



# **A Three-Day Effects of Mixed Pomegranate and Barberry Juice Consumption on Hemodynamic Parameters, and Blood PH Status following Force-Velocity based Exercises in Male Athletes**

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## **ABSTRACT**

**Introduction:** The effects of combined pomegranate and barberry juice supplementation, as well as the influence of exercise sequence on hematological, hemodynamic, and acid-base responses during and after sports preparation, remain unclear.

**Methods:** This study aimed to investigate hematological, hemodynamic, and acid-base responses to different sequences of high-intensity anaerobic-resistance exercises following the ingestion of a combined pomegranate and barberry juice supplement in athletes. A total of 12 athletes (mean age:  $24.33 \pm 0.78$  years; height:  $176.75 \pm 3.08$  cm; weight:  $73.93 \pm 3.71$  kg) participated in a double-blind crossover design. Each athlete received either the combined supplement (220 mL of pomegranate and barberry juice) or a placebo in four separate trials. The exercise protocol involved sequential anaerobic-resistance exercises performed in four different orders: (1) power-velocity-strength with supplement/placebo, and (2) power-strength-velocity with supplement/placebo.

**Results:** The results showed that, across all four trials, supplementation significantly decreased mean corpuscular hemoglobin concentration (MCHC;  $P = 0.007$ ) and increased respiratory rate ( $P = 0.024$ ) compared with placebo. Although no significant between-group differences were observed for other hematological variables (HCT, MCH, HGB, WBC, LYM, PLT), significant within-group changes were detected for each marker ( $P < 0.05$ ). Similarly, no significant between-group differences were found for hemodynamic indicators (HR, SBP, DBP, MAP, SaO<sub>2</sub>, BR), although significant within-group alterations were observed ( $P < 0.05$ ). Acid-base markers (LA, pH, HCO<sub>3</sub><sup>-</sup>) also showed no significant between-group differences, but significant within-group changes occurred ( $P < 0.05$ ).

**Conclusion:** In conclusion, supplementation with a combination of pomegranate and barberry juice appears to enhance athletic performance by reducing metabolite accumulation. Furthermore, the order in which exercises are executed influences physiological responses. These findings emphasize the importance of both nutritional supplementation and appropriate exercise sequencing in mitigating the adverse effects of anaerobic training.

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## **Introduction**

Hematological parameters such as hemoglobin (HGB) concentration, red blood cell (RBC) count, and hematocrit (HCT) are critical for maintaining adequate oxygen transport and carbon dioxide clearance during exercise. Alterations in these indices, driven by increased muscular oxygen demand and plasma volume shifts, can markedly influence athletic performance (1). The

structural characteristics of blood can vary depending on the type of physical activity. For instance, endurance training is well established to enhance RBC counts, while resistance training has also been identified as an effective strategy for improving RBC function (2, 3). Ahmadi Zadeh et al. reported changes in blood concentration following acute resistance training, attributing these to exercise-induced plasma volume

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alterations (2). Moreover, intense exercise has been shown to acutely stimulate circulating endothelial progenitor and angiogenic cells, potentially contributing to physiological adaptations through angiogenesis and tissue repair (4). Other investigations have examined the effects of exercise on hemoglobin levels, leukocyte counts, and platelets under varying durations and intensities, generally reporting increases in hemoglobin and platelet counts post-exercise (5). Despite significant advances in understanding exercise-induced hematological and hemodynamic adaptations, previous research indicates that these responses are strongly dependent on the type, duration, and intensity of exercise. Additionally, individual factors such as sex, nutrition, age, and environmental conditions further modulate these parameters (6).

In recent years, the consumption of antioxidant-rich supplements such as pomegranate juice and barberry has attracted increasing attention for their potential to reduce oxidative stress and enhance muscle recovery. Pomegranate juice is rich in bioactive compounds, including polyphenols and flavonoids, which help prevent free radical formation and mitigate oxidative damage induced by high-intensity exercise (7). Previous studies have reported that pre-exercise pomegranate supplementation can attenuate inflammatory markers, improve antioxidant capacity, and enhance muscular endurance (7). Moreover, supplementation has been associated with increases in RBC count and hemoglobin concentration (8). Barberry, containing active compounds such as berberine and berbamine, has been recognized for its anti-inflammatory and cardioprotective effects (9). Belyani et al. (2025) further demonstrated that pomegranate supplementation reduces oxidative stress and inflammation, with evidence suggesting accelerated recovery from exercise-induced muscle damage (EIMD), encompassing metabolic, mechanical, and neuromuscular domains (10). Another critical factor in athletic performance is the sequence of exercise execution. Performing resistance training before anaerobic exercise may help preserve energy stores in the initial stages and delay muscle glycogen depletion. Conversely, initiating training with anaerobic exercise can elevate lactate production, thereby impairing subsequent resistance performance (11).

Exercise sequence has also been shown to affect oxygen consumption, metabolite accumulation, and blood pH, all of which are central to optimizing performance and delaying fatigue (12). Exercise-induced muscle activity inevitably generates free radicals, with the magnitude and source of production varying by exercise type (13). Oxidative stress arises when reactive oxygen species exceed the capacity of endogenous antioxidant defenses, leading to cellular and tissue damage (14). Studies indicate that performing strength training before resistance exercise can further enhance anaerobic metabolism, elevating hydrogen ion concentration, lactate, heart rate, and ADP levels. These alterations impair muscle power output by reducing calcium binding to troponin and diminishing contractile force (15). These considerations raise two essential questions: (i) Can modifying the exercise sequence influence hematological, hemodynamic, and acid-base indices? and (ii) can supplementation with pomegranate and barberry mitigate these exercise-induced changes? Therefore, the primary aim of this study was to investigate whether pre-exercise consumption of combined pomegranate and barberry juice affects hematological, hemodynamic, and acid-base responses during resistance and anaerobic exercise.

## Materials & Methods

### Participants

Twelve athletes from the University of Guilan, whose characteristics are presented in Table 1, participated in this study. All participants were thoroughly informed about the research procedures before enrollment. Inclusion criteria consisted of a training history of more than six months, absence of medication use, and no musculoskeletal disorders. Written informed consent was obtained from all participants before participation.

