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# Title: Exercise-Specific Epigenetic Effects on Cardiovascular Health

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**Abstract**

Exercise is widely prescribed to prevent cardiovascular disease, which encompasses a heterogeneous spectrum of disorders involving vascular dysfunction, myocardial injury and remodeling, inflammation, and electrophysiological disturbances; however, its benefits are not uniformly dose-dependent. While aerobic and resistance training generally elicit sustained cardioprotective adaptations, evidence from ultra-endurance exercise suggests a distinct physiological regime in which repeated extreme load, prolonged duration and individual susceptibility may precipitate maladaptive remodeling. Here we synthesize an epigenetic remodeling framework to reconcile these divergent outcomes and to explain how exercise modality, intensity and exposure duration may influence the balance between cardiovascular adaptation and vulnerability.

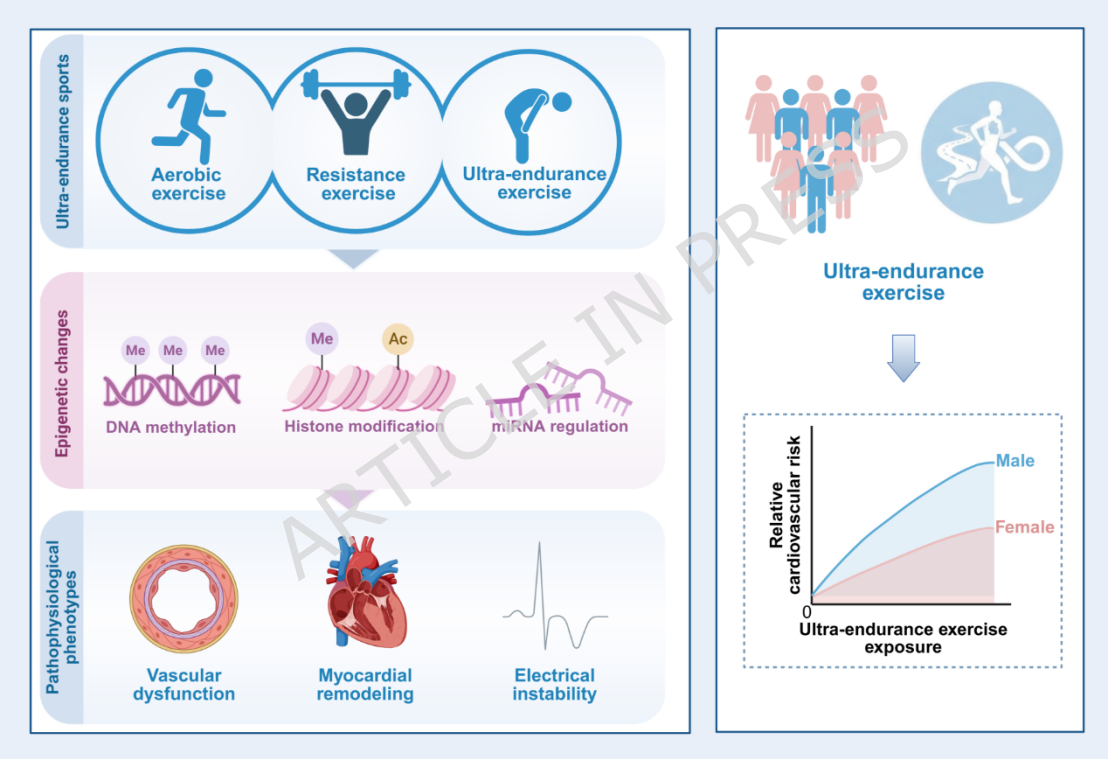
We systematically examine epigenetic pathways, including DNA methylation, histone modifications and non-coding RNA-mediated regulation, through which aerobic exercise and resistance training may modulate vascular function, myocardial metabolism, inflammation and fibrosis. We then contrast these signatures with those reported in ultra-endurance settings, highlighting mechanistic patterns associated with transient myocardial damage markers, arrhythmogenic substrates and adverse structural remodeling in susceptible individuals. We further discuss emerging sex-

specific epigenetic mechanisms that may contribute to differential cardiovascular disease trajectories under ultra-high-intensity endurance stress.

Finally, we outline key limitations in current evidence, including incomplete causal epigenetic chains, heavy reliance on experimental models, peripheral tissues and elite athlete cohorts, limited tissue-specific evidence and a paucity of longitudinal population studies. Addressing these gaps will be essential for translating epigenetic insights into risk-stratified and individualized exercise prescriptions that maximize cardioprotection while minimizing potential risk.

**Keywords:** Physical activity, Cardiovascular health, Epigenetic modifications, Types of physical activity, Sex differences

**Graphical Abstract**




## 1.Introduction

Cardiovascular disease (CVD) remains highly prevalent and causes significant disability and mortality, encompassing a heterogeneous spectrum of disorders involving vascular dysfunction, myocardial injury and remodeling, inflammation, and electrophysiological disturbances [1–5]. It is estimated that approximately 19.8 million people died from CVD in 2022, representing about 32% of all deaths globally that year[6]. Furthermore, CVD poses a significant public health challenge due to its increasing burden on healthcare systems. Despite the reduction in morbidity and mortality rates associated with drugs and interventions for CVD, its prevalence remains high,

necessitating effective prevention strategies to further reduce its incidence.

Increasing evidence suggests that an individual's lifestyle has a significant impact on the incidence of CVD[7], including diet, physical activity, psychosocial stress and sleep patterns. These factors can alter genetic predisposition, thereby affecting vascular function, myocardial remodeling, metabolic homeostasis and triggering inflammatory responses. In addition, coronary heart disease may be prevented if unhealthy lifestyle changes are made earlier, thereby significantly reducing the incidence and alleviating the burden of treatment.

Of all lifestyle factors, physical activity has long been believed that it can effectively protect cardiovascular health [8,9]. Regular aerobic exercise and resistance training can reduce the incidence and mortality of CVD by improving endothelial function, promoting beneficial metabolic adaptations, and enhancing myocardial function. These findings suggest that increased physical activity can enhance cardiovascular function, and prevent CVD. Increasing physical activity, typically aerobic exercise or resistance training, can restore cardiovascular health in the general population in a feasible, repeatable, and physiologically effective way.

However, as more and more people participate in ultra-endurance exercise such as marathons and triathlons [10,11], the physiological and cardiovascular health status, especially those professional athletes, contradicts this traditional view. New evidence suggests that extreme and prolonged exercise has different effects on different individuals and can sometimes even negatively influence the cardiovascular system[12,13]. These observations, along with recent large-scale population studies, indicate that the cardiovascular benefits of physical activity can vary with baseline cardiovascular risk stratification. Also, the benefits of high-intensity exercise may reach a plateau in high-risk individuals, suggesting that different forms or intensities of physical activity can trigger extremely different cardiovascular responses[14].

To address this issue, it is necessary to integrate exercise type, intensity, duration, and biological context to arrive at a suitable theory to explain it. Epigenetic regulation provides a plausible mechanistic framework because it can regulate the dynamic interaction between environmental exposure and gene expression without altering the DNA sequence[15]. Epigenetic remodeling enables the body to produce transient and sustained biological responses to exercise-related stimuli through mechanisms such as DNA methylation, histone modification, and non-coding RNA-mediated regulation.

A large body of evidence suggests that different exercise modalities lead to different epigenetic alterations in cardiovascular tissues, which may result in adaptive or maladaptive changes (e.g., exercise-related DNA methylation changes in inflammatory regulators after aerobic training[16], while epigenetic instability associated with oxidative stress is observed after ultra-

endurance exercise [17–20]). Additionally, sex-specific biological factors can further modulate epigenetic responses after extreme exercise, especially the differences in DNA methylation stability caused by sex, hormone-dependent chromatin regulation, and exercise-induced non-coding RNA responses. These differences make the possibility of having CVD different in men and women[21].

