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Effect of intensive training on immune system cells in elite female weightlifters

Mehmet Ali Karaselek[1*], Serkan Kuccukturk[2], Tugce Duran[3]

SUMMARY

OBJECTIVE: This study aimed to investigate the effects of intense weightlifting training on lymphocyte and natural killer cell subgroups, which are the major cells of the immune system, in elite female weightlifters.

METHODS: A total of 20 elite female weightlifters were evaluated using flow cytometry before training (pre-T), immediately after training (post-T), and after a 120-min rest period (rest-T).

RESULTS: Post-T and rest-T showed significant decreases in helper T (Th) and cytotoxic T compared with pre-T (p=0.045, p<0.001 and p=0.05, p<0.001, respectively). B and natural killer cells were higher in post-T and rest-T than in pre-T. The increase in B cells was significant in pre-T/rest-T (p<0.001) but not in pre-T/post-T (p=0.122). Intense training significantly increased natural killer cells in both post-T and rest-T (p<0.001). $CD56^{bright}$ and $CD56^{dim}$ natural killer cell subgroups were significantly lower in post-T and rest-T than in pre-T (p=0.005, p=0.006 and p<0.001, p=0.004, respectively).

CONCLUSION: This study shows that intense weightlifting alters peripheral lymphocyte and natural killer subgroup ratios, being the first investigation in this field.

KEYWORDS: CD4. CD8. CD19. NK.

INTRODUCTION

Weightlifting relies on strength, which is crucial for success[1]. Regular intense training enhances power generation[2]. Some studies show balanced exercise benefits immune cells[3-6]. Conversely, strenuous long-term exercise harms the immune system, increasing infection risk, especially in athletes[4-8].

Training affects lymphocytes, with lymphocytosis observed during and immediately after exercise, returning to pre-training levels within 24 h[3]. Some studies on weightlifting athletes reported decreased lymphocyte and leukocyte values post-training[9,10]. Lymphocytes include T and B cells, while natural killer (NK) cells are divided into $CD56^{bright}$ and $CD56^{dim}$ subsets. $CD56^{dim}$ cells are cytotoxic, and $CD56^{bright}$ cells secrete cytokines[11]. No study investigating peripheral lymphocyte and NK subgroups in male and female weightlifting athletes was found.

This study aimed to investigate the effect of 120-min weightlifting training (90–100% load) on CD4+, CD8+, CD19+ B, CD3-CD16+56+ NK cells, and subgroups ($CD56^{bright}$ and $CD56^{dim}$) in female weightlifters using flow cytometry.

METHODS

Participants

A total of 20 elite female weightlifters (≥18 years) who had been actively participating in national teams for the past 3 years were included. Exclusion criteria were <18 years of age, <3 years of sports experience, musculoskeletal issues, recent surgery/trauma, hematological/systemic disease, and medication affecting blood values. In addition, care was taken not to collect blood from the athletes during the menstrual period. For this purpose, the menstrual periods of the athletes were questioned and the blood required for the study was taken within 5–12 days, which is the earlier period in the menstrual cycle. The Institutional Review Board approved the study (2023/029; December 24, 2022), and written informed consent was obtained from participants.

Study design

In January 2023, 3 mL of K3 EDTA blood samples were collected three times: pre-training (pre-T; 60 min before), post-training (post-T; 10 min after), and rest period (rest-T; 120 min after) for analysis.

[1]Necmettin Erbakan University, Faculty of Medicine, Department of Internal Medicine – Konya, Turkey.
[2]Karamanoğlu Mehmetbey University, Faculty of Medicine, Department of Medical Biology – Karaman, Turkey.
[3]KTO Karatay University, Faculty of Medicine, Department of Medical Genetic – Konya, Turkey.
*Corresponding author: malikaraselek@gmail.com

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Complete blood count analysis

A complete blood count was performed with Cell-Dyn 1800 (Abbott Diagnostics, Abbott Park, IL, USA). White blood cell (WBC) (10^3/μL), neutrophil (10^3/μL), and lymphocyte (10^3/μL) counts were analyzed from each blood sample.

Flow cytometric peripheral lymphocyte and natural killer subgroup analysis

Peripheral lymphocyte and NK cell subgroup analysis involved gradient centrifugation using Ficoll-Hypaque for cell isolation. Surface staining was conducted with specific monoclonal antibodies (mAbs). For lymphocyte subsets, CD45, CD3, CD4, CD8, and CD19 mAbs were used, while, for NK and NK cell subsets, CD3, CD16, and CD56 mAbs were added. Following incubation and washing, cell count and analysis were performed using flow cytometry (BD Canto II) and the FACSDiva software. Absolute values were calculated using the [(WBCx1000)/% cell ratio] formula.

