The acute effects of simulated hypoxic training at different altitudes on oxidative stress and muscle damage in elite long-distance runners (#109713)

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The acute effects of simulated hypoxic training at different altitudes on oxidative stress and muscle damage in elite long-distance runners

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The objective of this study was to examine the effects of exercise at varying altitudes on oxidative stress and muscle damage. A total of twelve elite long-distance runners (mean age: 20.3 ± 1.5 years) from different branches participated in the study. The exercise protocol was the Bruce submaximal treadmill exercise test, which was conducted under three simulated hypoxic conditions (1700 m, 2450 m, and 3200 m) and one normoxic condition (sea level). All measurements took place at the same time of the day. After the exercise protocol, 5 ml venous blood samples were taken from the participants. Significant differences were observed in total oxidant status (TOS, p=0.017), malondialdehyde (MDA, p<0.001), total antioxidant status (TAS, p<0.001), and the oxidative stress index (OSI, p<0.001) across different altitudes. However, no significant difference was found in creatine kinase (CK, p=0.059) levels. Additionally, there were significant differences in the oxygen saturation measurement taken at the 3rd (p<0.001), 6th (p<0.001), 9th (p<0.001), and 12th (p<0.001) minutes following the exercise session. There was no difference in the pulse measurement taken at the 3rd and 12th minutes, but a difference was observed at the 6th and 9th minutes post-exercise (p<0.01). In conclusion, the study determined that exercises performed in a hypoxic environment at different altitudes

increased TAS and reduced OSI in elite long-distance runners.

The Acute Effects of Simulated Hypoxic Training at Different Altitudes on Oxidative Stress and Muscle Damage in Elite Long-Distance Runners

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Abstract

Background. The objective of this study was to examine the effects of exercise at varying altitudes on oxidative stress and muscle damage.

Methods. A total of twelve elite long-distance runners (mean age: 20.3 ± 1.5 years) from different branches participated in the study. The exercise protocol was the Bruce submaximal treadmill exercise test, which was conducted under three simulated hypoxic conditions (1700 m, 2450 m, and 3200 m) and one normoxic condition (sea level). All measurements took place at the same time of the day. After the exercise protocol, 5 ml venous blood samples were taken from the participants.

Results: Significant differences were observed in total oxidant status (TOS, $p=0.017$), malondialdehyde (MDA, $p<0.001$), total antioxidant status (TAS, $p<0.001$), and the oxidative stress index (OSI, $p<0.001$) across different altitudes. However, no significant difference was found in creatine kinase (CK, $p=0.059$) levels. Additionally, there were significant differences in the oxygen saturation measurement taken at the 3rd ($p<0.001$), 6th ($p<0.001$), 9th ($p<0.001$), and 12th ($p<0.001$), minutes following the exercise session. There was no difference in the pulse measurement taken at the 3rd and 12th minutes, but a difference was observed at the 6th and 9th minutes post-exercise ($p<0.01$).

Conclusions. In conclusion, the study determined that exercises per-formed in a hypoxic environment at different altitudes increased TAS and reduced OSI in elite long-distance runners.

Introduction

Exercise's metabolic effects and physiological responses largely depend on the biochemical reactions occurring in muscle cells. However, the physiological stress induced by exercise can also lead to side effects such as muscle damage and oxidative stress (Powers & Jackson, 2008; Quindry

et al., 2016). Oxidative stress has long been a hotspot of exercise-based research. In recent years, intensive research has been conducted to understand oxidative stress and the physiological responses of exercise under different conditions, including high altitude (Quindry et al., 2016; Li et al., 2024). Exercise adaptations to altitude increase with altitude, starting at approximately 1000–1500 m (Miller et al., 2013; Lukanova-Jakubowska et al., 2022). High-altitude adaptation is driven by various physiological and molecular mechanisms in response to hypoxia. The body compensates for reduced oxygen availability by increasing breathing and heart rate, while boosting erythrocyte production to enhance oxygen transport. On a molecular level, transcription factors like HIF-1 regulate energy metabolism and trigger antioxidant defenses to combat oxidative stress. These adaptations are crucial for both enhancing physical performance and preventing altitude-related illnesses (Mallet et al., 2023; Vignati et al., 2023). Current knowledge about overexertion and oxidative stress at high altitudes is generally derived from a limited number of field studies. Alongside field studies, well-controlled laboratory studies on exercise performance and blood biomarker changes in humans artificially exposed to hypoxia also contribute significantly to this field (Gore, Clark & Saunders, 2007; Millet et al., 2010; Millet, Faiss & Pialoux, 2012; Karayigit et al., 2022).

Exposure to high altitude, which is associated with decreased oxygen pressure, can cause oxidative/reductive stress, increased formation of reactive oxygen and nitrogen species (RONS), and associated oxidative damage to lipids, proteins, and DNA (Dosek et al., 2007; Powers & Jackson, 2008). Various RONS-producing systems are activated during high-altitude exposure, including the mitochondrial electron transport chain, xanthine oxidase, and nitric oxide synthase. High altitude appears to weaken both enzymatic and non-enzymatic antioxidant systems (Chao et al., 1999; Radak et al., 2014). The pattern of oxidative damage associated with high altitude

exposure is similar to ischemia/reperfusion injury. This adaptive process to the oxidative challenge requires a relatively long period (Cadenas, 2018; Zhang et al., 2019; Mallet et al., 2023). Physical exercise or increased physical activity at high altitudes may exacerbate the extent of oxidative threat (Maiti et al., 2006). The fact that acute exercise induces oxidative cell damage and contributes to systemic oxidative stress was first established by Dillard et al. (1978). Up-to-date investigators have clearly shown that acute exercise training increases oxidative stress levels predominantly with the skeletal muscle and blood (Powers, Smuder & Criswell, 2011; Powers, Radak & Ji, 2016). In this context, it is important to examine the effects of exercise performed at different altitudes on creatine kinase (CK) levels and oxidative stress. Different altitudes may have different effects on exercise performance and physiological responses (Wilber, 2007; Girard et al., 2020). The changing oxygen levels at different altitudes can cause changes in levels of muscle damage and oxidative stress. This study aims to investigate the acute effects of exercises performed at different altitudes on CK levels and oxidative stress in elite long-distance runners.

2. Materials and Methods

2.1 Participants

The study participants were 12 long-distance athletes between the ages of 20-24 years, residing in Van province of Turkey, at an altitude of 1700 m. Before the study, participants' health (health history questionnaire) and activity were assessed. All participants (1) were elite long-distance runners, (2) were free from chronic illness, (3) were free from musculoskeletal injuries for at least 6 months before the study, and (4) had no history of acute mountain sickness. Participants were informed about the risks, discomforts, and benefits associated with the study. Thereafter, all participants received detailed information about the study design, measurements, and procedures

and were required to give a written informed consent form. The procedures followed the Helsinki Declaration of 1975, as revised in 2000, and approval was received from the Ethics Committee of the local university.

