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Lymphocyte subpopulations in healthy Ukrainian men: local reference ranges, long temporal stability, and exercise-induced variability

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ABSTRACT

Reliable interpretation of lymphocyte immunophenotyping depends on population-specific reference ranges that account for demographic and environmental factors. However, such reference data are currently lacking for the Ukrainian population. This study aimed to establish local reference ranges for lymphocyte subpopulations in healthy adult men and to assess their temporal stability and variability following physical exercise.

Peripheral blood samples from 100 clinically healthy men aged 22–55 years were analyzed by flow cytometry to quantify T lymphocytes ($CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$), B lymphocytes ($CD3^-CD19^+$), and NK cells ($CD3^-CD56^+$, $CD3^-CD56^+NKp46^+$). Temporal stability was evaluated in 24 participants monitored during a one-year Antarctic expedition, while acute immune responses were assessed in 19 men before and after a modified Cooper test. The established reference ranges were as follows: $CD3^+$ (54.0–82.2%), $CD3^+CD4^+$ (29.3–54.0%), $CD3^+CD8^+$ (11.2–39.6%), $CD3^-CD19^+$ (3.7–20.1%), $CD3^-CD56^+$ (4.2–30.0%), and $CD3^-CD56^+NKp46^+$ (2.7–24.3%).

Comparison with published studies revealed differences in several parameters, whereas discrepancies relative to reference values used by local laboratories were observed across multiple measures. Following a one-year Antarctic expedition, lymphocyte subset distributions remained largely stable despite a significant expansion of monocytes, whereas short-term physical exercise elicited pronounced, subset-selective increases in NK cells and monocytes, accompanied by concurrent elevations in cortisol and testosterone levels. The differences observed across populations and laboratories underscore the need for harmonized, population-based reference intervals.

1. Introduction

Flow cytometric analysis of peripheral blood lymphocyte subpopulations is an essential component of clinical diagnostics and research, providing valuable insights into immune function in disorders such as primary and secondary immunodeficiencies, autoimmune diseases, malignancies, and infections (Preglej et al., 2023; Rawat et al., 2019). Accurate interpretation of these data requires reference ranges that are both analytically validated and population-specific, as lymphocyte distributions vary with demographic and environmental factors such as age, sex, geography, and exposure to pathogens (Yaman et al., 2005; Liu et al., 2022). According to established international guidelines, such as the CLSI C28-A3 guideline, reference intervals should be derived from well-characterized healthy reference individuals using standardized pre-analytical, analytical, and statistical approaches to ensure their clinical reliability and comparability (Clinical and Laboratory Standards Institute (CLSI), 2010).

Previous studies have demonstrated marked variability in lymphocyte subset reference values among populations worldwide (Yaman et al., 2005; Klose et al., 2007; Rudolf-Oliveira et al., 2015; Roszczyk et al., 2024). However, population-specific reference ranges have not yet been established for the Ukrainian population. The absence of population-specific reference intervals may compromise the accuracy of laboratory interpretation and clinical decision-making, particularly with regard to the assessment of immunodeficiency, immune dysregulation, and immune monitoring in routine clinical practice.

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An Antarctic expedition presents a major challenge to physical and emotional health, and therefore expedition participants are carefully selected from applicants who are healthy both physically and mentally. Accordingly, selected candidates can serve as a well-defined group of healthy reference individuals suitable for establishing population-specific reference intervals for lymphocyte subpopulations. Moreover, since polar explorers often remain in extreme environmental conditions for up to a year, this setting provides a unique opportunity to evaluate the impact of prolonged isolation, low temperatures, and limited daylight on immunological parameters and their temporal stability.

Immune cell populations are inherently dynamic and respond rapidly to physiological stimuli. Among these, physical activity is a well-recognized modulator of immune function (Dawod, 2025). Acute exercise can transiently alter leukocyte distribution, particularly affecting NK cells and other lymphocyte subsets (Shi et al., 2025), while hormonal mediators such as cortisol and testosterone also further influence these responses (Vancolen et al., 2023). The Cooper test is a standardized assessment of physical performance that evaluates general endurance and aerobic capacity. It typically involves performing a sequence of dynamic exercises—running, push-ups, squats, and other body-weight movements—performed at maximum effort within a defined period, providing an overall measure of physical fitness.

During the selection process for participants in the polar expedition, individuals who successfully pass the medical examination undergo a modified Cooper test as an additional assessment of cardiovascular fitness and overall physical endurance. Such short-term physical exertion in healthy men also provides a valuable opportunity to evaluate the impact of acute exercise on immunological parameters.

The purpose of this study was to establish locally derived reference ranges for major lymphocyte subpopulations and to evaluate their temporal stability and physiological responses to acute exercise.

2. Materials and methods

2.1. Subjects

The study enrolled 100 healthy adult men (mean age 39.5 ± 7.6 years; range 22–55 years) who applied for Antarctic expeditions and successfully passed comprehensive medical screening. Of these, 42 participants completed a one-year expedition, while the remaining individuals stayed at the Antarctic station for six months or less. Longitudinal immunological follow-up was conducted in 24 participants from the one-year expedition subgroup under continuous medical supervision. Immunophenotypic and serological assessments were performed between October 2020 and April 2025. Participants were recruited annually during October–November following comprehensive medical examinations, with supplementary recruitment each April after return from the preceding Antarctic expedition. All analyses were conducted using a uniform study protocol and identical reagent panels to ensure standardization and comparability.

Exclusion criteria included chronic diseases, recent infections, current or recent immunomodulatory therapy, and positive serological results for hepatitis B surface antigen (HBsAg), antibodies to hepatitis B core antigen (anti-HBc), antibodies to hepatitis C virus (anti-HCV), antibodies to human T-lymphotropic virus types I and II (anti-HTLV-I/II), syphilis, HIV-1/2 antigen/antibody combination assay, and antibodies to *Trypanosoma cruzi*.

Non-obesity was an inclusion criterion, and all participants had a body mass index (BMI) between 18.5 and 29.9 kg/m^2. According to self-reported data, 17.6% of participants were smokers, and 43.1% reported engaging in regular physical activity; serological analysis demonstrated CMV IgG seropositivity in 70% of participants, HSV-1/2 IgG seropositivity in 85%, and EBV nuclear antigen (NA) IgG seropositivity in 90%.

Samples exhibiting clot formation or hemolysis were also excluded.

To evaluate general physical fitness and endurance, participants performed a modified, circuit-based Cooper test. The test comprised four consecutive exercises, each performed for 10 repetitions: push-ups, squat thrusts (burpee-style knee-to-chest movements from the push-up position), abdominal crunches, and squats or squat jumps. Completion of all four exercises constituted one cycle. Participants were instructed to complete four consecutive cycles of this exercise sequence at maximal effort and within the shortest possible time. The test was designed to assess muscular strength, endurance, and overall physical performance capacity. Performance was classified based on total completion time as follows: excellent (≤ 7 min 30 s), satisfactory (7 min 30 s–9 min), and unsatisfactory (> 9 min). Blood samples were collected 30 min before the start of the test and 30 min after its completion.

The study protocol was reviewed and approved by the Biomedical Ethics Committee of the Institute of Pediatrics, Obstetrics and Gynecology and by the Clinical Immunology and Allergology Committee of the Ministry of Health of Ukraine. Written informed consent was obtained from all participants prior to study enrollment.

