


Cellular immune response to acute exercise: Comparison of endurance and resistance exercise

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Abstract

Objectives: Exercise-induced cellular mobilization might play a role in treatment and prevention of several diseases. However, little is known about the impact of different exercise modalities on immune cell mobilization and clinical cellular inflammation markers. Therefore, the present study aimed to investigate differences between acute endurance exercise (EE) and resistance exercise (RE) on cellular immune alterations.

Methods: Twenty-four healthy men conducted an acute EE (cycling at 60% of peak power output) and RE (five exercise machines at 70% of the one-repetition maximum) session lasting 50 minutes in randomized order. Blood samples were collected before, after and one hour after exercise cessation. Outcomes included counts and proportions of leukocytes, neutrophils (NEUT), lymphocytes (LYM), LYM subsets, CD4/CD8 ratio, and the clinical cellular inflammation markers NEUT/LYM ratio (NLR), platelets/LYM ratio (PLR), and systemic immune inflammation index (SII).

Results: Alterations in all outcomes were revealed except for CD8⁺ T cells, CD4/CD8 ratio, NLR, and PLR. EE induced a stronger cellular immune response and provoked alterations in more immune cell populations than RE. SII was altered only after EE.

Conclusion: An acute EE session causes a stronger mobilization of immune cells than RE. Additionally, SII represents an integrative marker to depict immunological alterations.

KEYWORDS

cellular immune system, endurance exercise, exercise, inflammation, resistance exercise

Abbreviations: 1RM, one-repetition maximum; ANCOVA, analysis of covariance; EE, endurance exercise; LEUK, leukocytes; LYM, lymphocytes; NEUT, neutrophils; NK cell, natural killer cell; NLR, neutrophil/lymphocyte ratio; PBMC, peripheral blood mononuclear cell; PLR, platelet/lymphocyte ratio; RE, resistance exercise; SD, standard deviation; SII, systemic immune-inflammation index; T_{reg}, regulatory T cell; $\dot{V}O_{2max}$, maximal oxygen uptake; $\dot{V}O_{2peak}$, peak oxygen uptake.

Marit Lea Schlagheck and David Walzik shared first authorship.

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1 | INTRODUCTION

Alterations of the immune system in response to acute and chronic exercise have frequently been investigated in both healthy athletes¹ and clinical populations.^{2,3} While evidence suggests that chronic exercise has an anti-inflammatory effect,^{1,4,5} the impact of a single bout of (acute) exercise remains strongly discussed.⁶ Beside alterations of the soluble components of the immune system (eg, acute phase proteins, complement proteins, cytokines), the acute immune response to exercise is also marked by changes in the cellular compartment. In this context, the most commonly described effect is an exercise-induced increase in leukocytes (leukocytosis), as similarly observed during infection or sepsis.⁷⁻⁹ Underlying mechanisms for this leukocytosis are of distinct nature. One assumption is that exercise-induced shear stress detaches immune cells from vessel walls, especially in secondary lymphoid tissues such as lung, spleen and liver, subsequently flushing them into circulation.¹⁰ Additionally, catecholamines released through exercise are thought to bind to β -adrenergic receptors on leukocytes (LEUK), enhancing LEUK mobilization into circulation.¹¹

Going deeper into leukocytosis, the elevated LEUK counts are mainly attributed to an increase in neutrophils (NEUT) and lymphocytes (LYM) during exercise. While NEUT tend to increase long into recovery, LYM decrease shortly after exercise cessation. Within 24 hours, baseline levels of both are usually restored.¹² However, the timeframe of immunological recovery can differ considerably in dependence of duration and intensity of the exercise modality applied.¹³ The acute exercise-induced lymphopenia is interpreted differentially: Early studies supposed a period of immunodepression (termed "open-window"), possibly explaining the higher susceptibility to upper respiratory tract infection observed in the early recovery period.¹⁴ However, this theory is mainly limited to small studies with professional athletes, which are frequently marked by methodological limitations. In contrast, a more recent interpretation proposes a redistribution of LYM to potential sites of infection (eg, mucosa, skin) rather than an actual loss, thereby augmenting immune surveillance and regulation.⁶ Supporting this theory, Baek et al recently revealed a reduced risk of opportunistic infections in trained mice even after acute exercise bouts.¹⁵

Alterations of the cellular compartment of the immune system in response to exercise are of major relevance in both healthy and clinical populations. While diseased populations might benefit from exercise-induced changes in immune cells (eg, increased natural killer [NK] cell migration to tumor tissue),^{3,16} in competitive sports the cellular immune response is usually utilized to depict transient immunoinflammatory processes and determine restoration of immunological homeostasis. Beside using classical blood count analyses and flow cytometry to determine alterations in immune cells, Joisten et al proposed to transfer clinically established cellular inflammation markers into exercise immunology using data solely from blood count.¹⁷ In this context, the NEUT/LYM ratio (NLR), the platelet/LYM ratio (PLR), and the systemic immune inflammation index (SII) represent integrative, cost- and time-efficient markers to detect cellular

