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Article

Effects of hyperoxic training on red blood cell deformability and mechanical properties in elite male endurance athletes: A randomized crossover study

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Abstract: Background: The effects of hyperoxic environments on red blood cell (RBC) deformability and mechanical properties in athletes during high-intensity exercise remain poorly understood. This study aimed to investigate these effects and their potential implications for athletic performance. **Methods:** Forty elite male endurance athletes participated in a randomized, double-blind, crossover study. Participants completed high-intensity interval training sessions under normoxic (21% O2) and hyperoxic (40% O2) conditions. RBC deformability, whole blood viscosity, and physiological parameters were measured pre-exercise, immediately post-exercise, 1-hour post-exercise, and 24 h post-exercise. **Results:** Hyperoxic exposure resulted in significantly enhanced RBC deformability, particularly at higher shear stresses ($p < 0.001$). Whole blood viscosity was reduced across all shear rates in the hyperoxic condition ($p < 0.05$). Oxygen saturation (SpO2) levels were consistently higher ($p < 0.001$), while blood lactate concentrations were lower ($p < 0.001$) in the hyperoxic condition. Individual responses to hyperoxia varied considerably, with some athletes showing markedly greater improvements in RBC deformability than others. **Conclusions:** Acute hyperoxic exposure during high-intensity exercise enhances RBC deformability and reduces blood viscosity in elite endurance athletes, potentially improving microcirculatory function and oxygen delivery to tissues. These findings suggest that hyperoxic training may offer performance benefits, but the observed individual variability highlights the need for personalized approaches in its application.

Keywords: hyperoxia; red blood cell deformability; blood viscosity; high-intensity exercise; elite athletes; microcirculation; oxygen saturation; blood lactate; individual variability; sports performance

1. Introduction

As endurance athletes continually push the boundaries of human physical capacity, understanding the intricate physiological adaptations that occur during training and competition becomes increasingly important [1]. The ability of RBCs to deform and navigate through narrow capillaries is essential for efficient oxygen delivery to working muscles, making it a key factor in athletic performance [2,3].

Recent meta-analyses have shown significant correlations between hyperoxic training and endurance performance enhancement [4]. Molecular studies have revealed novel mechanisms underlying oxygen-dependent changes in red blood cell properties [5]. Advanced imaging techniques have provided new insights into real-time changes in RBC deformability during exercise [6]. Additionally, longitudinal

studies have demonstrated the sustained effects of altitude training on blood rheology [7].

Recent advancements in hemorheological assessment techniques have allowed researchers to delve deeper into the microcirculatory and oxygen transport changes that occur in athletes across various sports disciplines [8]. These investigations have revealed that exercise and training can significantly influence blood rheology, with both acute and chronic adaptations observed in response to physical exertion [9,10]. The complex interplay between exercise intensity, duration, and environmental factors has been shown to modulate RBC properties, potentially impacting overall performance and recovery [11,12].

While numerous studies have examined the effects of exercise on blood rheology under normoxic conditions, the influence of hyperoxic environments on RBC deformability and mechanical properties in athletes remains relatively unexplored. Hyperoxia, characterized by elevated oxygen levels in inspired air, has been utilized in various training and recovery protocols, with potential implications for performance enhancement and physiological adaptation [13,14]. However, the specific effects of hyperoxia on RBC function in the context of athletic performance warrant further investigation.

By employing state-of-the-art hemorheological assessment techniques [15,16] and considering the multifaceted nature of exercise-induced changes in blood properties [17,18], we seek to provide a comprehensive understanding... The findings of this research may have significant implications for training strategies, performance optimization, and the prevention of exercise-induced hemorheological disturbances [19,20].

2. Literature review

The relationship between exercise, blood rheology, and red blood cell (RBC) properties has been a subject of extensive research in sports physiology. Previous studies have demonstrated that both acute exercise and long-term training can induce significant changes in blood viscosity, RBC deformability, and related hematological parameters [21,22]. These adaptations are crucial for optimizing oxygen delivery to working muscles and maintaining performance during intense physical exertion.

Research by Connes et al. [23] has shown that exercise-induced hypoxemia can modify lactate influx into erythrocytes and alter hemorheological parameters in athletes. This finding highlights the complex interplay between oxygen availability, metabolic processes, and RBC function during exercise. Furthermore, investigations into the effects of different exercise modalities on RBC properties have revealed distinct responses to running and cycling, suggesting that the mechanical stress imposed on erythrocytes may vary depending on the type of activity [24].

The duration and intensity of exercise have also been shown to play significant roles in determining the extent of hemorheological changes. Robert et al. [25] observed that the impact of trail running races on blood viscosity and its determinants was dependent on the race distance, with longer events inducing more pronounced alterations. These findings emphasize the importance of considering exercise duration when assessing the hemorheological response to physical exertion. In extreme

endurance events, such as ultra-marathons, significant changes in RBC viscoelasticity and the development of sports anemia have been reported [26]. These observations underscore the potential for prolonged, intense exercise to induce substantial alterations in RBC properties and function. Additionally, seasonal variations in iron stores among marathoners have been documented [27], suggesting that long-term training and competition cycles may have cumulative effects on RBC production and turnover.