2.2. Methods

Venous blood for immunological and serological analyses was obtained after an overnight fast. In participants who underwent the modified Cooper test (n = 19), blood samples were collected 30 min before exercise initiation and 30 min post-exercise. Venous blood samples were collected into a serum tube without anticoagulant (2.6 mL) and a lithium heparin tube (4.9 mL; Heparin-Li) (Primavette® S and V, KABE LABORTECHNIK, Nümbrecht, Germany) using 21-gauge needles (Sangocan®, KABE LABORTECHNIK, Nümbrecht, Germany).

Serum samples for biochemical analyses were obtained by centrifugation of whole blood without anticoagulant at 1500 ×g for 10 min, and the remaining serum after routine analyses was aliquoted and stored at −80 °C until further determination. Peripheral blood samples were processed using a single-platform flow cytometric approach on FACScan (Becton Dickinson Biosciences, San Jose, CA, USA) and Cytomics FC 500 (Beckman Coulter, Brea, CA, USA) flow cytometers.

Data analysis was performed using CellQuest (BD Biosciences, San Jose, CA, USA) and CXP software (Beckman Coulter, Brea, CA, USA). Immunophenotypic analysis was performed using the following panel of fluorochrome-conjugated monoclonal antibodies: BD Simultest Leucogate CD45-FITC/CD14-PE (REF 342408; BD Biosciences, San Jose, CA, USA), CYTO-STAT tetraCHROME CD45-FITC/CD56-RD1/CD19-ECD/CD3-PC5 monoclonal antibody reagent (REF 6607073; Beckman Coulter, Brea, CA, USA), CD8-FITC (REF A07756, clone B9.11; Beckman Coulter, Brea, CA, USA), CD4-PE (REF A07751, clone 13B8.2; Beckman Coulter, Brea, CA, USA), CD3-PC5 (REF A07749, clone UCHT1; Beckman Coulter, Brea, CA, USA), CD45-FITC (REF A07782, clone J33; Beckman Coulter, Brea, CA, USA), CD3-ECD (REF A07748, clone UCHT1; Beckman Coulter, Brea, CA, USA), CD56-PC7 (REF A21692, clone N901; Beckman Coulter, Brea, CA, USA), CD335-PE (Cat. 557,991, clone 9E2; BD Pharmingen, San Jose, CA, USA), and Flow-Count fluorospheres (REF 7547053; Beckman Coulter, Brea, CA, USA).

Briefly, whole blood (100 μL) was stained with the respective antibodies in accordance with the manufacturers' instructions. Following 30 min of incubation at room temperature in the dark, red blood cells (RBCs) were lysed using FACS Lysing Solution (BD Biosciences, San Jose, CA, USA) or OptiLyse C (Beckman Coulter, Brea, CA, USA) and washed with phosphate-buffered saline (PBS). Labeled lymphocytes were acquired on a FACScan flow cytometer (BD Biosciences, San Jose, CA, USA) or a Cytomics FC 500 (Beckman Coulter, Brea, CA, USA) following appropriate instrument setup, calibration, and compensation (Maecker and Trotter, 2006). Quality control of antibody staining, RBC lysis, instrument setup and performance, and data analysis was routinely performed using BD™ Multi-Check Control (BD Biosciences, San Jose, CA, USA).

Serum cortisol and testosterone levels, as well as IgG antibodies to cytomegalovirus (CMV), Epstein–Barr virus (EBV), single-stranded DNA (ssDNA), and double-stranded DNA (dsDNA), were measured by

enzyme-linked immunosorbent assay (ELISA) in accordance with the manufacturer's instructions (Xema, Kyiv, Ukraine). IgG antibodies to cardiolipin, phosphatidylserine, and phosphatidylethanolamine (aCL, aPS, and aPE) were also determined by ELISA, as previously described (Maecker and Trotter, 2006). Serum IgG antibodies to ssDNA, CMV, and EBV were additionally measured by ELISA using commercial kits (VitroTest, Kyiv, Ukraine).

2.3. Statistical analysis

The distribution of continuous variables was assessed using the Shapiro–Wilk normality test. Reference intervals for normally distributed variables were calculated as the mean ± 1.96 standard deviations (SD). For variables with non-normal distributions, reference intervals were estimated using the 2.5th and 97.5th percentiles. The coefficient of variation (CV, %) was additionally calculated to assess the variability of lymphocyte subsets. Comparisons of immunological parameters before and after short-term physical exercise were performed using paired-sample *t*-tests for normally distributed data or the Wilcoxon signed-rank test for non-normally distributed data.

Associations between immunological and hormonal parameters were evaluated using Pearson's or Spearman's correlation coefficients, depending on data distribution. Statistical significance was defined a priori at $p \leq 0.05$ (two-tailed). All statistical analyses were performed using SPSS software (version 17.0; SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Reference Ranges

The lymphocyte subsets evaluated in this study included $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$, $CD3^-CD19^+$ B cells, $CD3^-CD56^+$ NK cells, and $NKp46^+$ NK cells. In addition, the relative proportions of granulocytes, monocytes, and lymphocytes were assessed. Descriptive statistics, including the mean, median, standard deviation, and reference intervals, for all studied subsets are presented in Table 1. No significant correlations were observed between age and any lymphocyte subset. The established reference intervals were broadly consistent with previously published data, although some variability was noted (Table 2).

3.2. Changes in immunological parameters after a one-year expedition

Participants ($n = 24$) completed a one-year stay at the Antarctic station under continuous medical supervision, with no serious health complications reported during the observation period. Peripheral blood samples were collected on-site at quarterly intervals. No cases of primary infection or viral reactivation involving herpesviruses, including CMV and EBV, were identified, as confirmed by clinical monitoring. All participants experienced mild courses of COVID-19 only.

No autoimmune antibodies (aCL, ssDNA, or asDNA) were detected, and no clinical signs or symptoms of autoimmune disorders were observed. These findings indicate that the participants could be considered not only healthy control subjects at baseline but also a cohort free of clinically significant disease throughout the entire one-year observation period.

Immunological assessment following the one-year expedition demonstrated that the most consistent finding was a significant increase in monocyte counts (relative change [RCh] +61.2%, $p = 0.010$). In addition, trends toward higher absolute counts of T lymphocytes ($CD3^+$, $CD3^+CD4^+$, and $CD3^+CD8^+$) and B cells were observed; however, these changes did not reach statistical significance. Absolute NK-cell counts remained largely unchanged. Collectively, these data indicate a shift toward monocyte expansion accompanied by moderate, non-significant increases in T- and B-lymphocyte subsets following prolonged exposure to extreme environmental conditions (Table 3).

3.3. Immunological response to short-term exercise

Following the modified Cooper test, the most pronounced immunological changes were observed in the monocyte and NK cell compartments. Monocytes demonstrated a significant relative increase in both proportional representation and absolute counts (relative change [RCh] +30.3% and + 41.9%, respectively; $p = 0.001$ and $p = 0.002$), while NK cells also exhibited marked expansion (RCh +38.7% in relative frequency and + 61.0% in absolute numbers; $p = 0.001$). Other lymphocyte subsets, including $CD3^+CD8^+$ T cells, $CD3^+CD4^+$ T cells, and B cells, showed only minor changes that did not reach statistical significance (Table 3).

3.4. Comparison with reference values from local laboratories

Immunological parameters of the healthy male cohort were compared with reference intervals provided by two Ukrainian clinical laboratories. These reference values were not publicly available on the laboratories' official websites. Therefore, reference intervals were obtained from laboratory report forms of adult male patients who had undergone routine immunological testing and were kindly provided to us for analytical purposes.

