Lifestyle Exercise Attenuates Immunosenescence; Flow Cytometry Analysis

Anna Tylutka
University of Zielona Gora
Barbara Morawin
Univeristy of Zielona Gora
Artur Gramacki
University of Zielona Gora
Agnieszka Zembron-Lacny (a.zembron-lacny@cm.uz.zgora.pl)
https://orcid.org/0000-0001-7596-9850

Research article

Keywords: lymphocytes T, obesity, physical activity, successful ageing

Posted Date: July 27th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-47396/v1

Version of Record: A version of this preprint was published on March 22nd, 2021. See the published version at https://doi.org/10.1186/s12877-021-02128-7.

Abstract

It is widely well documented that ageing is accompanied by remodelling of the immune system including thymic atrophy and increased frequency of senescent T cells, thereby leading to immune compromise. The interaction of physical activity and the immune response is very complex and depends on the immune system status as well as the intensity, the type and the frequency of exercise. However, daily physical activity which particularly influences immunity and which declines dramatically with age, has not been widely reported in the studies. Therefore, this study was designed to explain whether physical activity sustained throughout life attenuates or reverses immunosenescence.

The study subjects (*n*=99) were recruited from the University of the Third Age; they were assessed for the presence of chronic diseases and their functional capacity (6-min walking test) and cardiorespiratory (6-min Åstrand test) fitness were evaluated. Eventually, 54 subjects of the mean age >65 years participated in the study. The baseline peripheral naïve and memory T cells were analysed by flow cytometry in 34 elderly subjects who had maintained a high level of physical activity (Nordic-walking, Tai-Chi, cycling, 2-4 times per week) for much of their adult lives, and in 20 elderly people who had not been involved in regular exercise. The elderly who demonstrated a superior gait speed >1.3 m/s had a significantly higher the $CD4^+$ naïve T lymphocyte population and a higher $CD4^+$naïve/$CD4^+$memory ratio compared to the inactive group. Above 50% of active individuals had the CD4/CD8 ratio ≥ 1 or ≤ 2.5 contrary to the inactive ones who demonstrated the values of CD4/CD8 ratio <1. Interestingly, the fat mass (FM) was positively correlated with $CD4^+$ and $CD4^+$memory in the inactive group which means that the number of $CD4^+$memory cells and $CD4^+$ T cells increased with the body fat content. Based on cytometry flow analysis, we concluded that major features of immunosenescence are contingent on lifestyle exercise in older adults.

Background

Ageing is accompanied by a decline in immune competence, termed immunosenescence, which is characterised by an increased risk of infections and chronic inflammatory diseases, poor vaccine efficacy, failure to maintain immunity to latent infections and increased autoimmunity. The mechanisms underlying this compromised immunity include involution of the thymus engaged in generation of T cells, with the most important change being the output of new naïve T cells ($CD45RA^+CD45RO^-CD62L^+CCR7^+$). The T cell pool is part of subpopulations of antigen-inexperienced naïve cells and antigen-experienced memory cells. The human immune compartment is composed of ~ 10^{12} T cells in total, ~10^{11} of which are naïve [1]. During the process of ageing, the population of naïve T cells decreases, while the population of memory T cells undergoes intensive proliferation [2, 3, 4]. The loss of naïve T lymphocytes of approximately 30% in older individuals is compensated by the expansion of T cells with $CD8^+CD45RO^+CD25^+$ phenotype. An increase in memory T cells enhances immunological memory of previously encountered antigens, thereby augmenting the existent immune protection. The remaining naïve T cell pool experiences a loss of T cell receptor (TCR) 'structural diversity': the number of distinct

TCR complexes present across the entire naïve pool [5, 6]. The diversity of T cell lymphocyte clones, associated with the different number of distinct TCR complexes among the cell population, ensures a suitable range of antigen specificity [1]. The number of naïve $CD4^+$ T cells is reduced at about 70 years of age whereas a decline in $CD8^+$ naïve cells occurs much earlier due to their sensitivity to apoptosis thereby increasing a risk of infectious diseases and contributing to the cardiovascular, metabolic, autoimmune, and neurodegenerative diseases [7].

As ageing is a natural process, the risk associated with relatively invasive surgical and immuno-therapeutic procedures, i.e. gene therapy, cytokine therapy, monoclonal antibody therapy, in otherwise healthy people is unlikely to be deemed acceptable. Of late, there has been some interest in the manipulation of certain lifestyle factors, such as an increasing physical activity level, as a way of moderating the effects of ageing on the immune system [8, 9]. In their latest research, Wong et al. [10] revealed an improved immunity response to the vaccination in more physically active elderly females. However, most studies concentrate on the effects of long-term exercise on immunity in master athletes, and they either disregard immunity response in active and inactive young participants or document the immune changes in response to an exercise training intervention in older adults [8, 11]. Daily physical activity which particularly affects immunity and dramatically declines with age has not been widely investigated yet.

The interaction of lifestyle exercise and the immune response is very complex and yet to be clarified. Regular physical activity including cardiovascular and resistance exercise has been associated with lower levels of pro-inflammatory cytokines, such as IL-6 and TNFα, and higher antioxidant capacity [12, 13], improved neutrophils chemotaxis [14], NK cell cytotoxicity and increased T-cell proliferation [15] as well as a greater post-vaccination response [10, 16]. However, current literature on immunosenescence is not able to determine which aspects of age-related immune changes are driven by exercise factors and which may be the consequence of a sedentary lifestyle. Future analyses can improve our understanding of the major features of immunosenescence and the impact of regular physical activity on the immune system in old age [17]. Therefore, the study was designed to evaluate the relationship between lifestyle exercise and numbers of naïve and memory $CD4^+$ and $CD8^+$ T cells as well as CD4/CD8 ratio in active compared to inactive older adults.

Material And Methods

Subjects (Table.1). Ninety-nine subjects were recruited from the University of the Third Age (U3A) (Fig. 1). The age of 60–90 years was the inclusion criterium. The exclusion criteria included: any experience in competitive sport, acute infectious diseases, oncologic diseases, neurodegenerative diseases, and an implemented pacemaker, based on the assessment of the responsible physician and the investigator. The current health status and lifestyle of the subjects were estimated by means of the health history questionnaire [18]. Eventually, the study included fifty-four subjects (females $n = 47$ males $n = 7$), aged 65–88 years, who represented the successful ageing according to the definition by Geard et al. [19]. On the basis of the assessment of functional and cardiorespiratory fitness, according to Åstrand [20], as well

as the gait speed measurement, according to Middleton et al. [21] and Studenski [22], thirty-four subjects (females n = 31, males n = 3) were included in the physically active group whereas the inactive group was composed of twenty subjects (females n = 16, males n = 4). All U3A students were informed of the aim of the study and gave their written consent for participation in the project. The protocol of the study was approved by The Bioethics Commission at Regional Medical Chamber Zielona Gora, Poland (N^{o}01/66/2017, N^{o}21/103/2018) in accordance with the Helsinki Declaration.

