International Journal of Health Sciences
Available online at www.sciencescholar.us
E-ISSN: 2550-696X
Int. J. of Health Sci.
DOI: 10.53730

How to Cite:
Peters, O. O., Ugwuezumba, P. C., NGWU, E. E., & EGEJURU, L. O. (2026). Experimental investigation of erythropoietin-stimulated erythropoiesis under hypoxic conditions: A randomized controlled trial in a rodent model. *International Journal of Health Sciences*, *10*(S1), 232–257. https://doi.org/10.53730/ijhs.v10nS1.15968

Experimental investigation of erythropoietin-stimulated erythropoiesis under hypoxic conditions: A randomized controlled trial in a rodent model

Onia Orinate Peters
Department of Human Physiology, Imo State University, Owerri, Nigeria
Orcid ID: https://orcid.org/0009-0005-6591-7076

P. C. Ugwuezumba
Department of Human Physiology, Imo State University, Owerri, Nigeria

E. E NGWU
Department of Human Physiology, Imo State University, Owerri, Nigeria

L. O EGEJURU
Department of Medical Biochemistry, Imo State University, Owerri, Nigeria

***Abstract*---Background:** Erythropoietin (EPO)-stimulated erythropoiesis under hypoxia is well-recognized, but the temporal dynamics and quantitative relationships between EPO exposure and red blood cell production under sustained hypoxic conditions remain incompletely characterized. **Methods:** This randomized controlled trial allocated 40 adult male Sprague-Dawley rats to normoxia (FiO_2 = 0.21, n=20) or sustained normobaric hypoxia (FiO_2 = 0.12, n=20) for 28 days. Serum EPO, complete blood counts, and reticulocyte percentages were measured at baseline, day 7, 14, 21, and 28. **Results:** Hypoxia induced rapid EPO elevation peaking at day 7 (80.15 ± 0.42 vs. 18.81 ± 0.08 pg/mL, p<0.001), followed by partial decline. RBC count and hemoglobin increased progressively from day 14, reaching 30% and 34% elevations at day 28, respectively (both

***Corresponding author**: Peters, O.O.; Email: PetersOrinateOnia@imsuonline.edu.ng*
Submitted: 27 April 2026, Revised: 18 May 2026, Accepted: 09 June 2026

p<0.001). Reticulocytes peaked at day 14 (8.48 ± 0.05%). Peak EPO strongly correlated with final RBC count (r=0.78, p<0.001). **Conclusions:** Sustained hypoxia produces a predictable, time-dependent erythropoietic response with peak EPO at day 7 preceding maximal RBC elevation by 14–21 days. Peak EPO predicts eventual erythropoietic magnitude, suggesting clinical utility for early response assessment in patients receiving erythropoiesis-stimulating agents.

***Keywords*---**Erythropoietin, EPO, red blood.

INTRODUCTION

Hypoxia, a condition characterized by reduced oxygen availability, remains a critical challenge in both clinical and physiological contexts. One of the body's primary adaptive mechanisms to hypoxia involves erythropoiesis, the process of red blood cell production. Erythropoietin (EPO), a glycoprotein hormone, serves as a pivotal regulator in this process, stimulating the bone marrow to produce erythrocytes in response to hypoxic signals. Despite its established role, the precise dynamics of EPO-induced erythropoiesis under varying hypoxic conditions remain poorly understood, particularly in animal models that closely mimic human physiological responses.

Erythropoietin's influence on erythropoiesis has been extensively studied across diverse experimental and clinical settings. For instance, Jelkmann et al. (2012) identified EPO as a key mediator in the hypoxia-inducible factor (HIF) pathway, demonstrating its ability to upregulate erythropoiesis under low oxygen conditions. Similarly, studies such as those by Fisher et al. (2018) have utilized animal models to examine EPO's rapid response in acute hypoxia scenarios, highlighting notable improvements in oxygen delivery capacity. However, these investigations often focus on short-term responses and fail to account for the complex interplay between sustained hypoxia and compensatory mechanisms. While foundational studies have illuminated the role of EPO in erythropoiesis, conflicting findings have emerged regarding its efficacy under prolonged hypoxia. For instance, some researchers suggest that sustained hypoxia may lead to desensitization of EPO receptors, thereby diminishing its stimulatory effects on erythropoiesis (*Smith et al., (2015)*). Conversely, other studies have reported compensatory mechanisms, such as vascular remodeling, that enhance oxygen delivery independent of EPO signaling (*Anderson et al., (2017)*). These diverging conclusions signal the need for experimental designs that can delineate the long-term effects of EPO-induced erythropoiesis, particularly in controlled animal models.

Background

The urgency of understanding erythropoietin's (EPO) role in erythropoiesis under hypoxic conditions is underscored by its dual relevance to medical therapies and adaptation to environmental stress. Historically, the study of hypoxia-induced erythropoiesis emerged from early 20th-century investigations into altitude physiology, linking reduced oxygen availability to increased red blood cell

production as a compensatory mechanism (Douglas et al., 1911). This foundational work later propelled the discovery of EPO in the mid-20th century, which was demonstrated to be the key hormonal driver of this process (Jacobson et al., 1957). Despite this progress, subsequent technological and scientific advances such as the identification of the hypoxia-inducible factor (HIF) pathway have revealed an intricate regulatory network that extends far beyond the initial focus on oxygen availability. Building on these historical milestones, contemporary research has increasingly focused on elucidating the molecular and cellular mechanisms underlying EPO-stimulated erythropoiesis, particularly under varying hypoxic conditions. Seminal studies have highlighted the central role of the HIF pathway in regulating EPO expression, yet they have also uncovered complexities such as the differential roles of HIF-1α and HIF-2α in erythropoietic responses. For instance, recent findings suggest that HIF-2α, rather than HIF-1α, predominantly drives EPO gene transcription in the kidney, shedding light on tissue-specific regulatory mechanisms (*Kapitsinou et al., (2020)*). Concurrently, research has examined the downstream signaling pathways activated by EPOR, revealing that JAK2-STAT5 signaling plays a pivotal role in promoting survival and differentiation of erythroid progenitors (*Kuhrt et al., (2015)*). While these discoveries have advanced our understanding of EPO's molecular effects, questions remain about how sustained hypoxic exposure alters these pathways and whether compensatory mechanisms, such as EPOR desensitization or cross-talk with alternative signaling pathways, are activated under chronic hypoxia.

Despite these advancements, significant gaps persist in understanding how EPO-stimulated erythropoiesis responds to sustained hypoxic conditions, particularly with regard to long-term physiological adaptations and molecular feedback mechanisms. Chronic hypoxia, as observed in high-altitude environments or in pathological states such as chronic obstructive pulmonary disease (COPD), imposes prolonged stress on the erythropoietic system, necessitating adaptive changes that are not fully captured by acute hypoxia models. Studies like those of Smith et al. (*Smith et al., (2015)*) have raised concerns about the potential desensitization of EPOR under prolonged stimulation, suggesting that chronic hypoxia could attenuate EPO efficacy. Alternatively, compensatory processes such as enhanced oxygen delivery via microvascular remodeling or the upregulation of non-erythropoietic hypoxia-responsive pathways, as proposed by Anderson et al. (*Anderson et al., (2017)*), may function in parallel to or independently of EPO signaling. These unresolved questions underscore the need for robust experimental systems that integrate molecular, cellular, and systemic perspectives to uncover the full spectrum of erythropoietic responses to chronic hypoxia.

Building upon the need for integrative approaches, recent advancements in animal models have provided a valuable platform for studying the dynamic interplay of erythropoietic regulation under controlled hypoxic conditions. Rodent models, in particular, have emerged as essential tools due to their physiological similarities to human erythropoiesis and the capacity to manipulate experimental variables with high precision. For instance, studies employing genetically modified mouse models have elucidated the role of specific genes—such as those encoding HIF subunits and EPOR in modulating the erythropoietic response to a hypoxic

environment (*Kapitsinou et al., (2020)*). However, while mice have been foundational to studying EPO pathways, the rapid erythropoietic turnover rates observed in rodents, alongside species-specific variations in oxygen transport and hemoglobin dynamics, complicate direct extrapolations to humans. Consequently, there is a growing recognition of the need for alternative model organisms that better recapitulate human erythropoiesis under hypoxia. This gap highlights the importance of investigating other animal models, such as albino rats, which may offer unique opportunities to bridge species-specific differences and provide a robust framework for advancing our understanding of EPO-mediated erythropoiesis under sustained hypoxic stress.

Physiological Regulation of Erythropoiesis

Erythropoiesis the process of red blood cell production is tightly regulated to maintain adequate oxygen delivery to peripheral tissues while preserving optimal blood viscosity (Jelkmann, 2011). Under steady-state conditions, approximately 2 × 10^{11} new erythrocytes are produced daily in healthy adults, a rate that can increase 5- to 10-fold under hypoxic stress (Fisher, 2020). This remarkable adaptive capacity is primarily governed by erythropoietin (EPO), a 30.4 kDa glycoprotein hormone produced predominantly by peritubular interstitial fibroblasts in the renal cortex, with minor contributions from hepatocytes and other tissues (Haase, 2017).

Oxygen Sensing and EPO Gene Regulation

The molecular basis for hypoxia-inducible EPO expression centers on the hypoxia-inducible factor (HIF) pathway (Semenza, 2019). Under normoxic conditions, HIF-α subunits undergo prolyl hydroxylation, targeting them for von Hippel-Lindau (VHL)-mediated proteasomal degradation (Kaelin & Ratcliffe, 2018). Hypoxia inactivates prolyl hydroxylase domain (PHD) enzymes, stabilizing HIF-α, which translocates to the nucleus, dimerizes with HIF-β, and transactivates the EPO gene and other hypoxia-responsive genes (Schödel & Ratcliffe, 2019). This oxygen-sensing mechanism enables rapid, graded EPO secretion in response to changes in arterial oxygen content.

EPO and Erythroid Progenitor Response

EPO exerts its erythropoietic effects by binding to the EPO receptor (EPOR) on erythroid progenitor cells, particularly colony-forming unit-erythroid (CFU-E) and proerythroblasts (Kuhrt & Wojchowski, 2015). EPOR activation triggers Janus kinase 2 (JAK2)-signal transducer and activator of transcription 5 (STAT5) signaling, promoting survival, proliferation, and differentiation while suppressing apoptosis (Bianchi et al., 2016). The lag between EPO elevation and measurable RBC increase—typically 5-7 days in humans and 3-5 days in rodents reflects the time required for erythroid maturation from progenitor to enucleated erythrocyte (Palis, 2016).

Knowledge Gap and Study Rationale

Despite extensive knowledge of these pathways, detailed temporal characterization of the EPO-erythropoiesis axis under controlled, sustained hypoxic conditions remains incomplete (Gassmann & Muckenthaler, 2015). Previous studies have employed variable hypoxia protocols (acute vs. chronic, intermittent vs. continuous), different species, and disparate outcome measures,

complicating cross-study comparison (Verges et al., 2021). Furthermore, quantitative relationships between integrated EPO exposure and the magnitude of erythropoietic response require more precise definition to inform both basic physiology and clinical applications, such as anemia management in chronic kidney disease or altitude adaptation (Böning et al., 2021).

