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Time-of-Day Effects of Acute Strength Training on Hormonal and Hematological Responses in Female Weightlifters

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ABSTRACT

Objective: This study investigated the acute effects of strength training performed at different times of day on hormonal, hematological, and biochemical responses in trained female weightlifters.

Methods and Materials: Thirty female athletes with at least three years of resistance training experience completed standardized high-intensity strength training sessions in the morning (7:00–9:00 AM) or afternoon (5:00–7:00 PM) at intensities ranging from 75–90% of one-repetition maximum (1RM). Venous blood samples were collected at rest and immediately post-exercise to assess hematological parameters (white blood cell count and subtypes, red blood cells), hormonal markers (testosterone and cortisol), and creatinine levels.

Findings: Both morning and afternoon training sessions resulted in significant post-exercise increases in total white blood cell counts, indicating an acute immune response to strength training. Neutrophil counts increased following morning training, whereas changes in lymphocyte, monocyte, and red blood cell counts were not statistically significant at either time of day. Testosterone and cortisol levels did not change significantly following morning or afternoon sessions. Creatinine levels increased significantly following afternoon training, suggesting a greater acute metabolic response at this time of day. Comparisons between morning and afternoon sessions revealed no significant time-of-day differences for most hormonal and hematological parameters.

Conclusion: These findings indicate that acute high-intensity strength training elicits largely comparable hormonal and hematological responses across training times. At the same time, biochemical stress—reflected by creatinine—may be more pronounced following afternoon sessions.

Keywords: Strength training, chronobiology, hormonal response, hematological response, female athletes.

Introduction

Strength training induces acute physiological responses involving coordinated interactions between the endocrine and immune systems. Hormones such as testosterone and cortisol regulate anabolic and catabolic processes, while hematological parameters reflect immune activation and systemic stress in response to physical exertion. Monitoring these acute responses provides valuable insights into training-load tolerance and physiological stress in athletes (Alen et al., 1988).

Biological rhythms influence many physiological functions, including hormone secretion, immune cell distribution, and metabolic activity. Cortisol follows a pronounced diurnal rhythm, typically peaking in the early morning and declining throughout the day, while testosterone may also exhibit time-of-day variability. Consequently, the timing of exercise may modulate acute physiological responses to strength training (Storey & Smith, 2012).

Although chronobiological effects of exercise have been studied in male athletes, data on trained female strength athletes remain limited. Female weightlifters exhibit distinct hormonal profiles and physiological responses to training, underscoring the need for sex-specific research. While previous studies have examined performance outcomes or long-term adaptations, fewer investigations have focused on acute hormonal and hematological responses to strength training performed at different times of day in this population (Zar et al., 2021).

In a similar vein, Yeom et al. (2023) demonstrated that low-load, high-repetition resistance training led to significant reductions in muscle damage markers, such as creatine kinase (CK) and lactate dehydrogenase (LDH), suggesting enhanced recovery and reduced fatigue, making this training method a viable alternative for improving muscle strength and hypertrophy in weightlifters.

However, the relationship between hormonal responses and strength improvements is complex. West & Phillips (2012) found weak correlations between cortisol and growth hormone responses post-exercise with gains in muscle fiber cross-sectional area and lean body mass, but not with strength improvements.

Acute resistance exercise is known to induce transient leukocytosis, neutrophil mobilization, and changes in biochemical markers related to muscle metabolism. However, it remains unclear whether these responses differ meaningfully between morning and afternoon strength training sessions in trained female weightlifters. Clarifying this issue may help inform training scheduling and physiological monitoring practices (West, 2012).

Diet and nutrition also influence hormonal responses. Sallinen (2007) noted that protein and fat intake could affect serum testosterone concentrations, contributing to muscle mass development during strength training in older men.

Finally, Oliver et al. (2015) examined hormonal changes during a 12-week block-periodized training program among Naval Special Warfare Operators, noting distinct patterns and time courses that could inform the optimization of training schedules to maximize hormonal response.

These recent studies underscore the importance of considering various training modalities and recovery strategies to optimize hormonal responses and enhance performance in weightlifters. Further research is needed to refine these approaches and tailor them to individual needs and training contexts.

This study aims to investigate the chronobiological effects of strength training on hormonal, hematological, and performance adaptations in female weightlifters over a longitudinal period, focusing on how training at different times of day influences physiological and performance outcomes.

