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Unveiling the role of exercise in modulating plasma heat shock protein 27 levels: insights for exercise immunology and cardiovascular health

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Abstract
Cardiovascular disease is one of the leading causes of mortality worldwide, primarily driven by atherosclerosis, a chronic inflammatory condition contributing significantly to fatalities. Various biological determinants affecting cardiovascular health across different age and sex groups have been identified. In this context, recent attention has focused on the potential therapeutic and preventive role of increasing circulating levels of heat shock protein 27 (plasma HSP27) in combating atherosclerosis. Plasma HSP27 is recognized for its protective function in inflammatory atherogenesis, offering promising avenues for intervention and management strategies against this prevalent cardiovascular ailment. Exercise has emerged as a pivotal strategy in preventing and managing cardiovascular disease, with literature indicating an increase in plasma HSP27 levels post-exercise. However, there is limited understanding of the impact of exercise on the release of HSP27 into circulation. Clarifying these aspects is crucial for understanding the role of exercise in modulating plasma HSP27 levels and its potential implications for cardiovascular health across diverse populations. Therefore, this review aims to establish a more comprehensive understanding of the relationship between plasma HSP27 and exercise.

Keywords Physical exercise · Chaperone · HSPB1 · Cardiovascular health

Introduction

Cardiovascular disease (CVD) remains a significant global health challenge and is persistently ranked among the leading causes of mortality worldwide. In the United States, the economic impact of CVD is staggering, with estimates around $351.2 billion, encompassing both direct healthcare costs and indirect expenses such as lost productivity. Over the past two decades, from 1996/97 to 2014/15, expenditures related to CVD have escalated significantly, reflecting an increasing burden on healthcare systems and economies. This burden disproportionately affects adults aged 45 to 64 years and those aged 65 years and above. Projections indicate a stable expenditure trend among adults aged 18 to 44 years by 2035, in contrast to anticipated rising costs among middle-aged and older adults [1].

Given this escalating economic strain, there is an urgent need to explore novel therapeutic targets for CVD. One promising candidate is circulating heat shock protein (HSP) 27. Plasma HSP27 (also known as HSPB1; its rodent analogue is HSP25) acts as a signaling mediator within the bloodstream, playing crucial roles in anti-inflammatory, antioxidant, antiapoptotic, and antiatherogenic processes [2–5]. Research suggests that HSP27 levels may decrease with age [6], and its interaction with estrogen implies a gender-specific protective role against atherosclerosis until menopause [7–10]. Atherosclerosis is a significant factor in various cardiovascular conditions [1, 4, 5], and the increased susceptibility to atherosclerosis among postmenopausal

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women [11–15] significantly contributes to cardiovascular disease after middle age [11].

Physical inactivity and sedentary behavior are well-established risk factors for cardiovascular disease. Regular physical exercise emerges as a critical strategy for both preventing and managing CVD across various age groups [16–19]. Recent studies highlight that exercise can also mitigate the adverse effects of menopause on cardiovascular health [20–25]. Structured physical activity, considering factors such as intensity, volume, and duration, improves endothelial function, slows atherosclerosis progression, and regulates the immune system [16, 17, 19, 26]. Although the molecular mechanisms behind these benefits are not fully understood, signaling molecules released during acute exercise, known as exerkines, are proposed to play a key role in enhancing inflammation and cardiovascular health through autocrine, paracrine, and endocrine pathways [27–31].

In this context, HSP27 has been identified as an exerkine [27]. Studies have consistently shown that plasma HSP27 levels increase significantly after exercise, with the molecule being released from various tissues, including muscles, kidneys, liver, and brain [27, 32–39]. Given its atheroprotective and anti-inflammatory effects, HSP27 could serve as a potential mediator of exercise's impact on cardiovascular health.

This review is structured into three main sections to address these topics comprehensively. The first section explores the role of plasma HSP27 in atherosclerosis and its relationship with age and menopause. The second section delves into advancements in understanding how exercise influences the release of HSP27 into circulation, including an analysis of its origin, targets, and the effects of sex and training variables. The third section examines the direct effects of exercise on plasma HSP27 levels, focusing on the magnitude, time course, and influencing factors such as sex, age, and training variables.

Literature search strategy and data analysis

In the first phase of this review, our primary objective was to analyze research studies focusing on the pivotal role of plasma HSP27 in the development of atherosclerosis. We also aim to establish the relationship between plasma HSP27 levels, age, and menopause. To achieve this, we reviewed a diverse array of study designs and experimental methodologies. Our investigation included cross-sectional and cohort studies to provide a comprehensive perspective on how menopause, plasma HSP27, and atherosclerosis interact.

In the second phase, we examined studies that employed exercise interventions—excluding those incorporating additional interventions such as temperature elevation or supplementary nutrition—to assess changes in HSP27 concentrations in various tissues and cells, which may serve as sources and targets of plasma HSP27. This phase involved a thorough investigation using various study designs and experimental approaches to explore the potential origins (muscle) and targets (immune cell) of plasma HSP27. We also assessed how sex and training variables influence HSP27 responses to exercise across different tissues and cells.

The third phase focused on evaluating the extent and duration of the plasma HSP27 response to different types, intensities, and durations of exercise. We also considered potential age and gender-specific effects on plasma HSP27 in relation to exercise. This phase involved a systematic review with specific exclusion criteria centered on human plasma studies, aiming to estimate combined effects for comparative purposes.

For the systematic review, we conducted a comprehensive literature search across multiple databases, including PubMed, Scopus, SPORTDiscus, and Web of Science. Our search strategy utilized terms such as "Exercise" or "Physical Activity" combined with "heat shock protein 27," "HSP27," or "HSBP1." We selected studies based on a detailed examination of titles, keywords, objectives, and methodological designs. We included studies where exercise or physical activity was an independent variable and circulating changes in HSP27 were observed (n = 9, Table 1), excluding those that did not meet these criteria or did not measure HSP27 in circulating forms (plasma or serum) (n = 133). Despite these stringent criteria, the limited number of relevant articles posed challenges in providing a comprehensive summary for some topics within this scoping review. Nonetheless, excluded articles helped shape the thematic focus of this review.

For studies meeting the inclusion criteria, we extracted and recorded data on participant demographics, exercise characteristics (e.g., type and intensity), and HSP27 values at various time points post-exercise. To facilitate comparison, post-exercise values were categorized by time intervals: immediate post-exercise (within 30 min), one hour (1 h), two to three hours (2–3 h), and 24 h post-exercise. Peak post-exercise values were aggregated to provide an overview of response magnitude. Additionally, studies were stratified by age groups: young (< 40 years old), middle-aged (40–59 years old), and older adults (> 59 years old).

