Strength endurance training but not intensive strength training reduces senescence-prone T-cells in peripheral blood in community-dwelling elderly women

Cao Dinh, Hung; Njemini, Rose; Onyema, Oscar Okwudiri; Beyer, Ingo; Liberman, Keliane; De Dobbeleer, Liza; Renmans, Wim; Vander Meeren, Sam; Jochmans, Kristin; Delaere, Andreas; Knoop, Veerle; Bautmans, Ivan

Published in:
The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences

DOI:
10.1093/gerona/gly229

Publication date:
2019

License:
Unspecified

Document Version:
Accepted author manuscript

Strength endurance training but not intensive strength training reduces senescence-prone T-cells in peripheral blood in community-dwelling elderly women

Hung Cao Dinh, MD[1,2,#], Rose Njemini, PhD[1,2,#], Oscar Okwudiri Onyema, PhD[1, 2], Ingo Beyer, PhD[1,2,3], Keliane Liberman MSc[1,2], Liza De Dobbeleer, MSc[1,2], Wim Renmans, BSc[4], Sam Vander Meeren, MD[4], Kristin Jochmans, PhD[4], Andreas Delaere, MSc [1,2], Veerle Knoop MSc[1,2], Ivan Bautmans, PhD[1,2,3,*]

[1]Frailty in Ageing Research Group, Vrije Universiteit Brussel, Laarbeeklaan 103, B-1090 Brussels, Belgium

[2]Gerontology Department, Vrije Universiteit Brussel, Laarbeeklaan 103, B-1090, Brussels, Belgium

[3]Department of Geriatric Medicine, Universitair Ziekenhuis Brussel, Laarbeeklaan 101, B-1090 Brussels, Belgium

[4]Laboratory of Hematology, Universitair Ziekenhuis Brussel, Laarbeeklaan 101, B-1090, Brussels, Belgium

#*These authors contributed equally to this manuscript*

***Corresponding author:**

Prof. Dr. Ivan Bautmans (PhD)

Vrije Universiteit Brussel

Head Gerontology (GERO) & Frailty in Ageing research (FRIA) departments

Laarbeeklaan 103, B-1090 Brussels (Belgium)

Tel. +32 2 477.42.07, E-mail. ivan.bautmans@vub.be

Abstract

Ageing is characterized by a progressive decline in immune function known as immunosenescence (IS). While the causes of IS are likely to be multi-factorial, an age-associated accumulation of senescent T-cells and decreased naïve T-cell repertoire are key contributors to the phenomenon. On the other hand, there is a growing consensus that physical exercise may improve immune response in ageing. However, the optimum training modality required to obtain beneficial adaptations in older subjects is lacking. Therefore, we aimed to investigate the effects of exercise modality on T-cell phenotypes in older women. 100 women (aged ≥65 years) were randomized to either intensive strength training (80% of one-repetition maximum (1RM)), strength endurance training (SET, 40% 1RM), or control (stretching exercise) for 2-3 times/week during 6 weeks. The T-cell percentages and absolute counts were determined using flow cytometry and hematology analyzer. C-reactive protein was measured using immunonephelometry. We report for the first time that 6 weeks of SET significantly decreased the basal percentage and absolute counts of senescence-prone T-cells, which was positively related to the number of training sessions performed. Conceivably, training protocols with many repetitions - at a sufficiently high external resistance - might assist the reduction of senescence-prone T-cells in older women.

Introduction

Ageing is accompanied by a progressive decline in immune function referred to as immunosenescence (IS) (1). Of all the immune compartments, that of the T-cells is among the most disturbed (2). With advancing age, naive T-cells are gradually being replaced by highly differentiated memory and senescent phenotypes. On the other hand, the thymus gland atrophy progresses rapidly until middle age, and only about 15% of functional thymic tissue remains at age 50, leading to a further reduction in the naive T-cell repertoire (3). While the potential causes of IS are likely to be multi-factorial, a restricted naïve T-cell repertoire in conjunction with an age-associated accumulation of senescent T-cells and/or thymic involution are major contributors to the phenomenon, which leads to increased susceptibility of older subjects to morbidity and mortality. Indeed, clinical evidence indicates that the ability to mount primary immune responses against novel antigens declines significantly with age (4), thereby decreasing the response to vaccines and increasing the vulnerability of older subjects to often severe infectious diseases. This liability is further complicated by the ageing related accumulation of senescent T-cells. Senescent T-cells are dysfunctional immune cells, which are viable but without division capacity, resist apoptosis, and secrete increased amounts of pro-inflammatory substances and matrix degrading enzymes (1,5). In this light, senescent T-cells can disturb the extra-cellular matrix, creating a pro-inflammatory micro-environment, favorable for the development of cancer (6). More generally, IS has been associated with the increased vulnerability of older subjects to inflammation-related conditions, such as cardiovascular diseases and cancer (7). In 2015, cardiovascular diseases and cancer were, respectively, the first and second most common causes of mortality worldwide (8). With the ageing of the population, the number of older adults presenting with IS has risen dramatically over the past decade. Therefore, IS in the geriatric population has progressively become a major health concern.

There are strong indications that physical exercise in older persons may improve the immune response to novel antigenic challenges (9), and prevent or at least slow down the age-related decline in immune function (10). Although the underlying mechanisms of exercise-induced immune response have not been completely elucidated, it has been demonstrated that regular bouts of exercise may improve immune function by lowering inflammation (11-13) via the induction of anti-inflammatory cytokines (14) and/or the inhibition of the expression of Toll-like receptors, with subsequent inhibition of the production of pro-inflammatory cytokines (15). Another obvious potential mechanism by which exercise can counteract IS and its associated diseases is by decreasing the numbers of senescent T-cells. Physical exercise has been shown to induce the mobilization of lymphocytes into the bloodstream (16), followed by a lymphocytopenia - whereby the lymphocytes egress to selected peripheral tissues (17) - with a subsequent reinforcement of immune surveillance and apoptosis of senescent T-cells (18). In this perspective, however, distinction should be made between the exercise-induced acute changes (sudden, temporary changes during or immediately following an exercise bout) and the long-term effects of exercise (changes in basal levels, when the acute exercise-induced effects have been washed out) on the immune response. It is also noteworthy that exercise-induced changes in immune response might be different according to the type of physical exercise, training load, as well as training modality (19).

Although the effects of single bouts of acute exercise on immunity have been well investigated in both young and older populations (20), studies examining the long-term adaptation effects of exercise training in older subjects are sparse (21). Further, research pertaining to resistance training - which is very well suited to older persons who are often in diverse challenging circumstances - has been largely unexploited in the context of the ageing immune system. More so, the optimum training modality required to obtain a beneficial immune response in older

subjects is yet unknown. Therefore, the present study aimed to investigate the impact of different resistance training modalities on markers of IS in older women.

Materials and methods

Participants and study design

The Senior Project Intensive Training (SPRINT) is an ongoing randomized controlled trial conducted by the Frailty in Ageing research department of Vrije Universiteit Brussel to evaluate the effects of resistance training at different intensities on the immune system in older persons. Recruitment was done by advertisement with flyers through day centers, health insurance companies, seniors associations, general practitioners, municipalities and other public places. Subjects were excluded when performing currently or within the past 6 months on a regular basis physical exercise at higher intensities than habitual daily activity (e.g. fitness classes, strengthening exercises, cycling club); when presenting contra-indication for any of the exercise interventions; when using corticosteroids; when being unable to understand or execute the exercise instructions due to cognitive impairment (mini mental state examination score < 24/30) (22) or physical disability. Comorbidity was not an exclusion criterion per se, except for acute uncontrolled conditions and/or acute inflammation (C-reactive protein (CRP) ≥ 10mg/L). One hundred apparently healthy older women (aged 65 years and over) living independently in the community were included in the present study (see Figure 1). The study protocol was approved by the local ethics committee in accordance with the Declaration of Helsinki and each participant gave a written informed consent.