In this review, we summarize the epigenetic pathways by which different exercise modalities affect cardiovascular health and disease incidence based on existing evidence. By comparing specific epigenetic changes under different exercise and elucidating the sex-specific mechanisms especially existing in ultra-endurance exercise, we believe this can help clarify the positive and negative impacts of various physical activities on cardiovascular health, thus fostering the development of more detailed and personalized exercise strategies. We also hope to promote this approach of using personalized exercise strategies to maintain cardiovascular health.

## 2. Distinct exercise modalities as non-equivalent biological stimuli

Aerobic exercise, resistance training, and ultra-endurance exercise differ significantly in intensity, duration, metabolic demand, and mechanical load, and these three types of exercise also have different effects on the body's physiological state. These differences may lead to significant differences in epigenetic phenomena within the cardiovascular system.

Aerobic exercise is a structured physical activity whose energy supply mainly depends on aerobic metabolism, and usually involves rhythmic, sustained contractions of large muscle groups. Typical forms of aerobic exercise include jogging, running, and cycling[22]. Resistance training is a training modality that enhances muscle strength and endurance by resisting external loads[23], including bodyweight training, resistance band training, and machine resistance training. Compared to the former two, ultra-endurance exercise is longer in duration and greater in intensity. Specifically, ultra-endurance exercise refers to physical activity lasting more than six hours, with an intensity of approximately 50% to 70% of maximum oxygen uptake, and a total energy expenditure typically exceeding 3,000 to 6,000 kcal. Common forms include ultramarathon trail running, triathlon, ultra-long-distance swimming, ultra-endurance cycling, and cross-country skiing[11].As shown in **Table 1**.

**Table 1 Exercise modality classification and representative examples (used in this review)**[11,22,23]

| Exercise | Operational | Typical load | Representative |
|---|---|---|---|

| modality | definition | profile | examples |
|---|---|---|---|
| Aerobic exercise | Rhythmic, continuous large-muscle activity primarily supported by aerobic metabolism | Moderate–moderate-to-vigorous intensity; minutes to hours | Running, cycling, continuous swimming, elliptical training |
| Resistance training | Muscle contractions against external or body-weight resistance to generate mechanical loading | High mechanical load; short, intermittent bouts | Free-weight training, machine resistance training, elastic-band and body-weight exercises |
| Ultra-endurance exercise | Prolonged endurance activity with extremely long duration and very high cumulative workload | Typically >6 h; ~50–70% $VO_2$max | Ultramarathon running, ultra-trail events, triathlon, ultra-distance cycling, long-distance open-water swimming |



A deeper exploration of the epigenetic mechanisms by which these three types of exercise affect individual physiology reveals significant differences between traditional exercise modalities (such as aerobic exercise and resistance training) and ultra-endurance exercise, not only in terms of exercise load but also at the cellular and molecular levels, specifically in the spatiotemporal distribution and metabolic characteristics of cellular stress, energy flux, and redox balance. Aerobic exercise and resistance training usually induce transient and reversible epigenetic modifications which are relevant with adaptive cardiovascular remodeling, thus promoting cardiovascular health. In contrast, ultra-endurance exercise can persistently modulate epigenetic modifications. The influence is so significant that the physiologic body cannot balance by itself, thus causing negative effects. In the following chapters, we will systematically compare specific epigenetic pathways of different exercise, which are crucial for the understanding of exercise's various influence on cardiovascular function.

## 3. Epigenetic architectures linking exercise modality to cardiovascular outcomes

Although epidemiological evidence consistently identifies physical activity as a key protective factor for cardiovascular health [24], accumulating data indicate that cardiovascular adaptation cannot be explained by exercise exposure alone. Instead, distinct exercise modalities engage qualitatively different epigenetic programs, through which metabolic, mechanical, and oxidative cues are translated into sustained gene regulatory changes that shape adaptive or maladaptive cardiovascular remodeling. In the following sections, we compare how aerobic exercise, resistance training, and ultra-endurance exercise influence cardiovascular outcomes by differentially regulating DNA methylation, histone modifications and non-coding RNA networks. As shown in **Table 2**.

**Table 2 Modality-specific epigenetic pathways linking exercise to cardiovascular outcomes**[16–20,25–37]

| Exercise modality | DNA methylation pathways | Histone modification pathways | miRNA-mediated pathways | Main molecular and functional relevance |
|---|---|---|---|---|
| Aerobic exercise | ↑ ASC[a] methylation; ↓ promoter methylation of PGC-1α[b] and PDK4[c] | Exercise-induced BHB[d] leading to HDAC[e] inhibition; activation of cardioprotective transcriptional programs | miR-21/miR-144 → PTEN[f]–PI3K[g]/Akt[h] axis; miR-27a/miR-27b and miR-143 → ACE[i]/ACE2 regulation | Attenuation of inflammatory signalling; enhancement of metabolic and mitochondrial regulation; promotion of physiological cardiac remodelling |
| Resistance training | Demethylation of GHRH[j] and FGF1[k] loci | Increased H3 acetylation and H3K4me1 at Myf6[k] and JUNB[l]; permissive histone marks at SOCS3[m] | miR-21-5p-mediated repression of TSP-1[n] | Skeletal muscle remodelling; restraint of inflammatory signalling; enhancement of vascular repair and homeostasis |
| Ultra-endurance exercise | ROS[o]-driven accumulation of 8-oxodG[p] and interference with DNMT[q] | SUV39H1-mediated H3K9me3 at the SIRT1[r] promoter | miR-125a-5p targeting Ninj1[s]; miR-223-3p targeting ACSL3[t], IGF1R[u] and | Impairment of antioxidant defence; limitation of vascular repair capacity; |

| | | | |
|---|---|---|---|
| | activity | PIK3C2A[v] | activation of myocardial injury-related processes |

a. ASC: apoptosis-associated speck-like protein containing a CARD

b. PGC-1α: peroxisome proliferator–activated receptor γ coactivator 1α

c. PDK4: pyruvate dehydrogenase kinase 4

d. BHB: β-hydroxybutyrate

e. HDAC□histone deacetylase

f. PTEN: phosphatase and tensin homolog

g. PI3K: phosphoinositide 3-kinase

h. Akt: protein kinase B

i. ACE: angiotensin-converting enzyme

j. GHRH: Growth hormone-releasing hormone

k. FGF1: fibroblast growth factor 1

l. JUNB: JunB proto-oncogene

m. SOCS3: suppressor of cytokine signaling 3

n. TSP-1: thrombospondin-1

o. ROS: oxygen species

p. 8-oxodG: 8-oxo-2′-deoxyguanosine

q. DNMT: DNA methyltransferase

r. SIRT1: sirtuin 1

s. Ninj1:Ninjurin1

t. ACSL3: acyl-CoA synthetase long-chain family member 3

u. IGF1R: insulin-like growth factor 1 receptor

v. PIK3C2A: phosphatidylinositol-4-phosphate 3-kinase catalytic subunit type 2 alpha

Before discussing modality-specific epigenetic mechanisms, it is important to clarify the evidentiary framework used in this review. The available literature spans several levels of evidence, including direct human exercise studies, observational studies in athletes or clinical populations, experimental animal models, cell-based mechanistic studies, and extrapolations from cardiovascular disease or oxidative stress models. These evidence types should not be interpreted as equivalent. Findings derived from human exercise interventions provide the most direct support for exercise-responsive epigenetic remodeling, whereas data from ischemia/reperfusion injury, myocardial infarction, oxidative stress, or other disease models are

used here primarily to support biological plausibility and to generate mechanistic hypotheses.