Twelve long-distance athletes participated in the study (age: 20.3 ± 1.5 years; body weight: 63.5 ± 15.2 kg; height: 171.8 ± 7.9 cm; body mass index: 21.4 ± 4.3; resting heart rate: 49.0 ± 3.0 bpm). To define the high-level status of the athletes in this study, we used the IAAF scoring tables to evaluate their performance based on season-best results from 2022 to 2023. This method provided an objective assessment of performance level, categorizing athletes as 'low,' 'medium,' or 'high' performers based on their scores. The use of IAAF scores ensured that only athletes reaching national and international competitive standards were classified as high-level (Spiriev, 2017).

2.2 Study design

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Van Yüzüncü Yıl University (protocol code TYD-2021-9553 and date of approval 15 May 2021). Participants in the study exercised under four different conditions: normoxic conditions (sea level), and simulated altitudes of 1700 m, 2450 m, and 3200 m. No acclimatization was required as the participants had been living at an altitude of 1700 m for an extended period. This study was conducted using a single-blind, balanced, randomized, and crossover design. Measurements were conducted on individuals at sea level to establish a baseline. This baseline was considered sufficient to observe potential differences at higher altitudes. Separate pre-test measurements were not taken at each altitude; therefore, sea level data served as the comparison baseline.

A quantitative method was used in the research, and the research model followed a single-group, two-treatment time series (temporarily applied) design, which is one of the quasi-experimental research models (Gliner, Morgan & Leech, 2017). To determine the necessary sample size within the scope of this research, a power analysis was conducted based on values recommended in the literature. Accordingly, using G*Power for Repeated Measures ANOVA (1-β power = 80%, alpha = 0.05, effect size = 0.40, correlation value = 0.7), it was determined that 8 participants would be needed (Cohen, 1992). Additionally, Suresh and Chandrashekara, (2012) suggest increasing the sample size by approximately 10% to account for potential withdrawals or missing data. Consequently, 12 athletes were included in this study. In this context, 12 athletes were included in the study. Each exercise session at a different altitude was separated by at least 7 days. The exercise protocol was the Bruce submaximal treadmill exercise test protocol under the four simulated hypoxic conditions (1700 m, 2450 m, and 3200 m) and normoxic conditions (sea level). Considering that excessive hypoxia (severe, > 3000 m) can cause adverse health effects such as high-altitude sickness, the levels of hypoxia selected in our study were chosen based on safety and practical reasons. This approach ensured that adequate metabolic stress was induced while minimizing the risk to participants (Millet et al., 2016; Strom et al., 2018; Burtscher et al., 2022).

Prior to the study, participants provided detailed information about their alcohol, caffeine, and smoking habits, as well as their use of medications and ergogenic aids, through a specially designed structured form aimed at assessing lifestyle factors that could influence oxidative stress levels. Additionally, participants were instructed to follow a standardized diet initiated three days before the first measurement, consisting of balanced meals with controlled portions of carbohydrates, proteins, and fats. This diet aimed to control the effects of consumed foods on

antioxidant levels, thereby preventing any distortion of the study data. To ensure consistency, each measurement was conducted at three-day intervals and at the same time throughout the study. All exercise sessions were conducted in a Hypoxico Everest Summit II Altitude Generator, with temperature and relative humidity maintained at 20°C and 40%, respectively. The physical and physiological characteristics of the study groups are shown in Table 1

.

--- INSERT TABLE 1 ABOUT HERE ---

2.3 Measurements

Before the exercise tests, a 15-minute classical warm-up protocol was applied under normoxic conditions. After the warm-up, the Bruce submaximal treadmill exercise test protocol was applied (Strom et al., 2018). The warm-up consisted of 5 minutes of low-intensity jogging at approximately 50-60% of maximum heart rate to increase heart rate and body temperature. This was followed by 5 minutes of dynamic stretching targeting key muscle groups involved in treadmill running, such as the quadriceps, hamstrings, calves, and gluteal muscles. The final 5 minutes included mobility drills like high knees and leg swings to enhance joint mobility and neuromuscular activation. At the end of Bruce's submaximal treadmill exercise protocol, a 5 mL venous blood sample was taken from the participants.

2.3.1 Bruce submaximal treadmill exercise test

In the 1st stage, participants walked for 3 minutes at a speed of 1.7 mph with a 10% incline. In the 2nd stage, they walked for 3 minutes at a speed of 2.5 mph with a 12% incline. In the 3rd stage, the exercise continued for 3 minutes at a speed of 3.4 mph with a 14% incline. In the 4th and final

stage, participants walked for another 3 minutes at a speed of 4.2 mph with a 16% incline. The exercise protocol was kept constant for each measurement day, and the ambient altitude was set in four different ways: 1st measurement at sea level (normoxic conditions), 2nd measurement at 1700 m, 3rd measurement at 2450 m, and 4th measurement at 3200 m. A Hypoxico Everest Summit II Altitude Generator was used to adjust the ambient partial pressure according to these altitudes (Harwood, Wright & Burnet, 2022). The Hypoxico Everest Summit II Altitude Generator simulates high-altitude conditions by lowering the oxygen concentration in the air using a filtration system. It mimics altitudes up to 8,848 meters, making it ideal for altitude training and research on physiological adaptations to hypoxia, such as enhanced endurance and reduced oxygen availability. Additionally, the athletes' oxygen saturation levels and heart rate variables were monitored under the supervision of a doctor throughout the exercise. This allowed for continuous real-time monitoring of physiological responses to ensure participant safety and the accuracy of data collection. To measure oxygen saturation and heart rate, the Masimo Radical-7 Pulse Oximeter and Polar H10 heart rate monitor were used. The Masimo Radical-7 is an advanced, clinically-approved pulse oximeter with an SpO2 accuracy of ± 2% for values between 70% and 100%. It can also provide accurate results even under low perfusion conditions. The Polar H10 is a chest-strap heart rate monitor that provides highly accurate ECG data.