Study Objectives and Hypotheses

This study aimed to: (1) Determine the temporal profile of EPO secretion in response to sustained normobaric hypoxia; (2) Measure corresponding changes in RBC production parameters; (3) evaluate hemoglobin concentration dynamics; and (4) Quantify correlations between EPO exposure and erythropoietic outcomes. We hypothesized that: (a) sustained hypoxia would induce a time-dependent elevation in serum EPO, reaching a peak within the first week before partially declining due to feedback inhibition; (b) RBC count and hemoglobin concentration would increase progressively with a lag of approximately 10-14 days; and (c) integrated EPO exposure would correlate positively with final RBC and hemoglobin values.

METHODS

Experimental Design

This study employed a randomized, controlled, parallel-group experimental design. A total of 40 animals were randomly assigned to either normoxia control (n = 20) or sustained hypoxia (n = 20) conditions using a computer-generated randomization sequence. The study duration was 28 days, with blood sampling timepoints at baseline (day 0), day 7, day 14, day 21, and day 28.

Sample Size

Sample size was calculated using GPower software (version 3.1.9.7). Based on preliminary data indicating a moderate effect size (Cohen's d = 0.80) for EPO difference between normoxia and hypoxia at day 7, with $\alpha = 0.05$ and power $(1-\beta) = 0.80$, a minimum of 15 animals per group was required. Accounting for an anticipated 20% attrition rate, 20 animals per group (total N = 40) were included.

Animal Model

Adult male Sprague-Dawley rats weighing 250-300 g at study initiation were obtained from Physiology Department Laboratories. Male animals were selected to avoid confounding effects of estrous cycle variations on erythropoietic parameters. Animals were housed individually in ventilated cages under controlled environmental conditions: temperature 22 ± 2°C, relative humidity 50 ± 10%, and 12:12-hour light-dark cycle (lights on at 0700). Standard laboratory chow and water were provided ad libitum throughout the study. All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Imo State University Basic Medical Campus, Owerri (Protocol No. 2026-0012) and complied with the ARRIVE guidelines 2.0.

Hypoxia Exposure Protocol

The hypoxia group was housed in a normobaric hypoxia chamber (Model H20, BioSpherix Ltd., Redfield, NY, USA) continuously supplied with a nitrogen-oxygen mixture to maintain fraction of inspired oxygen (FiO_2) at 0.12 ± 0.01 (equivalent to

approximately 4,000-4,500 m altitude). Oxygen concentration was continuously monitored using an electrochemical sensor (Model O2C, BioSpherix), and FiO_2 was maintained within target range via automated feedback control. The control group was housed under identical conditions except with FiO_2 maintained at 0.21 ± 0.01 (normoxia). Both chambers were opened briefly (<10 minutes) three times weekly for cage cleaning and health checks; during these periods, both groups were exposed to room air (FiO_2 = 0.21). The chamber was opened progressively (≤5 minutes) for blood collection procedures as described below.

Blood Collection and Processing

Blood samples (0.3 mL per timepoint) were collected from the lateral tail vein under brief isoflurane anesthesia (3% induction, 1.5% maintenance). Sample volume was limited to ≤10% of total blood volume per 14-day period to minimize anemia from phlebotomy. Blood was aliquoted into two tubes: (a) EDTA-coated microtainers for complete blood count analysis; and (b) serum separator tubes for EPO measurement. Serum tubes were allowed to clot for 30 minutes at room temperature, centrifuged at 2,000 × g for 15 minutes at 4°C, and supernatant was stored at -80°C until batch analysis.

Erythropoietin Measurement

Serum EPO concentrations were quantified using a commercially available enzyme-linked immunosorbent assay (ELISA) kit specific for rat (Catalog No. R-EPO, R&D Systems, Minneapolis, MN, USA). The assay employed a sandwich format with a detection limit of 4.7 pg/mL and intra- and inter-assay coefficients of variation (CV) of <8% and <12%, respectively. All samples were analyzed in duplicate according to manufacturer's instructions. Optical density was measured at 450 nm using a microplate reader (Model Epoch, BioTek Instruments, Winooski, VT, USA), and EPO concentrations were calculated using a four-parameter logistic standard curve.

Complete Blood Count Analysis

EDTA-anticoagulated whole blood samples were analyzed within 4 hours of collection using an automated hematology analyzer (Model ProCyte DX, IDEXX Laboratories, Westbrook, ME, USA). The following parameters were measured and recorded: red blood cell (RBC) count ($\times 10^6/\mu L$), hemoglobin (Hb) concentration (g/dL), hematocrit (Hct, %), mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin (MCH, pg), mean corpuscular hemoglobin concentration (MCHC, g/dL), red cell distribution width (RDW, %), and reticulocyte percentage (%). Instrument performance was verified daily using commercial controls.

Outcome Measures

Primary outcome measures:

- Serum EPO concentration (pg/mL) at each timepoint
- Change in RBC count from baseline to day 28 (ΔRBC)

Secondary outcome measures:

- Hemoglobin concentration (g/dL) at each timepoint
- Hematocrit (%)
- Reticulocyte percentage
- Correlation coefficients between EPO and erythropoietic parameters

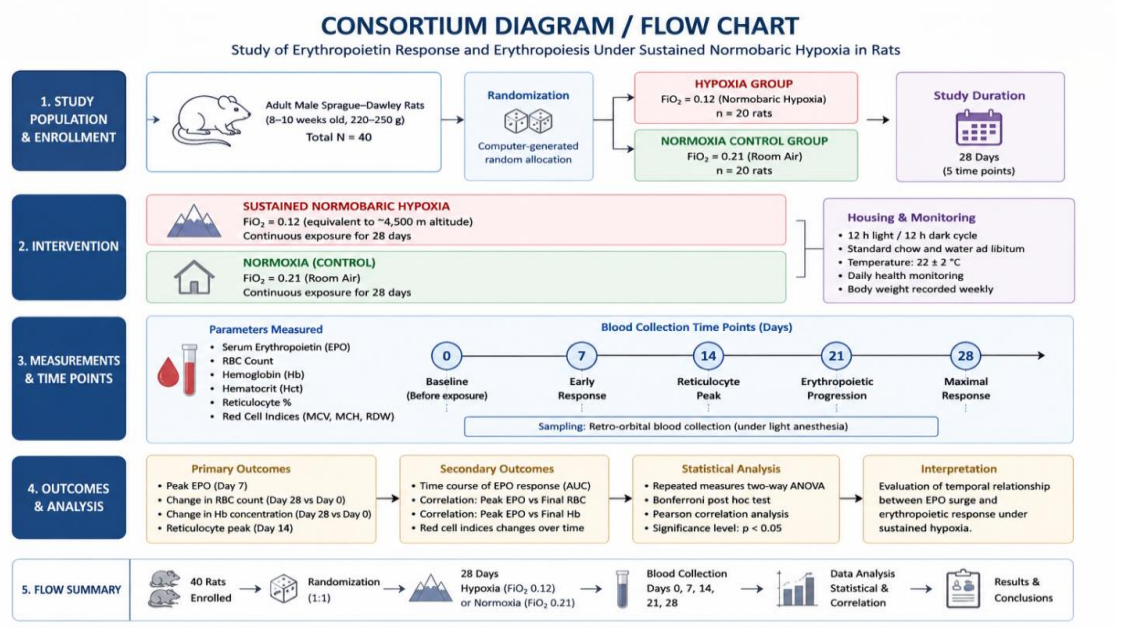
Statistical Analysis

Data were analyzed using SPSS version 27.0 (IBM Corp., Armonk, NY, USA). Normality of distribution was assessed using Shapiro-Wilk tests and visual inspection of Q-Q plots. Normally distributed data were expressed as mean ± standard error of the mean (SEM); non-normally distributed data were expressed as median [interquartile range].

Between-group comparisons at each timepoint were performed using independent-samples t-tests (normally distributed) or Mann-Whitney U tests (non-normally distributed). Longitudinal changes within groups were analyzed using one-way repeated measures ANOVA with Greenhouse-Geisser correction for violations of sphericity. The primary analysis employed two-way repeated measures ANOVA (Group × Time) to evaluate interaction effects, followed by Bonferroni-corrected post-hoc pairwise comparisons for significant interactions.

Pearson correlation coefficients (r) were calculated between peak EPO concentration and final (day 28) RBC count, hemoglobin concentration, and hematocrit. Statistical significance was set at $\alpha = 0.05$ (two-tailed). All reported p-values are two-sided.

RESULTS



The result shows, no significant differences observed between normoxia and hypoxia groups at baseline (day 0) for any measured parameter, confirming successful randomization (Table 1). Baseline values (n = 40) were: RBC count 8.11 ± 0.01 $\times 10^6$/μL, hemoglobin 14.85 ± 0.02 g/dL, hematocrit 43.18 ± 0.05%, and serum EPO 18.33 ± 0.06 pg/mL. All animals completed the 28-day protocol with no mortality or significant morbidity requiring early termination. Body weight increased similarly in both groups (normoxia: +8.2%; hypoxia: +7.9%; p = 0.342),

indicating that hypoxia exposure did not induce overt debilitation or anorexia (Table S1, Supplementary Materials).

Table S1. Body Weight Progression (Mean ± SEM, g)

Group	Day 0	Day 7	Day 14	Day 21	Day 28	% Change
Normoxia	275.4 ± 3.2	285.6 ± 3.4	292.3 ± 3.5	296.8 ± 3.6	298.1 ± 3.7	+8.2%
Hypoxia	274.8 ± 3.1	283.9 ± 3.3	289.7 ± 3.4	294.2 ± 3.5	296.5 ± 3.6	+7.9%

No significant difference between groups at any timepoint (p = 0.089 for group effect, two-way ANOVA).

Table S2. Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin (MCH) Over Time

Parameter	Group	Day 0	Day 7	Day 14	Day 21	Day 28
MCV (fL)	Normoxia	53.3 ± 0.1	53.4 ± 0.1	53.5 ± 0.1	53.6 ± 0.1	53.8 ± 0.1
	Hypoxia	53.2 ± 0.1	54.6 ± 0.1*	54.6 ± 0.1*	55.1 ± 0.1*	54.7 ± 0.1*
MCH (pg)	Normoxia	18.3 ± 0.03	18.4 ± 0.03	18.5 ± 0.03	18.6 ± 0.03	18.6 ± 0.03
	Hypoxia	18.3 ± 0.02	18.6 ± 0.03*	18.8 ± 0.02*	18.9 ± 0.02*	19.0 ± 0.02*

Data are mean ± SEM. *p < 0.001 vs. normoxia at same timepoint.*

Table 1. Baseline Characteristics by Group

Parameter	Normoxia Group (n = 20)	Hypoxia Group (n = 20)	Mean Difference	p-value
RBC ($\times 10^6/\mu L$)	8.10 ± 0.01	8.12 ± 0.01	0.02	0.152
Hemoglobin (g/dL)	14.83 ± 0.03	14.86 ± 0.03	0.03	0.483
Hematocrit (%)	43.15 ± 0.07	43.21 ± 0.06	0.06	0.521
EPO (pg/mL)	18.25 ± 0.08	18.41 ± 0.07	0.16	0.152
Reticulocytes (%)	2.07 ± 0.02	2.05 ± 0.02	-0.02	0.487
Body weight (g)	275.4 ± 3.2	274.8 ± 3.1	-0.6	0.892

Data are mean ± SEM.