Health categories and randomization

Participants were initially classified into health categories (see Table 1) based on a modified SENIEUR's protocol and according to the risk for complications during physical training as

described previously (23-25). Thereafter, they were randomly assigned to 6 weeks of either intensive strength training (IST), strength-endurance training (SET), or control (CON) (see Figure 1). Randomization was stratified for age (65-74 / ≥75 years) and health status (see Table 1). Group allocation was performed by a researcher, who was blinded for study outcomes and allocation sequence.

Training protocol

The purpose of this study was to evaluate the effects of 6 weeks resistance training – at different intensities – compared to control on the composition of blood T-cell subtypes in older women. Training took place at the exercise facilities of the Brussels Health Campus of the Vrije Universiteit Brussel on Technogym™ (Technogym, Gambettola, Italy) and Matrix® (Matrix, Wisconsin, USA) single station cable-type devices (for detailed specifications see manufacturers' websites www.technogym.com and www.world.matrixfitness.com). All training sessions were supervised by trained instructors to minimize the risk for injury and to make sure the participants used the proper technique and weights and performed the exercise throughout the entire range of motion. Each training session, regardless of the allocated training group, started with a warm-up of 10 exercises - that included mobility and activating exercises for both the upper and lower limbs (such as circular movements with the limbs, but no treadmill or cyclo-ergometer) - intended to prepare the joints and muscles for the up-coming exercises. Each warm-up exercise was performed without external resistance for a total of 15 repetitions per extremity or per exercise, duration of the complete warm-up was approximately 5 to 10 minutes. Six exercises (seated chest press, seated leg press, seated hip abduction, seated hip adduction, seated low row, and seated vertical traction) consisting of intensive sub-maximal muscle contractions with both concentric and eccentric components were performed in the IST and SET intervention groups. There was no predetermined sequence of the 6 exercises within

each training session, however, participants were instructed to alter exercises for upper and lower limbs. Participants were asked to exercise 2-3 times weekly, with a minimum of 1-day interval for recuperation. The exercise protocols for the IST and SET intervention groups were designed to be approximately equal in volume (% one-repetition maximum (1RM) - i.e. the maximum weight that can be moved once over the whole range of movement - multiplied by the number of sets and repetitions). For the IST intervention group, the large muscle groups were trained at 3 sets of 10 consecutive repetitions at 80% of 1RM. For the SET intervention group, the exercises were designed similarly as for the IST group, but with less intensive muscle contractions and a higher number of repetitions (2 sets of 30 consecutive repetitions at 40% of 1RM). The rest between sets was minimum 1 minute for the IST and SET groups. All exercise programs entailed an initial accommodation period of 2 weeks in which the target exercise intensity was progressively reached. Every 2 weeks, the individuals' 1RM was determined and exercise loads were adapted accordingly. A minimum of 24 hours of rest (i.e. no training) was scheduled before and after a 1RM test. For the 1RM determination the participant starts with a warming up of 20 repetitions at 30% of the estimated or previously assessed 1RM. Next, the participant is asked to perform 1 repetition at 70% of the estimated or previously assessed 1RM. This step is repeated with increasing load until the participant is unable to perform the exercise correctly in full range of motion. The 1RM is the highest load at which the participant was able to perform the exercise correctly in full range of motion. The load corresponding to 1RM was reached in maximum 4 to 5 steps.

The CON group performed a "placebo" flexibility training consisting of 3 sets of 10 to 12 sustained (30 sec) passive, static stretching exercises of the large muscle groups (for adherence purpose). Stretching exercises act principally by applying mechanical tension on the muscles and tendons, leading to improved range of motion (26). Therefore, this type of exercise - mainly

inducing a passive load on the muscles and tendons without muscle contractions or cardiovascular challenge - was chosen as a control intervention.

Anthropometric measurements

Weight was measured using a SECA balance, which was regularly calibrated to the nearest 0,1 kg. Height was determined using a SECA measuring rod to the nearest 0,1 cm. Body mass index was calculated using the measurements of height and weight as weight divided by height squared (weight (kg)/ height^2 (m^2)).

Flow cytometry analysis

Venous blood specimens were collected before and after 6 weeks (24h-48h after the last training session) in the morning for serum (stored at -80°C until analysis) and for EDTA anticoagulated blood. Peripheral blood leucocytes were recovered as described previously (27,28). Briefly, EDTA blood was exposed to lysis buffer for 10 min. After lysing the red blood cells, the blood leucocytes were centrifuged at 2,800 rpm for 4 min at room temperature. Thereafter, the cells were isolated, washed twice in PBS containing 1% BSA at 2,800 rpm for 3 min, and re-suspended in 200µl PBS containing 1% BSA.

Antibodies were initially titrated to determine the optimal conditions for flow cytometry analysis before staining. About 5×10^5 cells were stained with 3 µL each of PE-CY5-labelled anti-CD8 (Becton Dickinson, San Jose, CA, USA), PE-CY7-labelled anti-CD3 (Biolegend, San Diego, CA, USA), FITC-labelled anti-CD28 (Biolegend, San Diego, CA, USA), and PE-labelled anti-CD57 (Biolegend, San Diego, CA, USA). After 20 min incubation at room temperature in the dark, cells were washed at 2,800 rpm for 3 min, and 500 µL of FACS flow solution (Becton Dickinson, San Jose, CA, USA) was added.

The labelled samples were analyzed with a Coulter FC 500 flow cytometer (Beckman Coulter, Fullerton, CA, USA). Data acquisition was performed using the Coulter CXP software (Epics). The lymphocyte subpopulation was gated according to size and granularity in the forward vs. side scattergram, thereby excluding dead cells. Fluorescence-minus-one controls were used to distinguish positive from negative events and the various lymphocyte clusters were identified according to their expression of a combination of surface markers. The expression or non-expression of CD28 and CD57 are particularly useful in distinguishing between subsets of differentiated T-cells. Based on these surface markers, CD8– and CD8+ T-cells were separated into four distinct sub-populations including CD28+CD57–, CD28–CD57–, CD28–CD57+ and CD28+CD57+. We used the terminologies naive (CD28+CD57–, consisting predominantly of naive T-cells and perhaps some early differentiated T-cells), memory (CD28–CD57–), and senescence-prone (CD28–CD57+ and CD28+CD57+) phenotypes to define the distinct subsets as previously described (27,28). Absolute blood counts were measured using a dual platform methodology (flow cytometry and the Cell-Dyn Sapphire hematology analyzer (Abbott Diagnostics Division, Santa Clara, CA).

Serum CRP and CMV IgG determination

CRP was quantified by immunonephelometry using the high sensitivity CRP kit obtained from Dade Behring (Marburg GmbH, Germany). For CRP determination, the limit of detection was 0.175mg/L and the intra-assay and inter-assay coefficients of variation ranged from 3.1% to 4.4% and from 2.5% to 5.7%, respectively. Since Cytomegalovirus (CMV) sero-status can interfere with the impact of exercise on CD8+CD57+ cells, serum levels of CMV IgG were measured by a chemiluminescent microparticle immunoassay on the ARCHITECT iSystem (Abbott Diagnostics, Abbott Park, Ireland) with an assay sensitivity and specificity of 100% and 99%, respectively. Assays were regarded as positive if they had concentrations of 6.0 arbitrary units (AU)/mL or greater and negative if they had concentrations of less than 6.0 AU/mL. The detection limit of 6 AU/mL was based on the indications from the manufacturer

of the CMV IgG kit. The intra-assay and inter-assay coefficients of variation ranged from 4.39% to 5.67% and from 4.87% to 6.17%, respectively.

