Effect of exercise on acute senescent lymphocyte counts

Veiga Sardeli, Amanda; Mori, Marcelo Alves; Lord, Janet

DOI:
10.1159/000520528

Document Version
Peer reviewed version

Citation for published version (Harvard):
Veiga Sardeli, A, Mori, MA & Lord, J 2022, 'Effect of exercise on acute senescent lymphocyte counts: a systematic review and meta-analysis', *Gerontology*. https://doi.org/10.1159/000520528

Gerontology

Manuscript:	GER-2021-8-38/R1 RESUBMISSION
Title:	Effect of exercise on acute senescent lymphocyte counts: a systematic review and meta-analysis.
Authors(s):	Amanda Veiga Sardeli (Corresponding Author), Marcelo A. Mori (Co-author), Janet M. Lord (Co-author)
Keywords:	Aging, Cellular senescence, Exercise, Immunology, Immunosenescence
Type:	Meta-Analysis

Dear Monika Lechleitner,

Re: GER-2021-8-38 - Effect of exercise on acute senescent lymphocyte counts: a systematic review and meta-analysis.

Thank you for passing on the reviewers comments and for giving us the opportunity to revise the manuscript. We would like to thank the reviewers and section editor for their comments which helped us to improve the quality of our manuscript. We have revised our manuscript with all changes marked in red and our detailed response to each comment is described below.

We look forward to your response.

Yours sincerely

Section Editor comments:

This review and meta-analysis about the role of exercise on acute senescent lymphocyte counts is of clinical interest.

According to the statements of the reviewers there remain some minor points of concerns:

- *the authors have included several immunosenescence markers, however, p16 and p 21 were not included.*

Answer: We did not exclude studies using these classic markers, however, the only two studies testing the effects of exercise on SA-β-gal (Wu et al., 2018) and $p16^{INK4a}$ (Yang at al., 2018) did so in muscle and endothelial progenitor cells, respectively, which was out of the scope of our analysis. In the immune field the markers of senescence used tend to be different to non-immune cells and focus on the cell membrane phenotype, such as expression of CD57. We now include a comment on this point in the methods section (page 6, L 149).

- *did the authors consider the differences in the mode of exercise ? (such as bicycle versus treadmill)*

Answer: Originally we did include this variable but the results showed no difference between exercise type (treadmill vs cycling) and we decided not to show the data as the number of studies in the different categories were very unbalanced, ranging from 2 to 40 studies. We now mention this finding in the discussion section (page 13, L 347), but do not show the data, we hope this is acceptable.

- *units should be added to the data*

Answer: As stated in the methods (page 9, L 216) “We analysed the absolute cell count as the outcome measure, considering the standardized mean difference (SMD) and 95% of confidence interval between baseline levels and post exercise time-points since the units of measure were not consistent across studies”. This allowed us to overcome the issue of different units being used in different studies and instead bases the analysis on effect size.

- *in the introduction section the purpose of the review should be more clearly defined*

Answer: We have revised the paragraph to clarify the aim of the study and hope that this is now satisfactory (page 6, L 149).

Reviewer 1:

My only criticism is the very generous use of the term senescence. In the context of T cell differentiation, senescence is an ill-defined term and CMV-specific T cells in a 40 yo as included in this study does not necessarily have cellular senescence and it is not clear where effector functions of T cells ends and SASP start. Although transient loss of CD28 in effector T cells is normal in an immune response and not senescent (see for example the studies by Rafi Ahmed). the authors should use the introduction to discuss this issue and give a clear operative definition for the purpose of this paper.

Answer: This is a good point and the field of immunesenescence does have distinct features from senescence in non-immune cells. We have revised the introduction in order to clarify these issues in more detail (page 4, L 78).

Also, one of the limitations of the study that should be mentioned is that senescent cells in this study are a mixed bag. CD57 TEMRAs (representing the cell type that is closest to senescence) are not specifically identified in the published papers. Moreover, TEMRAs and CD8 EM that may be negative for CD27 or CD28 are quite different differentiation stages.

Answer: This is a valid comment, and we were of course aware of this limitation. We have now added a comment on this point to the limitations section in the discussion (page 14, L 379).

A minor issue is that data are given without units. I suppose that the included papers provide absolute numbers and not percentages and the unit is per ul.

Answer: This issue was also raised by the section editor and our response is shown above.

Reviewer 2:

Authors have included several immunosenescence markers, however, were classic senescence markers like p16, p19, p21 also looked at? For e.g. studies have shown that p16 and p21 expression was higher in CD28+ CD57+ senescent T cell populations.

Answer: This point was also raised by the section editor and our response is given above.

Was the mode of aerobic exercise – bicycle vs. treadmill – normalized in anyway? In table 1, Azali Alamdari et. al., and Turner use a treadmill in their study vs. other authors that use a *bicycle. Therefore, what was the rationale/parameter for clubbing both these modes under "aerobic exercise" for the meta-analysis?*

Answer: Please see the comment above to the section editor.

In table 1, how does intensity "until exhaustion" correspond to a VO2max value?

Answer: The studies classified as "until exhaustion" analysed the frequency of senescent lymphocytes immediately after a maximum test, which could be considered as 100% of effort, thus equivalent to VO2max intensity.

References:

Wu, J., Saovieng, S., Cheng, I. S., Liu, T., Hong, S., Lin, C. Y., Su, I. C., Huang, C. Y., & Kuo, C. H. (2019). Ginsenoside Rg1 supplementation clears senescence-associated beta-galactosidase in exercising human skeletal muscle. *Journal of Ginseng Research*, **43**, 580–588.

Yang, C., Jiao, Y., Wei, B., Yang, Z., Wu, J.-F., Jensen, J., Jean, W.-H., Huang, C.-Y., & Kuo, C.-H. (2018). Aged cells in human skeletal muscle after resistance exercise. *Aging (Albany NY)*, **10**, 1356–1365.

Meta-Analysis

Effect of exercise on acute senescent lymphocyte counts: a systematic review and meta-analysis.

Amanda V. Sardeli [a,b*], Marcelo A. Mori [c, d, e], Janet M. Lord [a, f]

[a] MRC-Versus Arthritis Centre for Musculoskeletal Ageing Research, Institute of Inflammation and Ageing, University of Birmingham, Birmingham, UK.

[b] Laboratory of Exercise Physiology (FISEX), University of Campinas, Campinas, Brazil.

[c] Department of Biochemistry and Tissue Biology, Institute of Biology, University of Campinas, Campinas, Brazil.

[d] Experimental Medicine Research Cluster, University of Campinas, Campinas, Brazil.

[e] Obesity and Comorbidities Research Center, University of Campinas, Campinas, Brazil.

[f] NIHR Birmingham Biomedical Research Centre, University Hospital Birmingham and University of Birmingham, Birmingham, UK.

Short Title: Effect of exercise on acute senescent lymphocyte counts

Corresponding Author:

Amanda Veiga Sardeli

Institute of Inflammation and Ageing

Queen Elizabeth Hospital

Mindelsohn Way

Birmingham, West Midlands, B15 2WB, UK

Tel: +44 (0)121 414 3344

E-mail: amandavsardeli@gmail.com

Number of Tables: 2.

Number of Figures: 5.

Word count: 4832.

Keywords: Exercise, Ageing, Cellular senescence, Immunosenescence, Immunology.

Abstract

Background: Highly differentiated, senescent lymphocytes are pro-inflammatory and contribute to age-related systemic inflammation, inflammageing. There are several reports of acute changes in senescent lymphocyte counts post-exercise which potentially has consequences for systemic inflammation. However, there is little consensus since the studies differ with respect to participants, exercise protocols, cellular markers assessed, and the time point of assessment post-exercise.

Objective: We performed a systematic review and meta-analysis to assess the impact of exercise on senescent lymphocyte counts in blood immediately, 1h and 2h post exercise.

Methods: The search was performed in PubMed (MEDLINE), Web of Science, Embase, Scopus and Cochrane, on January 11, 2021. The 13 studies selected tested aerobic exercise effects, mainly in young men. They assessed the counts of lymphocytes (CD4 T cells, CD8 T cells, NK cells), with the following immune cell marker combinations: KLRG1+, CD57+ (only NK cells), EMRA T cells (CD45RA+CCR7-CD28-CD27-), CD28-CD27-, KLRG1+CD28- and CD28-. Independent extraction of articles by 2 researchers.

Results: Standardized mean difference (SMD) and 95% confidence interval between baseline and post-exercise showed significant increases (SMD > 0.9, p<0.003) in all types of lymphocyte counts immediately post exercise. At 1h post exercise senescent CD4 T cells returned to baseline values (p=0.74), CD8 T cells were reduced (-0.26 [-0.41; -0.11], p=0.001), and senescent NK cells were raised (0.62 [0.14; 1.10], p=0.01) above baseline. By 2 hours post exercise, senescent CD4 T cells were reduced (-0.94 [-1.40; -0.48], p<0.001), CD8 T cells remained below baseline (-0.53 [-1.04; -0.009], p=0.04), and NK cells had returned to baseline values (-0.29 [-0.64; 0.07], p=0.11). The main determinants of heterogeneity between studies were cytomegalovirus (CMV) serostatus and the characteristics of exercise protocols. CMV+ individuals had a higher immediate lymphocytosis and 1h post lymphopenia than CMV- individuals. Exercise performed at higher intensities and shorter durations led to higher magnitude of change in senescent lymphocyte counts at all time-points.

Conclusion: The differing effects of exercise on senescent NK cells and CD4 and CD8 T cells suggest differing susceptibility to factors modulating lymphocyte extravasation such as adrenaline and exercise intensity.

Introduction

Immunosenescence, the gradual remodelling of the immune system, is an integral component of the ageing process [1]. Advanced age impairs innate immune responses, contributes to chronic low-grade inflammation (inflammageing) and reduces immunity, increasing the risk of infections, autoimmunity and overall poor health in the older adult [2,3]. Among the features associated with adaptive immunosenescence are the atrophy of the thymus, which reduces naïve T cell output, and the subsequent increased number of highly differentiated, senescent T cells in the circulation [3,4]. Senescent cells are one of the causes of detrimental effects to the body during ageing, contributing to chronic diseases, such as idiopathic pulmonary fibrosis, diabetes, and osteoarthritis [5]. It has been shown recently that mice with high levels of senescent T cells, due to dysfunctional mitochondria, enter premature senescence and a broad range of age-related diseases [6]. Immunosenescence, especially T cell senescence, may therefore be a major contributor to the ageing process.

Senescent cells undergo a state of cell quiescence with permanent cell cycle arrest induced by different sources of stress and damage to the cell. These cells produce a senescence-associated secretory phenotype (SASP), which is composed of pro-inflammatory cytokines, chemokines, growth factors and proteases. Cells releasing SASP alter the tissue microenvironment, affect neighboring cells, and are thus deleterious [7,8]. In the immune system there are some subtle differences. For example, T cells can have a functionally exhausted phenotype resulting from chronic stimulation, which is distinct from a senescent phenotype resulting from ageing or chronic infection. These phenotypes can be differentiated by cell surface markers [9]. We have therefore used the markers identified as relating to senescent T cells such as loss of CD28 and CD27 and expression of KLRG1 and CD57. Importantly, senescent T cells also produce a SASP that is highly pro-inflammatory and similar in content to that of non-immune senescent cells [10], therefore they are likely to contribute to inflammageing and tissue compromise during ageing.

The immunomodulatory effects of exercise have been widely explored and could be associated with the reduction in senescent cell counts [10], for example obese mice provided with an exercise wheel had reduced numbers of senescent cells in their adipose tissue [11]. Exercise has been reported to have a range of immune enhancing effects including reducing chronic low-grade inflammation [12], improving responses to vaccination [13], reducing the risk of infection [14,15], improving the immune response against viruses and bacteria and reducing the burden of latent viral infections [16–18]. Among the main physiological mechanisms mediating the immunomodulatory benefits of exercise are the reduction in body fat and the release of anti-inflammatory cytokines, such as interleukin-6 (IL-6) and IL-1RA, by the exercising muscle [12,19].

Recently, Duggal *et al*. [20] have reported the benefits of sustained physical activity in to old age on adaptive immune phenotype and immunosenescence. They reported that thymic health, as measured by the frequency of naïve T cells and recent thymic emigrants (RTE), was better preserved in older exercisers (cyclists) compared to inactive elders. Older cyclists also had significantly higher serum levels of the thymoprotective cytokine interleukin-7 (IL-7), higher B regulatory cell frequency, lower IL-6 and reduced Th17 polarization, all markers of an aged immune system. However, they also reported that the age-related increase in senescent T cells was not prevented in the cyclists [20].

Despite the chronic benefits of exercise being well established, whether acute exercise increases susceptibility to infection or confers immune protection is still a matter of debate [21]. However, an increase in lymphocyte counts in the blood (lymphocytosis), followed by a decrease (lymphocytopenia) post exercise has generally been reported [22]. Lymphocytes are proposed to migrate from the marginal pool, the spleen and lymph nodes in to the blood, as well as increased release from the bone marrow to produce the lymphocytosis. This migration is mediated by exercise-induced shear stress on blood vessels and catecholamines, as well as cortisol and to some extent cytokines such as IL-6 [23–25].

What is less clear is the impact of exercise on specific immune cell types and their differentiation state, notably senescent immune cells. This is important bearing in mind their pro-inflammatory nature and potential role in driving inflammageing and the aged phenotype [26]. Studies investigating senescent lymphocyte counts in circulation post-exercise have shown a variety of responses [27–31], including a reduction on leukocyte counts [32]. However, senescent, or highly differentiated lymphocytes appear to be more likely to increase in blood with exercise than lymphocytes in earlier stages of differentiation. This could be beneficial in leading to their subsequent removal by NK cells or CD8 T cells which can detect senescent cells and kill them by apoptosis [22,33].

Another important confounding factor in the various exercise intervention studies, is infection by cytomegalovirus (CMV) that increases with age and has deleterious effects on lymphocyte immunity, accelerating immunosenescence [22,34,35]. The higher baseline cell counts of senescent lymphocytes in CMV+ individuals could lead to higher magnitude of change in these individuals after exercise, and thus the CMV serostatus might be an important confounding factor between studies [27,28]. Other factors that may cause different results between studies are: the comparison between absolute cell counts and the frequency of cells in the circulation; the different types of lymphocytes assessed; the membrane markers used to identify cell senescence; characteristics of the study population (age, sex and physical activity level) and the exercise protocols used (type of exercise, volume and intensity).

To derive a consensus from the literature it is important to isolate the variety of confounding factors among the studies and to run a pooled effects meta-analysis. Thus, we aimed to identify the impact of acute exercise on the frequency of senescent T cells and NK cells, taking in to account variables such as CMV serostatus, age, training status and specifics of the exercise protocols.

Methods

This systematic review and meta-analysis was registered on PROSPERO under the number CRD42021267078, that can be assessed at https://www.crd.york.ac.uk/prospero/, and it was reported in accordance with the recommendations of Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) statement [36].

Search strategy

On January 11, 2021 the search was updated at PubMed (Medline), Web of Science, Embase, Scopus and Cochrane. It combined the synonyms of “senescent markers” and “exercise” according to each data base descriptor and field of search as detailed in the Supplementary material.

Eligibility criteria

Figure 1 shows the study selection process, completed by two independent reviewers. We included studies: (1) of acute interventional exercise; (2) with no associated intervention, i.e. exercise only group; (3) in humans from both sexes; (4) comparing resting and immediately, 1h and 2h post exercise condition; (5) assessing any bona fide markers of immunosenescence; (6) assessing CD4+ or CD8+ T cells, or NK lymphocytes; (7) written in English.

Immunosenescence cell markers

Markers of senescence traditionally used for non-immune cells, such as $p16^{ink4a}$ and SA-βGal have not been used in studies of immunosenescence which focus on cell membrane markers. We therefore selected several broadly accepted markers of immunosenescence to use in this study and the characteristics of each of them are described below.

CD57$^+$. CD57+ NK cells have been attributed a senescent-like phenotype due to their short telomeres and inability to proliferate [1,37,38].

CD28$^-$ CD27$^-$. CD27 and CD28 are costimulatory receptors and T cells lacking CD27 and CD28 are thought to be fully differentiated T cells exhibiting shorter telomere length [39]. When the expression of CD27 and CD28 is lost, there is no evidence of subsequent re-expression and the downregulation of these molecules are linked to dysfunctional T cells with a SASP secretome [40,41].

KLRG1$^+$. T-lymphocytes expressing KLRG1 have impaired capacity to proliferate, yet

maintain immediate effector cell capabilities such as the recognition and killing of target cells [42].

EMRA (CD45RA$^+$CCR7$^-$CD28$^-$CD27$^-$). EMRA, for terminally differentiated effector memory cells re-expressing CD45RA, have the key features of cell senescence, with low proliferation response and a highly inflammatory phenotype [10]. They also have high levels of DNA damage and loss of telomerase activity [43]. However, due to their ability to proliferate under specific conditions their phenotype is distinct from non-immune senescent cells which cannot proliferate [44].

Exclusion criteria

We excluded studies that: (1) did not have original data or did not undergo peer-review such as reviews, commentaries, editorials, letter to the editors, case reports or conference abstracts; (2) assessed other senescence markers such as telomere shortening, or telomerase activity; (3) had not tested exercise effects; (4) had not assessed immunosenescence in humans; (5) assessed senescence in other cells, besides lymphocytes and NK cells; (6) were not written in English.

Data collection and data items

Data collection was performed by two independent researchers. The means and a measure of dispersion of the senescent cell counts were extracted for each subgroup within studies. Mean, standard deviation (SD) and sample number (n) were used for the meta-analyses. Standard error (SE) was converted to SD by the equation $SD = SE \times (\sqrt{n})$, if SD was not provided in the original study.

For subgroup analysis we extracted information about participants sex, age, level of training, health condition and CMV serostatus, type of lymphocytes assessed, membrane markers used, unit of measurement, and the characteristics of the exercise bout such as intensity, volume, and type of equipment.

The sample of studies was classified as young, middle aged and old according to the mean age reported (young [<30yrs], middle aged [30-40yrs] and older adults [>50yrs].

The participants were considered “trained” when the studies classified them as elite athletes, trained, physically active, cyclists or when the VO_2max was above the 50% percentile according to their age [45]; they were considered “untrained” when the studies classified them as untrained, or doing no regular physical activity or sedentary. The studies that did not report the participant`s physical activity level or reported a too wide range of physical activity level among their participants were excluded from this subgroup analysis. Individuals undergoing exercise chronic intervention were considered trained [32,46]; while the individuals undergoing non-exercise intervention in Wang et. al. [32] were classified as untrained and the individuals undergoing non-exercise intervention in Azali Alamdari et al. [46] were excluded for training status analysis, since they were athletes at baseline.

Regarding health status, only Curran et al. [47] have included individuals with type I diabetes, while the other studies only included healthy participants.

The exercise intensity was classified according to the percentage of VO_2max described by the American college of Sports Medicine [48], in which 46-63% is moderate, 64-90% is vigorous and >91% is near maximum. The intensity reported on Ingram et al. study [49], in watts was estimated as 73.7% of maximum according to data from participants of a similar age. Another study tested different protocols according to their lactate threshold (5% under LT, 5% above LT and 15% above LT) in the same individuals and each of them were included in the meta-analysis as a separate study [27].The intensity was also converted to percentage according to Farina *et al.* [50], in which 5% <LT was considered 61.1%, 5%>LT was considered 71.1%, and 15% >LT was considered 81.1%. The studies reporting percentage of estimated maximum power or percentage of ventilatory threshold work rate, instead of VO_2max, were classified for subgroup analysis as these markers were proportionally equivalent.

The studies applying incremental maximum effort tests and other protocols expected to last less than 20 min were considered short, the ones applying 30 min duration were considered moderate and above this they were considered long duration.