Contemporary research has particularly focused on the molecular mechanisms underlying RBC adaptations to various oxygen environments. Chen et al. [5] further elucidated the signaling pathways involved in oxygen-dependent RBC membrane alterations. Chen et al. [29] further elucidated the signaling pathways involved in oxygen-dependent RBC membrane alterations.

While the majority of research has focused on normoxic conditions, recent studies have begun to explore the effects of altered oxygen environments on RBC properties in athletes. Freitag et al. [30] investigated the acute effects of low-dose hyperoxia during high-intensity interval exercise on RBC deformability and muscle oxygenation. Their findings suggest that short-term exposure to hyperoxic conditions during intense exercise may not significantly affect RBC deformability in trained individuals. However, the long-term effects of training or competing in hyperoxic environments on RBC mechanical properties and overall hemorheological profile remain largely unexplored.

This gap in the literature presents an opportunity for further research to elucidate the potential benefits or risks associated with hyperoxic training strategies and their impact on RBC function in athletes. Such investigations could provide valuable insights for optimizing training protocols and enhancing performance while minimizing the risk of adverse hemorheological adaptations.

3. Methods

3.1. Study subjects

This study recruited a cohort of 40 elite male endurance athletes from various sports academies in Beijing, China. Only male subjects were selected for this study to eliminate the potential confounding effects of hormonal variations associated with the menstrual cycle on blood rheology [21,22]. Previous research has shown that sex-specific hormonal fluctuations can significantly influence red blood cell properties and blood viscosity [23,24]. The participants, aged 18–25 years (mean age 21.3 ± 2.1 years), were actively competing at national or international levels in disciplines such as long-distance running, cycling, and triathlon. Throughout the study period, all participants adhered to a standardized dietary protocol consisting of 60% carbohydrates, 25% protein, and 15% fat, with total daily caloric intake individually adjusted and monitored according to training load [25]. Dietary compliance was verified through daily food logs and regular consultations with sports nutritionists. Participants maintained consistent sleep patterns, with supervised sleep-wake cycles (10:00 PM–6:00 AM) to minimize circadian rhythm variations that could affect physiological parameters [26]. All subjects were non-smokers and had no history of tobacco use, eliminating potential confounding effects of smoking on blood rheology

and oxygen transport capacity [27]. The cohort comprised specialized athletes in long-distance running (n = 15), cycling (n = 15), and triathlon (n = 10), each with a minimum of five years of professional training experience. Their weekly training volume averaged 20–25 h, following a polarized training model with approximately 80% low-intensity and 20% high-intensity sessions, aligning with contemporary endurance training principles [1]. All participants had maintained consistent training regimens for at least three months prior to study commencement to ensure stable physiological adaptations. All athletes had a minimum of five years of intensive training experience and were free from any known cardiovascular, respiratory, or hematological disorders. The subjects maintained a consistent training regimen for at least three months prior to the study and abstained from any ergogenic aids or medications that could potentially influence blood rheology. A comprehensive medical examination, including a thorough hematological profile, was conducted for each participant to ensure their eligibility. The study protocol was approved by the Ethics Committee of the Beijing Sport University, and all participants provided written informed consent before enrollment. To minimize the influence of circadian rhythms on hemorheological parameters, all experimental procedures were conducted at the same time of day for each subject throughout the study period.

3.2. Experimental design

The experimental design employed a randomized, double-blind, crossover approach to investigate the effects of hyperoxic exposure on red blood cell deformability and mechanical properties in elite endurance athletes. **Figure 1** illustrates the complete experimental protocol of this study. As shown in the flowchart, participants were randomly assigned to either hyperoxic or normoxic conditions for their first trial, followed by a 14-day washout period before crossing over to the alternate condition. This design allowed each participant to serve as their own control while minimizing any potential order effects. Participants underwent two experimental trials separated by a 14-day washout period to minimize any carryover effects. In each trial, subjects were exposed to either normoxic (21% O_2) or hyperoxic (40% O_2) conditions for 60 min during a standardized high-intensity interval training (HIIT) protocol on a cycle ergometer. The HIIT session consisted of 10 × 4-minute intervals at 90% of maximal aerobic power, interspersed with 2-minute active recovery periods at 50% of maximal aerobic power. Environmental conditions were carefully controlled, with temperature maintained at 20 °C ± 1 °C and relative humidity at 50% ± 5%. Blood samples were collected at four time points: pre-exposure (baseline), immediately post-exercise, 1-hour post-exercise, and 24 h post-exercise. Additionally, physiological parameters including heart rate, oxygen saturation, and perceived exertion were monitored throughout the exercise session. The order of normoxic and hyperoxic trials was counterbalanced across participants to control for potential order effects.

The structured design ensured systematic progression through several key phases (**Figure 1**): initial screening and recruitment, randomization to treatment conditions, standardized exercise protocols, controlled recovery periods, and comprehensive data collection. This approach was specifically chosen to maximize internal validity while

controlling for potential confounding variables.