To quantify effect size, we calculated the percent change from baseline. When graphical results were exclusively presented, visual data extraction was performed using WebPlotDigitizer software. For consistency, plasma HSP27 units were standardized to ng/dL. The average percentage change and 95% confidence intervals were calculated only for post-exercise peak responses, due to the availability of sufficient data.

Table 1 Characteristics of the studies and percentage increase in plasma HSP27 after exercise at various time points compared to baseline

Autorship	Sample	Protocol	Measure	Results (Percentage increase compared to baseline after exercise at various time points)			
				Post-exercise (up to 30 min after exercise)	1H	2-3H	24H
Brerro-Saby et al. [124]	3 men; 5 women, untrained, with an average age of 32 years	The subjects performed 4 tests in random order: (1) at rest, inhaling ambient air (normoxia) for a period of 50 min; (2) also at rest, breathing pure oxygen (normobaric hyperoxia) for a period of 50 min; (3) manual grip tests in normoxia; and (4) manual grip tests in normobaric hyperoxia. One week elapsed between successive sessions	Plasma	Normoxia + handgrip condition = ↑35% hyperoxia + handgrip = ↑ 123%	Hyperoxia + handgrip = ↑ 53%	N/A	N/A
Gmiat et al. [126]	14 women; untrained; assigned to the young group Y (n = 8) with an average age of 22 years or the middle-aged group M (n = 6) with an average age of 41	All participants completed a single session of high-intensity circuit training (HICT), using body weight as resistance, following the recommendations of the American College of Sports Medicine (ACSM). The training session consisted of performing ten circuit exercises, each for 30 s, with 10-s intervals between exercises. The complete circuit was repeated three times, with 2-min breaks between circuits. Participants were instructed to perform the exercises at maximum intensity	Serum	N/A	M = ↓ 64% Y = ↓ 16%	N/A	M = ↓ 4% Y = ↑42%

Table 1 (continued)

Autorship	Sample	Protocol	Measure	Results (Percentage increase compared to baseline after exercise at various time points)			
				Post-exercise (up to 30 min after exercise)	1H	2-3H	24H
Jammes et al. [35]	23 patients (group I and II) with chronic fatigue syndrome, 13 women and 10 men aged 39 ± 6 years, and 23 sedentary controls, 9 women and 14 men, with an average age of 39 ± 5 years *Due to their similarity, the groups I and II in the Jammes et al.'s study were combined into a single group. Additionally, to avoid potential confusion, the groups with infection were not included in the analysis	The exercise session was preceded by a 2-min cycling period at 0 W. The load was gradually increased (20 W per minute) until the subjects decided to terminate the exercise	Plasma	Sedentary group = ↑ 103% Fatigue group: ↑ 39%	N/A	N/A	N/A
Jammes et al. [120]	18 patients with chronic fatigue syndrome, 4 women and 14 men, with an average age of 38 years, and 9 controls, 4 women and 5 men, with an average age of 39 years	The protocol consisted of (i) a 30-min rest period, during which all variables were measured and samples of venous and arterial blood were collected, (ii) a 2-min workload period at 0 Watt used to achieve a cycling frequency of 60 revolutions per minute, (iii) a working period, and (iv) a 30-min recovery period. The working period began with a workload of 20 Watts, and the load was increased by 20 Watts every minute until the subject could not maintain the required pedaling rate	Plasma	Control group = ↑ 180% Fatigue group: ↑ 99%	Control group = ↑ 60% Fatigue group: ↑ 21%	N/A	N/A

Table 1 (continued)

Autorship	Sample	Protocol	Measure	Results (Percentage increase compared to baseline after exercise at various time points)			
				Post-exercise (up to 30 min after exercise)	1H	2-3H	24H
Kim et al. [125]	40 older women, aged 60 to 70 years, divided into aquatic exercise group (n = 14), and land-based exercise group (n = 14) *Due to the similarity of the measurements (first and last sessions) in the studies by KIM et al., the measurements taken after the exercises were combined for a better understanding of the post-exercise effect	The aquatic exercise was conducted twice a week for 16 weeks in a pool, under the following conditions: ambient temperature of 30–33 °C, humidity of 70–75%, water temperature of 29 ± 1 °C, and water depth of 1.2 m. Each exercise session was divided into 10 min of warm-up, 40 min of exercise, and 10 min of relaxation exercises. The exercise program was conducted twice a week for 16 weeks in an indoor gym, with an ambient temperature of 26 ± 1 °C and humidity of 40–50%. Each exercise session was divided into 10 min of warm-up, 40 min of exercise, and 10 min of relaxation exercises	Serum	Aquatic exercise group = ↑2.2% Land-based exercise group = ↑6.6%	N/A	N/A	N/A

Table 1 (continued)

Autorship	Sample	Protocol	Measure	Results (Percentage increase compared to baseline after exercise at various time points)			
				Post-exercise (up to 30 min after exercise)	1H	2-3H	24H
Kon et al. [121]	8 healthy men, with an average age of 23 years	High-Intensity Interval Exercise (HIE) under normoxic (NHIE) and hyperoxic (60% oxygen) (HHIE) conditions. The acute HIE consisted of four sets of all-out cycling for 30 s on a cycle ergometer, with resistance equivalent to 7.5% of the participant's body weight, with 4 min of passive rest between sets. In both NHIE and HHIE tests, participants wore a facial mask covering the nose and mouth before the experimental tests. In the HHIE test, participants received hyperoxic gas from the mask via a gas cylinder 10 min before the exercise test until immediately after the last (4th) set. In the NHIE test, participants received normoxic air (ambient air) from the mask	Serum	HHIE = ↑ 5% NHIE = ↑ 40%	HHIE = ↓37% NHIE = ↓10%	HHIE = ↓47% NHIE = ↓40%	N/A

Table 1 (continued)

Autorship	Sample	Protocol	Measure	Results (Percentage increase compared to baseline after exercise at various time points)			
				Post-exercise (up to 30 min after exercise)	1H	2-3H	24H
Micielska e al. [112]	33 healthy, inactive women; average age 38 years	Each HICT session consisted of 3 circuits with a 2-min break between them. Each HICT workout included 9 exercises using body weight as resistance, such as jumping jacks, push-ups, sit-ups, side plank, squats, plank, running in place, lunges, and push-ups with rotation. Participants in the CON group completed HICT twice, at the beginning and after 5 weeks. And the HICT group performed three circuits with the same nine body-weight exercises, three times a week, for five weeks	Serum	N/A	CON = ↓28% HICT = ↑15%	N/A	N/A
Periard et al. [122]	16 healthy, untrained men, with an average age of 26 years	All subjects were instructed and encouraged to exercise (bicycle) for as long as possible, without being aware of their heart rate and the time elapsed. Exhaustion was defined as the point at which (1) a subject voluntarily terminated the exercise, or (2) energy production could no longer be maintained at a cadence above 60 rotations per minute Exercise to exhaustion was conducted at 60% and 75% of VO2max in hot conditions	Plasma	60% of VO2max. = ↑ 29% 75% of VO2max. = ↑ 55%	N/A	N/A	60% of VO2max. = ↓ 7% 75% of VO2max. = ↓ 14%

