Effect of exercise on acute senescent lymphocyte counts

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

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Short Title: Effect of exercise on acute senescent lymphocyte counts

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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

125 comparison between absolute cell counts and the frequency of cells in the circulation; the different
126 types of lymphocytes assessed; the membrane markers used to identify cell senescence;
127 characteristics of the study population (age, sex and physical activity level) and the exercise protocols
128 used (type of exercise, volume and intensity).

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

133 Methods

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

138 *Search strategy*

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

142 *Eligibility criteria*

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

148 *Immunosenescence cell markers*

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

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

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

159 **KLRG1^{+}.** T-lymphocytes expressing KLRG1 have impaired capacity to proliferate, yet

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

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

166 *Exclusion criteria*

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

172 **Data collection and data items**

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

177 For subgroup analysis we extracted information about participants sex, age, level of training,
178 health condition and CMV serostatus, type of lymphocytes assessed, membrane markers used, unit
179 of measurement, and the characteristics of the exercise bout such as intensity, volume, and type of
180 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

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

215 **Statistical analysis**

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

219 The 3 main meta-analyses, for each time point (immediately, 1h and 2h post exercise) and
220 the subgroup analyses were performed using Comprehensive Meta-Analysis software, version
221 3.3.070. When there was statistical significance for heterogeneity, randomized effect models were
222 selected and when there was no significant heterogeneity, fixed effects were applied. The
223 inconsistency between studies was reported as a percentage (I^2), based on difference between
224 expected heterogeneity (df) and true heterogeneity (Q-value).

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

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

233 Results

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

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

241 *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

272 0.62 [0.14; 1.09], p=0.11). These analyses were homogeneous (p>0.53, I^2=0%), confirming that each
273 of these senescent cells have very consistent response 1h post exercise across the different studies.

274 Table 2 shows there was a significant reduction in the senescent lymphocyte count only in
275 CMV+ and not CMV- individuals, with significant difference between groups. Regarding each
276 subgroup of T lymphocyte there was no significant reduction for senescent CD4+ T or CD8+ cells in
277 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
278 trend of senescent CD4+ reduction in CMV+ individuals (SMD -0.35 [-0.74; 0.04], p=0.075), and
279 reduction of senescent CD8+ T cells in CMV+ (SMD -0.46 [-0.75; -0.18] p=0.001). Only vigorous
280 intensity and long duration exercise protocols led to significant reduction of senescent lymphocytes
281 (SMD -0.5 [-0.8; -0.2], p<0.001) while the other intensities and durations did not vary significantly
282 (p>0.16).

283 ***Please, insert Figure 3 here***

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

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

295 ***Please, insert figure 4 here***

296 Discussion

297 The main findings of the present meta-analysis were the significant increase in senescent CD8+, CD4+
298 and NK cell counts immediately post exercise followed by a reduction in senescent CD8+ T cells at 1h
299 and 2h post exercise, a reduction in senescent CD4+ T cells at 2h post exercise and maintenance of
300 increased NK senescent cells at 1h post exercise with a return to baseline at 2h post exercise (Figure
301 5). Although there is no consensus about the exact role of these redistributions of senescent
302 lymphocytes post exercise, it has been proposed that senescent lymphocytes are preferentially

303 recruited for immune surveillance and removal by NK and CD8+ T cells, resulting in an exercise-
304 induced senolytic effect [22].

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

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

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

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

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

334 [76]. Thus, it is believed that CMV$^+$ individuals have reduced sensitivity to β-adrenergic stimulation
335 and decreased β_2-adrenegic receptor expression to prevent CMV reactivation [58]. However, a
336 reduced β-adrenergic sensitivity of T cells in CMV$^+$ individuals is not supported by our analysis, and in
337 fact we saw a larger magnitude of changes in CMV+ individuals. Thus, we believe the expected higher
338 baseline senescent lymphocyte counts in CMV$^+$ individuals [28,77], especially for senescent CD8+ T
339 cells, explains the higher magnitude of change with exercise in this population.

