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Systematic review on the effects of physical exercise on cellular immunosenescence-related markers – an update

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ABSTRACT

Immunosenescence is a remodeling of the immune system occurring with aging that leads to an increased susceptibility to auto-immunity, infections and reduced vaccination response. A growing consensus supports the view that physical exercise may counteract immunosenescence and improve the immune response. Unfortunately, evidence regarding the effects of exercise on markers of cellular immunosenescence lacked uniformity at the time of an extensive literature review in 2016. Moreover, exercise-induced effects in older adults were underrepresented compared to young adults or completely lacking, such as for senescent T-cells and apoptosis of T-lymphocytes.

The aim of this systematic literature study was to collect and appraise newly available data regarding exercise-induced changes on immunosenescence-related markers of immune cells and compare this against data that was already available in 2016.

Systematically reviewing of newly available data in the field of exercise immunology provides additional evidence for the effect of exercise on immunosenescence-related cellular markers. Importantly, this review provides evidence for the effect of long-term exercise on senescent T-lymphocytes in older adults. Additionally, newly retrieved evidence shows an acute exercise-induced mobilization of naïve and memory cells in older adults. In general, data regarding long-term exercise-induced effects in older adults remain scarce. Noteworthy was the high number of articles describing exercise-induced effects on regulatory T-cells. However exercise-induced effects on this cell type are still inconclusive as some articles reported an exercise-induced up- or downregulation, while others reported no effects at all. Numerous studies on Natural Killer cell counts did not provide uniformity among data that was already available. Recent data regarding dendritic cells mostly described an increase after exercise. Overall, our literature update highlights the major influence of the type and intensity of exercise on immunosenescence-related markers, especially in older adults.

ABBREVIATIONS

APT	Aerobic physical training
CM	Central memory T-cell
CMV	Cytomegalovirus
CONT	Continuous exercise
CPET	Cardiopulmonary exercise testing
CpG	5'- C – phosphate- G-3'
DC	Dendritic cell
EM	Effector memory T-cell
EMRA	Effector memory T-cell re-expressing CD45RA
END	Endurance cycling protocol
GXT	Graded exercise rest
HE	Hypoxic exercise
HD	Hemodialysis
HIT	High intensity training
HIIT	High intensity interval training
HR	Heart rate
HSCT	Hematopoietic stem cell transplantation
IPAH	Idiopathic pulmonary arterial hypertension
IRB	Inspiratory resistive breathing
KIR	Killer immunoglobulin-like receptor
MAIT	Mucosal invariant T cells
MAX	Incremental maximal exercise
MICT	Moderate intensity continuous training
Min	Minute
NK	Natural killer
SPC	Senescent-prone T-cells
T1D	Type 1 diabetes
TCR	T-lymphocyte receptor
T-reg	Regulatory T-cell
VO_{2max}	Maximum oxygen consumption rate
W	Watt
WBE	Whole body exercise

INTRODUCTION

Immunosenescence is the process of remodeling of the immune system occurring with aging that leads to an increased susceptibility to auto-immunity and infections, but also to a reduced vaccination response [1, 2]. This remodeling can take place at different levels of the immune system and is, among others, characterized by aging-related alterations in immune cells, but also in lymphoid organs and circulating factors [3]. Major components of the innate system, such as dendritic cells (DC) and natural killer cells (NK) are affected by aging-related changes. Indeed, evidence suggests an impaired migration, phagocytosis capacity and T-cell presentation in DC and altered cell subsets and cytotoxic capacity of NK-cells [4]. Moreover, immunosenescence not only affects the innate, but also the more specific adaptive immunity. This is characterized by a shift in T cell phenotypes, namely from naïve - dealing with newly encountered antigens - to memory and senescent T-cells [2]. This decrease in naïve T-cells is believed to principally originate from thymic regression. Although this process alleviates the high energetic cost of generating immunocompetent cells [5, 6], it is also the root of a more restricted T-lymphocyte receptor (TCR) repertoire contributing to an increased susceptibility to new antigens and reduced vaccination responses. On the other hand, chronic antigenic stimulation (such as caused by Cytomegalovirus [7]) favors the balance towards the acquisition of a more senescent phenotype [8]. Having lost expression of co-stimulatory molecule CD28 and having shortened telomeres, these senescent immune cells are unable to divide and are resistant to apoptosis [9]. Although cellular senescence possibly acts as a suppressive mechanism for cancer, senescent T-cells also acquire a senescence-associated secretory phenotype (SASP) [10], associated with negative health repercussions. Indeed, SASP contributes to a low-grade pro-inflammatory state referred to as 'Inflammaging' through the secretion of pro-inflammatory cytokines [11-13]. This is highly relevant as many aging-associated chronic diseases are associated with this low-grade inflammatory profile [14, 15]. Furthermore, there are indications for the involvement of both immunosenescence and inflammaging in the development of frailty in older adults [5, 16].

Different approaches have already been proposed to try to tackle immunosenescence, physical exercise being one of them. Indeed, literature suggests that exercise may have an impact on several characteristics of immunosenescence, such as the shifts in T-cell subsets or vaccination response [17, 18]. As shown in animal studies, a mobilization of some of these immune cell subsets into the bloodstream is observed after acute exercise, followed by a subsequent post-exercise decrease. This is believed to represent a homing of cells to peripheral tissues, boosting immune surveillance and vigilance [19]. Additionally, acute and long-term exercise can also have repercussions on immunosenescence-related markers. It has for example been hypothesized recently that an acute bout of exercise may cause a mobilization of highly differentiated and senescent T cells from the peripheral tissues to the bloodstream, thereby possibly influencing their susceptibility to apoptosis-triggering pathways. This would create a vacant space enabling expansion of naïve T cells, ready to react to new antigenic threats [20]. Therefore, exercise interventions could be an affordable and minimally invasive way to alleviate detrimental aging-associated changes in the immune system and achieve appropriate immunization for e.g. influenza and other viruses.

Evidence regarding the effects of physical exercise on cellular immunosenescence-related markers has been extensively reviewed in 2016. Systematic screening of the literature demonstrated evidence for the ability of an acute bout of exercise to increase naïve, memory and senescent T-lymphocytes, but also apoptosis of lymphocytes in the circulation. Moreover, evidence regarding increased circulating levels of CD28+ in young and older populations after long-term exercise was also provided. However, effects of exercise in older adults remained unclear due to the low number of articles focusing on this population [18]. As the field of exercise immunology has been rapidly expanding over the last years,

the aim of this review was to provide an update on the effect of exercise on immunosenescence-related markers of immune cells (Table 1) by collecting and appraising new available data using an identical systematic literature review approach.

METHODS

Search strategy

Literature databases Pubmed and Web of Science were systematically screened using an interval of publication date between January 18th 2016 (last search by Cao Dinh et al.) and August 9th 2019. 2346 articles were retrieved using the same search key as described by Cao Dinh et al. [18]: ((''Exercise''[Mesh] OR physical exercise OR ''Motor Activity'' [Mesh] OR Physical Activity OR ''Physical Fitness'' [Mesh] OR Intensive strength training OR ''Resistance Training''[Mesh] OR Strength training OR ''Plyometric Exercise'' [Mesh] OR exhaustive exercise OR ''Physical Endurance'' [Mesh] OR ''Exercise Tolerance'' [Mesh] OR Aerobic fitness OR Aerobic training OR Strength endurance training OR Strengthening exercise)) AND (''Cell Physiological Processes'' [Mesh] OR immunosenescence OR ''Lymphocytes'' [Mesh] OR ''Monocytes'' [Mesh] OR ''Macrophages'' [Mesh] OR ''Dendritic Cells'' [Mesh] OR ''Killer Cells, Natural'' [Mesh] OR NK Cells OR ''Neutrophils'' [Mesh] OR ''Vaccines'' [Mesh] OR Cell surface marker) for PubMed and search key: TS = (Exercise OR physical exercise OR Motor Activity OR Physical Activity OR Physical Fitness OR Intensive strength training OR Resistance Training OR Strength training OR Plyometric Exercise OR exhaustive exercise OR Physical Endurance OR Exercise Tolerance OR Aerobic fitness OR Aerobic training OR Strength endurance training OR Strengthening exercise) AND TS = (Cell Physiological Processes OR immunosenescence OR Lymphocytes OR Monocytes OR Macrophages OR Dendritic Cells OR Killer Cells, Natural OR NKCells OR Neutrophils OR Vaccines OR Cell surface marker) for Web of Science. Articles were included when describing the interaction between physical exercise and cellular markers of immunosenescence in humans. Articles describing animal studies, monocyte and neutrophil subsets and cellular function were excluded, as well as studies in which no structured exercise program was provided or where the exercise intervention was not clearly provided. Screening of titles, abstracts and full-texts resulted in the inclusion of 55 articles (see figure 1).

Quality assessment

The NICE checklist for Randomized Controlled Trials (RCT) was used to assess the scientific quality of the studies [21].

Data extraction

The main participants' characteristics (age, sex, specific disease) were identified. Main study outcomes regarding exercise-induced changes in a selection of cellular immunosenescence-related markers (Table 1) were appraised. In table 1 we provide an overview of cell surface makers used to discriminate between innate and adaptive immune system subsets. The type and duration of physical exercise, as well as time of blood sampling and time of analyses (immediately or after cryopreservation) were also identified.

RESULTS AND DISCUSSION

Quality of study design

Most of the studies showed moderate to good quality (Table 2). In terms of selection bias, 4 studies did not describe the used randomization method. Concealment of allocation was also not always reported. Since former exercise training of participants could possibly influence the exercise interventions, disclosure of the trained or untrained condition of participants was estimated necessary to compare groups at baseline. Regarding performing bias, participants and investigators were not kept blinded to the treatment allocation due to the nature of the exercise interventions. Concerning attrition bias, almost all studies showed an equal length of follow-up. Most of the studies were not characterized by high dropout numbers. The availability of outcome data was however not always clear. Lastly, for detection bias, almost all studies had an appropriate length of follow-up. Precise definition of outcome and reliable measures were acceptable in all studies. Although 8 RCT's out of 11 reported that investigators were kept blinded to treatment allocation, most of the studies did not report whether investigators were kept blinded to confounding factors.

Participants

Out of the 55 articles included in this review, only 5 described exercise-induced effects in older adults (>65 years) (Table 4,5). Although most studies described a healthy population, several articles also described patients with type 1 diabetes or an impaired glucose tolerance, chronic kidney disease, idiopathic pulmonary arterial hypertension, chronic fatigue syndrome, multiple sclerosis, solid tumors, HIV, knee osteoarthritis or patients who have undergone a hematopoietic stem cell transplantation or are under haemodialysis or chemotherapy or have survived breast cancer.

Overview of the literature

Exercise-induced effects were divided into effects observed after an acute bout of exercise (in untrained or trained individuals) or after long-term exercise. Articles describing effects in young and middle-aged adults (<65 years) and older adults (> 65 years) were also separated. Noteworthy, a short overview of most important findings is provided at the end of each major section (acute and long-term exercise per age group).

Acute Exercise-Induced Effects

*Acute Exercise-Induced Effects in Young and Middle-aged Humans - **Untrained condition***

After systematically screening literature for recent studies regarding acute exercise-induced effects, 16 articles were retrieved describing effects in untrained individuals. Different types of exercise interventions were described. Curran et al. compared the exercise-induced T-cell mobilization in Type 1 diabetes patients (T1D) and a control group (CON) after a 30 min cycling bout. While cell counts of low differentiated phenotypes such as CD8+ naïve and central memory cells stayed unaffected by 30 min cycling as long as 1 hour post-exercise, the intervention induced a decrease in more differentiated CD8+ effector memory cells in T1D patients and a decrease in CD8+ EMRA in both groups 1 hour after exercise [22]. EMRA cells represent a subset of effector memory T-cells, re-expressing CD45RA. As terminally differentiated cells, they are characterized by low proliferative and functional capacity. Moreover, they have the shortest telomeres amongst T-cells and display senescence markers [23]. More specifically, exercise significantly mobilized the most differentiated (CD27-CD28-) EMRA's expressing CD69, a marker for activation and tissue-resident populations and CD11b, an adhesion marker involved in lymphocyte migration. EMRA expressing CD127, a marker needed for memory cell maintenance, and CD95, a marker for apoptosis on memory subsets, were not significantly mobilized.