Summary statements

- The systemic immune inflammation index represents an appropriate, integrative marker to detect cellular immune responses after acute exercise.
- An acute bout of endurance exercise provokes a stronger cellular immune response than an acute bout of resistance exercise.
- Since immune cells are a prominent target in the treatment and prevention of various clinical conditions, the mobilization of immune cells especially through endurance exercise might contribute to reaching the therapeutic goal.

immune changes. The value of these markers was confirmed by further investigations.¹⁸

However, in regard to dissimilar functional characteristics of LYM subsets, a closer and more differential look (eg, NK cells, T cells, B cells, and their respective subsets) appears to be necessary to investigate cellular immune responses to acute exercise. Moreover, exercise modalities such as type, duration, and intensity must be considered when interpreting the magnitude and duration of exercise-induced cellular immune responses.¹²

Concerning the impact of exercise type on cellular immune response, studies on endurance exercise (EE) outweigh those on resistance exercise (RE), as indicated by numerous investigations in humans.¹⁹ Considering the distinct immune cell subsets, evidence suggests an increase in T cells and their subsets (T helper cells (CD4⁺), cytotoxic T cells (CD8⁺)) during prolonged EE. The relatively smaller increase in CD4⁺ cells compared to CD8⁺ cells is often indicated by a smaller CD4/CD8 ratio during exercise. In the recovery period, the T-cell count declines while the CD4/CD8 ratio increases.⁹ Similarly, NK cells increase during EE and decline afterward.⁹ For B cells, results of investigations are inconsistent, although the majority of studies found minor²⁰ or no²¹ changes in B cell counts in response to prolonged EE.

Concerning exercise intensity, Jamurtas et al recently conducted a randomized crossover study with 12 healthy male participants and revealed that HIIT (4 × 30 seconds all-out sprint with 4 minutes of recovery between intervals) provokes a greater cellular immune response than moderate continuous EE (30 minutes cycling at 70% $\dot{V}O_{2max}$).²² Similar results were found by Wahl et al when comparing 4 × 30 seconds ("all-out") with 4 × 4 minutes (90%-95% of PPO) cycling exercise.¹⁸ McCarthy and Dale additionally obtained similar results with respect to exercise duration, with longer exercise at equal intensity resulting in higher LEUK concentrations.¹³

Cellular immune responses to RE are similar to EE with amplitude and duration of immunological alterations being protocol-dependent.²³⁻²⁵ While most of the studies investigated the cellular immune response in either EE or RE, to our knowledge only one study compared the two modalities directly.²⁶ Results showed that immune cell



subsets reacted delayed and less pronounced after RE (standard circuit of 5 exercises with 3 sets of 10 repetitions at 60%-70% of 1RM) compared to peak aerobic (5 minutes at 90% of maximal oxygen uptake [$\dot{V}O_{2max}$]) and prolonged aerobic exercise (2 hours at 60% of $\dot{V}O_{2max}$). However, the protocol applied is of little practical relevance, since acute sessions of 2 hours at 60% of $\dot{V}O_{2max}$ are rather rare in clinical context as well as competitive and recreational sport. Building upon this, the aim of this study was to investigate potential differences between a single bout of EE and RE on alterations in cellular immune homeostasis. For EE and RE, a duration of 50 minutes was chosen to mimic exercise sessions that are frequently used within a clinical setting, competitive sport and recreational activities. Against this background, we quantified counts and proportions of LEUK, LYM, and their subsets (T cells, NK cells, B cells) as well as CD4/CD8 ratio and cellular inflammation markers (NLR, PLR, SII) in 24 healthy young men. We hypothesize that EE induces a more pronounced alteration of immune cells than RE.

2 | MATERIALS AND METHODS

The study was approved by the local ethics committee of the German Sport University Cologne and complied with the current principles of the Declaration of Helsinki. Prior to study participation, each participant signed written informed consent. Methodological procedures were previously described extensively elsewhere.²⁷ In the following, experimental design will be briefly described.

2.1 | Participants

Participants who met the following criteria were included: (a) male, (b) aged between 20 and 35 years, (c) no contraindications to physical activity, and (d) no drug intake during the last six weeks. Therefore, 24 healthy men (mean age of 24.6 ± 3.9 years, mean weight of 83.9 ± 10.5 kg and mean height of 182.4 ± 6.2 cm [values as mean \pm SD]) volunteered to participate.