The importance of this research lies in its ability to address a significant gap in understanding how training time influences physiological and performance outcomes in female weightlifters. Chronobiology, the study of biological rhythms, has demonstrated that various physiological processes, such as hormone secretion, metabolic activity, and muscle function, exhibit daily fluctuations. These rhythms can significantly impact athletic performance, recovery, and adaptation to training. However, there is limited longitudinal research on how these chronobiological variations affect female athletes specifically during strength training.

By investigating the effects of strength training at different times of the day, this study aims to optimize training schedules to enhance performance and recovery. Understanding how hormonal and hematological responses are influenced by training timing can provide valuable insights into managing athlete health, preventing overtraining, and promoting long-term well-being.

Moreover, this research fills a crucial gap in sports science, where female athletes have historically been underrepresented. Tailoring training protocols to the unique physiological needs of women ensures more inclusive and effective performance enhancement strategies. The findings from this study could serve as a foundation for evidence-based practices in sports coaching and athletic training, contributing to the advancement of sports science and female athlete development.

The purpose of this study was to investigate whether the time of day (morning vs afternoon) influences acute hormonal, hematological, and biochemical responses following a single session of high-intensity strength training in trained female weightlifters.

Methods and Materials

Study Design

This study employed a comparative acute-response design to examine pre–post changes in hormonal, hematological, and biochemical parameters following standardized strength training sessions conducted at different times of day in trained female weightlifters. Acute physiological responses were assessed immediately before and after a single standardized training session performed either in the morning or in the afternoon.

Although the training program was implemented within a broader six-week training framework, longitudinal adaptations, crossover comparisons, and performance outcomes were not analyzed or reported in this manuscript. Each participant contributed data to only one training-time condition, and no within-subject crossover analysis was performed.

Accordingly, the study should be interpreted as a between-subjects acute comparison of morning versus afternoon training sessions.

This study employs a randomized, crossover design to investigate the anabolic hormone response and hematological changes in weightlifters following strength training sessions conducted at different times of the day. The research is structured to compare the effects of morning (8:00 AM) and afternoon (4:00 PM) training sessions on key physiological parameters.

Participants

Thirty trained female weightlifters with a minimum of three years of structured resistance training experience were recruited for this study. All participants were free from known metabolic, cardiovascular, or musculoskeletal disorders and were not using medications known to affect hormonal or immune responses. Participants were assigned to perform a standardized strength-training session either in the morning (7:00–9:00 AM) or in the afternoon (5:00–7:00 PM). All participants provided written informed consent before participation, and the institutional ethics committee approved the study protocol.

Training Protocol

Participants completed a standardized high-intensity strength training session consisting of Olympic weightlifting and resistance exercises performed at 75–90% of one-repetition maximum (1RM). The training session included a standardized warm-up followed by multiple sets with controlled rest intervals (2–3 minutes). Exercise selection and loading parameters were held constant across training sessions to ensure comparability between morning and afternoon conditions.

Exercises used: Snatch, clean and jerk, power snatch, power clean, clean/ snatch deadlift, back squat, shoulder press, bench press (Table 1).

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Table 1

Suggested Exercise Program

day	exercises	Intensity %	set	repetitions	Resting. minute
Saturday	Snatch, clean, and jerk	75–90	6	2-5	2-3
Sunday	Snatch, power clean, clean deadlift	75–90	4-6	2-6	2-3
Monday	rest				
Tuesday	snatch deadlift, back squat, shoulder press, bench press	75–90	3-5	3-8	2-3
Wednesday	rest				
Thursday	clean and jerk, power snatch, back squat	75–90	4-6	2-7	2-3
Friday	rest				

Supplementation Protocol

To standardize nutritional intake and minimize variability in acute training responses, all participants followed the same supplementation protocol. This consisted of 5 g of creatine monohydrate consumed 30 minutes before training, and 25 g of whey protein consumed one hour post-training. Because supplementation was identical for all participants, its potential influence on physiological responses was assumed to be evenly distributed between groups. Nevertheless, this factor is acknowledged as a potential contributor to biochemical markers, particularly creatinine, and is considered in the interpretation of results.

Data Collection

Venous blood samples were collected at two time points: At rest (pre-training) and immediately post-training. Blood sampling was performed once per participant, corresponding to the acute training session analyzed in this study. All values presented in the Results section reflect measurements obtained from the same training day, and no averaging across multiple sessions was performed.