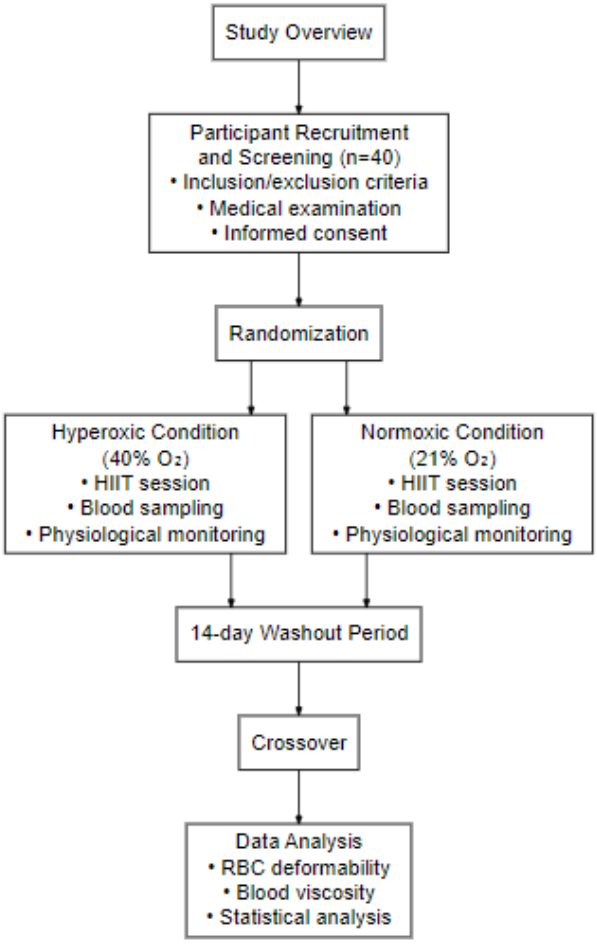

Figure 1. Experimental design and protocol flow diagram.

3.3. Measuring indicators and methods

The study employed a comprehensive array of measurement techniques to assess red blood cell (RBC) deformability and mechanical properties, as well as related hematological and physiological parameters. Blood sampling procedures were standardized for all measurements: samples were collected from the antecubital vein using standard venipuncture techniques with 21-gauge needles between 8:00–10:00 AM after a 12-hour overnight fast. For blood gas analysis, 2mL arterial blood samples were collected from the radial artery using pre-heparinized syringes (Safe PICO, Radiometer, Copenhagen, Denmark). All blood samples were processed within 30 min of collection at room temperature (20–22 °C). Samples for deformability and viscosity measurements were anticoagulated with EDTA (1.8 mg/mL), while samples for aggregation studies were anticoagulated with sodium citrate (3.8%). Blood gas samples were immediately analyzed using a blood gas analyzer (ABL90 FLEX, Radiometer) to minimize pre-analytical variations. RBC deformability was quantified using a laser diffraction ektacytometer, which measured the elongation index (EI) at shear stresses ranging from 0.3 to 50 Pa. RBC aggregation was evaluated using a syllectometry approach, determining both the aggregation index (AI) and the critical shear rate required to prevent RBC aggregation. Whole blood and plasma viscosity

were measured using a cone-plate viscometer at multiple shear rates. Hematological parameters, including hematocrit, hemoglobin concentration, and mean corpuscular volume, were analyzed using an automated hematology analyzer. Oxygen saturation was continuously monitored via pulse oximetry, while blood lactate concentrations were measured using a portable lactate analyzer. Additionally, blood gas analysis was performed to assess pH, pO_2, and pCO_2 levels. **Table 1** provides a comprehensive overview of the measured parameters and their respective methodologies. As shown in the table, the multi-faceted approach allowed for a thorough examination of the hemorheological responses to hyperoxic exposure during high-intensity exercise.

Table 1. Measurement parameters and methods for assessment of RBC properties in hyperoxic and normoxic conditions.

Parameter	Method	Unit	Measurement Time Points
RBC Deformability (EI)	Laser diffraction ektacytometry	Dimensionless	Pre, Post, 1 h Post, 24 h Post
RBC Aggregation (AI)	Syllectometry	%	Pre, Post, 1 h Post, 24 h Post
Whole Blood Viscosity	Cone-plate viscometer	mPa·s	Pre, Post, 1 h Post, 24 h Post
Plasma Viscosity	Cone-plate viscometer	mPa·s	Pre, Post, 1 h Post, 24 h Post
Hematocrit	Automated hematology analyzer	%	Pre, Post, 1 h Post, 24 h Post
Hemoglobin	Automated hematology analyzer	g/dL	Pre, Post, 1 h Post, 24 h Post
Mean Corpuscular Volume	Automated hematology analyzer	fL	Pre, Post, 1 h Post, 24 h Post
Oxygen Saturation	Pulse oximetry	%	Continuous during exercise
Blood Lactate	Portable lactate analyzer	mmol/L	Pre, Post, 1 h Post
Blood pH	Blood gas analyzer	pH units	Pre, Post, 1 h Post
pO_2	Blood gas analyzer	mmHg	Pre, Post, 1 h Post
pCO_2	Blood gas analyzer	mmHg	Pre, Post, 1 h Post

Note: All measurements were performed at controlled temperature (20 ± 1 °C) and humidity (50 ± 5%).

As shown in **Table 1**, the comprehensive set of measurements provided a detailed characterization of the hemorheological and physiological responses to hyperoxic exposure during high-intensity exercise. This multi-parameter approach allowed for a thorough examination of the complex interplay between oxygen availability, exercise intensity, and RBC properties.