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

351 It is unlikely that IL-6 released by muscle cells during exercise [85], explains the difference
352 between exercise protocols. It is known, that IL-6 attracts lymphocytes to the circulation together
353 with β-adrenergic signaling during exercise [67], however, there is a higher release of IL-6 within
354 exercise protocols with higher energetic demand, such as the higher volume and during regimens
355 [85–87] which do not agree with our findings.

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

359 *Limitations*

360 The first limitation of this study was that most studies included young individuals. The unbalanced
361 subgroup analysis suggested there is higher magnitude of change on senescent counts in young than
362 older or middle-aged individuals immediately and 1h post exercise. Whether it is a true effect is
363 unclear, it could be explained by reduced β_2-adrenergic receptor sensitivity with ageing [89], which in

364 turn increases the threshold for catecholamine-induced lymphocyte recruitment. In this way, it is
365 important to confirm these results with a larger sample of older adults.

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

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

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

382 *Conclusions*

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

393

Statements

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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.

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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.

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Figure Legends

651 Fig. 1. Flowchart of study selection.

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

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

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

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

677

Meta-Analysis

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

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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

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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.

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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.

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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+

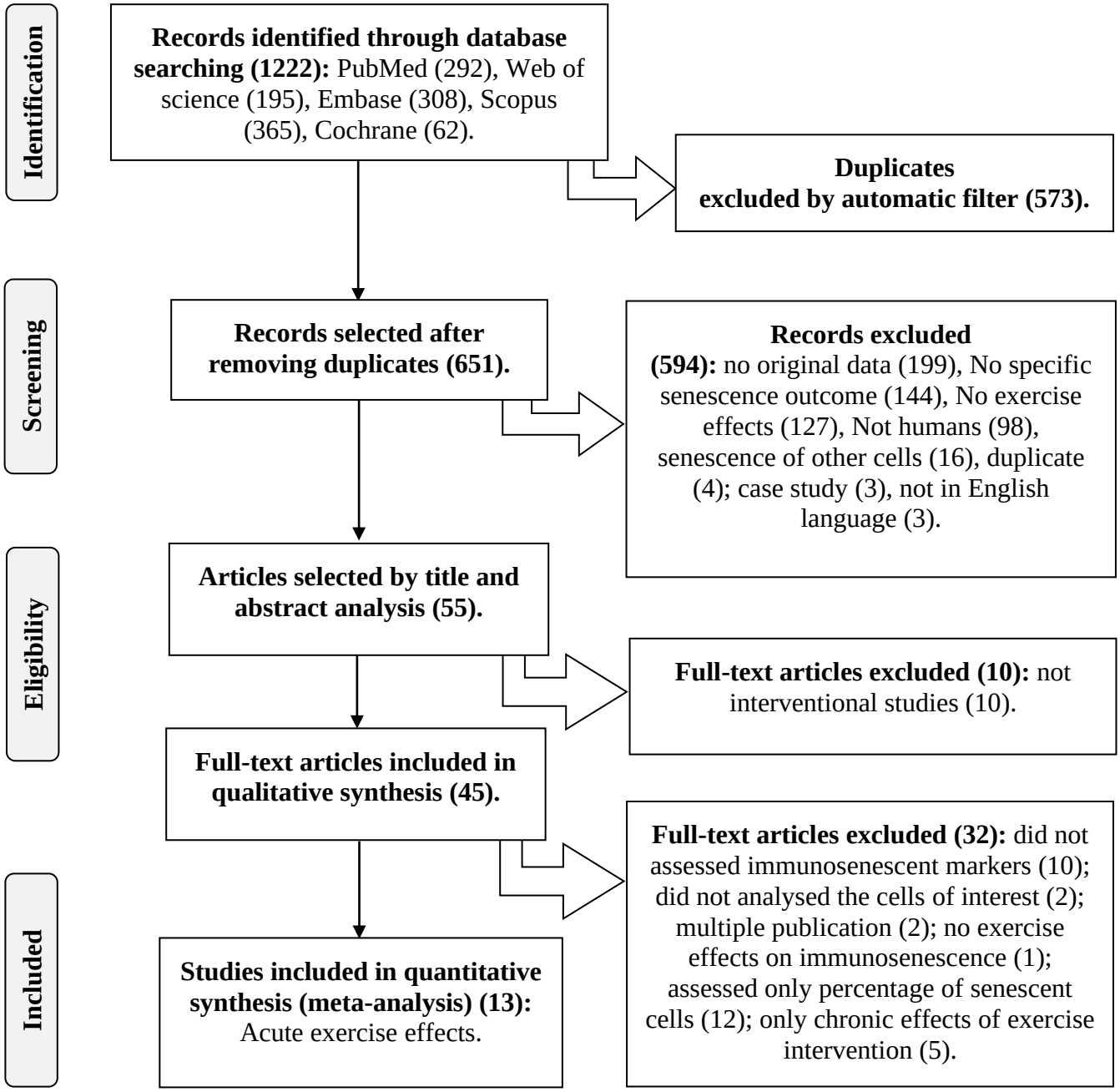
& Undisrupted sleep)											
Curran, 2020 [47]	0-15'' & 1'	M	2 Groups mean: 31 ± 7.15	NR	NA	Bicycle	80% VO_2max	30min	Vigorous & Moderate	NK	CD57+
Wang, 2009 [57] (HI & MI)	0-15'' & 2'	M	24.2 ± 1.2	UN	NA	Bicycle	Until exhaustion & 50% PD VO2max	Incremental & 30min	Maximum & short; Moderate & moderate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

