence of high blood volume. High blood volume enables greater venous return and ventricular filling, and is therefore a prerequisite for high cardiac output; however, as cardiac output increases, pulmonary capillary transit time decreases, which is believed to be one of the predominate factors leading to exercise-induced hypoxemia and desaturation in athletes [6, 31].

Another possible explanation for this finding is that desaturation during exercise may influence the regulation of thb. It is well documented that hypoxia can augment erythropoiesis and increase tHb [13]. Although very little research has been performed to directly study this issue, previous research indicates that hypoxemia during high-intensity exercise may interact with environmental hypoxia to create a larger erythropoietic stimulus in individuals who experience more severe EIAD. For example, in athletes with $S_a O_2$ at VO$_{2 \text{max}}$ below 91 %, three minutes of maximal aerobic exercise at sea level was found to elevate serum erythropoietin (EPO) for at least 24 h following exercise, and were found to increase reticulocyte count 96 h following exercise, whereas individuals with $S_a O_2$ at VO$_{2 \text{max}}$ above 91 % had no significant change in EPO or reticulocytes over time [29]. Additional research by the same group found that the increase in circulating EPO 24 h following high-intensity exercise was ~50 % higher when the exercise was performed in simulated normobaric hypoxia (2,100 meters compared to 1,000 meters) [30]. This finding was hypothesized to be a function of the significantly lower $S_a O_2$ during exercise in the simulated hypoxia compared to the control condition. To our knowledge, only one study has followed up on these results to examine whether high-intensity exercise performed in hypoxia can augment increases in hemoglobin mass over time compared to the equivalent training in normoxia. Brocherie et al. reported that 6 bouts of sprint interval training performed in hypoxia throughout a two-week live-high/train-low training camp increased tHb by 4 % on average, compared to a control group that lived high but performed sprint interval training in normoxia, which increased tHb only by 2.8 % (although these were not statistically different, in part due to low sample size).
[2]. Taken together, these results raise the possibility that individual variability in EIAD severity during exercise may be one factor that influences tHb in endurance athletes, especially over long durations of time or in athletes residing at moderate altitude. However, we do not have direct evidence to support this hypothesis, and therefore further experimental research is required to fully elucidate the relationship between EIAD and tHb.

Our finding that there was no difference in severity or prevalence of EIAD at moderate altitude between endurance-trained males and females is in contrast to our hypothesis. Previous work has identified that females experience more severe mechanical respiratory constraints during exercise compared to males [8, 14], which can exacerbate EIAD and has led to the hypothesis that females may be more likely than males to exhibit EIAD [5]. Our findings do not support this hypothesis. Our lab has previously published similar findings in endurance-trained male and female cyclists, where we reported no significant difference between males and females in $S_\text{O}_2$ at VO$_\text{max}$ at moderate altitude [3]. Interestingly, this study did find a statistically significant difference in the degree of desaturation in males versus females, but it was males, not females, that exhibited larger desaturation. One important caveat to this previous finding is that the statistical tests used for these comparisons did not take into account aerobic capacity, which is known to be a confounding variable for EIAD [5, 7, 8]. Taken together, these results raise the possibility that individual variability in EIAD during exercise could lead to further insights on this topic. Finally, it is difficult to determine causal relationships when looking at cross-sectional data. An additional factor that may influence the relationship between tHb and VO$_\text{max}$ is maximal cardiac output, and future research is therefore required on this subject.

Conclusions

At moderate altitude, over 90% of endurance-trained males and females experienced EIAD. Despite a wide range of exercise-induced desaturation values, taking into account $S_\text{O}_2$ at VO$_\text{max}$ did not improve the relationship between tHb and VO$_\text{max}$ at moderate altitude. This finding may be in part due to a relationship between oxyhemoglobin desaturation during exercise and tHb, which warrants further investigation. Future research is required to determine how other physiological parameters, such as cardiac output, influence the relationship between tHb and VO$_\text{max}$.

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Conflicts of interest

The authors declare that they have no conflict of interest.

References