Only Azali Alamdari et al.[46] had a control group, and thus, the change of control group was subtracted from the exercise change to increase the robustness of the analysis. Although Turner et al. [51] had also reported a control group, they did not present the effects of the control period on

CD28-CD27- markers, in this way the control group was not considered for analysis. Two studies presented acute exercise effects before and after a variety of chronic interventions [32,46] and thus we included only their post intervention session to avoid sample overlapping in the analysis.

Statistical analysis

We analysed the absolute cell count as the outcome measure, considering the standardized mean difference (SMD) and 95% of confidence interval between baseline levels and post exercise time-points since the units of measure were not consistent across studies.

The 3 main meta-analyses, for each time point (immediately, 1h and 2h post exercise) and the subgroup analyses were performed using Comprehensive Meta-Analysis software, version 3.3.070. When there was statistical significance for heterogeneity, randomized effect models were selected and when there was no significant heterogeneity, fixed effects were applied. The inconsistency between studies was reported as a percentage (I^2), based on difference between expected heterogeneity (df) and true heterogeneity (Q-value).

For subgroup analysis we tested the influence of the following confounding factors: sex (men and women); age (young [<30yrs], middle aged [30-40yrs] and older adults [>50yrs]); type of lymphocytes (CD4+, CD8+ and NK); type of senescence marker (KLRG1+, CD57+, EMRA [CD45RA+CCR7-CD28-CD27-], CD28-CD27-, KLRG1+CD28- and CD28-), level of training (trained and untrained); health condition (healthy and diseased); CMV serostatus (CMV+ and CMV-); exercise intensity (moderate, vigorous, near maximum); and exercise volume (short, moderate and long). Q tests were applied to group comparisons, considering 95% confidence.

Egger's tests were performed to check the risk of publication bias in each meta-analysis [52].

Results

We included thirteen studies [27–29,32,46,47,49,51,53–57] testing acute aerobic exercise effects on senescent T lymphocytes and NK cell counts (shown in Figure 1). It is noteworthy that some studies had to be excluded due to the absence of specific description of absolute senescent lymphocyte counts [30,58–64]. Most studies included, reported their results among different subgroups of individuals with different sex, ages, CMV serostatus, types of exercise protocols and time points of analysis that were analyzed as a sub-study.

please insert Figure 1 here

Study characteristics

Table 1 shows the characteristics of the studies included. Only Curran et al.[47] included a type I diabetes group, while the other studies only included healthy participants. While twelve studies tested exercise effects on males, just one tested exercise effects on participants from both sexes [27], and thus comparisons between men and women were not possible in subgroup analysis. One study included middle aged [28], two included older adults [28,29] and all of them (thirteen) tested young adults. Our analysis reported the effect of exercise on T CD4+, T CD8+ and NK cell counts. All studies tested the effects of aerobic exercise, the majority of them used bicycle, and a few used treadmill [46,51].

*****please insert Table 1 here*****

Syntheses of the results

Lymphocyte counts immediately post exercise. Figure 2 shows there were significant increases on senescent CD4 T cells (SMD 0.96 [0.67; 1.25], p<0.001), CD8 T cells (SMD 1.26 [0.93; 1.59], p<0.001) and NK cells counts immediately post exercise (SMD 1.04 [0.35; 1.72], p=0.003). However, all those analyses were heterogeneous, reinforcing the need for further subgroup analyses. Furthermore, the analysis of senescent CD4 T cells and CD8 T cell counts had significant risk of bias, evidencing that studies with low precision conduced the main effects.

Table 2 shows no effect of age (p= 0.46) or training status (p=0.35) on outcomes immediately post exercise. On the other hand, the intensity and duration of exercise protocols and CMV status influenced the post exercise senescent lymphocyte counts (Table 2). Specifically, the higher magnitude of increase in senescent lymphocytes were seen in the maximum intensity and short duration protocols (SMD 1.81 [1.45; 2.1], p<0.001) compared to the others (SMD <0.85, p<0.05). There was a trend to higher senescent lymphocyte counts in CMV positive participants compared to CMV- (p=0.09). The CMV status analysis for each subgroup of T lymphocyte showed higher increase in senescent CD8+ T cells for CMV+ (SMD 1.60 [0.73; 2.46], p<0.001) compared to CMV- (SMD 0.58 [0.33; 0.83], p<0.001), with no difference for senescent CD4+ T cells regarding CMV status (SMD CMV+: 0.42 [0.02; 0.82], p=0.038 and CMV-: 0.50 [0.18; 0.82], p=0.002).

Please, insert Figure 2 here

Lymphocyte counts one hour post exercise. Figure 3 shows senescent CD8+ T cell counts were lower compared to baseline levels (SMD -0.28 [-0.44; -0.13], p<0.001), while CD4+ T cell counts returned to baseline levels (SMD -0.13 [-0.37; 0.11], p=0.28) and NK cells were still above baseline values (SMD

0.62 [0.14; 1.09], p=0.11). These analyses were homogeneous (p>0.53, I^2=0%), confirming that each of these senescent cells have very consistent response 1h post exercise across the different studies.

Table 2 shows there was a significant reduction in the senescent lymphocyte count only in CMV+ and not CMV- individuals, with significant difference between groups. Regarding each subgroup of T lymphocyte there was no significant reduction for senescent CD4+ T or CD8+ cells in CMV- (SMD CD4+: 0 [-0.30; 0.31], p=0.97 and CD8+: -0.13 [-0.36; 0.09], p=0.25) while there was a trend of senescent CD4+ reduction in CMV+ individuals (SMD -0.35 [-0.74; 0.04], p=0.075), and reduction of senescent CD8+ T cells in CMV+ (SMD -0.46 [-0.75; -0.18] p=0.001). Only vigorous intensity and long duration exercise protocols led to significant reduction of senescent lymphocytes (SMD -0.5 [-0.8; -0.2], p<0.001) while the other intensities and durations did not vary significantly (p>0.16).

Please, insert Figure 3 here

Lymphocyte counts two hours post exercise. Figure 4 shows senescent CD4 T cells were reduced (SMD -0.94 [-1.40; -0.48], p<0.001), CD8 T cells remained below baseline (SMD -0.53 [-1.04; -0.009], p=0.04), and NK cells had returned to baseline values (SMD -0.29 [-0.64; 0.07], p=0.11). There was significant risk of publication bias for the analysis of senescent CD4 T cells (Egger test p-value <0.001), evidencing that studies with low precision conduced the main effects in this analysis.

All these three meta-analyses were heterogeneous, however, due to the low number of subgroups in these analyses, only training status, intensity and volume of exercise protocols were analyzed. No difference between trained and untrained individuals was noticed (p=0.81) and only maximum intensity and short duration protocols reduced senescent cell counts (SMD -0.7 [-1; -0.4], p<0.001), however, it is noteworthy there was very low number of studies in the other categories (Table 2).

Please, insert figure 4 here

Discussion

The main findings of the present meta-analysis were the significant increase in senescent CD8+, CD4+ and NK cell counts immediately post exercise followed by a reduction in senescent CD8+ T cells at 1h and 2h post exercise, a reduction in senescent CD4+ T cells at 2h post exercise and maintenance of increased NK senescent cells at 1h post exercise with a return to baseline at 2h post exercise (Figure 5). Although there is no consensus about the exact role of these redistributions of senescent lymphocytes post exercise, it has been proposed that senescent lymphocytes are preferentially recruited for immune surveillance and removal by NK and CD8+ T cells, resulting in an exercise-induced senolytic effect [22].

In fact, it is known that T-cells with high cytotoxic capabilities and tissue migration potential, which are characteristics of highly differentiated lymphocytes, are preferentially mobilised by acute stress and exercise [65]. These lymphocytes could be recruited due to their high β_2-adrenergic receptor expression [66] even though they have impaired replicability and co-stimulatory potential. Furthermore, in mice, NK cells are the main mediator of the antitumor effects of exercise. These effects depend on the mobilisation of these cells [67], which are the most responsive lymphocyte subset to acute exercise due to their high β-adrenergic receptor expression [68]. Mobilisation of the senescent, less functional form of these cells could be beneficial if they are then removed, improving the overall quality of the lymphocyte pool.

Following their mobilisation it is possible that T-lymphocytes egressing to the peripheral tissues may experience a pro-apoptotic environment [69], as Kruger et. al. [70] showed the number of highly differentiated CD3+ T cells remained reduced 3h and 24h post exercise. Another possibility could be the return of senescent cells to lymph nodes but most of these cells lack CCR7, a secondary lymphoid organ-homing marker, this is unlikely.

In theory, when senescent T-lymphocytes undergo apoptosis, a subsequent feedback loop could increase the output of naïve T-lymphocytes from the thymus, restoring the peripheral T-lymphocyte pool [22,59]. In fact, naïve lymphocytes counts are increasing 1h post exercise [30,59]. Furthermore, older adults involved in regular exercise have higher serum levels of the thymoprotective IL-7 and higher frequency of RTE than sedentary controls [20], which could be stimulated by senescent lymphocyte clearance post each exercise bout.

In an opposite way, exercise-induced hematopoiesis [25,71], could also affect the thymic feedback loop, increasing the stimuli for senescent lymphocyte removal. Cross-sectional studies showed physically active individuals have lower markers of senescent T lymphocytes [10,20] and master athletes have longer lymphocyte telomere length than untrained controls [72]. Nevertheless, it is noteworthy that highly differentiated, senescent lymphocytes are less sensitive to apoptotic signals [73,74], and the exercise effects on senescent cell apoptosis is still unknown.

Most studies tested the influence of CMV serostatus on exercise responses. CMV reactivation can be triggered through catecholamine-responsive elements [75] and stress hormone levels, which are known to correlate strongly with CMV reactivation in astronauts before and after spaceflight

[76]. Thus, it is believed that CMV^+ individuals have reduced sensitivity to β-adrenergic stimulation and decreased β_2-adrenegic receptor expression to prevent CMV reactivation [58]. However, a reduced β-adrenergic sensitivity of T cells in CMV^+ individuals is not supported by our analysis, and in fact we saw a larger magnitude of changes in CMV+ individuals. Thus, we believe the expected higher baseline senescent lymphocyte counts in CMV^+ individuals [28,77], especially for senescent CD8+ T cells, explains the higher magnitude of change with exercise in this population.

There was a greater magnitude of increase in senescent lymphocyte counts immediately post maximum intensity and short duration protocols and greater magnitude of reduction 2h post exercise compared to lower intensities and longer duration protocols. These differences could be explained by higher sympathetic activation and sustained release of epinephrine reported in higher intensities protocols [70,78,79]. However, there is also evidence that cortisol affects lymphocyte counts during exercise [70,80–83]. Exercise of high intensity leads to greater release of cortisol in to the blood and for a longer time and may explain the reduced cell counts at later time points since cortisol induces apoptosis in lymphocytes [84]. We also considered the type of exercise and whether this may make a difference to the senescent cell response. However, we found no difference at any of the time points between treadmill and cycling protocols (data not shown), though this is with the caveat that the number of studies per subgroup category varied greatly.

It is unlikely that IL-6 released by muscle cells during exercise [85], explains the difference between exercise protocols. It is known, that IL-6 attracts lymphocytes to the circulation together with β-adrenergic signaling during exercise [67], however, there is a higher release of IL-6 within exercise protocols with higher energetic demand, such as the higher volume and during regimens [85–87] which do not agree with our findings.

Finally, exercise hypoxia may explain at least part of the changes in T lymphocytes and NK counts with exercise, possibly mediated by the same neuroendocrinological factors released by other stress conditions (i.e.: catecholamines, cortisol) [57,88].

Limitations

The first limitation of this study was that most studies included young individuals. The unbalanced subgroup analysis suggested there is higher magnitude of change on senescent counts in young than older or middle-aged individuals immediately and 1h post exercise. Whether it is a true effect is unclear, it could be explained by reduced β_2-adrenergic receptor sensitivity with ageing [89], which in turn increases the threshold for catecholamine-induced lymphocyte recruitment. In this way, it is important to confirm these results with a larger sample of older adults.

Comparisons between men and women were also not possible due to the lack of studies in women. An exploratory analysis showed immediately post exercise there was a large ($p<0.001$) increase of senescent lymphocytes in men (1.23 [0.99; 1.47], $p<0.001$, k=44) compared to studies with mixed sex samples (0.48 [0.19; 0.78], $p<0.001$, k=6), while there was no difference between these subgroups 1h post. Future studies should test to what extent the results in men are also applied to women.

Another limitation was the lack of a control group, i.e. without exercise, within the original studies which precluded a proper risk of bias assessment. In the other hand, the comparison of the same participants along time removes the between subjects' effects, which in turn contributes to the isolation of exercise effects. Furthermore, we explored possible influences of the confounding factors in subgroup analysis. At last, it is noteworthy that only two studies investigated exercise effects on senescent NK cell counts, with is a limitation of the literature and future studies should fill these gaps to strengthen knowledge in the field.

Lastly, one additional issue was the use of markers to identify the different stages of T cell differentiation and their relation to T cell senescence. Thus, no studies enumerated CD57 TEMRA cells, the ones that are closest to a senescent phenotype.

Conclusions

Senescent lymphocyte counts change significantly in the acute response to aerobic exercise. However, a complex picture has emerged where senescent CD8+ cells had a higher immediate lymphocytosis and subsequent lymphopenia (1h and 2h post), senescent CD4+ T cells followed a similar profile but with lower magnitude of change, and senescent NK cells increased but had a delayed return to baseline levels. There was higher magnitude of lymphocytosis and lymphocytopenia for CMV+ individuals and near maximum intensity and short duration protocols. The differing effects of exercise on senescent NK cells and CD4+ and CD8+ T cells suggest differing susceptibility to factors modulating lymphocyte extravasation such as adrenaline that is also regulated by exercise intensity. More studies are needed for understanding exercise effects on senescent NK cells, in older adults and in women.

Statements

Acknowledgement

The authors thanks Diego Nacarato Pereira da Silva for the contribution in the selection of studies and data acquisition.

Statement of Ethics

An ethics statement is not applicable because this study is based exclusively on published literature.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This work was supported by the Newton International Fellowship generously awarded to Amanda Veiga Sardeli by the Academy of Medical Sciences through the UK Government's Newton Fund Programme [NIFR7\1031]. JML is supported by the NIHR Birmingham Biomedical Research Centre. The views expressed here are those of the authors and not necessarily those of the NIHR or the Department of Health and Social Care.

Author Contributions

All three authors have given substantial contributions to the conception and the design of the manuscript; AVS did the studies selection, data collection and analysis. AVS, MAM and JML interpretated the data. AVS did the first draft while MAM and JML reviewed it critically for important intellectual content. All authors read and approved the final version of the manuscript.

Data Availability Statement

The data in this study was obtained from the previous studies where specific restrictions for public sharing their data may apply according to each journal politics. Such dataset may be requested by the corresponding author e-mail.

References

1. Alpert A, Pickman Y, Leipold M, Rosenberg-Hasson Y, Ji X, Gaujoux R, et al. A clinically meaningful metric of immune age derived from high-dimensional longitudinal monitoring. Nat Med. Nature Publishing Group; 2019;25:487–95.

2. Franceschi C, Campisi J. Chronic inflammation (Inflammaging) and its potential contribution to age-associated diseases [Internet]. Journals Gerontol - Ser A Biol Sci Med Sci. Oxford University Press; 2014. p. S4–9.

3. Hazeldine J, Lord J, Hampson P. Immunesenescence and inflammaging: A contributory factor in the poor outcome of the geriatric trauma patient. Ageing Res Rev. Ageing Res Rev; 2015;24:349–57.

4. Majumdar S, Nandi D. Thymic Atrophy: Experimental Studies and Therapeutic Interventions [Internet]. Scand J Immunol. Blackwell Publishing Ltd; 2018. p. 4–14.

5. Muñoz-Espín D, Serrano M. Cellular senescence: from physiology to pathology. Nat Publ Gr. 2014;

6. Vasileiou PVS, Evangelou K, Vlasis K, Fildisis G, Panayiotidis MI, Chronopoulos E, et al. Mitochondrial Homeostasis and Cellular Senescence. Cells. Multidisciplinary Digital Publishing Institute (MDPI); 2019;8:686.

7. Coppé JP, Patil CK, Rodier F, Sun Y, Muñoz DP, Goldstein J, et al. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS Biol. PLoS Biol; 2008;6.

8. Campisi J. Aging, cellular senescence, and cancer [Internet]. Annu Rev Physiol. Annu Rev Physiol; 2013. p. 685–705.

9. Huff W, Kwon J, Henriquez M, Fetcko K, Dey M. The Evolving Role of CD8 + CD28 - Immunosenescent T Cells in Cancer Immunology. Int J Mol Sci. Int J Mol Sci; 2019;20.

10. Chen X, Yi Z, Wong GT, Hasan KMM, Kwan JS, Ma AC, et al. Is exercise a senolytic medicine? A systematic review. Aging Cell. Blackwell Publishing Ltd; 2020;

11. Schafer MJ, White TA, Iijima K, Haak AJ, Ligresti G, Atkinson EJ, et al. Cellular senescence mediates fibrotic pulmonary disease. Nat Commun. Nature Publishing Group; 2017;8:14532.

12. Gleeson M, Bishop N, Walsh N. Exercise Immunology. New York: Routledge/Taylor & Francis Group; 2013.

13. Pascoe AR, Fiatarone Singh MA, Edwards KM. The effects of exercise on vaccination responses: A review of chronic and acute exercise interventions in humans [Internet]. Brain Behav Immun. Academic Press Inc.; 2014. p. 33–41.

14. Pape K, Ryttergaard L, Rotevatn TA, Nielsen BJ, Torp-Pedersen C, Overgaard C, et al. Leisure-Time Physical Activity and the Risk of Suspected Bacterial Infections. Med Sci Sports Exerc. Lippincott Williams and Wilkins; 2016;48:1737–44.

15. Nieman DC, Wentz LM. The compelling link between physical activity and the body's defense system [Internet]. J Sport Heal Sci. Elsevier B.V.; 2019. p. 201–17.

16. Simpson RJ, Hussain M, Baker F, Bigley AB, Peek MK, Stowe RP. Cardiorespiratory fitness is associated with better control of latent herpesvirus infections in a large ethnically diverse community sample: Evidence from the Texas City Stress and Health Study. Brain Behav Immun [Internet]. Elsevier BV; 2017;66:e35.

17. Agha NH, Mehta SK, Rooney B V., Laughlin MS, Markofski MM, Pierson DL, et al. Exercise as a countermeasure for latent viral reactivation during long duration space flight. FASEB J. John Wiley and Sons Inc.; 2020;34:2869–81.

18. Grande AJ, Keogh J, Silva V, Scott AM. Exercise versus no exercise for the occurrence, severity, and duration of acute respiratory infections [Internet]. Cochrane Database Syst Rev. John Wiley and Sons Ltd; 2020.

19. Duggal NA, Niemiro G, Harridge SDR, Simpson RJ, Lord JM. Can physical activity ameliorate immunosenescence and thereby reduce age-related multi-morbidity? [Internet]. Nat Rev Immunol. Nature Publishing Group; 2019. p. 563–72.

20. 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 [Internet]. Blackwell Publishing Ltd; 2018;17.

21. 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 [Internet]. 2020;26:8–22.

22. Simpson RJ. Aging, persistent viral infections, and immunosenescence: Can exercise "make space"? Exerc Sport Sci Rev. Exerc Sport Sci Rev; 2011;39:23–33.

23. Agha NH, Baker FL, Kunz HE, Graff R, Azadan R, Dolan C, et al. Vigorous exercise mobilizes CD34+

hematopoietic stem cells to peripheral blood via the β 2 -adrenergic receptor. Brain Behav Immun. Academic Press Inc.; 2018;68:66–75.

24. Pedersen BK. Physical activity and muscle–brain crosstalk [Internet]. Nat Rev Endocrinol. Nature Publishing Group; 2019. p. 383–92.

25. McCarthy DA, Macdonald I, Grant M, Marbut M, Watling M, Nicholson S, et al. Studies on the immediate and delayed leucocytosis elicited by brief (30-min) strenuous exercise. Eur J Appl Physiol Occup Physiol. Springer-Verlag; 1992;64:513–7.