Tissue specificity is another important consideration. Exercise-responsive epigenetic signatures have been reported in skeletal muscle biopsies, circulating leukocytes, plasma or serum miRNAs, endothelial cells, and cardiomyocyte-based experimental systems. However, epigenetic regulation is highly cell-type and tissue dependent. Therefore, methylation changes in skeletal muscle or leukocytes, and circulating miRNA profiles, should not be assumed to directly reflect myocardial chromatin states or cardiomyocyte-specific gene regulation. Instead, these peripheral signatures may indicate systemic metabolic, inflammatory, vascular, or stress-related responses that are relevant to cardiovascular biology but require cautious interpretation when linked to myocardial remodeling or cardiovascular disease risk. Accordingly, where direct exercise-based human evidence is limited, the pathways summarized below should be interpreted as plausible mechanistic links rather than definitive causal chains.

### 3.1 Aerobic exercise establishes cardioprotective epigenetic patterning

#### 3.1.1 DNA methylation influencing inflammatory tone and metabolic control

Aerobic exercise may establish cardioprotective epigenetic adaptations through coordinated regulation of inflammatory signaling and metabolic homeostasis. Evidence from human studies indicates that aerobic exercise is associated with increased DNA methylation of the apoptosis-associated speck-like protein containing a CARD(ASC) gene, accompanied by reduced ASC expression[38]. ASC functions as a central adaptor protein in inflammasome-mediated activation of the pro-inflammatory cytokine interleukin-1β (IL-1β), and its methylation status is inversely related to IL-1β release[39]. Consistent with this association, aerobic exercise may attenuate inflammasome activation and downstream inflammatory signaling. Such anti-inflammatory effects may help reduce chronic inflammatory burden and preserve vascular and myocardial homeostasis, processes implicated in cardiovascular dysfunction. For example, in atherosclerotic vascular disease, the inflammasome–IL-1β axis contributes to plaque formation and progression through the promotion of vascular inflammation, endothelial dysfunction, and pathological vascular remodeling[40].

Aerobic exercise also promotes cardiovascular health through coordinated effects on cellular and systemic metabolism. In human skeletal muscle following acute aerobic exercise, DNA methylation at CpG sites within the promoter region of peroxisome proliferator–activated receptor γ coactivator 1α (PGC-1α) is reduced, accompanied by increased PGC-1α expression[16]. As a master regulator of mitochondrial biogenesis and oxidative metabolism, enhanced PGC-1α activity is linked to improved mitochondrial bioenergetic efficiency and systemic metabolic adaptation with relevance to cardiovascular function. Improved mitochondrial function is therefore

proposed to enhance cellular stress tolerance and functional reserve under conditions of increased metabolic demand. This observation is clinically relevant because the progression of heart failure is typically associated with impaired mitochondrial biogenesis and metabolic disturbances, and aerobic exercise–induced mitochondrial adaptations may partially offset these detrimental changes[41].

In addition to affecting mitochondrial biosynthesis, aerobic exercise can also regulate the epigenetics of key metabolic genes, thereby affecting glucose and lipid metabolism. In human skeletal muscle, DNA methylation levels of the pyruvate dehydrogenase kinase 4 (PDK4) gene decrease and PDK4 expression increases after acute aerobic exercise[16]. Since PDK4 coordinates substrate utilization and insulin sensitivity, exercise-associated epigenetic regulation of PDK4 may enhance metabolic flexibility and support glucose and lipid metabolism[16]. These metabolic adaptations may improve systemic metabolic homeostasis and thereby support vascular health. As shown in **(Fig. 1)**.

**Fig. 1 Aerobic exercise influences cardiovascular health by regulating DNA methylation**

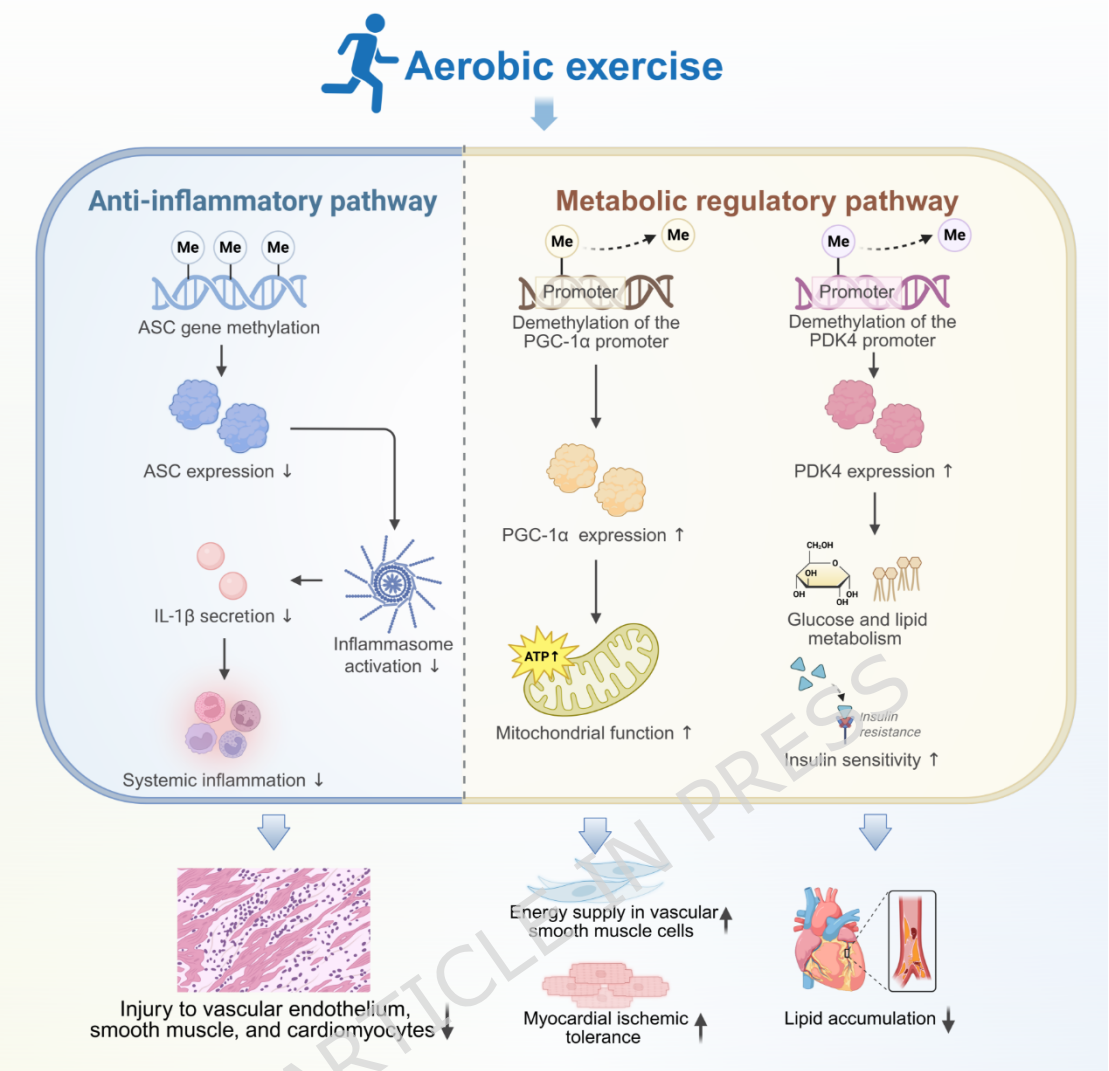



**Fig. 1** Abbreviations: ASC, apoptosis-associated speck-like protein containing a CARD; PDK4, pyruvate dehydrogenase kinase 4; IL-1β, interleukin-1β, a pro-inflammatory cytokine; PGC-1α, peroxisome proliferator–activated receptor γ coactivator 1α.