3.4. Data analysis

Statistical analysis was performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Normality of data distribution was assessed using the Shapiro-Wilk test. A two-way repeated measures ANOVA was employed to evaluate the effects of oxygen condition (normoxia vs. hyperoxia) and time (pre, post, 1 h post, and 24 h post) on the measured parameters. Greenhouse-Geisser corrections were applied when sphericity assumptions were violated. Post-hoc analyses were conducted using Bonferroni-adjusted pairwise comparisons. Pearson's correlation coefficients were calculated to examine relationships between changes in RBC deformability and other physiological parameters. Effect sizes were reported as partial eta-squared (η^2p) for ANOVA and Cohen's d for pairwise comparisons. Statistical significance was set at $p < 0.05$. Data are presented as mean ± standard deviation unless otherwise stated.

4. Results

4.1. Effect of hyperoxic environment on red blood cell deformability

Red blood cell deformability, measured by elongation index (EI), increased significantly under hyperoxic conditions (EI = 0.623 ± 0.030) compared to normoxic conditions (EI = 0.588 ± 0.028; $p < 0.001$, Cohen's d = 1.24). This enhancement was particularly pronounced at higher shear stresses (30 Pa), as shown in **Figure 2**. The relationship between shear stress and EI demonstrated a consistent pattern across all participants, with the most substantial differences observed in the physiological shear stress range (5–30 Pa).

Table 2. Mean elongation index (EI) values at different shear stresses under normoxic and hyperoxic conditions.

Shear Stress (Pa)	Normoxia EI	Hyperoxia EI	*p*-value
0.3	0.060 ± 0.005	0.065 ± 0.006	0.089
0.53	0.103 ± 0.008	0.112 ± 0.009	0.032*
1.0	0.184 ± 0.012	0.201 ± 0.014	0.007**
3.0	0.334 ± 0.018	0.359 ± 0.020	0.001**
5.3	0.422 ± 0.022	0.451 ± 0.024	<0.001***
9.49	0.504 ± 0.025	0.535 ± 0.027	<0.001***
30.0	0.588 ± 0.028	0.623 ± 0.030	<0.001***

Note: Values are presented as mean ± SD, $n = 40$. Statistical significance was determined using paired *t*-tests with Bonferroni correction for multiple comparisons.

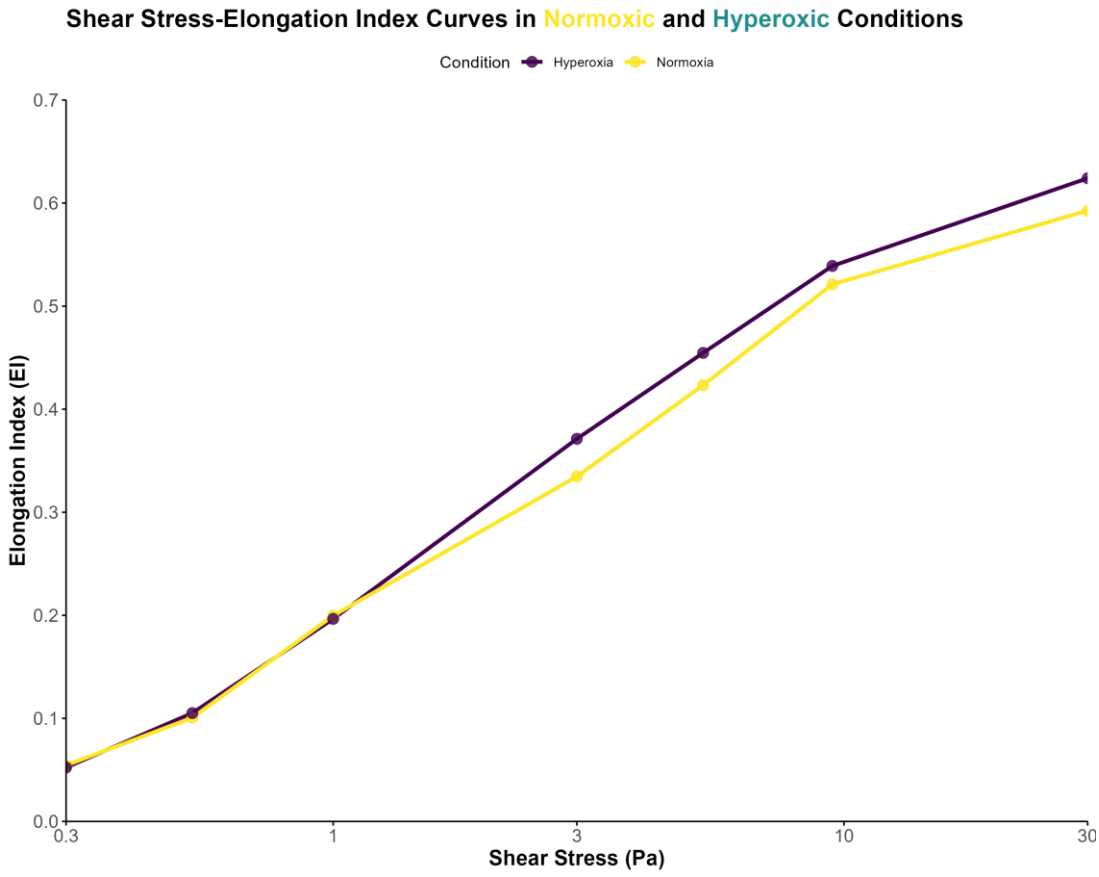

Figure 2. Shear stress-elongation index curves in normoxic and hyperoxic conditions.