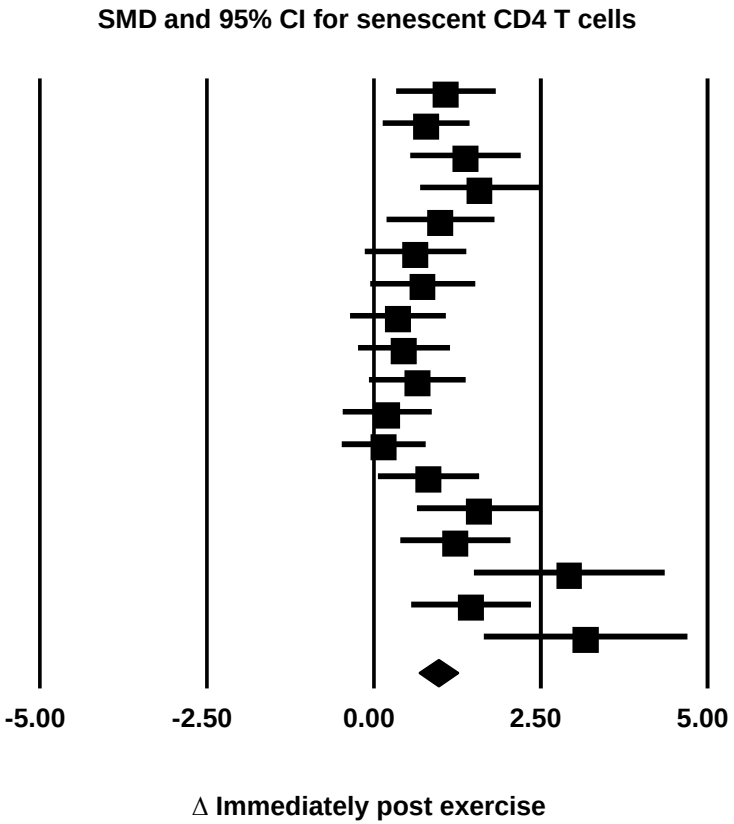
Time-point	Subgroups	Categories	K	SMD	LL	UL	p-value	Sample	p-diff
Immediately post exercise	**Training status**	TR	36	1.13	0.86	1.4	**<0.001**	345	0.35
		UN	8	1.38	0.92	1.84	**<0.001**	96	
	CMV status	CMV-	13	0.55	0.35	0.75	**<0.001**	120	0.09
		CMV+	9	1.09	0.55	1.63	**<0.001**	81	
	Age	Middle-aged	2	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