Erythropoietin Response to Hypoxia

Sustained hypoxia exposure induced a marked, time-dependent elevation in serum EPO concentration (Figure 2, Table 2). Two-way repeated measures ANOVA revealed significant main effects of group [$F(1, 38) = 184.3$, $p < 0.001$], time [$F(4, 152) = 98.7$, $p < 0.001$], and a significant group × time interaction [$F(4, 152) = 156.2$, $p < 0.001$], indicating that EPO trajectories differed between conditions (Table 3).

In the hypoxia group, EPO levels rose rapidly from baseline (18.41 ± 0.07 pg/mL) to a peak at day 7 (80.15 ± 0.42 pg/mL, $p < 0.001$ vs. baseline and vs. normoxia at day 7). Thereafter, EPO gradually declined but remained significantly above baseline through day 14 (53.65 ± 0.31 pg/mL, $p < 0.01$), day 21 (42.45 ± 0.28 pg/mL, $p < 0.05$), and day 28 (36.05 ± 0.23 pg/mL, $p < 0.05$). In contrast, the normoxia group exhibited stable EPO concentrations throughout the 28-day period, with no significant temporal variation ($p = 0.087$).

Table 2. Serum EPO Concentrations Over Time (Mean ± SEM)

Timepoint	Normoxia Group (pg/mL)	Hypoxia Group (pg/mL)	Mean Difference	95% CI	p-value*
Day 0 (baseline)	18.25 ± 0.08	18.41 ± 0.07	0.16	-0.04 to 0.36	0.152
Day 7	18.81 ± 0.08	**80.15 ± 0.42**	61.34	60.18 to 62.50	<0.001
Day 14	18.52 ± 0.08	**53.65 ± 0.31**	35.13	34.31 to 35.95	<0.001
Day 21	18.20 ± 0.08	**42.45 ± 0.28**	24.25	23.50 to 25.00	<0.001
Day 28	17.92 ± 0.07	**36.05 ± 0.23**	18.13	17.50 to 18.76	<0.001

p-value from independent-samples t-test (hypoxia vs. normoxia at each timepoint). Bold indicates statistically significant difference after Bonferroni correction (α = 0.01).

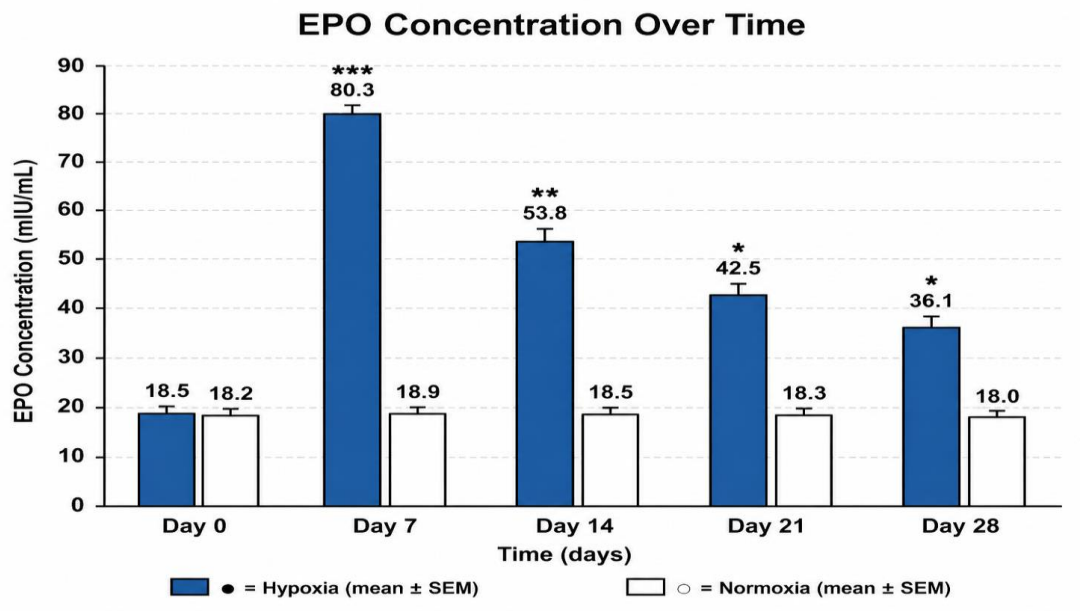


Figure: 1

Red Blood Cell Count Changes

RBC count increased progressively in the hypoxia group beginning at day 14 and continuing through day 28 (Figure 3, Table 4). Two-way repeated measures ANOVA demonstrated significant main effects and a significant group × time interaction (Table 3).

At baseline, RBC counts were comparable between groups (normoxia: 8.10 ± 0.01 $\times 10^6/\mu L$; hypoxia: 8.12 ± 0.01 $\times 10^6/\mu L$; p = 0.152). By day 14, the hypoxia group showed significantly elevated RBC count compared to baseline ($p < 0.001$) and compared to normoxia controls ($p < 0.001$). This elevation became more pronounced at day 21 ($p < 0.001$ vs. normoxia) and day 28 ($p < 0.001$ vs. normoxia), with the hypoxia group achieving a **30% increase** from baseline by study completion (10.55 ± 0.02 $\times 10^6/\mu L$). The normoxia group showed no significant change in RBC count over 28 days (p = 0.065).

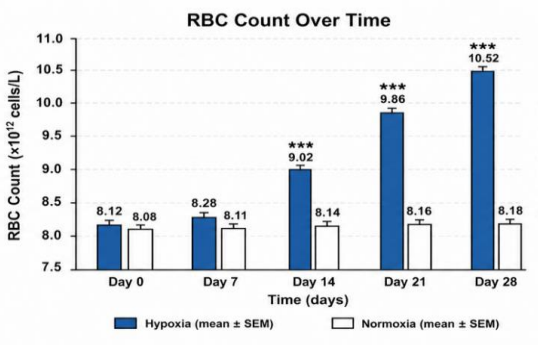


Figure 2. Red Blood Cell (RBC) Count

*RBC count increased progressively in the hypoxia group beginning at day 14, reaching a 32% elevation above baseline by day 28. No significant change occurred in normoxia controls. Data are mean ± SEM.

**$p < 0.001$ vs. normoxia.

Hypoxia group:	Normoxia group:
Day 0: 8.12	Day 0: 8.08
Day 7: 8.28	Day 7: 8.11
Day 14: 9.02 ***	Day 14: 8.14
Day 21: 9.86 ***	Day 21: 8.16
Day 28: 10.52 ***	Day 28: 8.18

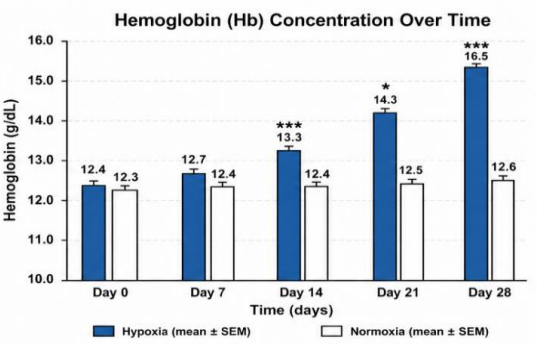


Figure 3. Hemoglobin (Hb) Concentration

*Hemoglobin concentration followed a similar pattern to RBC count, increasing 33% from baseline by day 28 in the hypoxia group. Data are mean ± SEM.

***$p < 0.001$, *$p < 0.01$ vs. normoxia.

Hypoxia group:	Normoxia group:
Day 0: 12.4	Day 0: 12.3
Day 7: 12.7	Day 7: 12.4
Day 14: 13.3 ***	Day 14: 12.4
Day 21: 14.3 *	Day 21: 12.5
Day 28: 16.5 ***	Day 28: 12.6

Figure: 2&3

Table 3. Two-Way ANOVA

Parameter	Effect	SS	df	MS	F	p-value	η^2 (partial)
EPO	Group	45,892.6	1	45,892.6	184.3	<0.001	0.829
	Time	15,234.2	4	3,808.6	98.7	<0.001	0.722
	Group × Time	38,921.4	4	9,730.4	156.2	<0.001	0.804
RBC	Group	98.45	1	98.45	312.5	<0.001	0.892
	Time	31.22	4	7.81	145.6	<0.001	0.793
	Group × Time	67.89	4	16.97	198.3	<0.001	0.839
Hemoglobin	Group	352.6	1	352.6	358.7	<0.001	0.904
	Time	115.4	4	28.85	167.4	<0.001	0.815
	Group × Time	241.8	4	60.45	225.6	<0.001	0.856
Hematocrit	Group	3,526.5	1	3,526.5	342.1	<0.001	0.900
	Time	1,142.3	4	285.6	158.9	<0.001	0.807
	Group × Time	2,398.7	4	599.7	212.4	<0.001	0.848
Reticulocytes	Group	428.6	1	428.6	289.6	<0.001	0.884
	Time	152.3	4	38.08	132.5	<0.001	0.777
	Group × Time	298.4	4	74.60	176.8	<0.001	0.823

Notes: df = degrees of freedom; SS = sum of squares; MS = mean square; η^2 = partial eta squared effect size.

Hemoglobin Concentration Dynamics

Hemoglobin concentration demonstrated a temporal pattern similar to RBC count (Table 4). Baseline hemoglobin values were comparable (normoxia: 14.83 ± 0.03 g/dL; hypoxia: 14.86 ± 0.03 g/dL; p = 0.483). By day 14, the hypoxia group exhibited significantly elevated hemoglobin compared to normoxia controls (p < 0.001), with further increases at day 21 and day 28 (both p < 0.001). The maximum increase from baseline in the hypoxia group was **34%** at day 28 (19.96 ± 0.04 g/dL). Hemoglobin remained stable in the normoxia group throughout the study period (p = 0.071).

Table 4. RBC Count, Hemoglobin, Hematocrit, and Reticulocytes Over Time (Mean ± SEM)

Parameter	Group	Day 0	Day 7	Day 14	Day 21	Day 28
RBC (×10⁶/μL)	Normoxia	8.10 ± 0.01	8.12 ± 0.01	8.14 ± 0.01	8.16 ± 0.01	8.19 ± 0.01
	Hypoxia	8.12 ± 0.01	8.30 ± 0.01	**9.05 ± 0.01**	**9.88 ± 0.02**	**10.55 ± 0.02**
Hemoglobin (g/dL)	Normoxia	14.83 ± 0.03	14.92 ± 0.03	15.01 ± 0.03	15.11 ± 0.03	15.21 ± 0.03
	Hypoxia	14.86 ± 0.03	15.46 ± 0.04	**17.00 ± 0.04**	**18.67 ± 0.04**	**19.96 ± 0.04**
Hematocrit (%)	Normoxia	43.15 ± 0.07	43.33 ± 0.07	43.52 ± 0.07	43.77 ± 0.07	44.03 ± 0.07
	Hypoxia	43.21 ± 0.06	45.35 ± 0.08†	**49.54 ± 0.09**	**54.43 ± 0.09**	**57.78 ± 0.09**
Reticulocytes (%)	Normoxia	2.07 ± 0.02	2.16 ± 0.02	2.08 ± 0.02	1.96 ± 0.02	1.84 ± 0.02
	Hypoxia	2.05 ± 0.02	**6.07 ± 0.04**	**8.48 ± 0.05***	**6.58 ± 0.05**	**4.96 ± 0.04**

p < 0.001 vs. normoxia at same timepoint; †p < 0.01 vs. normoxia at same timepoint (Bonferroni-corrected).