Statistical Analyses

Data were tested for normality using the Kolmogorov-Smirnov goodness of fit test. Most of the parameters were not normally distributed even after log-transformation and as such, nonparametric tests were applied during analysis. The Kruskal-Wallis test was employed to test for differences among the intervention groups. When a significant difference was detected, pairwise comparisons were performed using the Mann-Whitney U test. Wilcoxon's Signed Rank test was applied for assessing time-effects. Changes after 6 weeks intervention were calculated and compared among IST, SET and CON using Kruskal-Wallis test (and Mann-Whitney U test for post-hoc testing). Associations were explored by Spearman's rank correlation test. Statistical analysis was performed using IBM SPSS version 22.0. Statistical significance was set a priori at two-sided $p < 0.05$.

Results

The baseline characteristics of the cohort are illustrated in Table 2. As can be seen in the table, there was no difference in age, BMI, CRP or CMV IgG among the 3 intervention groups. Similarly, no significant difference in the percentage (see Table 2) or absolute counts (see Supplementary Table 1) of T-cell phenotypes was noticed among groups. On the other hand, we found significant associations between age and the percentage of the highly differentiated memory phenotypes in both the CD8+ and CD8– T cell subsets ($r = 0.250$, $p = 0.012$ and $r = 0.243$, $p = 0.015$; for CD8+CD28−CD57− and CD8−CD28−CD57−, respectively). Contrary wise, an inverse association was found between the proportion of naive cell phenotypes and age in both the CD8+ and CD8– T cell pools ($r = -0.329$, $p = 0.001$ and $r = -0.243$, $p = 0.015$; for CD8+CD28+CD57− and CD8−CD28+CD57−, respectively). Regarding the proportion of

senescence-prone phenotypes, we noticed a significant increase with age in the CD8– compartment ($r = 0.261$, $p = 0.009$), and a trend towards an age-related increase in the CD8+ compartment ($r = 0.174$, $p = 0.087$).

There was a significant decrease in the proportion of CD8+ senescent cells (CD8+CD57+, $p = 0.004$), rather due to the CD8+CD28–CD57+ variant ($p = 0.004$), as CD8+CD28+CD57+ did not change significantly. Figure 2 shows the training-induced changes in the percentage of senescence-prone cells between baseline and after six weeks training for the different intervention groups. Within group analysis revealed a significant training-induced decrease in the proportions of the CD8+ senescent phenotypes only in the SET intervention group (all $p < 0.05$, see Figure 2). Pairwise group comparisons indicated a significant decrease in the training-induced changes for CD8+CD57+ and CD8+CD28−CD57+ in the SET intervention group compared to the CON group ($p=0.036$, and $p=0.043$, respectively).

Regarding the CD8– T-cell compartment, we found a trend towards a decrease in the senescent cell phenotype CD8–CD57+ ($p=0.060$) (mostly due to the CD8–CD28–CD57+ sub-population ($p=0.067$)). Within group analysis revealed a significant training-induced decrease in the proportions of the senescent cell only in the SET intervention group (all $p < 0.05$, see Figure 2).

For the memory phenotypes, an overall significant increase in the percentage of cells was noticed within both the CD8+ and CD8– pools (respectively, $p=0.030$, and $p = 0.014$, see Figure 3). However, no significant changes were observed when investigating the groups separately. For the naïve sub-population, no significant difference was observed - within either the CD8+ or CD8– T-cell pools - from baseline to post exercise.

To understand if the exercise-induced changes in the proportion of T-cell phenotypes are reflected in their absolute numbers in peripheral blood, we also analyzed the absolute blood counts of the various T-cell subsets. We found a trend for a decrease in the absolute number of the CD8+ senescent phenotype CD8+CD57+ ($p = 0.057$) that was also observed in the CD8+CD28–CD57+ subtype ($p = 0.066$). Within group analysis revealed an exercise-induced decrease in the absolute number of all the senescent phenotypes within the CD8+ pool in the SET intervention group (all $p < 0.05$, Figure 4). Also, there was a time by group interaction effect for the CD8+CD28–CD57+ subset ($p = 0.047$), and a trend towards a time by group interaction effect for CD8+CD57+ ($p = 0.060$), with between-group pairwise comparisons portraying a significant decrease in the training-induced changes for CD8+CD57+ and CD8+CD28–CD57+ subtypes in the SET intervention group compared to the CON group ($p = 0.018$ and $p = 0.012$, respectively). Moreover, the SET group showed a trend towards a decrease for CD8+CD28+CD57+ senescent subtype ($p = 0.084$) compared to the CON group post-exercise. For the CD8– compartment, we found a trend for the absolute number of CD8–CD57+ senescent cells to decrease after exercise compared to pre-exercise counts ($p = 0.086$). There was no significant difference in the absolute number of T-cell subsets in the CD8– compartment post-exercise (see Figure 4). Similarly, no significant difference was found for the absolute blood counts of the naïve or memory T-cells after exercise relative to pre-exercise counts (see Figure 3).

The median (Interquartile range) number of training sessions were 13 (7), 14 (6), and 15 (5) for the IST, SET, and CON groups, respectively. There was no significant difference in the number of training sessions among the 3 groups. Notably, we observed an overall inverse association between the change in both the percentage and the absolute number of the CD8+CD28+CD57+

senescent phenotypes and the number of training sessions performed ($r = -0.318$; $p = 0.002$ and $r = -0.270$; $p = 0.014$ respectively).

Discussion

Although much effort has been devoted to exploring the impact of exercise on cellular senescence, studies pertaining to the long-term adaptation effects of training on immune response in older persons are rare. More so, most of the available longitudinal studies that have used an aerobic exercise intervention to improve immune responses in older subjects suggest that exercise training exerts little or no effect on immune function (21,29-31), leading to the misconception that exercise training does not result in major improvement of the senescent immune system. Several experimental variables can contribute to the lack of immunological responses to physical activity. The most obvious causes are related to the type, frequency, duration and intensity of exercise training (32). The current study is the first in older women, which has addressed specifically the effect of different resistance training intensities - while maintaining the same training volume - on resting T-lymphocyte phenotypes.

We found that 6 weeks of SET significantly lowered senescence-prone T-cells in older women. In an earlier study, Spielmann et al. demonstrated that aerobic fitness was associated with a lower age-related accumulation of senescent T-cells in healthy males (33). However, their study covered a large age range (18-61 years) and was not focused on older subjects. Also, in older persons aged 65 years and over, Silva et al. observed lower proportions of terminally differentiated T-cells among moderately trained (i.e. those who played volleyball, basketball, or running > 6 km 2-3 times/week) and intensely trained subjects (who trained ≥ 5 times/week, in total running > 50 km/week) (34). Notwithstanding, these exercise modalities are less practicable by an average older person unlike resistance training, which is more appealing and has been reported to be most efficient in promoting specific adaptations in several physiological

variables including muscle strength and function, body composition, metabolic and cardiovascular health of older subjects (35,36).