**Table 1.** Participant's characteristics Mean values and standard deviations, n: (12)

	Mean ± Sd
Age (y)	24.33 ± 0.78
Weight (kg)	73.93 ± 3.71
Height (cm)	176.75 ± 3.08
BMI (kg/m <sup>2</sup> )	23.14 ± 2.82

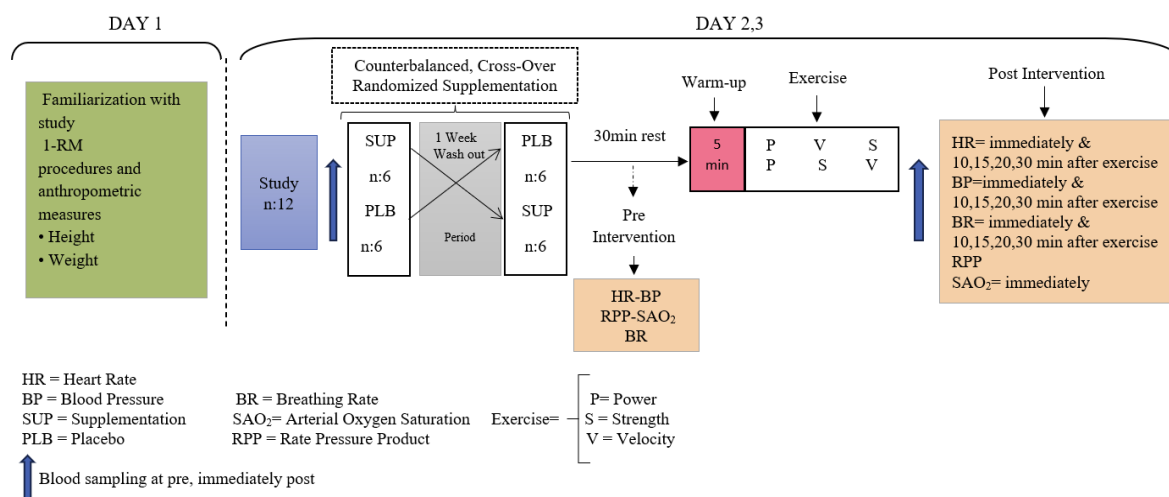
The study protocol was reviewed and approved by the Ethics Committee of the University of Guilan (IR/SSRI.REC.2023.13829.1974) and was

conducted at the Exercise Physiology Laboratory of the University of Guilan.

**Study Design**

This study was conducted using a randomized, double-blind, balanced, crossover, placebo-controlled design (PLB). Participants were evaluated in five stages. The initial stage included familiarization with the exercise protocol, determination of one-repetition maximum (1RM) in the squat exercise, and collection of anthropometric data (height, weight, and body composition). In the second stage, participants consumed 220 mL of pomegranate and barberry juice daily for three consecutive days, after which they performed the exercise protocol in the sequence of power, strength, and velocity. Following a one-week washout period, participants consumed a placebo for three consecutive days and then repeated the exercise sequence (power, strength, and velocity) under the same conditions as in Stage 2. After another one-week interval, participants again consumed the supplement for three consecutive days and subsequently performed the exercise protocol in a different order: power, velocity, and strength. Finally, after a further one-week washout, participants consumed the placebo for three consecutive days and repeated the same exercise

sequence as in Stage 4 (power, velocity, and strength). Blood samples were collected at two time points in each trial: immediately before and immediately after the exercise protocol, to analyze hematological indices and acid-base parameters. Hemodynamic parameters (heart rate, blood pressure, and respiratory rate) were assessed using laboratory-grade equipment at multiple time points: before exercise, immediately after, and at 10, 15, 20, and 30 minutes post-exercise. Arterial oxygen saturation (SaO<sub>2</sub>) was measured using an Lk-88 pulse oximeter at two time points: before and immediately after exercise. Hematological variables (HCT, MCH, MCHC, HGB, WBC, LYM, and PLT) and acid-base markers (La<sup>-</sup>, pH, and HCO<sub>3</sub><sup>-</sup>) were evaluated from venous blood samples (table 2 and 3). To minimize confounding factors, participants were instructed to refrain from physical activity for at least 48 hours before each trial and to fast (abstain from both food and fluids) for at least 12 hours before testing. Anthropometric measurements were also recorded. Height was measured using a wall-mounted stadiometer (Seca 222; accuracy 0.1 cm). Body weight was measured with a laboratory scale (Camry FB9003; accuracy 0.1 kg). (Figure 1).



**Figure 1.** Study Design. Supplement and placebo administrations were performed in a randomized, double-blind, crossover manner. Venous blood samples were collected at two time points—immediately before and immediately after exercise—to evaluate hematological and hemodynamic parameters. Hemodynamic variables were also measured twice at baseline (pre-exercise) and subsequently at 0, 10, 15, 20, and 30 minutes post-exercise.

**Table 2.** Mean and standard deviation of hematological and acidity variables in pre-tests and post-tests across four trials.

	Trial 1 (P-S-V + PLB)		Trial 2 (P-S-V + SUP)		Trial 3 (P-V-S + PLB)		Trial 4 (P-V-S + SUP)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
HCT (%)	40.11 ± 4.14	43.02 ± 4.08	39.46 ± 3.38	43.08 ± 3.30	39.62 ± 4.17	43.70 ± 4.53	40.10 ± 3.47	44.11 ± 3.40
MCH (pg)	29.44 ± 1.58	29.76 ± 1.64	28.98 ± 0.902	29.09 ± 0.942	29.21 ± 0.591	29.85 ± 1.29	28.90 ± 0.807	29.24 ± 0.799
HGB (g/dL)	13.57 ± 1.55	14.50 ± 1.62	13.42 ± 1.02	14.40 ± 1.22	13.45 ± 1.45	14.62 ± 1.62	13.37 ± 1.10	14.62 ± 1.12
MCHC (g/dL)	33.80±0.57 3	33.67±0.958	33.79 ± 0.448	33.43±0.841	33.95±0.681	33.45±0.750	33.34±0.620	33.10±0.757
WBC (×10 <sup>3</sup> cells/μL)	6.43 ± 1.41	9.61 ± 2.08	5.84 ± 1.27	9.00 ± 1.58	5.75 ± 1.35	9.11 ± 1.75	5.76 ± 1.02	8.80 ± 1.92
LYM (×10 <sup>3</sup> cells/μL)	37.75 ± 7.44	43.45 ± 7.50	38.27 ± 6.79	43.52 ± 6.41	35.74 ± 8.12	42.75 ± 9.38	40.15 ± 7.58	41.94 ± 9.03
PLT (×10 <sup>3</sup> cells/μL)	243.16±57.08	292.75 ±80.78	236.58 ±35.03	295.83 ±54.06	232.50±52.70	288.58±67.41	237.33±32.86	284.08±49.43
SaO <sub>2</sub> (%)	96.41 ± 2.60	96.16 ± 2.55	97.41 ± 1.16	96.25 ± 1.42	96.91 ± 1.50	96.58 ± 1.67	96.83 ± 0.937	97.00 ± 1.59
La <sup>-</sup> (mmol/L)	1.86 ± 0.288	10.66 ± 2.59	1.72 ± 0.597	10.50 ± 2.11	1.47 ± 0.298	11.64 ± 2.33	2.08 ± 0.693	8.91 ± 3.39
PH (unitless)	7.33 ± 0.02	7.22 ± 0.06	7.32 ± 0.02	7.22 ± 0.06	7.33 ± 0.01	7.20 ± 0.05	7.33 ± 0.01	7.23 ± 0.09
HCO <sub>3</sub> <sup>-</sup> (mmol/L)	28.22 ± 1.49	18.23 ± 3.36	26.84 ± 1.44	17.90 ± 2.89	27.42 ± 2.57	17.76 ± 2.96	26.51 ± 2.24	19.76 ± 2.99