Exercise did also mobilize CD11b+ EMRA, but not CD11b+ naïve and memory CD8+ subsets. [22]. Gustafson et al. observed a peak in lymphocytes already 2 to 5 minutes after incremental maximal cycling (max) and an endurance cycling protocol (end) before returning to baseline after 3 hours. However, this peak was only significant after maximal exercise. In contrast to the previous study, Gustafson et al. showed a significant decrease in the percentage of naïve cells and an increase in memory T-cells post-exercise, although this was not significant in CD8+ cells. More specifically, CD4+ and CD8+ central memory cells decreased while effector memory cells increased [24], contrasting with results from Curran et al [22]. In a next study, lymphocyte subset responses to 45 min of whole body exercise (WBE) and inspiratory resistive breathing (IRB) were examined. While WBE resulted in a decrease in the percentage of CD4+ memory cells post-exercise, IRB did not affect percentage of these cells. In contrast, the percentage of CD8+ memory cells was not influenced by WBE or IRB. Both WBE and IRB also had no effects on the percentage CD4+ naïve cells, but WBE and IRB increased the percentage of CD8+ naïve cells [25]. Furthermore, both acute high-intensity interval training (HIT) and continuous exercise (CONT) increased total cell numbers of naïve T cells immediately after exercise. Three hours post-exercise, these numbers decreased below pre-exercise levels. In contrast, senescent-prone T-cells (SPC) were increased both immediately and 3 hours after exercise. Although both naïve and senescent-prone subsets were mobilized after exercise, the increase in SPC was higher than in naïve cells. Compared to CONT, HIT mobilized higher counts of SPC [26]. Mobilization of these cells is likely associated to exercise intensity. It has been proposed in literature that these cells might be more often located at the vessel wall and in secondary lymphoid organs [19, 27]. Repeated sympathetic stimulation inducing catecholamine release might induce changes in adhesive interactions between the vessel walls and lymphocytes, thereby contributing to the mobilization to peripheral blood [28, 29]. Indeed, this study also showed that plasma levels of norepinephrine were significantly associated with the percentage of mobilized senescent-prone T-cells (and T-regs). Additionally, epinephrine was also associated with senescent-prone cells. Moreover, Krüger et al. also showed that HIT and CONT affected apoptosis of different T-cell subsets. While CONT induced a higher increase in the percentage of apoptotic naïve T-cells 3 hours after exercise compared with HIT, HIT induced a higher increase in percent apoptosis of senescent-prone T-cells immediately after exercise [26]. Exercise-induced mobilization of different T-cell phenotypes after HIT and CONT were also observed by Turner et al. Both interventions increased CD8+ naïve, central memory and effector memory counts immediately after exercise. No differences with baseline could be observed already 30 minutes after exercise. However, only CONT significantly increased counts of CD8+ EMRA after exercise before returning to baseline 30 min post-ex. In this study, no significant time x task interactions were found [30]. Surprisingly, results regarding mobilization of these different cell subsets after CONT (20 min cycling at 80% VO_2max) immediately after exercise strongly differed from results described by Curran et al. after 20 min cycling at 80% VO_2max, where no mobilization of CD8+ naïve, central and effector memory and EMRA cells could be observed immediately after exercise [22]. Interestingly, although a small subset of lymphocytes expressing cutaneous lymphocyte antigen (CLA) were mobilized after exercise, CLA- were the greatest contributors of this mobilization. This suggests that the skin is not a major origin or homing-destination of mobilized lymphocytes [30].

In the untrained, 3 studies were found describing effects of acute exercise on the CD4/CD8 ratio. As part of the 'immune risk phenotype', an inverted CD4/CD8 ratio has been identified as a predictor of non-survival [31]. A reduction in this ratio was found after a graded exercise test until volitional fatigue [32] and treadmill running at 40% VO_{2max} until exhaustion in the heat [33] immediately post-exercise. As CD4/CD8 ratio was not assessed during further recovery in these studies, no conclusion regarding possible lasting effects on the immune risk phenotype of participants after these interventions can be made. Asimakos et al. however not only sampled post-exercise, but also after 120 minutes recovery.

Although a decrease in the CD4/CD8 ratio was observed after inspiratory resistive breathing, a return to baseline levels was observed already after 120 minutes, precluding long-term detrimental effects on the immune system. Lastly, no significant post-exercise decrease could be observed after whole body exercise [25].

Although less described, exercise can also affect mobilization of mucosal associated invariant T (MAIT) cells. These unconventional cells can make up about 5% of total blood T cells, 10% of CD8+ T cells, and up to 45% of liver T cells [34]. Exhibiting innate-like effector responses, they have been shown to be involved in several infectious and non-infectious diseases [35]. In a study from Hanson et al., about 3% of CD3+ T cells were found to be MAIT cells. A graded exercise test (GXT) until volitional fatigue in recreationally active men induced an increase of these cells. Increased counts were believed to primarily arise from the increase in total lymphocyte number. Although this intervention did not affect the percentage of CD8+ MAIT cells, a major subpopulation of MAIT cells, absolute counts were increased post-exercise. This was also the case for CD4-CD8- MAIT cells in general. MAIT cells accounted for around 8 percent of cytotoxic T-lymphocytes. This proportion was not influenced by exercise. [32]. Similarly, MAIT cells were also increased after a moderate cycling exercise. However, this increase was smaller compared to the increase after maximal exercise. Percentage of MAIT also remained elevated 1 hour into recovery. The authors proposed that the activation of the sympathetic nervous system, with redirection from blood flow to active tissues instead op MAIT residential tissues and the expression of adhesion proteins (CD44) as a possible cause for this increase 1 hour post-exercise. Counts of CD8+ and CD4-CD8 MAIT cells were again elevated immediately post-exercise, although this was not reflected in the percentage of these cells. Counts of CCR4+, CCR5+ and CD69+ MAIT cells were increased post-exercise before returning to baseline after 1 hour. CCR6+ MAIT cells however only showed a trend to increase post-exercise. However, no significant changes in the proportion of MAIT cells within T-cells expressing these surface markers were observed [36].

T-cell phenotypes have long been known to be implicated in the process of cellular immunosenescence [2]. However, indications for the involvement of regulatory T-cells are relatively new as no articles described this cell population in the original review of 2016 [37]. Indeed, recent literature suggests that aging also affects T-reg frequencies, subset distribution and function. As essential regulators of both innate and adaptive immune responses, loss of T-regs could expose hosts to excessive immunity. In contrast, a gain of these cells could lead to immune failure in response to infections and malignancies. It has been suggested that naturally occurring T-regs would accumulate with age while inducible T-regs would be downregulated. Unfortunately, mechanisms are still not understood [4, 38]. In this review, four articles describing exercise-induced effects on T-regs in untrained young adults were found. A first article described a decrease in percentage T-regs after incremental maximal cycling. However, this was not significant after an endurance cycling protocol [24]. An increase in T-regs could also be observed immediately and 3 hours after HIT, but not after CONT. Moreover, this study also showed that plasma levels of norepinephrine were significantly associated with the percentage of mobilized T-regs, suggesting that mobilization of these cells is also, at least partly, dependent of exercise intensity. Lastly, only CONT induced apoptosis in Treg-cells three hours after intervention [26]. Curran et al. also showed effects of 30 min cycling on T-regs in type 1 diabetes patients and healthy controls. T-regs were mobilized overall and in the control group, but not in T1D patients, followed by a decrease below baseline levels 1 hour after exercise overall, but not in the CON or T1D group. While the same was observed for memory T-regs, naïve T-regs were not significantly mobilized [22]. Harbaum et al. also investigated the effects of cardiopulmonary exercise testing on T-regs in a study population consisting of idiopathic pulmonary arterial hypertension (IPAH) patients. At baseline, these patients showed elevated levels of T-regs [39], as expected from literature [40]. No effects on absolute counts and percent of T-regs in IPAH patients were found after an incremental cycling exercise, suggesting

that this intervention was able to counteract elevated levels of T-regs in those patients [39]. The available evidence regarding exercise-induced effects on T-regs in untrained participants is currently too sparse to draw strong conclusions.

Natural Killer (NK) cells are important regulators of the innate immune system. Characterized by an ability to kill virus infected or cancer cells without sensitization, they play an important role not only in the defense against viruses, but also in tumor immune-surveillance [41-43]. Schenk et al. investigated the effects of 15 a minutes graded exercise test (GXT) on a bicycle ergometer in five healthy women between the age of 50 and 60, who reflect a population with enhanced cancer risk. No statistically significant effects were observed in the proportions of $CD56^{bright}$ and $CD56^{dim}$ cells. Authors also investigated the effects of this intervention on promotor DNA methylation of KIR2DS4 and KIR3DL1 [44]. Within the innate immune system, modulation of the activity of NK cells is a result of a balance between activating and inhibiting receptors on the surface of NK cells, killer immunoglobulin-like receptor (KIR) being a large family of these receptors [45]. Epigenetic modification is partially responsible for the modulation of NK cell activity: hypomethylation of the promotor of KIR genes will lead to gene expression of these genes, while hypermethylation will counteract gene expression [46]. After the GXT, activating receptor KIR2DS4 showed a decreased promotor methylation and an enhanced gene expression. Regarding inhibiting receptor KIR3DL1, no significant changes in DNA promotor methylation or gene expression were found [44]. Although an enhanced gene expression of activating receptors could lead to a higher NK-cell activity and therefore could have repercussions on cancer risk, inclusion of only two KIR genes in this study did not allow to draw such conclusions. In a second (pilot) study, Schenk et al. described the effect of an incremental exercise test on a bicycle ergometer in 5 healthy women. This again showed no changes in the percentages of $CD56^{bright}$ and $CD56^{dim}$ cells after exercise. Interestingly, DNA methylation in NK cells was affected by the exercise intervention at 33 targets, corresponding to 25 genes having different roles in cell regulation [47]. Moreover, Park et al., who compared immunologic responses to a submaximal exercise on a cycle ergometer at sea level (normoxia) and at simulated 3000m (normobaric hypoxia condition at 14.5% O_2 concentration) in 10 young girls also found no effects of exercise and conditions on NK-cells [48]. The same was true for a next study assessing the effect of a cardiopulmonary exercise testing (CPET) in 16 patients with idiopathic pulmonary arterial hypertension (IPAH) and 10 healthy adults on NK-cells. No exercise-induced effects on levels of NK-cells could be found [39]. In a next study comparing the effect of low intensity strength training with blood flow restriction and high intensity strength training, a significant decrease after 24 hours in the percentage of NK-cells among total was observed only following high intensity strength training [49]. In contrast, Fuhro et al. were able to show that moderate intensity cycle-ergometer exercise between two sessions of hemodialysis (HD) in patients with chronic kidney disease could prevent decrease in NK cells. Indeed, while control HD therapy trial resulted in a decrease in frequency of NK, NK $CD56^{bright}$ and $CD56^{dim}$ subsets, HD therapy after intra-dialytic exercise trial did not [50]. Exercise interventions could help counteract HD-linked disorders, which are characterized by decreased NK cell counts [51]. Asimakos showed an increase in NK cells percentage after both inspiratory resistive breathing and whole body exercise. All subsets showed no differences with baseline levels 120 min after exercise. [25]. Moreover, an increase in percent and absolute counts of NK cells was also observed after treadmill running at 40% VO_{2max} in the heat [33]. Additionally, Gustafson et al. also reported an increase in NK cell counts 2 to 5 minutes after exercise. However, this peak in NK cell counts was only significant after the maximal exercise intervention and not after an endurance protocol, suggesting that NK cells sensitiveness to high workload regimens. $CD56^{br}CD16^{-}$ cells increased to a lesser extent than $CD56^{+}CD16^{+}$ NK cells. Remarkably, NK cells showed the greatest degree of change compared to other cell populations in both interventions [24]. Turner et al. also found increased NK-cells counts immediately after exercise after both HIIE (400% increase)

and CONT (600% increase) with significant differences between the two exercise interventions. The same was also true for $CD56^{dim}$ cells, with approximately a 550% increase after HIIE and a 725% increase after CONT. A smaller, but still significant, increase was also observed in $CD56^{bright}$ cell counts with 100 and 200% increase after HIIE and CONT, indicating that this subset might be less sensitive to exercise-induced responses. Moreover, no differences between the two exercise regimens were found for this subset. Rolland-Debord et al. also showed an increase in %CD16 and %CD56 cells after an incremental test on a cycle ergometer, with or without blood flow restriction. Similar peak values were found for both interventions, but the peak values during blood flow-restricted exercise were significantly greater than those at iso-workload. Interestingly, this increase was related to the concentration of (nor)epinephrine and to the global activation of both respiratory and expiratory muscles. Noteworthy, these different interventions were performed on consecutive days. Therefore, washout of effects from the previous intervention may not be completely guaranteed [52]. Contrasting results on NK cells between different exercise interventions suggests that exercise type and intensity is an important factor for the mobilization of these cells. Mobilization of NK cells to the peripheral blood in response to exercise is orchestrated by several mechanisms, such as increased shear stress and peripheral blood flow [53]. As blood flow and shear stress increase during exercise are intensity-dependent, the intensity of the exercise is an important factor to consider [54].

As a last paper in untrained, young participants, Brown et al. showed that 20 min of steady state cycling at 80% of VO_2max resulted in an increase of absolute counts of dendritic cells (DC, by 150%) followed by a decrease to baseline levels 30 min post-exercise. This was also observed in both plasmacytoid and myeloid DC's, with the exception of the CD1c-CD141+ subgroup. Remarkably, plasmacytoid DC mobilized to greater extent than myeloid DC. In myeoloid DC, a stepwise mobilization pattern was identified, with the largest magnitude of exercise-induced change observed in CD1c-CD141-, followed by CD1c+CD141-, CD1c+CD141+ and CD1c-CD141+ [55]. Previously, it has been shown that the majority of DC's and subpopulations also express a marker for apoptotic/necrotic cells, CD205 [56]. Intriguingly, Brown et al. found a trend for a larger exercise-induced mobilization of CD205- cells in most subpopulations. Regarding CD1c-CD141+ and CD1c+CD141+ cells however, the mobilization among CD205- cells was significantly greater than among CD205+ cells. On the contrary, this trend was reversed in plasmacytoid DC, where CD205+ cells tend to be more mobilized [55]. Plasmacytoid DC are involved in the antiviral and auto-immunity as they are effective sensors of intracellular viral or self DNA and RNA[57]. Myeloid DC are specialized in the processing and presentation of antigens to T-cells, bridging the gap between innate to adaptive immunity [58]. Therefore, preferential mobilization of plasmacytoid DC's might be related to their function, namely for their effector responses, for example against viruses [55].