Table 1
Anthropometrics and body composition in the elderly (mean ± SD).

	Active *n* **= 34**	**Inactive** *n* **= 20**	**Active *vs.* Inactive *p* level**	η^2
Age [yr.]	70.2 ± 5.8	73.5 ± 5.4	< 0.05	0.008
Weight [kg]	69.8 ± 11.8	67.1 ± 11.3	0.567	0.000
Height [cm]	160.3 ± 6.0	159.7 ± 7.2	0.573	0.040
BMI [kg/m^2]	27.1 ± 3.6	26.3 ± 4.1	0.529	0.006
FM [kg]	24.1 ± 5.8	22.0 ± 5.9	0.268	0.004
FM%	34.3 ± 4.7	32.7 ± 6.2	0.348	0.006
FFM [kg]	45.7 ± 7.7	45.1 ± 8.7	0.622	0.012
SBP [mmHg]	145.1 ± 19.2	151.3 ± 20.9	0.264	0.017
DBP [mmHg]	81.2 ± 11.6	79.2 ± 12.9	0.602	0.004
6MWT [m]	527 ± 52	388 ± 59	< 0.001	0.662
Gate speed [m/s]	1.5 ± 0.1	1.0 ± 0.1	< 0.001	0.662
VO_2max [mL/kg/min]	35.8 ± 5.7	32.8 ± 4.0	0.131	0.060

Abbreviations: BMI Body Mass Index, FM Fat Mass, FFM Fat-Free Mass, SBP systolic blood pressure, DBP diastolic blood pressure, 6MWT 6-min walking test, VO_2max maximal oxygen consumption. The measurements in groups are compared by the one-way ANOVA or the Manna-Whitney non-parametric test (if the normality assumption is violated).

Body composition. Body mass (BM) and body composition fat-free mass (FFM) and FM were estimated by a bioelectrical impedance method using Tanita Body Composition Analyser MC-980 (Japan) calibrated prior to each test session in accordance with the manufacturer's guidelines. Duplicate measures were taken with the participant in a standing position; the average value was used for the final analysis. The recurrence of measurement was 98%. The measurements were taken between 7:00 and 9:00 a.m., before blood sampling.

Functional fitness. The 6-min walking test (6MWT) was performed according to technical standards of European Respiratory Society and American Thoracic Society [23]. A marked walkway was laid out in a 50-m rectangular area (dimensions: 20 × 5 m), with cones placed at regular intervals to indicate the distance covered. The aim of the test was to walk as quickly and as far as possible over a span of six minutes. The subjects were allowed to self-pace (a preliminary trial was useful to practice pacing) and rest as needed. The total distance walked in the test was recorded and the 6MWT gait speed was then calculated by the following equation: 6MWT gait speed (m/s) = total distance(m)/360 s. The gait speed ranging from 1.3 to 1.8 m/s classified the older adults as active and the gait speed < 1.3 m/s classified them as inactive according to Middleton et al. [21].

Cardiorespiratory fitness. The measurement of maximal oxygen consumption (VO_2max) was performed via the indirect method known as the Åstrand-Ryhming bike test (6-min submaximal exercise test) which relies on the linear relationship between heart rate (HR) and VO_2 to predict maxVO_2 and which is recommended for both men and women of various ages [20]. Each subject performed a 6-min submaximal exercise test on a cycle ergometer eBike GE Healthcare (Germany). Initially, the study subjects rested for 15 minutes prior to the measurement of their resting HR. The seat height and handlebars were adjusted for each subject prior to the test. According to normative data for submaximal exercise test, the subjects who reached the values of VO_2max > 35 mL/kg/min were classified as active (high activity level) and the remaining ones were determined as inactive (average and low activity level).

Type and amount of physical activity. The type and weekly amount of physical activity was evaluated by Community Healthy Activities Model Program for Seniors (CHAMPS) [24]. The CHAMPS was originally designed to assess the types and intensity levels of physical activity including lighter (e.g. leisurely walking, water gymnastics, stretching, Tai-Chi) as well as more vigorous activities (e.g. dancing, cycling, swimming). Currently, the CHAMPS also includes a group of items related to a sedentary lifestyle e.g. sitting and chatting with friends.

Blood sampling. Blood samples were taken from the median cubital vein using S-Monovette-EDTA K_2 tubes (Sarstedt, Austria) for flow cytometry analysis and morphology and S-Monovette - serum tubes were used for other biochemical markers. Serum samples were left to clot for 45 min before centrifugation and then centrifuged at 3000 g and + 8^{o}C for 10 min. Aliquots of serum were stored at -80 °C.

Flow cytometry analysis. Cytometric analysis was performed using eight-parameter CyFlow Space Sorter flow cytometer by Sysmex Partec (Germany). For the analysis of immune cells, CyLyse kit by Sysmex (Germany) was used. 100 µl venous blood was mixed with fluorochrome labeled monoclonal antibodies (CD8 APC, CD4 FITC, CD45 RA Pacific Blue™ CD45RO PE) and incubated for 15 minutes in the dark at room temperature. After the incubation 100 µL of Leukocyte Fixation Reagent A was added and incubated again in the dark for 10 minutes. In the last step, 2.5 ml Erythrocytes Lysing Reagent B was added, mixed and incubated in the dark for 20 minutes and further measurements were made. T helper and cytotoxic lymphocytes were gated by positive surface staining for CD4 and CD8 and were expressed

as a percentage of gated lymphocytes. Memory and naïve subpopulations were gated by positive surface staining for CD45RO and CD45RA, respectively. The ratios of $CD4^+$naïve to $CD4^+$memory and $CD8^+$naïve to $CD8^+$memory, as prognostic markers of chronic diseases, were calculated according to Hang et al. [25]. Moreover, the CD4/CD8 ratio was calculated according to McBride and Striker [26] to express the Immune Risk Profile (IRP) associated with altered immune function. The reference values for IRP were adopted from Strindhall et al. study [27]. The ratios ≥ 1 or ≤ 2.5 are generally considered normal, however, a wide heterogeneity exists because of sex, age, ethnicity, genetics, environmental exposures and infections. The high or inverted CD4/CD8 ratio (< 1 or > 2.5) is regarded as an immune risk phenotype and is associated with immunosenescence and chronic inflammatory disease**s** [27].

Haematological variables. Peripheral blood morphology: white blood cell count (WBC), granulocytes (%GRA), lymphocytes (%LYM), mid absolute count (%MID), red blood cells count (RBC), haemoglobin (HB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT) were determined by using 3 diff BM HEM3 Biomaxima (Poland).

Biochemical markers. Total cholesterol (TC), high-density lipoproteins (HDL), low-density lipoproteins (LDL), triglycerides (TG) were determined by using BM200 Biomaxima (Poland). The non-HDL cholesterol was calculated by subtracting HDL from the total cholesterol concentration. The serum C-reactive protein (CRP) level was determined in duplicate by DRG ELISA kit (Poland). 0.001 mg/L was established as the detection limit and CV for the CRP kit was set at < 3%. The serum glucose was measured spectrophotometrically using Diaglobal spectrophotometer (Germany).

