Is stretching an appropriate control for studies on exercise immunology in older adults? A systematic review.

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ABSTRACT

Objective: Active muscle contraction is assumed to be essential in the anti-inflammatory effect of physical exercise, also in older adults. Although stretching does not involve active muscle contractions, its (anti)inflammatory effects remain unclear. This systematic review aims to determine whether stretching affects the inflammatory profile of older adults and if it can be considered as an appropriate control intervention for exercise immunology studies.

Methods: This systematic review was registered in PROSPERO (CRD42023388920) and conducted in accordance with PRISMA guidelines. PubMed and Web of Science were systematically screened for articles describing the effect of muscle stretching on the inflammatory profile (immune cell proportions, cytokines, oxidative markers, and inflammatory gene expression in muscle/immune cells) in older adults. A methodological quality assessment was performed using the ROB2 tool and effect sizes (ES) were calculated.

Results: Nine randomized controlled trials were included, all showing sufficient methodological quality and reporting effects on basal levels of inflammation. Muscle stretching had no effect on the number of naïve, memory, and senescence-prone T-cells or circulating inflammatory markers CRP and IL-6 neither on most studied oxidative stress markers (SOD, NO, VCAM, ICAM, PTX3, OX-LDL, MDA, HNE, nitrotyrosine, ox-LDL, and protein carbonyls). However, the oxidative stress marker LPO increased (ES=0.76) while CAT, ROS, fibrinogen, and MDA-LDL decreased (ES between -0.50 and -0.63) after stretching in older persons with chronic diseases. Contradictory results were found for TNF-alpha and gene expression levels. One study observed no changes in circulating TNF-alpha after stretching in healthy women, while another study showed an increase in muscle gene expression of TNF-alpha (ES=1.60) as well as circulating TNF-alpha (ES=0.64) in men with peripheral arterial disease. Regarding gene expression changes in pro/anti-inflammatory related genes, one study analysing RNA extracted from peripheral blood mononuclear cells showed stretching-induced increases (fold change≥1.5) or decreases (fold change≤0.67), while another study using RNA from buffy coat samples demonstrated no effect on gene expression.

Conclusion: Passive or active types of muscle stretching appears to be a suitable active control for exercise immunology studies in older populations. However, in populations with peripheral artery disease stretching may affect the inflammatory profile, possibly due to a higher overall inflammatory status.

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INTRODUCTION

The average age and life expectancy of men and women has increased drastically, giving rise to many novel healthcare challenges. Consequently, the incidence of late-life diseases tends to increase, resulting in a higher burden on scarce healthcare resources (1, 2). Older adults possess a higher risk of infection and the occurrence of chronic diseases (3, 4). An increased level of inflammation is associated with a higher incidence of illness and death (5). The phenomenon of age-associated elevations of pro-inflammatory markers, such as interleukin-6 (IL-6), Tumor Necrosis Factor-alpha (TNF-α) and C-reactive protein (CRP), in blood and tissue is defined as 'inflammageing' (5-7). This pro-inflammatory profile that emerges during ageing can partly be explained by a shift of immune cell phenotype towards a more senescent-prone cell (SPC) composition. These senescent-prone cells contribute to an accumulation of pro-inflammatory cytokines via their senescence-associated secretory phenotype (SASP), inducing an (unresolved) state of chronic inflammation, known as chronic low grade inflammatory profile (CLIP), even without the presence of antigenic stimuli (8-10). The transition from more naïve to memory- and senescent-prone T-cells is part of a gradual remodelling of the immune system during ageing, referred to as 'immunosenescence' (8, 9, 11). Immunosenescence is among others associated with a reduced vaccination response and increased susceptibility to infections (12-14). Moreover, increases in cytokines, such as IL-6 and CRP, are associated with frailty, cardiovascular diseases, sarcopenia, and other pathophysiological conditions (6, 10, 11). Additionally, the ageing process is characterised by an increase in reactive oxygen species (ROS) and decrease in anti-oxidant systems, leading to oxidative stress that can damage various cell components, exacerbating the ageing process and contributing to ageing-related diseases (15, 16).

Recent studies showed that physical exercise can exert beneficial effects on the inflammatory status, immunosenescence and oxidative stress-related markers in older persons (17-20). It has been shown that exercise can increase the number of naïve T-cells, enhance T-cell proliferation, and decrease the number of senescent-prone CD4+ and CD8+ T-cells. These changes in the immune system could therefore (at least partially) alleviate or counteract immunosenescence (17, 21, 22). Exercise can also decrease markers of oxidative stress and improve the anti-oxidant response (19, 20). Possible mechanisms have been identified by which aerobic or resistance exercises may counteract CLIP. Firstly, physical exercise involves muscle contraction and activates energy metabolism (23), thereby stimulating skeletal muscle fibers to produce myokines which can exert anti-inflammatory effects (24). Exercise-induced production of the myokine IL-6, secreted via a TNF-independent pathway, can induce secretion of anti-inflammatory cytokines IL-1ra and IL-10 by peripheral immune cells, which inhibit the release of the pro-inflammatory cytokine IL-1β (25-27). Secondly, physical exercise induces epinephrine and norepinephrine release by the adrenal medulla and sympathetic nerves. Activation of the β adrenoceptors expressed on T- and B-cells leads to cyclic adenosine monophosphate (cAMP) production and adenyl cyclase activation. This cascade can result in IL-10 secretion by T-cells (3, 27, 28). Moreover, physical exercise can also lead to a decrease in systemic inflammation through other mechanisms, including the loss of visceral fat mass (29).

Muscle stretching exercises are defined in the Medical Subject Headings database of the National Library of Medicine as "Exercises that stretch the muscle fibers with the aim to increase muscle-tendon flexibility, improve range of motion or musculoskeletal function, and prevent injuries. There are various types of stretching techniques including active, passive (relaxed), static, dynamic (gentle), ballistic (forced), isometric, and others" (30). This form of exercise seems more suitable as an active control intervention compared to a non-exercising control in exercise intervention studies. Indeed, muscle stretching allows to control for confounding factors related to the social environment of group sessions and personalised training follow-up by instructors, but also the possible exposure to pathogens during group trainings or via public transportation to the training facility. Moreover, this type of control offers the advantage of allowing the structure, timing, and follow-up of the program, as well as the duration of individual sessions and the amount of interaction between research staff and participants, to be kept consistent across both the intervention and control arms. These elements have all been identified as potential influencers of participant motivation, study adherence and completion, and self-reported outcomes (including increased daily physical activity when being randomized to a non-exercising control group) (31, 32). Although the use of muscle stretching as an active control group for exercise interventions seems promising, the effects of muscle stretching on the inflammatory profile in older adults has not yet been documented. The suitability of muscle stretching as a control for exercise immunology studies thus remains unclear. Therefore, the aim of this systematic review was to determine whether stretching affects the inflammatory profile of older adults and if it can be considered as an appropriate control intervention for exercise immunology studies.

METHODS

Search strategy and selection criteria

This systematic review was conducted in accordance with PRISMA guidelines, and the protocol is registered in Prospero (CRD42023388920). Two databases, PubMed and Web of Science, were systematically screened. The search key was built according to the PICO design (Population: adults aged ≥65 years old; Intervention: muscle stretching exercise; Comparison: no exercise or other forms of exercise; Outcomes: inflammatory status) including both MeSH and free terms (for the full search strings see supplement 1 & 2, for full PICO framework see Supplementary Table S1). The last search was performed on 24th of January 2024.