2.3.2 Blood sampling and assays

A 5 mL venous blood sample was collected from the participants' antecubital veins immediately after performing the Bruce submaximal treadmill exercise test protocol under normoxic conditions, as well as at 1700 m, 2450 m, and 3200 m altitudes. Blood samples were centrifuged at 3600 rpm at + 4° C for 5 min and then their serum was separated. Serum samples were stored at

-20º C until analyzed. Blood samples were analyzed at Van YYÜ Dursun Odabaş Medical Center. Then, the levels of CK-MM and oxidative stress markers such as malondialdehyde (MDA), total antioxidant status (TAS), and total oxidant status (TOS) were determined for each subject. CK-MM (Detection range 62.5–4000 pg/mL, sensitivity: 18.9 pg/mL, intra-assay CV: < 8%, inter-assay CV: < 10%) and MDA (range 0.3mmol/L-7mmol/L, sensitivity: 0.01mmol/L, intra-assay CV: < 7%, inter-assay CV: < 19%) levels in serum were measured using the ELISA method and TAS and TOS levels were measured using a new automatic colorimetric measurement method developed by Erel, (2005). This method determines TOS by measuring the oxidation of ferrous ion to ferric ion in the presence of oxidant molecules, with the ferric ion then forming a colored complex with xylenol orange. The color intensity is proportional to the total oxidant molecules in the sample, allowing for an accurate assessment of oxidative status (Erel, 2005). The increase in antioxidant molecules in response to the increase in oxidative molecules may evaluate TOS measurement alone in terms of oxidative damage misleading. By comparing the change in the TOS/TAS ratio, an accurate comparison is possible by eliminating the antioxidant reactive response that may be misleading for oxidative damage. The importance of the Oxidative stress index (OSI) is that it shows the general balance of oxidative stress and antioxidant status in a two-way manner by disabling reactive increases (Sırmatel et al., 2007). OSI was calculated using the TOS/TAS ratio. The selection of these parameters - heart rate, blood oxygen saturation, and four serum markers (TAS, TOS, MDA, CK) - was based on their established reliability in the literature for assessing oxidative stress and muscle damage under hypoxic conditions (Brancaccio, Maffulli & Limongelli, 2007; Mazzeo, 2008; Kaschina et al., 2011; Kong et al., 2022). Heart rate and oxygen saturation directly monitor cardiovascular and respiratory responses, essential for evaluating hypoxic adaptation (Richalet, Hermand & Lhuissier, 2024). TAS and TOS measure

224 oxidative and antioxidative balance, while MDA and CK-MM serve as key indicators of lipid
225 peroxidation, cellular damage, and muscle response to exercise (Spirlandeli, Deminice & Jordao,
226 2014; Janion et al., 2022).
227
228 *2.3.3 Statistical analysis*
229 Data analysis was done using the SPSS 25 package program. The normality of the data was
230 checked with the Kolmogorov-Smirnov and Shapiro-Wilk tests and the homogeneity with the
231 Levene test. A repeated measures ANOVA was used to compare tests applied at different times.
232 In cases where the sphericity assumption was not met, the Greenhouse-Geisser correction was
233 used. For values with significant differences as a result of repeated measurements, the Bonferroni
234 Post Hoc test was used to determine which data group caused the difference. The significance level
235 for analysis was evaluated as $p<0.05$. For altitudes where a significant difference was observed,
236 Cohen's d was calculated, presented with 95% confidence intervals, and classified as small (d =
237 0.2), medium (d = 0.5), and large (d ≥ 0.8) (Cohen, 1988).
238
239 **3. Results**
240 *3.1 Oxidative stress markers and creatin kinase*
241 Table 2 summarizes the results of TAS, TOS, MDA, OSI, and CK levels across different altitudes.
242 TAS levels were significantly different between the altitudes ($p<0.001$). TAS levels were
243 significantly higher in 1700 m, 2450, and 3200 m compared to the normoxia condition ($p<0.005$,
244 d= 1.72 [0.78-2.65], $p<0.001$, d= 2.94 [1.78-4.09], $p<0.001$, d=4.17 [2.74-5.60] respectively).
245 Additionally, 3200 m had significantly higher TAS level compared to 1700 m and 2450 m
246 ($p<0.001$, d= 1.66 [0.73-2.59], $p<0.05$; d= 1.0 [0.15-1.85] respectively). For the TOS levels the

only significant difference was detected between 2450 m and normoxic condition ($p<0.05$, d= 1.99 [1.01-2.97]. MDA levels showed significant differences across the altitudes ($p<0.001$). MDA levels were higher at 2450 m and 3200 m compared to the normoxic condition $p<0.001$, d= 1.49 [0.58-2.39], $p<0.005$, d=2.99 [1.82-4.15] respectively). OSI levels were significantly different between the altitudes ($p<0.001$). OSI was significantly higher under normoxic condition compared to 1700 m, 2450 m, and 3200 m ($p<0.001$, d= 1.54 [0.63-2.45], $p<0.001$, d= 4.10 [2.69-5.51], $p<0.001$, d=4.61 [3.08-6.14] respectively) (Figure 1). CK levels showed no differences between the altitudes ($p>0.05$) (Figure 2).

--- INSERT TABLE 2 ABOUT HERE ---

--- INSERT FIGURE 2 ABOUT HERE ---

3.2 Variations in oxygen saturation across different intervals

Table 3 summarizes the variations in oxygen saturation across different intervals and altitudes. Oxygen saturation (OS) exhibited significant differences between altitudes at the 3-minute measurement point ($p < 0.001$). OS was significantly lower in 2450, and 3200 m compared to the normoxic condition ($p<0.001$, d= 3.32 [2.08-4.55], $p<0.001$, d= 3.97 [2.59-3.35] respectively). Additionally, OS was significantly lower at 2450 m compared to 1700 m ($p<0.005$, d= 2.48 [1.41-3.54] and, 3200 m had significantly lower OS level compared to 1700 m and 2450 m ($p<0.001$, d= 3.58 [2.29-4.87], $p<0.001$; d= 2.27 [1.24-3.30] respectively). OS showed significant differences between altitudes at the 6-minute measurement point ($p < 0.001$). OS was significantly lower in 2450, and 3200 m compared to the normoxic condition ($p<0.001$, d= 2.16 [1.15-3.17], $p<0.001$, d= 5.91 [4.05-7.76] respectively). Additionally, OS was significantly lower at 2450 m compared

to 1700 m (p<0.001, d= 1.85 [0.89-2.80] and, 3200 m had significantly lower OS level compared to 1700 m and 2450 m (p<0.001, d= 5.63 [3.84-7.41], p<0.001; d= 3.21 [2.00-4.43] respectively). OS showed significant differences between altitudes at the 9-minute measurement point (p < 0.001). OS was significantly lower in 2450, and 3200 m compared to the normoxic condition (p<0.001, d= 2.90 [1.75-4.05], p<0.001, d= 6.42 [4.43-8.40] respectively). Additionally, OS was significantly lower at 2450 m compared to 1700 m (p<0.001, d= 2.38 [1.34-3.43] and, 3200 m had significantly lower OS level compared to 1700 m and 2450 m (p<0.001, d= 5.99 [4.11-7.86], p<0.001; d= 3.77 [2.44-5.11] respectively). Oxygen saturation (OS) exhibited significant differences between altitudes at the 12-minute measurement point (p<0.001). OS was significantly lower in 2450, and 3200 m compared to the normoxic condition (p<0.001, d= 2.50 [1.43-3.57], p<0.001, d= 5.42 [3.69-7.15] respectively). Additionally, OS was significantly lower at 2450 m compared to 1700 m (p<0.001, d= 2.38 [1.33-3.43] and, 3200 m had significantly lower OS level compared to 1700 m and 2450 m (p<0.001, d= 5.28 [3.58-6.97], p<0.001; d= 2.34 [1.30-3.37] respectively) (Figure 3).