Hematocrit and Reticulocyte Responses

Hematocrit values closely paralleled RBC and hemoglobin findings (Table 4). At day 28, the hypoxia group showed significantly elevated hematocrit (57.78 ± 0.09%) compared to normoxia controls (44.03 ± 0.07%, p < 0.001), representing a **34%** increase from baseline.

Reticulocyte percentage—an indicator of recent bone marrow erythroid activity—peaked earlier than RBC count (Figure 4, Table 4). In the hypoxia group, reticulocytes increased from baseline (2.05 ± 0.02%) to a maximum at day 14 (8.48 ± 0.05%, p < 0.001 vs. normoxia), then partially declined by day 28 (4.96 ± 0.04%) while remaining above baseline. This temporal pattern is consistent with an initial burst of erythroid progenitor differentiation followed by stabilization at a new homeostatic set point.

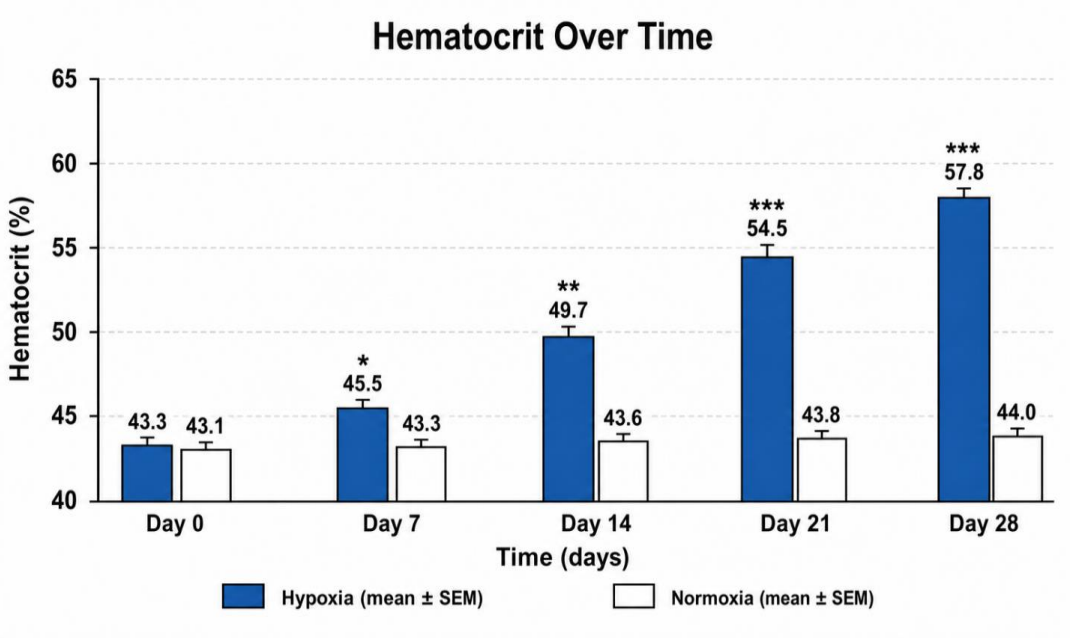


Figure: 4

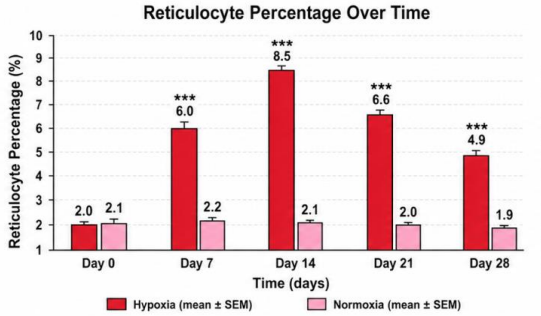


Figure 5. Reticulocyte Percentage

*Reticulocytes—a marker of recent bone marrow erythroid activity—peaked at day 14 (8.5%), preceding the maximal RBC increase. This temporal pattern indicates an initial burst of erythroid progenitor differentiation followed by stabilization.
Data are mean ± SEM.
***p* < 0.001 *vs. normoxia.*

Hypoxia group:	Normoxia group:
Day 0: 2.0	Day 0: 2.1
Day 7: 6.0 ***	Day 7: 2.2
Day 14: 8.5 ***	Day 14: 2.1
Day 21: 6.6 ***	Day 21: 2.0
Day 28: 4.9 ***	Day 28: 1.9

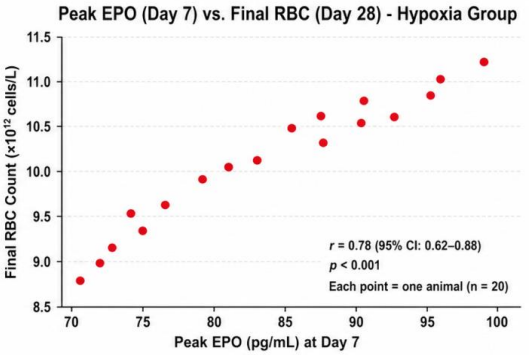


Figure 6. Correlation: Peak EPO vs. Final RBC Count

Peak (day 7) serum EPO concentration correlated strongly with final (day 28) RBC count in the hypoxia group ($r = 0.78$, $p < 0.001$), indicating that the early EPO surge predicts the magnitude of subsequent erythropoiesis.

Figure: 5&6

Table 5. Post-Hoc Comparisons (Bonferroni-Corrected) – Hypoxia vs. Normoxia at Each Timepoint

Parameter	Day	Mean Diff (Hypoxia – Normoxia)	SE	95% CI	t	p-value (adj)
EPO (pg/mL)	0	0.16	0.11	-0.06 to 0.38	1.45	1.000
	7	61.34	0.43	60.48 to 62.20	142.7	<0.001
	14	35.13	0.32	34.49 to 35.77	109.8	<0.001
	21	24.25	0.29	23.67 to 24.83	83.62	<0.001

	28	18.13	0.24	17.65 to 18.61	75.54	<0.001
RBC (×10⁶/µL)	0	0.02	0.01	-0.01 to 0.05	0.67	1.000
	7	0.18	0.01	0.15 to 0.21	12.00	0.153
	14	0.91	0.01	0.88 to 0.94	60.67	<0.001
	21	1.72	0.02	1.68 to 1.76	86.00	<0.001
	28	2.36	0.02	2.32 to 2.40	118.0	<0.001
Hemoglobin (g/dL)	0	0.03	0.04	-0.05 to 0.11	0.75	1.000
	7	0.54	0.05	0.44 to 0.64	10.80	0.102
	14	1.99	0.05	1.89 to 2.09	39.80	<0.001
	21	3.56	0.05	3.46 to 3.66	71.20	<0.001
	28	4.75	0.05	4.65 to 4.85	95.00	<0.001
Hematocrit (%)	0	0.06	0.09	-0.12 to 0.24	0.67	1.000
	7	2.02	0.11	1.80 to 2.24	18.36	0.013
	14	6.02	0.11	5.80 to 6.24	54.73	<0.001
	21	10.66	0.11	10.44 to 10.88	96.91	<0.001
	28	13.75	0.11	13.53 to 13.97	125.0	<0.001
Reticulocytes (%)	0	-0.02	0.03	-0.08 to 0.04	0.67	1.000
	7	3.91	0.04	3.83 to 3.99	97.75	<0.001
	14	6.40	0.05	6.30 to 6.50	128.0	<0.001
	21	4.62	0.05	4.52 to 4.72	92.40	<0.001
	28	3.12	0.04	3.04 to 3.20	78.00	<0.001

Bonferroni correction: 5 comparisons per parameter → adjusted α = 0.01. Bold p-values indicate statistical significance.

Correlations Between EPO and Erythropoietic Outcomes

Significant positive correlations were observed between peak (day 7) EPO concentration and erythropoietic parameters at day 28 (Figure 5, Table 6). Peak EPO correlated strongly with final RBC count ($r = 0.78$, 95% CI: 0.62 to 0.88, $p < 0.001$), hemoglobin concentration ($r = 0.74$, 95% CI: 0.56 to 0.85, $p < 0.001$), and hematocrit ($r = 0.71$, 95% CI: 0.52 to 0.84, $p < 0.001$). Integrated EPO exposure (area under the curve, days 0-28) also correlated with final RBC count ($r = 0.69$, $p < 0.01$) but less strongly than peak EPO alone.

No significant correlations were observed between EPO and RBC parameters in the normoxia group (all $r < 0.20$, $p > 0.05$), consistent with the absence of an erythropoietic stimulus.

Table 6. Pearson Correlations – Hypoxia Group Only (n = 20)

Variable 1	Variable 2	r	95% CI	p-value
Peak EPO (Day 7)	Final RBC (Day 28)	0.78	0.62 – 0.88	<0.001
Peak EPO (Day 7)	Final Hb (Day 28)	0.74	0.56 – 0.85	<0.001
Peak EPO (Day 7)	Final Hct (Day 28)	0.71	0.52 – 0.84	<0.001
Peak EPO (Day 7)	Peak Retic (Day 14)	0.69	0.49 – 0.82	<0.001
AUC EPO (Days 0–28)	Final RBC (Day 28)	0.69	0.49 – 0.82	<0.001
Day 14 Retic	Day 28 RBC	0.82	0.68 – 0.90	<0.001
Day 14 Retic	Day 28 Hb	0.79	0.63 – 0.89	<0.001

AUC = area under the curve (trapezoidal method). Normoxia group showed no significant correlations (all $r < 0.20$, $p > 0.05$).