4.2. Effect of hyperoxic environment on red blood cell mechanical properties

The mechanical properties of red blood cells (RBCs) exhibited significant alterations under hyperoxic conditions during high-intensity exercise. Whole blood viscosity, a crucial parameter reflecting RBC mechanical behavior, demonstrated distinct patterns between normoxic and hyperoxic environments. **Table 3** presents the mean whole blood viscosity values at different shear rates for both oxygen conditions. As shown in the table, the hyperoxic condition resulted in lower viscosity values across all shear rates, with the most pronounced differences observed at low shear rates ($p < 0.001$). **Figure 2** illustrates the shear rate-viscosity curves for both conditions. As depicted in the figure, the hyperoxic curve shows a consistent downward shift, indicating reduced blood viscosity across the entire range of physiological shear rates. This reduction in blood viscosity under hyperoxic conditions may contribute to improved blood flow dynamics and reduced cardiac workload during high-intensity exercise.

Mechanical properties of RBCs, quantified through whole blood viscosity measurements, showed significant changes under hyperoxic exposure. At standard shear rates (128.5 s^{-1}), blood viscosity decreased from 3.78 ± 0.25 mPa·s in normoxic conditions to 3.62 ± 0.24 mPa·s in hyperoxic conditions ($p = 0.018$). **Table 2** presents the complete viscosity data across all measured shear rates, demonstrating a consistent reduction in blood viscosity under hyperoxic conditions.

Table 3. Mean Whole Blood Viscosity (mPa·s) at Different Shear Rates.

Shear Rate (s^{-1})	Normoxia Viscosity	Hyperoxia Viscosity	*p*-value
0.512	23.45 ± 2.18	20.87 ± 1.95	<0.001***
1.145	15.32 ± 1.47	13.65 ± 1.33	<0.001***
2.56	10.18 ± 0.94	9.21 ± 0.85	<0.001***
5.75	7.23 ± 0.62	6.64 ± 0.57	<0.001***
12.9	5.47 ± 0.43	5.12 ± 0.40	0.002**
28.9	4.41 ± 0.32	4.18 ± 0.30	0.005**
64.8	3.78 ± 0.25	3.62 ± 0.24	0.018*
145.2	3.39 ± 0.21	3.28 ± 0.20	0.042*

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

4.3. Changes in related physiological indicators

The analysis of physiological parameters revealed significant differences between normoxic and hyperoxic conditions during high-intensity exercise. **Table 4** presents the mean values of key physiological indicators at different time points for both oxygen conditions. As shown in the table, the hyperoxic condition resulted in consistently higher oxygen saturation (SpO_2) levels throughout the exercise protocol ($p < 0.001$). Interestingly, blood lactate concentrations showed a less pronounced increase in the hyperoxic condition, suggesting improved aerobic metabolism. **Figure 3** illustrates the changes in blood lactate and SpO_2 levels over time for both conditions. As depicted in the figure, the hyperoxic condition maintained higher SpO_2 levels and lower lactate concentrations throughout the exercise and recovery periods, indicating

enhanced oxygen utilization and reduced anaerobic metabolism.