--- INSERT TABLE 3 ABOUT HERE ---

--- INSERT FIGURE 3 ABOUT HERE ---

3.3 Changes in heart rate (beat per minute) at different altitudes

Table 4 summarizes the changes in heart rate (beat per minute) at different altitudes. Heart rate (HR) exhibited significant differences between altitudes at the 3-minute measurement point (p<0.001). HR was significantly higher in 1700 m, 2450, and 3200 m compared to the normoxic condition (p<0.001, d= 2.66 [1.56-3.76], p<0.005, d= 1.51 [0.60-2.42], p<0.001, d= 2.84 [1.71-

3.98] respectively). Heart rate (HR) was significantly different between altitudes at the 6-minute measurement point ($p<0.001$). HR was significantly higher in 1700 m, 2450, and 3200 m compared to the normoxic condition ($p<0.001$, d= 4.13 [2.72-5.55], $p<0.001$, d= 2.17 [1.16-3.18], $p<0.001$, d= 5.60 [3.82-7.38] respectively). Also, HR was significantly higher in 3200 m compared to 1700 m and 2450 m ($p<0.001$, d= 1.31 [0.42-2.19], $p<0.001$; d= 1.43 [0.54-2.33] respectively). Heart rate (HR) was significantly different between altitudes at the 9-minute measurement point ($p<0.001$). HR was significantly higher in 1700 m, 2450, and 3200 m compared to the normoxic condition ($p<0.001$, d= 4.83 [3.25-6.42], $p<0.001$, d= 7.73 [5.40-10.06], $p<0.001$, d= 6.03 [4.15-7.92] respectively). HR was significantly lower at 1700 m compared to 2450 and 3200 m ($p<0.005$, d= 0.17 [-0.62-0.97], $p<0.001$; d= 1.23 [0.35-2.10] respectively). Also, HR was significantly higher in 3200 m compared to and 2450 m ($p<0.005$, d= 1.27 [0.39-2.14]). A significant difference was observed only between the normoxic condition and the 3200 m altitude at the 12-minute heart rate measurement point ($p<0.001$, d= 1.68 [0.75-2.61) (Figure 4).

--- INSERT TABLE 4 ABOUT HERE ---

--- INSERT FIGURE 4 ABOUT HERE ---

4. Discussion

While high altitude can change the oxidative stress response in different simulated altitudes, this study was conducted to determine how oxidative stress is affected under exercise conditions. Determining the relationship between muscle damage and high altitude will provide important information for athletes living and exercising at high altitudes, for athletes and coaches performing high altitude camps, and for optimizing training methods and recovery processes. The main

findings of the present study were that there were no differences in TOS, MDA, and CK variables. However, a significant difference was found in TAS and OSI variables. Creatine kinase (CK) levels might not change significantly in this study's context for several reasons, despite varying exercise intensities and altitudes. First, CK levels are known to reflect muscle damage mainly when exercises involve eccentric contractions or when the muscle activity is unusually intense or prolonged, leading to substantial muscle fiber stress and breakdown. However, submaximal treadmill protocols, like the Bruce protocol used here, typically exert relatively less eccentric stress on muscles, especially in trained athletes whose muscles are conditioned to such workloads. Consequently, CK elevations might remain minimal as muscle integrity is better preserved compared to more intense or eccentric exercises (e.g., downhill running or maximal resistance exercises) (Clarkson & Hubal, 2002; Latham et al., 2008). Additionally, long-distance runners, who formed the sample group, often develop a degree of physiological adaptation to frequent high-intensity training. These adaptations reduce CK response to exercise by enhancing muscle repair mechanisms and limiting muscle fiber damage under conditions that might otherwise elevate CK in untrained individuals. This resilience in elite athletes can attenuate CK fluctuations even when exercise is performed at higher altitudes, where muscle oxygenation might be more compromised (Brancaccio, Maffulli & Limongelli, 2007; Scalco et al., 2016). The possible lack of significant changes in MDA levels can be explained by various factors. Oxidative stress occurring during high-altitude exercise might be controlled by adaptive antioxidant defense mechanisms, which are often highly developed in elite endurance athletes. These adaptations increase resilience to oxidative stress and can prevent notable elevations in lipid peroxidation markers like MDA. Since MDA typically rises in response to heightened cellular damage, the robust antioxidant systems in such athletes might limit oxidative damage and, consequently, help stabilize MDA levels (Pialoux

et al., 2006; Furian, Tannheimer & Burtscher, 2022). Additionally, low-oxygen environments reduce muscle oxygenation, which can further trigger oxidative stress. However, endurance athletes who are adapted to high altitudes can maintain cellular homeostasis by upregulating antioxidant enzyme activities, effectively minimizing oxidative damage. This adaptive process helps counteract the harmful effects of free radicals and restricts shifts in MDA levels (Chapman, 2013).

León-López et al. (2018) investigated the effects of sea level and high-altitude training on oxidative stress and antioxidant enzyme activities in professional swimmers. They reported that high-altitude training led to an increase in markers of oxidative stress, such as protein oxidation (AOPP) and MDA, indicating elevated lipid peroxidation. However, they observed a concomitant increase in antioxidant enzyme activities, suggesting a compensatory response to alleviate oxidative damage. Similarly, Belviranlı et al. (2017) examined the effect of high-altitude training on oxidative stress and antioxidant defense markers in pentathlon athletes. They reported that altitude training increased oxidative stress assessed by MDA, a marker of lipid peroxidation, and non-enzymatic antioxidant levels measured by reduced glutathione (GSH). At the same time, it did not affect enzymatic antioxidant levels assessed by superoxide dismutase (SOD) activity.

Dosek et al. (2007) stated that oxidative stress, especially when induced by hypobaric hypoxia, causes structural changes and cellular damage in lipids, proteins, and DNA. Debevec, Millet and Pialoux, (2017) examined hypoxia-induced oxidative stress modeling with physical activity. They found that hypobaric hypoxia induces oxidative stress, as indicated by changes in oxidative stress markers. However, they also observed an upregulation of antioxidant defense mechanisms, suggesting an adaptive response to counteract oxidative damage. The results of our study show that TAS increases in direct proportion to altitude, while OSI decreases. This may be

362 due to the decrease in oxygen availability caused by low atmospheric pressure (Sinex & Chapman,
363 2015). This lack of oxygen triggers a physiological response in the body known as hypoxia. The
364 body adapts to hypoxic conditions by increasing antioxidant production to counteract oxidative
365 stress, which is caused by an imbalance between free radicals and antioxidants. Exercise-induced
366 oxidative stress occurs during physical activity because metabolic processes produce free radicals.