### 3.1.2 Histone acetylation shaping cardiometabolic transcriptional landscapes

Histone acetylation–dependent transcriptional reprogramming represents a potential epigenetic mechanism through which aerobic exercise may modulate myocardial remodeling, inflammation and cardiometabolic stress responses. Pharmacological inhibition of histone deacetylases (HDACs) confers broad cardioprotective effects by promoting histone acetylation and transcriptional reprogramming[27]. HDAC inhibitors promote the expression of myocardial protection-related genes by relieving histone deacetylase-mediated transcriptional repression. A prime example is the upregulation of the anti-hypertrophic transcription factor Krüppel-like factor 4(KLF4), which may inhibit hypertrophic signaling pathways and myocardial fibrosis

remodeling[27]. This regulatory pathway is particularly relevant to the development of pathological cardiac hypertrophy[42].

In addition, HDAC inhibitors disrupt the repressive complexes between HDAC and transcription factors such as YY1 and Nkx2.5, thereby limiting the activation of pathological gene programs[27]. This transcriptional alteration is accompanied by decreased expression of classic pathological markers, including B-type natriuretic peptide and sodium-calcium exchanger 1, which are associated with progression of heart failure, electrophysiological instability, and impaired contractile function. Collectively, these coordinated transcriptional effects may restrain pathological myocardial hypertrophy, mitigate interstitial fibrosis, and dampen chronic inflammatory signaling, reinforcing cardiovascular protection.

New evidence suggests that aerobic exercise is associated with the increase of hepatic ketogenesis, leading to the increased production of ketone bodies, particularly β-hydroxybutyrate(BHB) and its release in the whole body[25]. According to the pioneering work of Shimazu et al. [26]in cell and animal models, BHB is an endogenous histone deacetylase inhibitor, providing a mechanistic link between metabolic adaptation and epigenetic regulation. Through exercise-induced elevations in circulating BHB, aerobic exercise may converge on HDAC-sensitive epigenetic pathways, conceptually paralleling mechanisms described for pharmacological HDAC inhibition. This epigenetic signaling mechanism driven by metabolites may help explain how aerobic exercise modulates inflammation, metabolism, or myocardial stress response, which are all key factors influencing pathological remodeling and susceptibility to CVD. As shown in **(Fig. 2)**.

**Fig. 2 Aerobic exercise affects cardiovascular health through histone modification.**

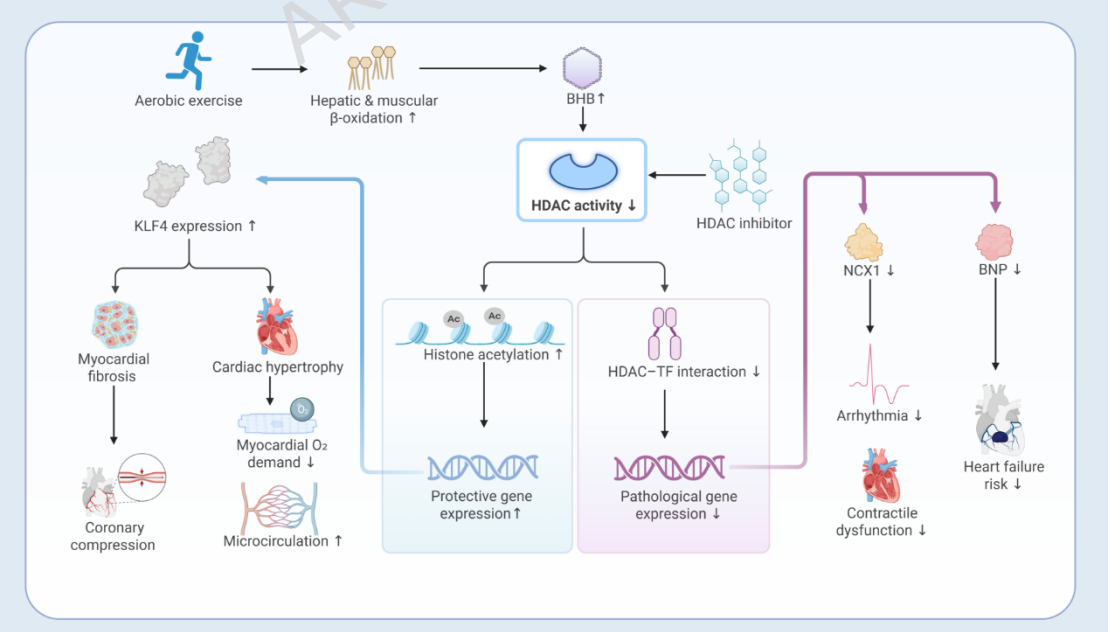


**Fig. 2** Abbreviations: HDAC, histone deacetylase; KLF4, Krüppel-like factor 4; BNP, B-type natriuretic peptide; NCX1, sodium–calcium exchanger 1; BHB, β-hydroxybutyrate.

Fig. 2a, b, and d illustrate cardioprotective mechanisms associated with pharmacological inhibition of histone deacetylases (HDACs). Fig. 2a summarizes key epigenetic effects of HDAC inhibition, including increased histone acetylation, relief of transcriptional repression, and altered HDAC–transcription factor interactions, leading to induction of protective gene expression and suppression of pathological markers. Fig. 2b and d depict representative downstream outcomes, such as reduced myocardial hypertrophy and fibrosis, improved coronary microcirculation, and attenuation of electrophysiological and contractile dysfunction.

Fig. 2c illustrates aerobic exercise–induced production of the endogenous HDAC inhibitor β-hydroxybutyrate (BHB), linking exercise-driven metabolic adaptation to the epigenetic and cardioprotective pathways shown in Fig. 2a, b, and d.

### 3.1.3 Aerobic exercise–encoded microRNA circuits governing physiological cardiac growth

Aerobic exercise is associated with the development of physiological cardiac hypertrophy, a process that has been linked to coordinated changes in microRNA (miRNA) expression[28]. Upregulated in response to aerobic exercise, miR-21 and miR-144 have been shown to act collaborately to target phosphatase and tensin homolog (PTEN). These regulatory relationships have been primarily characterized in animal models of aerobic exercise training and in mechanistic studies of cardiomyocytes, in which suppression of PTEN facilitates sustained activation of the phosphoinositide 3-kinase (PI3K)/ protein kinase B (Akt) signaling pathway. Activation of this pathway is widely recognized as an important regulator of cardiomyocyte survival and adaptive hypertrophic growth, contributing to enhanced myocardial tolerance to ischemic and hypoxic stress. Therefore, through this miRNA-mediated epigenetic regulatory axis, aerobic exercise may promote a form of cardiac remodeling that is functionally adaptive and cardioprotective [29]. For example, following ischemic injury (such as myocardial infarction), cardiomyocyte apoptosis and adverse remodeling are closely associated with the suppression of pro-survival signaling pathways, whereas sustained activation of the PI3K/Akt pathway has been shown to enhance cardiomyocyte survival and limit structural remodeling[43].