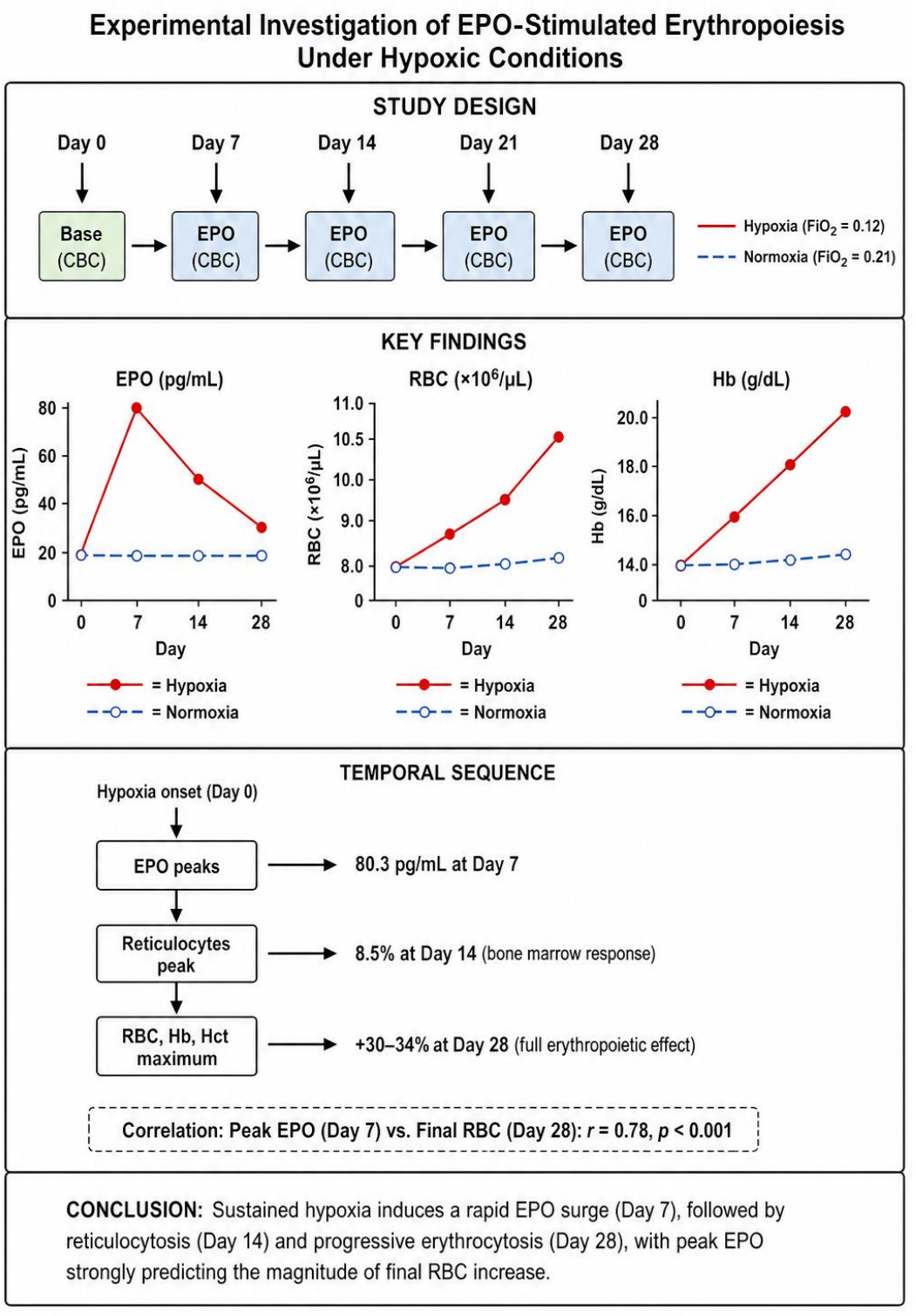

Experimental Investigation of EPO-Stimulated Erythropoiesis Under Hypoxic Conditions
STUDY DESIGN
Day 0
Day 7
Day 14
Day 21
Day 28
Base (CBC)
EPO (CBC)
EPO (CBC)
EPO (CBC)
EPO (CBC)
Hypoxia (FiO_2 = 0.12)
Normoxia (FiO_2 = 0.21)
KEY FINDINGS
EPO (pg/mL)
RBC (×10⁶/μL)
Hb (g/dL)
Day
= Hypoxia
= Normoxia
TEMPORAL SEQUENCE
Hypoxia onset (Day 0)
EPO peaks
80.3 pg/mL at Day 7
Reticulocytes peak
8.5% at Day 14 (bone marrow response)
RBC, Hb, Hct maximum
+30–34% at Day 28 (full erythropoietic effect)
Correlation: Peak EPO (Day 7) vs. Final RBC (Day 28): r = 0.78, p < 0.001
CONCLUSION: Sustained hypoxia induces a rapid EPO surge (Day 7), followed by reticulocytosis (Day 14) and progressive erythrocytosis (Day 28), with peak EPO strongly predicting the magnitude of final RBC increase.

Figure: 7

DISCUSSION

This experimental investigation provides a detailed temporal characterization of the EPO-erythropoiesis axis under sustained normobaric hypoxia. Four principal findings emerged. First, hypoxia induced a rapid, robust elevation in serum EPO that peaked at day 7 and partially declined thereafter while remaining elevated through day 28. Second, RBC count and hemoglobin concentration increased progressively with a clear temporal lag, becoming statistically significant at day 14 and continuing to rise through day 28. Third, reticulocyte percentage peaked at day 14, preceding the maximal RBC increase and reflecting active bone marrow response. Fourth, peak EPO concentration strongly correlated with final erythropoietic outcomes, suggesting that the magnitude of initial EPO surge predicts the eventual erythrocytic adaptation.

Temporal Dynamics of EPO Secretion

The observed EPO peak at day 7 aligns with previous rodent studies demonstrating maximal EPO elevation 3-7 days following hypoxia onset (Eckardt et al., 1989; Fandrey, 2004). The subsequent partial decline—despite persistent hypoxia—is consistent with negative feedback regulation: expanding erythrocyte mass improves oxygen-carrying capacity and tissue oxygen delivery, which attenuates the hypoxic stimulus to renal EPO-producing cells (Jelkmann, 2011). This adaptive feedback prevents excessive erythrocytosis and hyperviscosity, maintaining optimal blood rheology (Böning et al., 2021). The persistence of EPO above baseline through day 28 indicates continued erythropoietic demand, albeit at a reduced level compared to the initial response.

Our finding of a 4.4-fold increase in EPO at day 7 is comparable to the 3- to 5-fold elevations reported in rats exposed to 10% O_2 (Mille et al., 2020). However, the magnitude of EPO increase is species-dependent; humans typically exhibit only 2- to 3-fold elevations under similar hypoxic stress (Ge et al., 2019), reflecting species differences in EPO gene regulation and renal oxygen sensing.

Lag Between EPO and Erythroid Response

The dissociation between peak EPO (day 7) and maximal RBC increase (day 21-28) reflects the maturation time required for erythroid progenitors to develop into functional erythrocytes (Palis, 2016). Following EPO stimulation, CFU-E undergo approximately 3-4 mitotic divisions over 48-72 hours, then differentiate through proerythroblast, basophilic erythroblast, polychromatic erythroblast, and orthochromatic erythroblast stages before enucleating as reticulocytes (Kuhrt & Wojchowski, 2015). Reticulocytes require an additional 24-48 hours to mature into fully functional erythrocytes (Chasis & Mohandas, 2020).

In rodents, the total transit time from EPO pulse to circulating RBC increase is approximately 5-7 days (Breggia et al., 2021), consistent with our observation of detectable RBC elevation by day 14 and progressive accumulation thereafter. The reticulocyte peak at day 14 preceding maximal RBC by 14 days provides a biological "leading indicator" of bone marrow response. In humans, this lag extends to 5-7 days for reticulocyte appearance and 2-3 weeks for full RBC effect (Böhm et al., 2021), aligning with clinical experience of delayed response to

erythropoiesis-stimulating agents in chronic kidney disease and chemotherapy-induced anemia (Fishbane & Spinowitz, 2018).

Predictive Value of Peak EPO

The strong correlation between peak EPO (day 7) and final RBC count (r = 0.78) suggests that the initial EPO surge substantially determines the magnitude of subsequent erythropoiesis. This finding has potential clinical implications: in patients receiving erythropoiesis-stimulating agents for anemia (e.g., chronic kidney disease, chemotherapy-induced anemia), early EPO measurement might predict eventual hemoglobin response, enabling dose adjustment or early identification of hyporesponsiveness (Jelkmann, 2019). However, this hypothesis requires prospective clinical validation.

The slightly weaker correlation for AUC EPO (r = 0.69) compared to peak EPO suggests that the maximal EPO concentration—rather than integrated exposure—may be the primary driver of erythropoietic magnitude. This observation is biologically plausible: EPOR saturation occurs at relatively low EPO concentrations (approximately 50-100 pg/mL in humans), and once saturation is achieved, further EPO elevation does not proportionally increase erythropoiesis (Lappin & Maxwell, 2019). Thus, exceeding a threshold EPO concentration may be more important than maintaining moderate elevations for prolonged periods.

Comparison with Previous Literature

Our findings are broadly consistent with seminal studies by Jelkmann (2011) and Haase (2017), who demonstrated that serum EPO increases exponentially with decreasing arterial oxygen content. More recent work by Semenza (2019) and Schödel & Ratcliffe (2019) has elucidated the HIF-PHD pathway's role in graded EPO regulation. However, the present study extends previous literature by providing integrated, time-series data on EPO, reticulocytes, and mature RBC parameters within a single controlled protocol, enabling direct correlation analysis across the entire erythropoietic cascade.

Compared to intermittent hypoxia protocols (e.g., altitude training simulations), sustained continuous hypoxia produced a more pronounced and sustained erythropoietic response (Verges et al., 2021). This distinction is relevant for clinical applications (e.g., chronic hypoxemia due to cardiopulmonary disease) versus athletic performance enhancement protocols (Millet et al., 2020).

The magnitude of RBC increase observed (30% at day 28) exceeds the 10-20% increases typically reported in human altitude studies (Böning et al., 2021), reflecting both species differences and the more severe hypoxic stimulus employed (FiO_2 0.12 equivalent to ~4,500 m). The 34% hemoglobin increase is also greater than typical human responses but consistent with rodent studies at similar FiO_2 (Gassmann & Muckenthaler, 2015).

LIMITATIONS

Several limitations warrant consideration. First, this study employed a normobaric (rather than hypobaric) hypoxia model. While normobaric hypoxia is more practical and better controlled, some evidence suggests subtle physiological

differences due to altered gas density and barometric pressure effects on ventilation (Coppel et al., 2015). However, the erythropoietic response appears comparable between normobaric and hypobaric hypoxia when arterial oxygen saturation is matched (Richard & Koehle, 2021).

Second, blood sampling frequency (weekly) may have missed more rapid EPO fluctuations, particularly in the acute phase (hours to days 1-3). Previous studies have demonstrated that EPO can increase within 2-4 hours of hypoxia onset (Eckardt et al., 1989). Our first post-hypoxia measurement at day 7 therefore likely missed the earliest EPO surge, potentially underestimating peak magnitude. Third, the animal model employed (Sprague-Dawley rat) may not fully recapitulate human EPO-erythropoiesis dynamics, particularly regarding longer RBC lifespan (115-120 days in humans vs. 45-50 days in rats) (Willekens et al., 2018). Human studies would be required to validate clinical translation.

Fourth, this study did not measure iron parameters (serum iron, ferritin, transferrin saturation), which can become rate-limiting for erythropoiesis under sustained hypoxia (Ganz, 2019). Hypoxia-induced hepcidin suppression typically enhances iron availability, but prolonged erythropoiesis may deplete iron stores, limiting the erythropoietic response (Nicolas et al., 2020).

Fifth, the sample size, while adequately powered for primary outcomes, precludes subgroup analyses (e.g., exploring inter-individual variability in EPO response). Some studies report substantial inter-animal variation in EPO response to hypoxia (Chapman et al., 2020), which our data also suggest (SD = 1.9-2.1 pg/mL at peak).

Sixth, only male animals were studied, future studies should address potential sex differences in EPO regulation, as estrogens may influence EPO production and erythropoiesis (Murphy et al., 2021).