This comparison demonstrated that a substantial proportion of healthy individuals exhibited values outside the reported reference intervals (Table 4). The most pronounced discrepancies were observed for lymphocyte percentage, $CD3^+CD8^+$ T lymphocytes, and NK cells (Table 4). Using the reference intervals from Laboratory 1, 30% of participants fell outside the lymphocyte range, while 26% and 33% were outside the reference limits for $CD3^+CD8^+$ T lymphocytes and NK cells, respectively. According to the reference intervals from Laboratory 2, these proportions increased to 57% for lymphocytes and to 26% and 45% for $CD3^+CD8^+$ T lymphocytes and NK cells, respectively.

Further analysis of immunological parameters following short-term physical exercise revealed that, when local laboratory reference intervals were applied, the proportion of results falling outside the reference ranges exceeded 70% for certain parameters. In contrast, when

Table 1
Reference intervals for leukocyte subsets in healthy adult males ($n = 100$).

Parameter	Mean	Median	Max	Min	St.Deviation	Reference ranges		CV
Granulocytes, %	61.4	61.4	78.9	41.7	7.9	45.8	76.9	13
Monocytes, %	6.0	6.0	13.9	2.7	1.9	2.4	9.6	31
Lymphocytes, %	32.6	32.5	55.6	15.9	7.6	17.7	47.5	23
$CD3^+$T lymphocytes,%	68.1	69.2	82.8	48.9	7.2	54.0	82.2	11
$CD3^+CD4^+$T lymphocytes,%	41.7	41.5	57.8	24.5	6.3	29.3	54.0	15
$CD3^+CD8^+$T lymphocytes,%	25.4	24.5	48.5	12.1	7.2	11.2	39.6	28
CD4:CD8 ratio	1.8	1.7	4.0	0.5	0.7	1.7	1.9	39
B lymphocytes,%	11.9	11.3	27.3	4.7	4.2	3.7	20.1	35
NK lymphocytes,%	17.1	16.2	33.8	3.4	6.6	4.2	30.0	38
$NKp46^+$lymphocytes,%	13.5	13.0	24.7	3.0	5.5	2.7	24.3	41

St. Deviation – standard deviation.

Table 2
Comparison of observed reference ranges for lymphocyte subsets with literature data.

Parameter	Reference									
	Present study	(5)	(6)	(16)	(17)	(7)	(3)	(4)*	(18)*	(15)
$CD3^+$T lymphocytes,%	54.0–82.2	56–79	48.0–82.6	52.2–84.8	75–80	56.2–80.8	51.3–86.5	53.9–82.2	59.6–85.3	54–81
$CD3^+CD4^+$T lymphocytes,%	29.3–54.0	26–48	23.0–52.6	33.1–62.1	34–61	29.4–55.1	29.9–60.1	24.1–49.0	32.1–56.3	25–49
$CD3^+CD8^+$T lymphocytes,%	11.2–39.6	17–37	12.6–39.2	10.2–40.8	16–38	16.3–38.4	18.1–39.9	14.5–37.1	15.6–35.2	–
B lymphocytes,%	3.7–20.1	7–21	6.1–21.6	4.4–19.4	4–9	7.0–25.3	3.9–22.2	4.6–17.8	5.3–18.3	–
NK lymphocytes,%	4.2–30.0	8–30	5.0–31.3	5.2–32.9	2–27	6.2–25.4	–	4.5–33.2	–	–

* reference ranges were determined in mixed groups (men and women).

Table 3
Changes in leukocyte and lymphocyte subsets in response to a modified Cooper test and after 1-year polar expedition.

Parameter	Modified cooper test				Polar expedition			
	RCh,%	fold change	p	correl	RCh,%	fold change	p	correl
Leucocytes, abs	6,4	1,06	0,233	0,745	20,0	1,20	0,207	0,542
Lympocytes,%	4,6	1,05	0,260	0,859	18,1	1,18	0,096	-0,025
Lympocytes, abs	12,0	1,12	0,123	0,745	14,4	1,14	0,275	0,515
Granulocytes,%	-4,3	0,96	0,137	0,874	−5,1	0,95	0,061	0,082
Granulocytes, abs	1,3	1,01	0,464	0,840	24,7	1,25	0,276	0,465
Monocytes,%	30,3	1,30	0,001	0,461	38,7	1,39	0,069	0,336
Monocytes,abs	41,9	1,42	0,002	0,449	61,2	1,61	0,010	0,298
$CD3^+$T lymphocytes,%	-5,2	0,95	0,034	0,844	2,5	1,03	0,214	0,878
$CD3^+$T lymphocytes,abs	5,5	1,05	0,325	0,742	16,7	1,17	0,280	0,633
$CD3^+CD4^+$T lymphocytes,%	-11,2	0,89	0,003	0,755	2,9	1,03	0,381	0,757
$CD3^+CD4^+$T lymphocytes, abs	-5,1	0,95	0,289	0,718	17,4	1,17	0,400	0,630
$CD3^+CD8^+$T lymphocytes,%	0,7	1,01	0,428	0,938	7,8	1,08	0,243	0,939
$CD3^+CD8^+$T lymphocytes, abs	12,9	1,13	0,226	0,791	20,4	1,20	0,124	0,735
B lymphocytes,%	−10,1	0,90	0,062	0,842	12,5	1,13	0,136	0,258
B lymphocytes, abs	−1,8	0,98	0,388	0,941	26,9	1,27	0,261	0,319
NK lymphocytes,%	38,7	1,39	0,001	0,776	−8,6	0,91	0,190	0,774
NK lymphocytes, abs	61,0	1,61	0,001	0,603	6,8	1,07	0,319	0,523

RCh — relative change; abs — absolute count; correl — correlation coefficient;

Table 4
Comparison of deviations from reference ranges reported by two local laboratories and the current study.

Parameter	Lab #1			Lab #2			Current study	
	ref.ranges	a,%	b,%	ref.ranges	a,%	b,%	ref.ranges	b,%
Granulocytes,%	47–75	9.0	26.3	n/a	n/a	n/a	45.8–76.9	15.8
Monocytes,%	3–11	3.0	0.0	n/a	n/a	n/a	2.4–9.6	0.0
Lymphocytes,%	19–37	30.0	42.1	28–36	57.0	78.9	17.7–47.5	15.8
$CD3^+$T lymphocytes,%	60–80	17.0	47.4	61–85	17.0	47.4	54.0–82.2	15.8
$CD3^+CD4^+$T lymphocytes,%	33–52	14.0	36.8	35–55	17.0	52.6	29.3–54.0	10.5
$CD3^+CD8^+$T lymphocytes,%	19–35	26.0	36.8	19–35	26.0	36.8	11.2–39.6	15.8
B lymphocytes,%	7–19	12.0	15.8	7–17	15.0	21.1	3.7–20.1	5.3
NK lymphocytes,%	6–20	33.0	73.7	8–18	45.0	84.2	4.2–30.0	10.5

Ref. ranges – reference ranges
[a]Percentage of subjects with immunological parameters out of the reference range, blood samples were collected before completion of the modified Cooper test.
[b]Percentage of subjects with immunological parameters out of the reference range. Blood samples were collected 30 min following completion of the modified Cooper test.

reference intervals established in the present study were used, deviations for the same parameters after exercise did not exceed 20% (Table 4b).