In parallel, aerobic exercise has been linked to attenuation of pathological cardiac hypertrophy through coordinated regulation of renin–angiotensin system (RAS)–related miRNAs[28]. Evidence for this regulatory network is derived largely from aerobic training studies in rodents, in which exercise is associated with increased expression of miR-27a and miR-27b, which cooperatively target angiotensin-converting enzyme (ACE), potentially reducing local cardiac angiotensin II (Ang II) availability and restrained activation of Ang II–driven pro-fibrotic and pathological hypertrophic signaling pathways[30]. In addition, aerobic exercise is accompanied by downregulation of miR-143, which may relieve repression of its target gene angiotensin-converting enzyme 2 (ACE2). ACE2 catalyzes the conversion of

Ang II to angiotensin-(1–7), a peptide with well-established cardioprotective properties, thereby further counterbalancing the deleterious actions of Ang II[30]. Through miRNA-mediated rebalancing of the cardiac RAS, aerobic exercise may attenuate vasoconstrictive signaling, limit post-injury fibrotic remodeling, and preserve endothelial function. These effects are particularly relevant in the context of ischemic cardiomyopathy, where excessive local activation of Ang II is recognized as a key driver of myocardial fibrosis and adverse remodeling[44,45].

In summary, aerobic exercise may promote physiological cardiac hypertrophy and limit maladaptive remodeling through coordinated miRNA-mediated regulatory mechanisms, while supporting myocardial and vascular function. Through these integrated molecular and functional adaptive alterations, aerobic exercise may help shape a cardiovascular functional state with greater stress tolerance and lower susceptibility to pathological remodeling. As shown in **(Fig.5)**.

### 3.2 Resistance training reconfigures cardiovascular load through muscle-centred epigenetic control

#### 3.2.1 DNA methylation at growth-factor loci supporting muscle–vascular coupling

Resistance exercise–induced epigenetic adaptations may contribute to cardiovascular health through skeletal muscle–centered remodeling and improved muscle–vascular coupling. In human studies, resistance exercise has been associated with altered DNA methylation at growth-related loci, including growth hormone-releasing hormone (GHRH) and fibroblast growth factor 1 (FGF1), in circulating leukocytes[31]. Because GHRH is involved in growth hormone–dependent anabolic signaling[46] and FGF1 participates in muscle repair, regeneration and tissue remodeling[47], methylation changes at these loci may reflect or support molecular programs related to skeletal muscle adaptation after resistance exercise.

Improved skeletal muscle function provides a plausible peripheral pathway through which these epigenetic adaptations may influence cardiovascular regulation. Resistance training enhances muscle strength and contractile capacity, which can improve the skeletal muscle pump and support venous return, peripheral perfusion and local vascular shear stress. These hemodynamic effects may help reduce peripheral circulatory burden and promote endothelial homeostasis. In parallel, greater skeletal muscle mass and metabolic activity can improve glucose and lipid handling, thereby supporting systemic metabolic homeostasis and potentially reducing cardiovascular risk.

Together, these findings suggest that resistance exercise–associated methylation changes at growth-related loci may support skeletal muscle remodeling and provide a peripheral route linking resistance training to improved muscle–vascular coupling, metabolic homeostasis and

cardiovascular regulation.

### 3.2.2 Histone marks integrating muscle remodelling and inflammatory restraint

Resistance exercise has been associated with coordinated histone modifications at genomic loci critical for skeletal muscle remodeling, including myogenic factor 6 (Myf6), a regulator of muscle development, and JunB proto-oncogene (JUNB), a transcription factor implicated in hypertrophic growth[32]. These epigenetic modifications were identified in skeletal muscle biopsy samples obtained from human participants following resistance exercise interventions. After having resistance exercise, increased histone H3 acetylation has been observed at promoter and downstream regulatory regions of both genes[32], consistent with a more permissive chromatin configuration that facilitates transcription factor access. In parallel, enrichment of H3K4 monomethylation (H3K4me1), a hallmark of transcriptionally primed regulatory elements, further supports recruitment of transcriptional co-activators[32]. The convergence of these epigenetic modifications favors activation of Myf6 and JUNB expression, thereby supporting muscle repair and adaptive hypertrophic responses. By enhancing skeletal muscle function and reinforcing the muscle pump effect, these adaptations may contribute to improved systemic metabolic homeostasis and vascular function, both of which are widely recognized as important intermediates linking peripheral tissue adaptations to cardiovascular pathophysiological processes such as vascular dysfunction and dysregulated blood pressure control.

Besides genes directly involved in skeletal muscle function, resistance exercise–induced histone modifications also extend to regulatory pathways governing inflammatory control. Resistance exercise has been associated with altered histone acetylation and methylation at the locus of suppressor of cytokine signaling 3(SOCS3), a key negative regulator of cytokine signaling. These epigenetic changes at the SOCS3 locus were also observed in human skeletal muscle following resistance exercise[32]. The enrichment of permissive histone marks is consistent with chromatin relaxation and enhanced transcriptional activity, supporting increased expression of the cytokine signaling inhibitor SOCS3[32]. As a core anti-inflammatory regulator, SOCS3 can inhibit the interleukin-6-dependent JAK–STAT signaling pathway, thereby limiting the local inflammatory response after resistance exercise[33]. Although the anti-inflammatory effects of SOCS3 are primarily mediated through the regulation of cytokine signaling, its inhibition of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) pathways is considered an important mechanism in controlling systemic inflammation, and changes in systemic inflammatory status are widely recognized to contribute to multiple cardiovascular pathophysiological processes. Through these epigenetically mediated anti-inflammatory adaptations, resistance exercise may help establish a systemic inflammatory environment

characterized by cytokine signal restriction and improved immune homeostasis, which may be relevant to CVD risk. For example, in conditions such as hypertension and atrial fibrillation, chronic inflammatory activation and dysregulated cytokine signaling are recognized as key drivers of vascular dysfunction, vascular remodeling, and cardiac electrical remodeling. SOCS3-mediated modulation of inflammatory pathways may therefore help constrain pathological processes relevant to disease risk[48–50]. As shown in **(Fig.3)**.