P.S.V. power, strength, and velocity exercises

P.V.S. power, velocity, and strength exercises

SUP. Supplementation

PLB. Placebo

**Table 3.** Mean and standard deviation of hemodynamic indices at different time intervals in the four trials.

Trial	variables	pre	lp	P10	P15	P20	P30
Trial 1 (P-S-V + PLB)	HR (bpm)	77.16 ± 8.89	113.91 ± 11.58	104.41 ± 8.96	100.91 ± 10.52	97.50 ± 9.59	93.50 ± 9.90
	SBP (mmHg)	104.66 ± 8.59	112.16 ± 15.01	103.08 ± 17.42	103.08 ± 13.66	102.25 ± 11.24	106.33 ± 21.27
	DBP (mmHg)	64.25 ± 9.06	70.33 ± 16.83	61.33 ± 8.10	63.50 ± 10.73	64.83 ± 9.30	69.75 ± 15.86
	AMP (mmHg)	77.58 ± 8.01	84.13 ± 15.21	75.19 ± 10.06	76.56 ± 10.89	77.18 ± 7.32	81.82 ± 16.38
	RPP (mmHg-bpm)	8104.50±1358.18	12749.75±1950.00	10773.25±1887.21	10300±888.80	10000.00±1625.57	9987.66±2362.27
Trial 2 (P-S-V + SUP)	BR (br/min)	19.66 ± 2.67	26.50 ± 4.01	22.91 ± 4.20	22.33 ± 2.67	20.66 ± 3.11	20.33 ± 3.28
	HR (bpm)	77.33 ± 11.97	112.66 ± 10.42	99.08 ± 12.22	96.58 ± 12.36	94.91 ± 10.11	91.08 ± 9.74
	SBP (mmHg)	109.83 ± 12.26	107.91 ± 11.52	113.41 ± 16.26	107.25 ± 12.62	110.33 ± 13.80	107.00 ± 18.81
	DBP (mmHg)	60.08 ± 5.65	64.75 ± 7.93	64.75 ± 16.14	64.33 ± 10.26	68.41 ± 16.90	66.58 ± 13.03
	AMP (mmHg)	77.00 ± 5.75	78.99 ± 6.33	80.81 ± 14.54	78.49 ± 9.79	82.24 ± 15.10	79.92 ± 13.20
Trial 3 (P-V-S + PLB)	RPP (mmHg-bpm)	8466.66±1487.45	12119.66±1345.90	11220.91±2075.24	10344.66±1672.33	10443.33±1584.66	9808.83±2290.99
	BR (br/min)	21.83 ± 3.56	34.50 ± 5.97	27.00 ± 6.57	24.66 ± 5.54	24.16 ± 4.54	22.66 ± 3.93
	HR (bpm)	76.50 ± 12.90	111.25 ± 9.93	101.08 ± 9.64	98.50 ± 8.52	95.25 ± 7.62	91.66 ± 10.53
	SBP (mmHg)	107.41 ± 11.47	112.66 ± 13.43	109.58 ± 16.76	105.83 ± 11.35	106.41 ± 12.85	109.50±13.89
	DBP (mmHg)	66.00 ± 9.00	65.58 ± 8.86	67.08 ± 20.68	64.83 ± 11.06	64.66 ± 10.78	72.16 ± 16.10
Trial 4 (P-V-S + SUP)	AMP (mmHg)	79.66 ± 8.16	81.12 ± 7.78	81.10 ± 18.55	78.36 ± 10.17	78.44 ± 10.44	84.48 ± 14.29
	RPP (mmHg-bpm)	8190.33±1430.59	12511.66±1705.93	11079.33±1890.31	10486.33±1712.87	10152.41±1542.40	10057.25±1779.49
	BR (br/min)	20.83 ± 4.21	30.50 ± 6.82	25.00 ± 6.64	23.50 ± 5.53	23.00 ± 6.23	20.00 ± 4.00
	HR (bpm)	76.58 ± 10.80	111.75 ± 10.70	69.16 ± 11.40	98.00 ± 12.03	95.00 ± 9.89	92.66 ± 14.95
	SBP (mmHg)	108.00±13.03	113.83 ± 11.35	109.83 ± 11.67	102.66 ± 12.06	103.08 ± 7.58	106.08 ± 9.46
Trial 4 (P-V-S + SUP)	DBP (mmHg)	64.75 ± 11.55	64.33 ± 9.34	70.25 ± 17.38	64.58 ± 10.90	58.50 ± 7.56	63.50 ± 8.12
	AMP (mmHg)	79.02 ± 10.37	80.66 ± 7.89	83.31 ± 14.59	77.15 ± 10.41	73.21 ± 5.73	77.55 ± 4.79
	RPP (mmHg-bpm)	8011.33±1837.90	12690.50±1434.55	10516.41±1397.22	10037.66±1590.73	9800.08±1277.95	9771.00±1415.78
	BR (br/min)	20.83 ± 3.95	33.00 ± 9.92	26.50 ± 5.46	24.50 ± 6.21	24.66 ± 6.34	23.33 ± 6.62

P.S.V. power, strength, and velocity exercises ; P.V.S. power, velocity, and strength exercises;

SUP. Supplementation; PLB. placebo

## Procedures

### A: Randomization and Blinding

Randomization was conducted by the experimenter in a double-blind manner using a table of random numbers. Numbers were assigned to two groups (supplement and placebo), and participants were randomly allocated without knowledge of their group

assignment. A total of 15 trained young athletes were screened for eligibility. Of these, 12 met the inclusion criteria and were randomized into either the supplement or placebo condition. All 12 participants completed the study protocol, and no exclusions, injuries, or adverse events were reported. Data are therefore presented for all 12 participants (Figure 2).