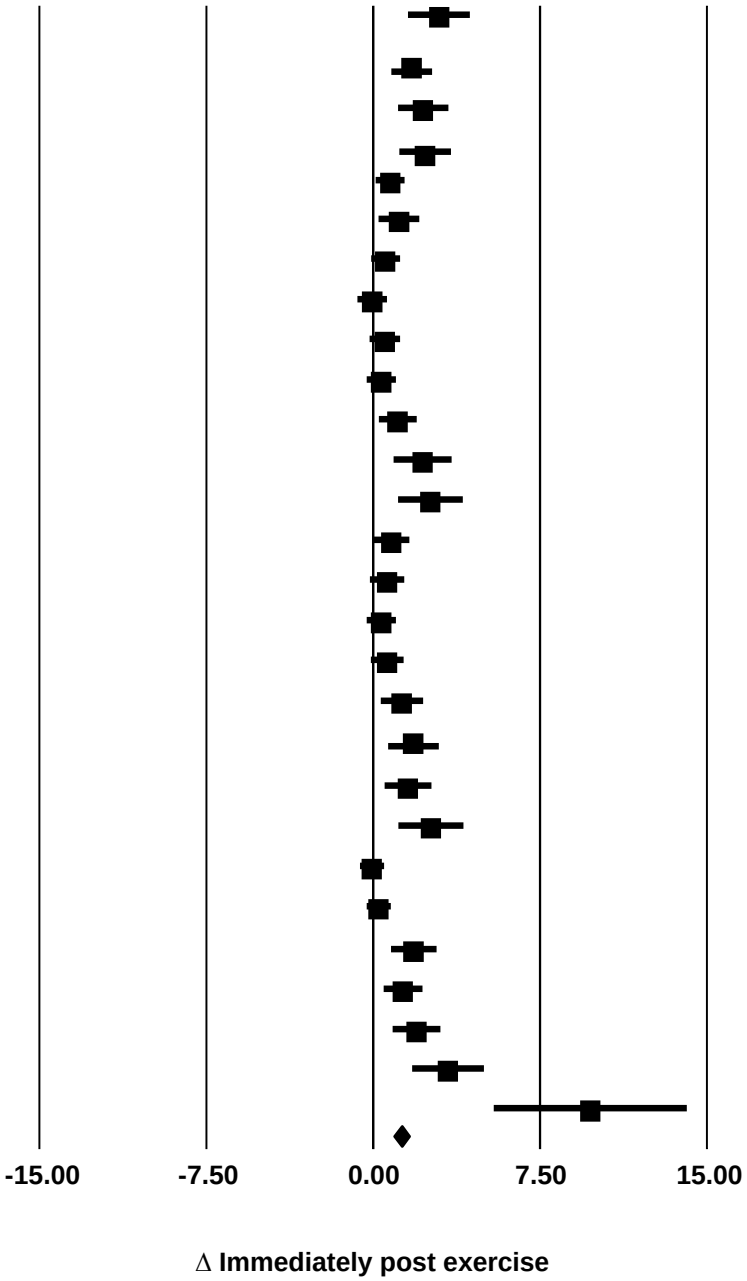
First author, year	Subgroup	SMD	LL	UL	p-Value	Total	Weight
Azali Alamdari, 2018 [46]	HC	1.078	0.335	1.821	0.004	11	6.03
Azali Alamdari, 2018 [46]	HE	0.784	0.137	1.431	0.018	12	6.68
Azali Alamdari, 2018 [46]	NC	1.372	0.549	2.195	0.001	11	5.51
Azali Alamdari, 2018 [46]	NE	1.585	0.697	2.472	0.000	11	5.13
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