Unlike the moderate-intensity SET, 6 weeks of IST at high-intensity did not influence significantly the blood counts of senescence-prone T-cells in older women. This could be attributed, at least in part, to exercise intensity-dependent mobilization of T-cells in combination with a myokine response during this kind of exercise. Firstly, acute exercise has been shown to induce the influx of lymphocytes – preferentially the late stage differentiated senescence-like phenotypes (37) - into the bloodstream. This exercise dependent mobilization of cells is driven in part by increased shear forces and blood pressure - all of which increase with increasing intensity of exercise (38,39). But, most importantly, lymphocytes are mobilized through stimulation of their beta-2-adrenergic surface receptors, by hormones released during exercise in an exercise intensity-dependent manner (high intensity - higher hormone levels) (40). In this perspective, a high intensity exercise can be expected to cause a more important mobilization of senescence-prone T-cells into blood than a moderate-intensity exercise (41,42). Secondly, after exercise, the cells are egressed into peripheral tissues, followed by their exposure to apoptotic stimuli with subsequent apoptosis of a portion of the senescent T-cells (17,41). Cell egress from blood is a tightly regulated process where chemokines interact with adhesion molecules expressed on leukocyte and endothelial cell membranes (43). Notwithstanding, the chemokine IL-8 has been reported to have inhibitory effect on the adherence of leukocytes on the endothelial lining and their subsequent emigration through the endothelial cell barrier (44,45). In a previous study - using a similar exercise approach - we found that the levels of IL-8 increased significantly after resistance training; interestingly, this was observed only in the male subjects of the high intensity intervention group (13). However, in the present study, we did not investigate chemokines (particularly, MCP-1, Rantes and MIP-1 alpha) or cortisol which are well known to influence lymphocyte egress from blood into

tissues (46). Blood levels of cortisol are known to increase with high intensity exercise and cortisol has been proposed to mediate lymphocytopenia. McGuigan et al. (47) reported that high intensity resistance exercise at 75% 1RM significantly increased salivary cortisol levels while lower intensity resistance exercise at 30% 1RM did not result in any significant change in cortisol levels. On the other hand, Dimitrov et al. (48) demonstrated that lymphocytopenia following cortisol infusion was restricted to naïve and lymphoid-homing CD8 T-cells, and that differentiated effector-memory cells were not affected. Moreover, Bosch et al. (49) showed that the frequency of CD8+ T-cells lacking surface expression of CD27 and CD28 was associated with higher overnight urinary cortisol secretion. Whether cortisol could have an effect on exercise-induced immune adaptations requires further investigation.

Thirdly, our observation could be related to the oxidative stress associated with the intensity of resistance exercise. Goto et al. reported that twelve weeks of cycling at high-intensity increased plasma levels of oxidative stress markers whereas cycling at moderate-intensity tended to decrease oxidative stress (50). This increase in oxidative stress with high-intensity exercise may favor the development of T-cell senescence, considering that activation of the P38 protein kinase signaling pathway by excess ROS can drive human T-cell senescence (51). Thus, assuming that the response to IST is similar to high-intensity cycling, this type of resistance training may be associated with increased T-cell senescence due to oxidative stress, higher release of senescent T-cells into the blood stream and diminished egress to peripheral tissues (due to higher IL-8 levels) reducing subsequent apoptosis. However, a positive effect after longer training periods may not be precluded, and more research is required in this area to elucidate the possible role played by the chemokines and oxidative stress in lymphocytes following resistance exercise at different intensities.

Regarding the exercise-induced changes in the memory T-cells, we found a significant increase in their proportion after 6 weeks intervention in the entire cohort. Within group analysis

revealed a significant training-induced increase only for the IST intervention group. In accordance with this finding, Silva et al. reported a higher proportion of highly differentiated effector memory cells in elderly with an intense exercise lifestyle, and they concluded that such effects may be beneficial for the immune response to known antigens in trained elderly (34). In the present study, however, there was no significant difference in the percentage or absolute blood counts of the naïve T-cell sub-populations in both the CD8– and CD8+ compartments. In support to this observation, Raso et al. (52) found no difference in the absolute blood counts of CD4+ or CD8+ T-cells expressing CD28 - a predominantly naïve cell phenotype - in sedentary older females (aged 60–77 years) after 12-months of moderate intensity resistance training. Similarly, Kapasi et al. (21) found no effect of 32-week endurance and resistance exercise in frail elderly subjects (mean(SD) age 87(8) years) on CD28 expression within CD4+ or CD8+ T-cells. On the other hand, Shimizu et al. (53) found no change in the number of CD4+CD28+ cells, and an increase in the number of CD8+ T-cells expressing CD28 after a 12-week endurance and resistance exercise in older persons aged 61–76 years. While further studies are required to elucidate the effects of resistance exercise on naïve T-cells, a reduction in senescent immune cells by itself is anticipated to have positive effects on the levels of inflammatory cytokines and related diseases.

Some studies have demonstrated sex-based differences in responses following exercise, even in older subjects (13). Regarding the association between sex and exercise-induced catecholamine – a key player in T-cell mobilization - several authors have reported a higher prevalence of catecholamine in men than women after exercise (54), though others found no sex difference (55) or even an increase in women compared to men (56). More so, reports from Grilo et al. in marine models support the existence of sex-differences in physiological responses to oxidative stress (57). On the other hand, at an advanced age there are more women than men and it is very challenging to recruit enough older male subjects. Taking this into consideration

and to avoid sex-related confounding effects in exercise-induced immune changes, it seemed sensible to start with women and, based on the findings, to proceed with the male individuals. Further research is highly required to elucidate eventual sex differences.

As expected, our findings support the current knowledge that T-cell repertoire are altered - with an expansion of senescent immune cells and a decrease in naïve T-cells - during the course of ageing (20,33,41). Indeed, there was a significant association between age and the percentage of the highly differentiated memory and senescence-prone phenotypes in both the CD8+ and CD8– T-cell compartments. Contrary wise, an inverse association was found between the proportion of naive phenotypes and age in both T-cell subsets. This age-dependent shift from T-cells exhibiting predominantly naive phenotypes to highly differentiated memory and senescent phenotypes might reflect lower numbers of haematopoietic stem cells, thymus involution and the influence of cumulative exposure to foreign infectious agents (58).

A factor that may weaken our study is the fact that we did not include a non-exercise control group - since participants in the CON group performed stretching exercise - which might have masked supplementary benefits resulting from training. On the other hand, this study design allowed to control for possible bias due to social interaction and mobility (subjects of the CON group came to the exercise center at a similar frequency as those in the SET & IST groups). The fact that the reduction in senescence-prone T-cells in the SET group was significantly different compared to CON (which showed no change over time), points therefore to mechanisms related to the SET resistance training intervention. Also, the authors acknowledge the limitation of not having a young control and male groups, which might have provided insight into the respective effects of ageing and sex on exercise-induced adaptations of immune cells. More so, by using nonparametric statistical methods, it is possible that we lost some statistical power in our analyses. Although this study has some limitations, it adds a highly

needed element in the current cellular concepts on exercise-induced immune response in older women. Thus far, the scant data in this field in older subjects have largely used the classical aerobic training, with results showing little or no beneficial long-term effects on immune response. Our study addresses a gap in the literature by comparing the effects of strength training at different training loads and repetitions on resting T-cell phenotypes. Our observation that SET, but not IST, reduces resting senescence-prone T-cell, will act as a guide for future experiments concerning T-cell response to exercise in older women. In this context, additional T-cell markers (e.g. CD45RA / CCR7 / CD27) might be useful to better define different naive and memory T-cell subsets and their response to exercise. Since reports addressing the immune response to resistance training in older individuals are lacking, more functional studies are highly needed to understand the significance of our observation in the context of immunosenescence. This could be done for instance by assessing the effects of SET on vaccine responses. Also, questions as to whether senescence-prone cells die or track to the marginal pool or tissues should be addressed in future studies.

Conclusion

Our results provide evidence that resistance exercise may have an anti-IS effect, once again showing the health benefits of resistance training among the aged. We observed that 6 weeks of SET, but not IST, significantly reduced senescence-prone T-cells compared to CON. Conceivably, training protocols with many repetitions - at a sufficiently high external resistance - may favour the reduction of senescence-prone cells in older women.