*Acute Exercise-Induced Effects in Young and Middle-aged Humans - **Trained condition***

Kostrzewa et al. studied the effects of a progressive efficiency test until exhaustion on a mechanical treadmill in soccer players. The same physical effort at two time- points, in spring and autumn during the beginning of preparatory phases to competition rounds, showed different exercise-induced effects. Percentages of CD4+ naïve T-helper cells were significantly higher after 17 hours recovery compared to pre-exercise levels in spring, but this was not significant in autumn. Overall, percentages of naïve T cells after exercise (post and recovery) were higher in spring compared to autumn. Percentages of effector and memory T-helper cells were decreased 17 hours post-exercise compared to pre-exercise in spring, but not in autumn [59]. The same experiment in other soccer players was repeated with a couple of years interval. Responses in CD4+ naïve T-cells in both experiments were similar, with the exception that the 17h post-exercise increase was now significant both in spring and

in autumn. Remarkably, percent CD4+ CM cells was increased 17 hours post-exercise in spring while a decrease was observed in the previous study. Regarding CD4+ EM cells, a decrease 17 hours post-exercise compared to baseline at both time points was now observed compared to only in spring in the first study. Regarding CD8+ subsets, only an increase in CM cells in spring was significantly different from baseline. Naïve and EM subsets showed no significant changes compared to baseline [60]. Exercise-induced effects after the same intervention were also studied in 3 elite karate athletes. In contrast to the two previous studies, percentage of naïve CD4+ cells decreased significantly directly after exercise and increased again 17 hours post-exercise. Here, the intervention also induced a significant upregulation of the percentage of CD8+ EM after exercise, but no longer after 17 hours. No significant changes could be observed for CD8+ CM cells, confirming the results observed in autumn in the previous study. Also in line with the previous studies, no significant changes could be observed for CD8+ naïve, CD4+ CM and EM [61]. Although the same exercise intervention was used in the three previous studies, differences in T-cell subset mobilization could still be observed. However, some differences might be attributed to the low number of participants included in the last study (n=3). In line with aforementioned studies, no effects on CD8+ naïve cells were found in another study investigating the effects of a 30 min cycling session in participants who regularly performed vigorous exercise. However, this intervention did induce a post-exercise increase in CD8+ CM, EM and EMRA counts before returning to baseline levels after 1 hour. Only more differentiated T-cell phenotypes thus seemed to be mobilized. Moreover, authors showed that this mobilization was largely dependent on cathecholamine signaling through the β_2 adrenergic receptors, corroborating earlier suppositions regarding the factors playing a role in exercise-induced lymphocyte mobilization [62]. Lavoy et al. determined the effect of exercise intensity on T-cell subsets in 17 cyclists asked to perform three 30 minutes cycling trials at intensities of -5% (low intensity), +5% (medium intensity) and +15% (high intensity) of blood lactate threshold. In contrast to previously mentioned studies, exercise led to a post-exercise increase in numbers of low (CD27+CD28+), medium (CD27+CD28-) and highly (CD27-CD28-) differentiated CD4+ and CD8+ T-cells. However, differences 1 hour post-exercise were found as counts of low and highly-differentiated CD4+ T-cells, but not medium differentiated, decreased below baseline levels. In contrast CD8+ subsets returned to baseline. As expected, exercise intensity had a significant impact of T-cells mobilization. Indeed, intensity significantly affected medium-differentiated CD4+ T-cells as well as low- and medium-differentiated CD8+ T-cells. This resulted in greater counts of these cells in the high intensity condition versus the low intensity condition, but also in low-differentiated CD8+ counts in the medium intensity condition compared to low intensity. Moreover, time x intensity interactions were also found for medium differentiated CD4 and CD8 subsets. Exercise at high intensity resulted in a larger ingress and egress compared to low intensity. Moreover, differences in CD4+ subsets ingress were found between low intensity and medium intensity and in the ingress and egress of CD8+ subsets when comparing medium and high intensity interventions. Interestingly, authors also showed an impact of cytomegalovirus (CMV)- serostatus on mobilization of these subsets [63]. CMV is a common virus affecting approximately 60 to 90% of all people. CMV infection is generally asymptotic or associated with mild symptoms in healthy people. However, persistent viral infection may cause an increase of (CD8+) memory T-cells with a more restricted repertoire. This so-called 'memory inflation' is likely associated to viral reactivation during life and is also associated with a decrease in the naïve subset [64, 65]. Indeed, LaVoy et al. showed that greater numbers of highly-differentiated subsets were found in CMV+ individuals. CMV x time effects were also observed for medium- and highly differentiated subsets. A greater ingress of medium-differentiated CD4+ and low-differentiated CD8+ T-cells was found in CMV- participants. In contrast, CMV+ participants had a greater ingress of highly differentiated CD4+ cells. Regarding the recovery, CMV- had a higher egress of medium-differentiated CD4+ cells while CMV+ had a higher egress of highly differentiated CD4+ cells. Lastly, interactions for intensity and CMV were also found: counts of

low-differentiated CD4+ and CD8+ cells were lower in the low intensity condition compared to medium intensity condition [63]. Zimmer et al. investigated the effect of a half marathon in 9 breast cancer survivors and a healthy control group. Naïve and memory CD4+ T-cells were elevated in respectively the control group and the patient group at baseline. Participation in a half marathon had no effects on the percentages of both subsets and neither time or time x group effects were observed [66].

Minuzzi et al. investigated the effects of a progressive exercise test to exhaustion on mobilization of senescent (KLRG1+) cells in master athletes and healthy controls. In general, KLRG1+ CD4+ and CD8+ cells increased 10 min post-exercise in the master athletes before returning to baseline after 1 hour. However, this increase was not statistically significant in healthy controls. There was also a significant difference between these two groups, master athletes showing reduced pre-exercise levels of KLRG1+ cells. No significant exercise-induced changes were observed for KLRG1+ naïve cells. While the intervention had no effects on KLRG1+ CD4+ CM cells, an increase in both the athletes and control group was observed in the CD8+ subset 10 min post-exercise. Both CD4+ and CD8+ KLRG1+ EM subsets were elevated after exercise in master athletes. Remarkably, after 1 hour a decrease was observed in the CD4+ EM subset in the control group whereas a decrease in the CD8+ EM subset was observed after 1 hour in master athletes. The exercise intervention had no effects on KLRG1+ EMRA cells in the control group. In athletes, an increase in KLRG1+ CD4+ EMRA+ cells was observed post-exercise. One hour after exercise, a decrease was observed in the CD8+ EMRA subset while no significant change was observed for the CD4+ subset in master athletes. Interestingly, differences between athletes and healthy controls could no longer be observed in EM and EMRA cells, with the exception of KLRG1+ EM cells one hour after exercise [67].

In trained individuals, exercise-induced effects on CD4/CD8 ratio seemed to differ from effects observed in the untrained. While most of the studies retrieved in the untrained showed a decrease in this ratio post-exercise, this was not the case in trained individuals. Morgado et al. showed that a high intensity swimming training session in athletes resulted in an increased CD4/CD8 ratio. Although this ratio was still elevated 2 hours post exercise, it returned to baseline levels after 24 hours [68]. A study from Wadley et al. however also showed a decrease in CD4/CD8 ratio immediately post-exercise in both trained and recreationally active men after a cycling exercise test to exhaustion. Moreover, this ratio was still significantly lower than baseline 15 minutes after exercise in recreationally active men. Although no further follow-up was performed during recovery, a significant increase after 15 minutes compared to immediately post-exercise seems to go against a permanent decrease of this ratio. Interestingly, at baseline, CD4/CD8 ratio was higher in recreationally active compared to the trained. Moreover, significant time x group effects were also found during the intervention [69]. This perhaps indicates prolonged effects of regular exercise in the trained. Lastly, while investigating the effect 300 countermovement jumps followed by passive recovery in a supine position, Joisten et al. found no significant changes in CD4/CD8 ratio 2 or 72 hours after recovery intervention [70]. Therefore, although various interventions contribute differently to the CD4/CD8 ratio immediately after exercise, a return to baseline levels a couple of hours into recovery suggests that possible harmful effects of a post-exercise decrease of the immune system are limited to a rather short time window.

Eight articles describing exercise-induced interventions on NK-cells in trained individuals were retrieved, of whom three described the effect of 30 min cycling. Remarkably, they all resulted in a post-exercise increase in absolute counts of NK cells. Firstly, Graff et al. showed this increase after a fixed intensity cycling trial at a power output corresponding to +10% of individual blood lactate threshold. They also found an increase in CD57+, but not in NKG2C+ NK-cells [62], two markers associated with the evolution to terminally differentiated NK cells [71] immediately after exercise. More specifically, NKG2C-CD57-, NKG2C-CD57+, NKG2C+CD57-, but not NKG2C+CD57+ counts increased immediately

after exercise and returned to baseline levels 1 hour after exercise. Interestingly, evidence was provided for a cathecholamine-induced preferential mobilization of NK-cells through β-2 adrenergic receptor signaling [62], as described earlier in this review. Secondly, Gupta et al. showed an increase (doubling) of the percentage NK-cells after cycling at 15% above calculated lactate threshold. Percent $CD56^{bright}$ cells decreased immediately after exercise and increased 1 hour later. On the contrary, the percentage of $CD56^{dim}$ cells increased post-exercise, but decreased below pre-exercise levels after 1 hour. Among these, cells positive for the inhibitory NKG2A receptor were at their lowest post-exercise, while the Immunoglobulin-like receptor (KIR, acquired during differentiation) positive cells were at their highest. Proportions of highly differentiated subsets ($CD56^{dim}$NKG2A-/KIR+) were at their highest post-exercise, while the percentage of low differentiated subsets were lower. The exercise intervention had no effect on percent hyporesponsive NKG2A-/KIR- cells [72]. Lastly, Rooney et al. showed an increase in NK cell counts after 30 min cycling at 80% of the age-predicted maximum heart rate. Moreover, authors characterized the early egress kinetics of these cells by sampling after 1, 2, 3, 4, 5 and 10 minutes recovery. Compared to the last minute of exercise, a decrease was already observed only after 2 minutes recovery. Moreover, NK-cells showed a more rapid egress compared to CD3+, CD4+ and CD8+ subsets. Interestingly, within the first 2 minutes of recovery, NK-cell egress was correlated with heart rate recovery. This study thus emphasizes the importance of an adequate sampling in function of the research interests, e.g. peak determination or start of egress of subsets from the peripheral blood [73]. Additionally, an increase in NK cell counts immediately after exercise could also be observed after a rowing exercise of 2000 meter [74]. Moreover, a significant increase in percentages of NK-cells could also be observed after a progressive efficiency test until exhaustion in one out of two preparatory phases of soccer competition rounds [59]. In contrast, a decrease in NK cell counts was observed after an acute swimming exercise. These lower cell counts were maintained two hours post exercise and returned to baseline values after 24 hours. Interestingly, NK cell counts were higher in males compared to females before the intervention and throughout the 24 hours after exercise [68]. Moreover, a decrease in the percentage NK-cells was observed after a half marathon in both breast cancer patients and healthy controls. This decrease was still present after 24 hours recovery [66]. A last study described the effect of 300 eccentric unilateral repetitions of knee extensors on NK cells. No significant effects could be observed immediately after exercise. However, a significant reduction was reached two hours after exercise, which disappeared after 1 day [75].

Regarding T-regs, participation in a marathon resulted in a decreased percentage of Treg cells in CD4+ cells already after 1 hour, returning to baseline levels after 1 day. The percentage Treg cells among total lymphocytes increased after 1 day. Meanwhile, absolute counts of Tregs decreased 1 hour after exercise but increased above pre-exercise levels after 1 day. Therefore, this increase after 1 day might serve as a compensation mechanism to avoid excessive cell damage after the marathon and to return to an anti-inflammatory environment. Interestingly, naïve and terminally differentiated subsets showed a different response to exercise. Indeed, while the naïve Treg population decreased after 1 hour but returned to baseline levels the next day, more mature Tregs showed no difference in percentage or counts after 1 hour, but substantially increased the day following the marathon [76]. In contrast, participation in a half marathon resulted in a decrease in percent T-regs in CD4+ T-cells after 24 hours recovery in both cancer patients and healthy controls [66]. Three studies also described T-reg responses to a shorter intervention, namely a progressive efficiency test to exhaustion. Two studies described no change in percent T-regs post-exercise. However, an increase was observed 17 hours later in karate athletes [61] and soccer players [60]. Lastly, Minuzzi et al. described effects of this test in master athletes and a sedentary control group. They showed no difference in % T-regs compared to the CD4+ T-cells or whole lymphocyte population. There was also an increase in absolute counts of Treg cells 10 minutes post-exercise in both groups, returning to pre-exercise levels after only one hour.