Training procedure

Athletes followed a 120-min training program comprising a 15-min warm-up (static flexibility, joint mobility, stretching, and balance exercises), a 90-min main training (3 sets of maximal repetitions at 90–100% load with auxiliary exercises for weightlifting movements), and a 15-min cool-down (static flexibility and stretching exercises).

Statistical analysis

Data normality was assessed with the Shapiro-Wilk test. Two-tailed tests were used with $p<0.05$ as the significance threshold. Mean and SEM were reported in the tables. One-way repeated-measures ANOVA was conducted to analyze the main effects across measurement points (pre-T, post-T, and rest-T), followed by Bonferroni correction for post-hoc comparisons. Statistical analyses were performed using the open-source jamovi statistical platform (Version 1.2.1.1) [The jamovi project 2021, Sydney, Australia, Jamovi. Retrieved from https://www.jamovi.org].

RESULTS

The study included 20 female elite weightlifters with a mean age of 18.47±1.61 years. Significant training-related changes were observed in WBC, lymphocyte, neutrophil, and peripheral lymphocyte/NK cell subgroups ($p<0.05$) (Table 1 and Figure 1).

Complete blood count analysis results

Pre-T/post-T and pre-T/rest-T comparisons showed significant increases in WBC, neutrophil, and lymphocyte counts. The elevation in WBC count was significant in pre-T/post-T and pre-T/rest-T ($p<0.001$), while the change between post-T/rest-T was not significant ($p=0.073$). Lymphocyte count significantly increased in all three comparisons ($p<0.001$; $p<0.001$; and $p=0.01$). Neutrophil count significantly changed in pre-T/post-T and pre-T/rest-T ($p=0.03$), but not in post-T/rest-T ($p=0.74$) (Tables 1 and 2).

Peripheral lymphocyte subgroup analysis results

Peripheral lymphocyte subgroup analysis compared changes in CD3+CD4+ Th, CD3+CD8+ CTLs, CD19+ B, and CD16+56+ NK cells during post-T and rest-T periods with pre-T. The cells significantly decreased in post-T and rest-T periods compared with basal values, with statistical significance in pre-T/post-T and pre-T/rest-T comparisons ($p=0.045$ and $p<0.001$, respectively). The change between post-T and rest-T was also statistically significant ($p<0.001$). CTLs decreased in post-T and rest-T, with non-significant reductions in pre-T/post-T and pre-T/rest-T ($p=1.0$ and $p=0.102$), but with a significant change between post-T and rest-T ($p<0.001$). B cells

Table 1. The results of the parameters measured before, after, and during the training period.

Parameters	Pre-T	Post-T	Rest-T	p-value
WBC (10^3/μL)	5.6±0.32	6.94±0.36	7.54±0.35	<0.001
Neutrophil (10^3/μL)	3.41±0.32	4.4±0.26	5.09±0.25	<0.001
Lymphocyte (10^3/μL)	1.5±0.11	1.92±0.12	1.81±0.08	0.003
CD3+CD4+ (10^3/μL)	2463.74±174.52	2339.04±200.92	2076.68±180.61	<0.001
CD3+CD8+ (10^3/μL)	1692.25±170.08	1654.31±113.89	1357.72±108.42	0.018
CD19+ (10^3/μL)	730±61.56	833.21±61.2	1913.16±102.43	<0.001
CD16+CD56+ (10^3/μL)	592.82±48.08	904.13±68.67	1225.02±77.32	<0.001
$CD56^{bright}$ (%)	1.60±0.39	0.18±0.02	0.22±0.02	<0.001
$CD56^{dim}$ (%)	2.84±0.46	0.34±0.06	1.07±0.24	<0.001

significantly increased in post-T and rest-T compared with pre-T, with significant increases in pre-T/post-T, pre-T/rest-T, and post-T/rest-T (p=0.012, p<0.001, and p<0.001, respectively) (Tables 1 and 2).

Natural killer cell subgroup analysis results

NK cells significantly increased due to training, showing statistical significance in all comparisons: pre-T/post-T, pre-T/rest-T, and post-T/rest-T (p<0.001, p<0.001, and p<0.001, respectively). $CD56^{bright}$ cell rates decreased in post-T compared with pre-T (p=0.005) and increased in rest-T compared with pre-T (p=0.006). $CD56^{dim}$ cell rates decreased in post-T compared with pre-T (p=0.005) and increased in rest-T compared with pre-T (p=0.004) (Tables 1 and 2).