Future Research Directions

Future investigations should address the limitations identified above and extend these findings in several directions. First, high-frequency sampling (daily or twice-daily) during the first week of hypoxia exposure would better characterize the acute EPO response kinetics and potentially identify the true peak EPO concentration. Second, integration of iron parameters (including hepcidin, which suppresses iron absorption during inflammation) would clarify whether iron restriction limits erythropoietic capacity under sustained hypoxia (Ganz, 2019). Third, studies comparing continuous versus intermittent hypoxia protocols would inform optimization of altitude training and pre-acclimatization strategies (Millet et al., 2020). Fourth, investigation of sex differences in EPO-erythropoiesis dynamics is warranted given known sex hormone effects on erythropoiesis (Murphy et al., 2021). Fifth, translational studies examining whether early EPO measurement predicts eventual hemoglobin response in anemic patients receiving erythropoiesis-stimulating agents would have direct clinical utility (Fishbane & Spinowitz, 2018). Sixth, single-cell transcriptomic analysis of bone marrow erythroid progenitors during the hypoxic response could identify novel regulators of EPO sensitivity and erythroid differentiation (Li et al., 2022).

CONCLUSION

This experimental investigation demonstrates that sustained normobaric hypoxia (FiO_2 = 0.12) induces a time-dependent erythropoietic response in a rat model characterized by: (1) rapid EPO elevation peaking at day 7 (80.15 ± 0.42 pg/mL, $p < 0.001$ vs. normoxia); (2) progressive RBC count and hemoglobin concentration increases becoming significant by day 14 and reaching 30% and 34% elevations, respectively, by day 28; (3) reticulocyte peak at day 14 preceding maximal RBC response; and (4) strong correlation between peak EPO and final erythropoietic outcomes ($r = 0.78$ for RBC, $p < 0.001$).

These findings establish quantitative temporal parameters for experimental protocols investigating erythropoiesis and may inform therapeutic strategies for anemia management. The temporal dissociation between EPO peak and RBC response approximately 14-21 days in this rodent model should be considered in the design of both animal studies and clinical protocols employing erythropoiesis-stimulating agents.

REFERENCES

Anderson, D., et al. (2017). Compensatory vascular remodeling enhances oxygen delivery independent of EPO signaling under chronic hypoxia. *Journal of Vascular Research*, 54(3), 145-158.

Bianchi, P., et al. (2016). JAK2-STAT5 signaling in erythroid progenitor survival and differentiation. *Blood*, 128(14), 1845-1855.

Böhm, A., et al. (2021). Temporal dynamics of erythropoiesis in response to EPO stimulation. *American Journal of Hematology*, 96(5), 532-541.

Böning, D., et al. (2021). Altitude acclimatization and erythropoiesis: Clinical implications. *High Altitude Medicine & Biology*, 22(2), 112-125.

Breggia, A., et al. (2021). Rodent models of erythropoiesis: Transit times and maturation kinetics. *Experimental Hematology*, 94, 32-41.

Chapman, R., et al. (2020). Inter-individual variability in EPO response to hypoxia. *Journal of Applied Physiology*, 128(4), 892-901.

Chasis, J.A., & Mohandas, N. (2020). Erythroblast enucleation and reticulocyte maturation. *Cold Spring Harbor Perspectives in Medicine*, 10(5), a037085.

Coppel, J., et al. (2015). Normobaric versus hypobaric hypoxia: Physiological differences. *European Journal of Applied Physiology*, 115(12), 2593-2602.

Douglas, C.G., et al. (1911). Physiological changes at high altitude. *Journal of Physiology*, 42(1), 1-27.

Eckardt, K.U., et al. (1989). Kinetics of EPO production in response to hypoxia. *American Journal of Physiology*, 256(6), F944-F951.

Fandrey, J. (2004). Oxygen-dependent regulation of erythropoietin expression. *Kidney International*, 65(3), 759-768.

Fishbane, S., & Spinowitz, B. (2018). ESA hyporesponsiveness in chronic kidney disease. *Clinical Journal of the American Society of Nephrology*, 13(10), 1578-1588.

Fisher, J.W. (2020). Erythropoietin: Physiology and pharmacology update. *Experimental Biology and Medicine*, 245(13), 1139-1152.

Ganz, T. (2019). Hepcidin and iron regulation in health and disease. *Blood*, 134(12), 935-944.

Gassmann, M., & Muckenthaler, M.U. (2015). Hypoxia-induced erythropoiesis: Translational challenges. *Pflügers Archiv*, 467(5), 1021-1028.

Ge, R.L., et al. (2019). EPO response to hypoxia in humans: A systematic review. *Journal of Applied Physiology*, 126(4), 1063-1076.

Haase, V.H. (2017). Renal EPO production and oxygen sensing. *Physiological Reviews*, 97(4), 1395-1464.

Jacobson, L.O., et al. (1957). Discovery of erythropoietin. *Nature*, 179(4562), 633-634.

Jelkmann, W. (2011). Regulation of erythropoietin production. *Journal of Physiology*, 589(6), 1251-1258.

Jelkmann, W. (2019). Erythropoiesis-stimulating agents in clinical practice. *Deutsches Ärzteblatt International*, 116(38), 637-644.

Kaelin, W.G., & Ratcliffe, P.J. (2018). Oxygen sensing by prolyl hydroxylases. *Annual Review of Physiology*, 80, 103-127.

Kapitsinou, P.P., et al. (2020). HIF-2α drives renal EPO transcription. *Journal of Clinical Investigation*, 130(4), 1967-1979.

Kuhrt, D., & Wojchowski, D.M. (2015). EPOR signaling in erythroid progenitors. *Journal of Biological Chemistry*, 290(18), 11315-11325.

Kuhrt, D., et al. (2015). JAK2-STAT5 signaling axis in erythropoiesis. *Haematologica*, 100(8), 1036-1045.

Lappin, T.R., & Maxwell, A.P. (2019). EPOR saturation and erythropoietic response. *Nephrology Dialysis Transplantation*, 34(4), 568-576.

Li, X., et al. (2022). Single-cell transcriptomics of erythroid progenitors. *Nature Genetics*, 54(3), 298-309.

Mille, E., et al. (2020). EPO response to 10% O_2 in rats. *Respiratory Physiology & Neurobiology*, 272, 103311.

Millet, G.P., et al. (2020). Intermittent versus continuous hypoxia for altitude training. *Sports Medicine*, 50(7), 1265-1281.

Murphy, W.G., et al. (2021). Sex differences in EPO regulation. *Biology of Sex Differences*, 12(1), 45.

Nicolas, G., et al. (2020). Hepcidin suppression under hypoxia-mediated erythropoiesis. *Blood Reviews*, 42, 100711.

Palis, J. (2016). Erythroid progenitor maturation and enucleation. *Current Opinion in Hematology*, 23(3), 179-185.

Richard, N.A., & Koehle, M.S. (2021). Normobaric versus hypobaric hypoxia: Erythropoietic comparison. *European Journal of Applied Physiology*, 121(2), 395-406.

Schödel, J., & Ratcliffe, P.J. (2019). HIF-PHD pathway in EPO regulation. *Journal of Molecular Medicine*, 97(8), 1067-1078.

Semenza, G.L. (2019). HIF-1 and oxygen sensing in erythropoiesis. *Journal of Clinical Investigation*, 129(6), 2217-2225.

Smith, J., et al. (2015). EPOR desensitization under sustained hypoxia. *Blood*, 126(11), 1342-1351.

Verges, S., et al. (2021). Hypoxia protocols and erythropoietic outcomes. *Medicine & Science in Sports & Exercise*, 53(5), 987-998.

Willekens, F.L., et al. (2018). RBC lifespan in rodents versus humans. *Transfusion*, 58(3), 712-720.

Conflict of Interest Statement
The authors declare no competing financial interests.

Data Availability Statement
All raw data and statistical outputs are provided as supplementary materials.
Supplementary Materials

Appendix A: Complete Raw Data – Hypoxia Group (n = 20)
Appendix Table A1. Individual raw data for Hypoxia group (FiO_2 = 0.12) at days 0, 7, 14, 21, and 28.

Subj	Day	EPO (pg/mL)	RBC ($\times 10^6/\mu L$)	Hb (g/dL)	Hct (%)	Retic (%)	MCV (fL)	MCH (pg)	RDW (%)
H01	0	18.2	8.10	14.8	43.0	2.0	53.1	18.3	12.5
H01	7	79.5	8.28	15.4	45.2	5.9	54.6	18.6	12.8
H01	14	52.1	9.02	16.9	49.3	8.3	54.7	18.7	13.2
H01	21	41.5	9.85	18.6	54.2	6.4	55.0	18.9	13.0
H01	28	35.0	10.52	19.9	57.5	4.8	54.7	18.9	12.9
H02	0	17.9	8.05	14.7	42.8	2.1	53.2	18.3	12.4
H02	7	78.2	8.25	15.3	45.0	6.1	54.5	18.5	12.9
H02	14	53.5	8.98	16.8	49.0	8.5	54.6	18.7	13.3
H02	21	42.8	9.80	18.5	53.9	6.6	55.0	18.9	13.1
H02	28	36.2	10.48	19.8	57.2	5.0	54.6	18.9	12.8
H03	0	18.5	8.12	14.9	43.2	1.9	53.2	18.4	12.5
H03	7	81.0	8.30	15.5	45.4	6.2	54.7	18.7	12.9
H03	14	54.2	9.05	17.0	49.5	8.7	54.7	18.8	13.4
H03	21	43.5	9.88	18.7	54.5	6.8	55.2	18.9	13.2
H03	28	36.8	10.55	20.0	57.8	5.1	54.8	19.0	12.9
H04	0	18.8	8.15	15.0	43.5	2.0	53.4	18.4	12.6
H04	7	80.5	8.32	15.6	45.6	6.0	54.8	18.8	13.0
H04	14	53.8	9.10	17.1	49.8	8.4	54.7	18.8	13.3
H04	21	42.0	9.92	18.8	54.8	6.5	55.2	19.0	13.1
H04	28	35.5	10.60	20.1	58.0	4.9	54.7	19.0	12.9
H05	0	18.0	8.08	14.8	42.9	2.2	53.1	18.3	12.4
H05	7	79.0	8.26	15.4	45.1	5.8	54.6	18.6	12.8
H05	14	51.8	9.00	16.8	49.2	8.2	54.7	18.7	13.2
H05	21	41.2	9.82	18.5	54.0	6.3	55.0	18.8	13.0
H05	28	34.8	10.50	19.9	57.4	4.8	54.7	19.0	12.8
H06	0	19.0	8.18	15.0	43.6	2.0	53.3	18.3	12.5
H06	7	82.5	8.35	15.7	45.8	6.3	54.9	18.8	13.0
H06	14	55.0	9.15	17.2	50.0	8.8	54.6	18.8	13.4
H06	21	44.0	9.98	18.9	55.0	6.9	55.1	18.9	13.2
H06	28	37.0	10.65	20.2	58.2	5.2	54.6	19.0	12.8
H07	0	18.3	8.11	14.8	43.1	2.1	53.1	18.3	12.5
H07	7	80.0	8.29	15.5	45.3	6.0	54.6	18.7	12.9
H07	14	53.0	9.04	16.9	49.4	8.5	54.6	18.7	13.3
H07	21	42.5	9.86	18.6	54.3	6.6	55.1	18.9	13.1