Study selection

Intervention studies (both randomized controlled trials (RCT),- and non-randomized controlled trials) with participants aged 65 years or older in which muscle stretching exercises were performed as experimental or control intervention were eligible for inclusion. Studies describing a combination of muscle

	P: Older adults	I: Muscle stretching	C	O: Inflammatory profile
MeSH terms	Aged	Exercise, muscle stretching Range of motion, articular		Inflammation Cytokines Immunity, cellular
Free terms	Aged Older adults Older person Older people Elderly	Muscle stretching exercise Articular range of motion "flexibility" AND "exercise" OR "flexibility" AND "training" "joint" AND "flexibility" "PNF" AND "stretching" Proprioceptive neuromuscular facilitation stretching Passive stretching Relaxed stretching Static-passive stretching Isometric stretching Active stretching Static-active stretching Ballistic stretching Dynamic stretching		Inflammation "inflammatory" AND "profile" Inflammatory Cellular immunity "immune" AND "response" "immune" AND "reaction" Cytokines Chemokine "tumor" AND "necrosis" AND "factor" "C-reactive protein"

Supplementary Table S1: Search strategy according to PICO

Supplementary Table S2: Quality assessment output – Risk of Bias

stretching with aerobic or resistance exercises, as well as yoga or Pilates, were excluded. Moreover, only studies written in English and investigating the effects of muscle stretching exercises on the inflammatory status – i.e. immune cell proportions, cytokines, oxidative stress markers, and inflammatory gene expression in muscle or immune cells - were eligible. Review articles, case reports, and animal studies were excluded. There were no restrictions on the publication date.

Firstly, two reviewers (LS and EM), who were blinded for each other's results, screened titles and abstracts for eligibility of the studies using 'Rayyan web application for systematic reviews' (33). Next, three reviewers (LS, EM and AG) performed a full-text review to further assess eligibility. A fourth reviewer (IB) was consulted to resolve disagreements.

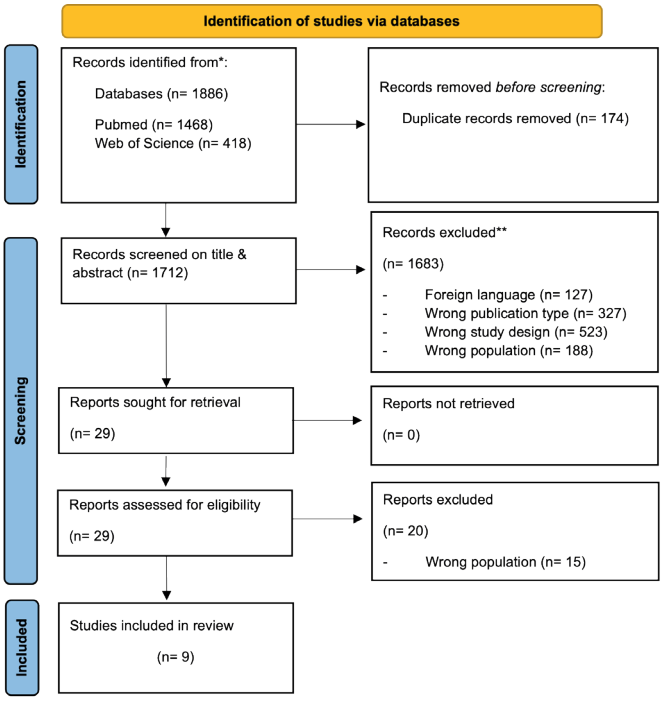

Figure 1. Flowchart Inclusion of articles

Data extraction

The main characteristics of participants (age, gender, and number of participants) and exercise interventions (training period, frequency and duration of sessions, specific type of muscle stretching exercises, number of repetitions, and duration of individual exercises) were appraised. All data regarding the inflammatory status of older adults were extracted. The outcomes were divided into three categories: 1) circulating inflammatory and oxidative stress markers (Table 1), 2) inflammation related gene expression in muscle or immune cells (Table 2), and 3) absolute counts or percentages of circulating immune cells (Table 3).This review also determined whether studies examined the effects of muscle stretching exercise on acute effects (i.e., sampling within 24h after the last exercise session) or basal levels (i.e., sampling minimum 24h after the last exercise session). The effect size (ES) of muscle stretching on outcomes of the inflammatory profile was calculated. Graph data was digitized utilizing the online tool Webplotdigitizer version 4.6 (34). When feasible, an estimate of the ES was derived following Cohen's D criteria (Small ES: d≥0.2, medium ES: d≥0.5, and large ES: d≥0.8). Only within-group comparisons were made, and the ES was determined using Hedges' average. Authors were contacted when data was unavailable. For illustrative purposes and to be able to appraise the ES of muscle stretching versus other types of (contractile) exercise, the calculated ES of muscle stretching was compared to the ES of exercise interventions extracted from recently published literature, with a preference for systematic reviews with meta-analyses or systematic reviews where ES, standardized mean difference (SMD) or weighted mean difference (WMD) were calculated. When no systematic reviews were available, recent research articles were used to compare the ES (Table 4-6).

Quality assessment of risk of bias

The quality of the included articles was assessed using the Cochrane Risk of Bias for randomized trials tool (ROB2) (35) by one reviewer (AG), which was verified by two independent reviewers (EM and LS).

RESULTS

Study selection

The medical databases PubMed and Web of Science yielded 1468 and 418 articles respectively, of which 174 articles were identified as duplicates. From these, 1683 articles were excluded after title and abstract screening due to the use of foreign languages (n=127) or wrong publication type (n=327), study design (n=523), population (n=188), intervention (n=483) or outcome (n=35) . An additional 20 articles were excluded based on full-text screening after which nine articles were included in this systematic review (Fig. 1).

Study characteristics

All included studies were randomized controlled trails (RCTs). In most studies (36-43), muscle stretching exercises were performed as an active control intervention for aerobic (36, 38, 41) or resistance (40, 42, 43) exercise interventions. De Paulo et al. (37) compared a combined aerobic with resistance program to a control muscle stretching exercise intervention. Rodrigues-Krause et al. (39) compared three interventions: a dancing intervention, an aerobic intervention, and a stretching intervention. Only one study (44) examined the effect of a stretching exercise program compared to a non-exercising control group. All studies reported 'pre-post intervention effects'. One study reported only within-group effects (44). Five studies (36, 37, 39, 42, 43) also investigated 'between group-time effects' and 'group*time effects'. One study mentioned 'within' and 'between effects' (38). Liberman et al. (40) and Iyalomhe et al. (41) reported fold changes in gene expression patterns (respectively absolute FC ≤0.67 or ≥1.5; and ≥2 considered as significant).

Participants' characteristics

In total, the studies included 163 participants. Interestingly, the effects of muscle stretching exercises on the inflammatory status of older persons were most frequently investigated in women, with 109 out of 158 participants (69%) being female. Iyalomhe et al. (41) investigated the effects in n=5 participants of both genders, however, without specifying the exact number of men and women. Most studies included healthy participants. In contrast, Andrade-Lima et al. (38) included men with peripheral artery disease (PAD) and intermittent claudication (IC) symptoms, Serra et al. (36) observed stroke survivors, Kato et al. (44) investigated chronic heart failure patients with an implantable defibrillator and De Paulo et al. (37) examined breast cancer survivors undergoing aromatase inhibitor therapy.