Table 1 (continued)

Autorship	Sample	Protocol	Measure	Results (Percentage increase compared to baseline after exercise at various time points)			
				Post-exercise (up to 30 min after exercise)	1H	2-3H	24H
Tan et al. [123]	11 healthy recreational male athletes between 21 and 27 years * Due to confounding factors and the difficulty in establishing the value of HSP27, the immersion group was not used in the analysis	An exercise test (ET) was conducted at 75% VO_2 max with a 1% treadmill incline until volitional exhaustion	Plasma	↑160%	N/A	N/A	N/A

First section

The role of plasma HSP27 in atherosclerosis

Atherosclerosis is a chronic inflammatory disease characterized by the formation of atherosclerotic plaques within the smooth muscle wall of blood vessels [3, 17, 40, 41]. The pathogenesis of atherosclerosis begins with the subendothelial accumulation of low-density lipoprotein cholesterol (LDL-c), which leads to endothelial cell dysfunction. This dysfunction is marked by increased production of reactive oxygen species, an imbalance between nitric oxide and prostaglandins, elevated levels of vasoconstrictors, and reduced shear stress, all contributing to a pro-atherogenic environment [3, 17, 40, 41].

The pro-atherogenic environment activates several inflammatory pathways, including the nuclear factor kappa B (NF-κB) pathway. This pathway triggers the expression of pro-inflammatory cytokines, leading to further endothelial dysfunction [3, 17, 40, 41]. Elevated expression of adhesion molecules such as VCAM-1, ICAM-1, P-selectin, and E-selectin, as well as pro-inflammatory endothelial cell receptors, facilitates the recruitment and migration of immune cells into the vascular tissue. Monocytes are recruited, differentiate into pro-inflammatory macrophages, and proliferate within the intima [3, 17, 40, 41]. These macrophages ingest oxidized LDL-c and other lipids, transforming into foam cells. The accumulation of foam cells, coupled with the ongoing recruitment of monocytes and oxidation of LDL-c, leads to the formation of atherosclerotic plaques in the subendothelial space. Foam cells release pro-inflammatory cytokines, exacerbating plaque progression, instability, and the formation of necrotic cores, alongside extracellular matrix degradation [3, 17, 40, 41].

According to Polly Matzinger's danger theory, innate immune responses can be triggered by molecules released from stressed or damaged tissues, known as "damage-associated molecular patterns" (DAMPs) [42]. Initially, heat shock proteins (HSPs) were considered DAMPs because they are released from stressed or injured tissues. HSPs are molecular chaperones that assist in protein transport, folding, and assembly within cells, prevent protein denaturation, and eliminate misfolded proteins. They can also prevent cell death by autophagy [4, 43, 44]. However, during stressful conditions, HSPs can enter the bloodstream, bind to immune and epithelial cell receptors, and activate an immune-metabolic response [4]. Despite this, the lack of a clear molecular pattern and potential inhibition of inflammatory signaling by certain HSP receptors have led some researchers to question their classification as DAMPs [2, 45–47]. Additionally, studies that

have removed contaminants do not support the hypothesis that HSPs have a pro-inflammatory function [2, 47–49]. Consequently, while HSPs were initially thought to be DAMPs, their unique properties and conflicting research findings have led to a reassessment of their role. Nonetheless, their function in protein transport and maintaining cellular function under stress remains critical.

In vivo and in vitro studies of human arteries have suggested a link between decreased levels of HSP27 and the progression, complexity, and instability of atherosclerotic plaques [2, 3, 5, 9]. HSP27 functions as a chaperone and antioxidant and plays a role in inhibiting apoptosis and remodeling the cytoskeleton. A reduction in HSP27 may favor smooth muscle proliferation and atherosclerotic plaque formation [2–5]. Elevated HSP27 levels can upregulate hepatic LDL-c receptor expression, reducing cholesterol concentrations [5, 7, 50]. Additionally, HSP27 regulates macrophage activity by preventing their accumulation, modulating the expression of class A scavenger receptors, promoting anti-inflammatory signaling, and reducing foam cell formation [2, 3, 5]. In animal models of inflammatory atherogenesis ($ApoE^{-/-}$ mice), overexpression of HSP27 has resulted in up to a 35% reduction in atherosclerotic burden, supporting its protective role [8, 10, 51, 52]. These studies suggest that increased plasma HSP27 concentrations correlate with reduced atherosclerotic burden. Furthermore, estrogen-treated macrophages increase HSP27 expression in vitro, and the addition of recombinant HSP27 (rHSP27) reduces foam cell formation, lowers interleukin (IL)-1β release, and increases IL-10 expression [2, 3, 53, 54]. HSP27 can also competitively bind to class A scavenger receptors, reduce oxidized LDL accumulation, and stimulate granulocyte–macrophage colony-stimulating factor, which promotes the expression of ABC transporters involved in reverse cholesterol transport [2, 3].

Animal studies have shown that mice lacking the HSPB1 gene (encoding HSP27) and receiving bone marrow transplants from HSP27-overexpressing donors exhibit significant increases in blood HSP27 levels and reductions in cholesterol and necrotic tissue [52]. Similarly, subcutaneous injections of recombinant HSP27 in HSPB1-deficient mice (C57BL/6, $ApoE^{-/-}$ littermates) produced comparable results [52]. Recent research has also demonstrated that vaccination with rHSP25, the rodent equivalent of human HSP27, reduces atherogenesis and inflammatory markers in $ApoE^{-/-}$ mice by increasing HSP25 antibody levels [50]. The HSP27 immune complex, consisting of antibodies and antigens, plays a critical role in reducing inflammation and atherogenesis. This complex facilitates signaling through HSP27 receptors, modulating inflammation, cholesterol uptake, and HSP27 internalization [55]. These findings indicate that both endogenous and exogenous HSP27 can significantly reduce atherosclerotic lesion formation, resulting in more stable and less inflamed plaques. Therefore, plasma HSP27, or its immune complex, may be more critical in managing atherosclerosis and inflammation compared to locally produced HSP27 within smooth muscle or endothelial cells [2, 3]. Observational studies also suggest that blood HSP27 concentrations are lower in individuals with atherosclerosis compared to healthy controls [52, 56, 57], and lower HSP27 levels are predictive of adverse clinical outcomes related to atherosclerosis, such as heart attacks, strokes, and cardiovascular death over a five-year period [52].