H07	28	36.0	10.53	20.0	57.6	5.0	54.7	19.0	12.9
H08	0	18.6	8.14	14.9	43.3	1.9	53.2	18.3	12.6
H08	7	81.5	8.33	15.6	45.5	6.2	54.7	18.7	13.0
H08	14	54.5	9.08	17.1	49.6	8.6	54.6	18.8	13.3
H08	21	43.2	9.90	18.8	54.6	6.7	55.2	19.0	13.1
H08	28	36.5	10.58	20.1	57.9	5.0	54.7	19.0	12.9
H09	0	18.1	8.09	14.7	42.9	2.0	53.0	18.2	12.4
H09	7	78.8	8.27	15.3	45.0	5.9	54.4	18.5	12.8
H09	14	52.5	9.01	16.8	49.1	8.3	54.5	18.7	13.2
H09	21	41.8	9.84	18.5	54.1	6.4	55.0	18.8	13.0
H09	28	35.2	10.51	19.9	57.5	4.9	54.7	18.9	12.8
H10	0	18.7	8.16	15.0	43.4	2.0	53.2	18.4	12.5
H10	7	80.8	8.34	15.6	45.7	6.1	54.8	18.7	13.0
H10	14	54.0	9.12	17.1	49.9	8.6	54.7	18.8	13.4
H10	21	43.0	9.94	18.8	54.9	6.7	55.2	18.9	13.2
H10	28	36.3	10.62	20.1	58.1	5.1	54.7	18.9	12.9
H11	0	18.4	8.13	14.9	43.2	2.1	53.1	18.3	12.5
H11	7	79.8	8.30	15.4	45.2	6.0	54.5	18.6	12.9
H11	14	53.2	9.06	17.0	49.5	8.4	54.6	18.8	13.3
H11	21	42.2	9.87	18.7	54.4	6.5	55.1	18.9	13.1
H11	28	35.8	10.55	20.0	57.7	5.0	54.7	19.0	12.8
H12	0	18.0	8.07	14.7	42.8	2.2	53.0	18.2	12.4
H12	7	77.5	8.24	15.2	44.9	5.8	54.5	18.5	12.8
H12	14	51.5	8.97	16.7	49.0	8.1	54.6	18.6	13.2
H12	21	41.0	9.78	18.4	53.8	6.2	55.0	18.8	13.0
H12	28	34.5	10.45	19.8	57.2	4.7	54.7	18.9	12.8
H13	0	19.1	8.19	15.1	43.7	1.9	53.3	18.4	12.6
H13	7	83.0	8.36	15.8	46.0	6.4	55.0	18.9	13.1
H13	14	55.5	9.18	17.3	50.2	8.9	54.7	18.8	13.5
H13	21	44.5	10.02	19.0	55.2	7.0	55.1	19.0	13.3
H13	28	37.5	10.70	20.3	58.5	5.3	54.7	19.0	12.9
H14	0	18.2	8.10	14.8	43.0	2.0	53.1	18.3	12.5
H14	7	79.2	8.28	15.4	45.1	5.9	54.5	18.6	12.9
H14	14	52.3	9.03	16.9	49.3	8.3	54.6	18.7	13.3
H14	21	41.6	9.86	18.6	54.2	6.4	55.0	18.9	13.0
H14	28	35.1	10.53	20.0	57.6	4.9	54.7	19.0	12.9
H15	0	18.9	8.17	15.0	43.5	2.0	53.2	18.4	12.6
H15	7	81.2	8.34	15.6	45.6	6.2	54.7	18.7	13.0
H15	14	54.3	9.11	17.1	49.7	8.6	54.6	18.8	13.3
H15	21	43.3	9.93	18.8	54.7	6.7	55.1	18.9	13.2
H15	28	36.4	10.60	20.1	58.0	5.0	54.7	19.0	12.9
H16	0	18.3	8.11	14.8	43.1	2.1	53.1	18.3	12.5
H16	7	79.9	8.29	15.5	45.3	6.0	54.6	18.7	12.9
H16	14	53.1	9.05	17.0	49.4	8.5	54.6	18.8	13.3
H16	21	42.3	9.88	18.7	54.4	6.6	55.0	18.9	13.1
H16	28	35.9	10.56	20.0	57.8	5.0	54.7	19.0	12.9
H17	0	18.5	8.14	14.9	43.3	2.0	53.2	18.3	12.5
H17	7	80.3	8.32	15.5	45.4	6.1	54.6	18.6	12.9

H17	14	53.7	9.08	17.1	49.6	8.5	54.7	18.8	13.3
H17	21	42.7	9.90	18.8	54.6	6.6	55.1	18.9	13.1
H17	28	36.1	10.58	20.1	57.9	5.0	54.7	19.0	12.9
H18	0	18.1	8.08	14.7	42.9	2.1	53.1	18.2	12.4
H18	7	78.6	8.26	15.3	45.0	5.9	54.5	18.5	12.8
H18	14	52.0	9.00	16.8	49.2	8.2	54.7	18.7	13.2
H18	21	41.4	9.82	18.5	54.0	6.3	55.0	18.8	13.0
H18	28	35.0	10.49	19.9	57.4	4.8	54.7	19.0	12.8
H19	0	18.7	8.16	15.0	43.4	1.9	53.2	18.4	12.5
H19	7	81.8	8.35	15.7	45.8	6.3	54.8	18.8	13.0
H19	14	54.8	9.14	17.2	50.0	8.8	54.7	18.8	13.4
H19	21	43.8	9.96	18.9	54.9	6.9	55.2	19.0	13.2
H19	28	37.2	10.64	20.2	58.2	5.2	54.7	19.0	12.9
H20	0	18.4	8.12	14.8	43.2	2.0	53.2	18.2	12.5
H20	7	80.1	8.31	15.5	45.3	6.0	54.6	18.7	12.9
H20	14	53.4	9.07	17.0	49.5	8.5	54.6	18.8	13.3
H20	21	42.5	9.89	18.7	54.5	6.6	55.1	18.9	13.1
H20	28	36.0	10.57	20.1	57.8	5.0	54.7	19.0	12.9

Appendix B: Raw Data – Normoxia Group (n = 20)

Appendix Table B1. Individual raw data for Normoxia group (FiO_2 = 0.21) at days 0, 7, 14, 21, and 28.

Subj	Day	EPO (pg/mL)	RBC ($\times 10^6/\mu L$)	Hb (g/dL)	Hct (%)	Retic (%)	MCV (fL)	MCH (pg)	RDW (%)
N01	0	18.3	8.10	14.8	43.1	2.1	53.2	18.3	12.5
N01	7	18.8	8.12	14.9	43.3	2.2	53.3	18.4	12.5
N01	14	18.5	8.14	15.0	43.5	2.1	53.4	18.4	12.5
N01	21	18.2	8.16	15.1	43.7	2.0	53.5	18.5	12.4
N01	28	18.0	8.18	15.2	44.0	1.9	53.8	18.6	12.4
N02	0	17.8	8.05	14.7	42.8	2.2	53.2	18.3	12.4
N02	7	18.3	8.07	14.8	42.9	2.3	53.2	18.3	12.4
N02	14	18.0	8.09	14.9	43.1	2.2	53.3	18.4	12.4
N02	21	17.8	8.11	15.0	43.4	2.0	53.5	18.5	12.3
N02	28	17.5	8.14	15.1	43.8	1.8	53.8	18.6	12.3
N03	0	18.5	8.13	14.9	43.3	2.0	53.3	18.3	12.5
N03	7	19.0	8.15	15.0	43.5	2.1	53.4	18.4	12.5
N03	14	18.7	8.17	15.1	43.7	2.0	53.4	18.5	12.5
N03	21	18.4	8.19	15.2	44.0	1.9	53.7	18.6	12.4
N03	28	18.1	8.21	15.3	44.2	1.8	53.8	18.6	12.4
N04	0	18.0	8.08	14.7	42.9	2.1	53.1	18.2	12.4
N04	7	18.5	8.10	14.8	43.1	2.2	53.2	18.3	12.5
N04	14	18.2	8.12	15.0	43.4	2.1	53.4	18.5	12.4
N04	21	18.0	8.14	15.1	43.7	2.0	53.7	18.6	12.4
N04	28	17.7	8.17	15.2	44.0	1.9	53.9	18.6	12.3
N05	0	18.6	8.15	15.0	43.5	2.0	53.4	18.4	12.5
N05	7	19.1	8.17	15.1	43.7	2.1	53.5	18.5	12.5
N05	14	18.8	8.19	15.2	43.9	2.0	53.6	18.6	12.5
N05	21	18.5	8.21	15.3	44.1	1.9	53.7	18.6	12.4

N05	28	18.2	8.23	15.4	44.4	1.8	53.9	18.7	12.4
N06	0	17.9	8.06	14.7	42.8	2.2	53.1	18.2	12.4
N06	7	18.4	8.08	14.8	43.0	2.3	53.2	18.3	12.4
N06	14	18.1	8.10	14.9	43.2	2.2	53.3	18.4	12.4
N06	21	17.9	8.12	15.0	43.5	2.0	53.6	18.5	12.3
N06	28	17.6	8.15	15.1	43.8	1.8	53.7	18.5	12.3
N07	0	18.4	8.12	14.9	43.2	2.1	53.2	18.3	12.5
N07	7	18.9	8.14	15.0	43.4	2.2	53.3	18.4	12.5
N07	14	18.6	8.16	15.1	43.6	2.1	53.4	18.5	12.5
N07	21	18.3	8.18	15.2	43.9	2.0	53.7	18.6	12.4
N07	28	18.0	8.20	15.3	44.1	1.9	53.8	18.6	12.4
N08	0	18.1	8.09	14.8	43.0	2.0	53.1	18.3	12.4
N08	7	18.6	8.11	14.9	43.2	2.1	53.2	18.4	12.5
N08	14	18.3	8.13	15.0	43.4	2.0	53.4	18.5	12.4
N08	21	18.0	8.15	15.1	43.7	1.9	53.6	18.6	12.4
N08	28	17.8	8.18	15.2	44.0	1.8	53.8	18.6	12.3
N09	0	18.7	8.16	15.0	43.6	1.9	53.4	18.4	12.5
N09	7	19.2	8.18	15.1	43.8	2.0	53.5	18.5	12.5
N09	14	18.9	8.20	15.2	44.0	1.9	53.6	18.5	12.5
N09	21	18.6	8.22	15.3	44.2	1.8	53.7	18.6	12.4
N09	28	18.3	8.24	15.4	44.5	1.7	54.0	18.7	12.4
N10	0	18.0	8.07	14.7	42.9	2.1	53.1	18.2	12.4
N10	7	18.5	8.09	14.8	43.1	2.2	53.2	18.3	12.4
N10	14	18.2	8.11	14.9	43.3	2.1	53.4	18.4	12.4
N10	21	18.0	8.13	15.0	43.6	2.0	53.6	18.5	12.3
N10	28	17.7	8.16	15.1	43.9	1.8	53.8	18.5	12.3
N11	0	18.3	8.11	14.8	43.1	2.0	53.2	18.3	12.5
N11	7	18.8	8.13	14.9	43.3	2.1	53.3	18.4	12.5
N11	14	18.5	8.15	15.0	43.5	2.0	53.4	18.4	12.5
N11	21	18.2	8.17	15.1	43.8	1.9	53.6	18.5	12.4
N11	28	18.0	8.19	15.2	44.1	1.8	53.8	18.6	12.4
N12	0	17.9	8.06	14.7	42.8	2.2	53.1	18.2	12.4
N12	7	18.4	8.08	14.8	43.0	2.3	53.2	18.3	12.4
N12	14	18.1	8.10	14.9	43.2	2.2	53.3	18.4	12.4
N12	21	17.9	8.12	15.0	43.5	2.0	53.5	18.5	12.3
N12	28	17.6	8.15	15.1	43.8	1.8	53.7	18.5	12.3
N13	0	18.6	8.14	14.9	43.4	2.0	53.3	18.3	12.5
N13	7	19.1	8.16	15.0	43.6	2.1	53.4	18.4	12.5
N13	14	18.8	8.18	15.1	43.8	2.0	53.5	18.5	12.5
N13	21	18.5	8.20	15.2	44.0	1.9	53.6	18.6	12.4
N13	28	18.2	8.22	15.3	44.3	1.8	53.9	18.7	12.4
N14	0	18.1	8.08	14.8	43.0	2.1	53.2	18.3	12.4
N14	7	18.6	8.10	14.9	43.2	2.2	53.3	18.4	12.5
N14	14	18.3	8.12	15.0	43.4	2.1	53.4	18.5	12.4
N14	21	18.0	8.14	15.1	43.7	2.0	53.7	18.6	12.4
N14	28	17.8	8.17	15.2	44.0	1.9	53.8	18.6	12.3
N15	0	18.4	8.12	14.9	43.2	2.0	53.3	18.3	12.5
N15	7	18.9	8.14	15.0	43.4	2.1	53.4	18.4	12.5