**Fig.3 Resistance exercise influences cardiovascular health through histone modifications**

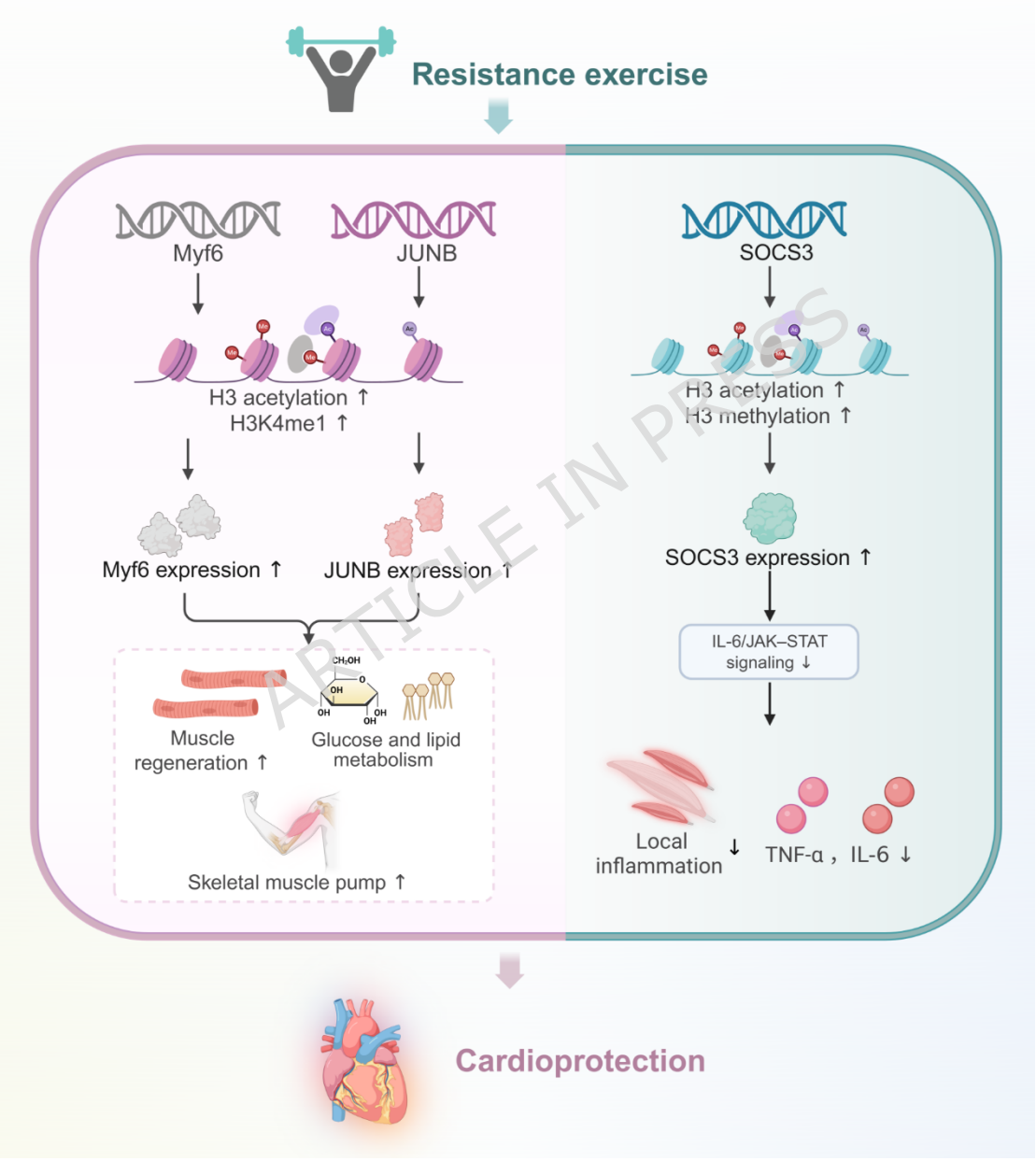



**Fig.3** Abbreviations: Myf6, myogenic factor 6; JUNB, JUNB proto-oncogene; SOCS3, suppressor of cytokine signaling 3; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; H3 acetylation, histone H3 acetylation; H3K4me1, monomethylation of lysine 4 on histone H3.

### 3.2.3 MicroRNA axes governing vascular homeostasis in resistance training

In addition to its effects on skeletal muscle remodeling, resistance training may also engage miRNA-dependent pathways relevant to vascular homeostasis. One of these pathways involves miR-21-5p and its downstream target, thrombospondin-1 (TSP-1), an extracellular matrix protein that is widely involved in vascular injury and maladaptive remodeling. The vascular effects of TSP-1 mentioned above are mainly based on mechanistic studies of endothelial cells and endothelial progenitors, as well as supplemental evidence from animal models. TSP-1 has been implicated in detrimental vascular effects through several mechanisms. First, it represses the proliferation of endothelial cell, as well as migration, and tubular formation by interacting with some surface receptors such as CD47 and CD36. At the same time, it also hinder the recruitment of endothelial progenitors to the sites of vascular injury[51]. These effects may compromise vascular repair capacity and contribute to pathological vascular remodeling characterized by luminal narrowing and structural vascular alterations. Additionally, TSP-1 activates the signaling pathways related to senescence, including the p53-p21 axis, which promotes the senescence of endothelial cell and progenitor cell [52]. Cellular senescence is accompanied by reduced regenerative potential, decreased vascular compliance, and a pro-inflammatory secretory phenotype, characterized by increased production of cytokines such as TNF-α and IL-6, which together may contribute to vascular dysfunction.

Resistance exercise is associated with upregulation of miR-21-5p in human resistance exercise interventions, and miR-21-5p has been shown to suppress TSP-1 expression. Through this miRNA-mediated modulation of TSP-1, resistance exercise may facilitate endothelial progenitor cell–dependent vascular repair and support the preservation of vascular integrity[34,35]. Collectively, regulation of the miR-21-5p–TSP-1 axis may represent a plausible epigenetic mechanism by which resistance exercise supports long-term cardiovascular homeostasis and constrains disease-promoting vascular alterations, particularly those related to endothelial dysfunction and impaired vascular repair, which are central features in vascular pathological remodeling and cardiovascular dysfunction. In the context of diabetic vascular complications, impaired endothelial repair capacity and defective vascular regeneration are recognized as key mechanisms driving vascular dysfunction and the progression of tissue ischemia. Modulation of endothelial repair through the miR-21-5p–TSP-1 axis may therefore be relevant to limiting these pathological changes [53,54]. As shown in **(Fig.5)**.

### 3.3 Limits of epigenetic stability in ultra-endurance exercise

Redox regulation provides a mechanistic bridge between exercise exposure, epigenetic remodeling and cardiovascular outcomes. Nuclear factor erythroid 2–related factor 2 (Nrf2) is a redox-sensitive transcription factor

that coordinates antioxidant and cytoprotective responses[55]. Under basal conditions, Nrf2 is retained by Kelch-like ECH-associated protein 1 (Keap1), which promotes Cullin 3-dependent ubiquitination and proteasomal degradation of Nrf2[56]. During transient oxidative or electrophilic stress, modification of reactive cysteine residues within Keap1 stabilizes Nrf2, allowing its nuclear translocation, interaction with small Maf proteins and binding to antioxidant response elements[57,58]. This activates a coordinated transcriptional program involving antioxidant enzymes, glutathione synthesis and recycling, NADPH regeneration, mitochondrial redox buffering and inflammatory restraint[59].

This pathway helps explain why ROS generated by exercise can have different biological consequences depending on exercise load and duration. During moderate exercise, transient ROS production may act as a hormetic signal that activates Nrf2-dependent antioxidant adaptation rather than causing persistent oxidative injury[60]. In ultra-endurance exercise, prolonged metabolic demand, repeated contraction cycles, fluctuating tissue perfusion and systemic inflammatory activation may impose a greater cumulative redox burden. Human ultramarathon studies have reported increases in lipid peroxidation and oxidative stress biomarkers, although these responses vary with exercise duration, training status, sampling time and biomarker selection[61]. Therefore, ultra-endurance exercise should not be interpreted as uniformly causing irreversible oxidative injury. Rather, in susceptible individuals or under repeated extreme exposure with insufficient recovery, ROS production may exceed or dysregulate Nrf2-mediated buffering capacity. Insufficient redox compensation may then permit accumulation of oxidative DNA lesions. Because the Nrf2/Keap1 axis itself can be regulated by DNA methylation, histone modifications and non-coding RNAs[62], this pathway may represent a bidirectional redox–epigenetic node linking exercise-induced oxidative stress to epigenetic stability or instability.

### 3.3.1 Methylation instability driven by sustained oxidative stress

Ultra-endurance exercise is associated with a significant elevation in systemic reactive oxygen species (ROS) production in studies of ultra-endurance athletes[17,63]. ROS comprise a heterogeneous group of oxygen-derived reactive molecules and free radicals that, when generated in excess, can overwhelm redox homeostasis and induce oxidative damage to cellular macromolecules, including DNA. A hallmark lesion of oxidative DNA damage is the oxidation of guanine residues to form 8-oxo-2′-deoxyguanosine (8-oxodG), which is widely used as a molecular indicator of oxidative stress–induced genomic injury. Under conditions of physiological homeostasis, damaged 8-oxodG bases are selectively recognized and excised by 8-oxoguanine DNA glycosylase (OGG1). Subsequent restoration of DNA integrity is achieved through the base excision repair (BER) pathway, thereby limiting the accumulation of oxidative lesions[18].