2.2 | Study design

A randomized crossover study design was applied. The participants visited the laboratory on a total of three occasions. At baseline, strength and endurance capacities were assessed. During the second and third visits, the participants conducted a single bout of EE or RE, respectively. The sequence of the sessions (EE + RE or RE + EE) was randomized using a minimization procedure by Pocock and Simon²⁸ with the stratification factors smoking status, relative peak oxygen uptake ($\dot{V}O_{2peak}$) and a total strength value (see Baseline testing). The wash-out period between both exercise sessions was at least 48 hours.

2.3 | Baseline testing and randomization

At baseline, strength and endurance capacities were assessed. Strength capacity was measured via 1RMs on five exercise machines (Cybex International) in the following order: Chest press, Lat pull, Leg curl, Leg extension, and Back extension. A total strength value was calculated using the mean of all five 1RMs. To assess participants' $\dot{V}O_{2peak}$, an incremental cardiopulmonary exercise test on a bicycle ergometer (Ergoline) with spirometry (Cortex, MetaLyzer 3B-R2) was conducted. The obtained results were used to determine the exercise intensity of the interventions.

2.4 | Interventions

Both exercise sessions started between 8:00 AM and 11:00 AM and lasted 50 minutes. Participants received a standardized breakfast one hour beforehand. EE consisted of a five-minute warm-up at 50 Watt on a cycle ergometer, followed by 45 minutes at 60% of the previously measured peak power output. For RE, participants performed a five-minute warm-up at 50 Watt on a cycle ergometer, followed by a specific warm-up (15 repetitions at 30% of 1RM) at the five machines used at baseline. After that, the participants performed four sets of 8-10 repetitions at 70% of 1RM at each machine. If participants were not able to execute the required 8-10 repetitions with the determined weight, the weight was reduced in consultation with the participant and based on the subjective perception in the following set. On average, participants achieved the aspired repetitions at 70% of the assessed 1RM. Recovery time between each machine and set was one minute. Venous blood samples were collected immediately before (t_0), immediately after (t_1), and one hour after (t_2) each exercise session to isolate peripheral blood mononuclear cells (PBMC).

2.5 | Blood sampling and PBMC isolation

Blood samples were drawn into K2 EDTA. A blood count was performed from EDTA blood using a hematology analyzer (Sysmex Europe GmbH), and further PBMCs were isolated. Therefore, blood was diluted with PBS and layered carefully on top of a LYM separation medium (PromoCell GmbH) and centrifuged for 30 minutes at 800 g. Afterward, PBMCs were washed with PBS, suspended in Gibco™ Recovery™ Cell Culture Freezing Medium (Thermo Fischer Scientific) and frozen at -80°C overnight, using a freezing aid. Afterward, PBMCs were stored at -150°C until flow cytometry analysis. The blood count was used for calculation of the cellular inflammation markers NLR (neutrophil counts [$10^3/\mu\text{L}$]/LYM counts [$10^3/\mu\text{L}$]), PLR (platelet counts [$10^3/\mu\text{L}$]/LYM counts [$10^3/\mu\text{L}$]), and SII (platelet counts [$10^3/\mu\text{L}$] \times NEUT counts [$10^3/\mu\text{L}$]/LYM counts [$10^3/\mu\text{L}$]).



	RE + EE (n = 13)	EE + RE (n = 11)	Overall (n = 24)
$\dot{V}O_{2peak}$ (mL/kg/min)	48.8 ± 8.0	47.8 ± 6.9	48.3 ± 7.4
1RM chest press (kg)	109.7 ± 25.1	116.8 ± 17.6	113.3 ± 22.1
1RM lat pull (kg)	99.5 ± 22.2	103.5 ± 21.4	102.0 ± 21.5
1RM leg curl (kg)	72.5 ± 10.1	76.8 ± 16.7	74.1 ± 13.2
1RM leg extension (kg)	115.0 ± 26.1	122.7 ± 23.8	120.9 ± 24.8
1RM back extension (kg)	100.3 ± 21.6	100.0 ± 20.1	100.9 ± 20.4
Total strength value (kg)	99.7 ± 17.9	104.5 ± 14.2	101.9 ± 16.2

Note: Values are presented as mean ± standard deviation.

Abbreviations: 1RM, one repetition maximum; EE, endurance exercise; RE, resistance exercise; Total strength value, mean of all 1RM tests; $\dot{V}O_{2peak}$, peak oxygen uptake.