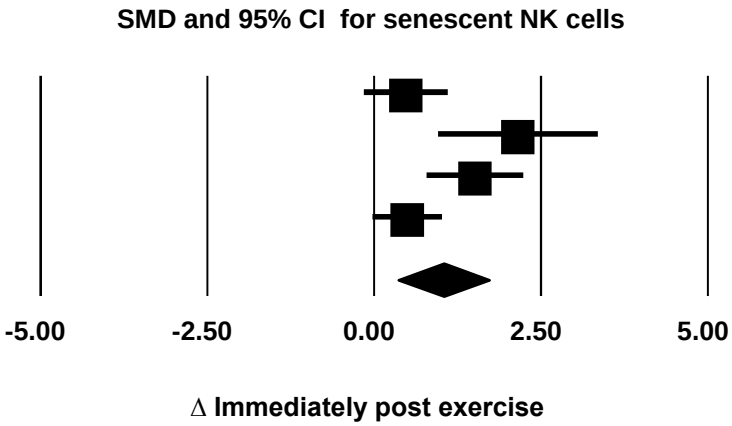
First author, year	Subgroup	SMD	LL	UL	p-Value	Total	Weight
Azali Alamdari, 2018 [46]	HC	2.968	1.594	4.342	0.000	11	2.75
Azali Alamdari, 2018 [46]	HE	1.739	0.842	2.636	0.000	12	3.75
Azali Alamdari, 2018 [46]	NC	2.247	1.138	3.356	0.000	11	3.28
Azali Alamdari, 2018 [46]	NE	2.336	1.195	3.476	0.000	11	3.22
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

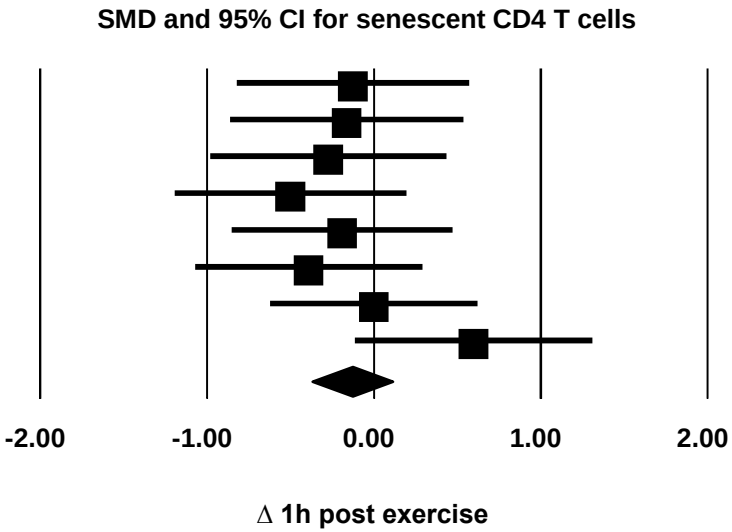
First author, year	Subgroup	SMD	LL	UL	p-Value	Total	Weight
Curran, 2020 [47]	Control	0.476	-0.147	1.100	0.134	11	27.56
Curran, 2020 [47]	TD1	2.155	0.964	3.346	0.000	9	17.13
Wang, 2009 [57]	HI	1.512	0.795	2.230	0.000	16	25.66
Wang, 2009 [57]	MI	0.499	-0.021	1.018	0.060	16	29.65
Summarized random effects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