367 Regular exercise at higher altitudes can lead to adaptations in the body's antioxidant
368 defense system, resulting in increased TOS and decreased OSI. Collectively, findings from these
369 studies (Dosek et al., 2007; Belviranlı et al., 2017; Debevec, Millet & Pialoux, 2017; León-López
370 et al., 2018; Elmas & Elmas, 2020) suggest that high-altitude exercise can induce oxidative stress,
371 as evidenced by changes in oxidative stress markers such as MDA. However, it is important to
372 note that these changes are often accompanied by a simultaneous upregulation of antioxidant
373 defense mechanisms, reflected by increased antioxidant enzyme activities and enhanced TAS.
374 These adaptive responses may contribute to the restoration of redox balance and the overall
375 improvement in athletic performance observed in athletes performing high-altitude exercises. It is
376 worth noting that the interaction between oxidative stress and performance improvements in high-
377 altitude exercise requires further investigation. The exact mechanisms underlying the relationship
378 between oxidative stress, antioxidant responses, and athletic performance adaptations in response
379 to high-altitude exercise have not been fully elucidated. Future studies should focus on elucidating
380 the signaling pathways involved in adaptation to oxidative stress and identifying strategies to
381 optimize the antioxidant defense system during high-altitude exercise.

382 In terms of saturation measurements, a significant difference was found between the 3rd,
383 6th, 9th, and 12th min. Many studies have examined the relationship between high-altitude
384 exercise and oxygen saturation levels. Chapman et al. (2011) investigated changes in oxygen

saturation during high-altitude endurance training. They observed that as athletes train at higher altitudes, oxygen saturation levels tend to decrease due to reduced oxygen availability. However, Chapman et al. (2016) indicated that athletes can adapt to high altitudes through acclimatization and adaptation processes. These adaptations include improvements in oxygen-carrying capacity and enhanced oxygen utilization efficiency. As a result, athletes may experience reduced declines in oxygen saturation levels over time as they adapt to the challenges posed by high-altitude training.

Additionally, Siebenmann et al. (2012) conducted a study examining the effects of high-altitude training on oxygen saturation and performance in endurance athletes. Initially, exposure to high altitude led to a decrease in oxygen saturation, among athletes. However, with continued training and acclimatization, athletes experienced increased saturation values and improvements in performance. The result of our study indicated that saturation values decrease with increasing altitude. This decrease may be attributed to reduced ambient oxygen availability during high-altitude exercises, with saturation values often referred to as oxygen saturation. Oxygen saturation is defined as the percentage of hemoglobin in the blood that is carrying oxygen (Collins et al., 2015). As individuals ascend to higher altitudes, the partial pressure of oxygen decreases, leading to a reduction in oxygen saturation levels. It is important to note that responses to high-altitude exercise and saturation levels can vary significantly among individuals due to factors such as genetic predisposition, fitness level, training altitude, and acclimatization time (Siebenmann et al., 2012; Elmas & Elmas, 2020). Furthermore, altitude-related conditions such as acute mountain sickness or high-altitude pulmonary edema can further affect saturation levels.

Regarding heart rate measurements, was observed in pulse values taken at the 3rd 6th 9th and 12th min. Bahenský and Grosicki (2021) investigated the effects of high-altitude training on

heart rate variability (HRV) in young athletes. The authors reported that high altitude training led to changes in HRV and autonomic modulation of heart rate. Specifically, they observed a decrease in parasympathetic activity and an increase in sympathetic activity, indicating a general shift towards sympathetic dominance. These changes in HRV may reflect adaptations in cardiovascular regulation in response to the hypoxic environment at high altitudes. Saunders et al. (2009) investigated the heart rate response to submaximal exercise at different altitudes in elite runners. They found that as altitude increased, there was a progressive increase in heart rate during submaximal exercise. This elevated heart rate response can be attributed to the reduced availability of oxygen at higher altitudes, leading to an increased sympathetic drive as an attempt to compensate for the reduced oxygen supply. These findings suggest that heart rate may serve as an indicator of the physiological strain imposed by high-altitude exercise. Similarly, Chapman et al. (2014) conducted a study examining the effects of altitude training on heart rate in athletes. They observed an increase in heart rate during exercise at higher altitudes compared to sea level. Additionally, they noted that heart rate recovery after exercise was slower at higher altitudes, indicating a prolonged sympathetic response. These findings further support the idea that high-altitude exercise causes changes in heart rate dynamics, potentially due to hypoxic stress imposed on the cardiovascular system.

Javaloyes et al. (2021) investigated HRV in trained cyclists during altitude training. They reported changes in HRV patterns, with decreases in both time domain and frequency domain measurements of HRV. These changes indicate a decrease in para-sympathetic activity and an altered sympathovagal balance. They suggested that these adaptations in heart rate dynamics reflect the body's physiological response to hypoxic stress and the increased demand on the cardiovascular system during high-altitude exercise. These studies demonstrate that high-altitude

exercise elicits specific heart rate responses in athletes. The results of our study show that as altitude increases, pulse values also increase. Observed increases in heart rate during exercise, along with changes in HRV, indicate adaptations in sympathetic and parasympathetic cardiovascular control mechanisms. This may be due to reduced oxygen availability and the physiological adjustments required to meet the increased oxygen demand during high-altitude exercise. Heart rate responses observed during high-altitude exercises are important indicators of the physiological strain and adaptation processes that occur in athletes training at high altitudes Bonato, Goodman and Tjh (2023). Monitoring heart rate during training sessions at different altitudes can provide valuable information about cardiovascular responses and help optimize training protocols for athletes in high-altitude environments (Yu et al., 2022; Feng et al., 2023).

4.1. Limitations

This study has certain limitations. First, all participants underwent the four altitude conditions in a fixed order, from sea level to the highest altitude. The lack of a randomized or counterbalanced order may introduce potential order and training effects, which could have been minimized with a random order. However, our study aimed to observe altitude adaptation in a natural progression. Future studies may benefit from employing a randomized order to mitigate these potential effects. Additionally, only a select set of biomarkers (TAS, TOS, MDA, CK) were used to assess oxidative stress and muscle damage. These biomarkers were chosen based on their reliability and relevance to hypoxic conditions; however, a broader range of markers, such as inflammatory or metabolic indicators, could provide a more comprehensive view of the physiological responses. Future research could include additional biomarkers to expand the understanding of hypoxic adaptation. Furthermore, participants in this study resided at an altitude of 1700 m, which may have influenced

their physiological responses compared to those at lower altitudes. To improve generalizability, future studies could consider recruiting participants residing at sea level or lower altitudes.

4.2 Future perspectives

Changing oxygen levels at altitudes causes muscle damage and changes in oxidative stress levels. In the conclusion of the study, it was determined that exercises performed in a hypoxic environment at different altitudes increased the TAS and reduced the OSI in athletes. However, the adaptation response and subsequent recovery processes that occur after high-altitude exposure appear to contribute to enhanced antioxidant defense systems. These findings emphasize the importance of maintaining redox balance during high altitude training and provide insight into the mechanisms underlying the physiological adaptations observed in athletes. However, there is a need for more comprehensive studies on the subject, especially on the molecular changes that occur in this process, with different protocols, taking into account performance markers.