Intervention characteristics

The diversity in number, duration, and intensity of muscle stretching exercise sessions was high. All articles examined the effects of muscle stretching exercises on basal levels (i.e., sampling minimum 24h after the last exercise session) of inflammation. However, in one article the sampling time was not clear since the authors only mentioned that blood was taken after 12h fasting at baseline, and after 12, 24 and 36 weeks of intervention (37). Throughout these studies, the exercises consisted of similar whole body muscle stretching exercises. Four studies (38, 40, 42, 43) included passive stretching exercises for large muscle groups throughout the whole body. These muscle stretching exercises mainly induced a passive load without muscle contraction or cardiovascular challenge. Only one study described the use of active stretches (36). Four studies did not describe whether the stretching exercises was passive or active (37, 39, 41, 44). In one study (36), dynamic balance exercises were included in the stretching exercise protocol and participants received recommendations and education on cardiovascular disease risk factors and strategies to promote physical activity (36). One study included relaxation exercises in the stretching group (37), and in another study participants also performed breathing exercises (39). Heterogeneity within the frequency and duration of intervention was also observed. The shortest intervention period was 4 weeks; however, participants of this study trained every day (7x/week) (44). In another study the participants trained for 8 weeks, but only once a week (39). The longest intervention was 9 months (36 weeks), two times a week(37).

Quality assessment

All RCTs were assessed for their methodological quality. A summary of the risk of bias assessment for the nine included articles (36-44) can be retrieved in Supplementary Table 2. Six studies (37-40, 42, 43) showed a low risk of bias, while the risk of bias was of concern for three studies (36, 41, 44). Due to the nature of the interventions, participants and supervisors were aware of their assigned intervention group in all studies. Six studies (37-40, 42, 43) extensively described participant follow-up and withdrawal, unlike the other studies (41, 44), which did not address deviations or intervention compliance, resulting in some concerns regarding risk of bias. In one article, information on missing values or drop-outs was not mentioned (36).

Muscle stretching exercise effects on the inflammatory profile in older adults

Effect sizes of the muscle stretching effects were calculated and are shown in Table 4, 5 and 6. In general, most of the effect sizes for muscle stretching were small to medium.

Muscle stretching exercise effects on circulating cytokines, cell adhesion and oxidative stress markers in older adults (Table 1 and 4)

Muscle stretching showed no significant effects on circulating levels of CRP (36-39, 44) or IL-6 (36, 38). after muscle stretching. In contrast, muscle stretching increased circulatory TNF-α concentrations ($P<0.05$) in men after a 12-week training program (38), but not in women after an 8-week program (39).

Three studies (36, 38, 44) investigated the effect of muscle stretching on oxidative stress markers in older adults, two of which also examined its impact on cell adhesion markers (38, 44). Andrade-Lima et al. (38) observed no differences for plasma vascular cell adhesion molecule 1 (VCAM 1), intercellular adhesion molecule (ICAM), nitric oxide (NO) and superoxide dismutase (SOD) between baseline and post-intervention values. However, a decrease in catalase (CAT) and an increase in lipid peroxidation (LPO) was observed after stretching ($P<0.05$). Moreover, the effect on muscle related markers SOD, LPO and CAT was investigated in homogenized muscle tissue, revealing an increase in LPO ($P<0.05$), no effects on in CAT ($P > 0.05$), and no effect on SOD ($P>0.05$). In the study of Kato et al. (44) no difference in circulating pentraxin 3 (PTX3) was found after four weeks of daily stretching interventions, while a decrease of fibrinogen, malondialdehyde-modified low-density lipoprotein cholesterol (MDA-LDL), and ROS was observed ($P<0.05$). Fibrinogen and PTX3 were measured as vascular inflammation

Table 1. Effects of muscle stretching exercise on circulatory interleukins, cytokines, and oxidative stress markers

Article ID	Study design	Participants	Intervention	Group type	Acute/basal effects	Sample type	Assessment method	Outcome
Andrade-Lima et al. (2021)	RCT	Men with PAD and IC symptoms (n = 16, 69 ± 3 years[a])	2 to 3 sets of 20 stretching exercises for all body segments. These exercises were executed in 30 minutes with a passive technique and maximal stretch was maintained for 20s (2x/week for 12 weeks)	Intervention control group (for aerobic exercise intervention)	Basal effects pre-post training period	Blood plasma	ELISA (CRP, IL-6, TNF-α, VCAM, ICAM SOD, CAT, LPO) and chemiluminescence (NO)	CRP ↔ ES=0.40, IL-6 ↔ ES=0.01, TNF-α ↑ ES=0.64, VCAM ↔ ES=0.68, ICAM ↔ ES=0.34, NO ↔ ES=-1.29, SOD ↔ ES=-0.67, CAT ↓ ES=-0.63, LPO ↑ ES=0.76
						Muscle biopt	Inhibition of xanthine/xanthine oxidase driven cytochrome c (SOD), rate of H2O2 decomposition (CAT), index of oxidative injury following FOX2 (LPO)	SOD ↔ ES=-0.24, CAT ↔ ES=-0.23, LPO ↑ ES=1.11.
De Paulo et al. (2018)	RCT	Postmenopausal breast cancer survivors undergoing aromatase inhibitor therapy (n = 18, 66.6 ± 9.6 years[b])	45-minute sessions of stretching and relaxation exercises in seated or lying position. Each exercise lasted 10-15s and were selected so little stimulus was applied to the musculoskeletal system (2x/week for 36 weeks)	Intervention control group (for resistance plus aerobic exercise intervention)	No specific information - Effects pre-, 12 weeks, 24 weeks, and post training period	Blood serum	ELISA (CRP)	CRP ↔ pre-, 12 weeks ES=0.21, 24 weeks ES=-0.15, and 36 weeks ES=-0.15 post int.
Kato et al. (2017)	RCT	Patients (80% male) with chronic heart failure (n = 25, 70 ± 11 years[b])	2 sets of 7 stretches, without pain, were performed in 20 minutes: wrist dorsiflexion, wrist palmar flexion, trunk rotation, close-legged trunk flexion, open-legged trunk flexion, hip extension, and ankle dorsiflexion. Each stretch was followed by 20s of relaxation (7x/week for 4 weeks)	Intervention group (control sedentary group)	Basal effects pre-post training period	Blood serum	ELISA (hs-CRP, PTX3, fibrinogen, ROS, MDA-LDL)	hs-CRP ↔ ES=0.21, PTX3 ↔ ES=0.08, fibrinogen ↓ ($p < 0.01$) ES=-0.60, ROS ↓ ($p < 0.01$) ES=-0.59, MDA-LDL ↓ ES=-0.50
Rodrigues-Krause et al. (2018)	RCT	Women who were not engaged in any type of regular exercise training during the last 6 months (n = 10, 61-70 years[c])	2 sets of stretching exercises were executed standing or on the floor: lateral flexion of cervical spine, horizontal adduction of shoulder, knee flexion, calf stretch, and breathing exercises. Each position was maintained for 10s and sessions lasted for 60 minutes (1x/week for 8 weeks)	Intervention control group (for dancing intervention and aerobic exercise intervention)	Basal effects pre-post training period	Blood plasma	ELISA (CRP, TNF-α)	hs-CRP ↔, TNF-α ↔.
Serra et al. (2022)	RCT	Chronic stroke survivors > 6 months with self-reported mild to moderate hemiparetic gait (n = 19: 13 men and 6 women), 68 ± 2 years[d])	3 sets of 30-60 seconds of static, active stretches for neck, triceps, posterior deltoids/rhomboids, hip flexors/extensors/adductors, and gastrocnemius/soleus performed supine or seated. 3 sets of balance exercises were also executed: seated shoulder shrugs and circles, standing weight shifts, tandem/semi-tandem stance progressing to single-leg stance, heel/toe walking, and standing calf raises. Each session lasted 50 minutes (2x/week for 6 months). General recommendations and education aimed at promoting physical activity were also provided.	Intervention control group (for aerobic exercise intervention)	Basal effects - Effects pre-post training period	Blood plasma	Beckman AU480 chemistry analyzer (hs-CRP, IL-6) and ELISA (nitrotyrosine, ox-LDL, protein carbonyls, HNA and MDA adducts)	hs-CRP ↔ ES=-0,14, IL-6 ↔ ES=0,35, nitrotyrosine ↔ ES=0,02, ox-LDL ↔ ES=-0,08, protein carbonyls ↔ ES=-0,06, HNE adducts ↔ ES=0,04, MDA adducts ↔ES= 0,16.