26. Desdín-Micó G, Soto-Heredero G, Aranda JF, Oller J, Carrasco E, Gabandé-Rodríguez E, et al. T cells with dysfunctional mitochondria induce multimorbidity and premature senescence. Science (80-). American Association for the Advancement of Science; 2020;368:1371–6.

27. LaVoy EC, Hussain M, Reed J, Kunz H, Pistillo M, Bigley AB, et al. T-cell redeployment and intracellular cytokine expression following exercise: effects of exercise intensity and cytomegalovirus infection. Physiol Rep. American Physiological Society; 2017;5.

28. Spielmann G, Bollard CM, Bigley AB, Hanley PJ, Blaney JW, LaVoy ECP, et al. The effects of age and latent cytomegalovirus infection on the redeployment of CD8+ T cell subsets in response to acute exercise in humans. Brain Behav Immun. Academic Press Inc.; 2014;39:142–51.

29. Ross M, Ingram L, Taylor G, Malone E, Simpson RJ, West D, et al. Older men display elevated levels of senescence-associated exercise-responsive CD28null angiogenic T cells compared with younger men. Physiol Rep. American Physiological Society; 2018;6.

30. Simpson RJ, Cosgrove C, Ingram LA, Florida-James GD, Whyte GP, Pircher H, et al. Senescent T-lymphocytes are mobilised into the peripheral blood compartment in young and older humans after exhaustive exercise. Brain Behav Immun. Brain Behav Immun; 2008;22:544–51.

31. Hanson ED, Sakkal S, Que S, Cho E, Spielmann G, Kadife E, et al. Natural killer cell mobilization and egress following acute exercise in men with prostate cancer. Exp Physiol. Blackwell Publishing Ltd; 2020;105:1524–39.

32. Wang JS, Chen WL, Weng TP. Hypoxic exercise training reduces senescent T-lymphocyte subsets in blood. Brain Behav Immun [Internet]. Academic Press; 2011;25:270–8.

33. Ovadya Y, Landsberger T, Leins H, Vadai E, Gal H, Biran A, et al. Impaired immune surveillance accelerates accumulation of senescent cells and aging. Nat Commun 2018 91. Nature Publishing

Group; 2018;9:1–15.

34. Koch S, Larbi A, Özcelik D, Solana R, Gouttefangeas C, Attig S, et al. Cytomegalovirus infection: A driving force in human T cell immunosenescence. Ann N Y Acad Sci. Blackwell Publishing Inc.; 2007. p. 23–35.

35. Pawelec G, Larbi A, Derhovanessian E. Senescence of the Human Immune System. J Comp Pathol. W.B. Saunders Ltd; 2010;142.

36. Moher D, Liberati A, Tetzlaff J, Altman DG, Altman D, Antes G, et al. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. PLoS Med. 2009.

37. Brenchley JM, Karandikar NJ, Betts MR, Ambrozak DR, Hill BJ, Crotty LE, et al. Expression of CD57 defines replicative senescence and antigen-induced apoptotic death of CD8+ T cells. Blood. Blood; 2003;101:2711–20.

38. Lopez-Vergès S, Milush JM, Pandey S, York VA, Arakawa-Hoyt J, Pircher H, et al. CD57 defines a functionally distinct population of mature NK cells in the human CD56dimCD16+ NK-cell subset. Blood. American Society of Hematology; 2010;116:3865–74.

39. Appay V, Van Lier RAW, Sallusto F, Roederer M. Phenotype and function of human T lymphocyte subsets: Consensus and issues [Internet]. Cytom Part A. Cytometry A; 2008. p. 975–83.

40. Henson SM, Akbar AN. KLRG1-more than a marker for T cell senescence [Internet]. Age (Omaha). Age (Dordr); 2009. p. 285–91.

41. Plunkett FJ, Franzese O, Finney HM, Fletcher JM, Belaramani LL, Salmon M, et al. The Loss of Telomerase Activity in Highly Differentiated CD8 + CD28 − CD27 − T Cells Is Associated with Decreased Akt (Ser 473) Phosphorylation . J Immunol. The American Association of Immunologists; 2007;178:7710–9.

42. Voehringer D, Koschella M, Pircher H. Lack of proliferative capacity of human effector and memory T cells expressing killer cell lectinlike receptor G1 (KLRG1). Blood. Blood; 2002;100:3698–702.

43. Callender LA, Carroll EC, Beal RWJ, Chambers ES, Nourshargh S, Akbar AN, et al. Human CD8 + EMRA T cells display a senescence-associated secretory phenotype regulated by p38 MAPK. Aging Cell. Blackwell Publishing Ltd; 2018;17.

44. Zhou D, Borsa M, Simon AK. Hallmarks and detection techniques of cellular senescence and cellular ageing in immune cells [Internet]. Aging Cell. Blackwell Publishing Ltd; 2021. p. e13316.

45. Kaminsky LA, Arena R, Myers J. Reference standards for cardiorespiratory fitness measured with cardiopulmonary exercise testing data from the fitness registry and the importance of exercise national database. Mayo Clin Proc. Elsevier Ltd; 2015;90:1515–23.

46. Azali Alamdari K, Bashiri J. Effects of hypobaric Endurance Training on Graded Exercise Induced Lymphocyte Mobilization, Senescence and Their Surface Thiol Levels in Elite Male Athletes. Int J Appl Exerc Physiol. International Society of Communication and Development Between Universities (ISCDBU); 2018;7:48–55.

47. Curran M, Campbell JP, Powell E, Chikhlia A, Narendran P. The mobilisation of early mature CD56dim-CD16bright NK cells is blunted following a single bout of vigorous intensity exercise in Type 1 Diabetes. Exerc Immunol Rev [Internet]. 2020;26:116–31.

48. Garber CE, Blissmer B, Deschenes MR, Franklin BA, Lamonte MJ, Lee IM, et al. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: Guidance for prescribing exercise. Med Sci Sports Exerc. Med Sci Sports Exerc; 2011;43:1334–59.

49. Ingram LA, Simpson RJ, Malone E, Florida-James GD. Sleep disruption and its effect on lymphocyte redeployment following an acute bout of exercise. Brain Behav Immun. Academic Press Inc.; 2015;47:100–8.

50. Farina D, Ferguson RA, Macaluso A, De Vito G. Correlation of average muscle fiber conduction velocity measured during cycling exercise with myosin heavy chain composition, lactate threshold, and VO2max. J Electromyogr Kinesiol. J Electromyogr Kinesiol; 2007;17:393–400.

51. Turner JE, Aldred S, Witard OC, Drayson MT, Moss PM, Bosch JA. Latent Cytomegalovirus infection amplifies CD8 T-lymphocyte mobilisation and egress in response to exercise. Brain Behav Immun. Brain Behav Immun; 2010;24:1362–70.

52. Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple , graphical test measures of funnel plot asymmetry. BMJ. 1997;315:629–34.

53. Curran M, Campbell J, Drayson M, Andrews R, Narendran P. Type 1 diabetes impairs the mobilisation of highly-differentiated CD8+T cells during a single bout of acute exercise. Exerc

Immunol Rev [Internet]. 2019;25:64–82.

54. Krzywkowski K, Petersen EW, Ostrowski K, Kristensen JH, Boza J, Pedersen BK. Effect of glutamine supplementation on exercise-induced changes in lymphocyte function. Am J Physiol - Cell Physiol. American Physiological Society; 2001;281.

55. Lavoy EC, Bigley AB, Spielmann G, Rector JL, Morrison MR, O'Connor DP, et al. CMV amplifies T-cell redeployment to acute exercise independently of HSV-1 serostatus. Med Sci Sports Exerc. Med Sci Sports Exerc; 2014;46:257–67.

56. Bigley AB, Lowder TW, Spielmann G, Rector JL, Pircher H, Woods JA, et al. NK-cells have an impaired response to acute exercise and a lower expression of the inhibitory receptors KLRG1 and CD158a in humans with latent cytomegalovirus infection. Brain Behav Immun [Internet]. Academic Press; 2012;26:177–86.

57. Wang JS, Wu CK. Systemic hypoxia affects exercise-mediated antitumor cytotoxicity of natural killer cells. J Appl Physiol. J Appl Physiol (1985); 2009;107:1817–24.

58. Bigley AB, Rezvani K, Pistillo M, Reed J, Agha N, Kunz H, et al. Acute exercise preferentially redeploys NK-cells with a highly-differentiated phenotype and augments cytotoxicity against lymphoma and multiple myeloma target cells. Part II: Impact of latent cytomegalovirus infection and catecholamine sensitivity. Brain Behav Immun. Academic Press Inc.; 2015;49:59–65.

59. Brown FF, Bigley AB, Sherry C, Neal CM, Witard OC, Simpson RJ, et al. Training status and sex influence on senescent T-lymphocyte redistribution in response to acute maximal exercise. Brain Behav Immun. Academic Press Inc.; 2014;39:152–9.

60. Bruunsgaard H, Jensen M, Schjerling P, Halkjaer-Kristensen J, Ogawa K, Skinhøj P, et al. Exercise induces recruitment of lymphocytes with an activated phenotype and short telomeres in young and elderly humans. Life Sci. Life Sci; 1999;65:2623–33.

61. JS W, TP W. Hypoxic exercise training promotes antitumour cytotoxicity of natural killer cells in young men. Clin Sci (Lond). Clin Sci (Lond); 2011;121:343–53.

62. JP C, NE R, VE B, M T, JJ van Z, MT D, et al. Acute exercise mobilises CD8+ T lymphocytes exhibiting an effector-memory phenotype. Brain Behav Immun. Brain Behav Immun; 2009;23:767–75.

63. Minuzzi L, Rama L, Bishop N, Rosado F, Martinho A, Paiva A, et al. Lifelong training improves anti-

inflammatory environment and maintains the number of regulatory T cells in masters athletes. Eur J Appl Physiol. Eur J Appl Physiol; 2017;117:1131–40.

64. Simpson RJ, Cosgrove C, Chee MM, McFarlin BK, Bartlett DB, Spielmann G, et al. Senescent phenotypes and telomere lengths of peripheral blood T-cells mobilized by acute exercise in humans. Exerc Immunol Rev [Internet]. 2010;16:40–55.

65. Anane LH, Edwards KM, Burns VE, Drayson MT, Riddell NE, van Zanten JJCSV, et al. Mobilization of γδ T lymphocytes in response to psychological stress, exercise, and β-agonist infusion. Brain Behav Immun. Brain Behav Immun; 2009;23:823–9.

66. Fan X, Wang Y. β2 Adrenergic receptor on T lymphocytes and its clinical implications. Prog Nat Sci. Science Press; 2009. p. 17–23.

67. Pedersen L, Idorn M, Olofsson GH, Lauenborg B, Nookaew I, Hansen RH, et al. Voluntary running suppresses tumor growth through epinephrine- and IL-6-dependent NK cell mobilization and redistribution. Cell Metab. Cell Press; 2016;23:554–62.

68. Dimitrov S, Lange T, Born J. Selective Mobilization of Cytotoxic Leukocytes by Epinephrine. J Immunol. The American Association of Immunologists; 2010;184:503–11.

69. Krüger K, Lechtermann A, Fobker M, Völker K, Mooren FC. Exercise-induced redistribution of T lymphocytes is regulated by adrenergic mechanisms. Brain Behav Immun [Internet]. Academic Press; 2008;22:324–38.

70. Krüger K, Alack K, Ringseis R, Mink L, Pfeifer E, Schinle M, et al. Apoptosis of T-Cell Subsets after Acute High-Intensity Interval Exercise. Med Sci Sports Exerc. Lippincott Williams and Wilkins; 2016;48:2021–9.

71. Bujko K, Cymer M, Adamiak M, Ratajczak MZ. An Overview of Novel Unconventional Mechanisms of Hematopoietic Development and Regulators of Hematopoiesis – a Roadmap for Future Investigations. Stem Cell Rev Reports. Springer; 2019;15:785–94.

72. Abrahin O, Cortinhas-Alves EA, Vieira RP, Guerreiro JF. Elite athletes have longer telomeres than sedentary subjects: A meta-analysis. Exp Gerontol [Internet]. Elsevier Inc.; 2019;119:138–45.

73. Schirmer M, Vallejo AN, Weyand CM, Goronzy JJ. Resistance to apoptosis and elevated expression of Bcl-2 in clonally expanded CD4+CD28- T cells from rheumatoid arthritis patients. J Immunol [Internet]. 1998;161:1018–25.

74. Vallejo AN, Schirmer M, Weyand CM, Goronzy JJ. Clonality and Longevity of CD4 + CD28 null T Cells Are Associated with Defects in Apoptotic Pathways . J Immunol. The American Association of Immunologists; 2000;165:6301–7.

75. Prösch S, Wendt CEC, Reinke P, Priemer C, Oppert M, Krüger DH, et al. A novel link between stress and human cytomegalovirus (HCMV) infection: Sympathetic hyperactivity stimulates HCMV activation. Virology [Internet]. Academic Press Inc.; 2000;272:357–65.

76. Mehta SK, Stowe RP, Feiveson AH, Tyring SK, Pierson DL. Reactivation and Shedding of Cytomegalovirus in Astronauts during Spaceflight. J Infect Dis. Oxford Academic; 2000;182:1761–4.

77. Spielmann G, McFarlin BK, O'Connor DP, Smith PJW, Pircher H, Simpson RJ. Aerobic fitness is associated with lower proportions of senescent blood T-cells in man. Brain Behav Immun. Brain Behav Immun; 2011;25:1521–9.

78. Fisher JP, Young CN, Fadel PJ. Autonomic adjustments to exercise in humans. Compr Physiol. Wiley-Blackwell Publishing Ltd; 2015;5:475–512.

79. Fritsche A, Stumvoll M, Grub M, Sieslack S, Renn W, Schmülling RM, et al. Effect of hypoglycemia on β-adrenergic sensitivity in normal and type 1 diabetic subjects. Diabetes Care. American Diabetes Association Inc.; 1998;21:1505–10.

80. Liston A, Gray DHD. Homeostatic control of regulatory T cell diversity [Internet]. Nat Rev Immunol. Nat Rev Immunol; 2014. p. 154–65.

81. Krüger K, Mooren FC. T cell homing and exercise. Exerc Immunol Rev [Internet]. 2007;13:37–54.

82. Dimitrov S, Benedict C, Heutling D, Westermann J, Born J, Lange T. Cortisol and epinephrine control opposing circadian rhythms in T cell subsets. Blood. Blood; 2009;113:5134–43.

83. Benschop RJ, Oostveen FG, Heijnen CJ, Ballieux RE. β 2-Adrenergic stimulation causes detachment of natural killer cells from cultured endothelium. Eur J Immunol. Eur J Immunol; 1993;23:3242–7.

84. Navalta JW, Sedlock DA, Park KS. Effect of exercise intensity on exercise-induced lymphocyte apoptosis. Int J Sports Med. Int J Sports Med; 2007;28:539–42.

85. Pizza FX, Mitchell JB, Davis BH, Starling RD, Holtz RW, Bigelow N. Exercise-induced muscle damage: effect on circulating leukocyte and lymphocyte subsets. Med Sci Sport Exerc [Internet].

1995;27:363–70.

86. Pedersen BK. Muscle as a secretory organ. Compr Physiol. Compr Physiol; 2013;3:1337–62.

87. Pedersen BK, Febbraio MA. Muscles, exercise and obesity: Skeletal muscle as a secretory organ [Internet]. Nat Rev Endocrinol. Nature Publishing Group; 2012. p. 457–65.

88. Akbar AN. The convergence of senescence and nutrient sensing during lymphocyte ageing [Internet]. Clin Exp Immunol. Blackwell Publishing Ltd; 2017. p. 4–5.

89. Feldman RD, Limbird LE, Nadeau J, Robertson D, Wood AJJ. Alterations in Leukocyte β-Receptor Affinity with Aging. N Engl J Med. Massachusetts Medical Society; 1984;310:815–9.

Figure Legends

Fig. 1. Flowchart of study selection.

Fig. 2. Forest plot of standardized mean difference (SMD) and 95% confidence interval for the overall effect immediately post exercise compared to baseline values. CMV: Cytomegalovirus; H-AT: hypoxic-absolute exercise; HC: hypobaric control; H-C: hypoxic resting; HE: hypobaric exercise; HI: High intensity; H-RT: hypoxic-relative exercise; HSV1: herpes simplex virus 1; LL: Lower limit of 95% confidence interval; LT: Lactate threshold; MI: Moderate intensity. NC: normobaric control; N-C: normoxic resting; NE: normobaric exercise; N-T: normoxic exercise; TD1: Type 1 diabetes; TR: Trained; UL: Upper limit of 95% confidence interval; UN: Untrained.

Fig. 3. . Forest plot of standardized mean difference (SMD) and 95% confidence interval for the overall effect 1h post exercise compared to baseline values. CMV: Cytomegalovirus; H-AT: hypoxic-absolute exercise; HC: hypobaric control; H-C: hypoxic resting; HE: hypobaric exercise; HI: High intensity; H-RT: hypoxic-relative exercise; HSV1: herpes simplex virus 1; LL: Lower limit of 95% confidence interval; LT: Lactate threshold; MI: Moderate intensity. NC: normobaric control; N-C: normoxic resting; NE: normobaric exercise; N-T: normoxic exercise; TD1: Type 1 diabetes; TR: Trained; UL: Upper limit of 95% confidence interval; UN: Untrained.

Fig. 4. Forest plot of standardized mean difference (SMD) and 95% confidence interval for the overall effect 2h post exercise compared to baseline values. CMV: Cytomegalovirus; H-AT: hypoxic-absolute exercise; HC: hypobaric control; H-C: hypoxic resting; HE: hypobaric exercise; HI: High intensity; H-RT: hypoxic-relative exercise; HSV1: herpes simplex virus 1; LL: Lower limit of 95% confidence interval; LT: Lactate threshold; MI: Moderate intensity. NC: normobaric control; N-C: normoxic resting; NE: normobaric exercise; N-T: normoxic exercise; TD1: Type 1 diabetes; TR: Trained; UL: Upper limit of 95% confidence interval; UN: Untrained.

Fig 5. The figure summarizes the lymphocytes count fold change from baseline to each time point for the senescent cells: CD8 T cells (in blue), CD4 T cell (in dark pink) and NK cells (in light pink and black centre). The position of the cells represents the SMD of each meta-analysis at each time-point post exercise.

Meta-Analysis

Effect of exercise on acute senescent lymphocyte counts: a systematic review and meta-analysis.

Amanda V. Sardeli [a,b*], Marcelo A. Mori [c, d, e], Janet M. Lord [a, f]

[a] MRC-Versus Arthritis Centre for Musculoskeletal Ageing Research, Institute of Inflammation and Ageing, University of Birmingham, Birmingham, UK.

[b] Laboratory of Exercise Physiology (FISEX), University of Campinas, Campinas, Brazil.

[c] Department of Biochemistry and Tissue Biology, Institute of Biology, University of Campinas, Campinas, Brazil.

[d] Experimental Medicine Research Cluster, University of Campinas, Campinas, Brazil.

[e] Obesity and Comorbidities Research Center, University of Campinas, Campinas, Brazil.

[f] NIHR Birmingham Biomedical Research Centre, University Hospital Birmingham and University of Birmingham, Birmingham, UK.

Short Title: Effect of exercise on acute senescent lymphocyte counts

Corresponding Author:

Amanda Veiga Sardeli

Institute of Inflammation and Ageing

Queen Elizabeth Hospital

Mindelsohn Way

Birmingham, West Midlands, B15 2WB, UK

Tel: +44 (0)121 414 3344

E-mail: amandavsardeli@gmail.com

Number of Tables: 2.

Number of Figures: 5.

Word count: 4832.

Keywords: Exercise, Ageing, Cellular senescence, Immunosenescence, Immunology.