3.5. Impact of short-term physical activity on serum cortisol and testosterone levels

Following short-term physical exercise, all participants demonstrated significant increases in serum cortisol and testosterone levels. Cortisol concentrations increased 1.6-fold ($p = 0.013$), while testosterone levels increased approximately twofold ($p = 0.001$) (Fig. 1). When participants were stratified according to physical test performance (successful completion: ≤ 7 min 30 s and 7 min 30 s–9 min; unsuccessful completion: > 9 min), a significant association between changes in NK-cell parameters and cortisol levels was identified. In the subgroup of men who did not successfully complete the physical test, the exercise-induced increase in cortisol levels showed a strong positive correlation with increases in both the relative proportion of NK cells ($r = 0.71$) and their absolute counts ($r = 0.91$, $p = 0.002$) (Fig. 2a). In contrast, no such correlation was observed among men who successfully completed the test ($r = 0.03$) (Fig. 2b). Notably, although NK cell levels increased after exercise in both groups, post-exercise cortisol concentrations were comparable between groups.

4. Discussion

Clinical diagnoses and research outcomes become more informative and reliable when interpreted against locally established reference intervals. Numerous studies have demonstrated that the distribution of lymphocyte subsets varies according to age, sex, and geographic region

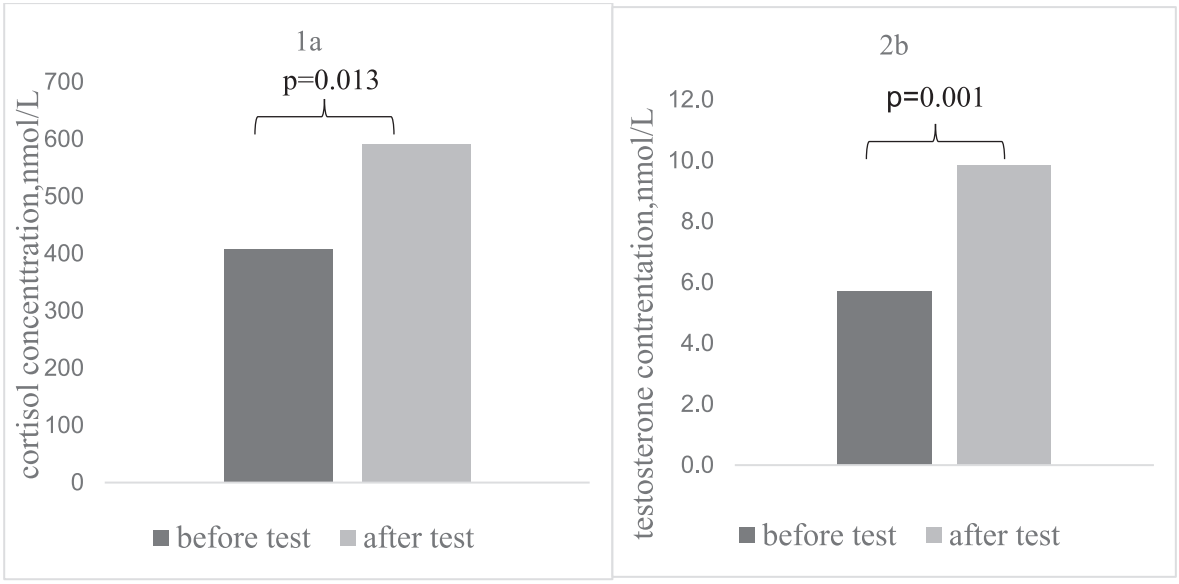
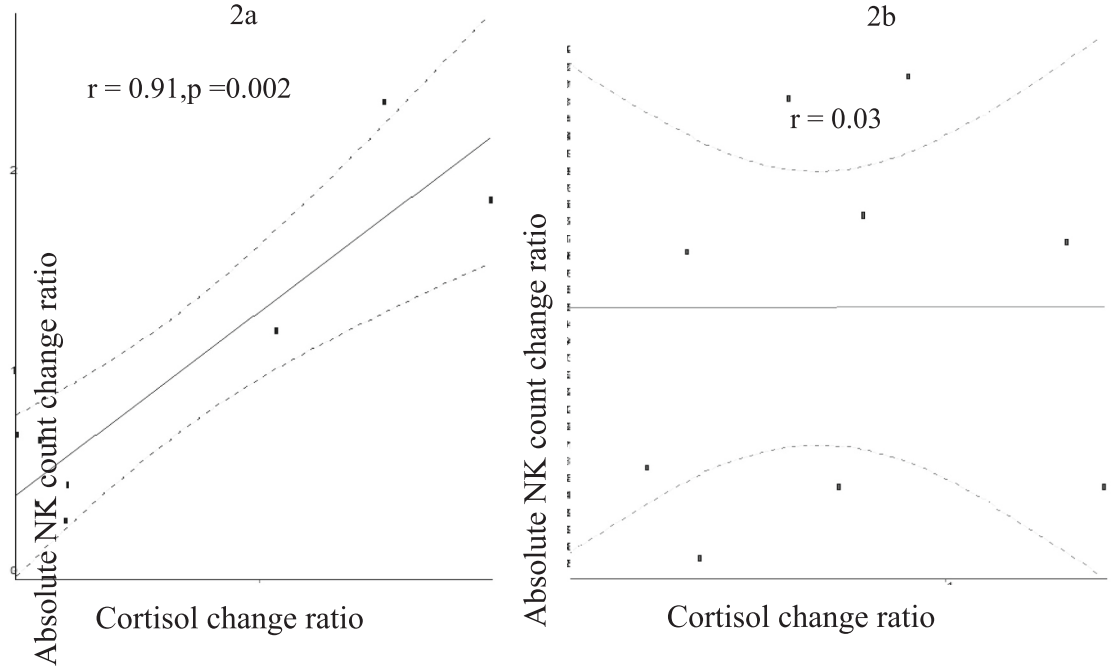


Fig. 1. Hormonal responses to short-term physical exercise.



(a) participants who failed the physical test; (b) participants who successfully completed the test

Fig. 2. Correlation between NK cell and cortisol fold changes in males participants stratified by physical test performance. (a) participants who failed the physical test; (b) participants who successfully completed the test.

(Preglej et al., 2023; Rawat et al., 2019; Yaman et al., 2005). Therefore, the regular assessment and establishment of population-specific reference intervals are essential for accurate interpretation of immunological results. In addition, exposure to infectious agents may substantially influence immune parameters. We have previously demonstrated that seropositivity for human CMV is associated with distinct immune alterations and may result in an accentuated immune phenotype in a subset of infected individuals, with marked differences observed between populations from different countries (Dons'koi et al., 2020; Fowler et al., 2022). Taken together, these observations further highlight the importance of deriving reference values from well-characterized local populations.

In the absence of population-specific immunological reference data for Ukraine, the present study evaluated peripheral blood lymphocyte subpopulations in clinically healthy men aged 22–53 years who had undergone comprehensive medical screening prior to participation in a polar expedition. These data allowed us to establish reference intervals for major lymphocyte subsets in peripheral blood, including NKp46^{+} NK

cells.