↑: significant increase ($P < 0.05$), ↓: significant decrease ($P < 0.05$), ↔: no significant differences observed, CAT: catalase, CRP: c-reactive protein, ELISA: enzyme-linked immunosorbent assay, FOX2: ferrous oxidation xylenol orange technique, HNE: hydroxynonenal, hs-CRP: high sensitive c-reactive protein, IC: intermittent claudication, ICAM: intercellular adhesion molecule, int: intervention, IL-6: interleukin-6, ox-LDL: oxidized low-density lipoprotein, LPO: lipid peroxidation, MDA: malondialdehyde, MDA-LDL: serum malondialdehyde-modified low-density lipoprotein cholesterol, NO: nitric oxide, PAD: peripheral artery disease, PTX3: plasma pentraxin 3, RCT: randomized controlled trial, ROS: Reactive oxygen species, SOD: superoxide dismutase, TNF-α: tumor necrosis factor α, VCAM: vascular cell adhesion molecule 1.

[a] Age in mean ± SE. [b] Age in mean ± SD. [c] Age in range. [d] Age in mean ± SEM. Acute effects: sampling within 24h after the last exercise session, basal levels: sampling minimum 24h after the last exercise session

Table 2. Effects of muscle stretching exercise on inflammatory related gene expression

Article ID	Study design	Participants	Intervention	Group type	Acute/basal effects	Sample type	Assessment method	Outcome
Andrade-Lima et al. (2021)	RCT	Men with PAD and IC symptoms (n = 16, 69 ± 3 years[a])	2 to 3 sets of 20 stretching exercises for all body segments. These exercises were executed in 30 minutes with a passive technique and maximal stretch was maintained for 20s (2x/week for 12 weeks)	Intervention control group (for aerobic exercise intervention)	Basal effects pre-post training period	Muscle biopsy: mRNA samples of tissue samples from gastrocnemius muscle of the leg	Real time PCR (CRP, IL-6, TNF-α, VCAM, ICAM)	CRP ↔ ES=-0.25, IL-6 ↔ ES=0.00, TNF-α ↑ ES=1.60, VCAM ↑ ES=3.77, ICAM ↑ ES=2.67
Iyalomhe et al. (2015)	RCT	Older adults with mild cognitive impairment (n = 5, 70 ± 9 years[a])	Training consisted of static maintaining exercise positions (15-30s) to produce a slight pull on muscles, but not induce pain. Stretches were directed at tight muscles (e.g., hamstrings, hip flexors, calves, and chest) and were repeated 3-5 times for a total of about 40 minutes (3x/week for 6 months)	Intervention control group (for aerobic exercise intervention)	Basal effects pre-post training period	Heparinized Blood: RNA-samples from Buffy coat samples	Micro-array analysis	Training-induced gene expression profile ↔ (fold change ≥ 2 at p < 0.01)
Liberman et al. (2022)	RCT	Women who did not regularly perform physical exercises at higher intensities than habitual daily activity within the past 6 months (n = 5, 69,45 +/-2,62 years[a])	3 sets of 30s passive, static stretching exercises of large muscle groups were performed, mainly inducing a passive load on muscles without causing muscle contractions or cardiovascular challenge (3x/week for 3 months)	Intervention control group (for intensive strength training group IST and strength endurance training group SET)	Basal effects pre-post training period	Blood: RNA-samples of peripheral mononuclear blood cells (PBMC)	RNA-sequencing (Illumina NovaSeq 6000 system)	23 pro-inflammatory genes ↑, 9 pro-inflammatory genes ↓, 6 anti-inflammatory genes ↑, 2 anti-inflammatory genes ↓. 1 gene with unknown inflammatory profile ↑, 3 genes with unknown inflammatory profile ↓

↑: significantly upregulated (P < 0.05), ↓: significantly downregulated (P < 0.05), ↔: no significant differences observed, CAT: catalase, CRP: c-reactive protein, ICAM: intercellular adhesion molecule, eNOS: endothelial nitric oxide synthase, IL-6: interleukin-6, int.: intervention, LPO: lipid peroxidation, NO: nitric oxide, RCT: randomized controlled trial, SOD: superoxide dismutase, TNF-α: tumor neerosis factor α, VCAM: vascular cell adhesion molecule 1

[a] Mean ± standard deviation.

Acute effects: sampling within 24h after the last exercise session, basal levels: sampling minimum 24h after the last exercise session

Table 3. Effects of muscle stretching exercise on absolute cell count or percentage of T-cell subsets

Article ID	Study design	Participants	Intervention	Group type	Acute/basal effects	Sample type	Assessment method	Outcome
Cao Dinh et al. (2019)	RCT	CMV+ and CMV- women who did not regularly perform physical exercises at higher intensities than habitual daily activity within the past 6 months (n = 32, 65 years or more)	3 sets of 30s passive, static stretching exercises of large muscle groups were performed, mainly inducing a passive load on muscles without causing muscle contractions or cardiovascular challenge (2-3/week for 6 weeks)	Intervention control group (for intensive strength training group and strength endurance training group)	Basal effects pre-post training period	EDTA anticoagulated blood; Blood serum	Flow cytometry analysis (percentage of T-cell subtypes) and Cell-Dyn Sapphire haematology analyser (absolute counts)	#/% CD8+ naive T-cell subset (CD28+CD57-) ↔ ES=0.18/ES=-0.06, #/%memory CD8+ T-cell subset (CD28-CD57-) ↔ ES=0.28/ES=0.11, #/%SPC CD8+ T-cell subset (CD57+) ↔ ES=0.06/ES=-0.04), #/%SPC CD8+ T-cell subset (CD28-CD57+) ↔ ES=0.07/ES=-0.04), #/%SPC CD8+ T-cell subset (CD28+CD57+) ↔ ES=-0.04/ES=-0.03), #/% naive CD8- subset (CD28+CD57-) ↔ ES=0.45/ES=-0.01, #/% memory CD8- T-cell subset (CD28-CD57-) ↔ ES=0.06/ES=0.15, #/% SPC CD8- T-cell subset (CD57+) ↔ ES=-0.17/ES=-0.15, #/%SPC CD8+ T-cell subset (CD28-CD57+) ↔ ES=-0.17/ES=-0.18), #/%SPC CD8+ T-cell subset (CD28+CD57+) ↔ ES=-0.09/ES=-0.02) (for CMV+)
Cao Dinh et al. (2018)	RCT	CMV+ and CMV- women who did not regularly perform physical exercises at higher intensities than habitual daily activity within the past 6 months (n = 33 70.31 ± 5.15 years)	3 sets of 10-12 (30s) passive, static stretching exercises of large muscle groups were performed, mainly inducing passive load on muscles without causing muscle contractions or cardiovascular challenge (2-3/week for 6 weeks)	Intervention control group (for intensive strength training group and strength endurance training group)	Basal effects pre-post training period	EDTA anticoagulated blood; Blood serum	Flow cytometry analysis (percentage of T-cell subtypes) and Cell-Dyn Sapphire haematology analyser (absolute counts)	#/% naïve CD8+ T-cell subset (CD28+CD57-) ↔ ES=0.23/ES=-0.02, #/% memory CD8+ T-cell subset (CD28-CD57-) ↔ ES=0.19/ES=0.03, #/%SPC CD8+ T-cell subset (CD57+) ↔ ES=0.09/ES=-0.07, #/%SPC CD8+ T-cell subset (CD28-CD57+) ↔ ES=0.09/ES=-0.07, #/%SPC CD8+ T-cell subset (CD28+CD57+) ↔ ES=0.03/ES=-0.03, #/% naïve CD8- T-cell subset (CD28+CD57-) ↔ ES=0.16/ES=-0.02, #/%memory CD8- T-cell subset (CD28-CD57-) ↔ES=0.09/ES=0.10, #/% SPC CD8- T-cell subset (CD57+) ↔ ES=-0.05/ES=-0.08, #/%SPC CD8- T-cell subset (CD28-CD57+) ↔ ES=-0.06/ES=-0.10, #/%SPC CD8- T-cell subset (CD28+CD57+) ↔ ES=0.03/ES=0.07, (for both CMV+ and CMV- population)