5. Conclusion

In conclusion, it was determined that exercises performed in a hypoxic environment at different altitudes increased TAS and reduced the OSI in elite long-distance runners. These findings suggest that altitude training not only elicits specific cardiovascular adaptations but also enhances the body's antioxidant defense mechanisms, thereby mitigating oxidative stress. This dual benefit underscores the potential of high-altitude training to improve athletic performance and overall physiological resilience. Monitoring and understanding these changes can help optimize training strategies, ensuring athletes achieve peak performance while maintaining health and well-being in high-altitude environments.

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Authors' Contributions

Mücahit Sarıkaya, Beyza Öğe and Nuri Mert Embiyaoğlu designed and supervised the study; Mücahit Sarıkaya, Beyza Öğe, Nuri Mert Embiyaoğlu, Muzaffer Selçuk, Vedat Çınar, Salih Öner, Yıldırım Gökhan Gencer, Mehdi Aslan, Mustafa Sencer Ulema, Yunus Emre Yarayan, and Kadir Keskin collected the data; Mücahit Sarıkaya, Beyza Öğe, and Sameer Badri Al-Mhanna carried out the statistical analyses; Beyza Öğe, Nuri Mert Embiyaoğlu, and Alexios Batrakoulis drafted the manuscript; Nouf H. Alkhamees, Bodor Bin Sheeha, Gerasimos Grivas, and Alexios Batrakoulis reviewed and edited the manuscript. All authors read and approved the final version of the manuscript.

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Data Availability Statement

The datasets generated and/or analysed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Declarations

Ethics Approval and consent to participate

The study was approved by Van Yüzüncü Yıl University Non-Interventional Clinical Research Ethics Committee (2021/05-15) and supported by Van YYÜ Scientific Research Projects Coordination with project number TYD-2021-9553. Participants were informed about the study in advance and their informed written consent was obtained.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Figure captures

Figure 1. Changes in oxidative stress markers and CK levels across different altitudes. A) Total Antioxidant Status (TAS) levels, B) Total Oxidant Status (TOS) levels, C) Malondialdehyde (MDA) levels, D) Oxidative Stress Index (OSI) levels. Data are presented as mean ± standard deviation. Statistical significance between groups is indicated by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

Figure 2. Changes in Creatine Kinase (CK) levels across different altitudes. Data are presented as mean ± standard deviation. Statistical significance between groups is indicated by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, and ns for non-significant differences.

Figure 3. Changes in oxygen saturation (%) across different altitudes at various measurement intervals (3 min, 6 min, 9 min, and 12 min). Data are presented as mean ± standard deviation. Statistical significance between groups is indicated by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 4. Changes in heart rate (BPM) across different altitudes at various measurement intervals (3 min, 6 min, 9 min, and 12 min). Data are presented as mean ± standard deviation. Statistical significance between groups is indicated by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

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726

Table 1(on next page)

Participants’ baseline characteristics

Table 1. Participants' baseline characteristics.

Variables	n	Average ± SD	Min	Max
Age (years)	12	20.3 ± 1.5	18	23
Body Weight (kg)	12	63.5 ± 15.2	51.0	98.5
Height (cm)	12	171.8 ± 7.9	155.5	184.0
BMI (kg/m^2)	12	21.4 ± 4.3	17.70	32.00
Resting Heart Rate (bpm)	12	49.0 ± 3.0	45	52

Table 2(on next page)

Changes in oxidative stress markers and CK at different altitudes (mean±SD)

Total Antioxidant Level (TAS), Total Oxidant Level (TOS), Malondialdehyde (MDA), Creatine Kinase (CK) and Oxidative Stress Index (OSI). a: significant differences between normoxic condition and 1700 m; b: significant differences between normoxic condition and 2450 m; c: significant differences between normoxic condition and 3200 m; d: significant differences between 1700 m and 2450 m; e: significant differences between 1700 m and 3200 m; f: significant differences between 2450 m and 3200 m. *: significant differences at 0.05 level; **: significant differences at 0.01 level; ***: significant differences at <0.001 level.

Table 2. Changes in oxidative stress markers and CK at different altitudes (mean±SD).

Oxidative Stress Markers and CK	Normoxic Condition	1700 m (%Change)	2450 m (%Change)	3200 m (%Change)	*p*-value
TAS (mmol /L)[a,b,c,e,f]	5.83 ± 1.25	18.64 ± 10.45 (219%)	25.87 ± 9.56 (343%)	35.70 ± 10.04 (512%)	0.000***
TOS (µmol /L)[b]	59.97 ± 5.35	49.90 ± 15.67 (-17%)	42.86 ± 10.89 (-28%)	49.23 ± 12.04 (-18%)	0.017*
MDA (mmol/L)[b,c]	5.06 ± 1.11	7.91 ± 1.85 (56%)	9.62 ± 4.18 (90%)	8.96 ± 1.47 (77%)	0.000***
OSI [a,b,c,e]	10.77 ± 2.83	4.62 ± 4.86 (-57%)	1.95 ± 1.10 (-82%)	1.44 ± .42 (-87%)	0.000***
CK (pg/mL)	12[illegible]4 ± 9.77	196.18 ± [illegible].61 (56%)	157.39 ± 35.61 (25%)	184.65 ± 60.93 (47%)	0.059

Total Antioxidant Level (TAS), Total Oxidant Level (TOS), Malondialdehyde (MDA), Creatine Kinase (CK) and Oxidative Stress Index (OSI). a: significant differences between normoxic condition and 1700 m; b: significant differences between normoxic condition and 2450 m; c: significant differences between normoxic condition and 3200 m; d: significant differences between 1700 m and 2450 m; e: significant differences between 1700 m and 3200 m; f: significant differences between 2450 m and 3200 m. *: significant differences at 0.05 level; **: significant differences at 0.01 level; ***: significant differences at <0.001 level.

Table 3(on next page)

Changes in oxygen saturation (%) at different altitudes (mean±SD)

m: meter; a: significant differences between normoxic condition and 1700 m; b: significant differences between normoxic condition and 2450 m; c: significant differences between normoxic condition and 3200 m; d: significant differences between 1700 m and 2450 m; e: significant differences between 1700 m and 3200 m; f: significant differences between 2450 m and 3200 m. *: significant differences at 0.05 level; **: significant differences at 0.01 level; ***: significant differences at <0.001 level.

Table 3. Changes in oxygen saturation (%) at different altitudes (mean±SD).