Statistical analysis. Statistical analyses were performed using the R system, version 3.6.1 [28]. The assumptions for the use of parametric or non-parametric tests were checked using the Shapiro-Wilk and the Levene tests to evaluate the normality of the distributions and the homogeneity of variances, respectively. The significant differences in mean values between the groups (Active *vs.* Inactive) were assessed by the one-way ANOVA. If the normality and homogeneity assumptions were violated, the Manna-Whitney non-parametric test was used. Additionally, eta-squared (η^2) was used as a measure of effect size which is indicated as having no effect if $0 \leq \eta^2 < 0.01$, a minimum effect if $0.01 \leq \eta^2 < 0.06$, a moderate effect if $0.06 \leq \eta^2 < 0.14$, and a strong effect if $\eta^2 \geq 0.14$ [29, 30]. Pearson's correlation coefficients were calculated to describe the relationships between a physical activity level and immune cells counts. Statistical significance was set at $p < 0.05$.

Results

Body composition (Table.1). The body mass index (BMI) in the physically active elderly ranged from 19.4 to 35.0 kg/m^2 whereas in the physically non-active elderly their BMI fell within 19.0 to 37.0 kg/m^2 range. Jointly, in both groups, approximately 20% were classified as obese (BMI > 30 kg/m^2). The BMI value highly correlated with fat mass content in active ($r = 0.862$, $p < 0.001$) and inactive individuals ($r = 0.857$, $p < 0.001$). In the physically inactive group, the high fat content significantly reduced the maximal oxygen

consumption which was confirmed by inverse correlation between FM and VO_2max (r=-0.618, $p < 0.05$). Moreover, the high fat content correlated with $CD4^+$ (r = 0.494, $p < 0.05$) and $CD4^+$memory T cells (r = 0.484, $p < 0.05$) in the inactive subjects. This shows that a low level of everyday activities impairs immunity more considerably in the obese than in the slim elderly.

Functional and cardiorespiratory fitness (Table 1). The result of the 6MWT was by 36% higher in active than inactive group. High physical fitness level was confirmed by VO_2max value which was elevated by 10% in the active individuals. Above 95% of the study subjects achieved a normal gait speed ≥1 m/s according to reference values by Middleton et al. [21]. Interestingly, the elderly who demonstrated a superior gait speed ≥1.3 m/s demonstrated a significantly higher $CD4^+$ naïve T lymphocyte population and a higher $CD4^+$naïve/$CD4^+$memory ratio compared to the inactive group. The value η^2 indicated a strong effect of lifestyle exercise on the result of the gait speed test and a moderate effect on VO_2max value.

Flow cytometry analysis. $CD4^+$ and $CD8^+$ T cells were analysed within naïve and memory subpopulations in the study groups of active and inactive older adults. The percentage of $CD4^+$ and $CD8^+$ cells showed a tendency towards low values in physically active compared to inactive elderly subjects but the differences did not reach the level of significance (Fig. 2a and 2d). The active study group of older adults was found to exhibit a decreased number of both $CD4^+$ and $CD8^+$ memory T cells (Fig. 2c and 2f). However, the percentage of $CD4^+$ naïve cells (Fig. 2b) and the $CD4^+$ T naïve/ memory ratio (Fig. 2h) were significantly higher in the physically active group compared to the inactive one. There were no statistically significant differences between males and females in either of the groups (active *vs.* inactive). The value η^2 indicated a moderate impact of lifestyle exercise on the $CD4^+$ naïve T cells as well as on the ratio of $CD4^+$ naïve to $CD4^+$ memory. The CD4/CD8 ratio was observed to be higher in the active than inactive individuals (Fig. 2g). 52.94% of the physically active older adults demonstrated CD4/CD8 ratio ≥ 1 or ≤ 2.5, 44.11% of them exhibited the CD4/CD8 ratio > 2.5, and the ratio < 1 was recorded only in 2.94%. The CD4/CD8 ratio ≥ 1 or ≤ 2.5 was detected in approximately 45% of the inactive older adults, whereas 40% of them exhibited the CD4/CD8 ratio > 2.5, and only 15% showed the CD4/CD8 ratio < 1. This shows that inactive lifestyle especially shifts the values of CD4/CD8 ratio < 1. In the physically active subjects with normal BMI (BMI < 24.9 kg/m^2) and the overweight ones (BMI < 29.9 kg/m^2), no value of CD4/CD8 ratio < 1 was identified which was the case in the physically active group with obesity (BMI > 30 kg/m^2). 42.85% of the active obese subjects demonstrated the CD4/CD8 ratio ≥ 1 or ≤ 2.5, the CD4/CD8 ratio > 2.5 was found in 28.55% of the group and only 14.28.% exhibited the CD4/CD8 ratio < 1. This indicates that high fat content increases IRP regardless of the physical activity level. However, approximately 60% of our physically inactive subjects with overweight and obesity demonstrated the CD4/CD8 ratio > 2.5 and the CD4/CD8 ratio < 1 was identified in only 20% of the group. The results clearly indicate that a low level of lifestyle exercise results in a higher IRP in older adults which, in turn, is related to immunosenescence and chronic inflammatory diseases [27].

Haematological variable (Table 2). The immune cell numbers (lymphocytes, %LYM, %GRA, %HCT, MCV, MCHC, MCH) were found to be at similar levels in all subjects. However, there were significant differences between PLT in the active compared to inactive subjects. Moreover, an increasing trend in HB, RBC and HCT was observed in the physically active group. The value η^2 indicated a moderate influence of lifestyle exercise on HB level, which suggests that a protective effect of exercise is also exerted on hematopoietic processes in the elderly. There were no statistically significant differences in HB, RBC and HCT between males and females in both groups (active *vs*. inactive).

Table 2
Haematological variables in the elderly (mean ± SD).