TABLE 1 Baseline performance results of study participants separated by exercise sequence

2.6 | Flow cytometry

PBMCs were thawed and two different panels were stained. For description of LYM subsets, PBMCs were stained with anti-CD3 PE-Cy7, anti-CD8 PE, anti-CD4 APC, anti-CD16 PE, anti-CD19 APC, and anti-CD56 APC-Cy7 (BD Bioscience). LYM were gated by their size and granularity. T cells were gated as CD3⁺ and further divided into CD4⁺ and CD8⁺. CD3⁻ LYM were onwards divided into CD16⁺ NK cells and CD19⁺ B cells. NK cells were subsequently divided into CD56^{bright} and CD56^{dim}. To investigate T_{regs}, PBMCs were stained with anti-CD3 PE-Cy7, anti-CD4 APC-Cy7, anti-CD127 PE, and anti-CD25 APC (BD Bioscience). T_{regs} were gated as CD3⁺ CD4⁺ CD25⁺ CD127^{dim}. Moreover, ratios of CD4⁺/CD8⁺ and CD56^{bright}/CD56^{dim} were calculated. A detailed description of the applied flow cytometry processing is presented in the Appendix (Flow cytometry procedures).

2.7 | Statistical analysis

Statistical analysis was proceeded using SPSS statistics 25 (IBM®). For all parametric procedures, level of significance was set at $P \leq .05$. Since immunological alterations concerning the investigated outcomes usually return to baseline within 24 hours following an acute bout of exercise, a wash-out period of at least 48 hours was applied. Subsequently, data of equal training modalities were pooled for further analysis. Due to the great number of participants and the following robustness of the ANCOVA,²⁹ an implementation of testing for normal distribution has been waived. Finally, to observe time and interaction (time × group) effects of EE and RE, baseline adjusted ANCOVAs for within and between group comparisons were executed. In the event of a violation of Mauchly's test of Sphericity,

Greenhouse-Geisser correction was applied for interpretation. In case of significant results, post hoc analyses according to Bonferroni were conducted and Cohen's *d* effect sizes were calculated. Detailed ANCOVA results of all outcomes (*P* value, *F* value, degrees of freedom, partial eta squared [η_p^2]), Cohen's *d* for significant post hoc time effects and raw data (as mean ± SD) are given in the appendix (see Appendix, Tables S1, S2 and S3).

3 | RESULTS

3.1 | Baseline characteristics

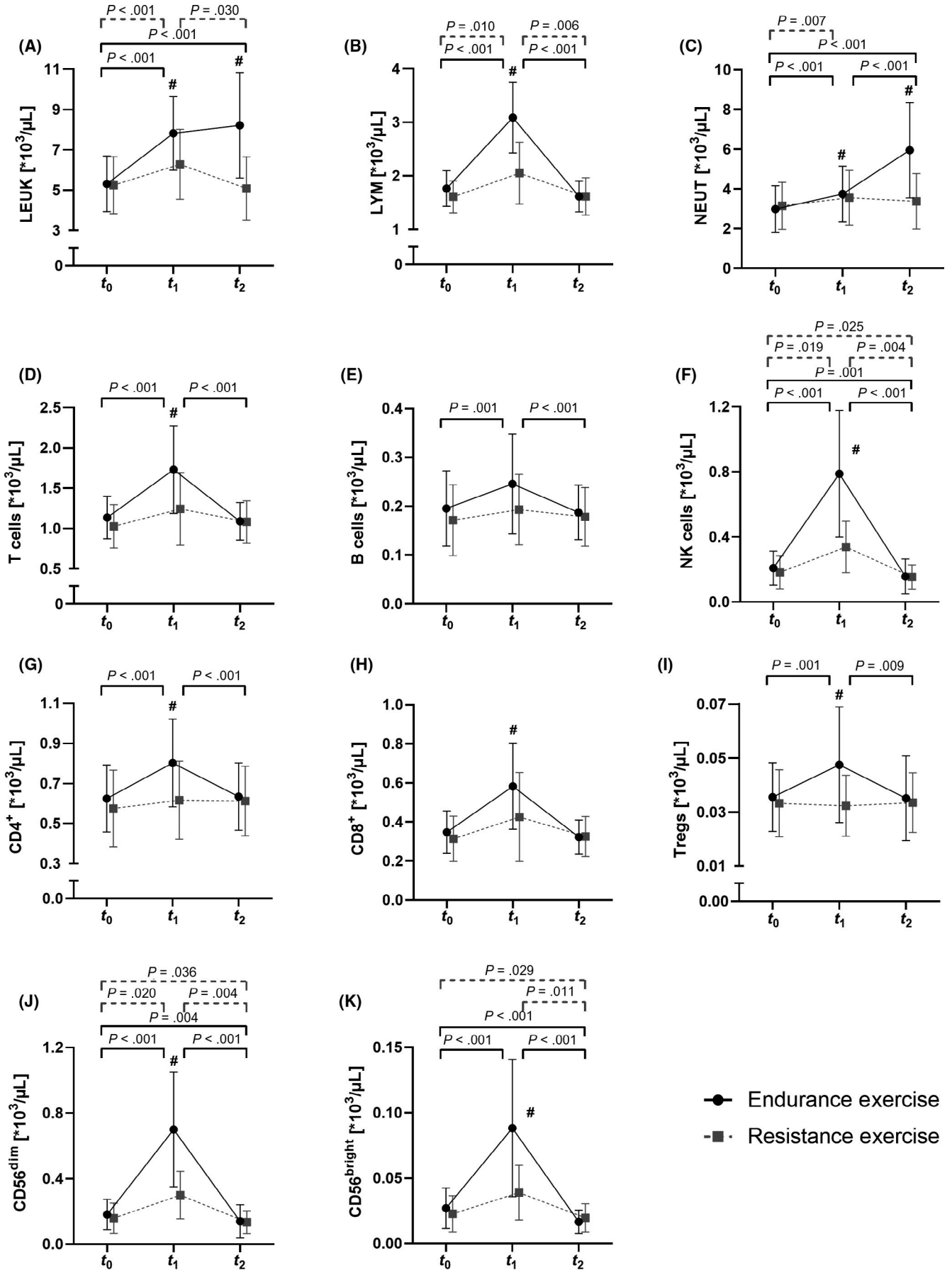
Results of the performance tests of all participants ($n = 24$) are reported in Table 1.