The percentages of $CD3^+$ and $CD3^+CD4^+$ T lymphocytes exhibited the lowest coefficients of variation among all parameters analyzed. Comparison with previously published studies demonstrated that the reference intervals for $CD3^+$ lymphocytes established in the present study were consistent with those reported for most male cohorts (Yaman et al., 2005; Klose et al., 2007; Roszczyk et al., 2024; Thakar et al., 2011; Bisset et al., 2004), with the exception of one study (Rudolf-Oliveira et al., 2015), which described a broader reference range. Our findings were also consistent with the data reported by Liu et al. (Liu et al., 2022), who analyzed a mixed-sex population. The low inter-individual variability and cross-population consistency observed for $CD3^+$ lymphocytes suggest that this subset is relatively insensitive to geographic or nutritional influences.

Reference intervals for $CD3^+CD4^+$ T lymphocytes were likewise in agreement with several previously published datasets (Yaman et al., 2005; Roszczyk et al., 2024). However, in contrast to total $CD3^+$ T lymphocytes, $CD3^+CD4^+$ cells demonstrated greater variability across studies. These discrepancies were primarily attributable to differences in the lower limits of the reported reference intervals, which were lower in some cohorts (Klose et al., 2007; Rudolf-Oliveira et al., 2015; Thakar et al., 2011) and higher in others (Bisset et al., 2004; Valiathan et al., 2014)

Reference intervals for $CD3^+CD8^+$ T lymphocytes differed more markedly from those reported in other populations (Yaman et al., 2005; Liu et al., 2022; Klose et al., 2007; Roszczyk et al., 2024; Valiathan et al., 2014; Rovati et al., 2014), particularly with respect to the lower reference limit, indicating substantial inter-study heterogeneity for this parameter. Among all lymphocyte subsets analyzed, B cells and NK cells exhibited the highest coefficients of variation. Reference intervals for B cells in the present study were comparable to those reported by Yaman et al. (Yaman et al., 2005), but differed from several other datasets primarily due to lower lower-limit values (Liu et al., 2022; Roszczyk et al., 2024; Bisset et al., 2004; Valiathan et al., 2014; Rovati et al., 2014). The reference intervals established for NK cells were broadly consistent with most published studies (Liu et al., 2022; Rudolf-Oliveira et al., 2015; Bisset et al., 2004; Phan et al., 2017), although notable discrepancies were observed when compared with selected cohorts (Yaman et al., 2005; Roszczyk et al., 2024; Valiathan et al., 2014)

NKp46 is a key activating receptor expressed on natural killer (NK) cells and plays a central role in the recognition and elimination of target cells (Hadad et al., 2015). Our previous studies further highlighted the functional relevance of NKp46, demonstrating that the frequency of $NKp46^+$ NK cells has prognostic value for identifying impaired or abnormal NK-cell cytotoxicity (Dons'koi et al., 2019). In the present study, reference intervals for $NKp46^+$ NK lymphocytes were largely consistent with previously published data (Apoil et al., 2017), although a higher mean frequency of these cells was observed. In contrast, our findings differed from those reported by (Phan et al., 2017), who described a substantially broader reference range. Such discrepancies may be attributable to differences in the age distribution of study populations, as NKp46 expression on NK cells is known to decline with advancing age, while showing no significant sex-related differences (Apoil et al., 2017).

Although the cohort of 100 clinically healthy participants is substantial and comparable to sample sizes used in previously published immunophenotyping studies, it does not fully satisfy the Clinical and Laboratory Standards Institute (CLSI) C28-A3 guideline, which recommends a minimum of 120 reference individuals for robust reference interval estimation. Accordingly, the relatively smaller sample size may reduce the precision of the estimated lower and upper reference limits, particularly at the extreme percentiles (Clinical and Laboratory Standards Institute (CLSI), 2010).

Nevertheless, the impact of this limitation is partly mitigated by the high homogeneity of the study population and the use of standardized pre-analytical and analytical procedures, both of which are known to reduce biological and technical variability. Because the cohort consisted of clinically healthy adult men of European ancestry who were in good physical condition, had normal body weight, and had no history of chronic disease, the findings may not be fully generalizable to the broader male population in Ukraine.

Accordingly, this cohort should be regarded as a well-defined subgroup of healthy men, and reference intervals applicable to the general male population in Ukraine are likely to differ and may exhibit broader ranges. Nevertheless, comparison of immunological parameters obtained in this healthy cohort with reference values currently used by local laboratories revealed that more than 50% of results fell outside laboratory-defined limits. These findings underscore substantial limitations in the clinical applicability of existing laboratory reference intervals. Together with the unclear origin and validation of these reference values, such discrepancies may increase the risk of misclassification and potential overdiagnosis.

Beyond the establishment of baseline reference intervals, this study also investigated dynamic changes in immune cell populations under distinct physiological conditions. Immunological parameters were assessed following participants' return from a one-year Antarctic expedition, reflecting long-term environmental exposure, while short-term physical exercise was used as a standardized acute stressor to evaluate immune reactivity.

Prolonged polar exposure was associated with a persistent and statistically significant expansion of monocytes, whereas only moderate, non-significant trends toward increased T- and B-lymphocyte subsets were observed, indicating selective adaptation of the innate immune compartment alongside relative stability of adaptive immune cell populations under chronic environmental stressors, including cold exposure, circadian disruption, and social isolation (Shephard, 1998).The relative stability of NK-cell counts observed under these conditions further supports their predominant role in acute, rather than long-term, immune modulation. Although vitamin D is a key immunomodulatory factor and its deficiency commonly develops during Antarctic residence (Martens et al., 2020; Cabalín et al., 2021), all participants in the present study received continuous vitamin D supplementation, and post-expedition levels exceeded baseline values. Thus, vitamin D–related deficiency is unlikely to have contributed to the observed immunological changes. Further studies are needed to clarify the observed immunological changes and their mechanisms. Consistent with this interpretation, our previous studies demonstrated that distinct immune accentuations may serve as prognostic markers of individual adaptation to Antarctic conditions, underscoring the value of immunological monitoring for predicting resilience in extreme environments (Dubrovskyi et al., 2025).

Physical activity is a well-established modulator of immune function, with its effects determined by the type, intensity, and duration of exercise (Dawod, 2025). Acute, short-term exercise ($\leq$ 30 min) is typically associated with transient increases in circulating lymphocyte subsets, including $CD4^+$ and $CD8^+$ T cells, $CD19^+$ B cells, and NK cells, which generally return to baseline levels within 10–30 min after exercise cessation. In contrast, prolonged or sustained exercise requires a longer recovery period for leukocyte counts to normalize (Pedersen and Hoffman-Goetz, 2000).

The present study evaluated the effects of acute short-term physical exercise (a modified Cooper test) on immunological and endocrine parameters. Both absolute and relative immunological measures, together with serum testosterone and cortisol concentrations, were assessed 30 min before and 30 min after exercise in a subgroup of 19 men.

No significant changes in total leukocyte counts were observed; however, both the relative proportion and absolute counts of monocytes increased significantly following physical exertion. It is well established that leukocyte subpopulations exhibit distinct mobilization and recovery kinetics after exercise (Neves et al., 2015); thus, inter-study variability may partly reflect differences in the timing of post-exercise blood sampling.

In our study short-term physical exercise induced an increase in NK cells and monocytes. According to existing evidence, the primary mechanism underlying the rapid post-exercise elevation in circulating immune cell counts involves acute sympathetic nervous system activation, mediated predominantly by catecholamine release, β_2-adrenergic receptor (β_2-AR) signaling, and exercise-induced hemodynamic shifts. β_2-adrenergic pathways play a particularly important role in the selective mobilization of NK cells and specific monocyte subsets into the peripheral circulation.