	Reference values	Active n = 34	Inactive n = 20	Active *vs.* Inactive *p* level	η^2
WBC [10^3/μL]	5.0–11.6	6.7 ± 2.1	6.5 ± 2.0	0.740	0.055
Lymphocytes [10^3/μL]	1.3 - 4.0	2.3 ± 0.6	2.2 ± 0.9	0.119	0.002
Granulocytes [10^3/μL]	2.4–7.6	3.9 ± 1.6	3.8 ± 1.4	0.851	0.076
LYM %	19.1–48.5	35.5 ± 7.6	33.7 ± 9.0	0.486	0.003
GRA %	43.6–73.4	57.2 ± 9.2	57.2 ± 9.0	0.981	0.000
RBC [10^3/μL]	F 4.0–5.5 M 4.5–6.6	4.8 ± 0.3	4.7 ± 0.3	0.209	0.054
HB [g/dL]	F 12.5–16.0 M 13.5–18.0	13.9 ± 0.7	13.7 ± 0.8	0.513	0.108
HCT [%]	F 37–47 M 40.0–51.0	39.5 ± 2.0	38.6 ± 2.4	0.201	0.031
MCV [fL]	F 80–95 M 80–97	81.9 ± 3.0	81.7 ± 2.6	0.950	0.048
MCH [pg]	F 27.0–32.0 M 26.0–32.0	28.7 ± 1.3	29.0 ± 1.1	0.395	0.054
MCHC [g/dL]	F 32.0–36.0 M 31.0–36.0	35.1 ± 0.8	35.5 ± 0.6	0.330	0.018
PLT [10^3/μL]	150–400	270 ± 60	236 ± 39	< 0.01	0.031

Abbreviations: WBC white blood cells, LYM lymphocytes, GRA granulocytes, RBC red blood cells HB haemoglobin, HCT hematocrit, MCV mean corpuscular volume, MCH mean cells haemoglobin, MCHC corpuscular/cellular haemoglobin concentration, PLT platelets, F female, M male. The measurements in groups are compared by the one-way ANOVA or the Manna-Whitney non-parametric test (if the normality assumption is violated.

Biochemical markers (Table 3). The glucose and TG concentrations did not exceed the limits of the reference values in most study subjects. TC concentration was found to fall within the range of 157 to 372 mg/dL in the physically active subjects whereas in the inactive group it amounted to the values from 162 to 394 mg/dL. High TC concentration > 200 mg/dL was observed in approximately 87% of the study

subjects and only 5 subjects took lipid-modifying drugs. Similar observations were made with regard to the changes in LDL, HDL and non-HDL concentrations. CRP level tended to reach higher values in the active than inactive subjects but remained within the reference range. However, a lower CRP concentration (2.62 ± 1.88 mg/L) was found in the elderly who demonstrated a superior gait speed ≥1.4 m/s when compared to the other active subjects (4.19 ± 2.75 mg/L). CRP concentration was inversely correlated with the results of the 6MWT or gait speed test (r= -0.350, $p < 0.05$) in the active older adults, which clearly indicates that lifestyle exercise diminishes the systemic inflammatory response.

Table 3
Lipoprotein-lipid profile, glucose and C-reactive protein in the elderly (mean ± SD).

	Reference values	Active *n* = 34	Inactive *n* = 20	Active *vs.* Inactive *p* level	η^2
Glucose [mg/dL]	60–115	98.6 ± 17.1	97.6 ± 20.3	0.452	0.000
TG [mg/dL]	< 150	90.2 ± 25.7	83.7± 26.0	0.375	0.000
TC [mg/dL]	< 200	262.4 ± 52.6	251.3 ± 59.1	0.534	0.013
LDL [mg/dL]	< 130	147.1 ± 40.3	149.1 ± 50.8	0.889	0.000
HDL [mg/dL]	desirable > 60	82.2 ± 16.0	81.1 ± 11.8	0.603	0.023
non-HDL [mg/dL]	< 130	180.2 ± 54.7	170.2 ± 65.5	0.599	0.047
CRP [mg/L]	0.068–8.2	3.21 ± 2.50	2.84 ± 3.14	0.121	0.004

Abbreviations: TC total cholesterol, TG triglycerides, LDL low density lipoprotein, HDL high density lipoprotein, CRP C-reactive protein. The measurements in groups are compared by the one-way ANOVA or the Manna-Whitney non-parametric test (if the normality assumption is violated).

Discussion

Regular exercise has a profound effect on the normal functioning of the immune system. It is generally accepted that prolonged periods of high-intensity exercise can depress immunity, while regular exercise of moderate-intensity produces a range of beneficial effects [31]. Physical activity maintained for much of adult lives enhances immune function and effectively increases vaccine response in “at-risk” patients. Exercise-induced improvements in immunity can be related to reduction in inflammation, maintenance of thymic mass, alterations in the composition of memory and naïve lymphocyte T cells, enhanced immunosurveillance, and/or the amelioration of psychological stress. Indeed, exercise is a powerful behavioural intervention that has the potential to improve immune and health outcomes in the elderly, the obese, and patients with cancer and chronic viral infections such as HIV [9, 32]. The benefits of regular physical activity undertaken by the elderly are much less documented than the effects of regular exercise on the immune system in young individuals [33]. Three randomized perspective trials were conducted to explain the effect of aerobic exercise on the immune system of the elderly, where Nieman et al. [34] found that a 3-month moderate aerobic exercise programme did not cause a significant increase in T

lymphocyte mitogenesis. On the other hand, the effect of resistance training on the immune function has also been poorly investigated and most researchers agreed that 8-12-week training had minimal effects on innate or acquired immunity in elderly people [35]. It is well known that physical activity does not only exert anti-inflammatory effects but also positively affects the metabolic health in the elderly. The results of CHAMPS questionnaire in our study confirmed the activity of our recruited seniors in everyday life, where seniors who were physically active were much more likely to lead a more active life, by being engaged in Nordic walking, swimming, intensive walks, and Tai-Chi, than inactive elderly subjects. In our study, the active elderly covered a much longer distance in the 6MWT compared to the inactive seniors. In addition, the active elderly demonstrated the gait speed of 1.5 ± 0.1 m/s that was significantly higher in comparison to the non-active group whose gait speed amounted to 1.0 ± 0.1 m/s, which can be indicative of better functional fitness of the active elderly. It is believed that a low intensity of everyday activity and a sedentary lifestyle constitute an independent risk of diseases known as „sedentariness" (such as diabetes, obesity or metabolic syndrome). A longitudinal Canadian study of 17,013 people aged over 12 years demonstrated that those who were inactive for a longer time span were 50% more likely to die prematurely in comparison with those who spent shorter periods of time in a sitting position [36].

In addition to beneficial effects on the immune system, physical activity of the elderly throughout lifetime also favourably affects their well-being and facilitates daily functioning in society [37]. Their good functional status could be related to their participation in various physical and health education programmes at the University of the Third Age. Additionally, seniors who have been active all their lives display elevated levels of IL-7 and IL-15 which play an important role in T cells proliferation [38]. The active older adults in our study were observed to have a statistically significantly increased number of blood $CD4^+$ naïve T cells in comparison to the inactive seniors. Lifestyle exercise in older adulthood may lead to rejuvenation of the immune system and regular exercise through adulthood has been proven to exert a positive effect on thymic output. According to Weyh et al. [38], this may be associated with elevated IL-15 levels that affect the immune homeostasis which is caused by the induction of a better survival rate of naïve T-cells. Similar observations were made by Duggal et al. [17] who also reported the frequency of $CD4^+$naïve T cells to be lower in healthy sedentary older adults in comparison with young donors. Moreover, a higher frequency of CD4 + naïve T cells was identified in amateur non-elite cyclists, who were also included in that research, than in sedentary older adults, but the frequency was lower than in the young subjects.