Plasma HSP27 and age

A study has demonstrated an inverse relationship between plasma levels of HSP27 and age, indicating that the expression of this heat shock protein decreases with aging [6]. Although the literature on the relationship between age and HSP27 plasma is still limited, some evidence suggests that other circulating heat shock proteins, such as HSP60 and HSP70, also decrease with advancing age [58]. Aging contributes to reduced levels of HSPs due to the decreased ability of the heat shock transcription factor, the master regulator of the heat shock response, to bind to HSP genes during periods of stress. This decreased binding ability compromises the cellular stress response and the expression of HSPs [46, 59, 60]. The global decrease in plasma HSPs may impair the body's ability to handle cellular and inflammatory stresses, contributing to the higher incidence of aging-related diseases [46, 60]. Therefore, understanding changes in the levels of HSPs with aging can provide valuable insights for developing therapeutic interventions aimed at mitigating the impacts of aging on cardiovascular health. For instance, potential therapeutic interventions could include strategies to enhance heat shock transcription factor 1 activity or directly increase the expression of HSPs.

Plasma HSP27 and menopause

The relationship between plasma HSP27 levels and aging is complex and multifaceted, significantly influenced by the menopausal transition. Canadian researchers have uncovered a complex interaction between HSP27, estrogen, and estrogen receptor β, which may explain the sex-dependent nature of HSP27's protective effects against atherosclerosis [7–10]. HSP27 acts as a co-repressor of estrogen signaling when binding to estrogen receptor β but is also released into the bloodstream in response to estrogenic stimulation. Studies have shown that the loss of estrogen in rats leads to reduced basal levels of HSP27 protein [61], and similar reductions in HSP27 concentrations have been observed in women undergoing premature surgical menopause [62]. Furthermore, comparisons between pre- and post-menopausal

women reveal a decrease in plasma HSP27 levels [2, 3]. These findings suggest that the reduction of HSP27 due to estrogen loss during menopause may contribute to the development of atherosclerosis. However, a nested case–control study of healthy women who developed cardiovascular disease (CVD) found that circulating HSP27 concentrations were inversely related to age but not to other established cardiovascular risk factors or future cardiovascular events [6]. Although drawing definitive conclusions about the relationship between menopausal transition, CVD, and HSP27 based solely on this nested case–control study is challenging, some limitations have been identified [2, 3]. For instance, the women in the study were, on average, 61 years old and postmenopausal at baseline, suggesting that atherogenesis may have already reached a moderately advanced stage, along with low concentrations of HSP27. These human studies, along with previous in vitro and animal research, indicate that HSP27 plays an important role in atherosclerosis, particularly in women undergoing menopause. Therefore, developing therapeutic interventions aimed at increasing plasma HSP27 levels could be beneficial for mitigating the cardiovascular impacts of menopause.

Second section

Exercise-induced plasma HSP27 origin

A growing body of evidence indicates that HSP27 levels rise in skeletal muscle following exercise [63–65], 52; [66–70]. Additionally, a reduction in HSP27 levels in muscle has been observed following decreased mechanical and neural activity, such as hindlimb suspension and spaceflight. However, these levels recover with reloading [71–73]. Notably, two studies [74–76] failed to detect an increase in HSP27 expression in muscle. The authors of these studies attributed the lack of positive results to methodological inadequacies, including insufficient exercise intensity, inadequately trained experimental groups, and a low number of biopsies, which may have missed the peak HSP27 elevation. Moreover, research in rodents has revealed notable disparities in HSP27 expression across different muscle fiber types [71, 72, 77]. Thus, variations in biopsy orientation and location before and after exercise could introduce confounding variables, potentially affecting result accuracy. Overall, the evidence supports that skeletal muscle can synthesize HSP27 during exercise.

Several mechanisms for the extracellular release of HSP27 have been reported [2]. Specifically, HSP27 is released from tissues via extracellular vesicles (exosomes), a process potentially dependent on intracellular calcium concentration [27]. While HSP27 has been suggested as a myokine [27], it remains unclear whether intact muscle cells, without exercise-induced damage, release HSP27 into circulation.

It is also noteworthy that other HSPs have been demonstrated to be released from various tissues, including the liver and brain, during exercise [32, 33, 37–39]. This suggests that the exercise-induced increase in plasma HSP27 concentrations may have origins beyond muscle tissue. Some rodent studies indicate that activation of skeletal muscle afferents (groups III and IV) triggers an increase in HSP27 levels not only in skeletal muscle but also in respiratory muscles, kidneys, and the brain [34, 36, 78]. Therefore, it is likely that the rise in plasma HSP27 following exercise originates from both muscle tissue and various other sources.

Exercise-induced plasma HSP27 targets

The observed increases in plasma concentrations of heat shock proteins (HSPs), including HSP27, following exercise suggest that HSP27 is released from various tissues, such as muscle, liver, and brain, to perform systemic functions [33, 34, 36, 38, 39]. Studies involving macrophages treated with recombinant HSP27 (rHSP27) have demonstrated a reduction in foam cell formation and a decrease in the release of IL-1β, a pro-inflammatory cytokine, while increasing IL-10, an anti-inflammatory cytokine [8, 10, 51, 52, 54]. These findings suggest that HSP27 may inhibit atherogenesis and inflammation by acting as an immunomodulator in cells associated with atherosclerosis [8, 10, 51, 52]. Although the exact mechanisms are not yet fully understood, HSP27 can function as a free extracellular protein, as part of a larger protein complex (through interactions with HSP27 antibodies), and/or as molecular cargo on the surface or within exosomes once outside the cell [2, 3, 5].

Hematopoietic cells are also known to respond to stress, leading to investigations into the role of HSP27 in leukocytes following exercise. Research suggests that exercise results in increased expression of HSP27 in leukocytes [79–83]. Notably, exercise can induce the expression of HSP27 on the surface of leukocytes (monocytes and granulocytes) [81, 83]. Given the identification of several HSP27 receptors, including CD91, CD40, CD36, CD14, toll-like receptors (TLRs), and scavenger receptor-A [2], and the ability of HSPs to be released from tissues via extracellular vesicles (exosomes) [2] and to bind to receptors through receptor-mediated endocytosis [84–87], it is plausible that the increase in HSP27 on immune cells' surfaces after exercise originates from extracellular sources.

Extracellular HSP27 can bind to surface receptors, such as TLRs, on distant target cells [2], suggesting that the increase in HSP27 following exercise might target leukocytes and exert immunomodulatory effects. HSP27 interacts with TLRs, such as TLR2, TLR3, and TLR4, which can promote NF-κB transcriptional activation and, consequently, the

expression and secretion of cytokines dependent on this activation in various cell types and conditions [2, 53, 54]. TLR activity is generally associated with inflammatory signaling, but its action is context-dependent. For example, TLR4/MyD88 signaling results in pro-inflammatory cytokine production, while TLR3 and TLR4/TRAM/TRIF/TRAF3 signaling leads to the production of interferon β (associated with the innate immune response) and the release of anti-inflammatory cytokines such as IL-10 [88]. The secretion of IL-10 initiates the resolution of inflammation [89]. Thus, TLR3 and TLR4 may act protectively on metabolism by enhancing the immune response [88]. The observed increase in IL-10 in the presence of HSP27 [53, 54] supports the possibility that TLR3 and TLR4 are likely targets of HSP27 [2, 3]. Therefore, the expression of cytokines by cells in response to HSP27 is contingent upon the type and concentration of receptors within the cell, as well as the cell's differentiation state [53, 90, 91]. Cytokine responses to HSP27 are observed in differentiated cells, but not in naïve cells [53].