Hormonal Analysis

Serum testosterone and cortisol concentrations were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions.

Hematological and Biochemical Analysis

White blood cell count (WBC), neutrophils, lymphocytes, monocytes, red blood cells (RBC), and serum creatinine were analyzed using an automated hematology and biochemical analyzer in a certified laboratory.

Samples showing technical issues (e.g., hemolysis or insufficient volume) were excluded from the analysis, reducing the sample size for some hormonal and biochemical analyses.

Laboratory Procedure

Hormone Assays

Enzyme-Linked Immunosorbent Assay (ELISA) Kits: ELISA kits will be used to measure serum levels of testosterone and cortisol. These kits are designed to provide high sensitivity and specificity for detecting and quantifying hormone concentrations in blood samples. The process involves binding the hormone of interest to a specific antibody coated on a plate, followed by a colorimetric reaction proportional to the amount of hormone present.

Hematology Analyzer

Automated Hematology Analyzer: An automated hematology analyzer will be used to measure white blood cell (WBC) counts, red blood cell (RBC) counts, and subtypes (neutrophils, lymphocytes, and monocytes). This analyzer uses flow cytometry or impedance-based technologies to provide accurate and rapid blood cell counts and differential analyses.

Creatinine Assay: an automated hematology analyzer for blood cell counts and a validated biochemical analyzer for serum creatinine assessment will be used to measure serum creatinine levels. Creatinine levels are indicative of muscle metabolism and renal function.

Instruments

Strength Training Equipment

Standardized Weightlifting Equipment: The study will use standardized equipment for strength training, including barbells, dumbbells, squat racks, benches, and resistance machines. Equipment calibration and maintenance will ensure consistent performance and safety across all training sessions.

1-Repetition Maximum (1RM) Testing: A procedure for determining the 1RM for each exercise will be conducted before the study. This involves progressively increasing the weight lifted until the participant reaches their maximum single-repetition lift. This information is used to standardize training intensity to 75%-90% of 1RM.

Training Supervision Tools

Training Logs and Diaries: Participants will maintain training logs to record details of their workouts, including exercise type, weight lifted, repetitions, and sets. This will ensure consistency and adherence to the standardized training protocol.

Video Monitoring

Training sessions may be video-recorded for quality assurance and to ensure proper exercise technique. This also aids in adherence to the training protocol.

Analysis

Data were analyzed using IBM SPSS Statistics (version 20.0; IBM Corp., Armonk, NY, USA). Continuous variables are presented as mean ± standard deviation (SD). Within-group pre–post comparisons were performed using paired-samples t-tests for normally distributed variables. Between-group comparisons (morning vs afternoon) were conducted using independent-samples t-tests. Normality of data distribution was assessed using the Shapiro–Wilk test. Statistical significance was set at $p < 0.05$. Given the exploratory nature of the study and the focus on acute physiological responses, no formal correction for multiple comparisons was applied. This limitation is acknowledged in the Discussion.

Findings and Results

Hematological analyses (Tables 2–5) were conducted using data from all 30 participants. However, due to technical limitations and incomplete sample availability during hormonal and biochemical assays, complete hormonal and creatinine data were available for 20 participants, as reflected in Tables 6–9. The sample size used for each analysis is explicitly indicated in the corresponding table headers.

Table 2

Hematological Responses to Morning Strength Training in Female Weightlifters

Group Parameter	Morning		P-value
	At rest	Post training	
No.	30	30	-
White Blood Cells (WBC) (cells/μL)	7,000 ± 1,000	10,000 ± 1,500	0.0006
Neutrophils (cells/μL)	4,000 ± 800	6,500 ± 1,200	0.009
Lymphocytes (cells/μL)	2,500 ± 500	2,200 ± 400	0.072
Monocytes (cells/μL)	500 ± 100	700 ± 150	0.124
Red Blood Cells (RBC) (million cells/μL)	5.2 ± 0.3	5.4 ± 0.4	0.362

Both morning and afternoon strength training sessions elicited a clear acute leukocytosis, as reflected by significant increases in total white blood cell (WBC) counts from pre- to post-training (Tables 2 and 3). In the morning session, WBC counts increased significantly ($p = 0.0006$), accompanied by a statistically significant rise in neutrophil counts ($p = 0.009$). In contrast, the afternoon session also demonstrated a significant increase in WBC counts ($p = 0.0003$), while the corresponding increase in neutrophils approached statistical significance but did not reach it ($p = 0.052$), indicating a trend-level response.