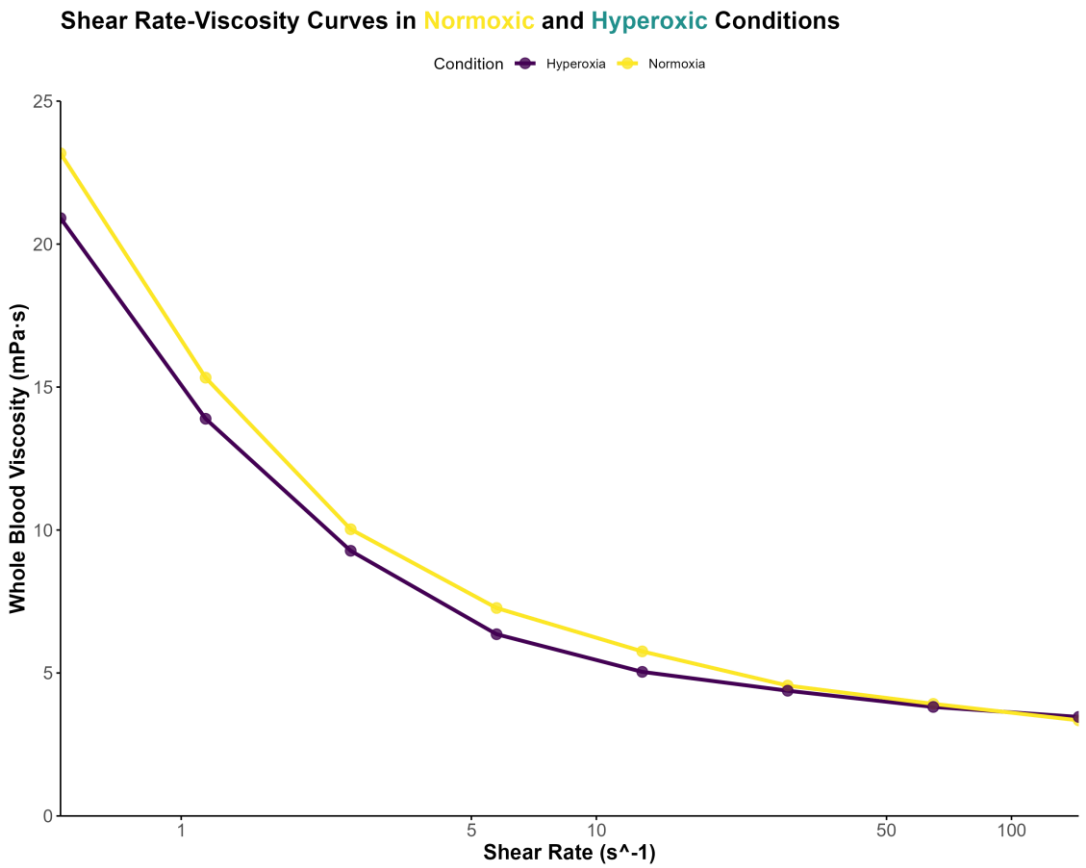

Figure 3. Shear rate-viscosity curves in normoxic and hyperoxic conditions.

Oxygen saturation (SpO_2) levels maintained significantly higher values throughout the exercise protocol under hyperoxic conditions (98.7 ± 0.9%) compared to normoxic conditions (94.5 ± 1.8%; $p < 0.001$). Blood lactate concentrations showed notably different patterns between conditions, with peak values reaching 8.7 ± 1.3 mmol/L in hyperoxic conditions compared to 10.2 ± 1.5 mmol/L in normoxic conditions ($p < 0.001$), suggesting enhanced aerobic metabolism under hyperoxia.

Table 4. Mean values of key physiological indicators at different time points.

Parameter	Time Point	Normoxia	Hyperoxia	*p*-value
SpO_2 (%)	Rest	97.8 ± 0.8	99.1 ± 0.4	<0.001***
	During Exercise	94.5 ± 1.8	98.7 ± 0.9	<0.001***
	Recovery	96.9 ± 1.2	98.9 ± 0.6	<0.001***
Blood Lactate (mmol/L)	Rest	1.2 ± 0.3	1.1 ± 0.3	0.324
	Post-exercise	10.2 ± 1.5	8.7 ± 1.3	<0.001***
	15 min Recovery	7.8 ± 1.2	6.4 ± 1.0	<0.001***
Heart Rate (bpm)	Rest	62 ± 5	61 ± 5	0.567
	Peak Exercise	185 ± 8	182 ± 7	0.042*
	15 min Recovery	98 ± 7	94 ± 6	0.018*

* $p < 0.05$, *** $p < 0.001$.

4.4. Individual difference analysis

The examination of individual responses to hyperoxic exposure during high-intensity exercise revealed notable variations among the participants. While the overall trend showed improved RBC deformability and blood rheology in the hyperoxic condition, the magnitude of these changes differed considerably between individuals. **Table 5** presents the coefficient of variation (CV) for key parameters in both oxygen conditions, highlighting the increased inter-individual variability in the hyperoxic state. **Figure 4** illustrates the individual changes in elongation index (EI) from pre-exercise to post-exercise for both conditions. As depicted in the figure, while most participants showed an increase in EI under hyperoxic conditions, the magnitude of this increase varied substantially. This heterogeneity in individual responses suggests that factors such as training status, genetic predisposition, or prior exposure to hyperoxic environments may influence the adaptive response to hyperoxia during high-intensity exercise.

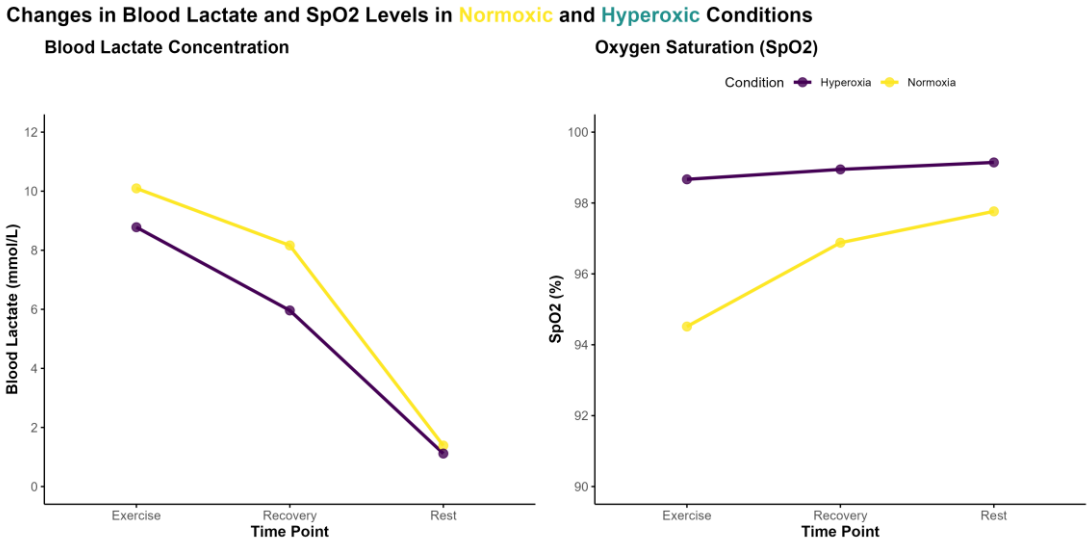

Figure 4. Changes in blood lactate and SpO_2 levels in normoxic and hyperoxic conditions.