Understanding the role of plasma HSP27 in monocyte signaling, particularly in relation to IL-1β, is crucial. Patients with cardiovascular disease (CVD) and cardiometabolic disorders often exhibit increased levels of certain monocyte subpopulations, such as intermediates (CD14 + + CD16 +) and non-classical monocytes (CD14 + CD16 + +) [92, 93]. IL-1β has been identified as a significant contributor to these pathological conditions [94–96]. HSP27 has been implicated in the differential regulation of IL-1β in monocyte subsets, mediating lower IL-1β production in non-classical monocytes (CD14 + CD16 + +) by increasing IL-1β mRNA decay rates [97]. These potential immunomodulatory mechanisms of HSP27 support findings that report a reduction in IL-1β by the cell in the presence of HSP27 [2, 3]. These findings suggest that HSP27 may target immune cells [2, 3], contributing to the well-documented anti-inflammatory effect of exercise [17, 98].

Our review identified a biphasic model in the HSP27 response (presented in Sect. "First section"), which aligns with the transient and time-dependent redistribution of immune cells to peripheral tissues following exercise [98]. Research indicates that various forms of exercise, including high-intensity interval training, low-intensity aerobic exercise, and resistance training, are associated with leukocytosis, characterized by increases in lymphocytes and monocytes [99–102]. High-intensity and short-duration exercises increase both absolute and relative numbers of intermediate (CD14 + + CD16 +) and non-classical (CD14 + CD16 + +) monocytes, while moderate-intensity and long-duration exercises increase classical monocytes (CD14 + + CD16-) [99, 103–106]. Post-exercise, immune cell numbers typically return to baseline levels within about an hour. Notably, monocyte populations, particularly non-classical monocytes, exit peripheral circulation faster than other leukocyte populations [99, 100, 107]. This is followed by a significant reduction in immune cell numbers 1–2 h post-exercise, with a return to baseline levels within 24 h, especially after high-intensity exercise. This biphasic phenomenon, initially identified as the "open window" hypothesis, was once associated with immune suppression and increased infection risk. However, recent advances in exercise immunology have clarified that the dramatic reductions in lymphocyte numbers and functions 1–2 h after exercise reflect a transient, time-dependent redistribution of immune cells to peripheral tissues, resulting in heightened immune surveillance and regulation rather than immune suppression [98].

In summary, the results collectively indicate that HSP27 may specifically target immune system cells, particularly intermediate (CD14 + + CD16 +) and non-classical (CD14 + CD16 + +) monocytes, acting as an immunomodulator in these cells associated with atherosclerosis. Further research is essential to determine whether the exercise-induced increase in plasma HSP27 can reduce IL-1β expression in different monocyte subpopulations. This research will enhance our understanding of the role of HSP27 in immune system modulation and may offer insights into potential therapeutic approaches for managing cardiovascular disease and cardiometabolic disorders.

HSP27 response following exercise in muscle and immune cells in men and women.

Skeletal muscle

Most studies have demonstrated that concentrations of muscle HSP27 increase immediately or within a few hours following exercise. These increases have been observed predominantly in male samples [67, 108, 109], with limited research conducted on women. However, a study exclusively focused on women found that resistance exercise resulted in both acute and chronic increases in muscle HSP27 levels [74, 75]. Additionally, a study with a predominantly female sample observed an increase in muscle HSP27 after eccentric exercise [64]. Furthermore, Gjøvaag and Dahl [110] found no significant differences in the increase of muscle HSP27 between men and women after undergoing different resistance training protocols lasting 5–8 weeks [110]. Overall, young men and women appear to exhibit similar responses to exercise in terms of muscle HSP27.

Leukocytes

Research has shown that exercise can increase HSP27 gene and protein expression in leukocytes in both men [37, 39; [79, 83, 111]) and women [112]. However, there is a scarcity of research on HSP27 in women. One study conducted by Micielska et al. [112] reported that high-intensity circuit

training led to increased HSP27 gene expression (mRNA) in leukocytes in young women, and this increase was correlated with higher levels of plasma HSP27 (serum) 24 h after exercise [112]. In men, increases in HSP27 gene and protein expression in leukocytes, particularly in monocytes and granulocytes, have been observed immediately after or within a few hours (1–3 h after exercise 37, 39; [79, 83]). One study [111] found that the expression of the HSP27 gene increased after 3 h, and the HSP27 protein increased after 24 h in peripheral blood mononuclear cells following 30 min of aerobic exercise at 70% of the maximal heart rate. Furthermore, some studies have reported that the increase in HSP27 expression in leukocytes was sustained for up to 24 h after exercise.

The impact of exercise variables on HSP27 response in both muscle and immune cells.

Skeletal muscle

Mechanical stress serves as a well-established stimulus for eliciting HSP27 responses in skeletal muscle. Resistance exercise and eccentric muscle actions are notably effective in augmenting HSP27 content within skeletal muscle, as demonstrated in various studies [63, 109, 110, 113, 114]. These exercises, particularly eccentric exercise, are commonly associated with muscle microtrauma and damage. For example, Feasson et al. [113] documented elevated HSP27 concentrations in the vastus lateralis following 30 min of downhill running, a prototypical eccentric exercise known to induce muscle damage [113]. Conversely, Morton et al. [115] observed no notable increase in HSP27 concentrations subsequent to 45 min of running on a treadmill with a positive incline, a concentric exercise typically devoid of muscle damage [115].

Low-intensity resistance exercise (load approximately 50% of one repetition maximum) and treadmill running and cycling alone may not increase HSP27 concentrations in skeletal muscle, as indicated by previous studies [63, 76, 108, 115]. However, when combined with blood flow restriction (vascular occlusion), these exercises have been shown to elevate HSP27 concentrations [67, 74, 75, 116]. Additionally, evidence suggests that HSP27 is released from the human myocardium after ischemia [117, 118], which is also induced by brief ischemia caused by vascular occlusion. Oxidative and metabolic stresses, such as muscle glycogen reduction, are also prominent during the use of vascular occlusion [63–65, 67, 69, 70, 119].

Overall, the integration of both cellular stress (metabolic and oxidative) induced by vascular occlusion and mechanical stress induced by high-load and eccentric-action exercise can provoke an increase in HSP27 in skeletal muscle.