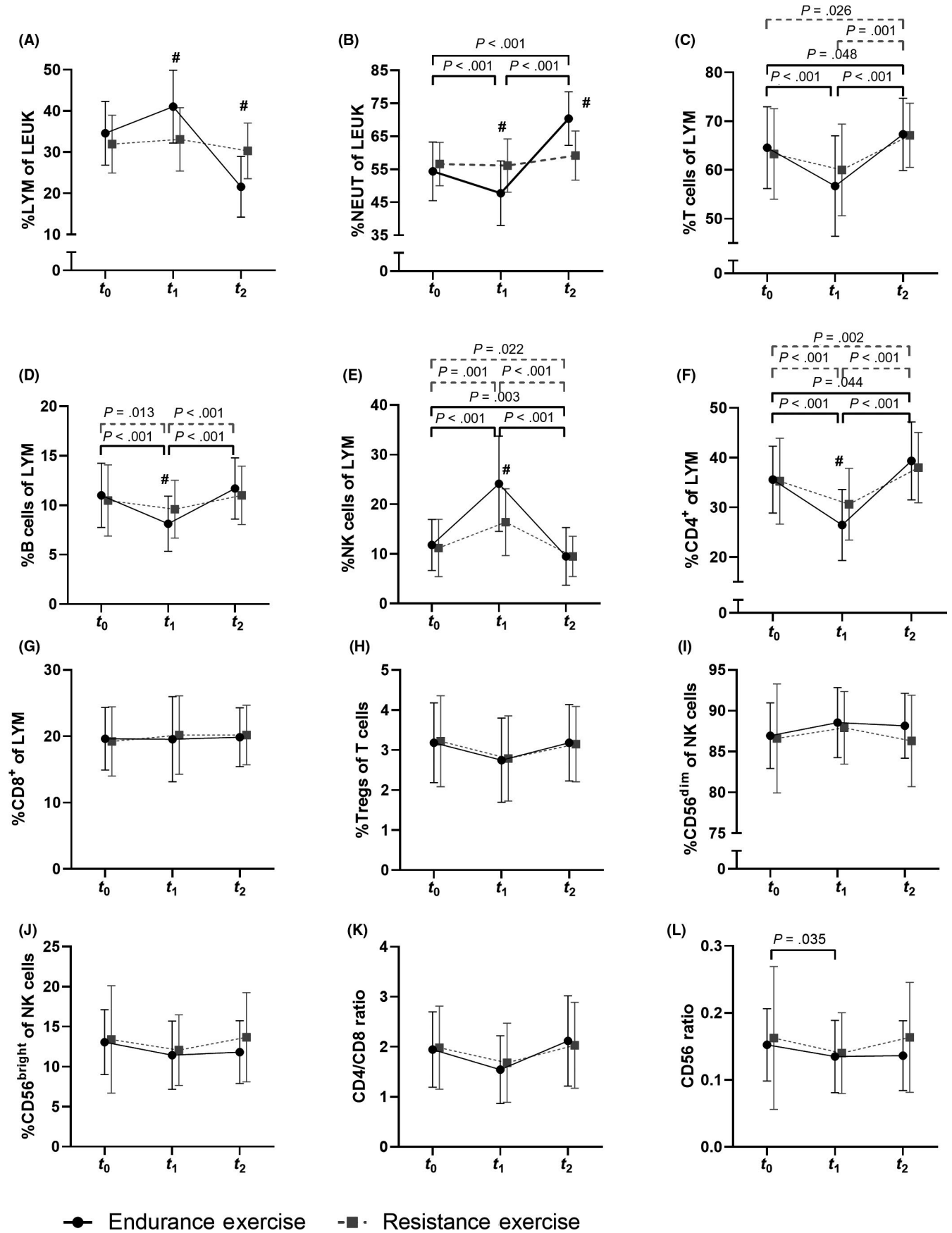
More detailed anthropometric and demographic characteristics as well as complete results of performance tests are reported elsewhere.²⁷