↑: significant increase ($P < 0.05$), ↓: significant decrease ($P < 0.05$), ↔: no significant difference observed., #: absolute counts; %: percentages, CMV: cytomegalovirus serostatus (+ = positive; - = negative), EDTA: Ethylenediamine tetra acetic acid, int: intervention, RCT: randomized controlled trial, SPC: senescence-prone T-cells (CD8+/CD8-CD57+, CD8+/CD8-CD28-CD57+, CD8+/CD8-CD28+CD57+).
Acute effects: sampling within 24h after the last exercise session, basal levels: sampling minimum 24h after the last exercise session

Table 4. Overview of the effect sizes related to the exercise effects on circulatory interleukins, cytokines, and oxidative stress markers after a flexibility intervention calculated where available

↑: significantly upregulated ($P < 0.05$), ↓: significantly downregulated ($P < 0.05$), ↔: no significant differences, *Flexibility training*, *Resistance exercise*, *Aerobic exercise*, *Combined training*, (m) muscle biopsy, * SMD, ° WMD, a. Bautmans et al., 2021, Exp. Gerontology; 146: 111236. b. Salimans et al., 2022, Exp Gerontology; 164, 111822. c. Lin et al., 2015, J. Am. Heart Assoc.; 4. d. Thompson et al., 2020, J Sports Sci; 38: 814-826 e. Koh et al., 2018, J Inflamm Res; 11:296-306. f. Powers et al, 2023, Antioxidants (Basel); 12: 39., g. Ye et al., 2021, Front Physiol; 12: 701151. h. Wang et al., 2023, Open Life Sci.; 18: 668. ., j. Radak et al., 2003, Eur J Clin Invest; 33: 726-730. k. Zempo-Miyaki et al., 2016, J Hum Hypertens; 30: 521-526 l. Takashima et al. 2014, Circ J.; 78: 2682-2687 , m: Kawamoto et al. 2015, Endocrine; 48: 871-877 **Bold arrows** represent populations with chronic diseases, negative effect sizes indicate smaller expression or levels after the intervention compared pre-intervention. CRP: C-reactive protein, ICAM: intercellular adhesion molecule, IL-6: interleukin-6, LPO: lipid peroxidation, MDA: malondialdehyde, MDA-LDL: serum malondialdehyde-modified low-density lipoprotein cholesterol, NO: nitric oxide, Ox-LDL: oxidized low-density lipoprotein, PTX3: plasma pentraxin 3, ROS: reactive oxygen species, SOD: superoxide dismutase, TNF-α: tumor necrosis factor α, VCAM: vascular cell adhesion molecule 1

Biomarker		Large	Medium	Small	Negligible	Small	Medium	Large
	NO ES	**ES ≤ -0,8**	**-0.8 < ES ≤ -0.5**	**-0.5 < ES ≤ -0.2**	**-0.2 < ES < 0.2**	**0.2 ≤ ES < 0.5**	**0.5 ≤ ES < 0.8**	**ES ≥ 0.8**
CRP	↓a, ↓a, ↓a, ↓a, ↔, ↔a			↓a,↓a, ↔,↓a	↔	↔,↔,↔,↔, ↔a		
IL6	↑a, ↓a, ↓a			↓a	↔	↔a, ↔	↔a, ↔a	
TNF alfa	↔, ↓a, ↓a, ↓a	↓a	↓a		↔b	↔a	↔	
Catalase	↑a		→	↔ (m)				
Fibrinogen			↓, ↓*d,↓*d	↓°c↓°c				
ROS	↔b		→					
MDA-LDL	↓l, ↓m		→					
VCAM	↓e, ↔e, ↔e						↔	
ICAM	↓e, ↔e, ↔e					↔		
SOD	↓(m) f		↔	↓a		↔ (m)	↔*g	
LPO		↓*g						↔, ↑(m)
MDA		↓*g,↓*h, ↓*h	↓*h			↔		
NO		↔						↔*g
Nitrotyrosine	↑j				↔			
Protein carbonyls	↑j				↔			
PTX3	↑k				↔			
ox-LDL		↔*g			↔			
HNE					↔			

Table 5. Effects of muscle stretching exercise on inflammatory related gene expression

Biomarker																								
	NO ES	<-1	-1	-0.9	-0.8	-0.7	-0.6	-0.5	-0.4	-0.3	-0.2	-0.1	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	>1.0
CRP*										↔														
IL6*													↔											
TNF alfa*																								↑
VCAM*																								↑
ICAM*																								↑
eNOS*																↔								
TIMM23	↔																							
APOBEC3C	↔																							
S100A12	↔																							
C1D	↔																							
CHMP5	↔																							
PCMTD2	↔																							
LYZ	↔																							
SNORD115_25	↔																							
IL1A	↑																							
CX3CL1	↑																							
IL1B	↑																							
CXCL2	↑																							
CCL3	↑																							
EREG	↑																							
BDKRB1	↑																							
THY1	↑																							
LTB4R2_A	↓																							
KLK15	↓																							
CD68	↑																							
RGS1	↑																							
CPA2	↓																							
REN	↔																							
CCL19	↔																							
PTGS2	↔																							
PGK1	↔																							
NCAM1	↔																							
COL3A1	↔																							
IL1RL1	↔																							
CCL2	↑																							
PLA2G5	↑																							
CXCL10	↑																							
CCL4	↑																							
DNM1P46	↓																							
SLC2A4	↓																							
HIF3A	↓																							
LRP2	↓																							
BDKRB2	↓																							
IL17A	↓																							
TERC	↑																							
CXCL11	↑																							
CD160	↔																							
KLK3	↔																							
LTC4S	↔																							
PTGER3	↔																							
CRISP3	↔																							
CCR3	↔																							
COL1A1	↔																							
LTB4R2	↔																							
NR4A2	↔																							
EPHB2	↔																							
IL5	↔																							
PDE4C	↔																							
TREM1	↔																							
IL1R2	↔																							
DUSP1	↔																							
COL4A1	↔																							
CSF3R	↔																							
CXCR1	↔																							
BMX	↔																							
MMP9	↔																							
PRKAA2	↔																							
IL3	↔																							
KLK2	↑																							
IL6	↑																							
HTR3B	↑																							
PLA2G2D	↑																							
CD69	↑																							
PLA2G2A	↑																							
TNFSF10	↑																							
IL1RAPL2	↑																							
IL9	↑																							
NFKBIA	↑																							
MXRA5	↓																							
FRZB	↓																							
HSPA1A	↔																							
FOXP3	↔																							
PTGIS	↔																							
FCGR3B	↑																							
IL13	↑																							
HRH1	↔																							
FGF21	↔																							
IL4	↔																							
ARNT2	↔																							
ASPN	↔																							
CSF3	↔																							
THBD	↔																							
HSPA1B	↔																							
SOD2	↔																							
HSPA1L	↔																							
CA4	↔																							
HSPA6	↔																							
AGTR1	↔																							
CSF2	↔																							
IGF2	↔																							
IL2	↔																							
FGF6	↔																							
SOCS3	↑																							
CACNA2D1	↑																							
CYP1A2	↓																							
MYH6	↓																							
PRM1	↔																							
RPL3L	↔																							
PTGFR	↔																							
DAAM2	↔																							
CYP7A1	↔																							
OLIG2	↓																							