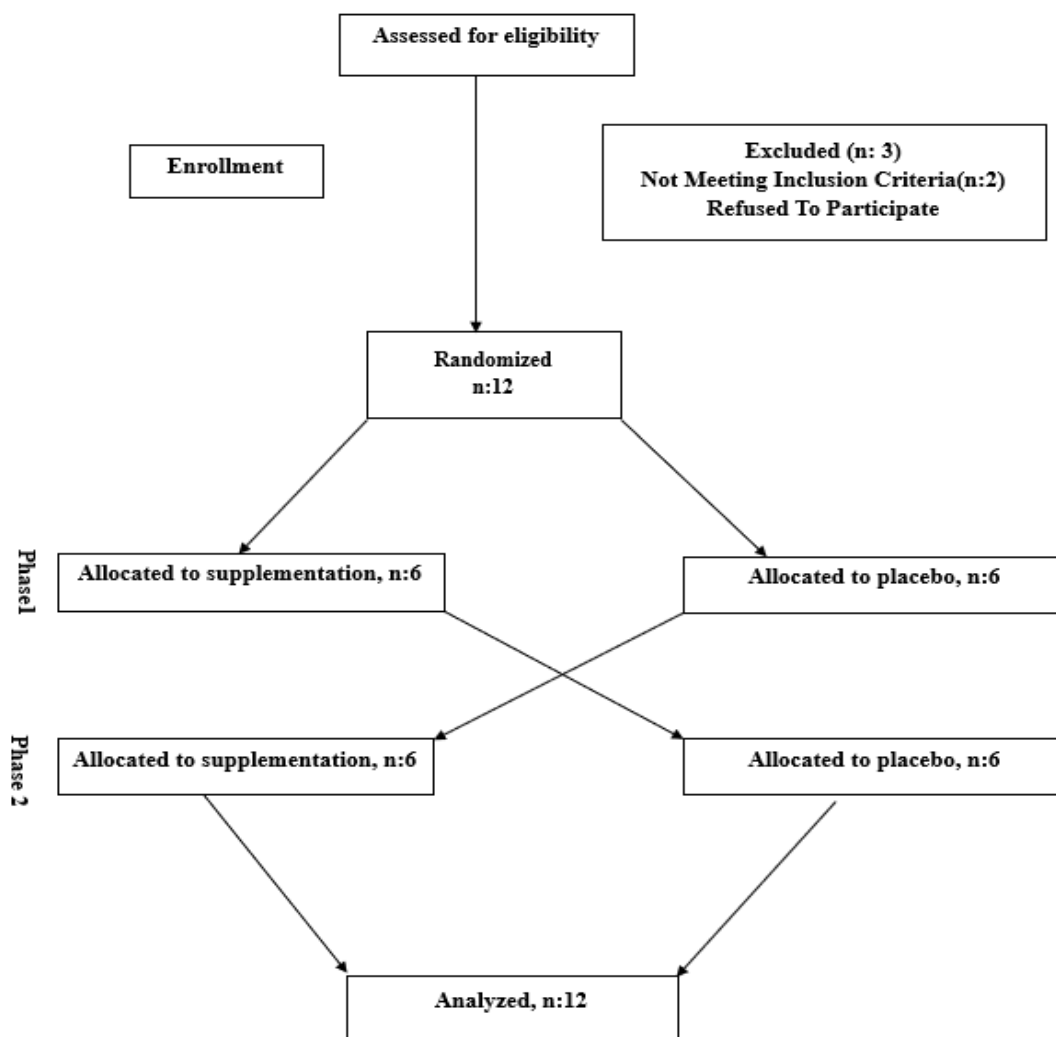


Figure 2. Flowchart diagram of participants' recruitment and allocation.

### B: Pomegranate and Barberry Juice Supplementation

Supplementation Protocol. Supplementation was administered over three days. The supplement consisted of a natural combination of pomegranate juice and barberry. The placebo was prepared by dissolving food coloring in water to match the appearance of the supplement. Participants consumed 220 mL of

either the supplement or placebo once daily, in a fasting state (8, 16). The final dose was ingested immediately before the exercise protocol

### C: Exercise Protocol

Before initiating the exercise protocol, participants completed a 10-minute warm-up consisting of 5 minutes of treadmill running followed by full-body stretching. Subsequently,

they performed the assigned exercise sequences according to their group allocation (power, strength, velocity, or the reverse order). For the power training, the jump test was employed. In this test, athletes were required to perform intermittent vertical jumps of at least 30 cm for 30 seconds (6 sets of 10 repetitions until fatigue), with a 1-minute rest interval between sets. Strength training was conducted using the barbell squat exercise, based on each participant's one-repetition maximum (1-RM). Training intensity ranged from 55% to 100% of 1-RM across five sets, progressing from a single repetition to volitional fatigue. Speed training was assessed using a 60-yard sprint (17, 18). A schematic representation of the exercise protocol is provided in Figure 1.

#### **D: Blood Sampling and Analysis**

Blood samples (7 mL) were collected twice—before and immediately after the training session—from the brachial vein of the participants' left arm while seated. The samples were drawn by a trained laboratory technician and transferred into test tubes and specialized syringes. Plasma was separated from other blood components by centrifugation, after which serum was extracted and stored at  $-70^{\circ}\text{C}$  for subsequent analyses. Blood lactate ( $\text{La}^-$ ) concentration was determined using a commercial Byrex Fars kit (BXC0622, Iran) with a sensitivity and accuracy of 2 mg/dL, measured on a BT3000 biochemical analyzer. Hematological parameters, including HCT, MCH, MCHC, HGB, WBC, LYM, and PLT, were analyzed using a Sysmex KX-21N hematology analyzer (Sysmex, Japan). Blood acid-base parameters, including pH (accuracy 0.01) and bicarbonate concentration (measured within the physiological range of 22–26 mEq/L), were assessed using the GASTAT 720 analyzer (Techno Media, Japan).