Minutes	Normoxic Condition	1700 m (%Change)	2450 m (%Change)	3200 m (%Change)	*p*-value
3 min[b,c,d,e,f]	96.08 ± 1.00	94.50 ± .91 (-2%)	90.08 ± 2.35 (-6%)	80.58 ± 5.42 (-16%)	0.000***
6 min[b,c,d,e,f]	94.75 ± .97	93.83 ± 1.12 (-1%)	88.08 ± 4.25 (-7%)	72.92 ± 5.13 (23%)	0.000***
9 min[b,c,d,e,f]	94.83 ± 1.11	93.92 ± 1.62 (-1%)	87.83 ± 3.22 (-7%)	72.33 ± 4.83 (-24%)	0.000***
12 min[b,c,d,e,f]	95.00 ± 1.48	9[illegible] ± 1.72 (-3%)	85.42 ± 5.21 (-10%)	72.75 ± 5.61 (-23%)	0.000***

m: meter; a: significant differences between normoxic condition and 1700 m; b: significant differences between normoxic condition and 2450 m; c: significant differences between normoxic condition and 3200 m; d: significant differences between 1700 m and 2450 m; e: significant differences between 1700 m and 3200 m; f: significant differences between 2450 m and 3200 m. *: significant differences at 0.05 level; **: significant differences at 0.01 level; ***: significant differences at <0.001 level.

Table 4(on next page)

Changes in heart rate (beat per minute) at different altitudes

a: significant differences between normoxic condition and 1700 m; b: significant differences between normoxic condition and 2450 m; c: significant differences between normoxic condition and 3200 m; d: significant differences between 1700 m and 2450 m; e: significant differences between 1700 m and 3200 m; f: significant differences between 2450 m and 3200 m.*: significant differences at 0.05 level; **: significant differences at 0.01 level; ***: significant differences at <0.001 level.

Table 4. Changes in heart rate (beat per minute) at different altitudes.

Minutes	Normoxic Condition	1700 m (%Change)	2450 m (%Change)	3200 m (%Change)	*p*-value
3 min [a,b,c]	73.67 ± 1.78	9[illegible] ± 13.47 (5%)	94.08 ± 18.95 (28%)	104.42 ± 15.16 (42%)	0.000***
6 min [a,b,c,e,f]	73.92 ± 1.31	112.33 ± 13.06 (52%)	105.08 ± 20.20 (42%)	130.17 ± 14.33 (76%)	0.000***
9 min [a,b,c,d,e,f]	73.75 ± 1.91	128.25 ± 15.82 (74%)	130.58 ± 10.21 (77%)	148.75 ± 17.47 (102%)	0.000***
12 min [c]	131.25 ± 22.36	152.50 ± 23.15 (16%)	148.00 ± 16.29 (13%)	162.67 ± 15.50 (24%)	0.001***

a: significant differences between normoxic condition and 1700 m; b: significant differences between normoxic condition and 2450 m; c: significant differences between normoxic condition and 3200 m; d: significant differences between 1700 m and 2450 m; e: significant differences between 1700 m and 3200 m; f: significant differences between 2450 m and 3200 m.*: significant differences at 0.05 level; **: significant differences at 0.01 level; ***: significant differences at <0.001 level.

Figure 1

Changes in oxidative stress markers and CK levels across different altitudes.

A) Total Antioxidant Status (TAS) levels, B) Total Oxidant Status (TOS) levels, C) Malondialdehyde (MDA) levels, D) Oxidative Stress Index (OSI) levels. Data are presented as mean ± standard deviation. Statistical significance between groups is indicated by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

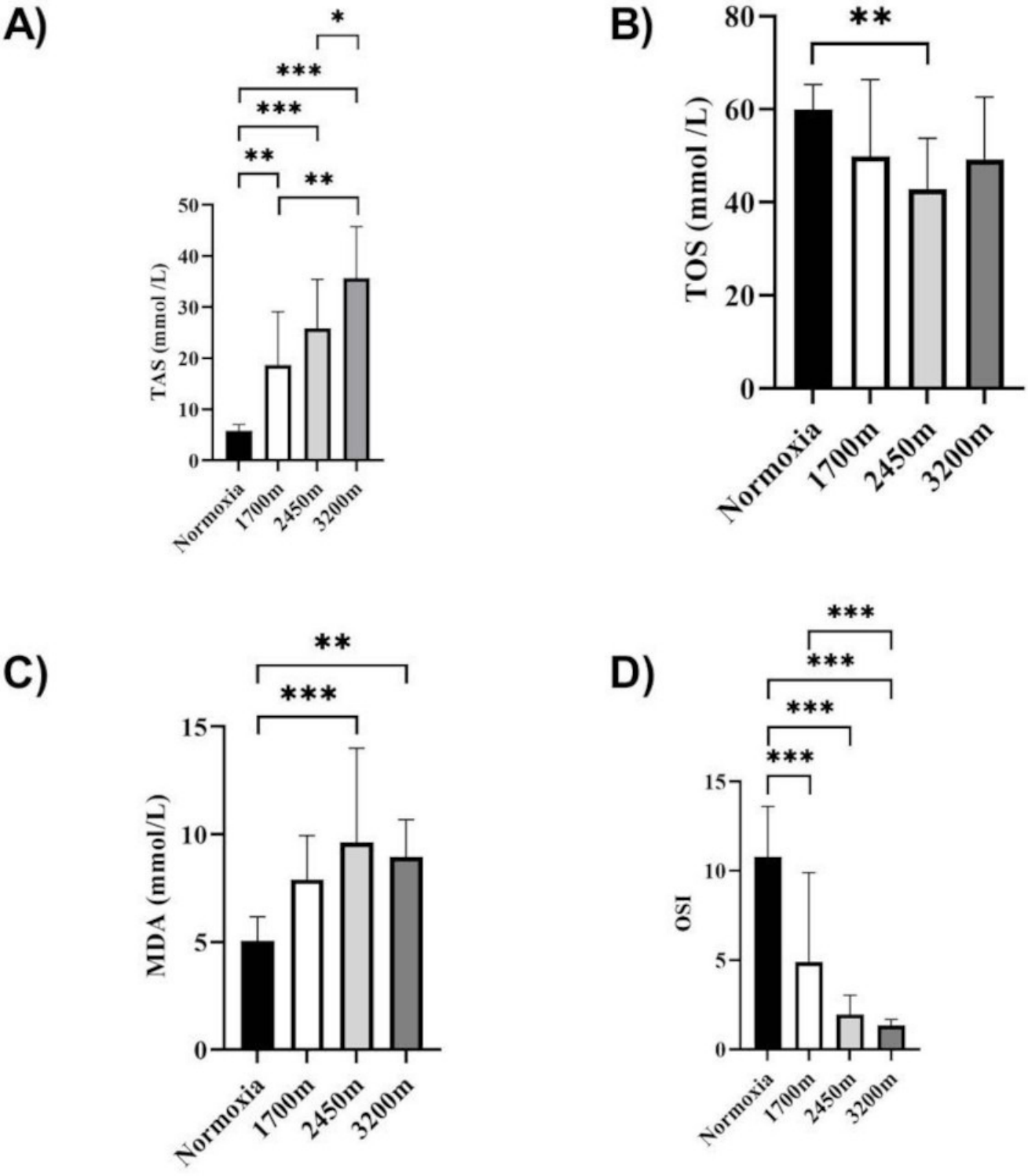
A)
*
***
***
**
**
TAS (mmol /L)
50
40
30
20
10
0
Normoxia
1700m
2450m
3200m
B)
**
TOS (mmol /L)
80
60
40
20
0
Normoxia
1700m
2450m
3200m
C)
**
***
MDA (mmol/L)
15
10
5
0
Normoxia
1700m
2450m
3200m
D)
***
***
***
***
OSI
15
10
5
0
Normoxia
1700m
2450m
3200m

Figure 2

Changes in Creatine Kinase (CK) levels across different altitudes.