3.2 | Blood count outcomes

ANCOVA results revealed significant time and interactions effects for all absolute outcomes (Figure 1A-C). Regarding relative values, ANCOVA results revealed significant effects over time for NEUT. Interaction effects were detected for both, LYM and NEUT. Concerning the clinical inflammation markers, significant time effects were only detected for SII. Interaction effects were found in all three of them. Significant results of post hoc tests according to Bonferroni are displayed in Figures 1, 2, and 3. In general, the measured outcomes reacted stronger after EE compared to RE.

FIGURE 1 Impact of an acute bout of endurance vs resistance exercise on immune cell counts. #Significant interaction effect ($P \leq .05$); Significant time effects ($P \leq .05$) are indicated above each graph; Black shading indicates endurance exercise; Gray shading indicates resistance exercise; T₀ Baseline; T₁ Postexercise; T₂ 1 h follow-up; Values are presented as mean ± standard deviation; A, Leukocytes; B, Lymphocytes; C, Neutrophils; D, T cells; E, B cells; F, Natural killer cells; G, T helper cells; H, Cytotoxic T cells; I, Regulatory T cells; J, CD56^{dim} cells; K, CD56^{bright} cells





3.3 | Flow cytometry outcomes

ANCOVA results revealed significant effects over time for all



FIGURE 2 Impact of an acute bout of endurance vs resistance exercise on proportions of immune cells and immune cell ratios. #Significant interaction effect ($P \leq .05$); Significant time effects ($P \leq .05$) are indicated above each graph; Black shading indicates endurance exercise; Gray shading indicates resistance exercise; T_0 Baseline; T_1 Postexercise; T_2 1 h follow-up; Values are presented as mean \pm standard deviation; A, Lymphocytes out of leukocytes; B, Neutrophils out of leukocytes; C, T cells out of lymphocytes; D, B cells out of lymphocytes; E, Natural killer cells out of lymphocytes; F, T helper cells out of lymphocytes; G, Cytotoxic T cells out of lymphocytes; H, Regulatory T cells out of T cells; I, CD56^{dim} cells out of natural killer cells; J, CD56^{bright} cells out of natural killer cells; K, CD4/CD8 ratio; L, CD56 ratio

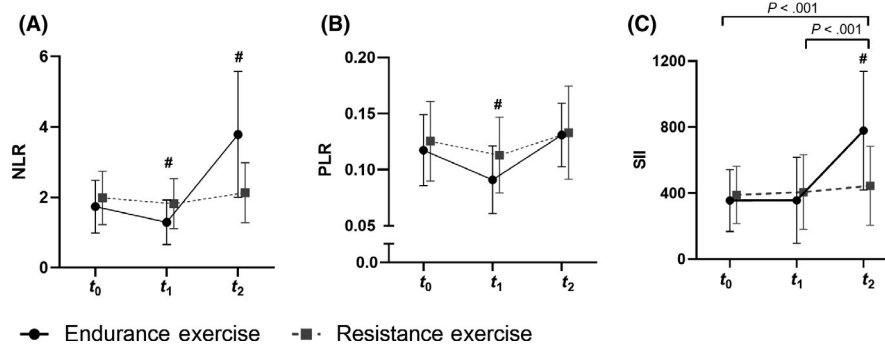


FIGURE 3 Impact of an acute bout of endurance vs resistance exercise on clinical cellular inflammation markers. #Significant interaction effect ($P \leq .05$); Significant time effects ($P \leq .05$) are indicated above each graph; Black shading indicates endurance exercise; Gray shading indicates resistance exercise; T_0 Baseline; T_1 Postexercise; T_2 1 h follow-up; Values are presented as mean \pm standard deviation; A, Neutrophil/lymphocyte ratio; B, Platelet/lymphocyte ratio; C, Systemic immune-inflammation index

absolute flow cytometry outcomes except CD8⁺. Interaction effects were found for all parameters. Concerning relative values, ANCOVA showed significant time effects ($P \leq .05$) for all immune cells. Interaction effects were detected for B, NK, and CD4⁺ cells. Concerning ratios of cell populations, ANCOVA results only revealed a significant time effect for CD56 ratio. For all other parameters, neither time nor interaction effects were detected. Similar to blood count outcomes, EE represented the greater stimulus compared to RE. Significant results of post hoc tests according to Bonferroni are displayed in Figures 1, 2, and 3.