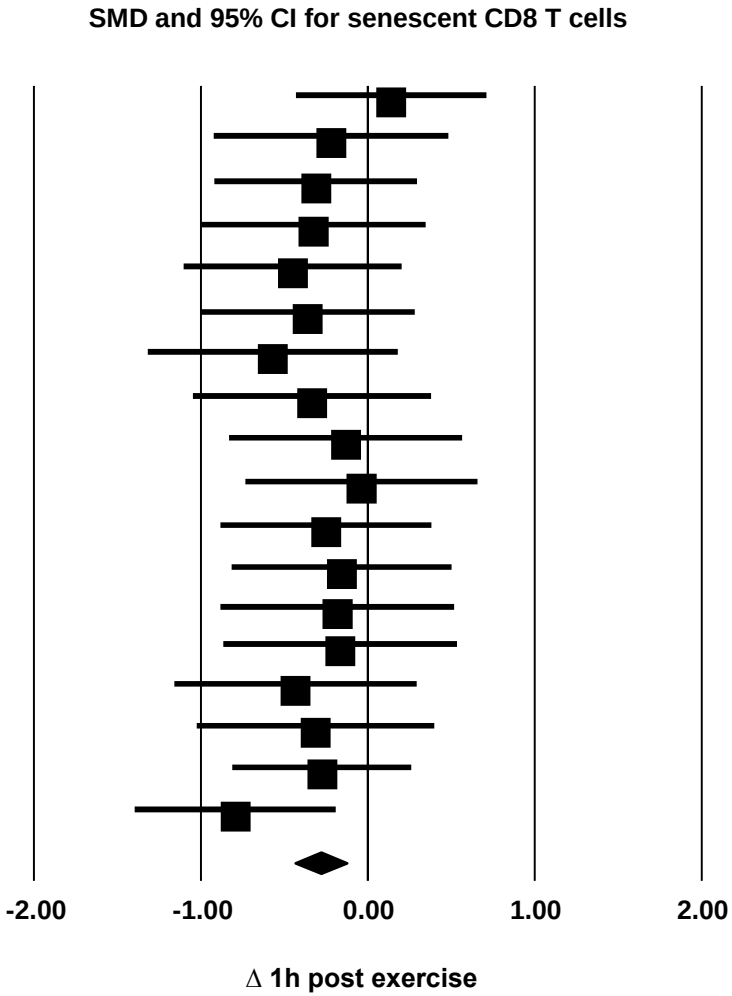
First author, year	Subgroup	SMD	LL	UL	p-Value	Total	Weight
Lavoy, 2017 [27]	CMV-/+15%LT	-0.124	-0.820	0.571	0.726	8	11.96
Lavoy, 2017 [27]	CMV-/+5%LT	-0.163	-0.860	0.535	0.647	8	11.89
Lavoy, 2017 [27]	CMV-/-5%LT	-0.272	-0.978	0.433	0.449	8	11.62
Lavoy, 2017 [27]	CMV+/+15%LT	-0.499	-1.192	0.194	0.158	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

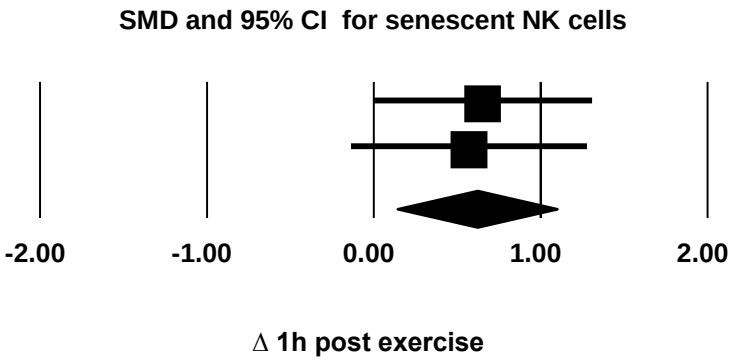
First author, year	Subgroup	SMD	LL	UL	p-Value	Total	Weight
Bigley, 2012 [56]	CMV-	0.140	-0.429	0.709	0.629	12	7.38
Bigley, 2012 [56]	CMV+	-0.219	-0.920	0.482	0.540	8	4.85
Curran, 2019 [53]	Control	-0.311	-0.916	0.294	0.314	11	6.52
Curran, 2019 [53]	TD1	-0.325	-0.995	0.345	0.342	9	5.31
Ingram, 2015 [49]	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**



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

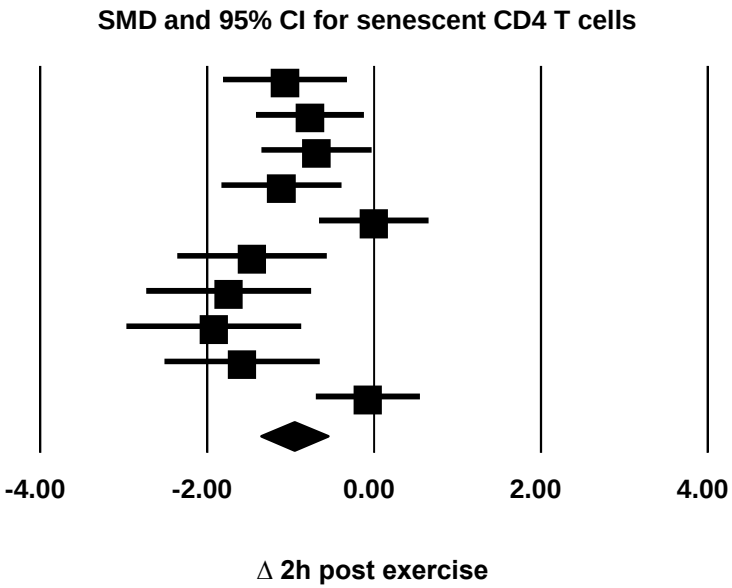
First author, year	Subgroup	SMD	LL	UL	p-Value	Total	Weight
Curran, 2020 [47]	Control	0.655	0.003	1.306	0.049	11	53.94
Curran, 2020 [47]	TD1	0.571	-0.133	1.276	0.112	9	46.06
Summarized fixed effects		**0.616**	**0.138**	**1.095**	**0.012**	**20**	**100**