Table 3

Hematological Responses to Afternoon Strength Training in Female Weightlifters

Group Parameter	Afternoon		P-value
	At rest	Post training	
No.	30	30	-
White Blood Cells (WBC) (cells/μL)	7,500 ± 1,200	10,500 ± 1,700	0.0003
Neutrophils (cells/μL)	4,300 ± 900	6,800 ± 1,300	0.052
Lymphocytes (cells/μL)	2,600 ± 550	2,300 ± 450	0.216
Monocytes (cells/μL)	550 ± 120	750 ± 170	0.325
Red Blood Cells (RBC) (million cells/μL)	5.3 ± 0.3	5.5 ± 0.4	0.169

Lymphocyte, monocyte, and red blood cell (RBC) counts did not change significantly following either morning or afternoon training sessions. These findings suggest that the acute immune response to high-intensity strength training was primarily driven by leukocyte and neutrophil mobilization, with no meaningful alterations in erythrocyte parameters.

Table 4

Comparison of Hematological Parameters at Rest: Morning vs. Afternoon in Female Weightlifters

Group Parameter	At rest		P-value
	Morning	Afternoon	
No.	30	30	-
White Blood Cells (WBC) (cells/µL)	7,000 ± 1,000	7,500 ± 1,200	0.852
Neutrophils (cells/µL)	4,000 ± 800	4,300 ± 900	0.436
Lymphocytes (cells/µL)	2,500 ± 500	2,600 ± 550	0.448
Monocytes (cells/µL)	500 ± 100	550 ± 120	0.453
Red Blood Cells (RBC) (million cells/µL)	5.2 ± 0.3	5.3 ± 0.3	0.198

Comparisons of hematological parameters between morning and afternoon sessions at rest (Table 4) and post-training (Table 5), performed using independent-samples statistical testing, revealed no statistically significant between-group differences for any hematological variable. The high p-values observed (e.g., WBC at rest, p = 0.852) indicate substantial overlap between morning and afternoon measurements, suggesting that baseline hematological status and post-exercise immune responses were broadly comparable across training times.

Overall, these results indicate that while strength training induces an acute hematological response characterized by leukocytosis, the magnitude of this response does not differ significantly between morning and afternoon sessions, except for a more robust neutrophil response following morning training.

Table 5

Comparative Analysis of Hematological Responses Post Training: Morning vs. Afternoon in Female Weightlifters

Group Parameter	Post training		P-value
	Morning	Afternoon	
No.	30	30	-
White Blood Cells (WBC) (cells/µL)	10,000 ± 1,500	10,500 ± 1,700	0.446
Neutrophils (cells/µL)	6,500 ± 1,200	6,800 ± 1,300	0.182
Lymphocytes (cells/µL)	2,200 ± 400	2,300 ± 450	0.568
Monocytes (cells/µL)	700 ± 150	750 ± 170	0.439
Red Blood Cells (RBC) (million cells/µL)	5.4 ± 0.4	5.5 ± 0.4	0.446

Table 6

Hormonal and Biochemical Responses to Morning Strength Training in Female Weightlifters

Group Parameter	Morning		P-value
	At rest	Post training	
No.	30	30	***
Testosterone (nmol/L)	24 ± 4	26 ± 5	0.1221
Cortisol (nmol/L)	500 ± 100	600 ± 120	0.2361
Creatinine (mg/dL)	1.0 ± 0.1	1.1 ± 0.1	0.1258

Hormonal responses to acute strength training are presented in Tables 6 and 7. Testosterone and cortisol levels did not change significantly from pre- to post-training in either the morning or afternoon sessions. These findings indicate that a single high-intensity strength training session did not elicit marked acute alterations in anabolic or catabolic hormone concentrations in this cohort. In contrast, serum creatinine levels increased significantly following afternoon training (p = 0.0003), whereas no significant change was observed following morning training. This finding suggests a greater acute metabolic or muscular load associated with afternoon sessions, and represents the only biochemical parameter showing a statistically significant time-specific response.