#### **Statistical Analysis**

All participants who completed the study were included in the final data analysis. Statistical analyses were conducted using SPSS software (version 26.0, IBM Corp., Armonk, NY, USA). The required sample size was estimated a priori using G\*Power software. Data are presented as mean  $\pm$  standard deviation (SD). The normality of distribution was assessed using the Shapiro-Wilk test. For hematological and acid-base parameters, a two-way repeated-measures

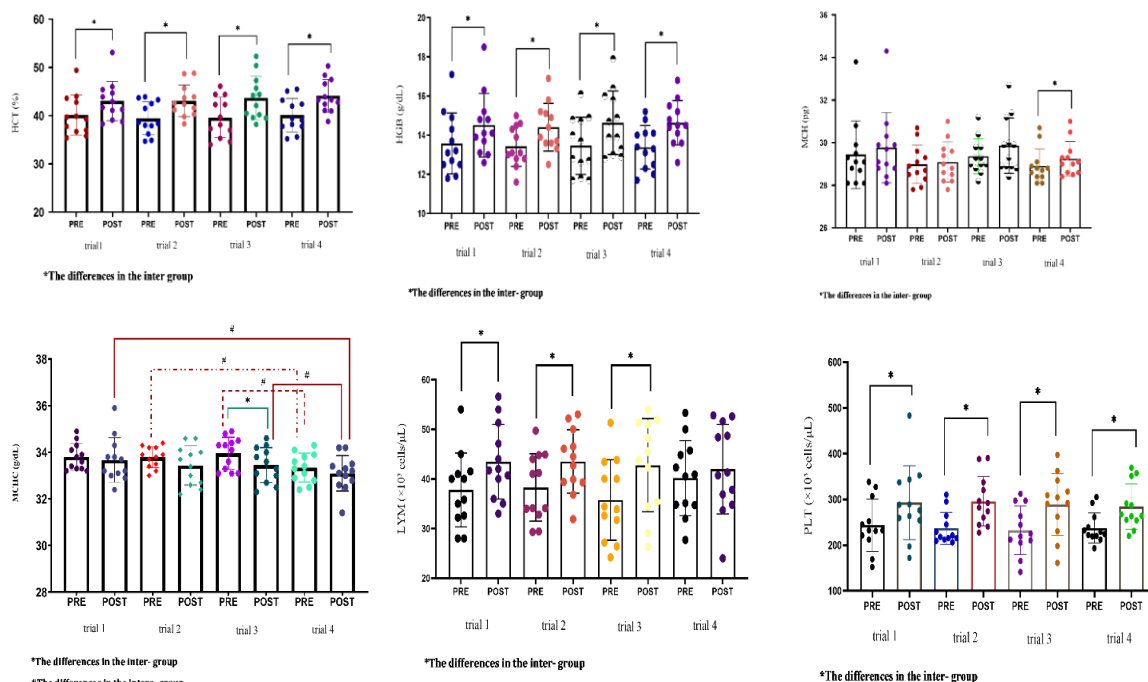
ANOVA ( $2 \times 4$ ; group  $\times$  time) was performed. For hemodynamic indicators, a six-way repeated-measures ANOVA ( $6 \times 4$ ; group  $\times$  time) was applied. When a significant main effect was observed, post hoc pairwise comparisons were conducted using the Bonferroni correction for multiple comparisons. Statistical significance was set at  $P \leq 0.05$ .

#### **Results**

The results showed a significant group  $\times$  time interaction effect for hematocrit (HCT) ( $f = 0.664$ ,  $p = 0.033$ ). Within-group analysis revealed significant differences across trial phases ( $f = 147.217$ ,  $p = 0.001$ ). HCT levels increased during the second and third trials, coinciding with supplement consumption, and reached their highest level in the fourth trial (power-velocity-strength training with supplementation). For mean corpuscular hemoglobin (MCH), no significant interaction effect between group and time was observed ( $f = 0.647$ ,  $p = 0.489$ ). However, within-group analysis indicated significant differences across trial phases ( $p = 0.001$ ). Specifically, the mean red blood cell count increased more during the third trial (power-velocity-strength training with placebo) compared with the other trials (Figure 3). Similarly, no significant group  $\times$  time interaction effect was found for hemoglobin (HGB) concentration ( $F = 3.015$ ,  $p = 0.068$ ). Nevertheless, within-group analysis revealed significant differences across trial phases ( $p = 0.001$ ), with hemoglobin levels increasing more in the fourth trial (power-velocity-strength training with supplementation) compared with the other trials. No significant group  $\times$  time interaction effect was observed for mean corpuscular hemoglobin concentration (MCHC) ( $F = 1.082$ ,  $p = 0.370$ ). A significant difference was detected in both the between-group ( $p = 0.007$ ) and within-group ( $p = 0.025$ ) analyses. This difference reflected a decrease in mean hemoglobin concentration across all four trials, with a more pronounced reduction in the second and fourth trials, both associated with supplement consumption (Figure 3). For white blood cell (WBC) count, no significant group  $\times$  time interaction effect was observed ( $F = 0.25$ ,  $p = 0.860$ ). However, a significant between-group difference was found ( $p = 0.001$ ). The most significant increase in WBC count occurred in the first trial (power-strength-velocity exercises

with placebo) compared with the other trials. A significant group  $\times$  time interaction effect was found for lymphocyte (LYM) count ( $F = 4.882$ ,  $p = 0.006$ ). Within-group analysis also revealed significant differences across trial phases ( $p = 0.003$ ). Lymphocyte counts increased in all four trials, with the first trial (power–strength–velocity exercises with placebo) showing the largest increase compared with the others. No

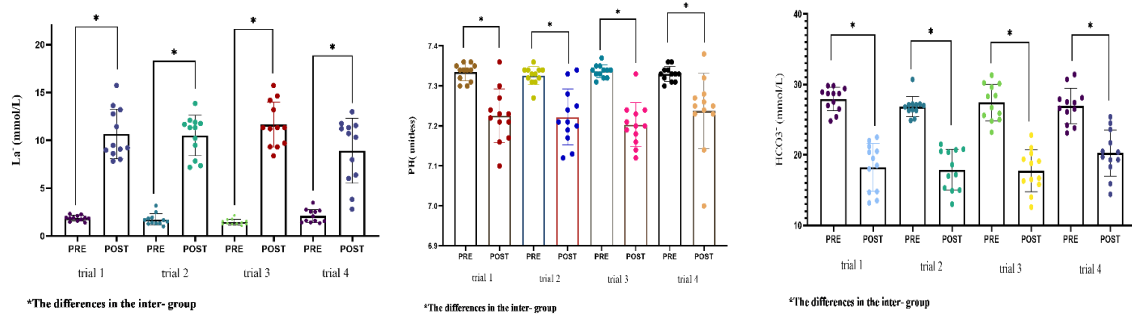
significant group  $\times$  time interaction effect was observed for platelet (PLT) count ( $F = 0.769$ ,  $p = 0.488$ ). However, within-group analysis indicated significant differences ( $p = 0.001$ ). Platelet counts increased in all four trials, with the second trial (power–strength–velocity exercises with supplementation) demonstrating the most significant increase (Figure 3).