In ultra-endurance exercise, the production of excessive ROS may exceed the repair capacity of the endogenous antioxidant system and DNA repair pathways, thereby favoring the accumulation of oxidative DNA lesions such as 8-oxodG. Experiments have shown that the continuous accumulation of 8-oxodG can interfere with local DNA methylation patterns, and its steric hindrance can prevent DNA methyltransferases (DNMTs) from approaching adjacent cytosine residues[19], thereby may leading to local DNA hypomethylation and abnormal activation of genes that are normally repressed in transcription. In addition, cell studies have shown that the binding of OGG1 to 8-oxodG damage can promote the recruitment of DNA demethylase ten-eleven translocation 1 (TET1) through protein-protein interactions, potentially enhancing active demethylation[20]. These mechanisms collectively link oxidative DNA damage to epigenetic modification instability and the dysregulation of DNA methylation profiles under excessive oxidative stress.

In human patients with CVD, altered DNA methylation of pro-inflammatory genes, including TNF-α and ASC, has been demonstrated, and these alterations may contribute to inflammation and impair exercise capacity[39,64]. These changes of DNA methylation may facilitate inflammatory activation by relieving the methylation at key regulatory sites, therefore making these genes shift from a normally state of repression to a less repressed or unrepressed state. Parallelly, excessive oxidative stress has been associated with aberrant downregulation of methylation at the promoter region of the PGC-1α gene, leading to its overactivation. This dysregulation of PGC-1α perturbs the balance between metabolic regulation and inflammatory control, fostering a pro-inflammatory metabolic environment that exacerbates systemic inflammation. Together, oxidative DNA damage–driven disruption of DNA methylation homeostasis provides a mechanistic framework linking excessive ROS exposure to epigenetic instability characterized by aberrant inflammatory activation and metabolic dysregulation. This mechanism also suggests that, in certain myocardial disorders such as dilated cardiomyopathy, abnormal DNA methylation patterns are closely linked to dysregulation of inflammation- and metabolism-related gene expression and myocardial structural remodeling. Under sustained excessive ROS exposure, accumulation of 8-oxodG induced by ultra-endurance exercise–associated exercise, together with its interference with DNMT binding and TET1 recruitment, may drive similar methylation disturbances in susceptible individuals, thereby increasing vulnerability to adverse myocardial remodeling phenotypes[65]. As shown in **(Fig.4)**.

**Fig.4 Ultra-endurance exercise affects cardiovascular health through DNA methylation**

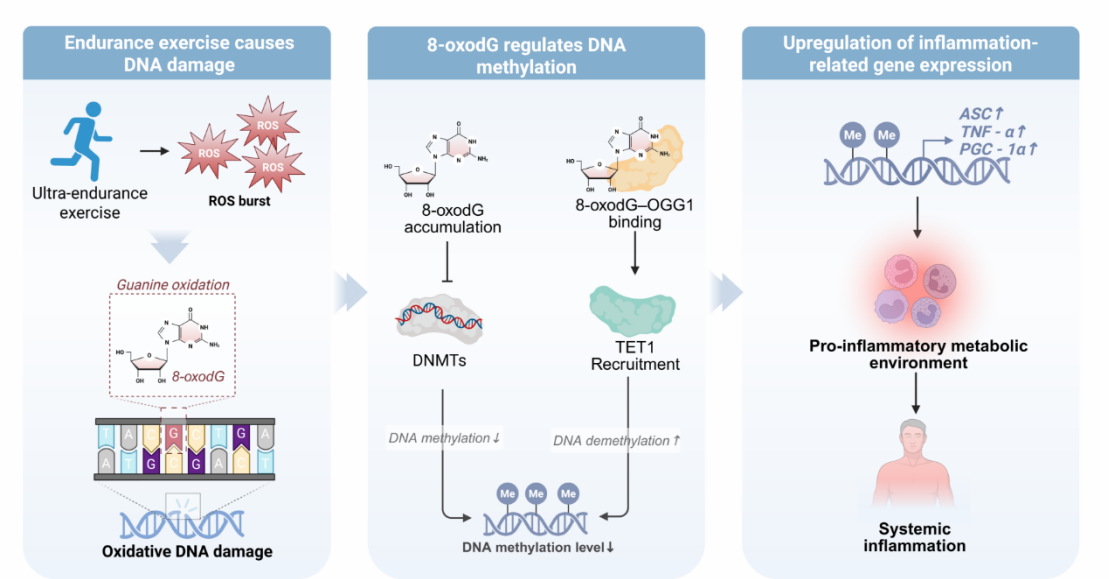



**Fig.4** Abbreviations: ROS, reactive oxygen species; 8-oxodG, 8-oxo-2′-deoxyguanosine; OGG1, 8-oxoguanine DNA glycosylase; BER, base excision repair.

### 3.3.2 Repressive histone methylation undermining antioxidant transcriptional programs

Ultra-endurance exercise is associated with high oxidative stress, and experimental models of cardiac oxidative injury suggest that excessive ROS can disrupt epigenetic regulation, including activation of the histone methyltransferase SUV39H1. Under conditions of excessive oxidative stress, SUV39H1 activation may promote repressive histone modifications that impair cardiomyocyte antioxidant defenses and increase susceptibility to myocardial injury. Experimental evidence from murine and cardiomyocyte-based models demonstrates that oxidative stress markedly enhances SUV39H1 activity, which specifically targets the promoter region of sirtuin 1 (SIRT1), a key antioxidant regulatory gene in the cardiovascular system. In these models, SUV39H1 catalyzes trimethylation of histone H3 at lysine 9 (H3K9me3)[36], a repressive modification that compresses chromatin structure, thereby restricting access of transcription factors and RNA polymerase to the SIRT1 promoter. This may reduce SIRT1 expression, which further impairs the activity of downstream antioxidant enzymes such as SOD1 and SOD2, thereby limiting cardiomyocyte ROS-scavenging capacity.

In cell and animal models, the accumulation of ROS, including superoxide anions ($O_2^-$) and hydrogen peroxide ($H_2O_2$), has been demonstrated to primarily damage cardiomyocyte membranes, structural proteins, and DNA, thus fostering cell necrosis and apoptosis[66]. Furthermore, excessive ROS can disrupt endothelial function, activate the NF-κB inflammatory pathway[67], and promote the release of pro-inflammatory cytokines such as TNF-α and IL-6. These oxidative and inflammatory responses have been consistently demonstrated in both experimental and translational studies.

Mechanistically, these processes are closely associated with cardiomyocyte injury and structural remodeling. When sustained, they may contribute to adverse myocardial remodeling and electrical instability[68]. For example, in arrhythmogenic cardiomyopathy, persistent oxidative stress is considered a key driver of cardiomyocyte injury, fibrofatty replacement, and electrical remodeling. Evidence suggests that disruption of antioxidant regulatory networks and aberrant chromatin modifications further exacerbate structural remodeling and the development of arrhythmogenic phenotypes. Therefore, if sustained oxidative stress during ultra-endurance exercise engages similar SUV39H1–H3K9me3–SIRT1 regulatory pathways, it may weaken antioxidant defense and increase vulnerability to adverse myocardial remodeling and arrhythmias in susceptible individuals[69].

### 3.3.3 MicroRNA shifts modulating vascular repair and myocardial integrity

Multiple studies have shown that miR-125a-5p and miR-223 are significantly upregulated in ultra-endurance athletes, including ultramarathon runners[70]. Although these miRNAs act on different downstream targets, their cardiovascular functions have been characterized mainly in vascular, macrophage, ischemic injury and cell-based models. Therefore, the following miRNA pathways should be interpreted as hypothesis-generating links between ultra-endurance-associated circulating miRNA changes and cardiovascular repair or injury pathways, rather than as established causal mechanisms in athletes. These circulating miRNA changes may reflect systemic vascular, inflammatory or stress-related responses rather than direct myocardial epigenetic regulation.