During the past decade, three prospective cohort studies with the participation of Swedes, Dutch and Belgians were performed to assess the IRP in the elderly defined by the CD4/CD8 ratio [39]. The results revealed some common features of immunosenescence but many differences between studies could also be noticed. The inconsistencies may be ascribed to a large number of factors, including gender, age, nutrition, amount of physical activity or fat content, which can all affect the ratio, and also to the fact that the values ≥ 1 or ≤ 2.5 are commonly used as the reference values in healthy individuals [26]. The CD4/CD8 ratio can also be a useful marker to determine the body response to lifestyle exercise. Researchers have not yet unequivocally established whether the CD4/CD8 ratio increases or decreases

with age. Neither are they unanimous as to whether the rise or the decline in the ratio is more favourable to maintain the longevity of the elderly. The CD4/CD8 ratio was found to increase with age in OCTO/NONA surviving participants over 100 years of age [27]. On the other hand, the analysis by Vasson et al. [40] showed a decreasing trend of the CD4/CD8 with age in Spanish and French population. In our study, we searched for the answer whether lifestyle exercise had an effect on the CD4/CD8 ratio. Interestingly, the CD4/CD8 ratio was found to fall within the range of the reference values in 52.94% of the group of older active participants. Our study group of active older adults was classified as representing healthy ageing. The frequency of the CD4/CD8 ratio is also contingent on the body fat content and in all our study subjects (both active and inactive), high fat content shifted the CD4/CD8 ratio < 1.

Ageing is commonly accompanied by obesity, especially abdominal obesity, which is often associated with health problems and with an increased risk of infections. Adipose tissue acts as a 'link' between nutrition, metabolism and the proper functioning of the immune system in healthy individuals. Age-related changes in adipose tissue significantly contribute to age-associated metabolic dysfunction and other health issues [41]. Furthermore, ageing does not only induce adiposity but it also causes changes in body composition, such as the loss of muscle mass, muscle fat infiltration or bone loss [42], and the ensuing decline in physical performance adversely affects the quality of life [43]. Changes in body composition are a significant risk factor for developing impaired physical performance, however, there is little evidence that the FFM and FM indices are associated with physical disability in seniors, women and men alike [42].

It is worth mentioning that visceral adipose tissue contains the major immune cells like: macrophages and T lymphocytes which play a critical role in immunometabolic homeostasis. The changes in adipose tissue T cells in obesity have been well documented in mice and humans [41]. In obese individuals the percentage of T lymphocytes of both CD4 and CD8 cells in adipose tissue is on the increase, secreting pro-inflammatory cytokines such as: IFN-γ (Th1) and IL-17 (Th17). The increased proportion of inflammatory $CD4^+$ T cells in obese individuals results in a decrease in the number of T_{reg} [44].

To date only a limited number of studies have analysed the composition of the peripheral blood of the immune system in obesity. In our study, a positive correlation between $CD4^+$ cells and FM was observed in the inactive seniors. This finding suggests that the $CD4^+$ T cell pool is on the rise along with the increase in the body fat. The results are consistent with the ones obtained by van der Weerd et al. [45], who reported an increase in the number of $CD4^+$ T cells in obese people. Womack et al. [46] analysed both $CD4^+$ and $CD8^+$ T cells in African women and they also recorded an increased T cell pool in the obese study subjects. However, Tanaka et al. [47] observed a decrease in the T lymphocyte ($CD4^+$ and $CD8^+$) population in the peripheral blood of 34 obese individuals as compared to 50 non-obese subjects. Contrastingly, O'Rourke et al. [48] demonstrated an elevated number of $CD4^+$ T lymphocytes and a reduced number of $CD8^+$ T cells in morbidly obese women compared to healthy, normal-weight controls. A potential mechanism related to such divergent results is a consequence of gender and age diversity of

the study population as well as a small sample size. Therefore, the relationship between body mass and the $CD4^+$ T cell pool invites additional consideration. What calls for further investigation is also the level of leptin, a hormone that is involved in the development of thymus gland especially in the differentiation of thymocytes from double positive cells to single positive $CD4^+$ and $CD8^+$ cell [49]. One of potential mechanisms of leptin has been proven to increase the production of naïve T cells ($CD4^+CD45RA^+CD45RO^-$) and to inhibit the proliferation of memory T cells ($CD4^+CD45RO^+CD45RA^-$) [50]. It is still not entirely clear how obesity-induced metabolic dysfunction affects and changes the imbalance in the naïve and memory cells population. Naïve T cells remain at rest and their activation is contingent on the energy demand for phosphorylation. After activation, the metabolic signature changes to provide support for increased glycolysis to fulfil cellular energy requirements [51]. According to Pearce et al. [52], the development and survival of memory T cells is reliant on fatty-acid oxidation (FAO), and memory T cell formation is accompanied by another shift to FAO. In our research we also demonstrated a positive correlation between FM and $CD4^+$memory T cell pool. Yang et al. [53, 54] observed that obesity accelerated the age-related reduction of T-cell receptor, which was connected with reduced thymopoiesis. Obesity was observed to lead to reduction in peripheral naïve T cells with increased frequency of effector-memory cells. The precise role of leptin and the association between FM and immune cells, T cells in particular, in older individuals shall be analysed in further investigation in our cohort.

Appropriate strategies to counteract immunosenescence should be implemented and one of them should take into account the beneficial effect of lifestyle exercise on subpopulations of the immune system [55]. To date, numerous studies have confirmed a favourable impact of physical activity on the immune system including an enhanced proliferative capacity of T cells and magnified cytotoxic activity of NK cells. The extent of exercise-induced changes to the immune system in the elderly is quite diverse and depends on the type of physical activity as well as the volume and intensity of exercise. Future analyses will allow to understand the role which physical activity sustained throughout life plays on prevention of immunosenescence and lifestyle diseases [56].

Conclusions

In this study we demonstrated for the first time that major features of immunosenescence are driven by lifestyle exercise in older adults. Physical activity sustained throughout life enhances the immune system by increasing the percentage of naïve T cell population and by reversing the $CD4^+$ naïve/memory ratio. Interestingly, the beneficial changes in the Immune Risk Profile, defined as the CD4/CD8 ratio, were observed at the values of ≥ 1 or ≤ 2.5 in ~60% of physically active seniors regardless of their body weight, which classified them as successfully ageing seniors, contrary to the inactive and obese older adults who demonstrated the CD4/CD8 ratio < 1.

Limitations

The limitations of the study include a relatively small number of subjects especially male individuals. Moreover, lack of information on the diet and exposure to pathogens throughout life of study participants may also have affected the disproportions in the populations of the analysed cells.