Leukocytes

Several studies have examined the impact of exercise on HSP27 concentrations on the surface of leukocytes, including monocytes and granulocytes [80, 81, 83]. Whitham et al. [83] observed an immediate increase in HSP27 concentrations on the surface of granulocytes after progressive and maximal exercise testing on cycle ergometers, which persisted even 24 h later. Additionally, they noted a rise in HSP27 concentrations on granulocytes immediately following a 110-min exercise session at 65% of the peak power achieved in the maximal progressive test [81, 83]. Similarly, Fehrenbach et al. [80] found elevated HSP27 concentrations on the surface of monocytes and granulocytes following a half marathon, although the increase in monocytes was immediate, while in granulocytes, it was observed 24 h later. Notably, the response of HSP27 on the surface of monocytes was inconclusive in the study by Fehrenbach et al. [81], with only half of the sample showing an increase. Despite some variability in the findings, moderate to high-intensity exercise and exercise until exhaustion (voluntary withdrawal) can lead to an increase in HSP27 concentrations on the surface of leukocytes.

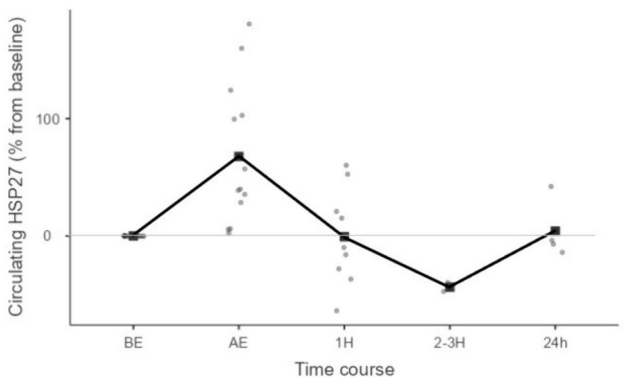

Fig. 1 Time course of plasma HSP27 after exercise. *Note* This figure presents the variation of plasma HSP27 in percentage, *BE* before exercise (baseline); *AE* after exercise (up to 30 min); One hour after exercise (1H); two to three hours after exercise (2-3H); twenty-four hours after exercise (24H)

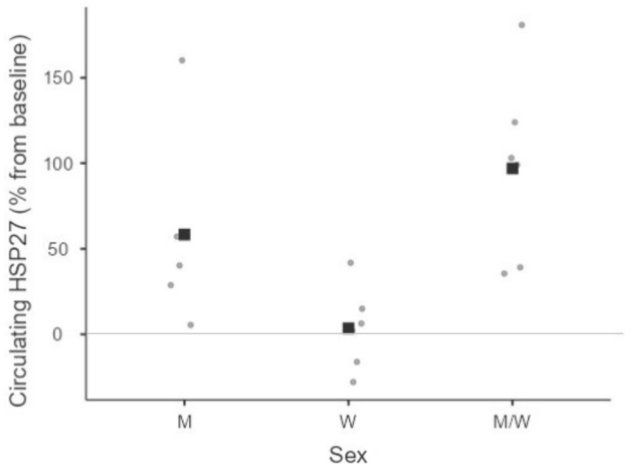

Fig. 2 Plasma HSP27 after exercise grouped by sex. *Note* This figure presents the HSP27 variation in percentage grouped by sex. *M* men; *W* women; *M/W* men and women

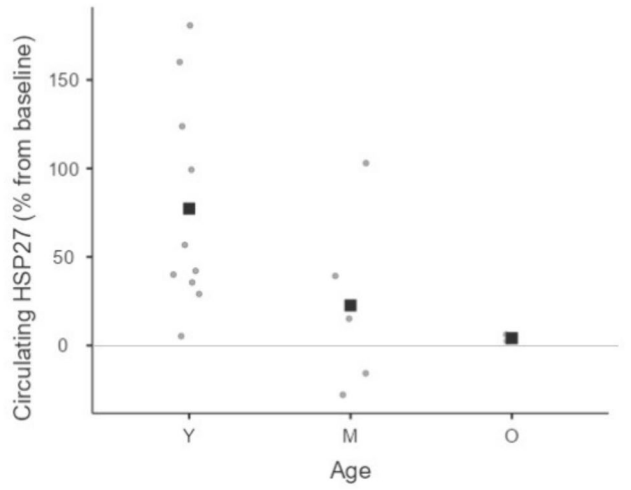

Fig. 3 Plasma HSP27 after exercise grouped by age group. *Note* This figure presents the plasma HSP27 variation in percentage grouped by age. *Y* young; *M* middle age; *O* old age

Third section

Plasma HSP27

Available published evidence for effect acute of exercise on plasma HSP27 concentration

Table 1 presents a summary of the main findings from eight studies examining plasma HSP27 concentration following various forms of exercise. Among the studies, four involved young participants, four involved middle-aged individuals, and one involved older subject. Additionally, three studies focused exclusively on men, three on women, and three on both genders. The types of exercise varied and included maximal exercise tests (4 studies), high-intensity interval exercise (2 studies), manual grip exercises (1 study), moderate-intensity continuous exercise (1 study), and circuit weight loss body exercises (1 study). Only one study considered the participants as athletes, while all other studies involved untrained individuals. Furthermore, with the exception of one study, all participants exercised to the point of voluntary exhaustion or near it. In studies involving different groups without confounding factors, such as immersion in hot water, the responses from each group were considered as a sampling unit, resulting in a total of n = 17.

Magnitude of the response and course time of plasma HSP27

An increase in plasma HSP27 concentrations has been documented immediately following exercise [35, 120–123], as well as at various time points thereafter, 10 min [124], 30 min [125], 60 min [126], and 24 h post-exercise [112]. These investigations have demonstrated that HSP27 concentrations returned to baseline levels after 60 min in some instances [121], while in other studies, it remained elevated for up to 24 h [122]. For instance, one study reported that plasma HSP27 levels increased within 10 min following maximal isometric exercise and remained elevated for 30 min thereafter [124]. Other studies, such as the one conducted by Jammes et al. [120], observed a peak immediately after maximal progressive exercise, with sustained maintenance of these elevated levels for up to 60 min, though without further measurements.