Funding

This study was funded by a grant from the People's Committee of Hochiminh City, Vietnam (grant number 35-QĐ/BTCTU) to [Hung Cao Dinh] and the scientific fund Willy Gepts from

the University Hospital (UZ Brussel) of the Vrije Universiteit Brussel (grant number WFWG17).

Conflict of interest

All authors certify that they comply with the ethical guidelines for publishing in the Journal of Gerontology: Biological Sciences. None of the authors have any conflict of interest with any entity with regard to this study. The authors have no other conflict of interest to declare.

References

1. Yuan J, Liu Y, Wang J, et al. Long-term persistent organic pollutants exposure induced telomere dysfunction and senescence-associated secretary phenotype. *J Gerontol A Biol Sci Med Sci.* 2018. doi: 10.1093/gerona/gly002
2. Saule P, Trauet J, Dutriez V, Lekeux V, Dessaint JP, Labalette M. Accumulation of memory T cells from childhood to old age: central and effector memory cells in CD4(+) versus effector memory and terminally differentiated memory cells in CD8(+) compartment. *Mechanisms of ageing and development.* 2006;127:274-281. doi: 10.1016/j.mad.2005.11.001
3. Palmer DB. The effect of age on thymic function. *Frontiers in immunology.* 2013;4:316. doi: 10.3389/fimmu.2013.00316
4. Fagnoni FF, Vescovini R, Passeri G, et al. Shortage of circulating naive CD8+ T cells provides new insights on immunodeficiency in aging. *Blood.* 2000;95:2860-2868. doi:
5. van Deursen JM. The role of senescent cells in ageing. *Nature.* 2014;509:439-446. doi: 10.1038/nature13193
6. Davalos AR, Coppe JP, Campisi J, Desprez PY. Senescent cells as a source of inflammatory factors for tumor progression. *Cancer metastasis reviews.* 2010;29:273-283. doi: 10.1007/s10555-010-9220-9
7. Castle SC. Clinical relevance of age-related immune dysfunction. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2000;31:578-585. doi: 10.1086/313947
8. Luscher TF. Prevention: some important steps forward, but many unmet needs in a world with cardiovascular disease as the leading cause of death. *European Heart Journal.* 2016;37:3179-3181. doi: 10.1093/eurheartj/ehw566

9. Kohut ML, Cooper MM, Nickolaus MS, Russell DR, Cunnick JE. Exercise and psychosocial factors modulate immunity to influenza vaccine in elderly individuals. *The journals of gerontology Series A, Biological sciences and medical sciences.* 2002;57:M557-562. doi: 10.1093/gerona/57.9.M557

10. Chin APMJ, de Jong N, Pallast EG, Kloek GC, Schouten EG, Kok FJ. Immunity in frail elderly: a randomized controlled trial of exercise and enriched foods. *Medicine and science in sports and exercise.* 2000;32:2005-2011. doi: 10.1097/00005768-200012000-00006

11. Liberman K, Forti LN, Beyer I, Bautmans I. The effects of exercise on muscle strength, body composition, physical functioning and the inflammatory profile of older adults: a systematic review. *Curr Opin Clin Nutr Metab Care.* 2017;20:30-53. doi: 10.1097/MCO.0000000000000335

12. Forti LN, Van Roie E, Njemini R, et al. Effects of resistance training at different loads on inflammatory markers in young adults. *European journal of applied physiology.* 2017;117:511-519. doi: 10.1007/s00421-017-3548-6

13. Forti LN, Van Roie E, Njemini R, et al. Load-Specific Inflammation Mediating Effects of Resistance Training in Older Persons. *J Am Med Dir Assoc.* 2016;17:547-552. doi: 10.1016/j.jamda.2016.02.010

14. Elosua R, Bartali B, Ordovas JM, et al. Association between physical activity, physical performance, and inflammatory biomarkers in an elderly population: The InCHIANTI study. *J Gerontol a-Biol.* 2005;60:760-767. doi: DOI 10.1093/gerona/60.6.760

15. McFarlin BK, Flynn MG, Campbell WW, et al. Physical Activity Status, but not Age, Influences Inflammatory Cytokine Production and Toll-like Receptor 4. *Medicine and*

science in sports and exercise. 2006;38:S308-S308. doi: Doi 10.1249/00005768-200605001-02204

16. Gleeson M, Bishop NC. The T cell and NK cell immune response to exercise. *Ann Transplant.* 2005;10:43-48. doi:

17. Kruger K, Lechtermann A, Fobker M, Volker K, Mooren FC. Exercise-induced redistribution of T lymphocytes is regulated by adrenergic mechanisms. *Brain Behavior and Immunity.* 2008;22:324-338. doi: 10.1016/j.bbi.2007.08.008

18. Mooren FC, Kruger K. Apoptotic lymphocytes induce progenitor cell mobilization after exercise. *J Appl Physiol (1985).* 2015;119:135-139. doi: 10.1152/japplphysiol.00287.2015

19. Cao Dinh H, Beyer I, Mets T, et al. Effects of Physical Exercise on Markers of Cellular Immunosenescence: A Systematic Review. *Calcif Tissue Int.* 2017;100:193-215. doi: 10.1007/s00223-016-0212-9

20. Simpson RJ, Cosgrove C, Ingram LA, et al. Senescent T-lymphocytes are mobilised into the peripheral blood compartment in young and older humans after exhaustive exercise. *Brain, behavior, and immunity.* 2008;22:544-551. doi: 10.1016/j.bbi.2007.11.002

21. Kapasi ZF, Ouslander JG, Schnelle JF, Kutner M, Fahey JL. Effects of an exercise intervention on immunologic parameters in frail elderly nursing home residents. *The journals of gerontology Series A, Biological sciences and medical sciences.* 2003;58:636-643. doi: 10.1093/gerona/58.7.M636

22. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res.* 1975;12:189-198. doi: 10.1016/0022-3956(75)90026-6

23. Bautmans I, Lambert M, Mets T. The six-minute walk test in community dwelling elderly: influence of health status. *BMC Geriatr.* 2004;4:6. doi: 10.1186/1471-2318-4-6

24. Forti LN, Njemini R, Beyer I, et al. Strength training reduces circulating interleukin-6 but not brain-derived neurotrophic factor in community-dwelling elderly individuals. *Age.* 2014;36:9704. doi: 10.1007/s11357-014-9704-6

25. Beckwée D, Bautmans I, Scheerlinck T, Vaes P. Exercise in knee osteoarthritis–preliminary findings: Exercise-induced pain and health status differs between drop-outs and retainers. *Experimental gerontology.* 2015;72:29-37. doi: 10.1016/j.exger.2015.09.009

26. Johnson E, Bradley B, Witkowski K, et al. Effect of a static calf muscle-tendon unit stretching program on ankle dorsiflexion range of motion of older women. *J Geriatr Phys Ther.* 2007;30:49-52. doi:

27. Onyema OO, Njemini R, Bautmans I, Renmans W, De Waele M, Mets T. Cellular aging and senescence characteristics of human T-lymphocytes. *Biogerontology.* 2012;13:169-181. doi: 10.1007/s10522-011-9366-z

28. Cao Dinh H, Bautmans I, Beyer I, et al. Association between immunosenescence phenotypes and pre-frailty in older subjects: Does cytomegalovirus play a role? . *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences.* 2018;In Press. doi:

29. Woods JA, Ceddia MA, Wolters BW, Evans JK, Lu Q, McAuley E. Effects of 6 months of moderate aerobic exercise training on immune function in the elderly. *Mechanisms of ageing and development.* 1999;109:1-19. doi: 10.1016/S0047-6374(99)00014-7