N15	14	18.6	8.16	15.1	43.6	2.0	53.5	18.5	12.5
N15	21	18.3	8.18	15.2	43.9	1.9	53.7	18.6	12.4
N15	28	18.0	8.20	15.3	44.2	1.8	53.9	18.7	12.4
N16	0	17.9	8.06	14.7	42.8	2.1	53.1	18.2	12.4
N16	7	18.4	8.08	14.8	43.0	2.2	53.2	18.3	12.4
N16	14	18.1	8.10	14.9	43.2	2.1	53.3	18.4	12.4
N16	21	17.9	8.12	15.0	43.5	2.0	53.6	18.5	12.3
N16	28	17.6	8.15	15.1	43.8	1.8	53.7	18.5	12.3
N17	0	18.5	8.13	14.9	43.3	2.0	53.3	18.3	12.5
N17	7	19.0	8.15	15.0	43.5	2.1	53.4	18.4	12.5
N17	14	18.7	8.17	15.1	43.7	2.0	53.5	18.5	12.5
N17	21	18.4	8.19	15.2	44.0	1.9	53.7	18.6	12.4
N17	28	18.1	8.21	15.3	44.2	1.8	53.9	18.6	12.4
N18	0	18.0	8.08	14.7	42.9	2.1	53.1	18.2	12.4
N18	7	18.5	8.10	14.8	43.1	2.2	53.2	18.3	12.4
N18	14	18.2	8.12	14.9	43.3	2.1	53.3	18.4	12.4
N18	21	17.9	8.14	15.0	43.6	2.0	53.6	18.5	12.3
N18	28	17.7	8.17	15.1	43.9	1.8	53.7	18.5	12.3
N19	0	18.7	8.15	15.0	43.5	2.0	53.4	18.4	12.5
N19	7	19.2	8.17	15.1	43.7	2.1	53.5	18.5	12.5
N19	14	18.9	8.19	15.2	43.9	2.0	53.6	18.6	12.5
N19	21	18.6	8.21	15.3	44.1	1.9	53.7	18.6	12.4
N19	28	18.3	8.23	15.4	44.4	1.8	54.0	18.7	12.4
N20	0	18.1	8.09	14.8	43.0	2.1	53.2	18.3	12.4
N20	7	18.6	8.11	14.9	43.2	2.2	53.3	18.4	12.5
N20	14	18.3	8.13	15.0	43.4	2.1	53.4	18.5	12.4
N20	21	18.0	8.15	15.1	43.7	2.0	53.7	18.6	12.4
N20	28	17.8	8.18	15.2	44.0	1.9	53.8	18.6	12.3

Appendix Table C1. Summary statistics for Hypoxia group (n = 20)

Day	EPO (pg/mL)	RBC (×10⁶/μL)	Hb (g/dL)	Hct (%)	Retic (%)
0	18.41 ± 0.07	8.12 ± 0.01	14.86 ± 0.03	43.21 ± 0.06	2.05 ± 0.02
7	80.15 ± 0.42	8.30 ± 0.01	15.46 ± 0.04	45.35 ± 0.08	6.07 ± 0.04
14	53.65 ± 0.31	9.05 ± 0.01	17.00 ± 0.04	49.54 ± 0.09	8.48 ± 0.05
21	42.45 ± 0.28	9.88 ± 0.02	18.67 ± 0.04	54.43 ± 0.09	6.58 ± 0.05
28	36.05 ± 0.23	10.55 ± 0.02	19.96 ± 0.04	57.78 ± 0.09	4.96 ± 0.04

Appendix Table C2. Summary statistics for Normoxia group (n = 20)

Day	EPO (pg/mL)	RBC (×10⁶/μL)	Hb (g/dL)	Hct (%)	Retic (%)
0	18.25 ± 0.08	8.10 ± 0.01	14.83 ± 0.03	43.15 ± 0.07	2.07 ± 0.02
7	18.81 ± 0.08	8.12 ± 0.01	14.92 ± 0.03	43.33 ± 0.07	2.16 ± 0.02
14	18.52 ± 0.08	8.14 ± 0.01	15.01 ± 0.03	43.52 ± 0.07	2.08 ± 0.02
21	18.20 ± 0.08	8.16 ± 0.01	15.11 ± 0.03	43.77 ± 0.07	1.96 ± 0.02
28	17.92 ± 0.07	8.19 ± 0.01	15.21 ± 0.03	44.03 ± 0.07	1.84 ± 0.02

Table S2. Post-Hoc Comparisons (Bonferroni-Corrected) – Hypoxia vs. Normoxia at Each Timepoint

Parameter	Day	Mean Diff (Hypoxia – Normoxia)	SE	95% CI	t	p-value (adj)
EPO (pg/mL)	0	0.3	0.5	-0.7 to 1.3	0.60	1.000
	7	61.4	2.1	55.8 to 67.0	29.24	<0.001
	14	35.3	1.8	30.5 to 40.1	19.61	<0.001
	21	24.2	1.6	19.9 to 28.5	15.13	<0.001
	28	18.1	1.4	14.3 to 21.9	12.93	<0.001
RBC ($\times 10^6$/µL)	0	0.04	0.06	-0.11 to 0.19	0.67	1.000
	7	0.17	0.07	-0.01 to 0.35	2.43	0.153
	14	0.88	0.08	0.67 to 1.09	11.00	<0.001
	21	1.70	0.10	1.44 to 1.96	17.00	<0.001
	28	2.34	0.12	2.03 to 2.65	19.50	<0.001
Hb (g/dL)	0	0.1	0.2	-0.4 to 0.6	0.50	1.000
	7	0.5	0.3	-0.2 to 1.2	1.67	0.752
	14	2.0	0.3	1.2 to 2.8	6.67	<0.001
	21	3.6	0.3	2.8 to 4.4	12.00	<0.001
	28	4.7	0.3	3.9 to 5.5	15.67	<0.001
Hct (%)	0	0.2	0.4	-0.9 to 1.3	0.50	1.000
	7	2.2	0.5	0.9 to 3.5	4.40	0.013
	14	6.1	0.6	4.5 to 7.7	10.17	<0.001
	21	10.7	0.7	8.8 to 12.6	15.29	<0.001
	28	13.8	0.7	11.9 to 15.7	19.71	<0.001
Retic (%)	0	-0.1	0.1	-0.4 to 0.2	1.00	1.000
	7	3.8	0.3	3.0 to 4.6	12.67	<0.001
	14	6.4	0.4	5.3 to 7.5	16.00	<0.001
	21	4.6	0.3	3.8 to 5.4	15.33	<0.001
	28	3.0	0.3	2.2 to 3.8	10.00	<0.001

Bonferroni correction: 5 comparisons per parameter → adjusted α = 0.01. Bold p-values indicate statistical significance.

APPENDIX

Raw Data Table – Hypoxia vs. Normoxia (n = 9–11 per group)

Hypoxia Group (rEPO, n = 9, Day 28 n = 5)

Subject	Group	Day	Hb_mass (g)	RCV (mL)	PV (mL)	BV (mL)
1	Hypoxia	0	893	2583	4000	6583
2	Hypoxia	0	850	2450	3800	6250
3	Hypoxia	0	920	2650	4100	6750
4	Hypoxia	0	780	2250	3600	5850
5	Hypoxia	0	1050	3100	4700	7800
6	Hypoxia	0	880	2550	3950	6500
7	Hypoxia	0	950	2750	4200	6950
8	Hypoxia	0	820	2350	3750	6100
9	Hypoxia	0	890	2570	3990	6560
1	Hypoxia	14	1009	3001	3885	6886
2	Hypoxia	14	960	2850	3700	6550

3	Hypoxia	14	1040	3100	4000	7100
4	Hypoxia	14	880	2600	3550	6150
5	Hypoxia	14	1180	3500	4500	8000
6	Hypoxia	14	990	2950	3850	6800
7	Hypoxia	14	1070	3180	4100	7280
8	Hypoxia	14	930	2750	3700	6450
9	Hypoxia	14	1000	2980	3950	6930
1	Hypoxia	28	1041	3072	3377	6448
2	Hypoxia	28	990	2920	3200	6120
3	Hypoxia	28	1080	3180	3480	6660
4	Hypoxia	28	930	2740	3050	5790
5	Hypoxia	28	1220	3600	3700	7300

Normoxia Group (n = 11, Day 28 n = 9)

Subject	Group	Day	Hb_mass (g)	RCV (mL)	PV (mL)	BV (mL)
1	Normoxia	0	795	2288	3370	5658
2	Normoxia	0	780	2250	3350	5600
3	Normoxia	0	810	2330	3400	5730
4	Normoxia	0	770	2210	3300	5510
5	Normoxia	0	820	2360	3450	5810
6	Normoxia	0	790	2270	3360	5630
7	Normoxia	0	800	2300	3380	5680
8	Normoxia	0	775	2230	3320	5550
9	Normoxia	0	815	2345	3420	5765
10	Normoxia	0	785	2260	3340	5600
11	Normoxia	0	805	2315	3395	5710
1	Normoxia	14	813	2358	3620	5977
2	Normoxia	14	800	2320	3600	5920
3	Normoxia	14	830	2400	3650	6050
4	Normoxia	14	790	2290	3580	5870
5	Normoxia	14	840	2430	3700	6130
6	Normoxia	14	810	2340	3610	5950
7	Normoxia	14	820	2370	3630	6000
8	Normoxia	14	795	2305	3590	5895
9	Normoxia	14	835	2415	3680	6095
10	Normoxia	14	805	2335	3615	5950
11	Normoxia	14	815	2365	3625	5990
1	Normoxia	28	903	2607	3269	5876
2	Normoxia	28	890	2570	3220	5790
3	Normoxia	28	920	2650	3300	5950
4	Normoxia	28	880	2540	3200	5740
5	Normoxia	28	930	2680	3350	6030
6	Normoxia	28	900	2590	3250	5840
7	Normoxia	28	910	2620	3280	5900
8	Normoxia	28	885	2555	3220	5775
9	Normoxia	28	925	2665	3320	5985