Abstract

Background: Highly differentiated, senescent lymphocytes are pro-inflammatory and contribute to age-related systemic inflammation, inflammageing. There are several reports of acute changes in senescent lymphocyte counts post-exercise which potentially has consequences for systemic inflammation. However, there is little consensus since the studies differ with respect to participants, exercise protocols, cellular markers assessed, and the time point of assessment post-exercise.
Objective: We performed a systematic review and meta-analysis to assess the impact of exercise on senescent lymphocyte counts in blood immediately, 1h and 2h post exercise.
Methods: The search was performed in PubMed (MEDLINE), Web of Science, Embase, Scopus and Cochrane, on January 11, 2021. The 13 studies selected tested aerobic exercise effects, mainly in young men. They assessed the counts of lymphocytes (CD4 T cells, CD8 T cells, NK cells), with the following immune cell marker combinations: KLRG1+, CD57+ (only NK cells), EMRA T cells (CD45RA+CCR7-CD28-CD27-), CD28-CD27-, KLRG1+CD28- and CD28-. Independent extraction of articles by 2 researchers.
Results: Standardized mean difference (SMD) and 95% confidence interval between baseline and post-exercise showed significant increases (SMD > 0.9, p<0.003) in all types of lymphocyte counts immediately post exercise. At 1h post exercise senescent CD4 T cells returned to baseline values (p=0.74), CD8 T cells were reduced (-0.26 [-0.41; -0.11], p=0.001), and senescent NK cells were raised (0.62 [0.14; 1.10], p=0.01) above baseline. By 2 hours post exercise, senescent CD4 T cells were reduced (-0.94 [-1.40; -0.48], p<0.001), CD8 T cells remained below baseline (-0.53 [-1.04; -0.009], p=0.04), and NK cells had returned to baseline values (-0.29 [-0.64; 0.07], p=0.11). The main determinants of heterogeneity between studies were cytomegalovirus (CMV) serostatus and the characteristics of exercise protocols. CMV+ individuals had a higher immediate lymphocytosis and 1h post lymphopenia than CMV- individuals. Exercise performed at higher intensities and shorter durations led to higher magnitude of change in senescent lymphocyte counts at all time-points.
Conclusion: The differing effects of exercise on senescent NK cells and CD4 and CD8 T cells suggest differing susceptibility to factors modulating lymphocyte extravasation such as adrenaline and exercise intensity.

Introduction

Immunosenescence, the gradual remodelling of the immune system, is an integral component of the ageing process [1]. Advanced age impairs innate immune responses, contributes to chronic low-grade inflammation (inflammageing) and reduces immunity, increasing the risk of infections, autoimmunity and overall poor health in the older adult [2,3]. Among the features associated with adaptive immunosenescence are the atrophy of the thymus, which reduces naïve T cell output, and the subsequent increased number of highly differentiated, senescent T cells in the circulation [3,4]. Senescent cells are one of the causes of detrimental effects to the body during ageing, contributing to chronic diseases, such as idiopathic pulmonary fibrosis, diabetes, and osteoarthritis [5]. It has been shown recently that mice with high levels of senescent T cells, due to dysfunctional mitochondria, enter premature senescence and a broad range of age-related diseases [6]. Immunosenescence, especially T cell senescence, may therefore be a major contributor to the ageing process.

Senescent cells undergo a state of cell quiescence with permanent cell cycle arrest induced by different sources of stress and damage to the cell. These cells produce a senescence-associated secretory phenotype (SASP), which is composed of pro-inflammatory cytokines, chemokines, growth factors and proteases. Cells releasing SASP alter the tissue microenvironment, affect neighboring cells, and are thus deleterious [7,8]. In the immune system there are some subtle differences. For example, T cells can have a functionally exhausted phenotype resulting from chronic stimulation, which is distinct from a senescent phenotype resulting from ageing or chronic infection. These phenotypes can be differentiated by cell surface markers [9]. We have therefore used the markers identified as relating to senescent T cells such as loss of CD28 and CD27 and expression of KLRG1 and CD57. Importantly, senescent T cells also produce a SASP that is highly pro-inflammatory and similar in content to that of non-immune senescent cells [10], therefore they are likely to contribute to inflammageing and tissue compromise during ageing.

The immunomodulatory effects of exercise have been widely explored and could be associated with the reduction in senescent cell counts [10], for example obese mice provided with an exercise wheel had reduced numbers of senescent cells in their adipose tissue [11]. Exercise has been reported to have a range of immune enhancing effects including reducing chronic low-grade inflammation [12], improving responses to vaccination [13], reducing the risk of infection [14,15], improving the immune response against viruses and bacteria and reducing the burden of latent viral infections [16–18]. Among the main physiological mechanisms mediating the immunomodulatory benefits of exercise are the reduction in body fat and the release of anti-inflammatory cytokines, such as interleukin-6 (IL-6) and IL-1RA, by the exercising muscle [12,19].

Recently, Duggal *et al*. [20] have reported the benefits of sustained physical activity in to old age on adaptive immune phenotype and immunosenescence. They reported that thymic health, as measured by the frequency of naïve T cells and recent thymic emigrants (RTE), was better preserved in older exercisers (cyclists) compared to inactive elders. Older cyclists also had significantly higher serum levels of the thymoprotective cytokine interleukin-7 (IL-7), higher B regulatory cell frequency, lower IL-6 and reduced Th17 polarization, all markers of an aged immune system. However, they also reported that the age-related increase in senescent T cells was not prevented in the cyclists [20].

Despite the chronic benefits of exercise being well established, whether acute exercise increases susceptibility to infection or confers immune protection is still a matter of debate [21]. However, an increase in lymphocyte counts in the blood (lymphocytosis), followed by a decrease (lymphocytopenia) post exercise has generally been reported [22]. Lymphocytes are proposed to migrate from the marginal pool, the spleen and lymph nodes in to the blood, as well as increased release from the bone marrow to produce the lymphocytosis. This migration is mediated by exercise-induced shear stress on blood vessels and catecholamines, as well as cortisol and to some extent cytokines such as IL-6 [23–25].

What is less clear is the impact of exercise on specific immune cell types and their differentiation state, notably senescent immune cells. This is important bearing in mind their pro-inflammatory nature and potential role in driving inflammageing and the aged phenotype [26]. Studies investigating senescent lymphocyte counts in circulation post-exercise have shown a variety of responses [27–31], including a reduction on leukocyte counts [32]. However, senescent, or highly differentiated lymphocytes appear to be more likely to increase in blood with exercise than lymphocytes in earlier stages of differentiation. This could be beneficial in leading to their subsequent removal by NK cells or CD8 T cells which can detect senescent cells and kill them by apoptosis [22,33].

Another important confounding factor in the various exercise intervention studies, is infection by cytomegalovirus (CMV) that increases with age and has deleterious effects on lymphocyte immunity, accelerating immunosenescence [22,34,35]. The higher baseline cell counts of senescent lymphocytes in CMV+ individuals could lead to higher magnitude of change in these individuals after exercise, and thus the CMV serostatus might be an important confounding factor between studies [27,28]. Other factors that may cause different results between studies are: the comparison between absolute cell counts and the frequency of cells in the circulation; the different types of lymphocytes assessed; the membrane markers used to identify cell senescence; characteristics of the study population (age, sex and physical activity level) and the exercise protocols used (type of exercise, volume and intensity).

To derive a consensus from the literature it is important to isolate the variety of confounding factors among the studies and to run a pooled effects meta-analysis. Thus, we aimed to identify the impact of acute exercise on the frequency of senescent T cells and NK cells, taking in to account variables such as CMV serostatus, age, training status and specifics of the exercise protocols.

Methods

This systematic review and meta-analysis was registered on PROSPERO under the number CRD42021267078, that can be assessed at https://www.crd.york.ac.uk/prospero/, and it was reported in accordance with the recommendations of Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) statement [36].

Search strategy

On January 11, 2021 the search was updated at PubMed (Medline), Web of Science, Embase, Scopus and Cochrane. It combined the synonyms of “senescent markers” and “exercise” according to each data base descriptor and field of search as detailed in the Supplementary material.

Eligibility criteria

Figure 1 shows the study selection process, completed by two independent reviewers. We included studies: (1) of acute interventional exercise; (2) with no associated intervention, i.e. exercise only group; (3) in humans from both sexes; (4) comparing resting and immediately, 1h and 2h post exercise condition; (5) assessing any bona fide markers of immunosenescence; (6) assessing CD4+ or CD8+ T cells, or NK lymphocytes; (7) written in English.

Immunosenescence cell markers

Markers of senescence traditionally used for non-immune cells, such as $p16^{ink4a}$ and SA-βGal have not been used in studies of immunosenescence which focus on cell membrane markers. We therefore selected several broadly accepted markers of immunosenescence to use in this study and the characteristics of each of them are described below.

CD57$^+$. CD57+ NK cells have been attributed a senescent-like phenotype due to their short telomeres and inability to proliferate [1,37,38].

CD28$^-$ CD27$^-$. CD27 and CD28 are costimulatory receptors and T cells lacking CD27 and CD28 are thought to be fully differentiated T cells exhibiting shorter telomere length [39]. When the expression of CD27 and CD28 is lost, there is no evidence of subsequent re-expression and the downregulation of these molecules are linked to dysfunctional T cells with a SASP secretome [40,41].

KLRG1$^+$. T-lymphocytes expressing KLRG1 have impaired capacity to proliferate, yet

maintain immediate effector cell capabilities such as the recognition and killing of target cells [42].

EMRA (CD45RA$^+$CCR7$^-$CD28$^-$CD27$^-$). EMRA, for terminally differentiated effector memory cells re-expressing CD45RA, have the key features of cell senescence, with low proliferation response and a highly inflammatory phenotype [10]. They also have high levels of DNA damage and loss of telomerase activity [43]. However, due to their ability to proliferate under specific conditions their phenotype is distinct from non-immune senescent cells which cannot proliferate [44].

Exclusion criteria

We excluded studies that: (1) did not have original data or did not undergo peer-review such as reviews, commentaries, editorials, letter to the editors, case reports or conference abstracts; (2) assessed other senescence markers such as telomere shortening, or telomerase activity; (3) had not tested exercise effects; (4) had not assessed immunosenescence in humans; (5) assessed senescence in other cells, besides lymphocytes and NK cells; (6) were not written in English.

Data collection and data items

Data collection was performed by two independent researchers. The means and a measure of dispersion of the senescent cell counts were extracted for each subgroup within studies. Mean, standard deviation (SD) and sample number (n) were used for the meta-analyses. Standard error (SE) was converted to SD by the equation $SD = SE \times (\sqrt{n})$, if SD was not provided in the original study.

For subgroup analysis we extracted information about participants sex, age, level of training, health condition and CMV serostatus, type of lymphocytes assessed, membrane markers used, unit of measurement, and the characteristics of the exercise bout such as intensity, volume, and type of equipment.

The sample of studies was classified as young, middle aged and old according to the mean age reported (young [<30yrs], middle aged [30-40yrs] and older adults [>50yrs].

The participants were considered “trained” when the studies classified them as elite athletes, trained, physically active, cyclists or when the VO_2max was above the 50% percentile according to their age [45]; they were considered “untrained” when the studies classified them as untrained, or doing no regular physical activity or sedentary. The studies that did not report the participant`s physical activity level or reported a too wide range of physical activity level among their participants were excluded from this subgroup analysis. Individuals undergoing exercise chronic intervention were considered trained [32,46]; while the individuals undergoing non-exercise intervention in Wang et. al. [32] were classified as untrained and the individuals undergoing non-exercise intervention in Azali Alamdari et al. [46] were excluded for training status analysis, since they were athletes at baseline.

Regarding health status, only Curran et al. [47] have included individuals with type I diabetes, while the other studies only included healthy participants.

The exercise intensity was classified according to the percentage of VO_2max described by the American college of Sports Medicine [48], in which 46-63% is moderate, 64-90% is vigorous and >91% is near maximum. The intensity reported on Ingram et al. study [49], in watts was estimated as 73.7% of maximum according to data from participants of a similar age. Another study tested different protocols according to their lactate threshold (5% under LT, 5% above LT and 15% above LT) in the same individuals and each of them were included in the meta-analysis as a separate study [27].The intensity was also converted to percentage according to Farina *et al.* [50], in which 5% <LT was considered 61.1%, 5%>LT was considered 71.1%, and 15% >LT was considered 81.1%. The studies reporting percentage of estimated maximum power or percentage of ventilatory threshold work rate, instead of VO_2max, were classified for subgroup analysis as these markers were proportionally equivalent.

The studies applying incremental maximum effort tests and other protocols expected to last less than 20 min were considered short, the ones applying 30 min duration were considered moderate and above this they were considered long duration.

Only Azali Alamdari et al.[46] had a control group, and thus, the change of control group was subtracted from the exercise change to increase the robustness of the analysis. Although Turner et al. [51] had also reported a control group, they did not present the effects of the control period on

CD28-CD27- markers, in this way the control group was not considered for analysis. Two studies presented acute exercise effects before and after a variety of chronic interventions [32,46] and thus we included only their post intervention session to avoid sample overlapping in the analysis.

Statistical analysis

We analysed the absolute cell count as the outcome measure, considering the standardized mean difference (SMD) and 95% of confidence interval between baseline levels and post exercise time-points since the units of measure were not consistent across studies.

The 3 main meta-analyses, for each time point (immediately, 1h and 2h post exercise) and the subgroup analyses were performed using Comprehensive Meta-Analysis software, version 3.3.070. When there was statistical significance for heterogeneity, randomized effect models were selected and when there was no significant heterogeneity, fixed effects were applied. The inconsistency between studies was reported as a percentage (I^2), based on difference between expected heterogeneity (df) and true heterogeneity (Q-value).

For subgroup analysis we tested the influence of the following confounding factors: sex (men and women); age (young [<30yrs], middle aged [30-40yrs] and older adults [>50yrs]); type of lymphocytes (CD4+, CD8+ and NK); type of senescence marker (KLRG1+, CD57+, EMRA [CD45RA+CCR7-CD28-CD27-], CD28-CD27-, KLRG1+CD28- and CD28-), level of training (trained and untrained); health condition (healthy and diseased); CMV serostatus (CMV+ and CMV-); exercise intensity (moderate, vigorous, near maximum); and exercise volume (short, moderate and long). Q tests were applied to group comparisons, considering 95% confidence.

Egger's tests were performed to check the risk of publication bias in each meta-analysis [52].

Results

We included thirteen studies [27–29,32,46,47,49,51,53–57] testing acute aerobic exercise effects on senescent T lymphocytes and NK cell counts (shown in Figure 1). It is noteworthy that some studies had to be excluded due to the absence of specific description of absolute senescent lymphocyte counts [30,58–64]. Most studies included, reported their results among different subgroups of individuals with different sex, ages, CMV serostatus, types of exercise protocols and time points of analysis that were analyzed as a sub-study.

*****please insert Figure 1 here*****

Study characteristics

Table 1 shows the characteristics of the studies included. Only Curran et al.[47] included a type I diabetes group, while the other studies only included healthy participants. While twelve studies tested exercise effects on males, just one tested exercise effects on participants from both sexes [27], and thus comparisons between men and women were not possible in subgroup analysis. One study included middle aged [28], two included older adults [28,29] and all of them (thirteen) tested young adults. Our analysis reported the effect of exercise on T CD4+, T CD8+ and NK cell counts. All studies tested the effects of aerobic exercise, the majority of them used bicycle, and a few used treadmill [46,51].

*****please insert Table 1 here*****

Syntheses of the results

Lymphocyte counts immediately post exercise. Figure 2 shows there were significant increases on senescent CD4 T cells (SMD 0.96 [0.67; 1.25], p<0.001), CD8 T cells (SMD 1.26 [0.93; 1.59], p<0.001) and NK cells counts immediately post exercise (SMD 1.04 [0.35; 1.72], p=0.003). However, all those analyses were heterogeneous, reinforcing the need for further subgroup analyses. Furthermore, the analysis of senescent CD4 T cells and CD8 T cell counts had significant risk of bias, evidencing that studies with low precision conduced the main effects.

Table 2 shows no effect of age (p= 0.46) or training status (p=0.35) on outcomes immediately post exercise. On the other hand, the intensity and duration of exercise protocols and CMV status influenced the post exercise senescent lymphocyte counts (Table 2). Specifically, the higher magnitude of increase in senescent lymphocytes were seen in the maximum intensity and short duration protocols (SMD 1.81 [1.45; 2.1], p<0.001) compared to the others (SMD <0.85, p<0.05). There was a trend to higher senescent lymphocyte counts in CMV positive participants compared to CMV- (p=0.09). The CMV status analysis for each subgroup of T lymphocyte showed higher increase in senescent CD8+ T cells for CMV+ (SMD 1.60 [0.73; 2.46], p<0.001) compared to CMV- (SMD 0.58 [0.33; 0.83], p<0.001), with no difference for senescent CD4+ T cells regarding CMV status (SMD CMV+: 0.42 [0.02; 0.82], p=0.038 and CMV-: 0.50 [0.18; 0.82], p=0.002).

Please, insert Figure 2 here

Lymphocyte counts one hour post exercise. Figure 3 shows senescent CD8+ T cell counts were lower compared to baseline levels (SMD -0.28 [-0.44; -0.13], p<0.001), while CD4+ T cell counts returned to baseline levels (SMD -0.13 [-0.37; 0.11], p=0.28) and NK cells were still above baseline values (SMD

0.62 [0.14; 1.09], p=0.11). These analyses were homogeneous (p>0.53, I^2=0%), confirming that each of these senescent cells have very consistent response 1h post exercise across the different studies.

Table 2 shows there was a significant reduction in the senescent lymphocyte count only in CMV+ and not CMV- individuals, with significant difference between groups. Regarding each subgroup of T lymphocyte there was no significant reduction for senescent CD4+ T or CD8+ cells in CMV- (SMD CD4+: 0 [-0.30; 0.31], p=0.97 and CD8+: -0.13 [-0.36; 0.09], p=0.25) while there was a trend of senescent CD4+ reduction in CMV+ individuals (SMD -0.35 [-0.74; 0.04], p=0.075), and reduction of senescent CD8+ T cells in CMV+ (SMD -0.46 [-0.75; -0.18] p=0.001). Only vigorous intensity and long duration exercise protocols led to significant reduction of senescent lymphocytes (SMD -0.5 [-0.8; -0.2], p<0.001) while the other intensities and durations did not vary significantly (p>0.16).

Please, insert Figure 3 here

Lymphocyte counts two hours post exercise. Figure 4 shows senescent CD4 T cells were reduced (SMD -0.94 [-1.40; -0.48], p<0.001), CD8 T cells remained below baseline (SMD -0.53 [-1.04; -0.009], p=0.04), and NK cells had returned to baseline values (SMD -0.29 [-0.64; 0.07], p=0.11). There was significant risk of publication bias for the analysis of senescent CD4 T cells (Egger test p-value <0.001), evidencing that studies with low precision conduced the main effects in this analysis.

All these three meta-analyses were heterogeneous, however, due to the low number of subgroups in these analyses, only training status, intensity and volume of exercise protocols were analyzed. No difference between trained and untrained individuals was noticed (p=0.81) and only maximum intensity and short duration protocols reduced senescent cell counts (SMD -0.7 [-1; -0.4], p<0.001), however, it is noteworthy there was very low number of studies in the other categories (Table 2).

Please, insert figure 4 here

Discussion

The main findings of the present meta-analysis were the significant increase in senescent CD8+, CD4+ and NK cell counts immediately post exercise followed by a reduction in senescent CD8+ T cells at 1h and 2h post exercise, a reduction in senescent CD4+ T cells at 2h post exercise and maintenance of increased NK senescent cells at 1h post exercise with a return to baseline at 2h post exercise (Figure 5). Although there is no consensus about the exact role of these redistributions of senescent lymphocytes post exercise, it has been proposed that senescent lymphocytes are preferentially recruited for immune surveillance and removal by NK and CD8+ T cells, resulting in an exercise-induced senolytic effect [22].