Table 7

Hormonal and Biochemical Responses to Afternoon Strength Training in Female Weightlifters

Group Parameter	Afternoon		P-value
	At rest	Post training	
No.	20	20	***
Testosterone (nmol/L)	20 ± 3	22 ± 4	0.162
Cortisol (nmol/L)	300 ± 80	400 ± 100	0.3321
Creatinine (mg/dL)	1.0 ± 0.1	1.2 ± 0.1	0.0003

Between-session comparisons at rest (Table 8) revealed a pronounced and statistically significant difference in cortisol concentrations, with higher levels in the morning than in the afternoon (p = 0.0001). This finding is consistent with the well-established circadian rhythm of cortisol secretion. Testosterone and creatinine levels at rest did not differ significantly between time points.

Table 8

Comparative Analysis of Hormonal and Biochemical Parameters at Rest: Morning vs. Afternoon in Female Weightlifters

Group Parameter	At rest		P-value
	Morning	Afternoon	
No.	20	20	***
Testosterone (nmol/L)	24 ± 4	20 ± 3	0.1362
Cortisol (nmol/L)	500 ± 100	300 ± 80	0.0001
Creatinine (mg/dL)	1.0 ± 0.1	1.0 ± 0.1	0.8652

Post-training comparisons between morning and afternoon sessions (Table 9) showed no statistically significant differences in testosterone or creatinine concentrations. Cortisol levels post-training demonstrated a trend toward higher values following morning training (p = 0.058), but this did not reach statistical significance.

Table 9

Comparative Analysis of Hormonal and Biochemical Responses Post Training: Morning vs. Afternoon in Female Weightlifters

Group Parameter	Post training		P-value
	Morning	Afternoon	
No.	20	20	***
Testosterone (nmol/L)	26 ± 5	22 ± 4	0.2398
Cortisol (nmol/L)	600 ± 120	400 ± 100	0.058
Creatinine (mg/dL)	1.1 ± 0.1	1.2 ± 0.1	0.1852

Discussion and Conclusion

The present study examined acute hormonal, hematological, and biochemical responses to standardized high-intensity strength training performed at different times of day in trained female weightlifters. The principal findings were: (i) strength training induced a clear acute leukocytosis irrespective of training time, (Einiö et al.) neutrophil mobilization was statistically significant following morning training and trend-level following afternoon training, (iii) testosterone and cortisol did not exhibit significant acute post-exercise changes, (iv) serum creatinine increased significantly only following afternoon training, and (v) cortisol concentrations at rest were markedly higher in the morning than in the afternoon, reflecting a robust circadian effect.

Both morning and afternoon sessions elicited significant increases in total white blood cell counts, consistent with well-documented exercise-induced leukocytosis. This response is typically attributed to catecholamine-mediated demargination and acute immune cell redistribution rather than inflammation per se. The significant increase in neutrophils observed following morning training, contrasted with a trend-level response in the afternoon, suggests a potential time-dependent modulation of innate immune mobilization. However, no formal time × session interaction was tested.

Importantly, between-session comparisons at rest and post-training revealed no statistically significant differences in hematological parameters, indicating that baseline immune status and overall acute immune responses were comparable across training times. Thus, while subtle differences in neutrophil responsiveness may exist, the present findings do not support a clinically meaningful divergence in hematological stress between morning and afternoon strength training.

Previous work by Banitalebi and colleagues examining immune responses in elite athletes demonstrated training-induced leukocyte fluctuations that varied with training load and recovery status. However, those studies were conducted over longer monitoring periods and in mixed-sex or male-dominant samples. The current findings extend this literature by demonstrating that, in trained female weightlifters, acute immune responses to strength training are largely preserved across time of day, with only minor differences in magnitude (Banitalebi Dehkordi et al., 2022).

Supporting our results, Bachero-Mena et al. (2017) investigated the effects of strength training on blood parameters in high-level athletes over an entire athletic season. Their study found increases in certain hematological parameters and potential immune system suppression throughout the season. This longitudinal perspective reinforces the importance of monitoring hematological and immune responses to strength training and emphasizes the acute effects observed in our study.

Revealing no significant differences between morning and afternoon measurements. The mean white blood cell (WBC) count at rest was 7,000 ± 1,000 cells/μL in the morning, with neutrophil counts recorded as 4,000 ± 800 cells/μL in the morning and 4,300 ± 900 cells/μL in the afternoon. Lymphocyte counts remained consistent throughout the day, indicating stable immune readiness. Monocyte counts were 500 ± 100 cells/μL in the morning and 550 ± 120 cells/μL in the afternoon, reflecting a stable baseline. Red blood cells (RBC) were 5.2 ± 0.3 million cells/μL in the morning and 5.3 ± 0.3 million cells/μL in the afternoon, suggesting that the oxygen transport capacity remains unchanged irrespective of the time of training. These findings indicate that the immune response to exercise is consistent throughout the day.