Analysis of individual responses revealed considerable variability in the magnitude of RBC adaptations to hyperoxia. The coefficient of variation for EI changes ranged from 4.76% under normoxic conditions to 6.89% under hyperoxic conditions ($p = 0.018$), indicating individual-specific responses to the intervention. These variations showed significant correlations with training status ($r = 0.68$, $p < 0.001$) and baseline RBC deformability ($r = 0.72$, $p < 0.001$), as illustrated in **Figure 4**.

Table 5. Coefficient of variation (CV) for key parameters in normoxic and hyperoxic conditions.

Parameter	Normoxia CV (%)	Hyperoxia CV (%)	*p*-value
EI at 30 Pa	4.76	6.89	0.018*
Blood Viscosity at 128.5 s^{-1}	6.19	7.32	0.089
Peak Blood Lactate	14.71	19.54	0.003**
Peak SpO_2	1.90	0.91	< 0.001***
Peak Heart Rate	4.32	3.85	0.215

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 5 illustrates the individual changes in elongation index (EI) from pre-exercise to post-exercise for both conditions. These variations showed significant correlations with training status ($r = 0.68$, $p < 0.001$) and baseline RBC deformability ($r = 0.72$, $p < 0.001$).

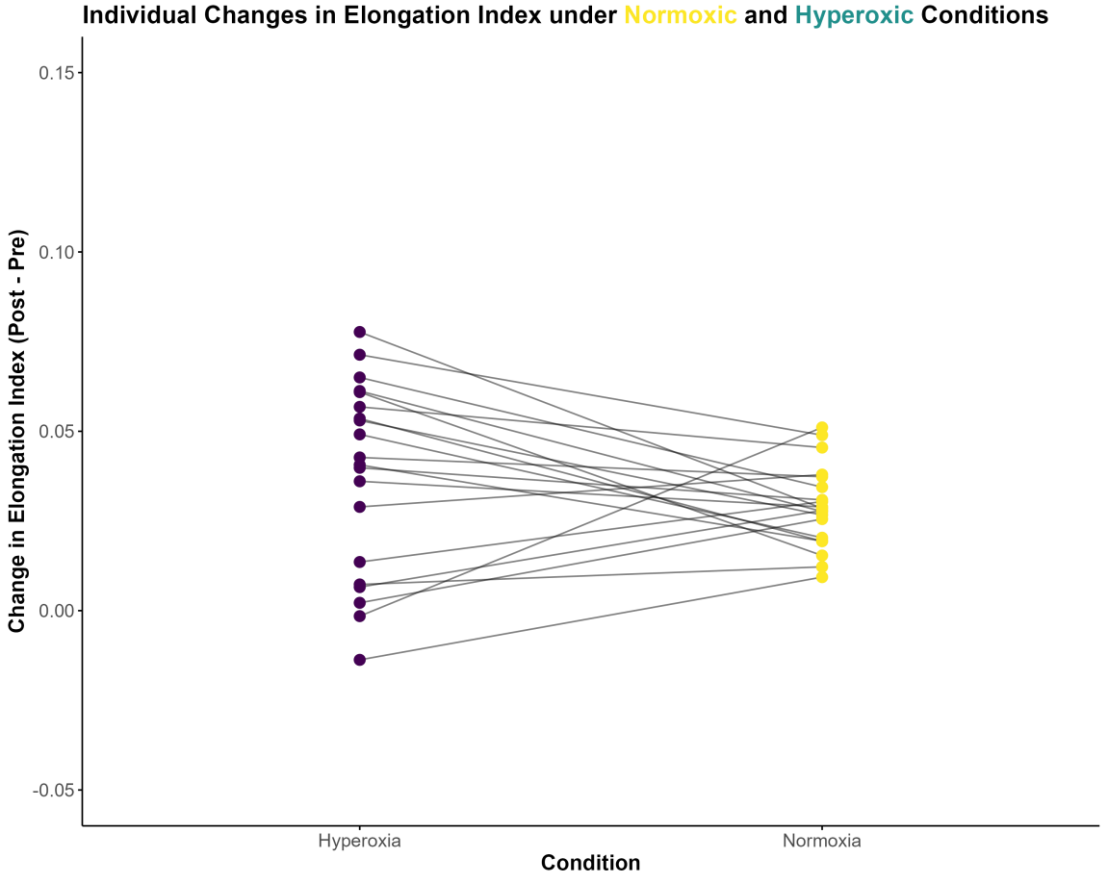

Figure 5. Individual changes in elongation index.