↑: significantly upregulated ($P < 0.05$), ↓: significantly downregulated ($P < 0.05$), ↔: no significant differences, *Flexibility training* **Bold arrows** represent populations with chronic diseases, negative effect sizes indicate smaller expression or levels after the intervention compared pre-intervention. CRP: C-reactive protein, ICAM: intercellular adhesion molecule, IL-6: interleukin-6, TNF-α: tumor necrosis factor α, VCAM: vascular cell adhesion molecule 1, No ES were calculated because of fold change values, the fold changes ≤0.67 or ≥1.5 were clinically relevant, with a downregulation at ≤0.67 and an upregulation at ≥1.5, values between 0.67 and 1.5 were indicated as no effect. *in muscle.

Table 6. Overview of the effect sizes related to the exercise effects on absolute cell count or percentage of T-cell subsets

*↑: significantly upregulated ($P < 0.05$), ↓: significantly downregulated ($P < 0.05$), ↔: no significant differences, Flexibility training, Resistance exercise, Aerobic exercise, Combined training, (m) muscle biopsy, **Bold arrows** represent populations with chronic diseases, negative effect sizes indicate smaller expression or levels after the intervention compared pre-intervention., SPC: senescent-prone cell*

Cell subsets																							
	NO ES	**<-1**	**-1**	**-0.9**	**-0.8**	**-0.7**	**-0.6**	**-0.5**	**-0.4**	**-0.3**	**-0.2**	**-0.1**	**0**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1.0**	**>1.0**
# Naive CD8+ T-cell (CD28+CD57-) subset	↔, ↔, ↔, ↔														↔ ↔									
% Naive CD8+ T-cell (CD28+CD57-) subset	↔, ↔, ↔, ↔											↔	↔											
# memory CD8+ T-cell (CD28-CD57-) subset	↔, ↔, ↔, ↔														↔	↔								
% memory CD8+ T-cell (CD28-CD57-) subset	↔, ↔, ↔, ↔												↔	↔										
# SPC CD8+ T-cell (CD8+CD57+) subset	↓,↓, ↔, ↔													↔ ↔										
% SPC CD8+ T-cell (CD8+CD57+) subset	↓,↓, ↔, ↔											↔	↔											
# SPC CD8+ T-cell (CD8+CD28-CD57+) subset	↓, ↓, ↔, ↔													↔ ↔										
% SPC CD8+ T-cell (CD8+CD28-CD57+) subset	↓, ↓, ↔, ↔											↔	↔											
# SPC CD8+ T-cell (CD8+CD28+CD57+) subset	↔, ↓, ↔, ↔												↔ ↔											
% SPC CD8+ T-cell (CD8+CD28+CD57+) subset	↔, ↔, ↔, ↔												↔ ↔											
# naïve CD8- T-cell (CD28+CD57-) subset	↔, ↔, ↔, ↔														↔			↔						
% naïve CD8- T-cell (CD28+CD57-) subset	↔, ↔, ↔, ↔												↔ ↔											
# memory CD8- T-cell (CD28-CD57-) subset	↔, ↔, ↔, ↔													↔ ↔										
% memory CD8- T-cell (CD28-CD57-) subset	↔,↔, ↔, ↔													↔	↔									
# SPC CD8- T-cell (CD8-CD57+) subset	↔,↓, ↔, ↔										↔	↔												
% SPC CD8- T-cell (CD8-CD57+) subset	↓,↓, ↔, ↔										↔	↔												
# SPC CD8- T-cell (CD8-CD28-CD57+) subset	↔, ↔, ↔, ↔										↔	↔												
% SPC CD8- T-cell (CD8-CD28-CD57+) subset	↓, ↓, ↔, ↔										↔	↔												
# SPC CD8- T-cell (CD8-CD28+CD57+) subset	↓, ↔, ↔, ↔											↔	↔											
% SPC CD8- T-cell (CD8-CD28+CD57+) subset	↓, ↔, ↔, ↔												↔	↔										

parameters (44). Serra et al. (36) assessed blood plasma levels of nitrotyrosine, oxidized low-density lipoprotein (ox-LDL), protein carbonyls, hydroxynonenal (HNE) and malondialdehyde (MDA) adducts, but no significant effects were identified.

Muscle stretching exercise effects on inflammation related gene expression (Table 2 and 5)

Andrade-Lima et al. (38) investigated gene expression changes in muscle tissue after 12 weeks stretching intervention. No change could be observed for CRP, IL-6, and eNOS, while an increase in TNF-α, VCAM and ICAM was observed post-intervention. Iyalomhe et al. (41) analyzed changes in training-induced gene expression profile after six months of stretching intervention in RNA purified from blood samples. This study found six unique (TIMM23, APOBEC3C, S100A12, C1D, CHMP5, PCMTD2) and two non-unique (LYZ, SNORD115_25) non-overlapping genes differentially expressed after stretching intervention (fold change ≥1.5), however these changes were not statistically significant (P>0.01). The study conducted by Liberman et al. (40) investigated the effects of a 3-month stretching intervention on 407 inflammation related genes in peripheral blood mononuclear cells. In total, 23 pro-inflammatory genes were upregulated (fold change ≥1.5) and nine were downregulated (fold change ≤0.67). Similarly, six anti-inflammatory genes were upregulated (fold change ≥1.5) and two were downregulated (fold change ≤0.67) after the intervention compared to baseline expression. Additionally, this study found expression changes for four genes of which the inflammatory roles have not yet been fully determined (40).

Muscle stretching exercise effects have no effect on absolute T-cell count and percentage of T-cell subsets (Table 3 and 6)

Two studies investigated the effects of a six-week stretching intervention on absolute T-cell counts and percentage of T-cell subsets (42, 43). A distinction was made between CD8+ and CD8- T-cell subsets, which were further subdivided into three separate groups: a naïve T-cell subpopulation (CD28+CD57-), a memory T-cell subpopulation (CD28-CD57-), and a senescent-prone T-cell subpopulation (CD28-CD57+ or CD28+CD57+). No significant changes in absolute cell count or percentage of different T-cell subsets were observed after the stretching intervention, regardless of the participants' cytomegalovirus (CMV) serostatus (42).

GENERAL DISCUSSION

In this systematic literature review, we verified the suitability of muscle stretching as an appropriate control group for exercise immunology studies by appraising the impact of muscle stretching on inflammation in an older population. Traditionally, non-active or usual care groups have often been used as controls in exercise immunology studies. However, whether these non-active groups adequately represent and account for the social and physical environment intrinsically linked to exercise interventions can be questioned. These include possible confounding effects of the social environment, such as training in group settings or receiving attention from instructors, as well as effects of the physical environment, such as pathogen exposure that may arise from training in a group setting or taking (public) transport to the training facilities. Muscle stretching is considered as a suitable control intervention because it includes all the conditions of an exercise intervention group, but without (intensive) active muscle contractions. However, using muscle stretching as a control group for exercise immunology studies implies that stretching does not affect the inflammatory profile.