The studies reviewed indicate an average increase of approximately 53% (95% CI 22% to 84%, n = 17, Table 1) in plasma HSP27 levels following exercise, with values ranging from over 180% to no response. This variability appears to be influenced by the type of exercise regimen, age, sex (discussed in subsequent sections of this article), and the timing of post-exercise blood sample collection (time course of HSP27, Fig. 1). Although studies are limited, some research suggests that HSP responses may differ between athletes and non-athletes, with athletes showing increased responses [82, 127]. Despite one of the highest responses being observed in a study with athletes (160%) [123], the highest HSP27 response (180%) was recorded in a study involving middle-aged non-athlete men [120]. Furthermore, although there are limited studies on the topic, a group of studies [35, 120] has demonstrated that the presence of stress factors seems to inhibit the plasma HSP27 response to exercise. Given the protective function of plasma HSP27, this area warrants further attention. Finally, the diverse exercise protocols used in the studies made it impossible to determine the potential impact of exercise duration on the HSP27 response in

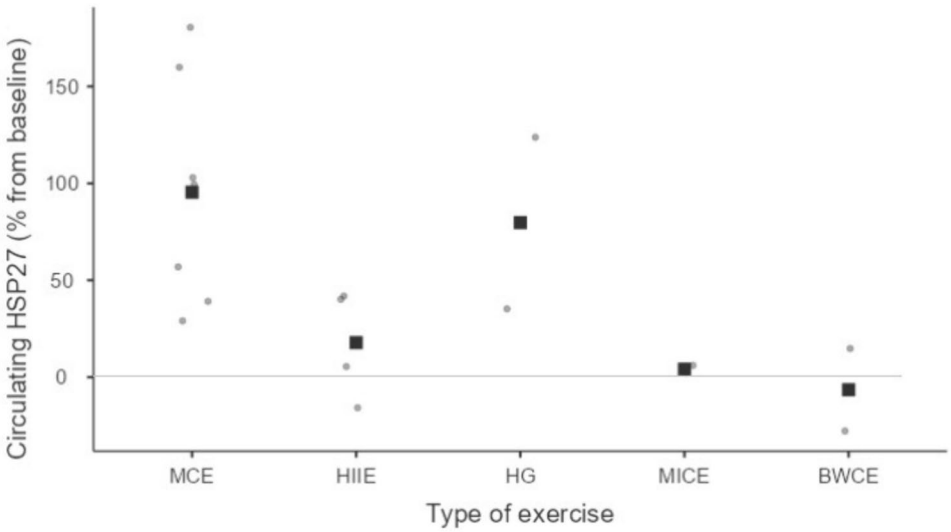

Fig. 4 Plasma HSP27 after exercise grouped by type of exercise. *Note* This figure presents the plasma HSP27 variation in percentage grouped by exercise. *MCE* maximal continuous exercise; *HIIE* high intensity interval training; *HG* hand grip; *MICE* moderate-intensity interval exercise; *BWCE* circuit training with body weight

plasma. Therefore, further research is necessary to establish whether the increase in plasma HSP27 response is linked to the duration of training.

Concerning the time course of HSP27, research suggests a biphasic response over time (Fig. 1): an initial post-exercise increase (65%), followed by a return to baseline levels in approximately one hour (7%), a subsequent decrease below baseline values around three hours (-44%), and ultimately, a return to baseline values within 24 h (~ 7%). The response exhibited a biphasic pattern similar to the one observed in immune cell training, as discussed earlier. Given that HSP can bind to receptors and undergo spontaneous internalization through receptor-mediated endocytosis [84, 86, 87], it is conceivable that the biphasic effect observed for HSP27 is linked to the biphasic effect seen in immune cells. This strengthens the notion that immune cells could be potential targets of HSP27 following exercise. However, the interpretation of the temporal response of plasma HSP27 in these studies is complicated by the limited number of post-exercise blood collection points. Therefore, caution must be exercised regarding this relationship, and further studies are needed to clarify it.

Difference between men and women in the acute response of Plasma HSP27

Research indicates that exercise-induced increases in plasma HSP27 concentrations are observable in both men [121–123] and women [112, 125, 126], as well as in mixed-sex studies [35, 120, 124]. Upon segregating and analyzing these studies by sex, a discrepancy in the average response magnitude of plasma HSP27 between men and women was identified (Fig. 2). The higher response observed in men may be primarily associated with a study involving athletes [123]. As previously discussed, athletes may exhibit greater responses than non-athletes. Excluding the athlete study from the analysis diminished the difference in responses between men and women. Furthermore, in studies that exclusively involved men, all participants were young. In contrast, the group consisting solely of women included studies involving older individuals. Mixed-sex studies showed average responses similar to those observed in groups consisting only of men. Consequently, the difference in responses between men and women may be attributable to other factors such as age or fitness level.

Exercise-induced plasma HSP27 and aging

It has been suggested that increasing plasma exerkines has potential implications for age-related health problems [128]. However, there has been limited research on the impact of aging on the responses to exercise in relation to HSP27 concentration. The studies found for this review, when grouped by age group, showed that exercise-induced increases in plasma HSP27 concentrations can be observed in young [121, 122, 124, 126, 129] and middle-aged individuals [112, 126, 129], but not in older adults [125] (Fig. 3). However, only one study evaluating the response of plasma HSP27 in the older adults was found in our review. Moreover, it is important to note that the study sample consisted only of women, which could potentially introduce confounding factors due to the absence of estrogen [2, 3]. As the source of plasma HSP27 may be muscle, muscle HSP27 concentrations have been the target of investigation in the context of aging. Although studies in this area are scarce, a recent study by Cumming et al. [66] discovered that HSP27 concentrations in muscle are similar in old (75 ± 5 years) and young (26 ± 5 years) subjects at baseline, but increase with resistance training only in young subjects. In this study, around 75% of the