Previous studies have demonstrated that the preferential mobilization of NK cells, non-classical monocytes, and differentiated $CD8^+$ T-cell subsets during exercise is largely dependent on catecholamine signaling via the β_2-AR. Moreover, exercise selectively mobilizes lymphocyte and monocyte subpopulations characterized by high expression of surface adhesion molecules and phenotypes associated with enhanced cytotoxicity, prior antigen exposure, inflammatory potential, and antigen-presenting capacity (Millard et al., 2013; Crisafulli et al., 2013; Graff et al., 2018a; Campbell and Turner, 2018).

In the present study, acute short-term physical activity was associated with significant increases in serum testosterone and cortisol concentrations. While the cortisol responses observed here were largely consistent with those reported in comparable studies, post-exercise testosterone dynamics appeared to be more heterogeneous. Although several investigations have described reductions in testosterone levels following physical exercise, accumulating evidence indicates that testosterone responses are highly context-dependent and vary according to exercise intensity, duration, timing of blood sampling, and the baseline physiological status of participants.

Specifically, acute high-intensity exercise has consistently been shown to elicit a transient increase in circulating testosterone concentrations immediately after exercise or during the early recovery phase, an effect thought to be mediated by catecholamine release, exercise-induced plasma volume shifts, and acute metabolic stress (Riachy et al., 2020; D'Andrea et al., 2020).

In contrast, prolonged endurance exercise has more frequently been associated with reductions in testosterone concentrations, particularly when assessed during later stages of recovery and in the context of sustained cortisol elevation (Taipale and Häkkinen, 2013) Moreover, studies using delayed post-exercise sampling have demonstrated declines in testosterone levels 120–180 min after high-intensity exercise, supporting the concept of a biphasic hormonal response, characterized by an early transient increase followed by a subsequent (Dote-Montero et al., 2021).

Given that the modified Cooper test used in the present study constitutes a brief, high-intensity aerobic challenge, the observed post-exercise increase in testosterone concentrations is consistent with studies focusing on early post-exercise assessments and may additionally reflect the relatively high physical fitness of the study participants.

Glucocorticoid and adrenergic receptors are widely expressed on immune cells and play an essential role in the regulation of their functional activity. Glucocorticoid receptors are differentially expressed across immune cell subsets, including NK cells, T and B lymphocytes, and monocytes, and this heterogeneity underlies cell type-specific sensitivity to cortisol-mediated regulation of immune activation, trafficking, and survival (Cain and Cidlowski, 2017; Sharma et al., 2020). Glucocorticoids modulate lymphocyte trafficking, including that by NK cells, affecting their immune functions. Still, cortisol, which increased in our study, is unlikely to serve as the primary and the main trigger of early NK cell leukocytosis; rather, it may modulate the subsequent redistribution and homing phases by influencing chemokine receptor expression and the migratory readiness of NK cells and monocytes. This is consistent with evidence suggesting that acute exercise–induced NK cell redistribution is not fully explained by cortisol alone but instead arises through multifactorial mechanisms involving adrenergic signaling combined with glucocorticoid modulation (Capellino et al., 2020; Okutsu et al., 2014).

According to our data, among men who did not successfully complete the physical test, exercise-induced fluctuations in cortisol levels were closely associated with NK-cell mobilization, whereas no such association was observed in men who completed the test successfully. These observations partially support previous evidence indicating that acute exercise–induced redistribution of NK cells cannot be fully explained by cortisol responses alone. Instead, they suggest that habitual physical training status may play a key role in modulating immune responsiveness to physical exertion and acute hormonal fluctuations.

Concomitant increases in testosterone concentrations may further modulate the functional profiles of NK cells and monocytes through androgen receptor–mediated signaling. Androgen receptors are expressed in multiple immune cell types, including myeloid populations, and are known to regulate transcriptional programs involved in inflammation and cytokine production (Ainslie et al., 2024). Emerging evidence further indicates that the effects of testosterone on monocyte- and NK-associated pathways are highly context dependent, suggesting that even short-term hormonal fluctuations may fine-tune immune responses to acute physical stress (Lakshmikanth et al., 2024). Taken together, the concomitant elevation of cortisol and testosterone observed in our study likely reflects a coordinated activation of two stress-response axes, with early mobilization of NK cells and monocytes primarily driven by β_2-adrenergic-mediated redistribution, while cortisol and testosterone may act as modulatory factors shaping migratory and functional properties during the post-exercise phase (Lakshmikanth et al., 2024; Graff et al., 2018b).

The immunological changes observed in the present study represent a part of a coordinated systemic response aimed at maintaining homeostasis under stress conditions. Experimental evidence indicates that the duration of stress exposure is a critical determinant of stress-induced immune dysregulation. While acute stress has been reported to prepare the organism to cope with challenges and may exert adaptive or even beneficial effects, chronic stress is consistently associated with detrimental consequences for immune function.

Prolonged exposure to stress hormones, particularly glucocorticoids and catecholamines, suppresses T-cell activation and proliferation, impairs NK-cell cytotoxic activity, and promotes a shift toward immunosuppressive phenotypes, thereby increasing susceptibility to infections and chronic inflammatory conditions (Balcerowska and Kwaśnik, 2025). Numerous mechanistic pathways underlying stress-induced immune dysregulation have been described at the molecular and cellular levels, providing insight into how sustained stress responses contribute to an elevated risk of pathological conditions (Balcerowska and Kwaśnik, 2025; Li et al., 2025; Alotiby, 2024).

Taken together, these findings underscore that stress-induced immune dysregulation represents a key mechanism contributing to the development of pathological conditions, including increased susceptibility to infections, chronic low-grade inflammation, and immune-mediated diseases. Accordingly, elucidating the mechanisms that link stress responses to immune imbalance is essential for identifying individuals at risk and for defining potential targets for prevention and early intervention.

This study established population-specific reference intervals for peripheral blood lymphocyte subpopulations in adult males. Comparison with previously published datasets demonstrated that reference intervals for $CD3^+$ T lymphocytes and NK cells were largely consistent with those reported in most male cohorts, whereas other lymphocyte subsets showed substantial inter-study variability.

In addition to defining baseline reference values, the study examined dynamic, context-dependent changes in immune parameters following both short-term and long-term physiological stressors, including a modified Cooper test and a one-year Antarctic expedition. Short-term physical activity exerted a pronounced effect on selected immune cell subsets, most notably inducing significant increases in NK-cell and monocyte counts, accompanied by elevations in circulating testosterone and cortisol concentrations. Importantly, exercise-related alterations in

immunological parameters remained detectable 30 min after exertion, a factor that should be considered when formulating clinical recommendations for blood sampling.

In contrast, prolonged exposure to Antarctic conditions over one year was associated with no significant long-term changes in major immune cell populations, particularly within the NK- and T-cell compartments. This indicates that, unlike the pronounced and transient alterations observed after short-term physical activity, chronic environmental exposure was characterized by relative stability of selected immunological parameters, consistent with long-term physiological adaptation.

Comparison of immunological parameters in healthy male subjects with reference intervals currently applied by local clinical laboratories revealed that a substantial proportion of results fell outside these ranges, indicating limited accuracy and potential clinical misclassification associated with existing laboratory norms. The often unclear origin and validation of such reference values further amplify the risk of overdiagnosis.