All articles described the long-term effects (≥24 hours after the last training session) of muscle stretching exercise programs on basal levels of inflammation. Those studies investigating the concentration of CRP (36-39, 44) and IL-6 (36, 38) in serum or plasma, observed no changes after a muscle stretching training period. In contrast, resistance and aerobic training decreases CRP-levels after long-term training. In general, the ES for resistance and for aerobic exercise are greater than the ES for muscle stretching, suggesting the appropriateness of stretching as a control intervention for those exercise programs (Table 4-6). However, Andrade-Lima et al. (38) reported a significant increase of circulatory TNF-α with an ES of 0.64 after 12 weeks (2x/week) stretching in older men with PAD or IC. This was not found by Rodrigues-Krause et al. (39) in healthy older women after muscle stretching (combined with breathing exercises) once a week for 8 weeks. These differences might be explained by an increased baseline inflammatory status of patients with PAD or IC, as inflammation contributes to disease initiation and progression(45). Indeed, Signorelli et al. previously showed that patients suffering from PAD exhibit higher resting levels of TNF-α compared to healthy controls(46). In PAD patients, higher TNF-α levels were associated with lower ankle-brachial index levels, reflecting a higher disease severity(47). Therefore, the increase in TNF-α concentration in this population after muscle stretching might reflect the disease progression over time rather than the effect of the muscle stretching intervention. Alternatively, it could be hypothesized that in the context of arterial wall inflammation, which was shown to be increased in PAD patients(48), the mechanical stress on inflamed endothelium due to muscle stretching could potentially increase inflammatory cytokine release such as TNF-α. However, this hypothesis is unlikely since the expression of ICAM of VCAM, of which TNF- α is an upstream regulator in the endothelial inflammation cascade(49), remained unchanged after muscle stretching in PAD patients(38). More research on the topic is needed. In contrast to muscle stretching, a resistance training program with the same duration and frequency in maintenance haemodialysis patients with sarcopenia, who exhibit a comparable elevated inflammation status at baseline, induced the opposite effect. In general, resistance training was shown to reduce levels of TNF-α in healthy older adults (ES up to -0.76, which is larger than the ES observed for stretching in men with PAD and IC (ES=0.64)). The same decrease could be observed after combined training (50). Long-term resistance training was shown to decrease IL-6 in healthy older adults and obese older women. In contrast, no significant changes in IL-6 were observed in healthy older adults and an increase was observed in haemodialysis patients with sarcopenia. Furthermore, long-term combined training also decreased IL-6 levels (50) (Table 4). Overall, these data suggest that a muscle stretching intervention does not affect the protein expression levels of CRP and IL-6 in older participants, regardless of their health status. However, TNF-α levels could be influenced by muscle stretching in participants with an increased inflammatory status at baseline, albeit with a smaller ES compared to resistance or combined exercise.

Stretching can be a potential active control group for resistance exercise regarding antioxidant markers, such as CAT and SOD. Andrade-Lima et al. (38) was the only group reporting on the effects of muscle stretching on mediators that can control oxidative stress. The authors showed a decrease in circulating but not in muscle CAT, an enzyme mitigating oxidative stress by decomposing hydrogen peroxide to water and oxygen (51), in men with PAD and IC symptoms after 12-weeks of muscle stretching exercises twice a week. Resistance exercise in a healthy population for 12 weeks at a ratio of three sessions a week was shown to increase in circulating CAT (no ES reported) (50). Regarding the effect of exercise on muscle CAT, Samjoo et al. (52) showed no effect of endurance training on muscle CAT in sedentary obese men. Andrade-Lima et al. (38) also showed no effect of stretching on circulating or muscle SOD. SOD has important antioxidant properties since it mediates the production of H2O2 from superoxide anion, which can be further catalyzed to water and oxygen by catalase (53). In comparison, Bautmans et al. (50) reported that in healthy older adults, resistance exercise could decrease circulating SOD after 12 weeks consisting of three sessions a week, while aerobic exercise was shown to increase circulating SOD with a medium effect (20). The differences between the directions of effects after aerobic and resistance exercise suggests that the type and intensity of exercise programs are crucial determinants for the outcome of exercise on oxidative stress (54). Regarding muscle SOD, a recent review shows that endurance exercise can decrease SOD in skeletal muscle of obese sedentary men and that lifelong exercise can increase muscle antioxidant enzymes (55).

Our review shows that the effects reported for muscle stretching on oxidative stress markers might be influenced by the health state of participants. However, stretching exercise can be used as an active control group in healthy older adults to control for confounding effects such as social environment. In participants with specific disease characteristics, caution is needed due to the limited extrapolation of the results. While Andrade-Lima et al. (38) and Serra et al. (36) observed no effects on oxidative markers MDA, ox-LDL, Nitrotyrosine, protein carbonyls, and HNE after muscle stretching, Kato et al. (44) in contrast, showed a decrease of oxidative stress, as measured by ROS and MDA-LDL after four weeks of daily muscle stretching for 20 minutes in participants with chronic heart failure. In addition, Kato et al. (44) showed a decrease in MDA-LDL, an oxidized form of LDL after muscle stretching. These effects of muscle stretching were similar to effects observed after exercise, as a 12 week intervention (three session per week lasting 120 minutes each) of Nordic walking in older adults, or 6 months of bicycle ergometer and walking for at least twice a week in cardiac rehabilitation, also showed a decrease on MDA-LDL level (56, 57). No effects were observed in MDA and HNE levels after muscle stretching (36). However, MDA levels decreased after both aerobic exercise and resistance exercise (20, 58). The use of stretching as an active control for exercise effects on HNE remains unclear.

Andrade-Lima et al. (38) showed no effects on NO in men with PAD and IC symptoms after stretching exercises, while LPO levels in both blood and muscle tissue were increased. The review of Ye et al. (20) showed increased levels of NO in older adults after aerobic exercise, as well as reduced levels of circulating oxidant marker LPO. LPO, a marker for oxidative stress related to lipid peroxidation, was increased after muscle stretching in men with PAD and IC symptoms. This increase can be related to the disease onset and progression (38, 45). The increase of NO after exercise was confirmed by the review of Arefirad et al. (59), which also indicated higher levels of NO after aerobic exercise compared to a control group for healthy older adults, obese older adults, and older adults with diabetes.

No changes were observed in ox-LDL, protein carbonyls, and nitrotyrosine after muscle stretching in chronic stroke survivors (36). For ox-LDL, an oxidant marker, no differences were observed after aerobic exercise (20). The oxidative changes in LDL modify both protein and lipid components in a complex process, resulting in the formation of ox-LDL. Ox-LDL contributes to inflammation and is related to the development and progression of atherosclerosis (60). Protein carbonyls are used as marker for protein damage by ROS (36). Radak et al. (61), showed increased serum nitrotyrosine and protein carbonyls levels after an extreme exhaustive aerobic exercise training program (4-day super marathon).

The cell adhesion molecules VCAM-1 and ICAM-1 in the circulation were unchanged after muscle stretching as shown by Andrade-Lima et al. (38) in men with PAD and IC symptoms. Both VCAM and ICAM glycoproteins play a role in the influx of leucocytes to the tissue via adhesion and transmigration through the endothelium, and they are associated with inflammation (62). Koh et al. (63) showed that resistance exercise also did not affect these cell adhesions molecules. In contrast, low-to-moderate intensity aerobic exercise was able to lower cell adhesion molecule levels, while high intensity aerobic exercise was only associated with an acute peak of these molecules, before returning to pre-exercise levels (38, 63).