Furthermore, no effects on percentage of naïve or memory could be observed [77], contrasting with results from Clifford et al [76]. There were also no effects on the percentage of senescent KLRG1+ T-regs. Noteworthy, KLRG1 expression was found in 33% and 50% in the master athletes and control group respectively. However, it's expression on T-regs was rather low, with around 2% expression in master athletes and 3,1% in the control group at baseline. Proportions of naïve, memory and total T-regs at baseline were similar between both groups [77]. Additionally, Svendsen et al. showed no effect on T-regs after 75 min cycling in normoxia or hypoxia as long as two hours post-exercise [78]. Moreover, a rowing performance test (2000m) in elite rowers also did not result in significant effects on T-reg counts 1 min and 24 hours after [74]. A last study showed a significant increase in percent CD4+CD25+CD39+ memory Treg both in low and highly active men after a high intensity interval exercise. Interestingly, this increase was significantly higher in high physically active men. Noteworthy, a positive correlation with $V0_2max$ was found at baseline, suggesting a possible effect of aerobic fitness status on immunoregulatory cells [79].

Lastly, Lackermair et al. compared the influence of a polyphenol-rich died versus placebo on DC in 100 male endurance athletes after a marathon run. In the placebo-group, the percentage of myeloid DC increased significantly one hour after the marathon and was still elevated after 72 hours. In contrast, the percentage of plasmocytoid DC decreased 1 hour after the marathon and gradually recovered, but was still incomplete, from 24 to 72 hours post-marathon [80]. This contrasts with the increase in plasmacytoid DC's observed post-exercise in the untrained. However, Brown et al. only described effects on DC until 30 minutes after exercise [55], whereas Lackermair et al. sampled as long as 72 hours after exercise [80].

Taken together, 37 articles describing acute-exercised effects in trained and untrained young adults were retrieved in this literature update. Despite the high number of retrieved articles, a great heterogeneity in exercise-induced effects still persists for different senescence-associated markers. A great variety in types of exercises described (e.g. the effect of 30 min cycling VS participation in a half marathon on T-regs), but also differences in study design (e.g. the absence or presence of control groups) and the levels of described subpopulations (e.g. considering total T-regs or subdividing this cell type into naïve and memory types) most certainly contribute to these differences. However, most newly retrieved evidence was able to confirm the previously observed acute-exercise induced mobilization of senescent T-cells in the blood circulation and suggest a mobilization of DC's in young to middle-aged adults (Table 3).

Acute Exercise-Induced Effects in older adults

Only one study describing the effects of an acute exercise bout in older adults was found. Van der Geest et al. studied the acute effects of participating in the Nijmegen Four Days Marches (30 km/day) in octogenarians. Although the participants were physically fit, 14 out of 20 suffered from at least one disease, such as cardiovascular diseases or cancer. The walking exercise did not alter the CD4/CD8 ratio. Exercise increased the cell counts of naïve, central memory, effector memory and terminally differentiated CD4+ T-cells. Interestingly, these changes appeared to be linked to CMV seropositivity. However, a significant increase in naïve CD4+ T-cells was also found in CMV-negative participants. Both recent thymic emigrants (CD31+) and central naïve (CD31-) cells were mobilized, but mobilization of naïve CD4+ T-cells in CMV- participants seemed to be caused by the CD31- subset. Regarding CD8+ T-cells, there was a significant increase in the naïve and effector memory subsets, but not in the central memory and terminally differentiated subset counts. Similar to what was observed in younger adults, these changes were again associated with CMV status. Walking also influenced regulatory T-cells, in particular naïve T-regs, as exercise increased those cell counts. Although this increase was not dependent on CMV-status, a more pronounced increase could be observed in CMV seropositive

participants. In contrast, CD45RA- memory T-reg counts remained stable. Interestingly, there was also a decrease in NK cells numbers, contrasting with the results of previous studies in older adults (Table 3). This decrease was caused by a decline in $CD56^{dim}$, but not $CD56^{bright}$ NK cells. Interestingly, this decrease was observed only in seronegative adults. Furthermore, in seronegative participants, no changes in the frequencies of activating and inhibitory receptors within $CD56^{dim}$ NK cells were observed. In seropositive participants, a downregulation of the expression of inhibitory receptors (KIR2DL1, KIR2DL2, KIR2DL3 and KIR3DL1) was observed, with the exception of increasing NKG2C subsets. Meanwhile, an increase in the percentage of NK cells expression activating receptor NKG2D was found while expression of four other activating receptors was unchanged. While NK cell counts in seropositive participants did not significantly change after post-exercise, the exercise intervention likely resulted in a less inhibited NK cell phenotype [81].

Although only one study was found describing acute exercise-induced effects in older adults, this study provided evidence for the -previously unreported- mobilization of naïve regulatory T-cells after exercise (Table 3). Overall, available evidence seems to suggest an increase in naïve and memory T-cells after acute exercise in older adults.

Long-term exercise-induced effects

Long-term exercise-induced effects *in Young and Middle-aged Humans*

Philippe et al. compared concentric (uphill walking) and eccentric (downhill walking) in 16 men with impaired glucose tolerance. Following 3 weeks of training at a frequency of 3 trainings a week at a pace perceived as somewhat hard, increases in percent CD8+ naïve cells, but not in CD4+ naïve cells were observed. Furthermore, increases in CD4+ and CD8+ central memory cells were observed after 3 weeks training. No changes were found in effector memory cells. In contrast, training induced decreases in percent CD4+ and CD8+ EMRA cells, suggesting a shift from more differentiated to less differentiated T-cell subsets. Overall, no differences between the concentric or eccentric intervention were found. Lastly, the influence of CMV-status was also investigated in this study population. However, in contrast to acute exercise-induced effects, no changes in T-cell population could be accounted by CMV-status [82]. From the 13 articles describing long term-exercise effects, only one described effects on exercise-induced redistribution of naïve, memory en EMRA T-cell subsets. However, this was a valuable contribution to the original review as no articles describing effects on naïve and memory T-cells after long-term exercise in young adults could be retrieved before 2016. Furthermore, first evidence regarding exercise-induced apoptosis after long-term exercise in young adults was provided by Schlabe et al. These authors described a decrease in the percentage apoptosis of CD4+ T-cells observed in 13 HIV-infected patients after 12 months of moderate endurance training as a preparation for a marathon. There was also an increase in CD4+ cell counts, which is suggested to be related to the reduced apoptosis-associated cell death in these cells [83].

Next, effects of long-term exercise on CD4/CD8 ratio were also provided. Philippe et al. showed an increase in this ratio after only 3 weeks of eccentric or concentric walking training at a frequency of 3 times a week [82]. If confirmed by other studies, aerobic exercise could thus reveal itself as an affordable and relatively fast way to counteract an inverted CD4/CD8 ratio, which is a predictor of premature death [31] and part of the immune risk profile. However, in elite judokas and swimmers, approximately 2 months respectively judo- and swimming training induced no changes in CD4/CD8 ratio, nor differences between groups [84]. Furthermore, Broadbent et al. also found no significant changes in the CD4/CD8 ratio in patients with chronic fatigue syndrome after performing graded and intermittent exercise [85].

Dungey et al. described the effects of 6 months cycling at a frequency of 3 times/week on T-regs of haemodialysis patients. They found a trend for an increase in the absolute counts of T-regs in exercising patients, while a decrease was found in non-exercising patients. Furthermore, the magnitude of change in exercising patients differed significantly from those who did not exercise. Given that a decreased T-reg capacity in HD patients possibly underlies chronic immune activation and inflammation in these patients, this change possibly reflected an enhanced anti-inflammatory activity. However, no changes were found in the percentages of T-regs of total CD4+ lymphocytes, indicating that this change may be due to differences in CD4+ cell counts [86]. Deckx et al. showed that 12 weeks of combined endurance and resistance training in multiple sclerosis patients had no effects on the percentage of T-regs [87]. Further research is needed to make conclusions regarding T-reg mobilization after long-term exercise. Deckx et al. also showed an increase in absolute counts of plasmacytoid and conventional DC. However, the increase in conventional DC was also observed in the non-exercising control group. Furthermore, there was an increase in counts of plasmacytoid DC expressing CD80 and CD62, indicative of an activated phenotype, in the exercising group. A decrease in CD86+ plasmacytoid DC was observed in both groups. No differences could be observed for CCR5 and CCR7 plasmacytoid DC. No changes were observed for counts of CD80+ and CD86+ and CD62L, CCR5, CCR7 conventional DC after exercise. Moreover, a positive correlation was found between Treg regulatory type 1 cells and counts of CD80+ plasmacytoid DC's [87]. This possibly suggests the involvement of these DC subsets in the development of T-regs, as stated earlier in literature [88].

Contrary to evidence available in 2016, most of newly retrieved data suggests that long-term exercise has no effect on NK-cells. Indeed, 2 weeks of STOTT Pilates, a branded contemporary approach to Pilates encompassing new insights on spinal rehabilitation, fascial integration, muscle conditioning and athletic performance [89] in 10 healthy women at a duration of 180 min a week had no effects on the percentage or absolute counts of NK cells [90]. Moreover, a 12 week home-based exercise program, consisting of aerobic, resistance and flexibility training, in thyroid cancer patients did not significantly affect NK counts. However, it did augment NK cell activity significantly [91]. Furthermore, 3 additional studies showed no effects of a resistance training program on NK cells. This was observed after 12 weeks of 3 sessions a week in healthy sedentary young males [92], but also when performed two times a week during chemotherapy in breast cancer patients. In contrast, endurance training in this population reduced absolute numbers of NK-cells. Although not significant, an almost significant trend to decrease was also observed in the non-exercising usual care group (p=.05) [93]. Next, Hagstrom et al. showed that after 16 weeks resistance training in breast cancer survivors, the difference in change between the exercising and non-exercising control group was not significant [94]. Twelve weeks of graded exercise intervention in patients suffering from chronic fatigue syndrome also did not result in significant differences in NK cell counts. However an increase was observed after intermittent exercise [85]. Chamorro-Viña et al. investigated the effect of 10 weeks of moderate intensity training, both strength and aerobic training for 60 min, 3 times a week on NK cell subsets in 3 children (3 other in the control group) having undergone a hematopoietic stem cell transplantation. This intervention resulted in a significant increase in the mean ratio (pre/post) of $CD56^{dim}$ in the intervention group. No significant differences were found for the mean ratio of $CD56^{bright}$ [95]. As the early immune reconstitution after HSCT is mainly characterized by less cytotoxic $CD56^{bright}$ NK subtypes, the authors suggest that moderate intensity exercise may help restore the observed imbalance in NK subpopulations by inhibiting immature, less cytotoxic NK cell subsets [96, 97]. Lastly, Schenk et al. investigated shifts in NK-cells and promotor of DNA methylation of specific KIR genes after 4 weeks of endurance interval exercise program in 9 healthy women compared to a passive control. They reported no effects of intervention on the percentages of $CD56^{dim}$ and $CD56^{bright}$, both acute (described earlier) and chronic exercise did not result in significant exercise-induced alterations. No time x group effects were found

either. After chronic exercise, a single CpG in the KIR2DS4 promotor had an increase in DNA methylation for the intervention group and a decrease for the passive control group, but no correlations with gene expression were found. No changes in DNA promotor methylation or gene expression were found for KIR3DL1. [44].

Two studies investigated the effects of long-term training on an acute exercise response. Patiño et al. investigated whether aerobic physical training (APT) conducted over a period of 6 months (30 min, 3x/week) could condition against strenuous exercise-induced changes in immune function. Therefore, previously untrained man followed and APT training during 6 months. At baseline and after 3 and 6 months APT-training, this intervention group and a sedentary control group (no APT) performed a cardiopulmonary exercise test (CET). Immediately after CET, an increase in NK-cells was observed at the 3 time points before returning to baseline levels after 24h in both intervention and control group. Furthermore, the overall APT program had no effect on resting levels of NK-cells before and 24 hours after the CET. Therefore, long-term APT training did not influence resting levels of NK cells or the mobilization of NK cells after an acute exercise bout [98]. Tsai et al. investigated the effect of 6 weeks of high-intensity interval training (HIIT), moderate-intensity continuous training (MICT) (5x/week for 60 min) or no intervention on hypoxic exercise (HE) test on lymphocytes subsets in healthy males. Acute HE before and after 6 months training decreased percentages of CD62L+ and CD28+ while increasing percentages of CD57+ lymphocytes. Only the hypoxic-exercise induced increase in CD57+ after 6 months HIIT training was not significant. After 6 weeks, HIIT training was shown to increase the percentages of CD28+ and decreased percentages of CD57 lymphocytes at rest and after HE. MICT induced the same changes, but only after HE. Additionally, none of the interventions changed the percentage of CD45RA+, CD45RO+ or CD11b+ lymphocytes at rest of after HE [99].

Taken together, 15 articles were retrieved describing the effect of long-term physical exercise in young to middle-aged adults. Importantly, these newly retrieved articles uncovered evidence for exercise induced-effects in a variety of subsets. Indeed, new evidence was provided for different cell types such as naïve and memory T-cells, but also for the CD4/CD8 ratio. Results however were still inconclusive as some studies reported an upregulation and others no effect. Moreover, previously lacking evidence was also provided for DC, suggesting an exercise-induced upregulation in plasmacytoid DC's. Many articles were retrieved describing effects on NK-cells, however without reporting conclusive results. Long-term exercise did not seem to influence T-regs, contrasting with results observed after acute exercise. Lastly, one article described a decrease in apoptosis of lymphocytes, again contrasting with results found in the same population after acute exercise (Table 3).