Taken together, these findings underscore the necessity of establishing well-characterized, population-specific immunological reference intervals and highlight the importance of inter-laboratory collaboration aimed not only at methodological harmonization but also at the standardization and validation of reference values.

4.1. Limitations

This study included a carefully selected cohort of applicants for Antarctic expeditions—physically fit individuals without chronic diseases—which may limit the applicability of the findings to the broader male population of Ukraine. Therefore, the derived reference ranges may be somewhat narrower than those observed in a more heterogeneous population. Further studies are needed to expand the reference population by including women, a wider age range, and individuals with varying levels of physical fitness in order to improve the applicability of these reference intervals.

CRediT authorship contribution statement

Dariia Zabara: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Formal analysis, Validation, Visualization, Writing – original draft, Writing – review & editing. **Iryna Kozeretska:** Conceptualization, Project administration, Resources, Writing – review & editing. **Ivan Klapoushenko:** Conceptualization, Project administration, Resources, Writing – review & editing. **Yaroslava Anoshko:** Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Ievgen Dubrovskyi:** Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Boris Dons'koi:** Supervision, Conceptualization, Data curation, Formal analysis, Investigation, Validation, Visualization, Methodology, Writing – review & editing.

Informed consent

Written informed consent was obtained from all participants prior to study enrollment.

Ethics statement

The study protocol was reviewed and approved by the Biomedical Ethics Committee of the Institute of Pediatrics, Obstetrics and Gynecology and by the Clinical Immunology and Allergology Committee of the Ministry of Health of Ukraine.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Ainslie, R.J., Simitsidellis, I., Kirkwood, P.M., Gibson, D.A., 2024. RISING STARS: Androgens and immune cell function. J. Endocrinol. 261 (3), e230398. https://doi.org/10.1530/JOE-23-0398.

Alotiby, A., 2024. Immunology of stress: a Review Article. J. Clin. Med. 13 (21), 6394. https://doi.org/10.3390/jcm13216394.

Apoil, P.A., Puissant-Lubrano, B., Congy-Jolivet, N., Peres, M., Tkaczuk, J., Roubinet, F., Blancher, A., 2017. Reference values for T, B and NK human lymphocyte subpopulations in adults. Data Brief 12, 400–404. https://doi.org/10.1016/j.dib.2017.04.019.

Balcerowska, M., Kwaśnik, P., 2025. The multifaceted impact of stress on immune function. Mol. Biol. Rep. 52 (1), 1008. https://doi.org/10.1007/s11033-025-11134-6.

Bisset, L.R., Lung, T.L., Kaelin, M., Ludwig, E., Dubs, R.W., 2004. Reference values for peripheral blood lymphocyte phenotypes applicable to the healthy adult population in Switzerland. Eur. J. Haematol. 72 (3), 204–212. https://doi.org/10.1046/j.0902-4441.2003.00199.x.

Cabalín, C., García-García, A., Valdés, A., et al., 2021. Vitamin D status and supplementation in Antarctica: a systematic review and meta-analysis. Nutrients 13 (5), 1499. https://doi.org/10.3390/nu13051499.

Cain, D.W., Cidlowski, J.A., 2017. Immune regulation by glucocorticoids. Nat. Rev. Immunol. 17 (4), 233–247. https://doi.org/10.1038/nri.2017.1.

Campbell, J.P., Turner, J.E., 2018. Debunking the myth of exercise-induced immune suppression: redefining the impact of exercise on immunological health across the lifespan. Front. Immunol. 9, 648. https://doi.org/10.3389/fimmu.2018.00648.

Capellino, S., Claus, M., Watzl, C., 2020. Regulation of natural killer cell activity by glucocorticoids, serotonin, dopamine, and epinephrine. Cell. Mol. Immunol. 17 (7), 705–711. https://doi.org/10.1038/s41423-020-0477-9.

Clinical and Laboratory Standards Institute (CLSI). Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; approved guideline—third edition (EP28-A3c). Wayne (PA): Clinical and Laboratory Standards Institute; 2010. (Reaffirmed 2020).

Crisafulli, A., Tocco, F., Melis, F., Milia, R., Concu, A., 2013. Natural killer cells responsiveness to physical exercise: a brief review. Open J Immunol 3, 190–200. https://doi.org/10.4236/oji.2013.34024.

D'Andrea S, Spaggiari G, Barbonetti A, Santi D. Endogenous transient doping: physical exercise acutely increases testosterone levels-results from a meta-analysis. J. Endocrinol. Invest. 2020 Oct;43(10):1349–1371. doi:https://doi.org/10.1007/s40618-020-01251-3. Epub 2020 Apr 15. PMID: 32297287.

Dawod, O.Y., 2025. Immune response modulation through physical activity: mechanisms and outcomes. South East Eur. J. Public Health 4079–4094. https://doi.org/10.70135/seejph.vi.4767.

Dons'koi, B.V., Osypchuk, D.V., Chernyshov, V.P., 2019. Enumeration of peripheral blood NKp46 positive NK lymphocytes reflects NK cytotoxic activity in vitro. J. Immunol. Methods 474, 112639. https://doi.org/10.1016/j.jim.2019.112639.

Dons'koi, B.V., Tutchenko, T.M., Chernyshov, V.P., Stepaniuk, K.S., 2020. HCMV seropositivity is associated with specific proinflammatory immune phenotype in women with implantation failure. Immunol. Lett. 217, 84–90. https://doi.org/10.1016/j.imlet.2019.11.008.

Dote-Montero, M., Carneiro-Barrera, A., Martinez-Vizcaino, V., Ruiz, J.R., Amaro-Gahete, F.J., 2021. Acute effect of high-intensity interval training on testosterone and cortisol levels in healthy individuals: a systematic review and meta-analysis. Scand. J. Med. Sci. Sports 31 (9), 1722–1744. https://doi.org/10.1111/sms.13999.

Dubrovskyi I, Zabara D, Anoshko Y, et al. Immune accentuations as a prognostic factor for adaptation in Antarctic conditions. Polar Biol. 2025;48:53.doi:https://doi.org/10.1007/s00300-025-03359-9.

Fowler, K., Mucha, J., Neumann, M., Lewandowski, W., Kaczanowska, M., Grys, M., et al., 2022. A systematic literature review of the global seroprevalence of cytomegalovirus: possible implications for treatment, screening, and vaccine development. BMC Public Health 22 (1), 13971. https://doi.org/10.1186/s12889-022-13971-7.

Graff, R.M., Kunz, H.E., Agha, N.H., Baker, F.L., Laughlin, M.S., Bigley, A.B., et al., 2018a. B_2-Adrenergic receptor signaling mediates the preferential mobilization of differentiated subsets of $CD8^+$ T cells, NK cells, and non-classical monocytes in response to acute exercise in humans. Brain Behav. Immun. 74, 143–153. https://doi.org/10.1016/j.bbi.2018.08.017.

Graff, R.M., Kunz, H.E., Agha, N.H., Baker, F.L., Laughlin, M., Bigley, A.B., Markofski, M. M., LaVoy, E.C., Katsanis, E., Bond, R.A., Bollard, C.M., Simpson, R.J., 2018b. β2-Adrenergic receptor signaling mediates the preferential mobilization of differentiated subsets of CD8+ T-cells, NK-cells and non-classical monocytes in response to acute exercise in humans. Brain Behav. Immun. 74, 143–153. https://doi.org/10.1016/j.bbi.2018.08.017.