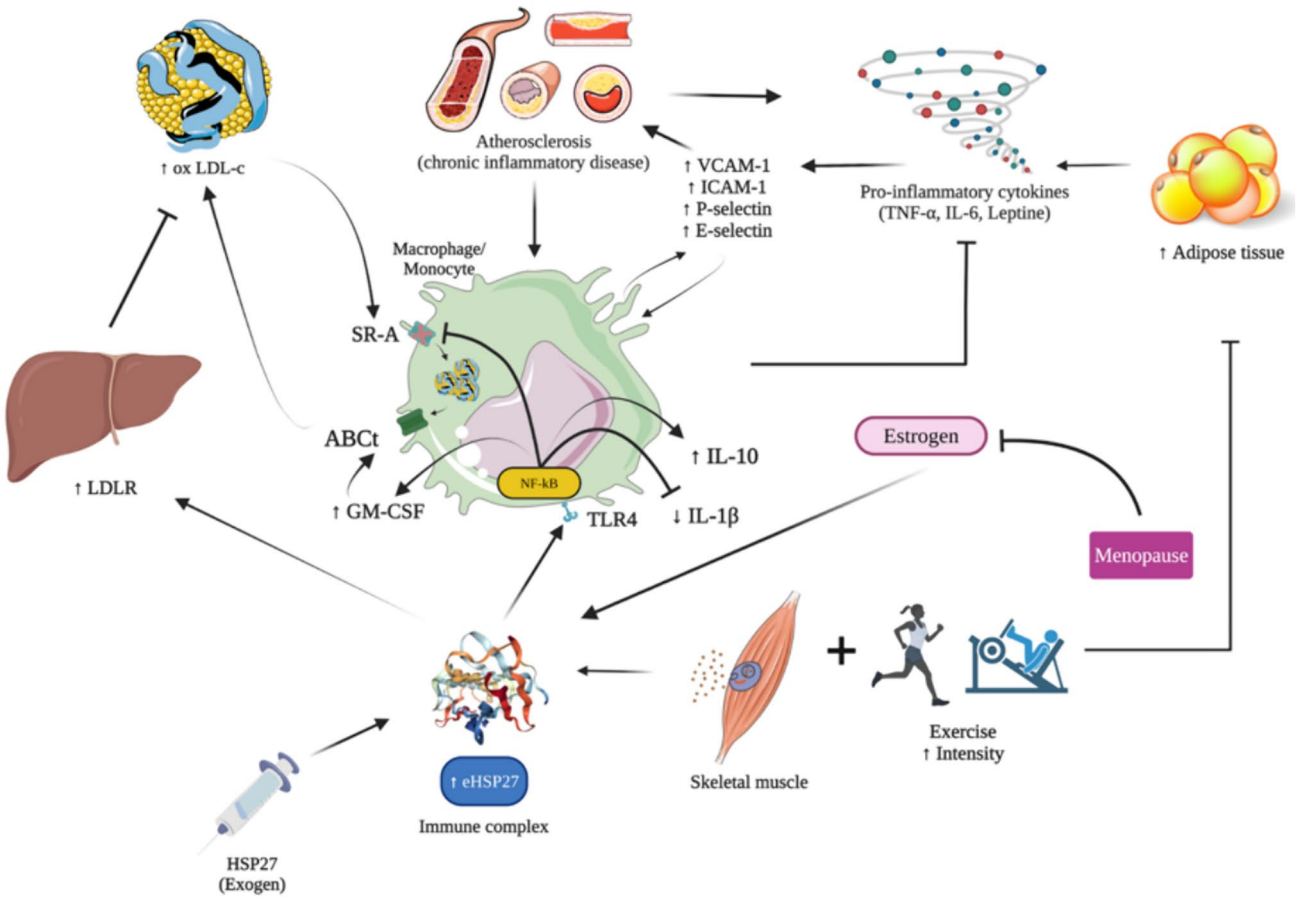

Fig. 5 Schematic diagram of plasma HSP27 as immunomodulator. *Note* We hypothesize that exercise can acutely increase plasma HSP27 levels (originating from muscle and other tissues) in women, regardless of menopausal status (pre- or post-menopausal). Plasma HSP27 seems to act as an immunomodulator in monocyte subpopulations (intermediate and non-classical), increasing IL-10 and decreasing IL-1β. *GM-CSF* Granulocyte Macrophage Colony-Stimulating Factor; *eHSP27* extracellular HSP27; *LDR* Low-Density Lipoprotein Receptor; *ox LDL* Oxidized Low-Density Lipoprotein; *VCAM-1* or Vascular Cell Adhesion Molecule 1; *ICAM-1*, Intercellular Adhesion Molecule 1. P-selectin is a cell adhesion molecule expressed on the surface of activated platelets and endothelial cells; E-selectin is an adhesion molecule expressed on the surface of endothelial cells; *ABCt* ATP-binding cassette transporters; *TLR4* Toll-Like Receptor 4; *SR-A* Scavenger Receptor Class A; *NF-κB* Nuclear Factor kappa B

older group sample consisted of women, while the young groups were mostly male. Furthermore, studies in rodents and birds have shown that the levels of HSP27 increase in the muscles of young animals (quails), but not in the muscles of older animals [71, 130, 131]. In addition, even in immune cells (peripheral blood mononuclear cells), a study found no increase in HSP27 expression after 1 and 25 h following a graded maximal stress test in older adults (70–75 years) [132]. Thus, there is emerging evidence suggesting a potential decrease in the plasma HSP27 response to exercise with aging. In women, this decrease may be linked to lower estrogen levels. However, the existing literature on this specific topic is limited. Further research is necessary to determine whether the reduced HSP27 responses to exercise in women are dependent on lower estrogen levels or age.

Effect of exercise variables on Plasma HSP27

In healthy individuals, plasma HSP27 concentrations increase following both maximal static exercises (without joint movement) [124] and dynamic exercises [120]. Additionally, plasma HSP27 concentrations have been observed to increase after treadmill running at moderate intensity (65–75% of maximal oxygen consumption) until voluntary interruption [122, 123] or for more than 40 min [125]. Plasma HSP27 concentrations also rise after high-intensity circuit training (using only body mass as resistance without external load) [112, 126] and maximal progressive [35, 120] or intermittent efforts [121]. Maximal exercise modalities, including maximal continuous exercise testing (MCET), high-intensity interval exercise (HIIE), and maximal static exercises like hand grip (HG), collectively

demonstrate superiority over moderate-intensity continuous exercise (MICE) or circuit training with body weight (BWCE) (Fig. 4).

The increase in plasma HSPs has been linked to exhaustive exercise, with the response depending on the exercise's intensity and duration [32, 122, 133]. During exhaustive exercise, multiple stressors such as muscular acidosis, hypoxia, ischemia, and reactive oxygen species stimulate muscle afferents (groups III and IV) [129, 134, 135]. Activation of muscle afferents leads to the generalized production of HSPs [34, 36], and hence, greater intensity of exercise appears to result in a greater response of plasma HSPs.

Conclusion

The reduction of HSP27 levels is associated with increased complexity and instability of atherosclerotic plaques, with HSP27 being an important modulator due to its anti-inflammatory, antioxidant, and anti-apoptotic functions. Advanced age and menopause contribute to decreased plasma levels of HSP27, increasing vulnerability to cardiovascular diseases. Increasing HSP27 levels may be a promising strategy for the prevention and management of atherosclerosis. The data shows that engaging in high-intensity physical exercise or exercising until close to exhaustion can acutely increase plasma levels of HSP27 in both young and middle-aged individuals, regardless of gender. However, further research is needed to determine if exercise can increase HSP27 levels in individuals with estrogen deficiency, such as menopausal women and the alder adults. The biphasic response of HSP27 levels to exercise, reflected in the response of leukocytes, suggests that these may be targets of plasma HSP27. Furthermore, HSP27 acts as an immunomodulator in monocyte subpopulations, particularly in intermediate and non-classical subsets, through TLR 3 and 4 pathways, increasing IL-10 levels and reducing IL-1β levels. These findings indicate that plasma HSP27 may modulate the immune system response to exercise-induced stress. The proposed hypothesis is represented in Fig. 5.

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Data Availability As this is a review article, all data used comes from articles available on the PubMEd platform and are listed in the references.

Declarations

Competing Interests The authors declare no competing interests.

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