Data are presented as mean ± standard deviation. Statistical significance between groups is indicated by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, and ns for non-significant differences.

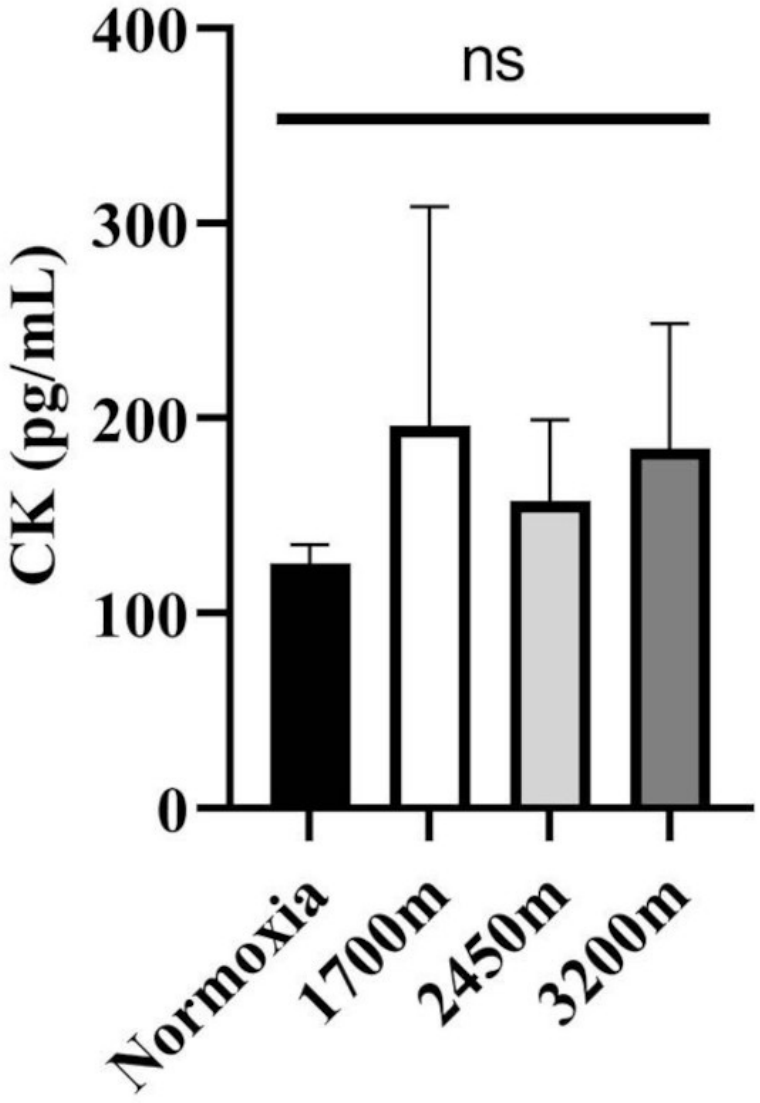

Figure 3

Changes in oxygen saturation (%) across different altitudes at various measurement intervals (3 min, 6 min, 9 min, and 12 min).

Data are presented as mean ± standard deviation. Statistical significance between groups is indicated by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

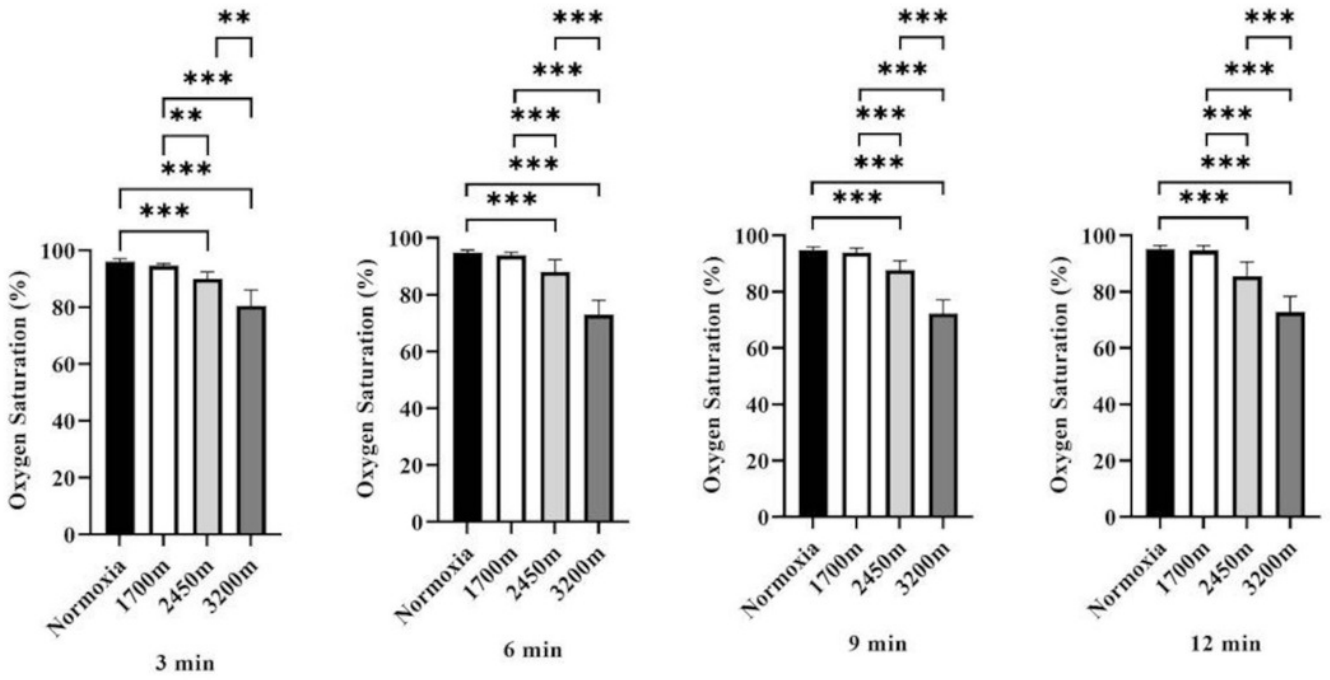

Figure 4

Changes in heart rate (BPM) across different altitudes at various measurement intervals (3 min, 6 min, 9 min, and 12 min).

Data are presented as mean ± standard deviation. Statistical significance between groups is indicated by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.