**Figure 3.** Mean  $\pm$  standard deviation of hematocrit (HCT), hemoglobin (HGB), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), white blood cells (WBC), lymphocytes (LYM), and platelets (PLT) measured before and immediately after exercise. Significant within-group differences were observed across all variables in the four trials, and significant between-group differences were detected in hemoglobin and MCHC values during the first and fourth trials.

No significant group  $\times$  time interaction effect was observed for pH ( $F = 1.727$ ,  $p = 0.180$ ); however, a significant between-group difference was detected ( $p = 0.001$ ). pH decreased across all four trials, with the reduction being less pronounced in the third trial (power–strength–velocity exercises with placebo) compared with the others. A significant group  $\times$  time interaction effect was observed for bicarbonate ( $\text{HCO}_3^-$ ) levels ( $F = 3.035$ ,  $p = 0.043$ ). Within-group analysis also revealed significant differences across trials ( $p = 0.001$ ). Bicarbonate levels decreased in all trials, with a more minor reduction in the third trial (power–strength–

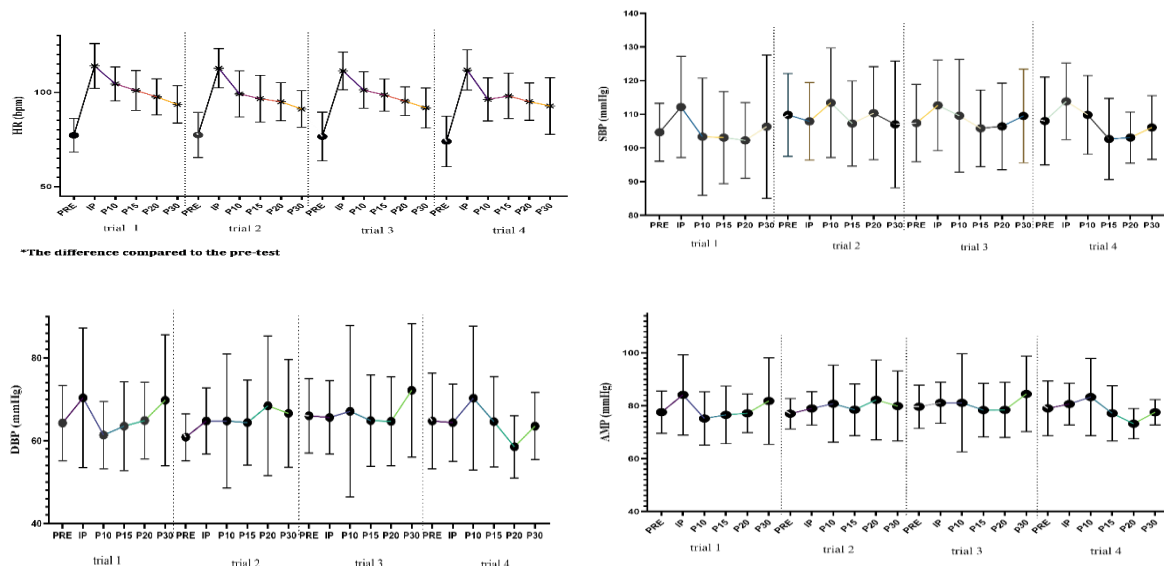
velocity exercises with placebo) compared with the others. For lactate ( $\text{La}^-$ ) concentration, a significant group  $\times$  time interaction effect was found ( $F = 4.597$ ,  $p = 0.009$ ), along with significant within-group differences across trials ( $p = 0.001$ ). Lactate levels increased in all four trials. The increase was most significant in the third trial (power–strength–velocity exercises with placebo), whereas in the fourth trial (power–velocity–strength exercises with supplementation), the increase was lower than in the other trials, suggesting a potential effect of supplementation (Figure 4).



**Figure 4.** Mean ± standard deviation of lactate (La<sup>-</sup>), pH, and bicarbonate (HCO<sub>3</sub><sup>-</sup>) levels before and after exercise, along with within-group variations across the four experimental trials.

No significant group × time interaction effect was observed for heart rate (HR) ( $F = 1.727, p = 0.180$ ); however, a significant between-group difference was detected ( $p = 0.001$ ). HR increased significantly immediately after exercise in all four trials. In the trials accompanied by supplement consumption, HR declined more rapidly and prominently during recovery. For systolic blood pressure (SBP), no significant group × time interaction effect was observed ( $F = 0.536, p = 0.917$ ). SBP increased immediately after exercise in all trials, decreased at 10, 15, and 20 minutes post-exercise, and then rose again at 30 minutes post-exercise. No significant group × time interaction effect was found for diastolic blood pressure (DBP)

( $F=1.078, p=0.838$ ). DBP increased immediately after exercise in all trials, decreased at 10 minutes, and showed an increasing trend at 15, 20, and 30 minutes post-exercise. Similarly, no significant group × time interaction effect was observed for mean arterial pressure (MAP) ( $F = 0.923, p = 0.540$ ). MAP increased immediately after exercise in all trials. In the first trial, MAP decreased at 10, 15, and 20 minutes post-exercise before increasing again at 30 minutes. In the second trial, MAP increased at 10 and 15 minutes, decreased at 20 minutes, and increased again at 30 minutes. In the third and fourth trials, MAP showed an upward trend both immediately and at 10 minutes post-exercise (Figure 5).



**Figure 5.** Mean and standard deviation of blood pressure (SBP, DBP, AMP) and heart rate (HR) variables at different time intervals: before, immediately after, and at 10, 15, 20, and 30 minutes post-exercise.

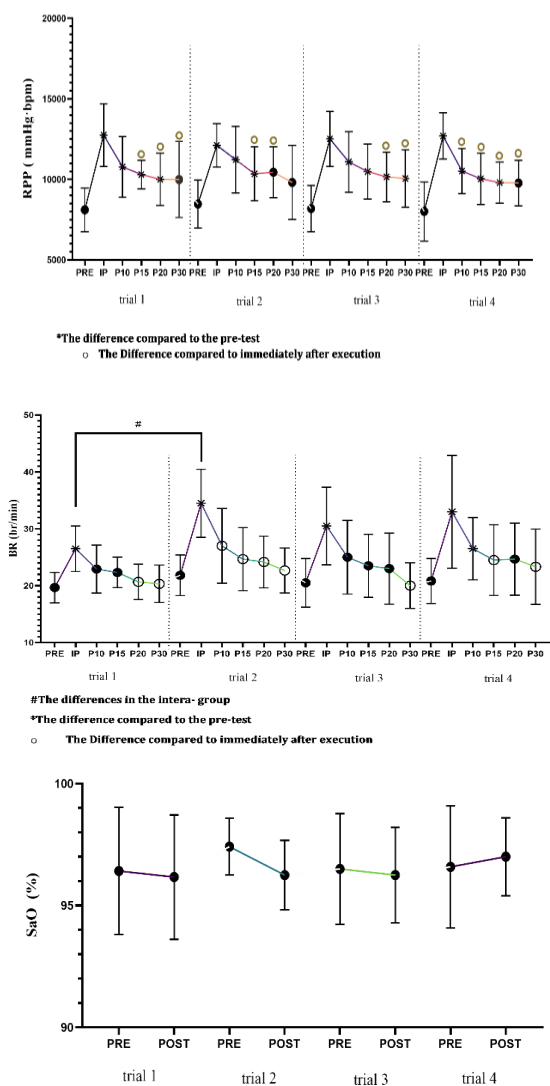
No significant group × time interaction effect was observed for the rate pressure product (RPP) ( $F = 0.348, p = 0.989$ ). RPP increased immediately after exercise in all trials and subsequently decreased. The first trial showed a greater immediate post-exercise increase compared with the other trials. For arterial oxygen saturation ( $SaO_2$ ), no significant group × time interaction effect was found ( $F = 0.664, p = 0.580$ ).