4 | DISCUSSION

The present study aimed to compare the cellular immune response provoked by two exercise sessions (EE and RE) of high practical relevance. Our findings strongly suggest that an acute bout of EE represents a more pronounced immune disturbance than RE, therefore confirming our hypothesis. In fact, in EE, alterations of immune cell counts were significantly greater concerning all cell populations measured except for B cells, although even in this case data revealed a similar tendency (see Figure 1). Against the background of shear stress- and catecholamine-induced mobilization of LEUK from marginal pools into circulation,^{10,11} it is not surprising that immune response was stronger in EE. While exercise load and blood flow were continuously high in EE, it greatly oscillated in RE due to its intermittent character. Confirming this potential explanation, Fry et al published a study, showing that similar to RE, aerobic intervals at 60% of the maximal exercise intensity fail to cause significant alterations in immune cell counts.³⁰ Assuming that EE represented a higher workload than RE, a greater release of catecholamines may

additionally be suspected since their secretion was shown to be intensity-dependent.³¹ Therefore, both mechanisms, release of catecholamines and increased shear stress, may have contributed to the stronger immune response following EE. Natale et al published a notable groundwork, already showing that EE has a greater impact on kinetics of LEUK, LYM, and LYM subset counts compared to RE.²⁶ However, the applied EE session consisted of two hours of prolonged running, while the duration of RE was not even reported. Since immunological alterations can differ considerably depending on exercise duration,¹² we assume that sessions of different duration are hardly comparable. Building upon this, we applied exercise modalities of same duration that could additionally be implemented in clinical context.

4.1 | Blood count outcomes

The onset of leukocytosis after an acute bout of exercise is supported by previous studies and well-recognized by now.^{7,9,23,32} Confirming these findings, the present study revealed an increase of LEUK counts immediately after both exercise modalities. However, in EE the increase was greater and lasted until one hour postexercise, whereas in RE, values returned to baseline within this timeframe. Previous investigations revealed that following EE (2 hours cycling at 65% $\dot{V}O_{2max}$) LEUK counts peak 30-120 minutes after exercise cessation and that elevated numbers are still present 24 hours afterward.⁹ For RE, evidence suggests a shorter and less pronounced leukocytosis.²⁶ However, intensity appears to be relevant as indicated by increased LEUK counts after exhaustive RE.³² Therefore, the different results obtained in the present study might be due to the less exhaustive character of the RE protocol applied.



Concerning NEUT counts, values increased into recovery after EE while in RE values were only altered immediately after exercise. Similar kinetics was also found for NEUT proportions. In general, the exercise-induced neutrophilia is thought to be mediated by a release of immature NEUT from the bone marrow under the influence of cortisol.¹¹ Since cortisol secretion was shown to be intensity-dependent (similar to catecholamines),³³ the sustained neutrophilia observed after EE in this study once again verifies the superior role of EE in provoking LEUK mobilization.

In contrast, LYM counts increased immediately after EE and RE and returned to baseline within one hour after exercise cessation. While the exercise-induced lymphocytosis is in line with previous investigations,^{20,34} the subsequent lymphopenia that is commonly observed remained absent. One possible explanation for this might be the short observation period of one hour postexercise. Confirming this assumption, McCarthy and Dale suggested a time-frame of 2–4 hours after exercise cessation for the most pronounced decrease in LYM.¹³ However, although LYM counts revealed no exercise-induced lymphopenia, relative values showed a tendency for this phenomenon (see Figure 2A).

The fact that EE represents the stronger stimulus for LEUK mobilization is also visible in the kinetics of the calculated inflammation markers (see Figure 3). Interestingly, significant changes in favor of EE became visible for NLR and SII only one hour after exercise cessation, suggesting a delayed response of these markers compared to immune cell response. However, only SII was sensible to immunological alterations over time, thereby underlining its superior role in revealing exercise-induced immune changes. A potential explanation for this is that SII takes more immune cell populations into consideration than NLR or PLR. While NLR considers the two major immune cell populations affected by exercise (NEUT and LYM), SII additionally regards platelet count.³⁵ PLR in contrast seems to be inadequate for detection of exercise-induced alterations in cellular immune homeostasis, although previous investigations showed alterations in PLR following intense exercise.¹⁸ Taken together, these results partially confirm the findings of Joisten et al who identified NLR and SII as cheap and time-efficient cellular inflammation markers after an acute bout of exercise.¹⁷