List Of Abbreviations

BM: Body Mass, BMI: Body Mass Index, CHAMPS: Community Healthy Activities Model Program for Seniors, CRP: C-reactive protein, DBP: diastolic blood pressure, FAO: fatty-acid oxidation, FFM: Fat Free Mass, FM: Fat Mass, GRA: granulocytes, HB: haemoglobin, HCT: haemotocrit, HDL: high density lipoprotein, HIV: human immunodeficiency virus, HR: heart rate, IRP: immune risk profile, LDL: low density lipoprotein, LYM: lymphocytes, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, MCV: mean corpuscular volume, MID: mid absolute count, NK: natural killer, PLT: platelets, RBC: red blood cells count, SBP: systolic blood pressure, TC: total cholesterol, TCR: T cell receptor, TG: triglycerides, U3A: University of the Third Age, VO2max: maximal oxygen consumption, WBC: white blood cells count, 6MWT: 6-minute walking test

Declarations

Ethics approval and consent to participate.

All subjects were informed of the aim of the study and gave their written consent for participation in the project. The protocol of the study was approved by The Bioethics Commission at Regional Medical Chamber Zielona Gora, Poland (N^{o}01/66/2017, N^{o}21/103/2018) in accordance with the Helsinki Declaration.

Consent for publication.

Not applicable.

Availability of data and materials.

Data will be made available on request.

Competing interests.

The authors declare that they have no competing interests.

Funding.

This work was supported by the funds for young investigators from the University of Zielona Gora (Nº 222267/E-545/S/2019) and the National Science Centre Poland (Nº 2016/21/N/NZ7/03329)

Authors' contribution.

AT, BM and AZL contributed to the research concept and design, data acquisition. AT analysed and interpreted the data and coordinated the preparation of the manuscript. AZL raised funds for research and revised the manuscript for its substantive content. AG performed the statistical analyses. All authors read and approved of the final manuscript.

Acknowledgements.

We are grateful for cooperation with University of the Third Age in Zielona Gora, and thank PhD Jolanta Chmielowiec, a nurse, for blood collection.

References

1. Lewkiewicz S, Chuang YL, Chou T. A mathematical model of the effects of aging on naive T cells populations and diversity. Bull Math Biol. 2019;81:2783-817.
2. Fagnoni FF, Vescovini R, Passeri G, Bologna G, Pedrazzoni M, Lavagetto G et al. Shortage of circulating naive CD8+ T cells provides new insights on immunodeficiency in aging. Blood. 2000;95:2860-8.
3. Globerson A, Effros RB. Aging of lymphocytes and lymphocytes in the aged. Immunol Today. 2000;21:515-21.
4. Tylutka A, Zembron-Lacny A. Immunological aging and clinical consequences. Post Hig Med Dosw. 2020;74:1-12 (in press).
5. Goronzy JJ, Lee WW, Weyland CM. Aging and T-cell diversity. Exp Gerontol. 2007;42:400-6.
6. Goronzy JJ, Fang F, Cavanagh MM, Qi Q, Weyand CM. Naıve T cell maintenance and function in human aging. J Immunol. 2015;194:4073-80.
7. Muller L, Pawelec G. Aging and immunity - impact of behavioral intervention. Brain Behav Immun. 2014;39:8-22.
8. Simpson RJ, Lowder TW, Spielmann G, Bigley AB, LaVoy EC, Kunz HK. Exercise and the aging immune system. Ageing Res Rev. 2012;11:404-20.
9. Simpson RJ, Campbell JP, Gleeson M, Krüger K, Nieman DC, Pyne DB, et al. Can exercise affect immune function to increase susceptibility to infection? Exerc Immunol Rev. 2020;26:8-22.
10. Wong GCL, Narang V, Lu Y, Camous X, Nyunt MSZ, Carre C, et al.. Hallmarks of improved immunological responses in the vaccination of more physically active elderly females. Exerc Immunol Rev. 2019;25:20-33.

11. Minuzzi LG, Rama L, Chupel MU, Rosado F, Dos Santos JV, Simpson R, et al. Effects of lifelong training on senescence and mobilization of T lymphocytes in response to acute exercise. Exerc Immunol Rev. 2018;24:72-84.
12. Gleeson M, Bishop NC, Stensel DJ, Lindley MR, Mastana SS, Nimmo MA. The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. Nature Rev. Immunol 2011;11:607-15.
13. Zembron-Lacny A, Dziubek W, Rynkiewicz M, Woźniewski M. Peripheral brain-derived neurotrophic factor is related to cardiovascular risk factors in active and inactive elderly men. Braz J Med Biol Res. 2016;49:e5253.
14. Bartlett DB, Fox O, McNulty CL, Greenwood HL, Murphy L, Sapey E, et al. Habitual physical activity is associated with the maintenance of neutrophil migratory dynamics in healthy older adults. Brain Behav Immun. 2016;56:12-20.
15. Woods JA, Davis JM, Smith JA, Nieman Exercise and cellular innate immune function. Med Sci Sports Exerc. 1999;31:57-66.
16. Kohut ML, Lee W, Martin A, Arnston B, Russell DW, Ekkekakis P, et al. The exercise-induced enhancement of influenza immunity is mediated in part by improvements in psychosocial factors in older adults. Brain Behav Immun. 2005;19:357-66.
17. Duggal NA, Pollock RD, Lazarus NR, Harridge S, Lord JM. Major features of immunesenescence, including reduced thymic output, are ameliorated by high levels of physical activity in adulthood. Aging Cell. 2018;17:e12750.
18. Durstine JL, Moore GE. ACSM's exercise management for persons with chronic diseases and disabilities. 2nd Champaign, IL: Human Kinetics 2003.
19. Geard D, Rebar AL, Dionigi RA, Reaburn PRJ. Testing a model of successful aging on masters athletes and non-sporting adults. Res Q Exerc Sport. 2020;6:1-10.
20. Åstrand PO. Human physical fitness with special reference to sex and age. Physiol Rev. 1956;36:307-35.
21. Middleton A, Fritz SL, Lusardi M. Walking speed: the functional vital sign. J Aging Phys Act. 2015;23:314-22.
22. Studenski S. Bradypedia: Is gait speed ready for clinical use? J Nutr Health Aging. 2009;13:878-80.
23. Holland AE, Spruit MA, Troosters T, Puhan MA, Pepin V, Saey D, et al. An official European Respiratory Society/American Thoracic Society technical standard: field walking tests in chronic respiratory disease. Eur Respir J. 2014;44:1428-46.
24. Stewart AL, Mills KM, Sepsis PG, King AC, McLellan BY, Roitz K, Ritter Evaluation of CHAMPS, a physical activity promotion program for older adults. Ann Behav Med. 1997;19:353-61.
25. Hang J, Huang J, Zhou S, Wu L, Zhu Y, Zhu L, et al. The clinical implication of CD45RA+ naïve T cells and CD45RO+ memory T cells in advanced pancreatic cancer: a proxy for tumor biology and outcome prediction. Cancer Med. 2019;8:1326–35.