$SaO_2$  decreased in the first, second, and third trials, whereas an increase was observed in the fourth trial (power-velocity-strength training with supplementation). For breathing rate (BR), no significant group × time interaction effect was observed ( $F = 1.131, p = 0.332$ ). However, significant between-group ( $p = 0.024$ ) and within-group ( $p = 0.001$ ) differences were detected. BR increased immediately after exercise in all trials, with the second trial showing the most significant post-exercise increase compared with the others (Figure 6).

### Discussion

The present study examined the effects of combined pomegranate juice and barberry supplementation on hematological indices, blood acidosis, and hemodynamic responses, as well as the influence of exercise sequence. Alterations in exercise order, when combined with supplementation, may differentially affect athletes' physiology, particularly with respect to hematology, acid-base balance, and post-exercise recovery. Two exercise sequences were employed: power-velocity-strength and power-strength-velocity.

The findings demonstrated that hematocrit levels increased during different stages of the experiment (trials 2, 3, and 4), with the most significant increase observed in trial 4 (power-velocity-strength sequence with supplementation). This elevation may reflect stimulation of red blood cell (RBC) production in response to combined resistance and speed training. Interestingly, in trial 3 (power-strength-velocity sequence with placebo), hematocrit also increased significantly, suggesting that exercise order alone can modulate physiological responses, particularly when stress is imposed on the hematopoietic system. Previous studies have reported that performing strength exercises at the beginning of a session exerts a stronger effect on hematocrit elevation (19). Resistance training appears to promote the mobilization and stimulation of red blood cells, thereby increasing hematocrit; however, this effect may be influenced by factors such as exercise type and intensity (20, 21). One of the key findings of the present study was the significant rise in hematocrit in the supplementation group, consistent with previous reports showing that pomegranate consumption increases HCT levels (22).



**Figure 6.** Mean ± standard deviation of rate pressure product (RPP), breathing rate (BR), and arterial oxygen saturation ( $SaO_2$ ) at baseline, immediately post-exercise, and at 10, 20, and 30 minutes post-exercise. A significant difference in breathing rate was observed between the first and second trials.

Furthermore, supplementation with pomegranate juice and barberry resulted in a greater increase in hemoglobin (HGB) during trial 4 (power-velocity-strength sequence with supplementation). This observation aligns with earlier studies suggesting that pomegranate, due to its potent antioxidant properties, may enhance hematological performance and reduce oxidative stress (23). The most pronounced increase in hemoglobin occurred in trial 4, specifically associated with the power-velocity-strength sequence combined with supplementation. In contrast, significant increases in hemoglobin were also observed in other trials following resistance and speed exercises with a placebo. These findings suggest that exercise sequence may play a role in modulating hemoglobin production. Strength exercises, when performed at the beginning of a training session, may further stimulate red blood cell and hemoglobin synthesis, leading to higher HGB levels (24). Conversely, when strength and speed exercises are performed after power training, hemoglobin production may be attenuated (24).

The sequence of exercises, when combined with Supplementation, may differentially influence blood acidosis indices such as lactate ( $\text{La}^-$ ), pH, and bicarbonate ( $\text{HCO}_3^-$ ). These effects could be attributed to variations in exercise type and intensity, as well as the biochemical properties of the supplements and their impact on metabolic processes. Regarding lactate and acid-base balance, the results showed that lactate levels increased in all trials. However, in trial 4 (power-velocity-strength sequence with supplementation), the magnitude of lactate accumulation was lower. This finding suggests that pomegranate and barberry supplementation may exert beneficial effects by reducing lactate buildup and attenuating exercise-induced acidosis. The reduced lactate response in trial four may reflect both the exercise sequence and the role of supplementation in enhancing recovery and limiting lactate production. The order of exercises appears to play a crucial role in lactate dynamics. Previous studies have reported that performing aerobic or strength training in different sequences can significantly alter lactate accumulation (8). These findings are consistent with prior research indicating that natural compounds such as pomegranate can reduce lactate production during high-intensity exercise. This effect is likely related to its

antioxidant properties, which help mitigate oxidative stress induced by strenuous activity. When power exercises are performed first, the body rapidly enters a state of acidosis; however, supplementation may modulate this response and attenuate lactate accumulation (8, 25). Previous studies have demonstrated that antioxidant or anti-inflammatory supplements can mitigate the negative effects of lactate accumulation (26). For example, pomegranate supplementation, recognized for its antioxidant properties, has been shown to reduce lactate production and facilitate recovery following high-intensity exercise (27). This effect may be particularly relevant in exercise modalities that produce high levels of lactate, such as speed and resistance training. Bicarbonate is a key marker in regulating acid-base balance. A reduction in bicarbonate levels typically reflects increased acid accumulation in the body, a phenomenon commonly observed after high-intensity exercise, especially when lactate accumulation is substantial. In the present study, bicarbonate levels decreased across all trial stages; however, in trial 3 (placebo condition), the reduction was less pronounced. This finding suggests that exercise sequence, in combination with supplementation, may differentially influence bicarbonate dynamics. Supporting this, prior research has reported that supplementation can enhance blood buffering capacity and attenuate exercise-induced acidosis, particularly when antioxidants are used (28). Pomegranate consumption, due to its high antioxidant content, may play an essential role in improving acid-base balance and maintaining bicarbonate levels (27). These results are consistent with previous findings showing that resistance and anaerobic exercise can reduce circulating bicarbonate and induce metabolic acidosis (29). In the present study, blood pH decreased across all stages; however, in trial 3 (power-strength-velocity sequence with placebo), the reduction was less pronounced compared with the other trials. This suggests that both exercise sequence and supplementation may modulate the extent of exercise-induced pH reduction. Previous research has shown that strength and speed exercises, particularly when performed at high intensity, contribute to excess acid production and reduced blood pH (30). Antioxidant supplementation, such as pomegranate, may help attenuate this decline by reducing oxidative

stress, limiting acid production, and maintaining blood pH within physiological ranges (31). When considering the role of exercise sequence, our results further suggest that ordering may influence acid–base responses. For instance, initiating a session with power exercises may accelerate lactate accumulation and pH reduction, whereas placing speed or strength exercises later in the sequence, in combination with supplementation, may allow natural antioxidants such as pomegranate to mitigate these alterations (31).