Hadad, U., Thauland, T.J., Martinez, O.M., Butte, M.J., Porgador, A., Krams, S.M., 2015. NKp46 clusters at the immune synapse and regulates NK cell polarization. Front. Immunol. 6, 495. https://doi.org/10.3389/fimmu.2015.00495.

Klose, N., Coulibaly, B., Tebit, D.M., Nauwelaers, F., Spengler, H.P., 2007. Immunohematological reference values for healthy adults in Burkina Faso. Int. J. Immunopathol. Pharmacol. 20 (4), 751–758. https://doi.org/10.1128/CVI.00044-07.

Lakshmikanth, T., Consiglio, C., Sardh, F., et al., 2024. Immune system adaptation during gender-affirming testosterone treatment. Nature 633, 155–164. https://doi.org/10.1038/s41586-024-07789-z.

Li, G., Wan, Y., Jiao, A., Jiang, K., Cui, G., Tang, J., Yu, S., Hu, Z., Zhao, S., Yi, Z., Long, L., Yang, Y., Cui, X., Qu, C., 2025. Breaking Boundaries: Chronic Diseases and the Frontiers of Immune Microenvironments. Med Research 1, 62–102. https://doi.org/10.1002/mdr2.70007.

Liu W, Xu J, Pu Q, Lan M, Zhang X, Gu Y, et al. The reference ranges and characteristics of lymphocyte parameters and the correlation between lymphocyte parameters and routine health indicators in adults from China. Immun. Ageing 2022;19(1):42. doi: https://doi.org/10.1186/s12979-022-00298-5.

Maecker, H.T., Trotter, J., 2006. Flow cytometry controls, instrument setup, and the determination of positivity. Cytometry A 69 (9), 1037–1042. https://doi.org/10.1002/cyto.a.20333.

Martens, P.J., Gysemans, C., Verstuyf, A., Mathieu, A.C., 2020. Vitamin D's effect on immune function. Nutrients 12 (5), 1248. https://doi.org/10.3390/nu12051248.

Millard, A.L., Valli, A., Aspinall, R., Burns, V.E., 2013. Brief exercise increases peripheral blood NK cell counts in healthy donors. Front. Immunol. 4, 125. https://doi.org/10.3389/fimmu.2013.00125.

Neves, P.R.D.S., Tenório, T.R.D.S., Lins, T.A., Muniz, M.T.C., Pithon-Curi, T.C., Botero, J. P., et al., 2015. Acute effects of high- and low-intensity exercise bouts on leukocyte counts. J. Exerc. Sci. Fit. 13 (1), 24–28. https://doi.org/10.1016/j.jesf.2014.11.003.

Okutsu, M., Ishii, K., Niu, K., & Nagatomi, R. (2014). Cortisol is not the primary mediator for augmented CXCR4 expression on natural killer cells after acute exercise. Journal of applied physiology (Bethesda, Md. : 1985), 117(3), 199–204. https://doi.org/10.1152/japplphysiol.00176.2014, J. Appl. Physiol.

Pedersen, B.K., Hoffman-Goetz, L., 2000. Exercise and the immune system: regulation, integration, and adaptation. Physiol. Rev. 80 (3), 1055–1081. https://doi.org/10.1152/physrev.2000.80.3.1055.

Phan, M.T., Chun, S., Kim, S.H., Ali, A.K., Lee, S.H., Kim, S., et al., 2017. Natural killer cell subsets and receptor expression in peripheral blood mononuclear cells of a healthy Korean population: reference range, influence of age and sex, and correlation between NK cell receptors and cytotoxicity. Hum. Immunol. 78 (2), 103–112. https://doi.org/10.1016/j.humimm.2016.11.006.

Preglej, T., Brinkmann, M., Steiner, G., Aletaha, D., Göschl, L., Bonelli, M., 2023. Advanced immunophenotyping: a powerful tool for immune profiling, drug screening, and a personalized treatment approach. Front. Immunol. 14, 1096096. https://doi.org/10.3389/fimmu.2023.1096096.

Rawat, A., Arora, K., Shandilya, J., Vignesh, P., Suri, D., Kaur, G., et al., 2019. Flow cytometry for diagnosis of primary immune deficiencies – a tertiary center experience from North India. Front. Immunol. 10, 2111. https://doi.org/10.3389/fimmu.2019.02111.

Riachy, R., McKinney, K., Tuvdendorj, D.R., 2020. Various factors may modulate the effect of exercise on testosterone levels in men. J Funct Morphol Kinesiol. 5 (4), 81. https://doi.org/10.3390/jfmk5040081.

Roszczyk A, Zych M, Sołdacki D, et al. Reference values of lymphocyte subsets from healthy polish adults. Cent Eur J Immunol 2024;49(1):26–36. doi:https://doi.org/10.5114/ceji.2024.136371.

Rovati, B., Mariucci, S., Poma, R., Tinelli, C., Delfanti, S., Pedrazzoli, P., 2014. An eight-colour flow cytometric method for detection of reference values of lymphocyte subsets in selected healthy donors. Clin. Exp. Med. 14 (3), 267–275. https://doi.org/10.1007/s10238-013-0239-4.

Rudolf-Oliveira, R.C.M., Gonçalves, K.T., Maringnago, M.L., et al., 2015. Determination of lymphocyte subset reference ranges in peripheral blood of healthy adults by flow cytometric method. Immunol. Lett. 163 (1), 96–101. https://doi.org/10.1016/j.imlet.2014.11.003.

Sharma, D., et al., 2020. Adrenergic regulation of immune cell function and inflammation. Semin. Immunopathol. 42 (3), 357–370. https://doi.org/10.1007/s00281-020-00829-6.

Shephard, R.J., 1998. Immune changes induced by exercise in an adverse environment. Can. J. Physiol. Pharmacol. 76 (5), 539–546. https://doi.org/10.1139/cjpp-76-5-539.

Shi X, Hu L, Nieman DC, Li F, Chen P, Shi H, et al. Exercise workload: a key determinant of immune health – a narrative review. Front. Immunol. 2025;16:1617261.doi:https://doi.org/10.3389/fimmu.2025.1617261.

Taipale, R., Häkkinen, K., 2013. Acute hormonal and force responses to combined strength and endurance loadings in men and women: the "order effect". PloS One 8 (2), e55051. https://doi.org/10.1371/journal.pone.0055051.

Thakar MR, Abraham PR, Arora S, Balakrishnan P, Bandyopadhyay B, Joshi AA, et al. Establishment of reference $CD4^+$ T cell values for adult Indian population. AIDS Res. Ther. 2011;8:35.doi:https://doi.org/10.1186/1742-6405-8-35.

Valiathan, R., Deeb, K., Diamante, M., Ashman, M., Sachdeva, N., Asthana, D., 2014. Reference ranges of lymphocyte subsets in healthy adults and adolescents with special mention of T cell maturation subsets in adults of South Florida. Immunobiology 219 (7), 487–496. https://doi.org/10.1016/j.imbio.2014.02.010.

Vancolen, S., Sébire, G., Robaire, B., 2023. Influence of androgens on the innate immune system. Andrology 11 (7), 1237–1244. https://doi.org/10.1111/andr.13416.

Yaman, A., Çetiner, S., Kibar, F., Taşova, Y., Seydaoglu, G., Dündar, I.H., 2005. Reference ranges of lymphocyte subsets of healthy adults in Turkey. Med. Princ. Pract. 14 (3), 189–193. https://doi.org/10.1159/000084638.