26. McBride JA, Striker R. Imbalance in the game of T cells: What can the CD4/CD8 T cell ratio tell us about HIV and health. PLoS Pathog. 2017;13:e1006624.
27. Strindhall J, Skog M, Ernerudh J, Bengner M, Löfgren S, Matussek A et al. The inverted CD4/CD8 ratio and associated parameters in 66-year-old individuals: the Swedish HEXA immune study. Age (Dordr.). 2012;35:985-91.
28. https://www.r-project.org
29. Cohen J. Statistical power analysis for the behavioral sciences (2nd ed.). Hillsdale, NJ: Erlbaum 1988.
30. Miles J, Shevlin M. Applying regression and correlation: a guide for students and researchers. Sage: London 2001.
31. Walsh NP, Gleeson M, Pyne DB, Nieman DC, Dhabhar FS, Shephard RJ, et al. Position statement. Part two: maintaining immune health. Exerc Immunol Rev. 2011;17:64-103.
32. Gleeson M, Bishop NC, Stensel DJ, Lindley MR, Mastana SS, Nimmo MA. The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. Nat Rev Immunol. 2011;11:607-15.
33. Dinh HC, Bautmans I, Beyer I, Onyema OO, Liberman K, De Dobbeleer L, et al. Six weeks of strength endurance training decreases circulating senescence-prone T-lymphocytes in cytomegalovirus seropositive but not seronegative older women. Immun Ageing. 2019;16:17.
34. Nieman DC, Henson DA, Gusewitch G, Warren BJ, Dotson RC, Butterworth DE et al. Physical activity and immune function in elderly women. Med Sci Sports Exer. 1993;25:823–31.
35. Abd El-Kader SM, Al-Shreef FM. Inflammatory cytokines and immune system modulation by aerobic versus resisted exercise training for elderly. Afr Health Sci. 2018;18:120–31.
36. Apostolopoulos V, Borkoles E, Polman R, Stojanovska L. Physical and immunological aspects of exercise in chronic diseases. Immunotherapy. 2014;6:1145-57.
37. Halaweh H, Dahlin-Ivanoff S, Svantesson U, Willén C. Perspectives of older adults on aging well: a focus group study. J Aging Res. 2018;2018:9858252.
38. Weyh C, KrügerK,Strasser B. Physical activity and diet shape the immune system during aging. Nutrients. 2020;12:622
39. Adriaensen W, Derhovanessian E, Vaes B, Van Pottelbergh G, Degryse JM, Pawelec G et al. CD4:8 ratio > 5 is associated with a dominant naïve T-cell phenotype and impaired physical functioning in CMV – seropositive very elderly people: results from the BELFRAIL study. J Gerontol A Biol Sci Med Sci. 2015;70:143-54.
40. Vasson MP, Farges MCH, Goncalves-Mendes N, Talvas J, Ribalta J, Winklhofer-Roob et al. Does aging affect the immune status. A comparative analysis in 300 healthy volunteers from France, Austria and Spain. Immun Ageing. 2013;10:38.
41. Antony AK, Lian ZWu H. T cells in adipose tissue in aging. Front Immunol. 2018; 9: 2945.

42. Ramírez Torres M, Ruiz Valenzuela RE, Esparza-Romero J, López Teros MT, Alemán-Mateo H. The Fat Mass Index, Not the Fat-Free Mass Index, Is Associated With Impaired Physical Performance in Older Adult Subjects: Evidence From a Cross-Sectional Study. Clin Nutr. 2019;38:877-82.
43. Kim S, Leng XI, Kritchevsky SB. Body composition and physical function in older adults with various comorbidities. Innov Aging. 2017;1:igx008.
44. Alwarawrah Y, Kiernan K, MacIver NJ. Changes in nutritional status impact immune cell metabolism and function. Front Immunol. 2018;9:1055.
45. van der Weerd K, Dik WA, Schrijver B, Schweitzer DH, Langerak AW, Drexhage HA,et al. Morbidly obese human subjects have increased peripheral blood CD4+ T cells with skewing toward a treg- and Th2-dominated phenotype. Diabetes. 2012;61:401-8.
46. Womack J, Tien PC, Feldman J, Shin JH, Fennie K, Anastos K, et al. Obesity and immune cell counts in women. Metabolism. 2007;56:998–1004.
47. Tanaka S, Isoda F, Ishihara Y, Kimura M, Yamakawa T. T lymphopaenia in relation to body mass index and TNF-alpha in human obesity: adequate weight reduction can be corrective. Clin Endocrinol (Oxf). 2001;54:347-54.
48. O'Rourke RW, Kay T, Scholz MH, Diggs B, Jobe BA, Lewinsohn DM, Bakke AC. Alterations in T-cell subset frequency in peripheral blood in obesity. Obesity Surgery. 2005;15:1463–8.
49. Francisco V, Pino J, Campos-Cabaleiro V, Ruiz-Fernández C, Mera A, Gonzalez-Gay MA, Gómez R, Gualillo O. Obesity, fat mass and immune system: role for leptin. Front Physiol. 2018;9:640.
50. Bouassida A, Zalleg D, Bouassida S, Zaouali M, Feki Y, Zbidi A, Tabka Z. Leptin, its implication in physical exercise and training: a short review. J Sports Sci Med. 2006;5:172–81.
51. Aguilar EG, Murphy WJ. Obesity induced T cell dysfunction and implications for cancer immunotherapy. Curr Opin Immunol. 2018;51:181–6.
52. Pearce EL, Walsh MC, Cejas PJ, Harms GM, Shen H, Wang LS, et al. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. Nature. 2009;460:103–7.
53. Yang H, Youm YH, Vandanmagsar B, Rood J, Kumar KG, Butler AA, Dixit VD. Obesity accelerates thymic aging. Blood. 2009;114:3803-12.
54. Yang H, Youm YH, Vandanmagsar, Ravussin A, Gimble JM, Frank Greenway, et al. Obesity increases the production of proinflammatory mediators from adipose tissue T cells and compromises TCR repertoire diversity: implications for systemic inflammation and insulin resistance. J immunol. 2010;185:1836-45.
55. Senchina DS, Kohut ML. Immunological outcomes of exercise in older adults. Clin Interv Aging. 2007;2:3-16.
56. Sellami M, Gasmi M, Denham J, Hayes JD, Stratton D, Padulo J, Bragazzi N. Effects of acute and chronic exercise on immunological parameters in the elderly aged: can physical activity counteract the effects of aging?Front Immunol. 2018;9:2187.