In fact, it is known that T-cells with high cytotoxic capabilities and tissue migration potential, which are characteristics of highly differentiated lymphocytes, are preferentially mobilised by acute stress and exercise [65]. These lymphocytes could be recruited due to their high β_2-adrenergic receptor expression [66] even though they have impaired replicability and co-stimulatory potential. Furthermore, in mice, NK cells are the main mediator of the antitumor effects of exercise. These effects depend on the mobilisation of these cells [67], which are the most responsive lymphocyte subset to acute exercise due to their high β-adrenergic receptor expression [68]. Mobilisation of the senescent, less functional form of these cells could be beneficial if they are then removed, improving the overall quality of the lymphocyte pool.

Following their mobilisation it is possible that T-lymphocytes egressing to the peripheral tissues may experience a pro-apoptotic environment [69], as Kruger et. al. [70] showed the number of highly differentiated CD3+ T cells remained reduced 3h and 24h post exercise. Another possibility could be the return of senescent cells to lymph nodes but most of these cells lack CCR7, a secondary lymphoid organ-homing marker, this is unlikely.

In theory, when senescent T-lymphocytes undergo apoptosis, a subsequent feedback loop could increase the output of naïve T-lymphocytes from the thymus, restoring the peripheral T-lymphocyte pool [22,59]. In fact, naïve lymphocytes counts are increasing 1h post exercise [30,59]. Furthermore, older adults involved in regular exercise have higher serum levels of the thymoprotective IL-7 and higher frequency of RTE than sedentary controls [20], which could be stimulated by senescent lymphocyte clearance post each exercise bout.

In an opposite way, exercise-induced hematopoiesis [25,71], could also affect the thymic feedback loop, increasing the stimuli for senescent lymphocyte removal. Cross-sectional studies showed physically active individuals have lower markers of senescent T lymphocytes [10,20] and master athletes have longer lymphocyte telomere length than untrained controls [72]. Nevertheless, it is noteworthy that highly differentiated, senescent lymphocytes are less sensitive to apoptotic signals [73,74], and the exercise effects on senescent cell apoptosis is still unknown.

Most studies tested the influence of CMV serostatus on exercise responses. CMV reactivation can be triggered through catecholamine-responsive elements [75] and stress hormone levels, which are known to correlate strongly with CMV reactivation in astronauts before and after spaceflight

[76]. Thus, it is believed that CMV^+ individuals have reduced sensitivity to β-adrenergic stimulation and decreased β_2-adrenegic receptor expression to prevent CMV reactivation [58]. However, a reduced β-adrenergic sensitivity of T cells in CMV^+ individuals is not supported by our analysis, and in fact we saw a larger magnitude of changes in CMV+ individuals. Thus, we believe the expected higher baseline senescent lymphocyte counts in CMV^+ individuals [28,77], especially for senescent CD8+ T cells, explains the higher magnitude of change with exercise in this population.

There was a greater magnitude of increase in senescent lymphocyte counts immediately post maximum intensity and short duration protocols and greater magnitude of reduction 2h post exercise compared to lower intensities and longer duration protocols. These differences could be explained by higher sympathetic activation and sustained release of epinephrine reported in higher intensities protocols [70,78,79]. However, there is also evidence that cortisol affects lymphocyte counts during exercise [70,80–83]. Exercise of high intensity leads to greater release of cortisol in to the blood and for a longer time and may explain the reduced cell counts at later time points since cortisol induces apoptosis in lymphocytes [84]. We also considered the type of exercise and whether this may make a difference to the senescent cell response. However, we found no difference at any of the time points between treadmill and cycling protocols (data not shown), though this is with the caveat that the number of studies per subgroup category varied greatly.

It is unlikely that IL-6 released by muscle cells during exercise [85], explains the difference between exercise protocols. It is known, that IL-6 attracts lymphocytes to the circulation together with β-adrenergic signaling during exercise [67], however, there is a higher release of IL-6 within exercise protocols with higher energetic demand, such as the higher volume and during regimens [85–87] which do not agree with our findings.

Finally, exercise hypoxia may explain at least part of the changes in T lymphocytes and NK counts with exercise, possibly mediated by the same neuroendocrinological factors released by other stress conditions (i.e.: catecholamines, cortisol) [57,88].

Limitations

The first limitation of this study was that most studies included young individuals. The unbalanced subgroup analysis suggested there is higher magnitude of change on senescent counts in young than older or middle-aged individuals immediately and 1h post exercise. Whether it is a true effect is unclear, it could be explained by reduced β_2-adrenergic receptor sensitivity with ageing [89], which in turn increases the threshold for catecholamine-induced lymphocyte recruitment. In this way, it is important to confirm these results with a larger sample of older adults.

Comparisons between men and women were also not possible due to the lack of studies in women. An exploratory analysis showed immediately post exercise there was a large ($p<0.001$) increase of senescent lymphocytes in men (1.23 [0.99; 1.47], $p<0.001$, k=44) compared to studies with mixed sex samples (0.48 [0.19; 0.78], $p<0.001$, k=6), while there was no difference between these subgroups 1h post. Future studies should test to what extent the results in men are also applied to women.

Another limitation was the lack of a control group, i.e. without exercise, within the original studies which precluded a proper risk of bias assessment. In the other hand, the comparison of the same participants along time removes the between subjects' effects, which in turn contributes to the isolation of exercise effects. Furthermore, we explored possible influences of the confounding factors in subgroup analysis. At last, it is noteworthy that only two studies investigated exercise effects on senescent NK cell counts, with is a limitation of the literature and future studies should fill these gaps to strengthen knowledge in the field.

Lastly, one additional issue was the use of markers to identify the different stages of T cell differentiation and their relation to T cell senescence. Thus, no studies enumerated CD57 TEMRA cells, the ones that are closest to a senescent phenotype.

Conclusions

Senescent lymphocyte counts change significantly in the acute response to aerobic exercise. However, a complex picture has emerged where senescent CD8+ cells had a higher immediate lymphocytosis and subsequent lymphopenia (1h and 2h post), senescent CD4+ T cells followed a similar profile but with lower magnitude of change, and senescent NK cells increased but had a delayed return to baseline levels. There was higher magnitude of lymphocytosis and lymphocytopenia for CMV+ individuals and near maximum intensity and short duration protocols. The differing effects of exercise on senescent NK cells and CD4+ and CD8+ T cells suggest differing susceptibility to factors modulating lymphocyte extravasation such as adrenaline that is also regulated by exercise intensity. More studies are needed for understanding exercise effects on senescent NK cells, in older adults and in women.

Statements

Acknowledgement

The authors thanks Diego Nacarato Pereira da Silva for the contribution in the selection of studies and data acquisition.

Statement of Ethics

An ethics statement is not applicable because this study is based exclusively on published literature.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This work was supported by the Newton International Fellowship generously awarded to Amanda Veiga Sardeli by the Academy of Medical Sciences through the UK Government's Newton Fund Programme [NIFR7\1031]. JML is supported by the NIHR Birmingham Biomedical Research Centre. The views expressed here are those of the authors and not necessarily those of the NIHR or the Department of Health and Social Care.

Author Contributions

All three authors have given substantial contributions to the conception and the design of the manuscript; AVS did the studies selection, data collection and analysis. AVS, MAM and JML interpretated the data. AVS did the first draft while MAM and JML reviewed it critically for important intellectual content. All authors read and approved the final version of the manuscript.

Data Availability Statement

The data in this study was obtained from the previous studies where specific restrictions for public sharing their data may apply according to each journal politics. Such dataset may be requested by the corresponding author e-mail.

References

1. Alpert A, Pickman Y, Leipold M, Rosenberg-Hasson Y, Ji X, Gaujoux R, et al. A clinically meaningful metric of immune age derived from high-dimensional longitudinal monitoring. Nat Med. Nature Publishing Group; 2019;25:487–95.

2. Franceschi C, Campisi J. Chronic inflammation (Inflammaging) and its potential contribution to age-associated diseases [Internet]. Journals Gerontol - Ser A Biol Sci Med Sci. Oxford University Press; 2014. p. S4–9.

3. Hazeldine J, Lord J, Hampson P. Immunesenescence and inflammaging: A contributory factor in the poor outcome of the geriatric trauma patient. Ageing Res Rev. Ageing Res Rev; 2015;24:349–57.

4. Majumdar S, Nandi D. Thymic Atrophy: Experimental Studies and Therapeutic Interventions [Internet]. Scand J Immunol. Blackwell Publishing Ltd; 2018. p. 4–14.

5. Muñoz-Espín D, Serrano M. Cellular senescence: from physiology to pathology. Nat Publ Gr. 2014;

6. Vasileiou PVS, Evangelou K, Vlasis K, Fildisis G, Panayiotidis MI, Chronopoulos E, et al. Mitochondrial Homeostasis and Cellular Senescence. Cells. Multidisciplinary Digital Publishing Institute (MDPI); 2019;8:686.

7. Coppé JP, Patil CK, Rodier F, Sun Y, Muñoz DP, Goldstein J, et al. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS Biol. PLoS Biol; 2008;6.

8. Campisi J. Aging, cellular senescence, and cancer [Internet]. Annu Rev Physiol. Annu Rev Physiol; 2013. p. 685–705.

9. Huff W, Kwon J, Henriquez M, Fetcko K, Dey M. The Evolving Role of CD8 + CD28 - Immunosenescent T Cells in Cancer Immunology. Int J Mol Sci. Int J Mol Sci; 2019;20.

10. Chen X, Yi Z, Wong GT, Hasan KMM, Kwan JS, Ma AC, et al. Is exercise a senolytic medicine? A systematic review. Aging Cell. Blackwell Publishing Ltd; 2020;

11. Schafer MJ, White TA, Iijima K, Haak AJ, Ligresti G, Atkinson EJ, et al. Cellular senescence mediates fibrotic pulmonary disease. Nat Commun. Nature Publishing Group; 2017;8:14532.

12. Gleeson M, Bishop N, Walsh N. Exercise Immunology. New York: Routledge/Taylor & Francis Group; 2013.

13. Pascoe AR, Fiatarone Singh MA, Edwards KM. The effects of exercise on vaccination responses: A review of chronic and acute exercise interventions in humans [Internet]. Brain Behav Immun. Academic Press Inc.; 2014. p. 33–41.

14. Pape K, Ryttergaard L, Rotevatn TA, Nielsen BJ, Torp-Pedersen C, Overgaard C, et al. Leisure-Time Physical Activity and the Risk of Suspected Bacterial Infections. Med Sci Sports Exerc. Lippincott Williams and Wilkins; 2016;48:1737–44.

15. Nieman DC, Wentz LM. The compelling link between physical activity and the body's defense system [Internet]. J Sport Heal Sci. Elsevier B.V.; 2019. p. 201–17.

16. Simpson RJ, Hussain M, Baker F, Bigley AB, Peek MK, Stowe RP. Cardiorespiratory fitness is associated with better control of latent herpesvirus infections in a large ethnically diverse community sample: Evidence from the Texas City Stress and Health Study. Brain Behav Immun [Internet]. Elsevier BV; 2017;66:e35.

17. Agha NH, Mehta SK, Rooney B V., Laughlin MS, Markofski MM, Pierson DL, et al. Exercise as a countermeasure for latent viral reactivation during long duration space flight. FASEB J. John Wiley and Sons Inc.; 2020;34:2869–81.

18. Grande AJ, Keogh J, Silva V, Scott AM. Exercise versus no exercise for the occurrence, severity, and duration of acute respiratory infections [Internet]. Cochrane Database Syst Rev. John Wiley and Sons Ltd; 2020.

19. Duggal NA, Niemiro G, Harridge SDR, Simpson RJ, Lord JM. Can physical activity ameliorate immunosenescence and thereby reduce age-related multi-morbidity? [Internet]. Nat Rev Immunol. Nature Publishing Group; 2019. p. 563–72.

20. 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 [Internet]. Blackwell Publishing Ltd; 2018;17.

21. 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 [Internet]. 2020;26:8–22.

22. Simpson RJ. Aging, persistent viral infections, and immunosenescence: Can exercise "make space"? Exerc Sport Sci Rev. Exerc Sport Sci Rev; 2011;39:23–33.

23. Agha NH, Baker FL, Kunz HE, Graff R, Azadan R, Dolan C, et al. Vigorous exercise mobilizes CD34+

hematopoietic stem cells to peripheral blood via the β 2 -adrenergic receptor. Brain Behav Immun. Academic Press Inc.; 2018;68:66–75.

24. Pedersen BK. Physical activity and muscle–brain crosstalk [Internet]. Nat Rev Endocrinol. Nature Publishing Group; 2019. p. 383–92.

25. McCarthy DA, Macdonald I, Grant M, Marbut M, Watling M, Nicholson S, et al. Studies on the immediate and delayed leucocytosis elicited by brief (30-min) strenuous exercise. Eur J Appl Physiol Occup Physiol. Springer-Verlag; 1992;64:513–7.

26. Desdín-Micó G, Soto-Heredero G, Aranda JF, Oller J, Carrasco E, Gabandé-Rodríguez E, et al. T cells with dysfunctional mitochondria induce multimorbidity and premature senescence. Science (80-). American Association for the Advancement of Science; 2020;368:1371–6.

27. LaVoy EC, Hussain M, Reed J, Kunz H, Pistillo M, Bigley AB, et al. T-cell redeployment and intracellular cytokine expression following exercise: effects of exercise intensity and cytomegalovirus infection. Physiol Rep. American Physiological Society; 2017;5.

28. Spielmann G, Bollard CM, Bigley AB, Hanley PJ, Blaney JW, LaVoy ECP, et al. The effects of age and latent cytomegalovirus infection on the redeployment of CD8+ T cell subsets in response to acute exercise in humans. Brain Behav Immun. Academic Press Inc.; 2014;39:142–51.

29. Ross M, Ingram L, Taylor G, Malone E, Simpson RJ, West D, et al. Older men display elevated levels of senescence-associated exercise-responsive CD28null angiogenic T cells compared with younger men. Physiol Rep. American Physiological Society; 2018;6.

30. Simpson RJ, Cosgrove C, Ingram LA, Florida-James GD, Whyte GP, Pircher H, et al. Senescent T-lymphocytes are mobilised into the peripheral blood compartment in young and older humans after exhaustive exercise. Brain Behav Immun. Brain Behav Immun; 2008;22:544–51.

31. Hanson ED, Sakkal S, Que S, Cho E, Spielmann G, Kadife E, et al. Natural killer cell mobilization and egress following acute exercise in men with prostate cancer. Exp Physiol. Blackwell Publishing Ltd; 2020;105:1524–39.

32. Wang JS, Chen WL, Weng TP. Hypoxic exercise training reduces senescent T-lymphocyte subsets in blood. Brain Behav Immun [Internet]. Academic Press; 2011;25:270–8.

33. Ovadya Y, Landsberger T, Leins H, Vadai E, Gal H, Biran A, et al. Impaired immune surveillance accelerates accumulation of senescent cells and aging. Nat Commun 2018 91. Nature Publishing

Group; 2018;9:1–15.

34. Koch S, Larbi A, Özcelik D, Solana R, Gouttefangeas C, Attig S, et al. Cytomegalovirus infection: A driving force in human T cell immunosenescence. Ann N Y Acad Sci. Blackwell Publishing Inc.; 2007. p. 23–35.

35. Pawelec G, Larbi A, Derhovanessian E. Senescence of the Human Immune System. J Comp Pathol. W.B. Saunders Ltd; 2010;142.

36. Moher D, Liberati A, Tetzlaff J, Altman DG, Altman D, Antes G, et al. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. PLoS Med. 2009.

37. Brenchley JM, Karandikar NJ, Betts MR, Ambrozak DR, Hill BJ, Crotty LE, et al. Expression of CD57 defines replicative senescence and antigen-induced apoptotic death of CD8+ T cells. Blood. Blood; 2003;101:2711–20.

38. Lopez-Vergès S, Milush JM, Pandey S, York VA, Arakawa-Hoyt J, Pircher H, et al. CD57 defines a functionally distinct population of mature NK cells in the human CD56dimCD16+ NK-cell subset. Blood. American Society of Hematology; 2010;116:3865–74.

39. Appay V, Van Lier RAW, Sallusto F, Roederer M. Phenotype and function of human T lymphocyte subsets: Consensus and issues [Internet]. Cytom Part A. Cytometry A; 2008. p. 975–83.

40. Henson SM, Akbar AN. KLRG1-more than a marker for T cell senescence [Internet]. Age (Omaha). Age (Dordr); 2009. p. 285–91.

41. Plunkett FJ, Franzese O, Finney HM, Fletcher JM, Belaramani LL, Salmon M, et al. The Loss of Telomerase Activity in Highly Differentiated CD8 + CD28 − CD27 − T Cells Is Associated with Decreased Akt (Ser 473) Phosphorylation . J Immunol. The American Association of Immunologists; 2007;178:7710–9.

42. Voehringer D, Koschella M, Pircher H. Lack of proliferative capacity of human effector and memory T cells expressing killer cell lectinlike receptor G1 (KLRG1). Blood. Blood; 2002;100:3698–702.

43. Callender LA, Carroll EC, Beal RWJ, Chambers ES, Nourshargh S, Akbar AN, et al. Human CD8 + EMRA T cells display a senescence-associated secretory phenotype regulated by p38 MAPK. Aging Cell. Blackwell Publishing Ltd; 2018;17.

44. Zhou D, Borsa M, Simon AK. Hallmarks and detection techniques of cellular senescence and cellular ageing in immune cells [Internet]. Aging Cell. Blackwell Publishing Ltd; 2021. p. e13316.

45. Kaminsky LA, Arena R, Myers J. Reference standards for cardiorespiratory fitness measured with cardiopulmonary exercise testing data from the fitness registry and the importance of exercise national database. Mayo Clin Proc. Elsevier Ltd; 2015;90:1515–23.

46. Azali Alamdari K, Bashiri J. Effects of hypobaric Endurance Training on Graded Exercise Induced Lymphocyte Mobilization, Senescence and Their Surface Thiol Levels in Elite Male Athletes. Int J Appl Exerc Physiol. International Society of Communication and Development Between Universities (ISCDBU); 2018;7:48–55.

47. Curran M, Campbell JP, Powell E, Chikhlia A, Narendran P. The mobilisation of early mature CD56dim-CD16bright NK cells is blunted following a single bout of vigorous intensity exercise in Type 1 Diabetes. Exerc Immunol Rev [Internet]. 2020;26:116–31.

48. Garber CE, Blissmer B, Deschenes MR, Franklin BA, Lamonte MJ, Lee IM, et al. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: Guidance for prescribing exercise. Med Sci Sports Exerc. Med Sci Sports Exerc; 2011;43:1334–59.

49. Ingram LA, Simpson RJ, Malone E, Florida-James GD. Sleep disruption and its effect on lymphocyte redeployment following an acute bout of exercise. Brain Behav Immun. Academic Press Inc.; 2015;47:100–8.

50. Farina D, Ferguson RA, Macaluso A, De Vito G. Correlation of average muscle fiber conduction velocity measured during cycling exercise with myosin heavy chain composition, lactate threshold, and VO2max. J Electromyogr Kinesiol. J Electromyogr Kinesiol; 2007;17:393–400.

51. Turner JE, Aldred S, Witard OC, Drayson MT, Moss PM, Bosch JA. Latent Cytomegalovirus infection amplifies CD8 T-lymphocyte mobilisation and egress in response to exercise. Brain Behav Immun. Brain Behav Immun; 2010;24:1362–70.

52. Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple , graphical test measures of funnel plot asymmetry. BMJ. 1997;315:629–34.

53. Curran M, Campbell J, Drayson M, Andrews R, Narendran P. Type 1 diabetes impairs the mobilisation of highly-differentiated CD8+T cells during a single bout of acute exercise. Exerc

Immunol Rev [Internet]. 2019;25:64–82.

54. Krzywkowski K, Petersen EW, Ostrowski K, Kristensen JH, Boza J, Pedersen BK. Effect of glutamine supplementation on exercise-induced changes in lymphocyte function. Am J Physiol - Cell Physiol. American Physiological Society; 2001;281.