Kato et al. (44) showed no effects in PTX3 marker and a decrease in fibrinogen after an intensive muscle stretching exercise (seven times weekly for four weeks) in patients with chronic heart failure (ES=-0.6). PTX3 was measured as a vascular inflammatory marker and fibrinogen is a blood clotting factor related to inflammatory processes and can be associated with cardiovascular diseases (44). Zempo-Miyaki et al. (64) observed increased PTX3 levels after an aerobic exercise intervention of eight weeks in older adults. The review of Lin et al. (65) showed a reduction in fibrinogen levels after vigorous exercise intervention in both diabetes and sedentary healthy older adults. Moreover, the review of Thompson et al. (66) observed also lower fibrinogen levels after cardiorespiratory aerobic exercise at both vigorous and moderate levels. At the moment, there is thus insufficient evidence for the use of stretching as a control for the effects of exercise on these markers of vascular inflammation, especially in populations with chronic disease.

Iyalomhe et al. (41) stated that no gene expression changes were observed for the investigated training-induced gene expression profile. On the contrary, Andrade-Lima et al. (38) and Liberman et al. (40) noticed changes in expression of several inflammatory related genes after muscle stretching. Andrade-Lima et al. (38) investigated mRNA levels of inflammatory biomarkers in muscle tissue samples, in contrast to Iyalomhe et al. (41) and Liberman et al. (40) who investigated mRNA levels

in blood, respectively buffy coat samples and in PBMCs. At first sight, this may indicate that muscle stretching exercise might alter the RNA expression of inflammation related genes in the blood circulation, but less in the muscle itself. The expression of pro- and anti-inflammatory genes was less pronounced in the flexibility group compared to the strength training (40) and aerobic training (41). Iyalomhe et al. (41) performed a pathway analysis on both inflammation and cancer related pathways and focused on the interaction within these pathways, in contrast to Liberman et al. (40) who excluded cancer-related pathways. This might explain the discrepancies in gene expression between Iyalomhe et al. (41) and Liberman et al. (40). More research on the effects of muscle stretching on inflammatory related gene expression is needed, since most assessed genes did not overlap, or contradicting results were reported (e.g. IL-6) within the included studies. Moreover, a sedentary control group was not present.

Lastly, muscle stretching had no effect on absolute cell count and percentage of different T-cell subsets in contrast to a resistance endurance training where a decrease in percentages and absolute counts was found after a six week program (42, 43). This suggests that muscle stretching is an appropriate control for studies investigating the effects of exercise on T-cell subsets.

In this systematic literature search we retrieved nine RCTs measuring the effect of muscle stretching exercise programs on the inflammatory profile in older persons. We found no major effects of muscle stretching exercise on the inflammatory status of (healthy) older adults. Most studies reported that muscle stretching has no effect on inflammatory markers and markers related to oxidative stress in older adults. Moreover, muscle stretching exercises had no effect on outcomes related to absolute cell count and percentage of T-cell subsets, except for populations with chronic diseases. Overall, the pro- and anti-inflammatory gene expression was less pronounced in the flexibility group compared to resistance training and aerobic training. However, inconclusive results were found for the effects on oxidative stress markers and gene expression profiles of inflammatory cells. Nonetheless, more dedicated research should be conducted within this area to confirm these findings and clarify the contradictory results. Overall, our systematic review provides evidence for the use of stretching as a suitable control group for exercise immunology studies in older adults. However, caution is warranted in populations with peripheral artery disease.

This systematic review has both strengths and limitations. The novelty of the present systematic review lies in the evaluation of the effects of stretching exercise on the immune system (17, 50, 67). The inclusion of RCTs is a major strength, however, only nine RCTs were identified. Of those, the quality assessment was of some concern in three articles. No meta-analysis was performed because of the heterogeneity regarding the study population (healthy vs disease), duration of intervention, outcome (cell count, gene expression, circulatory markers), and time of assessment. However, ES-values of the muscle stretching interventions were calculated where possible and compared to ES after other types of physical exercise. When the exact type, number and duration of the exercises were unclear, we contacted the authors, but unfortunately, not all authors responded to our inquiries. Only passive (n=4) and active (n=1) muscle stretching interventions were described in the nine articles included in this systematic review, with four studies not defining the type of muscle stretching used. Although this systematic review included 9 studies of which the majority (2/3) were of high quality, there are still some aspects that need investigation in more high qualitative intervention studies focusing on other types of stretching, dose-response aspects and differences according to presence of specific conditions.

CONCLUSION

This systematic review showed no major effects of passive and active muscle stretching exercise on the inflammatory status of older adults and therefore, suggest that a muscle stretching program is a suitable active control group for aerobic and resistance exercise programs. Nevertheless, it is important to take caution when considering populations with peripheral artery disease, as an exacerbation of their inflammatory profile could occur; although it remains unclear whether this exacerbation is due to the passive stretching intervention per se or rather reflecting the overall disease progression.

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Conflicts of Interest:
The authors declare that they have no conflicts of interest relevant to the content of this review.

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Data availability statement:
All data analyzed during this literature study are included in this article. Further inquiries can be directed to the corresponding author.

Author contribution:
EM and LS contributed to the literature search, data extraction, data analysis, and writing of the manuscript. AG contributed the literature search, data extraction, data analysis, and writing of the manuscript. IK, RN and JG reviewed the manuscript. IB designed the study, contributed to the data analysis and interpretation, and writing of the manuscript.

Supplementary materials

Supplement 1: Search key PUBMED:

((exercise, muscle stretching[MeSH Terms]) OR (range of motion, articular[MeSH Terms]) OR (muscle stretching exercise) OR (articular range of motion) OR ("flexibility" AND "exercise") OR ("stretching" AND "exercise") OR (stretching) OR (static stretching) OR ("flexibility" AND "exercise" OR "flexibility" AND "training") OR ("joint" AND "flexibility") OR ("PNF" AND "stretching") OR (proprioceptive neuromuscular facilitation stretching) OR (passive stretching) OR (relaxed stretching) OR (static-passive stretching) OR (isometric stretching) OR (active stretching) OR (static-active stretching) OR (ballistic stretching) OR (dynamic stretching)) AND ((Aged[MeSH Terms]) OR (aged) OR (older adults) OR (older person) OR (older people) OR (elderly)) AND ((inflammation[MeSH Terms]) OR (cytokines[MeSH Terms]) OR (immunity, cellular[MeSH Terms]) OR (inflammation) OR ("inflammatory" AND "profile") OR (inflammatory) OR (cellular immunity) OR ("immune" AND "response") OR ("immune" AND "reaction") OR (cytokines) OR (chemokine) OR ("tumor" AND "necrosis" AND "factor") OR ("C-reactive protein"))

Hits: 1468

Supplement 2: Search key Web of Science:

TS=((muscle stretching exercise) OR (articular range of motion) OR ("flexibility" AND "exercise") OR ("stretching" AND "exercise") OR (stretching) OR (static stretching) OR ("flexibility" AND "exercise" OR "flexibility" AND "training") OR ("joint" AND "flexibility") OR ("PNF" AND "stretching") OR (proprioceptive neuromuscular facilitation stretching) OR (passive stretching) OR (relaxed stretching) OR (static-passive stretching) OR (isometric stretching) OR (active stretching) OR (static-active stretching) OR (ballistic stretching) OR (dynamic stretching)) AND TS=((aged) OR (older adults) OR (older person) OR (older people) OR (elderly)) AND TS=((inflammation) OR ("inflammatory" AND "profile") OR (inflammatory) OR (cellular immunity) OR ("immune" AND "response") OR ("immune" AND "reaction") OR (cytokines) OR (chemokine) OR ("tumor" AND "necrosis" AND "factor") OR ("C-reactive protein"))

Hits: 418

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