4.2 | Flow cytometry outcomes

A second aim of this investigation was to give a comprehensive insight into exercise-induced changes in LYM subsets. As expected, T cell, B cell, and NK cell counts responded similar to overall LYM counts with an increase after exercise and a decrease within one hour postexercise (see Figure 3C–E). While T and B cells were only mobilized in response to EE, NK cells also reacted to RE, although once again EE represented the more effective stimulus. This is in line with previous investigations showing that among the mobilized LYM subsets, NK cells are most sensible to exercise stimuli.²¹ Thereby, our results suggest the potential application of RE or EE in clinical settings to provoke NK cell mobilization. In this context, a recent

mouse model showed promising effects of EE on NK cell mobilization with subsequent suppression of tumor growth.³⁶

Going into detail, NK and T-cell subsets (CD56^{dim}, CD56^{bright}, CD4⁺, CD8⁺, T_{reg}) showed similar kinetics as their parent cell populations. While CD56^{dim}, CD56^{bright}, CD4⁺, and T_{reg} counts reacted exactly in the same manner as their parent cells, CD8⁺ only showed a similar tendency. Compared to previous investigations this is surprising, since an increase in CD8⁺ was found after both EE and RE.^{9,26} However, the short duration of the EE and the intermitted character of the RE might account for these results. Concerning relative changes, only CD4⁺ showed changes over time, with values decreasing after both exercise stimuli and subsequently rising over baseline. This response might be attributable to the stronger response of NK cell compared to CD4⁺ counts. Since NK cells increase by a much greater extent than CD4⁺, the proportions of CD4⁺ decrease. Subsequently, the same reaction can be observed vice versa (see Figure 2E–F). These observations are supported by previous findings.³⁷ A special emphasis might be given to the increase of T_{reg} after EE. Since T_{reg} exert immunosuppressive effects on proliferation and activation of a wide range of immune cells (eg, T cells, B cells, NK cells), they became a popular target for prevention and treatment of autoimmune disease.^{38,39} The increase found after exercise might therefore promote these immunosuppressive effects. Additionally, a cohort study by Weinhold et al revealed higher proportions of T_{reg} in dependence of aerobic fitness (as indicated by rel. $\dot{V}O_{2peak}$).⁴⁰ These results are interpreted as an immunological shift toward anti-inflammatory properties through chronic accumulation of acute exercise stimuli, which might ultimately ameliorate the risk of chronic cardiovascular and metabolic diseases and their symptoms.⁵

Flow cytometry outcomes were additionally used to calculate the CD4⁺/CD8⁺ and the ratio of CD56^{bright} and CD56^{dim} NK cells. However, due to the similar kinetics of the underlying immune cell populations (CD4⁺, CD8⁺ and CD56^{dim}, CD56^{bright}, respectively) no changes of the associated ratios were found. This is surprising since previous studies revealed a decreased CD4/CD8 ratio immediately after exercise.^{9,21,26} A possible explanation for the lack of changes in CD4/CD8 ratio is the lacking increase of CD8⁺. While cell counts of T, B, and NK cells were only altered after EE, proportions of these cell populations were changed after EE and RE (see Figure 2C–E). This indicates that proportions depict exercise-induced changes in immune homeostasis more sensitively. Regarding relative values of NK and T-cell subsets, immunological disturbances were only visible in the kinetic of CD4⁺. All other immune cell populations (CD56^{bright}, CD56^{dim}, and CD8⁺) did not show any significant alterations. Taken together, our findings indicate that the determination of relative values of LYM subsets is of high interest, since it enables a deeper insight into exercise-induced changes in immune homeostasis.

5 | PERSPECTIVE

In line with previous findings, the results of the present study indicate that a single bout of EE drives stronger alterations of cellular immune homeostasis than RE.²⁶



In terms of intensity and duration, both exercise modalities were designed for potential implementation into clinical settings, competitive sports, and recreational activities. Nonetheless, a homogenous sample of healthy, young men was tested. Therefore, the transferability of the results to other populations is limited. However, the mobilization of immune cells induced by exercise might potentially be of relevance in clinical context for treatment and prevention of various diseases.¹ Further investigations examining different populations of age, sex, and state of health are strongly required. Moreover, limitations of the study include the selection of cycling as EE compared to a predominantly upper body workout as RE. However, the RE was designed to target major muscle groups. Similar to previous findings,^{17,18} we identified the SII as an appropriate, integrative marker to detect alterations in cellular immune homeostasis following an acute bout of EE, although a delayed response was observed compared to cellular components. Future studies may include additional measurement time points up to 24 hours postexercise to improve understanding of the precise time course of restoration of cellular immune homeostasis following exercise. Finally, we suggest the detection of relative values in addition to immune cell counts, in order to display immunological alterations more precisely.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data relevant to the study are included in the article or uploaded as supporting information.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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