Exercise-Induced Effects on Basal Levels in older adults

Only 4 articles described the effect of long-term exercise in older adults. Cao Dinh et al. were the first to show reductions in senescent phenotypes after 6 weeks training in community dwelling women aged 65 and over. Indeed, they showed that strength endurance training (SET), but not intensive strength training (IST) or flexibility training (CON) reduced proportions of CD8+CD57+ and CD8+CD28-CD57+ senescent-prone phenotypes after 6 weeks training. Moreover, the observed decreases were also significant when compared to CON. In the CD8- population, post-exercise decreases in percentage CD8-CD57+ and CD8-CD28-CD57+ SPC were also observed in the SET group. Focusing on absolute counts, all CD8+ senescence-prone phenotypes were decreased in the SET group after 6 weeks. A significant time by group interaction was also found for the CD8+CD28-CD57+ subset. Moreover, a significant decrease for CD8+CD57+ and CD8+CD28-CD57+ subtypes and a trend towards a decrease for the CD8+CD28+CD57+ were also found in SET compared to CON. No significant differences regarding absolute counts of CD8- SPC were found in the different intervention groups. Additionally, an overall significant increase after 6 weeks was observed for proportion of both CD8+ and CD8-

memory phenotypes. However, no changes were observed within the groups separately. Moreover, no significant differences were observed for naïve phenotypes. No changes were observed post-exercise for absolute counts of naïve and memory T-cells [100]. As expected from literature, CMV status also had an influence on exercise-induced T-cell mobilization. In the same population, six weeks of SET, but not IST or CON reduced absolute counts of CD8+CD57+, CD8+CD28-CD57+, CD8-CD57+, CD8-CD28+CD57+ senescent-prone phenotypes in CMV+ women. These decreases were also observed when comparing SET with CON. Moreover, no pre-to post- changes in absolute counts could be found in CMV- participants. Six weeks of training also reduced percentages of CD8+CD57+ and CD8+CD28-CD57+ SPC in the SET group only after exercise. Moreover, these changes were also observed when compared to the CON group. At last, 6 weeks of SET reduced all percentages of CD8- SPC in the CMV+ group. A significant inverse correlation was also found between the proportion of CD8- naïve and the decline in CD8- SPC was found. Similarly to absolute counts, percentages of T-cell phenotypes in the CMV seronegative group were not significantly altered [101]. In another study Gomes et al. investigated the effects of a 12-weeks walking training program (3x/week) in 16 older women with knee osteoarthritis. The training program had no effect on the percentages of CD4+CD28+ or CD8+CD28+ T-cells [102]. Since the exercise-intensity was different between these studies, effects of long-term exercise on CD28+ expression in older adults might strongly depend on the type and intensity of the training program. Intriguingly, a last study in previously sedentary older women, aerobic exercise showed a decrease of the CD4/CD8 ratio while resistance exercise did not after six months of training. However, this could be due to a premature blood sampling, too shortly after the last training session before the acute effects have been washed-out [103].

Although articles describing effects of exercise in older adults are still underrepresented, previously lacking evidence was finally provided for the effect of long-term exercise on senescent T-cells. More so, this new evidence suggests an intensity-dependent effect of exercise on these cells. New evidence showed no exercise-induced effects on the expression of CD28, supporting some of the previously retrieved evidence on this co-stimulatory molecule. Overall, more evidence is needed to provide a clear overview of exercise-induced effects in older adults.

The strength of this review lies within its thorough analysis of most recent available data originating from primary intervention studies. Results of this literature study were reported under the form of a systematic review; unfortunately the heterogeneous results precluded a meta-analysis. It is important to bear in mind that this in-depth analysis on the effect of exercise on immunosenescence-related markers of immune cells does not entirely cover the broad spectrum of immunosenescence, as this is a complex process that cannot be limited to fluctuations in the cellular composition of blood only. Indeed, immunosenescence is a long-term process consisting of whole-body, system-level changes in the function and effectiveness of a broad range of integrated immunological processes. Moreover, as retrieved studies describe indirect measurements (analysis of surface markers) and often report no clinical outcomes, research on the underlying mechanisms of action of exercise-induced effects is necessary to complement the findings of this review. For example, experimental evidence regarding the hypothetical induction of apoptosis targeting senescent cells after their exercise-induced mobilization [20] is still lacking and should therefore be examined in the future. This will allow us to draw stronger conclusions regarding the potential of exercise to prevent or counter immunosenescence and related negative outcomes, such as a reduced vaccination response. Exercise has already been shown to boost antibody and cell-mediated responses to vaccination, especially in older adults [104]. Although age-related changes in vaccination response can not be attributed to cell numbers alone [105], the exercise-induced increases in naïve T-cells in older adults might be beneficial to the vaccination response. Together with the exercise-induced increase in dendritic cells in younger adults, these naïve cells could potentially play a role in inducing a better vaccination response, as these

two cell types are essential to the immunization process [106]. Moreover, depletion of regulatory T-cells could perhaps contribute to vaccination responses, as the presence of these cells was in some cases shown to have counterproductive effects on vaccination [107]. However, as the exercise-induced effects on regulatory T-cells currently lack uniformity, potential implications on vaccination remain unclear. In older adults, exercise-induced mobilization of CD8+ cytotoxic T-cells, but also CD4+ helper T-cells and dendritic cells could potentially be of particular interest for the immunization process as these cell types were shown to be compromised in this population [108]. In general, more research on underlying mechanisms of exercise on immunosenescence-related cellular markers and associated effects on vaccination is needed. Therefore, burning research questions regarding the possible effect of exercise on apoptosis of senescent T-cells and implications of this – as hypothetically proposed by Simpson et al. [20] - newly created immune space on naïve T-cells counts and related antigen repertoire should be addressed in a near future. Moreover, it will be of particular interest to investigate the potential effect of the acute mobilization of immune cells following repetitive bouts of exercise on basal level changes. It can indeed be expected that repetitive bouts of exercise (e.g. 2-3 times per week during 6-12 weeks) and the accompanied acute mobilization of immune cells with a specific immunosenescence phenotype will finally induce the long-term adaptations in basal levels. Moreover, implications of exercise-induced mobilization of regulatory T-cells should also be investigated as this cell type appears to receive increasing attention in the context of immunosenescence. Finally, more research is still needed on the effect of exercise in older adults, especially regarding the exercise-induced effects on senescent T-cells, apoptosis of T-cells and dendritic cells.

CONCLUSION

This review confirms the considerable effects of physical exercise on cellular markers related to immunosenescence on immune cells. Although in general, data regarding exercise-induced effects in older adults remains scarce compared to younger adults, this review uncovers previously lacking data in older adults. Importantly, evidence was finally provided for the ability of long-term exercise to decrease senescent T-lymphocytes in older adults. Additionally, newly retrieved evidence reveals the ability of acute exercise to induce the mobilization of naïve and memory cells in older adults. Importantly current results highlight the major influence of the type (e.g. aerobic versus resistance training) and intensity of exercise on immunosenescence-related cellular markers, especially in older adults. Experimental evidence on underlying mechanisms however is still lacking and needs to be addressed in the future in order to develop optimal exercise regimens.

DECLARATION OF COMPETING INTEREST

None of the authors have any conflict of interest with any entity with regard to this study.

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Figure 1. Flow of the literature search

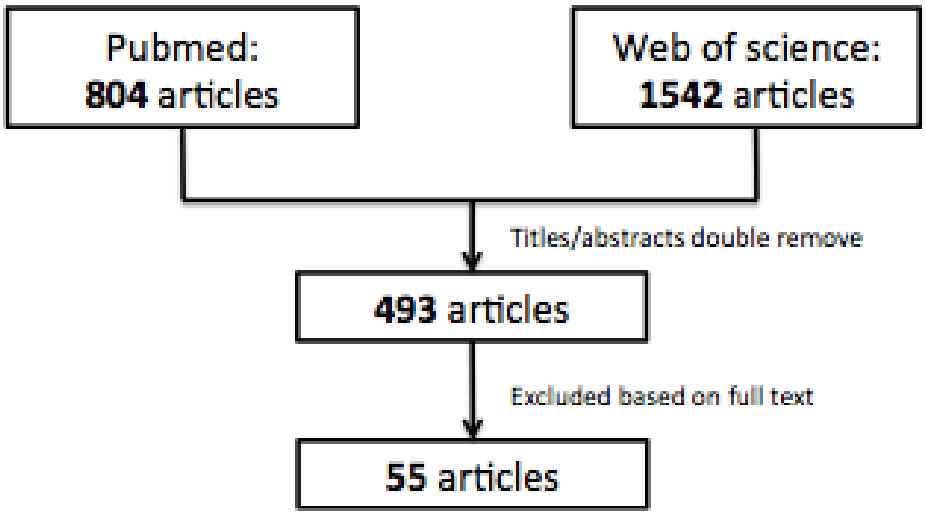
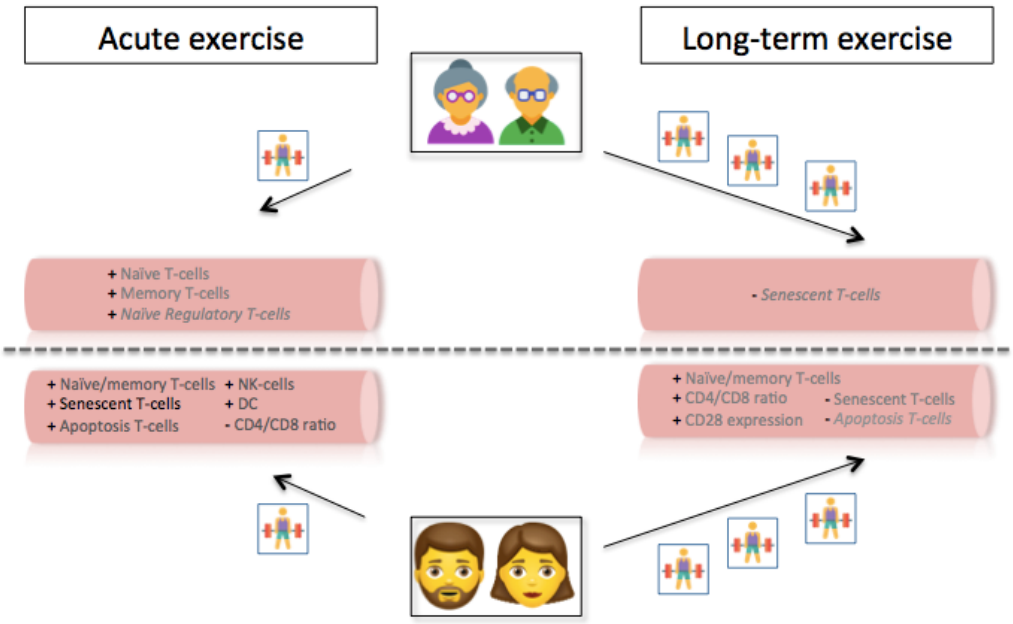


Figure 2. Graphic overview of the main findings in young and older adults



'+' indicates an increase in absolute counts or percentages after exercise; '-': indicates a decrease in absolute counts or percentages after exercise; color intensity of subset names refers to the amount of available evidence and the uniformity of results: light grey indicates a low amount of available evidence and/or a greater heterogeneity among results, dark grey indicates a high amount of available evidence and/or uniformity between results; cell types in *italic* refer to the need for further confirmation as only one article describing this effect could be retrieved; NK= Natural Killer cells, DC= Dendritic Cells; Icons retrieved by Icons8 (https://icons8.com)

Table 1. Overview of cell surface makers used to discriminate between A) innate and B) adaptive immune system subsets

A) Innate immunity components

NK cells [4, 8, 109]	CD3- (CD16+) CD56+
NK bright	CD3-CD56^{bright}(CD16 dim/-) *
NK dim	CD3-CD56^{dim}(CD16+) *
DC [4, 5, 8, 109]	
Myeloid	Lineage-HLA-DR+CD303-/BDCA1
Plasmacytoid	Lineage-HLA-DR+CD303+/BDAC2

* as obtained per gating on flow cytometry [110]

B) Adaptive immune components

Naïve T-cells [2, 4, 5, 8, 109]	CCR7+/CD62L+/CD197+ CD45RA+ (CD27+CD28+)/ CD28+CD57-
Memory T-cells [2, 4, 5, 8, 109]	CD45RO +
CM T-cells	CCR7+/CD62L+/CD197+ CD45RA- (CD27+CD28+)
EM T-cells	CCR7-/CD62L-/CD197- CD45RA- (CD27-CD28-)
EMRA T-cells	CCR7- CD45RA+ (CD27-CD28-)
SPC T-cells [8, 10, 111]	CD28-CD57+ / CD28+CD57+
MAIT cells [112]	Vα7.2+CD161+
Regulatory T-cells [4, 8, 38]	CD4+ (FoxP3+) CD25+ CD127-
Naïve	CD45RA+
Memory	CD4+CD25+CD39+
Terminally differentiated	CD45RA- / HLA-DR+

Table 2 Quality assessment (NICE)