30. Shinkai S, Kohno H, Kimura K, et al. Physical activity and immune senescence in men. *Medicine and science in sports and exercise.* 1995;27:1516-1526. doi:

31. Campbell PT, Wener MH, Sorensen B, et al. Effect of exercise on in vitro immune function: a 12-month randomized, controlled trial among postmenopausal women. *J Appl Physiol (1985).* 2008;104:1648-1655. doi: 10.1152/japplphysiol.01349.2007

32. Allison DJ, Ditor DS. The common inflammatory etiology of depression and cognitive impairment: a therapeutic target. *J Neuroinflammation.* 2014;11:151. doi: 10.1186/s12974-014-0151-1

33. Spielmann G, McFarlin BK, O'Connor DP, Smith PJ, Pircher H, Simpson RJ. Aerobic fitness is associated with lower proportions of senescent blood T-cells in man. *Brain, behavior, and immunity.* 2011;25:1521-1529. doi: 10.1016/j.bbi.2011.07.226

34. Silva LC, de Araujo AL, Fernandes JR, et al. Moderate and intense exercise lifestyles attenuate the effects of aging on telomere length and the survival and composition of T cell subpopulations. *Age (Dordr).* 2016;38:24. doi: 10.1007/s11357-016-9879-0

35. Cadore EL, Pinto RS, Bottaro M, Izquierdo M. Strength and endurance training prescription in healthy and frail elderly. *Aging Dis.* 2014;5:183-195. doi: 10.14336/AD.2014.0500183

36. de Cabo R, Carmona-Gutierrez D, Bernier M, Hall Michael N, Madeo F. The Search for Antiaging Interventions: From Elixirs to Fasting Regimens. *Cell.* 2014;157:1515-1526. doi: https://doi.org/10.1016/j.cell.2014.05.031

37. Simpson RJ, Cosgrove C, Chee MM, et al. Senescent phenotypes and telomere lengths of peripheral blood T-cells mobilized by acute exercise in humans. *Exercise immunology review.* 2010;16:40-55. doi:

38. Cinamon G, Shinder V, Alon R. Shear forces promote lymphocyte migration across vascular endothelium bearing apical chemokines. *Nat Immunol.* 2001;2:515-522. doi: 10.1038/88710

39. Wielemborek-Musial K, Szmigielska K, Leszczynska J, Jegier A. Blood Pressure Response to Submaximal Exercise Test in Adults. *Biomed Res Int.* 2016;2016:5607507. doi: 10.1155/2016/5607507

40. Kruger K, Lechtermann A, Fobker M, Volker K, Mooren FC. Exercise-induced redistribution of T lymphocytes is regulated by adrenergic mechanisms. *Brain Behav Immun.* 2008;22:324-338. doi: 10.1016/j.bbi.2007.08.008

41. Simpson RJ. Aging, persistent viral infections, and immunosenescence: can exercise "make space"? *Exercise and sport sciences reviews.* 2011;39:23-33. doi: 10.1097/JES.0b013e318201f39d

42. Campbell JP, Riddell NE, Burns VE, et al. Acute exercise mobilises CD8+ T lymphocytes exhibiting an effector-memory phenotype. *Brain Behav Immun.* 2009;23:767-775. doi: 10.1016/j.bbi.2009.02.011

43. Rot A, von Andrian UH. Chemokines in innate and adaptive host defense: basic chemokinese grammar for immune cells. *Annual review of immunology.* 2004;22:891-928. doi: 10.1146/annurev.immunol.22.012703.104543

44. Luscinskas FW, Kiely JM, Ding H, et al. In vitro inhibitory effect of IL-8 and other chemoattractants on neutrophil-endothelial adhesive interactions. *J Immunol.* 1992;149:2163-2171. doi:

45. Ley K, Baker J, Cybulsky M, Gimbrone M, Luscinskas F. Intravenous interleukin-8 inhibits granulocyte emigration from rabbit mesenteric venules without altering L-selectin expression or leukocyte rolling. *The Journal of Immunology.* 1993;151:6347-6357. doi:

46. Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell.* 1994;76:301-314. doi: 10.1016/0092-8674(94)90337-9

47. McGuigan MR, Egan AD, Foster C. Salivary Cortisol Responses and Perceived Exertion during High Intensity and Low Intensity Bouts of Resistance Exercise. *J Sports Sci Med.* 2004;3:8-15. doi:

48. Dimitrov S, Benedict C, Heutling D, Westermann J, Born J, Lange T. Cortisol and epinephrine control opposing circadian rhythms in T cell subsets. *Blood.* 2009;113:5134-5143. doi: 10.1182/blood-2008-11-190769

49. Bosch JA, Fischer JE, Fischer JC. Psychologically adverse work conditions are associated with CD8+ T cell differentiation indicative of immunesenescence. *Brain Behav Immun.* 2009;23:527-534. doi: 10.1016/j.bbi.2009.02.002

50. Goto C, Higashi Y, Kimura M, et al. Effect of different intensities of exercise on endothelium-dependent vasodilation in humans: role of endothelium-dependent nitric oxide and oxidative stress. *Circulation.* 2003;108:530-535. doi: 10.1161/01.CIR.0000080893.55729.28

51. Lanna A, Henson SM, Escors D, Akbar AN. The kinase p38 activated by the metabolic regulator AMPK and scaffold TAB1 drives the senescence of human T cells. *Nat Immunol.* 2014;15:965-972. doi: 10.1038/ni.2981

52. Raso V, Benard G, AJ DASD, Natale VM. Effect of resistance training on immunological parameters of healthy elderly women. *Medicine and science in sports and exercise.* 2007;39:2152-2159. doi: 10.1249/mss.0b013e318156e9fa

53. Shimizu K, Suzuki N, Imai T, et al. Monocyte and T-cell responses to exercise training in elderly subjects. *Journal of strength and conditioning research / National Strength & Conditioning Association.* 2011;25:2565-2572. doi: 10.1519/JSC.0b013e3181fc5e67

54. Friedlander AL, Casazza GA, Horning MA, et al. Training-induced alterations of carbohydrate metabolism in women: women respond differently from men. *J Appl Physiol (1985).* 1998;85:1175-1186. doi: 10.1152/jappl.1998.85.3.1175

55. Marliss EB, Kreisman SH, Manzon A, Halter JB, Vranic M, Nessim SJ. Gender differences in glucoregulatory responses to intense exercise. *J Appl Physiol (1985).* 2000;88:457-466. doi: 10.1152/jappl.2000.88.2.457

56. Lehmann M, Berg A, Keul J. Sex-related differences in free plasma catecholamines in individuals of similar performance ability during graded ergometric exercise. *Eur J Appl Physiol Occup Physiol.* 1986;55:54-58. doi: 10.1007/BF00422893

57. Grilo TF, Lopes AR, Sampaio E, Rosa R, Cardoso PG. Sex differences in oxidative stress responses of tropical topshells (Trochus histrio) to increased temperature and high pCO2. *Mar Pollut Bull.* 2018;131:252-259. doi: 10.1016/j.marpolbul.2018.04.031

58. Geiger H, de Haan G, Florian MC. The ageing haematopoietic stem cell compartment. *Nat Rev Immunol.* 2013;13:376-389. doi: 10.1038/nri3433

Table 1: Participants' health status

Parameter	Description *	Clinical examples	IST (n=31)	SET (n=33)	CON (n=36)
Health category					
A A1	Completely healthy; no medication		1 (3.23)	3 (9.09)	1 (2.78)
A2	Completely healthy; using only preventive medication	Hormonal replacement therapy, aspirin, ...	1 (3.23)	2 (6.06)	3 (8.33)
B B1	Functioning normally; presence of stabilized, non-cardiovascular disease; absence of cardiovascular abnormalities	Treated hypothyroidism, stable diabetes, ...	14 (45.16)	12 (36.36)	9 (25.00)
B2	Functioning normally; using medication with cardiovascular effect, no overt cardiovascular disease other than normalized arterial hypertension	Arterial hypertension, β blocking agent, ...	12 (38.71)	11 (33.33)	17 (47.22)
C	(history of) cardio-vascular pathology or abnormal ECG.	Bundle branch block, angina, CABG, ...	3 (9.67)	5 (15.16)	6 (16.67)
D	Presenting signs of acute or active disease at the moment of examination	Bronchospasm, swollen joints, influenza, ...	/	/	/
Age category					
65-74			27 (87.10)	28 (84.85)	30 (83.33)
≥ 75			4 (12.90)	5 (15.15)	6 (16.67)

Note: The values denote number (percentage). * Status after questioning, physical examination, ECG, and laboratory examination of blood, serum & urine; CABG= coronary artery bypass graft; IST= Intensive strength training; SET= Strength endurance training; CON= control.