55. Lavoy EC, Bigley AB, Spielmann G, Rector JL, Morrison MR, O'Connor DP, et al. CMV amplifies T-cell redeployment to acute exercise independently of HSV-1 serostatus. Med Sci Sports Exerc. Med Sci Sports Exerc; 2014;46:257–67.

56. Bigley AB, Lowder TW, Spielmann G, Rector JL, Pircher H, Woods JA, et al. NK-cells have an impaired response to acute exercise and a lower expression of the inhibitory receptors KLRG1 and CD158a in humans with latent cytomegalovirus infection. Brain Behav Immun [Internet]. Academic Press; 2012;26:177–86.

57. Wang JS, Wu CK. Systemic hypoxia affects exercise-mediated antitumor cytotoxicity of natural killer cells. J Appl Physiol. J Appl Physiol (1985); 2009;107:1817–24.

58. Bigley AB, Rezvani K, Pistillo M, Reed J, Agha N, Kunz H, et al. Acute exercise preferentially redeploys NK-cells with a highly-differentiated phenotype and augments cytotoxicity against lymphoma and multiple myeloma target cells. Part II: Impact of latent cytomegalovirus infection and catecholamine sensitivity. Brain Behav Immun. Academic Press Inc.; 2015;49:59–65.

59. Brown FF, Bigley AB, Sherry C, Neal CM, Witard OC, Simpson RJ, et al. Training status and sex influence on senescent T-lymphocyte redistribution in response to acute maximal exercise. Brain Behav Immun. Academic Press Inc.; 2014;39:152–9.

60. Bruunsgaard H, Jensen M, Schjerling P, Halkjaer-Kristensen J, Ogawa K, Skinhøj P, et al. Exercise induces recruitment of lymphocytes with an activated phenotype and short telomeres in young and elderly humans. Life Sci. Life Sci; 1999;65:2623–33.

61. JS W, TP W. Hypoxic exercise training promotes antitumour cytotoxicity of natural killer cells in young men. Clin Sci (Lond). Clin Sci (Lond); 2011;121:343–53.

62. JP C, NE R, VE B, M T, JJ van Z, MT D, et al. Acute exercise mobilises CD8+ T lymphocytes exhibiting an effector-memory phenotype. Brain Behav Immun. Brain Behav Immun; 2009;23:767–75.

63. Minuzzi L, Rama L, Bishop N, Rosado F, Martinho A, Paiva A, et al. Lifelong training improves anti-

inflammatory environment and maintains the number of regulatory T cells in masters athletes. Eur J Appl Physiol. Eur J Appl Physiol; 2017;117:1131–40.

64. Simpson RJ, Cosgrove C, Chee MM, McFarlin BK, Bartlett DB, Spielmann G, et al. Senescent phenotypes and telomere lengths of peripheral blood T-cells mobilized by acute exercise in humans. Exerc Immunol Rev [Internet]. 2010;16:40–55.

65. Anane LH, Edwards KM, Burns VE, Drayson MT, Riddell NE, van Zanten JJCSV, et al. Mobilization of γδ T lymphocytes in response to psychological stress, exercise, and β-agonist infusion. Brain Behav Immun. Brain Behav Immun; 2009;23:823–9.

66. Fan X, Wang Y. β2 Adrenergic receptor on T lymphocytes and its clinical implications. Prog Nat Sci. Science Press; 2009. p. 17–23.

67. Pedersen L, Idorn M, Olofsson GH, Lauenborg B, Nookaew I, Hansen RH, et al. Voluntary running suppresses tumor growth through epinephrine- and IL-6-dependent NK cell mobilization and redistribution. Cell Metab. Cell Press; 2016;23:554–62.

68. Dimitrov S, Lange T, Born J. Selective Mobilization of Cytotoxic Leukocytes by Epinephrine. J Immunol. The American Association of Immunologists; 2010;184:503–11.

69. Krüger K, Lechtermann A, Fobker M, Völker K, Mooren FC. Exercise-induced redistribution of T lymphocytes is regulated by adrenergic mechanisms. Brain Behav Immun [Internet]. Academic Press; 2008;22:324–38.

70. Krüger K, Alack K, Ringseis R, Mink L, Pfeifer E, Schinle M, et al. Apoptosis of T-Cell Subsets after Acute High-Intensity Interval Exercise. Med Sci Sports Exerc. Lippincott Williams and Wilkins; 2016;48:2021–9.

71. Bujko K, Cymer M, Adamiak M, Ratajczak MZ. An Overview of Novel Unconventional Mechanisms of Hematopoietic Development and Regulators of Hematopoiesis – a Roadmap for Future Investigations. Stem Cell Rev Reports. Springer; 2019;15:785–94.

72. Abrahin O, Cortinhas-Alves EA, Vieira RP, Guerreiro JF. Elite athletes have longer telomeres than sedentary subjects: A meta-analysis. Exp Gerontol [Internet]. Elsevier Inc.; 2019;119:138–45.

73. Schirmer M, Vallejo AN, Weyand CM, Goronzy JJ. Resistance to apoptosis and elevated expression of Bcl-2 in clonally expanded CD4+CD28- T cells from rheumatoid arthritis patients. J Immunol [Internet]. 1998;161:1018–25.

74. Vallejo AN, Schirmer M, Weyand CM, Goronzy JJ. Clonality and Longevity of CD4 + CD28 null T Cells Are Associated with Defects in Apoptotic Pathways . J Immunol. The American Association of Immunologists; 2000;165:6301–7.

75. Prösch S, Wendt CEC, Reinke P, Priemer C, Oppert M, Krüger DH, et al. A novel link between stress and human cytomegalovirus (HCMV) infection: Sympathetic hyperactivity stimulates HCMV activation. Virology [Internet]. Academic Press Inc.; 2000;272:357–65.

76. Mehta SK, Stowe RP, Feiveson AH, Tyring SK, Pierson DL. Reactivation and Shedding of Cytomegalovirus in Astronauts during Spaceflight. J Infect Dis. Oxford Academic; 2000;182:1761–4.

77. Spielmann G, McFarlin BK, O'Connor DP, Smith PJW, Pircher H, Simpson RJ. Aerobic fitness is associated with lower proportions of senescent blood T-cells in man. Brain Behav Immun. Brain Behav Immun; 2011;25:1521–9.

78. Fisher JP, Young CN, Fadel PJ. Autonomic adjustments to exercise in humans. Compr Physiol. Wiley-Blackwell Publishing Ltd; 2015;5:475–512.

79. Fritsche A, Stumvoll M, Grub M, Sieslack S, Renn W, Schmülling RM, et al. Effect of hypoglycemia on β-adrenergic sensitivity in normal and type 1 diabetic subjects. Diabetes Care. American Diabetes Association Inc.; 1998;21:1505–10.

80. Liston A, Gray DHD. Homeostatic control of regulatory T cell diversity [Internet]. Nat Rev Immunol. Nat Rev Immunol; 2014. p. 154–65.

81. Krüger K, Mooren FC. T cell homing and exercise. Exerc Immunol Rev [Internet]. 2007;13:37–54.

82. Dimitrov S, Benedict C, Heutling D, Westermann J, Born J, Lange T. Cortisol and epinephrine control opposing circadian rhythms in T cell subsets. Blood. Blood; 2009;113:5134–43.

83. Benschop RJ, Oostveen FG, Heijnen CJ, Ballieux RE. β 2-Adrenergic stimulation causes detachment of natural killer cells from cultured endothelium. Eur J Immunol. Eur J Immunol; 1993;23:3242–7.

84. Navalta JW, Sedlock DA, Park KS. Effect of exercise intensity on exercise-induced lymphocyte apoptosis. Int J Sports Med. Int J Sports Med; 2007;28:539–42.

85. Pizza FX, Mitchell JB, Davis BH, Starling RD, Holtz RW, Bigelow N. Exercise-induced muscle damage: effect on circulating leukocyte and lymphocyte subsets. Med Sci Sport Exerc [Internet].

1995;27:363–70.

86. Pedersen BK. Muscle as a secretory organ. Compr Physiol. Compr Physiol; 2013;3:1337–62.

87. Pedersen BK, Febbraio MA. Muscles, exercise and obesity: Skeletal muscle as a secretory organ [Internet]. Nat Rev Endocrinol. Nature Publishing Group; 2012. p. 457–65.

88. Akbar AN. The convergence of senescence and nutrient sensing during lymphocyte ageing [Internet]. Clin Exp Immunol. Blackwell Publishing Ltd; 2017. p. 4–5.

89. Feldman RD, Limbird LE, Nadeau J, Robertson D, Wood AJJ. Alterations in Leukocyte β-Receptor Affinity with Aging. N Engl J Med. Massachusetts Medical Society; 1984;310:815–9.

Figure Legends

Fig. 1. Flowchart of study selection.

Fig. 2. Forest plot of standardized mean difference (SMD) and 95% confidence interval for the overall effect immediately post exercise compared to baseline values. CMV: Cytomegalovirus; H-AT: hypoxic-absolute exercise; HC: hypobaric control; H-C: hypoxic resting; HE: hypobaric exercise; HI: High intensity; H-RT: hypoxic-relative exercise; HSV1: herpes simplex virus 1; LL: Lower limit of 95% confidence interval; LT: Lactate threshold; MI: Moderate intensity. NC: normobaric control; N-C: normoxic resting; NE: normobaric exercise; N-T: normoxic exercise; TD1: Type 1 diabetes; TR: Trained; UL: Upper limit of 95% confidence interval; UN: Untrained.

Fig. 3. . Forest plot of standardized mean difference (SMD) and 95% confidence interval for the overall effect 1h post exercise compared to baseline values. CMV: Cytomegalovirus; H-AT: hypoxic-absolute exercise; HC: hypobaric control; H-C: hypoxic resting; HE: hypobaric exercise; HI: High intensity; H-RT: hypoxic-relative exercise; HSV1: herpes simplex virus 1; LL: Lower limit of 95% confidence interval; LT: Lactate threshold; MI: Moderate intensity. NC: normobaric control; N-C: normoxic resting; NE: normobaric exercise; N-T: normoxic exercise; TD1: Type 1 diabetes; TR: Trained; UL: Upper limit of 95% confidence interval; UN: Untrained.

Fig. 4. Forest plot of standardized mean difference (SMD) and 95% confidence interval for the overall effect 2h post exercise compared to baseline values. CMV: Cytomegalovirus; H-AT: hypoxic-absolute exercise; HC: hypobaric control; H-C: hypoxic resting; HE: hypobaric exercise; HI: High intensity; H-RT: hypoxic-relative exercise; HSV1: herpes simplex virus 1; LL: Lower limit of 95% confidence interval; LT: Lactate threshold; MI: Moderate intensity. NC: normobaric control; N-C: normoxic resting; NE: normobaric exercise; N-T: normoxic exercise; TD1: Type 1 diabetes; TR: Trained; UL: Upper limit of 95% confidence interval; UN: Untrained.

Fig 5. The figure summarizes the lymphocytes count fold change from baseline to each time point for the senescent cells: CD8 T cells (in blue), CD4 T cell (in dark pink) and NK cells (in light pink and black centre). The position of the cells represents the SMD of each meta-analysis at each time-point post exercise.

Table 1. Characteristics of the studies included.

	Population characteristics					Exercise protocols					
First author, year (subgroup)	**Time points**	**Sex**	**Mean age ± SD, or range**	**Training status**	**CMV serostatus**	**Equipment**	**Intensity**	**Duration**	**Intensity & duration**	**Cells analysed**	**Senescent marker**
Azali Alamdari, 2018 [46] (post HC, HE, NC, NE)	0-15'' & 1'	M	4 Groups mean: 21.8 ± 1.58†	TR & NR*	NA	Treadmill	Until exhaustion	Incremental	Maximum & short	CD4+ & CD8+	KLRG1+
Krzywkowski, 2001 [54]	0-15'' & 2'	M	37 (25-48)	TR	NA	Bicycle	75% VO_2max	120min	Vigorous & long	CD4+ & CD8+	CD28-
Lavoy, 2017 [27]	0-15'' & 1'	B	30.9 ± 5.0	TR	CMV- & CMV+	Bicycle	-5% BLT, +5% BLT & +15% BLT	30min	Moderate & moderate	CD4+	CD28- CD27-
Ross, 2018 [29]	0-15'' & 1'	M	60-75 & 18-25	TR	CMV-	Bicycle	70% VO_2peak	30min	Vigorous & Moderate	CD4+ & CD8+	CD28-
Wang, 2011 [32] (post H-AT, H-C, H-RT, N-C, N-T)	0-15'' & 2'	M	5 groups mean: 22.46 ± 0.6†	UN & TR	NA	Bicycle	Until exhaustion	Incremental	Maximum & short	CD4+ & CD8+	KLRG1+
Bigley, 2012 [56]	0-15'' & 1'	M	2 Groups mean: 28.55 ± 5.35	NR	CMV- & CMV+	Bicycle	85% EMP	30min	Vigorous & Moderate	CD8+	KLRG1+
Curran, 2019 [53] (Control & TD1	0-15'' & 1'	M	2 Groups mean: 31 ± 7.15	NR	NA	Bicycle	80% VO_2max	30min	Vigorous & Moderate	CD8+	EMRA
Lavoy, 2014 [55] (HSV1+ & HSV1-)	0-15'' & 1'	M	4 Groups mean: 38.72 ± 15.22	TR	CMV- & CMV+	Bicycle	80-85% EMP	30min	Vigorous & Moderate	CD8+	KLRG1+CD28-
Spielmann, 2014 [28]	0-15'' & 1'	M	2 Groups mean (Older): 55.35 ± 4.1 2 Groups mean (Younger): 28.5 ± 4.9	TR	CMV- & CMV+	Bicycle	80-85% PP	30min	Vigorous & Moderate	CD8+	KLRG1+CD28-
Turner, 2010 [51]	0-15'' & 1'	M	35 ± 14	TR	CMV- & CMV+	Treadmill	80% VO_2max	60min	Vigorous & long	CD8+	CD28- CD27-
Ingram, 2015 [49] (Disrupted	0-15'' & 1'	M	27 ± 8	TR	NA	Bicycle	265 ± 27 Watts	55:12 min	Vigorous & long	CD8+	KLRG1+

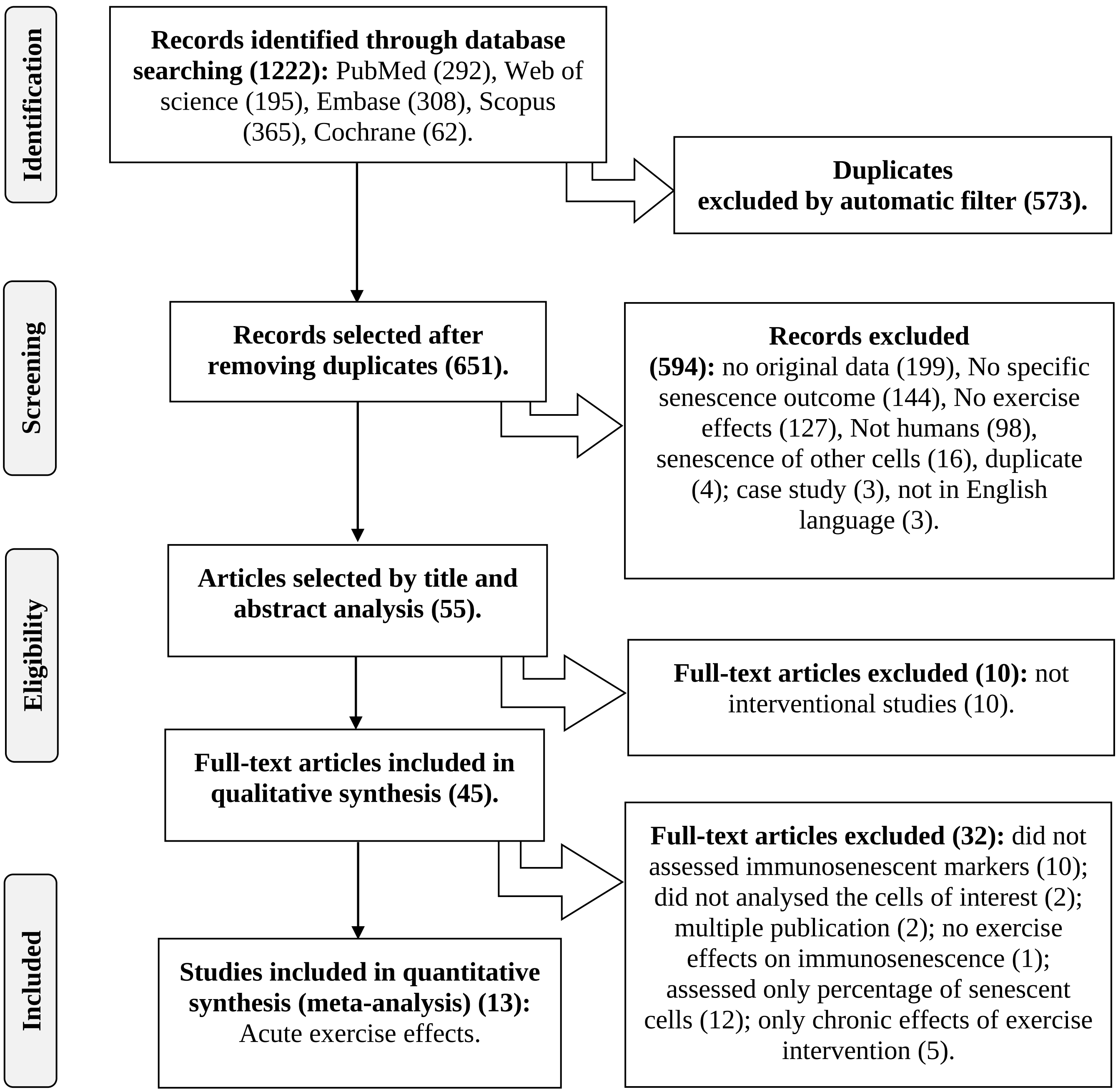
& Undisrupted sleep)											
Curran, 2020 [47]	0-15'' & 1'	M	2 Groups mean: 31 ± 7.15	NR	NA	Bicycle	80% VO_2max	30min	Vigorous & Moderate	NK	CD57+
Wang, 2009 [57] (HI & MI)	0-15'' & 2'	M	24.2 ± 1.2	UN	NA	Bicycle	Until exhaustion & 50% PD VO2max	Incremental & 30min	Maximum & short; Moderate & moderate	NK	CD57+

Legend: BLT: blood lactate threshold; C: Control; CMV: cytomegalovirus; EMP: estimated max power; EMRA: CD45RA+CCR7-CD28-CD27-; H-AT: hypoxic-absolute exercise; HC: hypobaric control; H-C: hypoxic resting; HE: hypobaric exercise; HI: High intensity; H-RT: hypoxic-relative exercise; HSV1: herpes simplex virus 1; MI: Moderate intensity. NA: not applicable (when did not use just one subgroup); NC: normobaric control; N-C: normoxic resting; NE: normobaric exercise; NR: not reported; ; NR*: groups undergoing control period (NC and HC) were not analysed for training status, since they were trained at baseline and it was not clear how much untrained they became after intervention; N-T: normoxic exercise; PP: peak power; TD1: Type 1 diabetes; TR: Trained; UN: Untrained; USA: United States of America; VTWR: ventilatory threshold work rate †Standard error.

Table 2. Subgroups comparison for the effects of exercise on aged immune cells.