	Selection bias			Performance bias			Attrition bias				
	S1	**S2**	**S3**	**P1**	**P2**	**P3**	**A1**	**A2**	**A3**	**A4**	**A5**
Abd El Kader et al. [103]	U	U	U	Y	N	N	U	U	U	12 in AE, 14 in RE	U
Broadbent et al. [85]	Y	Y	U	Y	N	N	Y	2 in GE and 1 in UC	Y	O	Y
Cao Dinh et al. [101]	Y	Y	Y	Y	N	N	Y	4 in IST, 3 in SET	Y	4 in IST, 6 in SET, 3 in CON	Y
Cao Dinh et al. [100]	Y	Y	Y	Y	N	N	Y	4 in IST, 4 in SET	Y	4 in IST, 7 in SET, 3 in CON	Y
Chamorro-Viña [95]	Y	Y	N	Y	N	N	Y	O	Y	O	Y
Deckx et al. [87]	Y	Y	U	Y	N	N	Y	9 in EX, 9 in CON	Y	9 in EX, 9 in CON	Y
Hagstrom et al. [94]	Y	Y	Y	Y	N	N	Y	1 in EX, 4 in CON	Y	1 in EX, 4 in CON[$]	7
Ibrahim et al. [92]	U	Y	Y	Y	N	N	Y	0	Y	0	Y
Schenk et al. [44]	U	U	U	Y	N	N	Y	0	Y	0	Y
Schmidt et al. [93]	Y	Y	U	N	N	N	y	3 in RT, 9 in ET, 2 in UC	Y	3 in RT, 9 in ET, 2 in UC	Y
Tsai et al. [99]	U	U	Y	Y	N	N	Y	0	Y	0	Y

Table 2 continued

	Detection bias				
	Appropriate length of follow-up	**Precise definition of outcome**	**Reliable outcome**	**Investigators blinded to treatment**	**Investigators blinded to confounding factors**
Abd El Kader et al. [103]	U	Y	Y	Y	Y
Broadbent et al. [85]	Y	Y	Y	Y	U
Cao Dinh et al. [101]	Y	Y	Y	Y	Y
Cao Dinh et al. [100]	Y	Y	Y	Y	Y
Chamorro-Viña [95]	Y	Y	Y	Y	Y
Deckx et al. [87]	Y	Y	Y	Y	U
Hagstrom et al. [94]	Y	Y	Y	Y	U
Ibrahim et al. [92]	U	Y	U*	Y	U
Schmidt et al. [93]	Y	Y	Y	Y	U
Schenk et al. [44]	U	Y	Y	U	U
Tsai et al. [99]	Y	Y	Y	U	U

S1: Appropriate Method of randomization, S2: Concealment of allocation, S3: Comparable groups at baseline, P1: Same care apart from intervention, P2: Participants blinded to treatment, P3: Carers blinded to treatment, A1: Equal length of follow-up , A2: How many participants did not complete (in each group), A3: Comparable availability of outcome data, A4: For how many participants were no outcome data available (in each group), A5: Comparable availability of outcome data, N: no, U: unclear, Y: Yes, AE: aerobic exercise, RE: resistance exercise, GE: graded exercise, UC: usual care, IST: intensive strength training, SET: strength endurance training, CON: control, EX: exercise intervention, RT: resistance training, ET: endurance training; $ Last observation carried forward method used; * Flow cytometry not described

Table 3 Summary of the effects of exercise on immunosenescence-associated cellular markers

Cell population	Acute exercise effects					Long-term exercise effects			
	Young/ middle-aged adults			*Older adults*		*Young/middle-aged adults*		*Older adults*	
CD4/CD8 ratio	↑(n=**1**) or ↔(n=**2**) or ↓(n=**5**)			↔(n=**1**)		↑(n=**2**) or ↔(n=**3**)		↔(n=**1**) or ↓(n=**1**)	
CD28+ expression on T-cells CD4+	↓(n=**1**)					↑(n=**1**) or ↔(n=**1**)	↑(n=1)	↑(n=1) or ↔(n=2+**1**)	
CD8+		↑(n=1)					↑(n=1)	↑(n=1) or ↔(n=2+**1**)	
Naïve T-cells CD3+CD4+	↑(n=**2**) or ↔ (n=**1**)	↑(n=1) or ↔(n=**6**) or ↓(n=**2**)		↑(n=1+**1**)		↔(n=**2**)	↔(n=**2**)	↔(n=1)	
CD3+CD8+		↑(n=2+**4**) or ↔(n=**6**)		↑(n=1+**1**)			↑(n=**2**)	↔(n=1)	
Memory T-cells CD3+CD4+CM	↔(n=**1**)	↑(n=1) or ↔(n=**2**) or ↓(n=**1**)	↔(n=**4**) or ↓(n=**1**)	↑(n=1)	↑(n=**1**)	↔(n=**2**)	↑(n=**2**)	↔(n=2)	
CD3+CD4+EM			↑(n=**1**) or ↔(n=**4**)		↑(n=**1**)		↔(n=**2**)		
CD3+CD8+CM		↑(n=3) or ↔(n=**2**)	↑(n=**4**) or ↔(n=**3**) or ↓(n=**1**)	↑(n=2)	↔(n=**1**)		↑(n=**2**)	↔(n=2)	
CD3+CD8+EM			↑(n=**5**) or ↔(n=**3**)		↑(n=**1**)		↔(n=**2**)		
Senescent T-cells CD4+	↑(n=**3**)	↑(n=3+**1**) or ↔(n=**1**)				↔(n=**1**) or ↓(n=**1**)	↓(n=1)	↔(n=**1**) or ↓(n=**1**)	
CD8+		↑(n=4+**1**) or ↔(n=**1**)						↔(n=**1**) or ↓(n=**1**)	
Regulatory T-lymphocytes naïve	↑(n=**2**) or ↔(n=**9**) or ↓(n=**2**)		↔(n=**2**) or ↓(n=**1**)	↑(n=**1**)		↔(n=**2**)			
memory			↑(n=**1**) or ↔(n=**3**)	↔(n=**1**)					
Apoptosis of T-lymphocytes	↑(n=6+**2**) or ↔(n=**4**)					↓(n=**1**)			
Dendritic cells (Myeloid)	↑(n=1+**2**)					↔(n=**1**)			
(Plasmacytoid)	↑(n=1+**1**) or ↓(n=**1**)					↑(n=**1**)			
Natural Killer Cells	↑(n=3+**11**) or ↔(n=**9**) or ↓(n=2+**2**)			↑(n=2) or ↓(n=**1**)		↑(n=2+**1**) or ↔(n=**8**) or ↓(n=**1**)		↑(n=1) or ↔(n=4)	

↑ = significant increase ($p<0.05$), ↓ = significant decrease ($p<0.05$), ↔ no significant difference observed, acute effects defined as the first change reported after end of intervention, Newly retrieved data depicted in **bold and are** underlined

Table 4 Intervention studies in young humans

References	Design	Participants	Intervention	Sampling type	Sampling time	Outcome	Effect
Effects of acute exercise in untrained condition							
Asimakos et al. [25]	CO, No non-ex con	6 healthy males (28- 37 years)	45 min. of Inspiratory resistive breathing (IRB) at 70% of maximum inspiratory pressure and Whole body exercise (WBE) (electrically braked cycle ergometer) at 70% VO_2max) (separated by 15 days)	F	Pre-ex Post-ex 2h post-ex	 CD4+/CD8+ ratio % Naïve CD4+ % Memory CD4+ % Naïve CD8+ % Memory CD8+ % NK CD4+/CD8+ ratio % Naïve CD4+ % Memory CD4+ % Naïve CD8+ % Memory CD8+ % NK	 = (WBE) / ↓ (IRB) = (WBE/IRB) ↓ (WBE) / = (IRB) ↑ (WBE/IRB) = (WBE/IRB) ↑ (WBE/IRB) = (WBE/IRB) = (WBE/IRB) = (WBE) / = (IRB) = (WBE/IRB) = (WBE/IRB) = (WBE/IRB)
Brown et al. [55]	Non-Con	9 healthy men (21,9 ± 3,6 years)	20 min. steady state cycling at 80% VO2 max	C	Pre-ex Last minute of intervention 30 min post-ex	 # DC # Plasmacytoid DC # Myeloid DC # DC # Plasmacytoid DC # Myeloid DC	 ↑ ↑ ↑ except CD1c-CD141+ = = =

References	Design	Participants	Intervention	Sampling type	Sampling time	Outcome	Effect
Curran et al. [22]	No non-ex con	12 men suffering from type 1 diabetes (T1D) (33,2 ± 9,7 years), 12 male controls (CON) (28,8 ± 4,6 years)	30 min cycling at 80% VO_2 max	F	Pre-ex Imm post-ex 1h post-ex	 # Naïve CD8+ # CM CD8+ # EM CD8+ # EMRA CD8+ # T-regs # Naïve T-regs # Memory T-regs # Naïve CD8+ # CM CD8+ # EM CD8+ # EMRA CD8+ # T-regs # Naïve T-regs # Memory T-regs	 = = = = = = = = = ↓ (T1D),= (CON) ↓ (T1D, CON) = = =
Dorneles et al. [49]	No non-ex con	31 untrained young men (18-30 years)	Low intensity strength training with blood flow restriction: four sets of 23 repetitions at 30% 1RM, 2 min interval (LI-BFR, n=15) and high intensity strength training: four session of 8 repetitions at 80% 1RM, 2 min interval (HI, n=16)	NR	Pre-ex Imm post-ex 24h post-ex	 % NK % NK	 = (LI-BRF, HI) = (LI-BFR), ↓ (HI)
Fuhro et al. [50]	CO	9 patients with chronic kidney disease (and overweight) undergoing hemodialysis (HD) (2 men, 7 women; 64,88 ± 1,98 years)	20 minutes intradialytic exercise on cycle ergometer at (very) hard intensity and control (conventional 4 hour HD session), 1week interval	F	Pre-HD Imm post-ex Post-HD	 % NK % NK ^{bright} % NK ^{dim} % NK % NK ^{bright} % NK ^{dim}	 = = = = (prevents ↓) = (prevents ↓) = (prevents ↓)

References	Design	Participants	Intervention	Sampling type	Sampling time	Outcome	Effect
Gustafson et al. [24]	CO, no non-ex con	15 healthy males (31 ± 4 years, 33% sedentary)	Visit 1 : incremental maximal cycling until exhaustion test (50W, 30W increase ervery 2 min), visit 2 : endurance cycling protocol (45min at 60% of max workload) (separated by 1 to 3 weeks)	F	Pre-ex 2-5min post 3h post 24h post	 % CD4+ Naïve (% of CD4) % CD4+ Memory %CD4+ CM % CD4+ EM % T-regs % CD8+ Naïve (% of CDB) % CD8+ memory % CD8+ CM % CD8+ EM # NK CD4+ T-cell subets CD8+ T-cell subsets NK	 ↓ (MAX) ↑ (MAX) ↓ (MAX) ↑ (MAX) ↓ (MAX) = = ↓ (MAX) ↑ (MAX) ↑ (MAX) = = =
Hanson et al. [32]	Non-Con	20 healthy males (28 ± 5 years), recreationally active (twice a week, >30 min)	Graded exercise test until volitional fatigue (31 min on average)	NR	Pre-ex Imm post-ex	 CD4/CD8 ratio % MAIT (of CD3+) % CD8+ MAIT # CD8+ MAIT % CD4-CD8- MAIT # CD4-CD8- MAIT % MAIT (of CD3+CD8+)	 ↓ ↑ = ↑ = ↑ =

Hanson et al. [36]	Non-Con	20 healthy males (28-35 years), recreationally active (twice a week, >30 min)	40 min moderate (submaximal) intensity cycling at 86% V_T	NR	Pre-ex Imm post-ex	% MAIT # MAIT % CD8+ MAIT # CD8+ MAIT % CD4-CD8- MAIT # CD4-CD8- MAIT	↑ ↑ = ↑ = ↑ = = ↑ =
					1h post-ex	% CCR4/5/6+ MAIT % CD69 MAIT # CCR4/5/CD69 MAIT # CCR6 MAIT % MAIT # MAIT % CD8+ MAIT # CD8+ MAIT % CD4-CD8- MAIT # CD4-CD8- MAIT % CCR4/5/6+ MAIT % CD69 MAIT # CCR4/5/6/CD69 MAIT	↑ = = = = = = = =

References	Design	Participants	Intervention	Sampling type	Sampling time	Outcome	Effect
Harbaum et al. [39]	No non-ex con	16 idiopathic pulmonary arterial hypertension patients (4 men, 12 women; 58 ± 16 years) and 10 healthy controls (4 men, 6 women; 58 ± 15 years)	Cardiopulmonary exercise testing as symptom-limited incremental cycling exercise (10 Watt/min increase)	F	Pre-ex Imm post-ex 1h post-ex	 % NK-cells % and # T-regs % NK-cells % and # T-regs	 = = = =
Krüger et al. [26]	CO, no non-ex con	23 males (25,7 ± 3,2)	High intensity training (HIT) (5x 3min interval at 90% peak power output) and continuous exercise (CONT) (30 min at 70% VO_2) matched for similar energy expenditure and duration on bicycle	NR	Pre-ex Post-ex 3h post-ex 24h post-ex	 # Naïve T-cells # SPC # T-regs % AP naïve T-cells % AP SPC % AP T-regs # Naïve T-cells # SPC # T-regs % AP naïve T-cells % AP SPC % AP T-regs # Naïve T-cells # SPC # T-regs % AP naïve T-cells % AP SPC % AP T-regs	 ↑ ↑ ↑ (HIT) = ↑ = ↓ ↑ ↑ (HIT) ↑ = ↑ (CONT) = = = = = =