DISCUSSION

Intense training affects weightlifters' immune system and its cells[12,13]. This study investigated changes in immune system cells during pre-T, post-T, and rest-T in weightlifting athletes using flow cytometry. Th and CTLs decreased in post-T and rest-T, while B and NK cells increased. Limited literature exists on peripheral lymphocyte subgroups in weightlifters, making our findings valuable for comparison with other sports studies.

The immune system plays a crucial role in protecting the body from microorganisms and maintaining its health. Exercise has been reported to have both positive and negative effects on the immune system, especially with intense and long-term exercise[14,15]. Exercise regulates the immune system by influencing leukocytes, which are the major immune cells[16]. Intense training generally

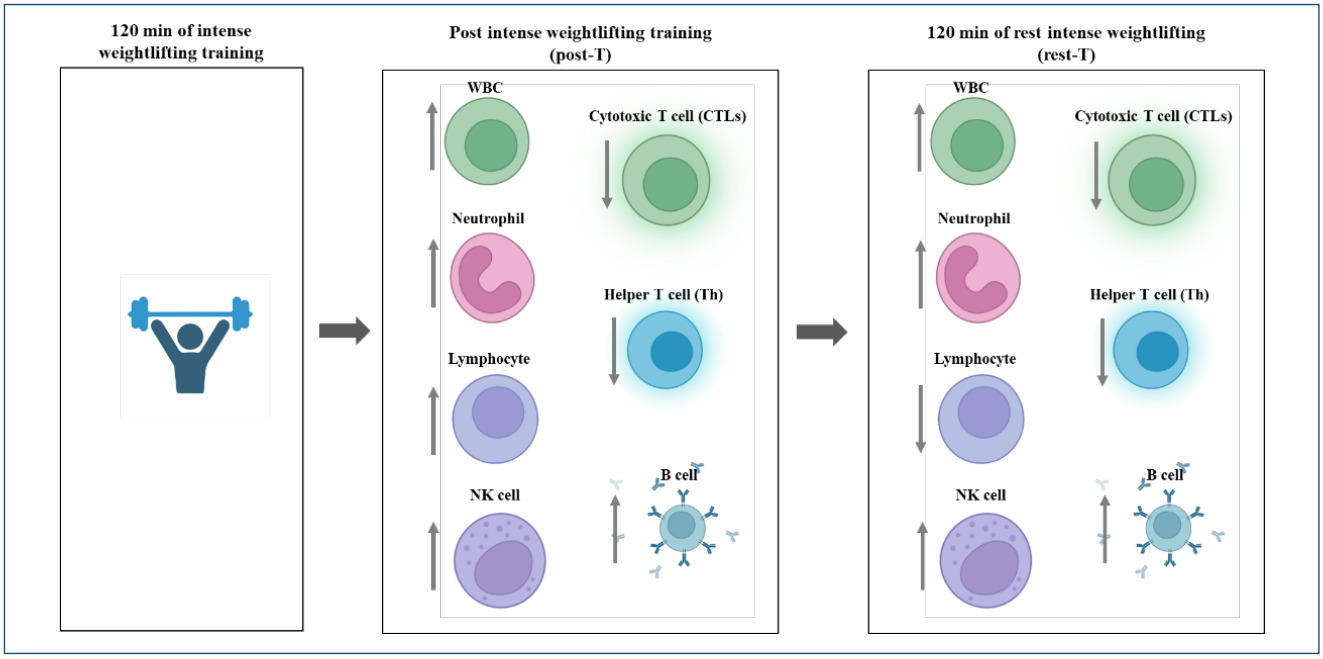

Figure 1. Change in peripheral lymphocyte subgroups due to training.

Table 2. Comparative statistical post-hoc analysis results of parameters.

Parameters	Pre-T/Post-T	Pre-T/Rest-T	Post-T/Rest-T
WBC ($10^3/\mu L$)	<0.001	<0.001	0.073
Neutrophil ($10^3/\mu L$)	0.001	<0.001	0.01
Lymphocyte ($10^3/\mu L$)	0.03	0.03	0.74
CD3+CD4+ ($10^3/\mu L$)	0.045	0.045	<0.001
CD3+CD8+ ($10^3/\mu L$)	1	0.102	<0.001
CD19+ ($10^3/\mu L$)	0.012	<0.001	<0.001
CD16+CD56+ ($10^3/\mu L$)	<0.001	<0.001	<0.001
$CD56^{bright}$	0.005	0.006	>0.05
$CD56^{dim}$	<0.001	0.004	0.008