Table 2. Overview of subjects' characteristics at baseline.

Parameter	IST (n = 31)	SET (n = 33)	CON (n = 36)	p value [a]
Age (years)	69.18 (5.12)	69.02 (6.05)	70.31 (5.15)	0.331
BMI (kg/m^2)	26.02 (8.42)	26.23 (4.90)	26.12 (5.94)	0.996
CRP (mg/l)	1.38 (2.15)	1.30 (1.60)	1.00 (2.08)	0.484
T-cell subset				
CMV serostatus, n (%)				
CMV+	24 (80.00)	19 (59.38)	20 (57.14)	0.113
CMV−	6 (20.00)	13 (40.62)	15 (42.86)	
CD8 + T-cell				
CD8+CD28+CD57− (naïve)	54.20 (27.00)	66.30 (25.50)	60.50 (34.42)	0.304
CD8+CD28−CD57− (memory)	28.00 (22.50)	24.70 (17.80)	25.75 (22.45)	0.665
CD8+CD57+ (SPC)	5.85 (12.40)	5.60 (9.65)	4.00 (12.30)	0.214
CD8+CD28−CD57+ (SPC)	5.65 (12.02)	5.20 (9.95)	2.90 (11.70)	0.209
CD8+CD28+CD57+(SPC)	0.30 (0.50)	0.20 (0.65)	0.20 (0.60)	0.813
CD8 – T-cell				
CD8−CD28+CD57− (naive)	97.00 (5.50)	97.80 (5.55)	98.25 (4.90)	0.751
CD8−CD28−CD57− (memory)	1.40 (3.40)	1.20 (4.25)	1.15 (2.80)	0.861
CD8−CD57+ (SPC)	0.90 (1.80)	0.50 (1.80)	0.20 (1.40)	0.242
CD8−CD28−CD57+ (SPC)	0.80 (1.60)	0.40 (1.65)	0.15 (1.30)	0.319
CD8−CD28+CD57+ (SPC)	0.10 (0.30)	0.10 (0.10)	0.10 (0.10)	0.533

Note: The values denote median (Interquartile range). BMI= body mass index; CRP= C- reactive protein; SPC= senescence-prone cells; IST= intensive strength training; SET= strength-endurance training; CON= control; Phenotype frequencies were expressed as percentages within the CD3+CD8+ or CD3+CD8− T-cells; [a] Results of Kruskal-Wallis test except for CMV serostatus which employed Chi-squared test.

Supplementary Table 1: Absolute counts of T-cell phenotypes at baseline.

T-cell subtype	IST (n = 31)	SET (n = 33)	CON (n = 36)	p value [a]
CD8 + cell				
CD8+CD28+CD57− (naive)	175 (133)	152 (128)	142 (71)	0.539
CD8+CD28−CD57− (memory)	100 (160)	50 (95)	60 (58)	0.199
CD8+CD57+ (SPC)	20.11 (35.15)	14.54 (34.05)	7.87 (40.13)	0.152
CD8+CD28−CD57+ (SPC)	18.81 (35.09)	13.70 (32.21)	7.43 (40.07)	0.135
CD8+CD28+CD57+ (SPC)	0.90 (1.38)	0.71 (1.06)	0.64 (1.49)	0.492
CD8 – cell				
CD8−CD28+CD57− (naive)	597 (307)	656 (310)	646 (392)	0.730
CD8−CD28−CD57− (memory)	10 (30)	10 (30)	10 (20)	0.998
CD8−CD57+ (SPC)	4.85 (17.25)	2.65 (11.19)	1.83 (11.68)	0.283
CD8−CD28−CD57+ (SPC)	4.01 (10.78)	1.82 (9.94)	1.08 (10.55)	0.389
CD8−CD28+CD57+ (SPC)	0.62 (1.46)	0.66 (0.91)	0.65 (1.31)	0.829

Note: The values denote median (Interquartile range) of the absolute number of cells per µL blood; SPC= senescence-prone cells; IST= intensive strength training; SET= strength-endurance training; CON= control; [a] Results of Kruskal-Wallis test.