References	Design	Participants	Intervention	Sampling type	Sampling time	Outcome	Effect
Park et al. [48]	CO, no non-ex con	10 healthy girls, untrained but physically active at a local tennis club (12,8 ± 1,0 years)	60 min of submaximal exercise on a cycle ergometer at sea level and at a simulated high altitude of 3000m (separated by min 1 week)	F	Pre Post	% NK-cells	=
Rolland-Debord et al. [52]	No non-exercising control	8 healthy individuals (5 men, 3 women; 31 ± 4 years)	Day 1: maximal incremental cycle exercise (familiarization), day 2: flow-limited incremental exercise on cycle ergometer, day 3: same exercise intervention as day 2, without flow limitation	NR	Baseline Iso-workload Exercise peak (symptom limitation)	 % CD16 % CD56	 ↑ ↑
Schenk et al. [44]	Non-Con	16 healthy women (53,44± 0,55)	Graded exercise test on bicycle ergometer until exhaustion (on average 15.5 min)	NR	Pre-ex 1 min post-ex	% $CD56^{bright}$ NK-cells % $CD56^{dim}$ NK-cells	= =
Schenk et al. [113]	Non-Con	5 healthy women (61,4 ± 8,0 years)	Incremental step test on bicycle ergometer (+-15 min)	NR	Pre-ex 1 min post-ex	% $CD56^{bright}$ NK-cells % $CD56^{dim}$ NK-cells	= =

References	Design	Participants	Intervention	Sampling type	Sampling time	Outcome	Effect
Turner et al. [30]	CO, no non-ex con	9 healthy males (22,1 ± 3,4 years)	Vigorous continuous (steady state) cycling (80% VO_2 max, 20 min) (CONT) and high intensity interval (90% VO2 max, 10x 1 min repetitions, 1 min recovery intervals at 40% VO_2 max between phases) (HIIT); separated by at least 3 days	C	Pre-ex Imm post-ex 30 min Post-ex	# Naïve CD8+ # CM CD8+ # EM CD8+ # EMRA CD8+ # CD56+ # $CD56^{bright}$ # $CD56^{dim}$ # Naïve CD8+ # CM CD8+ # EM CD8+ # EMRA CD8+ # NK # $CD56^{bright}$ # $CD56^{dim}$	↑ ↑ ↑ ↑ (CONT) ↑* ↑ ↑* = = = = = = =
Zheng et al. [33]	No non-ex control	13 healthy untrained young males (20,2 ± 1,1 years)	Treadmill running at 40% VO_{2max} to exhaustion in 38 ± 1°C, 60 ± 5% relative humidity and 20,8 % oxygen + placebo ingestion	NR	Pre-ex Imm post-ex	CD4/CD8 ratio # and % NK	↓ ↑
Effects of acute exercise in trained condition							

References	Design	Participants	Intervention	Sampling type	Sampling time	Outcome	Effect
Clifford et al. [76]	Non-Con	17 endurance trained athletes (12 men, 5 women; 40 ±12 years)	Marathon	C	Pre (previous week) Post-1h Post-1d	 % T-regs in tot. Lymph. %T-regs in CD4+ # T-regs CD45RA+ T-regs HLA-DR T-regs % T-regs in tot. Lymph. %T-regs in CD4+ # T-regs CD45RA+ T-regs HLA-DR T-regs	 = ↓ ↓ ↓ = ↑ = ↑ = ↑
Dorneles et al. [79]	Non-R	15 high physically active men (25,3 ± 1,4 years) and 15 low physically active men (26,1 ± 1,9 years)	High intensity interval exercise (10x 60sec at 85% Hrmax, 75sec at 50% Hrmax)	NR	Pre-ex Post-ex 1H post-ex	 % memory T-regs % memory T-regs	 ↑ ↑
Juszkiewicz et al. [74]	No non-ex con	10 members of the Polish Rowing Team (men; 20,5 ± 1,08)	Rowing performance test (2000m on rowing ergometer in shortest time possible)	NR	Pre-ex 1 min-post 24h-post	 # T-regs # NK-cells # T-regs # NK-cells	 = ↑ = =

References	Design	Participants	Intervention	Sampling type	Sampling time	Outcome	Effect
Graff et al. [62]	No non-ex con	14 participants performing regular vigorous activity (13 men and 1 women; 31± 6 years)	30 min fixed intensity leg cycling trial (power output: +10% of blood lactate threshold)	NR	3h pre-ex Pre-ex Post-ex 1h post-ex	 # CD8+ Naïve T-cells # CD8+ CM # CD8+ EM # CD8+ EMRA # NK-cells # CD57+ NK # NKG2C+ NK # CD8+ Naïve T-cells # CD8+ CM # CD8+ EM # CD8+ EMRA # NK-cells # CD57+ NK # NKG2C+ NK	 = ↑ ↑ ↑ ↑ ↑ = = = = = = = = =
Gupta et al. [72]	Non-Con	12 healthy, physically active adults (6 men, 6 women; 33 ± 7 years)	30 min cycling (at 115% of blood lactate threshold)	F	Pre-ex Post-ex 1h post-ex	 % NK-cells in tot. LYM. % $CD56^{dim}$ % $CD56^{bright}$ % NK-cells in tot. LYM. % $CD56^{dim}$ % $CD56^{bright}$	 ↑ ↑ ↓ = ↓ ↑
Joisten et al. [70]	No non-ex con	20 healthy male sport students (24,4 ± 2,2 years)	300 countermovement jumps without arm motion (every 8sec, maximal effort) + passive recovery (supine position)	NR	45min pre-ex 2h post-recovery 72h post-recovery	 CD4/CD8 ratio CD4/CD8 ratio	 = =

References	Design	Participants	Intervention	Sampling type	Sampling time	Outcome	Effect
LaVoy et al. [63]	No non-ex con	17 cyclists (13 men, 4 women, 30,9 ± 5 years) participating in road cycling events (group rides and organized races) ≥ 3 times/week for at least 12 months	30 steady-state cycling on indoor cycle ergometer at -5, +5 and +15% of blood lactate threshold	U	Pre-ex Post-ex 1h post-ex Effects : Time (T), Intensity (I), CMV	 # CD4+ CD27+CD28+ # CD4+ CD27+CD28- # CD4+ CD27-CD28- # CD8+ CD27+CD28+ # CD8+ CD27+CD28- # CD8+ CD27-CD28- # CD4+ CD27+CD28+ # CD4+ CD27+CD28- # CD4+ CD27-CD28- # CD8+ CD27+CD28+ # CD8+ CD27+CD28- # CD8+ CD27-CD28- # CD4+ CD27+CD28+ # CD4+ CD27+CD28- # CD4+ CD27-CD28- # CD8+ CD27+CD28+ # CD8+ CD27+CD28- # CD8+ CD27-CD28-	 ↑ ↑ ↑ ↑ ↑ ↑ ↓ = ↓ = = = T, I x CMV T, I, T x CMV, T x I T, CMV, T x CMV T, I, I x CMV T, I, T x CMV, T x I T, CMV, T x CMV

References	Design	Participants	Intervention	Sampling type	Sampling time	Outcome	Effect
Kostrzewa-Nowak et al. [60]	Non-Con	14 elite male soccer players (16-21 years)	Progressive efficiency test on mechanical treadmill (5 km/h + 2km/h every 3 min) until exhaustion in spring (S) en autumn (A) (preparatory phases to competition rounds)	F	Pre-ex Post-ex (5') 17h post-ex	% CD8+ naïve % CD8+ CM % CD8+ EM % CD4+ naïve % CD4+ CM % CD4+ EM % T-regs % CD8+ naïve % CD8+ CM % CD8+ EM % CD4+ naïve % CD4+ CM % CD4+ EM % T-regs	= ↑ (S), = (A) = = = = = = = = ↑ ↑ (S), = (A) ↓ ↑

References	Design	Participants	Intervention	Sampling type	Sampling time	Outcome	Effect
Kostrzewa-Nowak et al. [59]	Non-Con	14 elite soccer players (17 to 21 years)	Progressive efficiency test on mechanical treadmill (5 km/h + 2km/h every 3 min) until exhaustion in spring (S) en autumn (A) (preparatory phases to competition rounds)	F	Pre-ex Post-ex (5') 17h post-ex	 % NK-cells % Naïve (of CD4+) % CM (of CD4+) % EM (of CD4+) % NK-cells % Naïve (of CD4+) % CM (of CD4+) % EM (of CD4+)	 = (S), ↑ (A) = = = NR ↑ (S), = (A) ↓ (S), = (A) ↓ (S), = (A)

References	Design	Participants	Intervention	Sampling type	Sampling time	Outcome	Effect
Kostrzewa-Nowak et al. [61]	Non-Con	3 elite karate athletes (21 to 31 years)	Progressive test until exhaustion on mechanical treadmill (5 km/h + 2km/h every 3 min)	F	Pre-ex		
					Imm post-ex	% CD8+ naïve	=
						% CD8+ CM	=
						% CD8+ EM	↑
						% CD4+ naïve	↓
						% CD4+ CM	=
						% CD4+ EM	=
						% T-regs	=
					17h post-ex	% CD8+ naïve	=
						% CD8+ CM	=
						% CD8+ EM	=
						% CD4+ naïve	↑
						% CD4+ CM	=
						% CD4+ EM	=
						% T-regs	↑

References	Design	Participants	Intervention	Sampling type	Sampling time	Outcome	Effect
Lackermair et al. [80]	No non-exercising con	58 trained endurance athletes (41 ± 9,5 years)	Marathon run (+ placebo beverage)	NR	(4w pre-ex) 1w pre-ex 1h post-ex 24 post-ex 72h post-ex	 % Myeoloid DC % Plasmocytoid DC % Myeoloid DC % Plasmocytoid DC	 ↑ ↓ ↑ ↓
Minuzzi et al. [77]	Non-R	19 masters athletes (16 men, 3 women; 53,2 ± 9,08 years) and untrained control group (6 men, 4 women; 54,2 ± 5,94)	Incremental test to exhaustion of cycle ergometer	NR	Pre-ex 10min post-ex 1h post-ex	 % T-regs in CD4/LYM # T-regs % KLRG1+ T-regs % naïve T-regs % memory T-regs % T-regs in CD4/LYM. # T-regs % KLRG1+ T-regs % naïve T-regs % memory T-regs	 = ↑ = = = = = = = =

Minuzzi et al. [67]	Non-R	19 master athletes (MA, 15 men, 4 women; 53,5 ± 8,94 years, 20 years experience in training and competition) and healthy control (CON, 7 men, 3 women; 53,7 ± 6,04)	Progressive test to exhaustion on cycle ergometer (75W + 25W every 3 min until volitional fatigue)	NR	Pre-ex 10min post-ex 1h post-ex	% KLRG1+ CD4+ % KLRG1+ CD4+ naïve % KLRG1+ CD4+ CM % KLRG1+ CD4+ EM % KLRG1+ CD4+ EMRA % KLRG1+ CD8+ % KLRG1+ CD8+ naïve % KLRG1+ CD8+ CM % KLRG1+ CD8+ EM % KLRG1+ CD8+ EMRA % KLRG1+ CD4+ % KLRG1+ CD4+ naïve % KLRG1+ CD4+ CM % KLRG1+ CD4+ EM % KLRG1+ CD4+ EMRA % KLRG1+ CD8+ % KLRG1+ CD8+ naïve % KLRG1+ CD8+ CM % KLRG1+ CD8+ EM % KLRG1+ CD8+ EMRA	↑ (MA), = CON° = (MA, CON)° = (MA, CON)° ↑ (MA), = (CON) ↑ (MA), = (CON) = (CON), ↑ (MA)° = (MA, CON)° ↑ (MA, CON) ↑ (MA), = (CON) = (MA, CON) = (MA, CON)° = (MA, CON)° = (MA, CON)° = (MA), ↓ (CON)° = (MA, CON) = (MA, CON)° = (MA, CON)° = (MA, CON)° ↓ (MA), = (CON)° ↓ (MA), = (CON)

References	Design	Participants	Intervention	Sampling type	Sampling time	Outcome	Effect
Morgado et al. [68]	Non-C	65 competitive swimmers (35 men, 16,5 ± 2.1 years and 30 women, 15 ± 1,3 years)	High intensity swimming training session (50 min high intensity task, 500m recovery task)	NR	Pre Post 2h-post 24h-post	 # NK-cells CD4/CD8 ratio # NK-cells CD4/CD8 ratio # NK-cells CD4/CD8 ratio	 ↓ ↑ ↓ ↑ = =
Rooney et al. [73]	Non-C	11 healthy and physically active men (n=7) and women (n=4) (31± 4,4 years)	30 min steady state cycling (power output: 80% max heart rate)	NR	Pre 15m into ex 30 min into ex 1min-post 2min-post 3min-post 4min-post 5min-post 10min-post	 # NK-cells # NK-cells	 ↑ ↓ VS 30 min ↓ VS 30 min ↓ VS 30 min ↓ VS 30 min ↓ VS 30 min
Sakelliou et al. [75]	No non-ex con	10 healthy men (24,2 ± 2,1 years) who exercised regularly (≥ 3 times/ week) during the last 12 months prior to the study	300 eccentric unilateral repetitions (performed in 20 sets of 15 repetitions/set, 30 sec rest interval between sets) of knee extensors at a velocity of 30°/sec on isokinetic dynamometer + placebo ingestion after exercise during 8 days	C	Baseline Post-ex 2h post-ex 24h post-ex 2-8 days post	 % NK-cells	 = ↑ =