leads to leukocytosis, but the response of leukocyte subgroups may vary[13,17,18]. In our study, WBC values significantly increased from pre-T ($5.6x10^3/\mu L$) to post-T ($6.94x10^3/\mu L$) and rest-T ($7.54x10^3/\mu L$), aligning with literature findings. Lymphocyte count typically rises during and immediately after exercise and then returns to pre-exercise levels within 30–60 min[13,17,18]. A recent meta-analysis also reported an immediate increase in total lymphocyte count after exercise, followed by regression within 1–2 h[19]. In our study, lymphocyte count significantly increased in post-T ($1.92\times10^3/\mu L$) and rest-T ($1.81\times10^3/\mu L$) compared with pre-T ($1.5\times10^3/\mu L$). However, the decrease in rest-T was not statistically significant compared with post-T. While post-training lymphocytosis aligns with the literature, the sustained elevation in the resting period after training may be specific to weightlifting, as other sports studies showed different patterns.

A meta-analysis on training-related peripheral lymphocyte subgroups indicated that Th cells returned to basal values within 1 h after exercise (p=0.74), CTL cells decreased (p=0.001), and NK cells increased above basal values (p=0.01)[19]. Studies in the literature consistently report decreased T cell proliferation during and after exercise. Trained male athletes showed significant reductions in mitogen-stimulated T cell proliferation following increased treadmill exercise[20]. Similar findings were observed in female athletes who underwent 2.5 h of treadmill running and cycling training[21]. In another study, comparing jogging at 80% VO_{2max} for 45 min with the same exercise at 50% VO_{2max}, lymphocyte proliferation decreased by 50 and 25%, respectively[21]. The changes in immune cells and recovery time after exercise are closely linked to exercise duration and intensity. In our study, Th and CTL cells decreased in the post-T period and did not return to pre-T levels during the 120-min rest-T period. Although our findings align relatively well with the literature, they contradict the data showing no return to baseline within the 120-min period. This discrepancy may be attributed to the specific sport type and intensity, highlighting the different responses of immune system cells to different sports training.

Senescent T cells theoretically undergo apoptosis, while naive T cells from the thymus enter the periphery, maintaining the peripheral lymphocyte count. Naive lymphocytes increase 1 h after exercise, and after 2 h, they transition to the periphery, restoring the lymphocyte count[22]. Exercise intensity is believed to increase apoptosis rates in T cell subgroups, leading to decreased cell numbers, which aligns with the findings of our study[23]. Furthermore, our study demonstrates that the intensity of weightlifting training induces more substantial decreases in T cell subgroups, with varying recovery times for these cells.

Unlike Th cells, B cells exhibit an increased response to exercise. Limited literature is available on weightlifting athletes. Turner et al. found that B cells nearly doubled immediately after exercise in healthy non-athletes but returned to baseline within 60 min[24]. In athletes performing at maximum capacity, B cell counts doubled post-training and tripled after resting[22]. The impact of training on B cells remains unclear in the literature. Despite varying data, the increase in B cells during post-T and rest-T periods aligns relatively well with the existing literature, suggesting that the sport type and training intensity may contribute to these differences, similar to Th cells.

The effects of exercise on NK cell subgroups are still not clear, with limited studies available. Our study revealed an overall increase in NK cell count but a decrease in $CD56^{bright}$ and $CD56^{dim}$ cells. Elite swimmers demonstrated an increase in $CD56^{bright}$ and a decrease in $CD56^{dim}$ NK subgroups[25]. NK cell responses to training vary across studies, with some reporting an increase and others a decrease[19,25]. Therefore, our study contributes to understanding the impact of intense exercise on NK cell subgroups, highlighting their dynamic nature.

The decrease in immune system cells in weightlifting female athletes poses an infection risk. Unlike some sports, T cells did not fully recover within 120 min after weightlifting training. Thus, ensuring an adequate rest period, post-training can minimize the athletes' susceptibility to potential infections.

CONCLUSION

Regular training is crucial for athletes' success, but inadequate rest can lead to significant changes in immune system cells. While studies on immune system cells exist for various sports, none specifically focuses on weightlifting athletes. This is the first study to examine the effects of weightlifting on peripheral lymphocyte and NK cell subgroups. It revealed that the decrease in cells due to intense weightlifting training did not fully recover within 120 min of rest. Long-term follow-up studies investigating cell recovery times could greatly contribute to mitigating infection risks for athletes. Hence, this study has the potential to inform future research in this field.

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AUTHORS' CONTRIBUTIONS

MAK: Conceptualization, Methodology, Project administration, Writing – original draft, Writing – review & editing. **SK:** Formal Analysis, Methodology, Validation, Visualization. **TD:** Methodology, Validation, Visualization, Writing – review & editing.

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