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

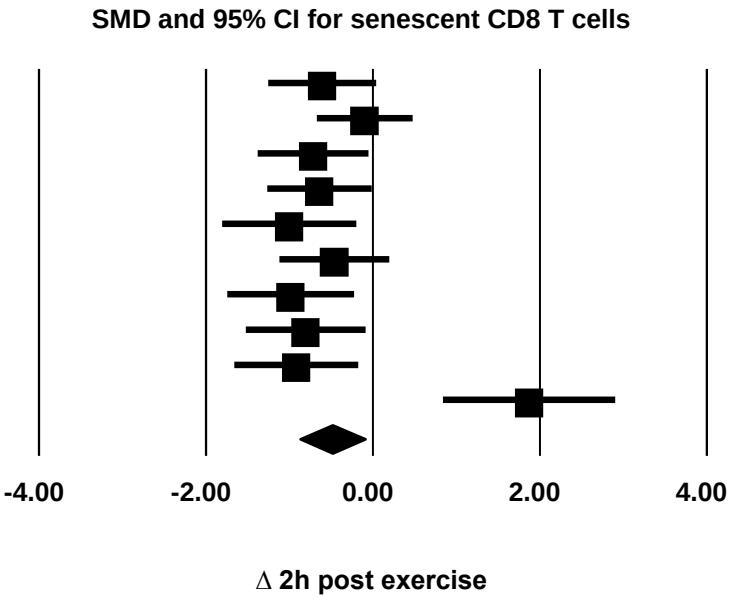
First author, year	Subgroup	SMD	LL	UL	p-Value	Total	Weight
Azali Alamdari, 2018 [46]	HC	-1.064	-1.804	-0.324	0.005	11	10.40
Azali Alamdari, 2018 [46]	HE	-0.764	-1.407	-0.121	0.020	12	11.37
Azali Alamdari, 2018 [46]	NC	-0.687	-1.344	-0.030	0.040	11	11.23
Azali Alamdari, 2018 [46]	NE	-1.108	-1.826	-0.389	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

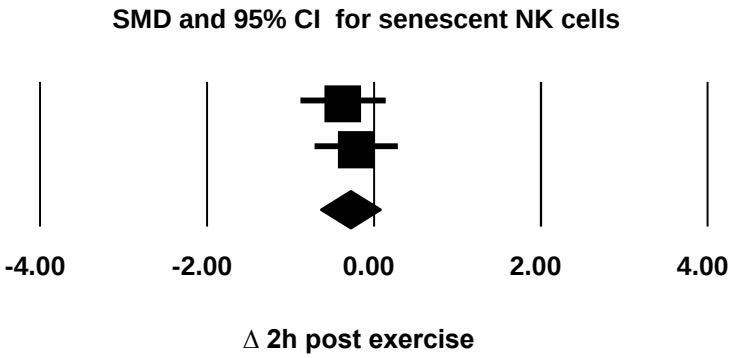
First author, year	Subgroup	SMD	LL	UL	p-Value	Total	Weight
Azali Alamdari, 2018 [46]	HC	-0.605	-1.248	0.038	0.065	11	10.67
Azali Alamdari, 2018 [46]	HE	-0.098	-0.665	0.469	0.735	12	11.40
Azali Alamdari, 2018 [46]	NC	-0.714	-1.376	-0.052	0.034	11	10.49
Azali Alamdari, 2018 [46]	NE	-0.641	-1.262	-0.020	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**