Figures

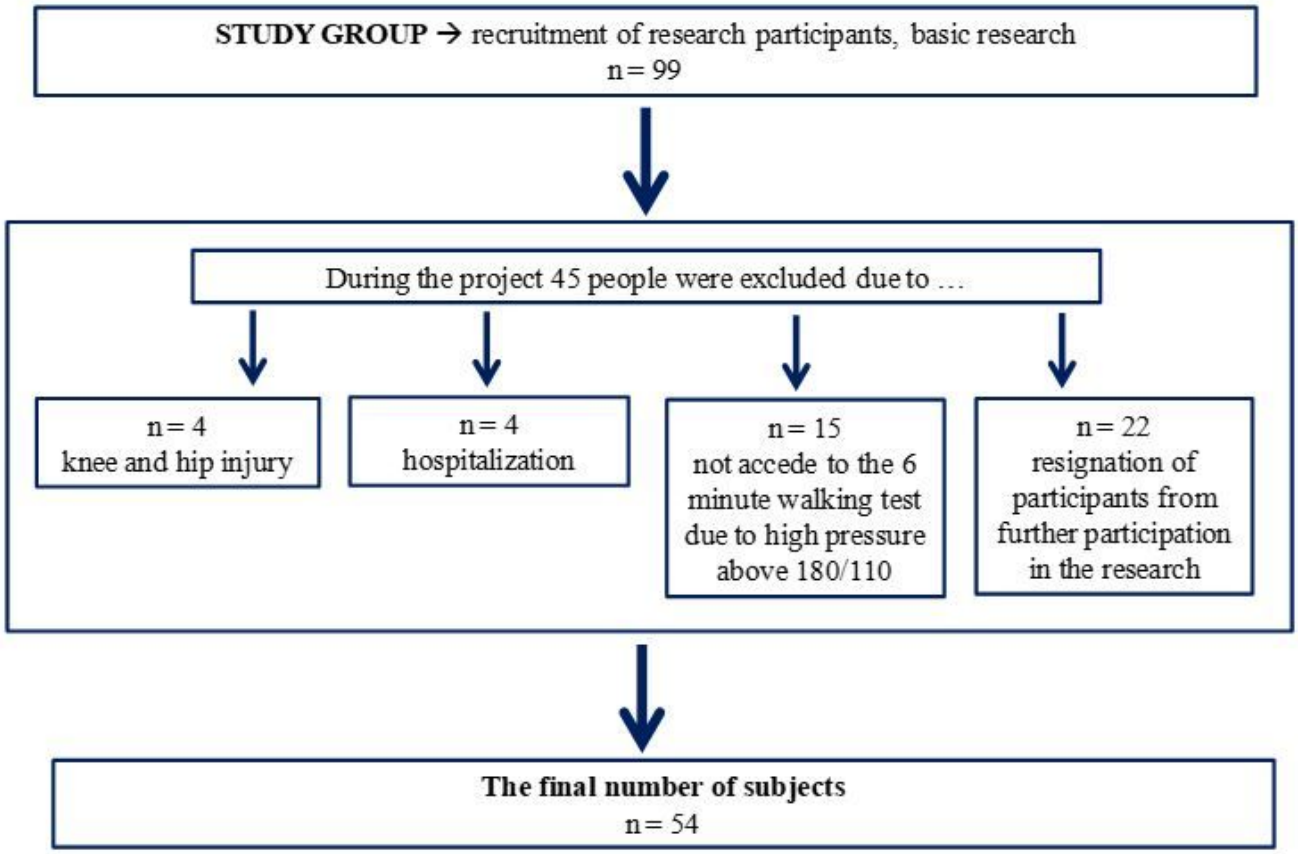

Figure 1

Group characteristics.

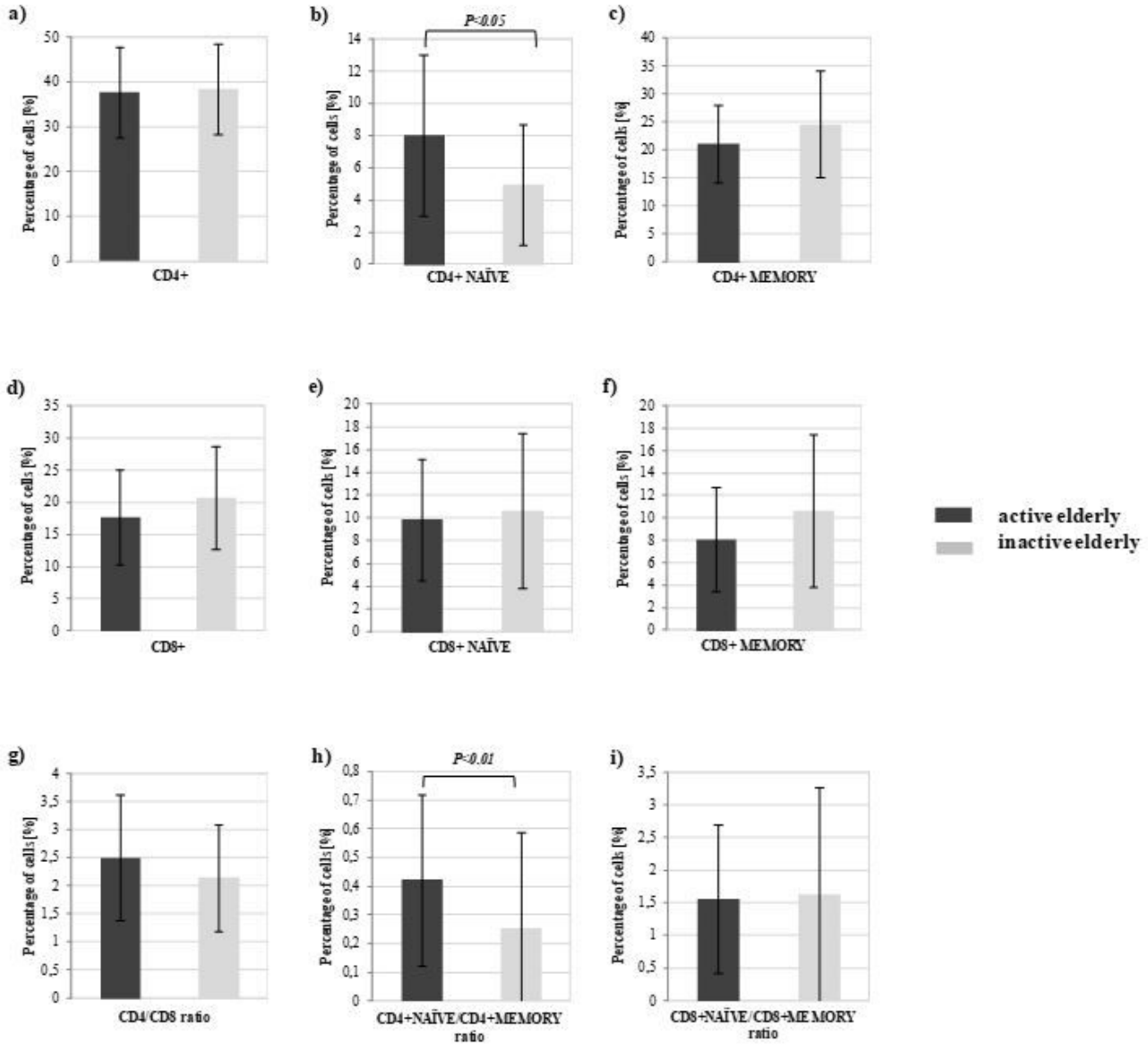

Figure 2

Changes in lymphocytes T: CD4+ and CD8+ naïve T cells, CD4+ and CD8+ memory T cells and the ratios: CD4/CD8 and CD4+ naïve / memory and CD8+ naïve / memory in active compared to inactive elderly.