Lundby et al. (2024) investigated the effects of eight weeks of heavy strength training on well-trained elite rowers, reporting significant increases in hemoglobin mass and VO2 peak. This improvement enhances exercise performance and oxygen transport capabilities. Their findings are consistent with our observation of stable RBC counts, indicating that oxygen transport capacity remains consistent between morning and afternoon sessions, regardless of the training time.

da Silva et al. (2021) studied resistance training in older hemodialysis patients with chronic kidney disease (CKD), noting that low hemoglobin concentrations impaired the effectiveness of resistance training in increasing fat-free mass. Their research underscores the importance of maintaining hematological health to optimize training outcomes, reinforcing the relevance of our findings on stable RBC counts.

Ammar et al. (2016) explored the relationship between muscle damage biomarkers and redox status in response to weightlifting at different times of day. They found higher levels of oxidative stress markers and specific biomarkers of muscle damage in the morning and evening sessions compared with the afternoon sessions. This suggests that while specific stress responses may vary by time of day, overall immune readiness remains consistent, aligning with our observation of stable immune responses.

Contrary to expectations based on chronobiological theory, neither testosterone nor cortisol showed significant acute pre-to-post-exercise changes in either training condition. These findings align with evidence suggesting that acute hormonal fluctuations following resistance exercise may be modest in trained athletes, particularly when training volume and rest intervals are tightly controlled.

A pronounced diurnal variation in cortisol concentrations was observed at rest, with significantly higher levels in the morning than in the afternoon. This finding reflects the well-established circadian rhythm of cortisol secretion and confirms that the study protocol was sensitive to baseline chronobiological variation. However, post-training cortisol levels did not differ significantly between morning and afternoon sessions, although a trend toward higher post-exercise cortisol following morning training was noted. This pattern may indicate that exercise-induced cortisol responses are partially constrained by baseline circadian amplitude rather than being strongly modulated by training time alone.

The results align with the observations of Cintineo et al. (2018), who emphasized that training volume is a critical factor influencing the training stimulus and subsequent metabolic and hormonal responses. Their study suggests that the intensity and volume of training sessions can significantly impact hormonal and biochemical outcomes. Therefore, the lack of significant changes in testosterone, cortisol, and creatinine levels in our study may be attributed to the specific training volume and intensity employed.

Duan & Lu (2024) conducted a randomized controlled trial comparing resistance training (RT) and aerobic training (AT) in obese men with type 2 diabetes mellitus. They observed greater adaptive hormonal responses, including changes in testosterone and cortisol levels, with RT compared to AT. Although their research focused on a different population, their findings highlight that variations in training type and intensity can influence hormonal responses. This underscores the potential for different outcomes in hormonal and biochemical parameters depending on the training regimen.

Serum creatinine increased significantly following afternoon training but not following morning training, making it the only biochemical parameter to show a statistically significant time-specific response. This finding suggests a greater acute metabolic or muscular load associated with afternoon sessions, potentially related to higher neuromuscular efficiency, greater force output, or cumulative daily activity before training.

Given that creatine supplementation was standardized across participants, differential effects of supplementation are unlikely to explain this observation. Instead, the creatinine response may reflect time-of-day variation in muscle metabolism or renal clearance, an area that warrants further investigation. Importantly, this result contradicts any blanket statement that “no biochemical changes occurred post-training” and underscores the need for a nuanced interpretation of time-specific responses.

The significant post-exercise increase in serum creatinine observed following afternoon training, but not morning sessions, aligns with existing evidence indicating a time-of-day effect on exercise-induced metabolic responses (Al-temimi et al., 2016) reported that serum creatinine and blood urea nitrogen levels were significantly higher following evening compared to morning exercise, suggesting a greater renal and metabolic response later in the day. This finding supports the current observation that afternoon training induces a more pronounced increase in serum creatinine, indicating elevated metabolic and muscular load during later sessions. Kim et al. (2023) further explained that diurnal variations in physiological and metabolic functions—such as increased body temperature, hormonal activity, and neuromuscular efficiency in the afternoon—enhance performance capacity and energy turnover. These circadian factors likely contribute to the heightened biochemical response observed in afternoon exercise. Chernozub et al. (2020) emphasized that serum creatinine is a sensitive biomarker of training intensity and adaptive muscular stress, increasing proportionally with load and exercise-induced metabolic demand. Thus, the elevated creatinine following afternoon training plausibly reflects greater acute muscular metabolism and cumulative physiological activation throughout the day.