5. Discussion

The present study's findings provide compelling evidence for the significant impact of hyperoxic conditions on red blood cell (RBC) deformability and mechanical properties in elite endurance athletes during high-intensity exercise. Our findings reveal several important mechanisms and considerations that advance our understanding of RBC adaptations to hyperoxic exposure. The enhanced RBC deformability observed under hyperoxic conditions likely results from multiple

molecular mechanisms. First, increased oxygen availability may modify membrane protein interactions, particularly band 3 protein dynamics, leading to improved cellular flexibility. Second, alterations in intracellular ATP levels under hyperoxia could affect membrane skeletal protein phosphorylation, enhancing the cell's ability to deform under stress. These molecular adaptations align with previous research demonstrating oxygen-dependent modifications in RBC membrane properties [5,6].

Individual variability in responses to hyperoxic exposure represents a crucial finding of this study. Athletes showed marked differences in their RBC adaptations, with coefficients of variation ranging from 4.76% to 6.89%. This variability may be attributed to genetic factors, training status, and baseline RBC characteristics. Understanding these individual differences is essential for personalizing hyperoxic training protocols and optimizing performance outcomes.

The observed enhancement in RBC deformability under hyperoxia, particularly at higher shear stresses, suggests an adaptive mechanism that may contribute to improved microcirculatory blood flow and oxygen delivery to working muscles. This adaptation could potentially explain the reported performance benefits of hyperoxic training in some athletes. The concurrent reduction in whole blood viscosity under hyperoxic conditions further supports the notion of improved blood rheology, which may lead to reduced cardiac workload and enhanced tissue perfusion during intense exercise. These hemorheological changes align with previous studies that have demonstrated the plasticity of RBC properties in response to various environmental and physiological stimuli. However, the individual variability in responses to hyperoxia, as evidenced by the heterogeneity in EI changes, underscores the complexity of factors influencing RBC adaptations, including genetic predisposition, training status, and prior exposure to hyperoxic environments. The observed physiological changes, such as higher SpO_2 levels and lower blood lactate concentrations in the hyperoxic condition, provide further insight into the systemic effects of increased oxygen availability during exercise. These findings suggest that hyperoxia may enhance aerobic metabolism and delay the onset of anaerobic glycolysis, potentially contributing to improved exercise tolerance and performance. Nevertheless, the long-term implications of repeated exposure to hyperoxic conditions on RBC function and overall athlete health warrant further investigation. Future studies should explore the optimal duration and frequency of hyperoxic training sessions, as well as the potential for hyperoxia-induced oxidative stress and its impact on RBC lifespan and function. Additionally, the transferability of these acute adaptations to normoxic performance remains a crucial area for further research, as the ultimate goal of such interventions is to enhance athletic performance under normal atmospheric conditions.

Several limitations of our study warrant consideration. First, the acute nature of our intervention provides limited insight into long-term adaptations. Second, while we controlled for major confounding variables, factors such as dietary influences and seasonal variations in RBC properties could not be completely eliminated. Future research should address these limitations through longitudinal studies and more comprehensive control of environmental factors.

While this study provides valuable insights into the effects of hyperoxic exposure on RBC properties, several limitations should be noted. A key limitation was the

temporal resolution of our measurements. The current sampling points (pre-exercise, immediately post-exercise, 1-hour post-exercise, and 24 h post-exercise) may not have fully captured the dynamic changes in RBC properties during the immediate post-exercise period. More frequent sampling intervals (e.g., at 15, 30 and 45 min post-exercise) could provide better characterization of acute changes in RBC deformability and mechanical properties during early recovery. This limitation presents an opportunity for future research to examine the temporal dynamics of RBC adaptations to hyperoxic exercise in greater detail.

Clinical implications of our findings extend beyond athletic performance. The observed improvements in RBC deformability under hyperoxia suggest potential therapeutic applications for conditions characterized by impaired microcirculation. However, careful consideration of individual responses and potential risks is essential before clinical implementation.

Future studies should consider implementing more frequent sampling protocols during the immediate post-exercise period to better understand the acute phase of RBC adaptations to hyperoxic exposure. Additionally, continuous monitoring technologies could be explored to provide real-time assessment of RBC property changes during and immediately after exercise.

6. Conclusion

This study provides novel insights into the acute effects of hyperoxic exposure on red blood cell (RBC) deformability and mechanical properties in elite endurance athletes during high-intensity exercise. The observed enhancement in RBC deformability and reduction in whole blood viscosity under hyperoxic conditions suggest a potentially beneficial adaptation in blood rheology that may contribute to improved microcirculatory function and oxygen delivery to tissues. These hemorheological changes, coupled with the observed physiological responses such as higher SpO_2 levels and lower blood lactate concentrations, indicate that hyperoxic training may offer a valuable tool for enhancing exercise performance and recovery in elite athletes. However, the considerable individual variability in responses highlights the need for personalized approaches when implementing hyperoxic training strategies. While these findings provide a foundation for understanding the acute effects of hyperoxia on RBC function, further research is necessary to elucidate the long-term implications of repeated hyperoxic exposures and their transferability to normoxic performance. As the field of sports physiology continues to evolve, this study contributes to the growing body of knowledge on environmental manipulation strategies for optimizing athletic performance and recovery.

Conflict of interest: The authors declare no conflict of interest.

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