LEGEND TO THE FIGURES

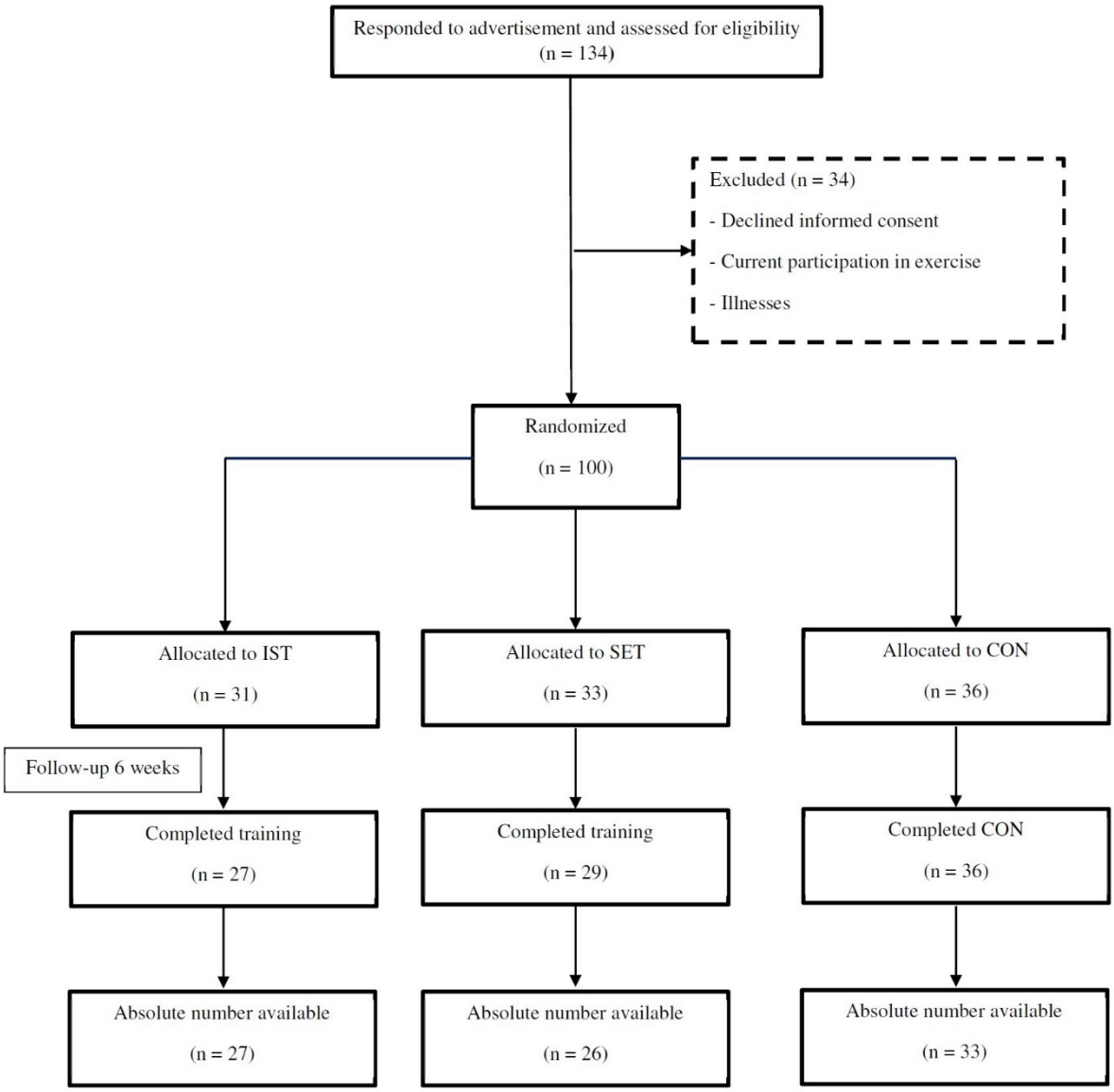

Figure 1. Flowchart of study participants

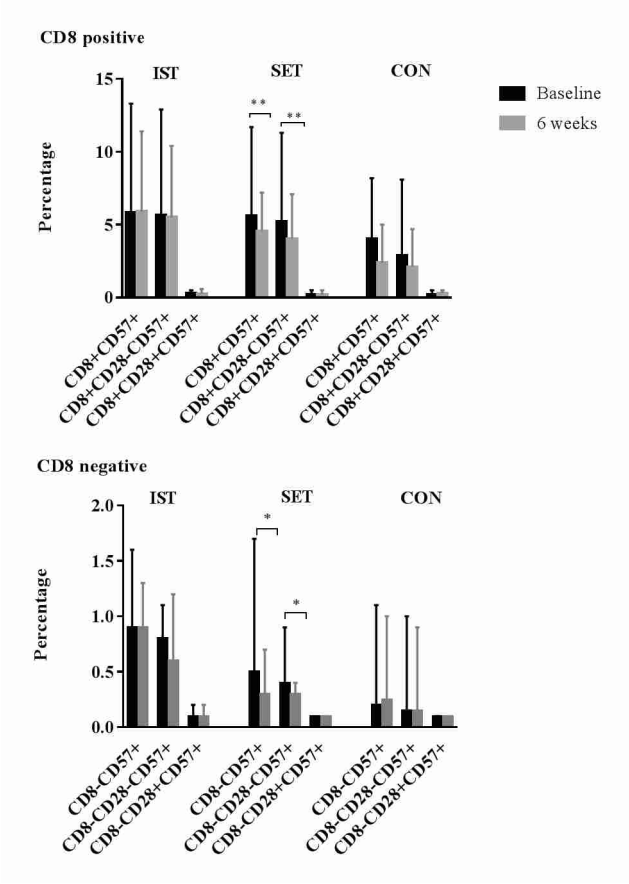

Figure 2. Change in the percentage of senescent phenotypes in peripheral blood at 6 weeks compared to baseline in the various intervention groups. Data are median values with error bars representing 95% confident intervals. IST= intensive strength training; SET= strength endurance training; CON= control. Results of Wilcoxon signed-rank test for difference between baseline and 6 weeks; *p <0.05, **p<0.01, decreased significantly after exercise compared to baseline.

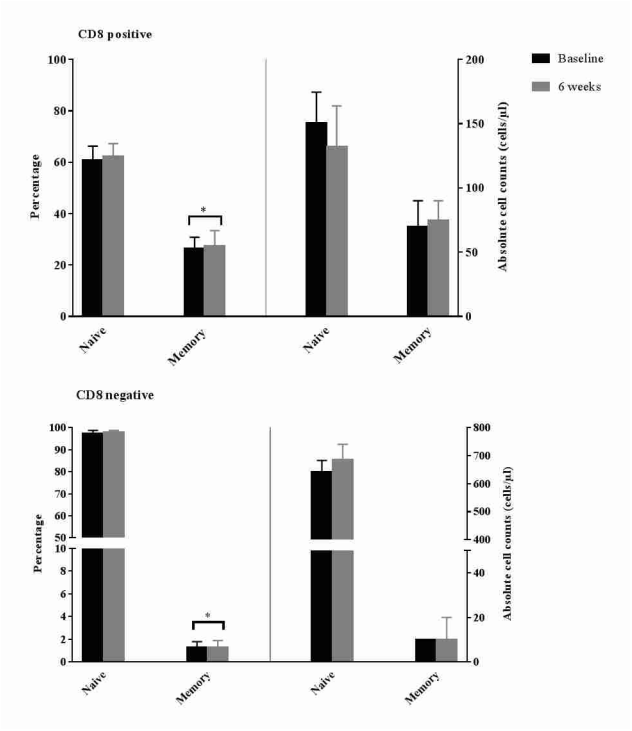

Figure 3. Change in the percentage and absolute cell counts of naive and memory phenotypes in peripheral blood at 6 weeks compared to baseline. Data are median values with error bars representing 95% confident intervals. Results of Wilcoxon signed-rank test for difference between baseline and 6 weeks; *p <0.05, decreased significantly after exercise compared to baseline.

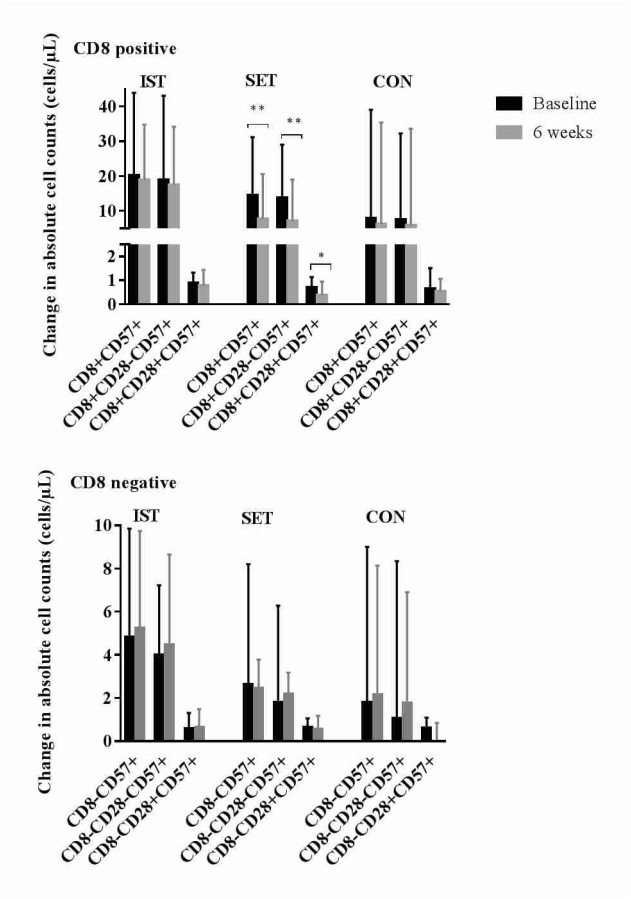

Figure 4. Change in the absolute cell counts of senescent phenotypes in peripheral blood at 6 weeks compared to baseline in the various intervention groups. Data are median values with error bars representing 95% confident intervals. IST= intensive strength training; SET= strength endurance training; CON= control. Results of Wilcoxon signed-rank test for difference between baseline and 6 weeks; *p <0.05, **p<0.01, decreased significantly after exercise compared to baseline.