Several studies have investigated time-of-day effects on exercise performance and physiological stress markers, providing conceptual context for the current findings. However, as the present study did not include direct measurements of performance outcomes, muscle damage biomarkers (e.g., CK, LDH), or aerobic capacity (e.g., VO_2peak), comparisons to these studies should be considered conceptual rather than confirmatory.

(Mirizio et al., 2020; Valenzuela et al., 2024) demonstrated that exercise performance peaks in the late afternoon, coinciding with higher body temperature, hormonal activation, and neuromuscular efficiency. Similarly, VO_2max and aerobic capacity are reported to be significantly higher in the evening compared with the morning, likely due to circadian variations in oxygen transport and metabolic enzyme activity.

In addition, markers of muscle fatigue and biochemical stress, such as CK and LDH, tend to exhibit higher values after afternoon exercise, reflecting greater muscular strain and metabolic turnover. Resistance exercise studies also suggest enhanced anabolic signaling responses later in the day, indicating time-dependent differences in the potential for muscle adaptation (Chtourou et al., 2013).

Collectively, these findings provide conceptual support for the notion that afternoon exercise induces greater physiological and metabolic stress; however, the absence of direct measures of performance or muscle damage in the current study necessitates cautious interpretation of these parallels.

This study is limited by its acute design, modest sample size for hormonal analyses, the absence of interaction modeling, and the lack of performance or recovery markers. Additionally, reliance on pre- and post-exercise measurements from a single session limits inference about cumulative or adaptive responses.

This study examined acute hormonal, hematological, and biochemical responses to a single session of high-intensity strength training performed at different times of day in trained female weightlifters. The findings demonstrate that acute strength training induces a robust immune response characterized by significant leukocytosis, regardless of training duration. Neutrophil mobilization was statistically significant following morning training and at a trend level following afternoon training, suggesting minor time-dependent differences in innate immune activation.

Acute testosterone and cortisol concentrations did not change significantly from pre- to post-exercise in either training condition. However, a pronounced diurnal variation in cortisol was observed at rest, with significantly higher morning values compared to afternoon values, consistent with established circadian physiology. In contrast to the hormonal findings, serum creatinine increased significantly only following afternoon training, indicating a time-specific acute metabolic response.

Collectively, these results indicate that while baseline circadian variation is evident—particularly for cortisol—acute post-exercise hormonal and hematological responses to high-intensity strength training are largely comparable between morning and afternoon sessions in trained female weightlifters. The only parameter demonstrating a clear time-of-day–specific post-exercise response was creatinine following afternoon training.

Based on the findings and limitations of the present study, the following recommendation is proposed: future studies should incorporate statistical models that explicitly test time-of-day × exercise interactions better to quantify the chronobiological modulation of acute physiological responses. Expanded Biochemical Profiling: Given the significant creatinine response observed following afternoon training, additional markers of muscle metabolism and recovery (e.g., creatine kinase, urea, myoglobin) should be assessed to clarify time-dependent metabolic stress. True Longitudinal Designs: Longitudinal studies incorporating repeated physiological and performance measurements across training phases are required to determine whether acute time-of-day responses translate into meaningful chronic adaptations.

Integration of Performance Outcomes: Future research should align physiological measurements with performance indicators such as maximal strength, power output, and fatigue resistance to establish functional relevance. Adequately Powered Hormonal Analyses: Larger sample sizes are needed to improve statistical power for hormonal outcomes and allow calculation of effect sizes and confidence intervals to support practical interpretation.

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Declaration of Interest

The authors of this article declared no conflict of interest.

Ethical Considerations

The study protocol adhered to the principles outlined in the Declaration of Helsinki, which provides guidelines for ethical research involving human participants. Ethical considerations in this study were that participation was entirely optional.

Transparency of Data

In accordance with the principles of transparency and open research, we declare that all data and materials used in this study are available upon request.

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Authors' Contributions

All authors equally contribute to this study.

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