Hemodynamic indices—including heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), and rate-pressure product (RPP)—are influenced by both exercise intensity and type, as well as nutritional supplementation. These variables reflect cardiovascular adaptations to metabolic demands during and after exercise. In the present study, HR increased immediately following exercise in all trials. Notably, in the supplementation trials, HR returned to baseline more rapidly and markedly, suggesting that both exercise sequence and supplementation may modulate the speed of post-exercise heart rate recovery. Performing power exercises at the beginning of the sequence appears to place greater initial stress on the cardiovascular system, resulting in higher HR at exercise onset. Conversely, when speed and strength exercises are positioned later in the sequence, different recovery dynamics may occur (10). Similar results have been reported in studies evaluating plant-based supplements and cardiovascular recovery. For example, pomegranate supplementation has been shown to accelerate post-exercise HR reduction compared with placebo following high-intensity exercise (10). These effects may be attributed to the antioxidant and anti-inflammatory properties of the active compounds in pomegranate and barberry. Previous studies have reported that antioxidant supplementation, such as pomegranate, can attenuate oxidative stress and thereby prevent excessive elevations in heart rate following high-intensity exercise (32). This effect appears particularly relevant when power exercises are performed at the beginning of the sequence, as supplementation may reduce the additional cardiovascular burden and shorten recovery time (33). With respect to blood pressure, similar patterns were observed across

all trials. SBP increased immediately after exercise in all four stages, consistent with the elevated oxygen and nutrient demands of skeletal muscle during resistance and high-intensity training. SBP subsequently declined during the 10-, 15-, and 20-minute recovery periods but rose again at 30 minutes post-exercise. Notably, in the supplementation trials, the decline in SBP during recovery was more rapid, suggesting a potential role of supplementation in accelerating blood pressure normalization. Previous research has similarly demonstrated that certain supplements, such as nitric oxide donors, can improve blood flow and reduce SBP during recovery (34, 35). These effects are particularly evident during high-intensity exercise, which imposes a substantial load on the cardiovascular system. In the present study, mean arterial pressure (MAP)—a general indicator of overall blood pressure status—increased following exercise. In the first trial, MAP decreased at 10, 15, and 20 minutes post-exercise, whereas in the other trials, particularly those involving supplementation, MAP rose more rapidly. Such changes in MAP, especially during resistance and speed exercises that exert higher cardiovascular stress, may be related to alterations in hydrostatic balance and vascular function. For other indices, such as breathing rate (BR) and arterial oxygen saturation (SaO<sub>2</sub>), the sequence of exercises appeared to have variable effects. In this study, BR was higher immediately after exercise in trial 2 (placebo condition), which may reflect the specific sequence and intensity of exercises performed. Previous studies have suggested that antioxidant supplementation can reduce the cardiovascular burden and consequently lower the rate-pressure product (RPP). This effect may be particularly beneficial for athletes engaged in high-intensity training (33, 36).

## Conclusion

This study demonstrated that supplementation with pomegranate and barberry, in combination with different sequences of resistance and anaerobic exercise, can significantly influence hematological, hemodynamic, and acid–base indices in athletes. Notably, supplementation increased hematocrit and hemoglobin levels in certain phases of the study, suggesting a potential enhancement in oxygen transport to tissues. In addition, supplementation was

associated with reduced lactate accumulation and improved hemodynamic recovery, including more favorable heart rate and blood pressure responses following exercise. The sequence of exercises also played an important role, as varying orders elicited distinct physiological responses. These findings indicate that the appropriate integration of supplementation with exercise type and sequence may enhance athletic performance, attenuate exercise-induced acidosis, and accelerate post-exercise recovery. Considering both training sequence and supplementation strategies may therefore provide more effective approaches for managing the physiological stress of high-intensity training. Future research should further investigate the long-term effects of pomegranate and barberry supplementation in athletes with different fitness levels and training backgrounds. Incorporating natural antioxidant-rich compounds such as pomegranate and barberry into athletes' diets may represent a practical nutritional strategy to optimize performance and recovery.

#### **Limitations and Suggestions for Future Research**

This study investigated the effects of pomegranate and barberry supplementation, combined with different exercise sequences, on hemodynamic indices and acid-base balance in athletes. The findings demonstrated that supplementation positively influenced hematological parameters such as hematocrit (HCT), hemoglobin (HGB), and platelets (PLT), while also reducing lactate accumulation and enhancing hemodynamic recovery, including more favorable heart rate and blood pressure responses after exercise. Furthermore, exercise sequence significantly affected these indices, particularly under high-intensity conditions, highlighting its role in modulating hemodynamic and acid-base responses. Overall, these results suggest that the appropriate integration of supplementation and exercise sequencing may improve athletic performance and accelerate recovery. Future research should employ larger sample sizes and more extended intervention periods to evaluate the long-term effects of supplementation on performance and recovery. Additionally, studies combining different exercise modalities and nutritional strategies, as well as assessing other physiological markers such as oxidative stress and neurocognitive

function, could provide deeper insights into the mechanisms involved. Finally, cellular and molecular investigations are warranted to better elucidate how the antioxidant properties of pomegranate and barberry contribute to these outcomes.

#### **Declarations**

##### **Conflict of Interest**

The authors declare that there is no conflict of interest.

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##### **Ethical Considerations**

This study was reviewed and approved by the Ethics committee of Sport sciences research institute (IR/SSRI.REC.2023.13829.1974), and study protocol were conducted at the exercise physiology laboratory of University of Guilan.

##### **Authors' Contribution**

HF, JM, HA: conceptualization, Methodology and Investigation; HF, JM: Data analysis, HF, JM: Writing original draft; JM: Supervision, Project administration; JM, HA: Review editing and final checking.

##### **Artificial Intelligence**

No AI platforms were used in the writing and design of this article.

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