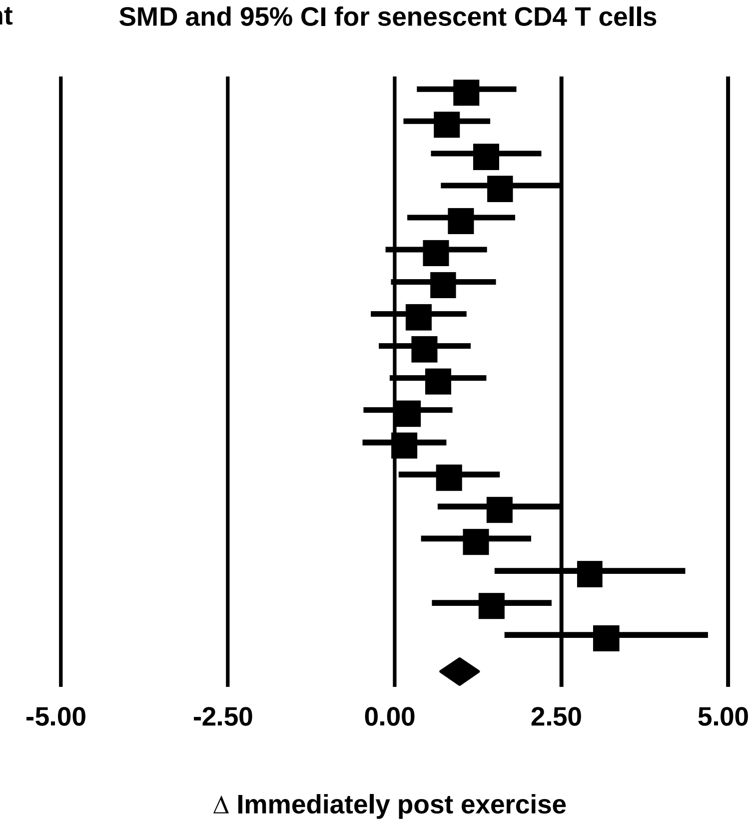
Time-point	**Subgroups**	**Categories**	**K**	**SMD**	**LL**	**UL**	**p-value**	**Sample**	p-diff
Immediately post exercise	**Training status**	TR	36	1.13	0.86	1.4	**<0.001**	345	0.35
		UN	8	1.38	0.92	1.84	**<0.001**	96	
	CMV status	CMV-	13	0.55	0.35	0.75	**<0.001**	120	0.09
		CMV+	9	1.09	0.55	1.63	**<0.001**	81	
	Age	Middle-aged	2	1.2	0.52	1.88	**0.001**	16	0.46
		Older adults	4	0.74	0.1	1.38	**0.02**	36	
		Young adults	44	1.14	0.91	1.37	**<0.001**	449	
	Intensity & duration	Vigorous & long[b]	6	0.4	0.14	0.66	**0.002**	66	**<0.001**
		Moderate & moderate	3	0.38	0.02	0.73	**0.04**	33	
		Vigorous & Moderate[ab]	22	0.85	0.61	1.1	**<0.001**	196	
		Maximum & short[a]	19	1.81	1.45	2.16	**<0.001**	206	
1h post exercise	**Age**	Middle-aged	2	-0.3	-0.8	0.17	0.2	16	0.81
		Older adults	4	-0.2	-0.5	0.13	0.23	36	
		Young adults	22	-0.2	-0.3	-0	**0.02**	209	
	CMV status	CMV-	13	-0.1	-0.3	0.1	0.37	120	0.02
		CMV+	9	-0.4	-0.7	-0.2	**<0.001**	81	
	Intensity & duration	Vigorous & long	4	-0.5	-0.8	-0.2	**<0.001**	48	0.09
		Moderate & moderate	2	-0.3	-0.8	0.16	0.18	17	
		Vigorous & Moderate[c]	22	-0.1	-0.3	0.04	0.16	196	
2h post exercise	**Training status**	TR	14	-0.6	-1	-0.3	**<0.001**	146	0.81
		UN	8	-0.6	-0.9	-0.4	**<0.001**	96	
	Intensity & duration	Vigorous & long	2	-0.4	-0.9	0.11	0.12	18	0.21
		Moderate & moderate	1	-0.2	-0.7	0.29	0.41	16	
		Maximum & short	19	-0.7	-1	-0.4	**<0.001**	208	

Legend: SMD: Standardized mean difference; K: number of study groups; LL: Lower limit of 95% confidence interval; UL: Upper limit of 95% confidence interval; p-value: p-value for significance (<0.05) change of senescent cell counts withing categories of subgroup; p-diff: p-value for significance (<0.05) change of senescent cell counts between categories of subgroup; TR: trained individuals; UN: untrained individuals; [a:] different of Moderate & moderate; [b:] Different of Maximum & short; [c]: different of Vigorous & long.


Identification
Records identified through database searching (1222): PubMed (292), Web of science (195), Embase (308), Scopus (365), Cochrane (62).
Duplicates excluded by automatic filter (573).
Screening
Records selected after removing duplicates (651).
Records excluded (594): no original data (199), No specific senescence outcome (144), No exercise effects (127), Not humans (98), senescence of other cells (16), duplicate (4); case study (3), not in English language (3).
Eligibility
Articles selected by title and abstract analysis (55).
Full-text articles excluded (10): not interventional studies (10).
Full-text articles included in qualitative synthesis (45).
Full-text articles excluded (32): did not assessed immunosenescent markers (10); did not analysed the cells of interest (2); multiple publication (2); no exercise effects on immunosenescence (1); assessed only percentage of senescent cells (12); only chronic effects of exercise intervention (5).
Included
Studies included in quantitative synthesis (meta-analysis) (13): Acute exercise effects.

2.a

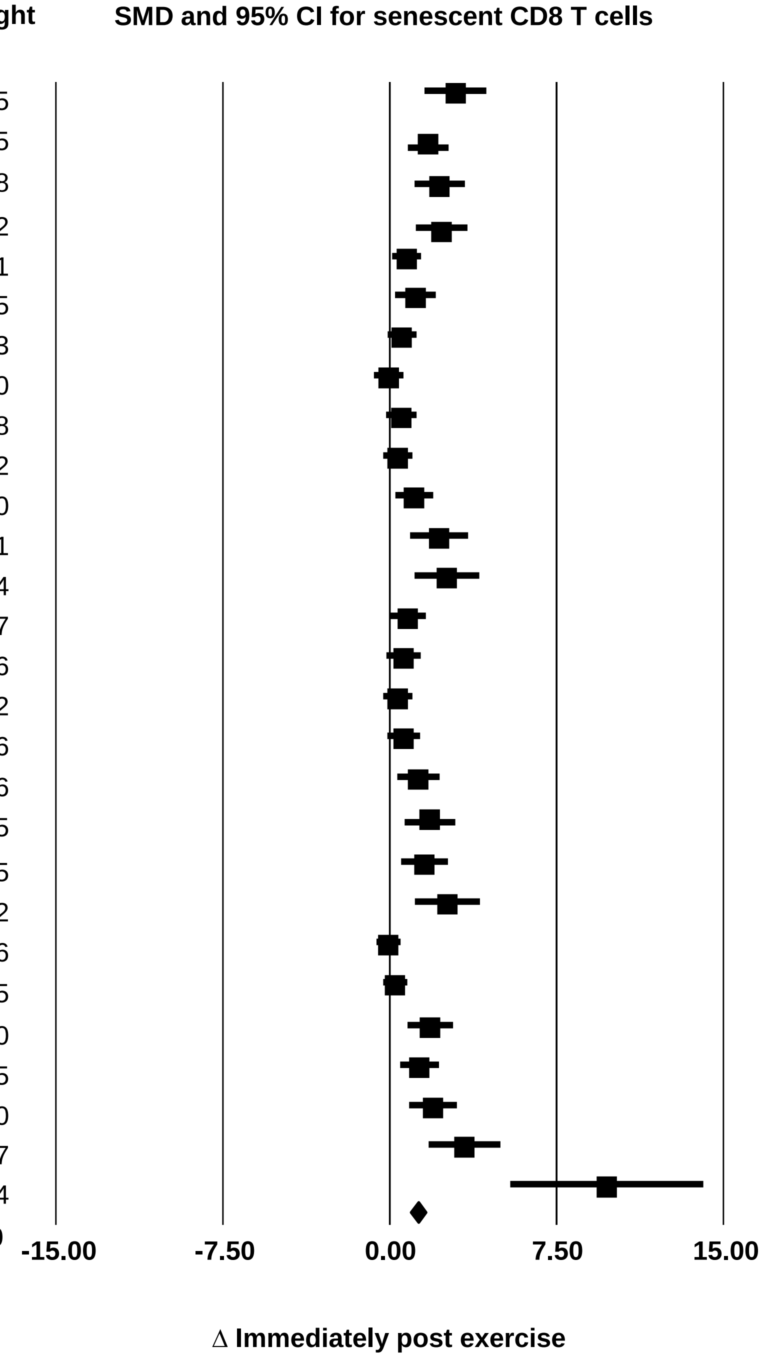
First author, year	Subgroup	SMD	LL	UL	p-Value	Total	Weight
Azali Alamdari, 2018 [46]	HC	1.078	0.335	1.821	0.004	11	6.03
Azali Alamdari, 2018 [46]	HE	0.784	0.137	1.431	0.018	12	6.68
Azali Alamdari, 2018 [46]	NC	1.372	0.549	2.195	0.001	11	5.51
Azali Alamdari, 2018 [46]	NE	1.585	0.697	2.472	0.000	11	5.13
Krzywkowski, 2001 [54]		1.000	0.200	1.800	0.014	9	5.66
Lavoy, 2017 [27]	CMV-/+15%LT	0.625	-0.133	1.382	0.106	8	5.93
Lavoy, 2017 [27]	CMV-/+5%LT	0.733	-0.048	1.513	0.066	8	5.78
Lavoy, 2017 [27]	CMV-/-5%LT	0.364	-0.352	1.080	0.319	8	6.21
Lavoy, 2017 [27]	CMV+/+15%LT	0.450	-0.235	1.136	0.198	9	6.41
Lavoy, 2017 [27]	CMV+/+5%LT	0.652	-0.067	1.372	0.076	9	6.18
Lavoy, 2017 [27]	CMV+/-5%LT	0.196	-0.463	0.856	0.560	9	6.59
Ross, 2018 [29]	Older	0.149	-0.474	0.773	0.638	10	6.85
Ross, 2018 [29]	Young	0.820	0.065	1.575	0.033	9	5.95
Wang, 2011 [32]	H-AT	1.579	0.650	2.508	0.001	10	4.90
Wang, 2011 [32]	H-C	1.222	0.403	2.041	0.003	10	5.54
Wang, 2011 [32]	H-RT	2.928	1.503	4.354	0.000	10	2.88
Wang, 2011 [32]	N-C	1.457	0.567	2.347	0.001	10	5.12
Wang, 2011 [32]	N-T	3.172	1.650	4.694	0.000	10	2.62
Summarized random effects		**0.961**	**0.675**	**1.247**	**0.000**	**174**	**100**



Heterogeneity tests: Q=39.18, df=17, p=0.001, I^2=56.62%;
Hypothesis test: Z=6.59, p<0.001; Egger test: p<0.001.

2.b

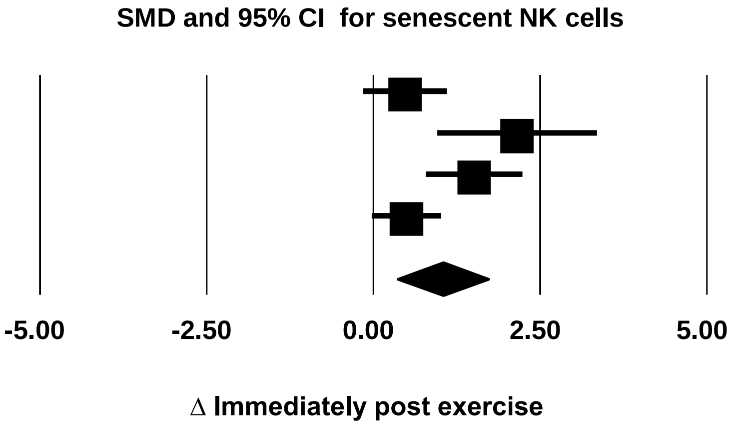
First author, year	Subgroup	SMD	LL	UL	p-Value	Total	Weight
Azali Alamdari, 2018 [46]	HC	2.968	1.594	4.342	0.000	11	2.75
Azali Alamdari, 2018 [46]	HE	1.739	0.842	2.636	0.000	12	3.75
Azali Alamdari, 2018 [46]	NC	2.247	1.138	3.356	0.000	11	3.28
Azali Alamdari, 2018 [46]	NE	2.336	1.195	3.476	0.000	11	3.22
Bigley, 2012 [56]	CMV-	0.772	0.128	1.417	0.019	12	4.31
Bigley, 2012 [56]	CMV+	1.164	0.267	2.062	0.011	8	3.75
Curran, 2019 [53]	Control	0.560	-0.075	1.196	0.084	11	4.33
Curran, 2019 [53]	TD1	-0.038	-0.691	0.616	0.910	9	4.30
Ingram, 2015 [49]	Disrupted sleep	0.527	-0.135	1.188	0.119	10	4.28
Ingram, 2015 [49]	Undisrupted sleep	0.368	-0.273	1.008	0.261	10	4.32
Krzywkowski, 2001 [54]		1.109	0.279	1.940	0.009	9	3.90
Lavoy, 2014 [55]	CMV+HSV1-	2.224	0.933	3.516	0.001	8	2.91
Lavoy, 2014 [55]	CMV+HSV1+	2.573	1.135	4.012	0.000	8	2.64
Lavoy, 2014 [55]	CMV-HSV1-	0.811	0.012	1.610	0.047	8	3.97
Lavoy, 2014 [55]	CMV-HSV1+	0.632	-0.127	1.391	0.103	8	4.06
Ross, 2018 [29]	Older	0.369	-0.271	1.010	0.258	10	4.32
Ross, 2018 [29]	Young	0.636	-0.080	1.352	0.082	9	4.16
Spielmann, 2014 [28]	CMV-/older	1.290	0.352	2.228	0.007	8	3.66
Spielmann, 2014 [28]	CMV-/young	1.804	0.681	2.927	0.002	8	3.25
Spielmann, 2014 [28]	CMV+/older	1.566	0.532	2.600	0.003	8	3.45
Spielmann, 2014 [28]	CMV+/young	2.597	1.148	4.046	0.000	8	2.62
Turner, 2010 [51]	CMV-	-0.047	-0.571	0.477	0.861	14	4.56
Turner, 2010 [51]	CMV+	0.249	-0.283	0.780	0.360	14	4.55
Wang, 2011 [32]	H-AT	1.825	0.813	2.837	0.000	10	3.50
Wang, 2011 [32]	H-C	1.343	0.488	2.198	0.002	10	3.85
Wang, 2011 [32]	H-RT	1.953	0.896	3.010	0.000	10	3.40
Wang, 2011 [32]	N-C	3.361	1.763	4.959	0.000	10	2.37
Wang, 2011 [32]	N-T	9.758	5.437	14.079	0.000	10	0.54
Summarized random effects		**1.261**	**0.926**	**1.596**	**0.000**	**275**	**100**



Heterogeneity tests: Q=111.94, df=27, $p<0.001$, I^2=75.88%;
Hypothesis test: Z=7.38, $p<0.001$; Egger test: $p<0.001$.

2.c

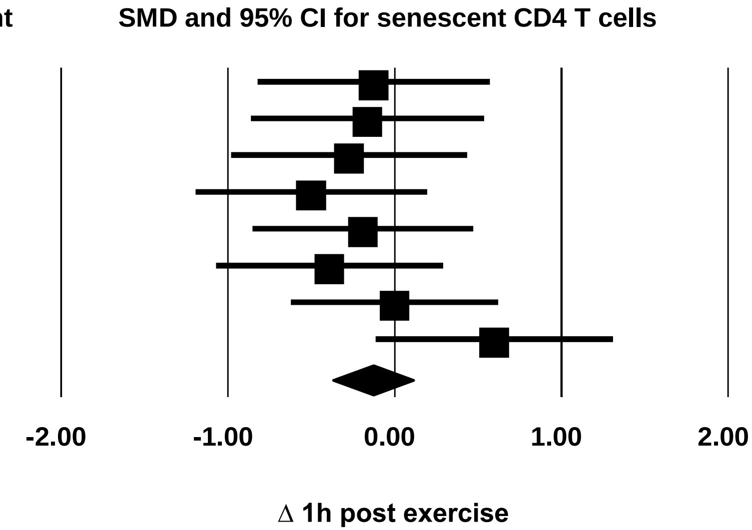
First author, year	Subgroup	SMD	LL	UL	p-Value	Total	Weight
Curran, 2020 [47]	Control	0.476	-0.147	1.100	0.134	11	27.56
Curran, 2020 [47]	TD1	2.155	0.964	3.346	0.000	9	17.13
Wang, 2009 [57]	HI	1.512	0.795	2.230	0.000	16	25.66
Wang, 2009 [57]	MI	0.499	-0.021	1.018	0.060	16	29.65
Summarized random effects		**1.036**	**0.354**	**1.719**	**0.003**	**52**	**100**



Heterogeneity tests: Q=11.02, df=3, p=0.011, I^2=72.79%;
Hypothesis test: Z=2.98, p=0.002; Egger test: p=0.13.

3.a

First author, year	Subgroup	SMD	LL	UL	p-Value	Total	Weight
Lavoy, 2017 [27]	CMV-/+15%LT	-0.124	-0.820	0.571	0.726	8	11.96
Lavoy, 2017 [27]	CMV-/+5%LT	-0.163	-0.860	0.535	0.647	8	11.89
Lavoy, 2017 [27]	CMV-/-5%LT	-0.272	-0.978	0.433	0.449	8	11.62
Lavoy, 2017 [27]	CMV+/+15%LT	-0.499	-1.192	0.194	0.158	9	12.06
Lavoy, 2017 [27]	CMV+/+5%LT	-0.189	-0.848	0.470	0.573	9	13.32
Lavoy, 2017 [27]	CMV+/-5%LT	-0.390	-1.067	0.288	0.260	9	12.60
Ross, 2018 [29]	Older	0.000	-0.620	0.620	1.000	10	15.06
Ross, 2018 [29]	Young	0.599	-0.111	1.308	0.098	9	11.50
Summarized fixed effects		**-0.132**	**-0.372**	**0.109**	**0.284**	**70**	**100**

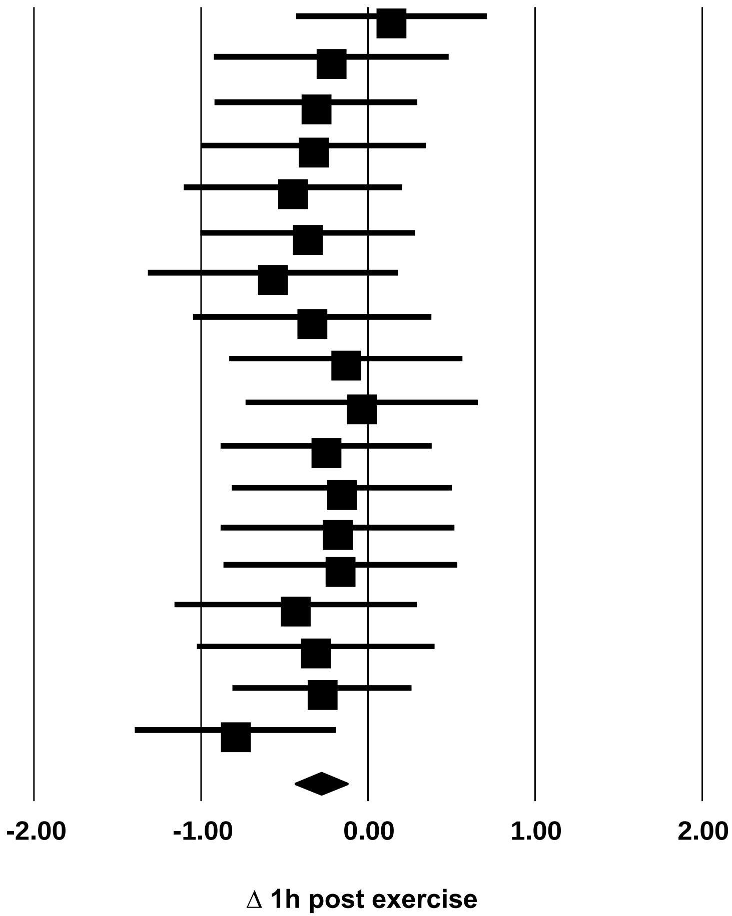


Heterogeneity tests: Q=6.07, df=7, p=0.53, I^2=0%;
Hypothesis test: Z=-1.07, p=0.28; Egger test: p=0.90.