References	Design	Participants	Intervention	Sampling type	Sampling time	Outcome	Effect
Svendsen et al. [78]	No non-ex con	12 endurance-trained men (28 ± 4 years)	75 min cycling at 70% altitude specific VO_{2max} in normoxia (N) and hypobaric hypoxia (H)	NR	Pre-ex Post-ex 2h post-ex	 % and # T-regs % and # T-regs	 = =
Wadley et al. [69]	No non-ex con	9 trained men (28 ± 5 years), 11 recreationally active men (27 ± 6 years)	Ramp exercise test to exhaustion on electromagnetically braked cycle ergometer (start at 50W, + 1W/6sec)	NR	Pre-ex During ex Post-ex (imm) 15min post-ex	CD4/CD8 ratio	↓ (RA), = (TR) ↓ (RA and TR) ↓ (RA), = (TR)
Zimmer et al. [66]	Non-R	9 breast cancer survivors (47 ± 7,36) and 9 healthy controls (47± 5,38 years)	Half marathon	NR	Pre 15min-post 24h-post	 % NK-cells % CD4+ Naïve T-cells % CD4+ Memory T-cells % T-regs % NK-cells % CD4+ Naïve T-cells % CD4+ Memory T-cells % T-regs	 ↓ = = = ↓ = = ↓
Effects of long-term exercise on acute exercise-induced response							

References	Design	Participants	Intervention	Sampling type	Sampling time	Outcome	Effect
Patiño et al. [98]	Non-R	Previously untrained males (18-25 years)	6 months of aerobic physical training (APT) or control group with cardiopulmonary exercise (CET) test at 0, 3 and 6 (T0, T3, T6) months of APT program		**Acute response** Pre CET 30s post CET 24h post CET **Baseline levels** Pre CET 24h post CET	# NK-cells # NK-cells # NK-cells # NK-cells	↑ at T1,T3,T6 (APT and CON) = at T1,T3,T6 (APT and CON) APT=CON at T1,T3,T6 APT=CON at T1,T3,T6

References	Design	Participants	Intervention	Sampling type	Sampling time	Outcome	Effect
Tsai et al. [99]	RCT	Sixty healthy sedentary males	Hypoxic exercise (HE, continuous exercise for 30 min) test before (T0) and after (T1) 6 weeks of high-intensity interval training (HIIT), moderate-intensity continious training (MICT) (5x/week) or no intervention (CON)	F	**Acute response** Pre-HE Post-HE **Baseline levels** Pre 6m-post	%CD28+ % CD57+ % CD45RA+ % CD45RO+ % CD62L+ % CD11a+ % CD28+ % CD57+ % CD45RA+ % CD45RO+ % CD62L+ % CD11a+	↓ all (T0/1) ↑ all (T0/1) exc. HIIT T1 (↑T0, =T1) = all (T0/1) = all (T0/1) ↓ all (T0/1) = all (T0/1) ↑ HIIT (rest/after HE), MICT (after HE) ↓ HIIT (rest/after HE), MICT (after HE) = = = =
Effects of long-term exercise							

References	Design	Participants	Intervention	Sampling type	Sampling time	Outcome	Effect
Broadbent [85]	RCT	24 participants with chronic fatigue syndrome receiving usual care or exercise intervention (7 men, 17 women; 50.9 ± 10 years) VS 18 sedentary non-CFS participants (5 men, 13 women; 50,6 ± 10 years)	12 weeks intermittent (IE) or graded (GE) cycling (3x/w)	NR	Pre-ex Post-ex	CD4+/CD8+ ratio # NK	= in all groups ↑ in IE
Chamorro-Viña [95]	RCT	6 children who have undergone Hematopoietic stem cell transplantation	10 week moderate intensity exercise (EP, strength and aerobic), 3 x 60min/week or usual care (UC)	F	Pre-ex 10w Post-ex (36h after last training)	mean ratio $CD56^{dim}$ (pre/post) mean ratio $CD56^{bright}$	↑ in EP =
Deckx et al. [87]	RCT	63 patients with multiple scleroris	Combined endurance and resistance training, (EX, 5 sessions/ 2 weeks) or sedentary control (CON)	NR	Pre-ex Post-ex (48h after ex)	# plasmacytoid DC # conventional DC % T-regs	↑ (EX), = (CON) ↑ (EX and CON) =
Dungey et al. [86]	Non-R	31 patients receiving haemodialysis (HD, 63,4 ± 13,7 years): 16 exercising (57,0 ± 10,5 years), 15 non-exercising (70,2 ± 13,7 years) , 16 healthy individuals (61,5 ± 10,9 years)	6 months of cycling, 3 times/week or usual care (UC) in HD patients + healthy control	NR	Prior to HD Pre-ex Post-ex (at least 48 hours after last session)	Δ # T-regs Δ % T-regs	EX $\neq$ non-EX =
Gronesova et al. [90]	Non-Con	10 healthy women (46 ± 3 years)	2 weeks STOTT Pilates (180 min/week)	F	Pre-ex 2w post-ex	% NK cells # NK cells	= =

References	Design	Participants	Intervention	Sampling type	Sampling time	Outcome	Effect
Ibrahim et al. [92]	RCT	22 healthy sedentary men (10 in control group, 22 ± 2,0 years and 12 in exercise group, 21 ± 2,0 years)	12 weeks of circuit training: 3 times/week; 2 circuits from week 1 to 6, 3 from week 9 to 12; 10 stations/circuit (30sec per station, 1 min rest between stations); 5 min rest between circuits; one type of exercise per station: heal raise/triceps extension/ standing chest fly/biceps curl with dumbbell, side lateral raise/leg abduction/ shoulder extension and flexion/half squat/leg curl with elastic band/ rope skipping	NR	Pre-ex Post-ex	# NK-cells	= (CON and EX)
Hagstrom et al. [94]	RCT	39 sedentary breast cancer survivors (51,9 ± 8,8 years)	16 weeks resistance training (RT; 60 min, 3 times/ week) or control (CON)	NR	Week 0 Week 17	Δ % NK cells EX-CON	=
Kim et al. [91]	Non-R	43 patients taking thyroid hormone replacement after thyroidectomy (7 men, 36 women; 50 ± 9,12 years)	12 weeks home exercise intervention (aerobic, resistance and flexibilty) or no intervention (CON)	NR	Pre-ex Post-ex	# NK cells	=

References	Design	Participants	Intervention	Sampling type	Sampling time	Outcome	Effect
Morgado et al. [84]	No non-ex con	30 elite male athletes: 16 judoists (22,9 ± 2,7 years) and 14 swimmers (17,9 ± 1,4 years)	± 2 months training for competition period; judoists: ± 4h, 6 days/week; swimmers: ± 13-15 hours pool + ± 4 hours dry land training/ week	F	Baseline Competitive period assessment	CD4/CD8 ratio	=
Philippe et al. [82]	No non-ex con	16 men with impaired glucose tolerance (57,0 ± 5,2 years)	3 weeks of concentric or eccentric endurance training (3x/ week)	C	Pre-ex Post-ex (min 1 day after last session)	CD4/CD8 ratio % CD4+ Naïve %CD8+ Naïve % CD4+/CD8+ CM %CD4+/CD8+ EM % CD4+/CD8+EMRA	↑ = ↑ ↑ = ↓
Schenk et al. [44]	RCT	9 healthy women (53,33 ± 0,99 years) in intervention group and 7 healthy women as passive control (53,57 ± 0,20 years)	4 weeks endurance interval exercise program (2-3x/week) on cross trainer	NR	Pre-ex Post-ex	% $CD56^{dim}$ NK % $CD56^{bright}$ NK	= =
Schmidt et al. [93]	RCT	81 women with breast cancer receiving chemotherapy	12 weeks supervised resistance training (RT) or endurance training (ET) compared with usual care (UC)	NR	Pre-ex 12w post-ex	# NK cells	↓ (ET), = (RT, UC)

References	Design	Participants	Intervention	Sampling type	Sampling time	Outcome	Effect
Schlabe et al. [83]	No non-ex con	13 HIV-infected patients (12 men, 1 women; on average 42 years)	Moderate endurance training program (3-4/week) to prepare for marathon	F	12m pre-marathon 9m pre-marathon 6m pre-marathon right before run	# CD4+ cells % annexin V CD4+	↑ ↓

Non-Con: Non-controlled intervention studies; RCT: Randomized Controlled Trial; Non= Non-Randomized intervention studies with control group; No non-ex con: No non-exer cising control, CO: cross-over design, Min= minute; VO_{2max}= maximal oxygen uptake ; V_T: Ventilatory threshold; W: Watt, 1-IRM= one-repetition maximum; Sec= second; Imm= immediately; ↑: increase with $p < .05$, ↓: decrease with $p < 0.05$, = : no significant change compared to baseline unless stated otherwise; * within group and compared to other group (between group), ° significant difference ($p<0.05$) between groups

Table 5 Intervention studies in older humans

References	Design	Participants	Intervention	Sampling type	Sampling time	Outcome	Effect
Effects of acute exercise in trained condition							
Van der Geest et al. [81]	Non-Con	20 older men and women (81,3 ± 1,9 years)	Participation in the 2013 Nijmegen Four Days Marches (30 km/day at self selected pace)	C	Pre-ex (12-36h) Post-ex (10 min)	CD4/CD8 ratio # CD4+ Naïve # CD4+ CM/EM # CD8+ Naïve/EM # CD8+ CM # Naïve T-regs # Memory T-regs # NK # $CD56^{dim}$ # $CD56^{bright}$	= ↑ ↑ ↑ = ↑ = ↓ ↓ =
Effects of long-term exercise							
Abd El-Kader et al. [103]	RCT	60 sedentary older women (61-67 years)	6 months aerobic (40 min on treadmill, 60-70% HR_{max} to 70-80% in last 3 months) or resistance (40 min, 60-80% 1-RM) exercise intervention (3x/ week)	NR	NR	CD4/CD8 ratio	↓ (aerobic), = (resistance)

References	Design	Participants	Intervention	Sampling type	Sampling time	Outcome	Effect
Cao Dinh et al. [101]	RCT	100 apparently healthy older women (65+)	6 weeks of Intensive strength training (IST, 3 sets of 10 repetitions at 80% IRM), strength endurance training (SET, 2 sets of 30 repetitions at 40% IRM), flexibility training (CON, 3 sets of passive, static stretching) (2-3x/week)	F	Pre-ex Post-ex (24-48h)	# CD8+CD57+ # CD8+CD28-CD57+ # CD8+CD28+CD57+ # CD8-CD57+ # CD8-CD28-CD57+ # CD8-CD28+CD57+ %CD8+CD57+ %CD8+CD28-CD57+ % CD8+CD28+CD57+ %CD8-CD57+ %CD8-CD28-CD57+ %CD8-CD28+CD57+	↓ (SET; CMV+)* ↓ (SET; CMV+)* = (SET; CMV+) ↓ (SET; CMV+)* = (SET; CMV+) ↓ (SET; CMV+)* ↓ (SET; CMV+)* ↓ (SET; CMV+)* = (CMV+) ↓ (SET; CMV+) ↓ (SET; CMV+) ↓ (SET; CMV+)

References	Design	Participants	Intervention	Sampling type	Sampling time	Outcome	Effect
Cao Dinh et al. [100]	RCT	100 apparently healthy older women (65+)	6 weeks (2-3x/week) of Intensive strength training (IST), strength endurance training (SET), flexibility training (CON)	F	Pre-ex Post-ex (24-48h)	%CD8+CD57+ %CD8+CD28-CD57+ % CD8+CD28+CD57+ %CD8-CD57+ %CD8-CD28-CD57+ %CD8-CD28+CD57+ #CD8+CD57+ #CD8+CD28-CD57+ # CD8+CD28+CD57+ #CD8-CD57+ #CD8-CD28-CD57+ #CD8-CD28+CD57+	↓ (SET)* ↓ (SET)* = ↓ (SET) ↓ (SET) = ↓ (SET)* ↓ (SET)* ↓ (SET) = = =
Gomes et al. [102]	Non-Con	16 older women (67 ± 4 years) with knee osteoarthritis	12 week walking program (3/week with, 30 min at start with 5 min increments every 2 weeks)	NR	Pre-ex Post-ex (1week after end of training program)	% CD4+CD28+ % CD8+CD28+	= =

Non-Con; Non-controlled intervention studies, RCT; Randomized Controlled Trial; Non= Non-Randomized intervention studies with control group; No non-ex con: No non-exercising control, CO: cross-over design, Min= minute; Sec= second; VO_{2max}= maximal oxygen uptake ; V_T= Ventilatory threshold; 1-IRM= one repetition maximum Sec= second; Imm= immediately; ↑: increase with $p < .05$, ↓: decrease with $p < .05$, = : no significant change compared to baseline unless stated otherwise; * within group and compared to control group (between group)