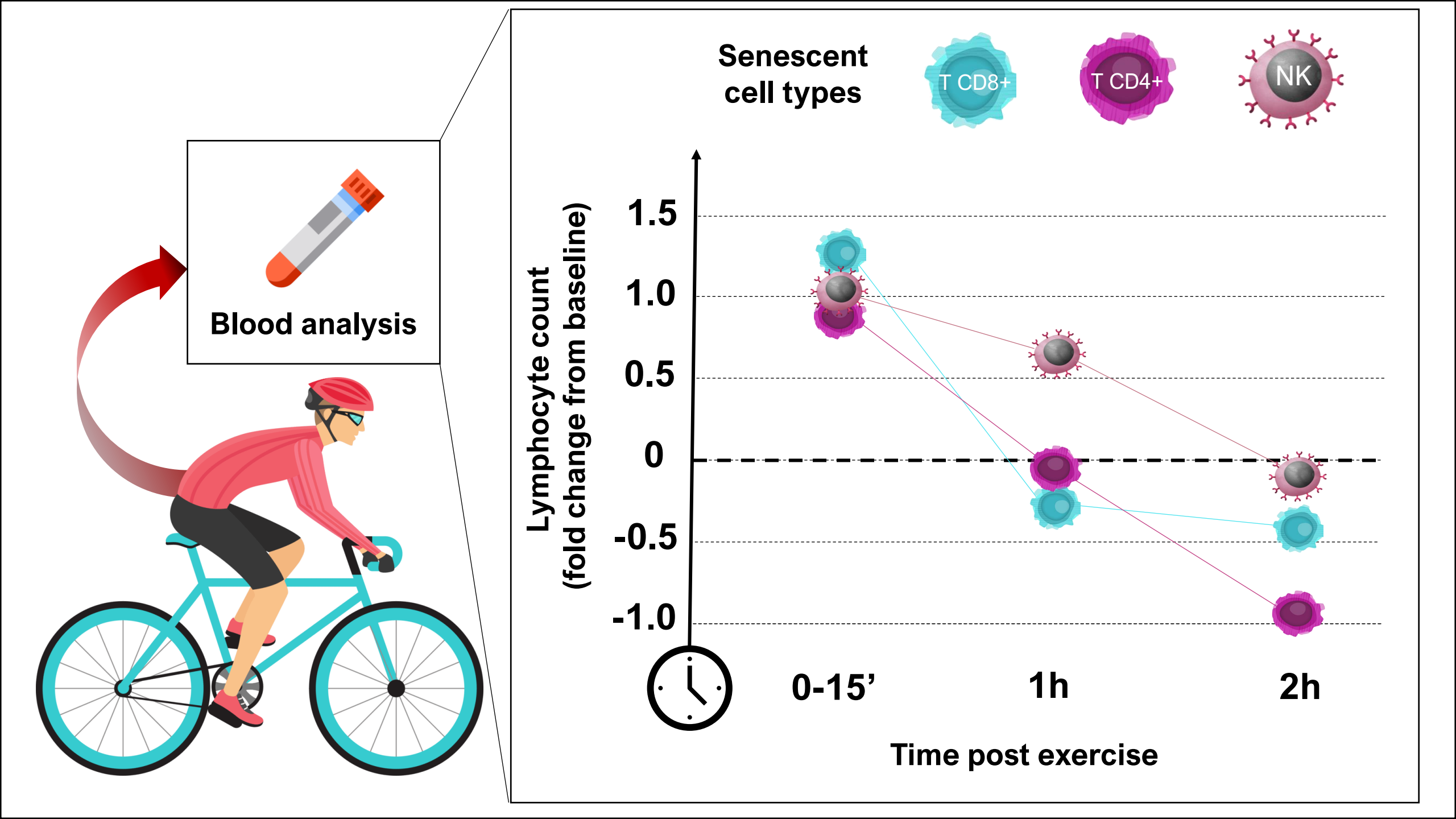
Heterogeneity tests: Q=28.05, df=9, p=0.001, I^2=67.92%;
Hypothesis test: Z=-2.44, p=0.015; Egger test: p=0.29.

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)