3.b

First author, year	Subgroup	SMD	LL	UL	p-Value	Total	Weight
Bigley, 2012 [56]	CMV-	0.140	-0.429	0.709	0.629	12	7.38
Bigley, 2012 [56]	CMV+	-0.219	-0.920	0.482	0.540	8	4.85
Curran, 2019 [53]	Control	-0.311	-0.916	0.294	0.314	11	6.52
Curran, 2019 [53]	TD1	-0.325	-0.995	0.345	0.342	9	5.31
Ingram, 2015 [49]	disrupted sleep	-0.450	-1.100	0.201	0.175	10	5.64
Ingram, 2015 [49]	undisrupted sleep	-0.361	-1.001	0.278	0.268	10	5.83
Lavoy, 2014 [55]	CMV+HSV1-	-0.569	-1.316	0.178	0.135	8	4.28
Lavoy, 2014 [55]	CMV+HSV1+	-0.333	-1.045	0.379	0.359	8	4.71
Lavoy, 2014 [55]	CMV-HSV1-	-0.132	-0.828	0.564	0.709	8	4.92
Lavoy, 2014 [55]	CMV-HSV1+	-0.038	-0.731	0.655	0.914	8	4.96
Ross, 2018 [29]	Older	-0.250	-0.880	0.379	0.436	10	6.02
Ross, 2018 [29]	Young	-0.156	-0.813	0.501	0.642	9	5.52
Spielmann, 2014 [28]	CMV-/older	-0.183	-0.882	0.516	0.608	8	4.89
Spielmann, 2014 [28]	CMV-/young	-0.165	-0.863	0.533	0.643	8	4.90
Spielmann, 2014 [28]	CMV+/older	-0.433	-1.157	0.292	0.242	8	4.54
Spielmann, 2014 [28]	CMV+/young	-0.313	-1.023	0.397	0.387	8	4.74
Turner, 2010 [51]	CMV-	-0.274	-0.808	0.259	0.314	14	8.38
Turner, 2010 [51]	CMV+	-0.793	-1.394	-0.193	0.010	14	6.61
Summarized fixed effects		**-0.283**	**-0.438**	**-0.129**	**0.000**	**171**	**100**

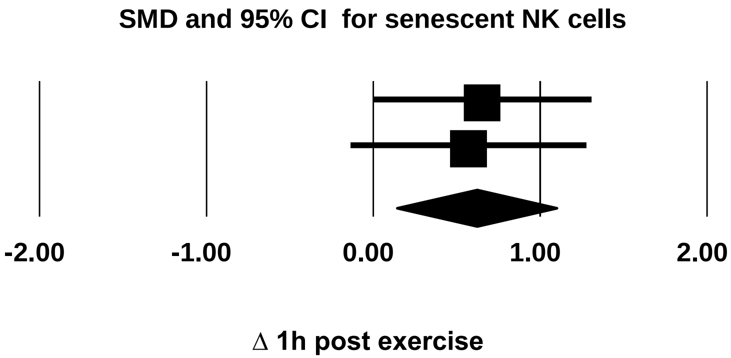
SMD and 95% CI for senescent CD8 T cells



Heterogeneity tests: Q=7.02, df=17, p=0.98, I^2=0%;
Hypothesis test: Z=-3.59, p<0.001; Egger test: p=0.69.

3.c

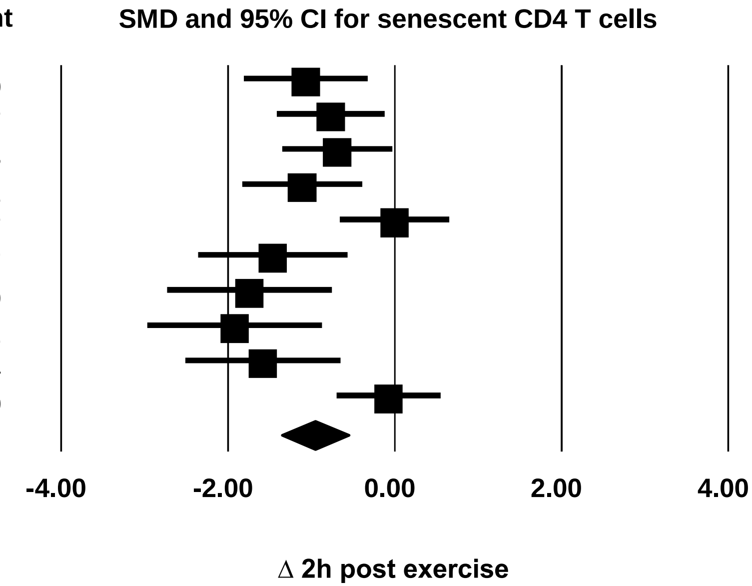
First author, year	Subgroup	SMD	LL	UL	p-Value	Total	Weight
Curran, 2020 [47]	Control	0.655	0.003	1.306	0.049	11	53.94
Curran, 2020 [47]	TD1	0.571	-0.133	1.276	0.112	9	46.06
Summarized fixed effects		**0.616**	**0.138**	**1.095**	**0.012**	**20**	**100**



Heterogeneity tests: Q=0.03, df=1, p=0.87, I^2=0%;
Hypothesis test: Z=2.53, p=0.011; Egger test: NA.

4.a

First author, year	Subgroup	SMD	LL	UL	p-Value	Total	Weight
Azali Alamdari, 2018 [46]	HC	-1.064	-1.804	-0.324	0.005	11	10.40
Azali Alamdari, 2018 [46]	HE	-0.764	-1.407	-0.121	0.020	12	11.37
Azali Alamdari, 2018 [46]	NC	-0.687	-1.344	-0.030	0.040	11	11.23
Azali Alamdari, 2018 [46]	NE	-1.108	-1.826	-0.389	0.003	12	10.61
Krzywkowski, 2001 [54]		0.000	-0.653	0.653	1.000	9	11.27
Wang, 2011 [32]	H-AT	-1.461	-2.352	-0.570	0.001	10	8.97
Wang, 2011 [32]	H-C	-1.739	-2.722	-0.757	0.001	10	8.20
Wang, 2011 [32]	H-RT	-1.917	-2.961	-0.873	0.000	10	7.71
Wang, 2011 [32]	N-C	-1.579	-2.508	-0.650	0.001	10	8.64
Wang, 2011 [32]	N-T	-0.073	-0.693	0.548	0.818	10	11.60
Summarized random effects		**-0.958**	**-1.361**	**-0.556**	**0.000**	**105**	**100**

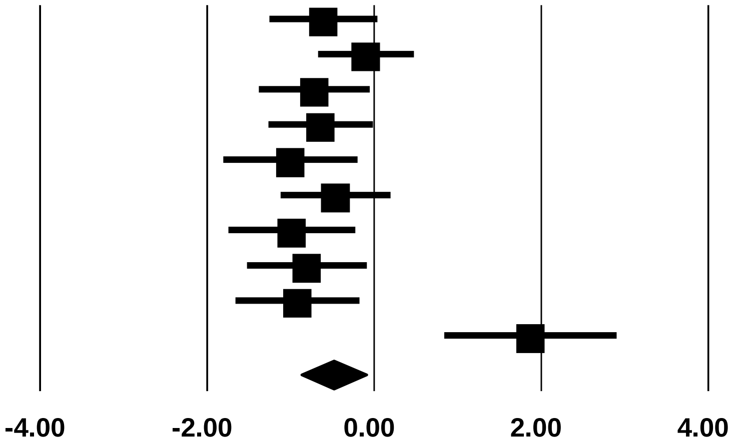


Heterogeneity tests: Q=24.97, df=9, p=0.003, I^2=63.96%;
Hypothesis test: Z=-4.67, p<0.001; Egger test: p<0.001.

4.b

First author, year	Subgroup	SMD	LL	UL	p-Value	Total	Weight
Azali Alamdari, 2018 [46]	HC	-0.605	-1.248	0.038	0.065	11	10.67
Azali Alamdari, 2018 [46]	HE	-0.098	-0.665	0.469	0.735	12	11.40
Azali Alamdari, 2018 [46]	NC	-0.714	-1.376	-0.052	0.034	11	10.49
Azali Alamdari, 2018 [46]	NE	-0.641	-1.262	-0.020	0.043	12	10.88
Krzywkowski, 2001 [54]		-1.000	-1.800	-0.200	0.014	9	9.22
Wang, 2011 [32]	H-AT	-0.459	-1.111	0.192	0.167	10	10.59
Wang, 2011 [32]	H-C	-0.983	-1.738	-0.228	0.011	10	9.62
Wang, 2011 [32]	H-RT	-0.805	-1.518	-0.092	0.027	10	10.01
Wang, 2011 [32]	N-C	-0.916	-1.655	-0.178	0.015	10	9.77
Wang, 2011 [32]	N-T	1.875	0.846	2.904	0.000	10	7.36
Summarized random effects		**-0.488**	**-0.880**	**-0.096**	**0.015**	**105**	**100**

SMD and 95% CI for senescent CD8 T cells

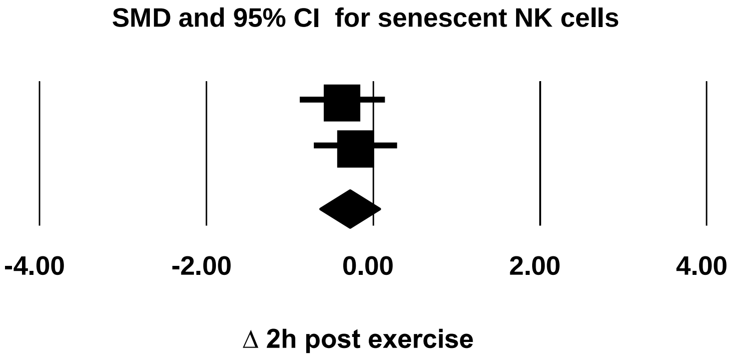


Heterogeneity tests: Q=28.05, df=9, p=0.001, I^2=67.92%;
Hypothesis test: Z=-2.44, p=0.015; Egger test: p=0.29.

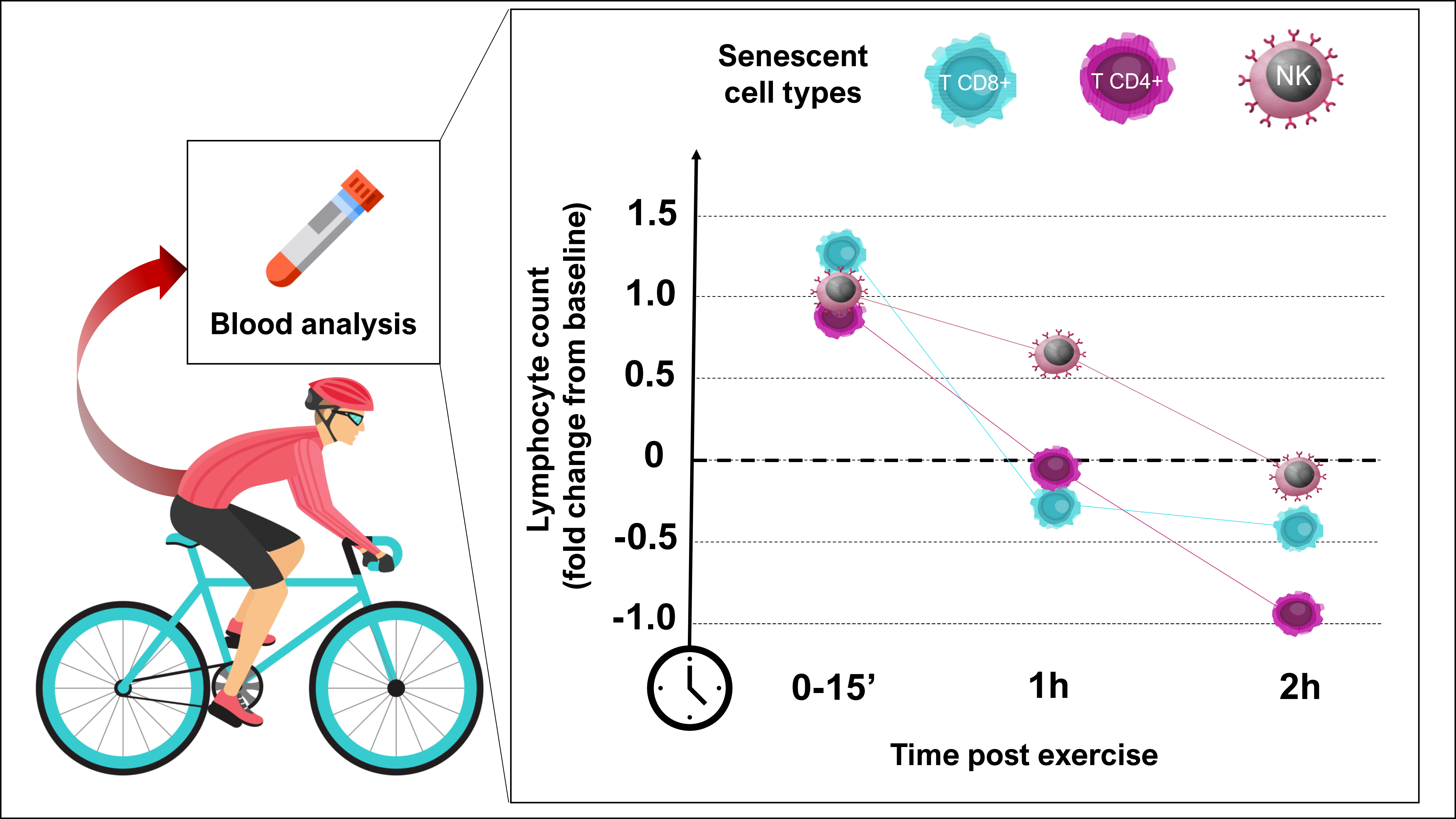
Δ 2h post exercise

4.c

First author, year	Subgroup	SMD	LL	UL	p-Value	Total	Weight
Wang, 2009 [57]	HI	-0.369	-0.876	0.137	0.153	16	48.88
Wang, 2009 [57]	MI	-0.208	-0.703	0.287	0.410	16	51.12
Summarized fixed effects		**-0.287**	**-0.641**	**0.067**	**0.112**	**32**	**100**



Heterogeneity tests: Q=0.20, df=1, p=0.65, I^2=0%;
Hypothesis test: Z=-1.59, p=0.11; Egger test: NA.



Blood analysis
Senescent
cell types
T CD8+
T CD4+
NK
Lymphocyte count
(fold change from baseline)
1.5
1.0
0.5
0
-0.5
-1.0
0-15’
1h
2h
Time post exercise

Supplementary Material

Pubmed Search

(“Cellular Senescence”[mh] OR “cell ageing”[tiab] OR “cellular ageing”[tiab] OR “cell aging”[tiab] OR “Senescence-Associated Secretory Phenotype”[tiab] OR “Senescence Associated Secretory Phenotype”[tiab] OR ("cellular"[tiab] AND "senescence"[tiab]) OR "cellular senescence"[tiab] OR "cell senescence"[tiab] OR “CD28 Antigens”[mh] OR “CD28”[tiab] OR “CD57 Antigens”[mh] OR “CD57”[tiab] OR “KLRG1 protein, human” [Supplementary Concept] OR “KLRG1”[tiab] OR “Immunosenescence”[mh] OR “Immunosenescence”[tiab]) AND ("exercise"[mh] OR "exercise"[tiab] OR "physical training"[tiab] OR "physical activity"[tiab]) NOT (Review [Publication Type] OR "Review Literature as Topic" [mh])

Web of Science Search

(TS= ("Cellular Senescence") OR TS= ("cell ageing") OR TS=("cellular ageing") OR TS=("cell aging") OR TS=("Senescence-Associated Secretory Phenotype") OR TS=("Senescence Associated Secretory Phenotype") OR TS=("cellular senescence") OR TS=("cell senescence") OR TS=("cell senescence") OR TS=("CD28 antigens") OR TS=(“CD57 Antigens”) OR TS=(“KLRG1 protein, human”) OR TS=(“Immunosenescence”) OR AB= ("Cellular Senescence") OR AB= ("cell ageing") OR AB=("cellular ageing") OR AB=("cell aging") OR AB=("Senescence-Associated Secretory Phenotype") OR AB=("Senescence Associated Secretory Phenotype") OR AB=("cellular senescence") OR AB=("cell senescence") OR AB=("cell senescence") OR AB=("CD28 antigens") OR AB=(“CD57 Antigens”) OR AB=(“KLRG1 protein, human”) OR AB=(“Immunosenescence”)) AND (TS=("exercise") OR AB=("exercise") OR AB=(“physical activity") OR AB=("physical training") OR TI=("exercise") OR TI=(“physical activity") OR TI=("physical training")) AND LANGUAGE: (English) AND TYPE OF DOCUMENT: (Article)

Embase Search

(('cell aging'/exp OR 'cell aging':ab,ti OR 'Senescence Associated Secretory Phenotype'/exp OR ‘CD28 antigen’/exp OR ‘CD57 antigen’/exp OR ‘klrg1 protein’/exp OR ‘immunosenescence’/exp OR cd28:ab,ti OR cd57:ab,ti OR klrg1:ab,ti OR immunosenescence:ab,ti OR ‘cellular aging':ab,ti OR ‘Senescence-Associated Secretory Phenotype’:ab,ti OR “Senescence Associated Secretory Phenotype’:ab,ti OR (‘cellular’:ab,ti AND ‘senescence’:ab,ti) OR ‘cellular senescence’:ab,ti OR ‘cell senescence’:ab,ti)) AND ('kinesiotherapy'/exp OR 'exercise'/exp OR 'exercise':ab,ti OR 'physical activity':ab,ti OR 'physical training':ab,ti) AND ([article]/lim OR [article in press]/lim OR [data papers]/lim) AND 'human'/de

Scopus Search

(TITLE-ABS-KEY(“Cellular Senescence”) OR TITLE-ABS-KEY(“cell ageing”) OR TITLE-ABS-KEY(“cellular ageing”) OR TITLE-ABS-KEY(“cell aging”) OR TITLE-ABS-KEY(“Senescence-Associated Secretory Phenotype”) OR TITLE-ABS-KEY(“Senescence Associated Secretory Phenotype”) OR TITLE-ABS-KEY("cellular senescence") OR TITLE-ABS-KEY("cell senescence") OR TITLE-ABS-KEY(“CD28”) OR TITLE-ABS-KEY(“CD57”) OR TITLE-ABS-KEY(“KLRG1”) OR TITLE-ABS-KEY(“Immunosenescence”)) AND (TITLE-ABS-KEY("exercise") OR TITLE-ABS-KEY("physical activity") OR TITLE-ABS-KEY("physical training")) AND (LIMIT-TO (DOCTYPE , "ar")) AND (LANGUAGE (DOCTYPE , "ar"))

Cochrane Search

((“Cellular Senescence”):ti,ab,kw OR (“cell ageing”):ti,ab,kw OR (“cellular ageing”):ti,ab,kw OR (“cell aging”):ti,ab,kw OR (“Senescence-Associated Secretory Phenotype”):ti,ab,kw OR (“Senescence Associated Secretory Phenotype”):ti,ab,kw OR ("cell senescence"):ti,ab,kw OR ("CD28"):ti,ab,kw OR ("CD57"):ti,ab,kw

OR ("KLRG1"):ti,ab,kw OR ("Immunosenescence"):ti,ab,kw) AND (("exercise"):ti,ab,kw OR ("physical training"):ti,ab,kw OR ("physical activity"):ti,ab,kw)