In mouse models and macrophage-based experimental systems, miR-125a-5p binds to the 3′ untranslated region (3′ UTR) of the adhesion molecule Ninjurin1 (Ninj1) in a dose-dependent manner, directly inhibiting its expression[37]. If exercise-associated increases in miR-125a-5p are accompanied by suppression of Ninj1, mechanisms characterized in macrophage-based models suggest that macrophage adhesion to extracellular matrix components and vascular endothelial cells may be weakened. Such impaired macrophage adhesion could limit macrophage recruitment to sites of vascular injury or inflammation, potentially delaying debris clearance and vascular repair.

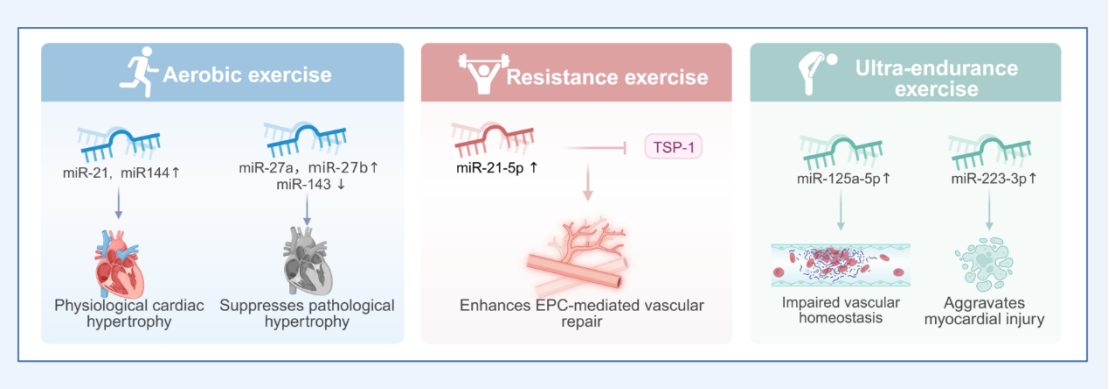
Simultaneously, by downregulating Ninj1 in animal models, experiments have shown that downregulation of Ninj1 can indirectly inhibit pro-angiogenic and anti-inflammatory mediators, including stem cell factor (SCF) and vascular endothelial growth factor receptors 1 and 2 (VEGFR1/2)[37]. Impaired macrophage adhesion and recruitment disrupt the normal transmission of inflammatory signals, while inhibition of SCF and VEGFR signaling further impairs macrophage function and endothelial repair. Experimental vascular disease models support an association between the miR-125a-5p–Ninj1 axis, vascular permeability and delayed repair,

suggesting a plausible link to adverse vascular remodeling.

Ultra-endurance exercise has also been associated with a significant increase in circulating miR-223-3p levels in human endurance athletes[70]. The downstream effects of miR-223-3p have been more extensively characterized in experimental models of myocardial infarction and ischemic cardiac injury than in exercise intervention studies. At the cardiomyocyte level, studies involving mouse models of ischemic cardiac injury, platelet-cardiomyocyte co-culture systems, and cardiomyocyte lines have shown that elevated miR-223-3p levels can directly inhibit acyl-CoA synthetase long-chain family member 3 (ACSL3) expression[71], leading to reduced stearate-phosphatidylcholine synthesis. Such alterations may impair membrane integrity, promote lipid peroxidation and increase susceptibility to cardiomyocyte ferroptosis in ischemic injury models. Excessive ferroptotic signaling may further aggravate myocardial injury in ischemic settings.

Meanwhile, animal and endothelial cell studies suggest that miR-223-3p impairs post-ischemic vascular repair by targeting the insulin-like growth factor 1 receptor (IGF1R)[72], which hinders the migration of endothelial cell and inhibiting the formation of new blood vessels in ischemic cardiac regions. Insufficient perfusion maintains myocardial ischemia and further exacerbates cardiomyocyte loss. Furthermore, a rat model of ischemic cardiac injury showed that miR-223-3p regulates the apoptosis signaling pathway by modulating phosphatidylinositol-4-phosphate 3-kinase catalytic subunit type 2 alpha (PIK3C2A) expression, leading to altered activation of the PI3K/Akt pathway[73]. Under ischemic conditions, altered activation of this pathway may disturb apoptotic balance and contribute to myocardial injury progression. Therefore, these findings support a potential role for miR-223-3p in myocardial injury pathways characterized in experimental ischemic cardiac injury models. However, the exercise-related upregulation of circulating miR-223-3p in humans should be interpreted as a potential biomarker or mechanistic clue rather than direct evidence that ultra-endurance exercise induces these downstream injury pathways. As shown in **(Fig.5)**.

**Fig.5 Exercise influences cardiovascular health by regulating miRNA levels**



## 4. Sex-specific divergence in epigenetic and cardiovascular remodeling under ultra-endurance exercise

### 4.1 Divergent structural, functional and electrical cardiac phenotypes between sexes

Epidemiological and imaging studies in human cohorts indicates that cardiovascular risks following ultra-endurance exercise are higher in men than in women[21,74,75]. With respect to ventricular structural remodeling, men more frequently develop concentric hypertrophy, with left ventricular wall thickness in some individuals approaching thresholds considered pathologically relevant[74,76]. In addition, ventricular dimensional indices and the proportion of individuals meeting diagnostic features consistent with arrhythmogenic right ventricular remodeling are higher in men. By contrast, women predominantly exhibit eccentric, physiologically adaptive hypertrophy without abnormal increases in wall thickness[74]. These sex-specific remodeling patterns are considered markers of differential cardiovascular adaptation and may reflect a greater susceptibility to maladaptive remodeling in men following prolonged ultra-endurance exercise.

Gender differences also affect cardiac function. Men show a slight decrease in both left and right ventricular ejection fractions, more pronounced functional impairment after exercise, and worse myocardial strain indices. In contrast, women generally maintain more stable systolic function and exhibit better strain curves[74,75]. Regarding diastolic function, men show a significant decrease in the E/A ratio and increased passive myocardial stiffness after exercise, while women maintain relatively good diastolic function indices and have lower myocardial stiffness[74]. Such functional alterations are indicative of sex-dependent differences in cardiac reserve and recovery capacity following extreme endurance loading.

Differences in electrical remodeling further distinguish the cardiovascular responses of men and women. Men exhibit a higher prevalence of electrocardiographic abnormalities, including sinus bradycardia, incomplete right bundle branch block, and greater post-exercise QTc prolongation while women more often display normal electrocardiograms, and anterior T-wave inversions are largely considered physiological variants.[74] Consistent with these findings, the incidence of exercise-related sudden death in men is approximately 2.3-fold higher than in women, with arrhythmogenic syndromes representing a major cause and atrial fibrillation occurring more frequently[75]. By comparison, exercise-related sudden death in women is rare, whereas the risk of ischemic cardiac events remains substantially higher in men.[74] As shown in **Table 3**.

**Table 3. Sex-specific cardiovascular adaptations and risks following ultra-endurance exercise**[74]

| Dimension | Male | Female |
|---|---|---|

| | | |
|---|---|---|
| Ventricular remodeling | Predominantly concentric hypertrophy; increased wall thickness; higher prevalence of ARVC-like criteria | Predominantly eccentric hypertrophy; physiological remodeling; no pathological wall thickening |
| Hypertension and myocardial infarction risk | Elevated | Lower |
| Systolic function | Mild reduction in LVEF and RVEF; impaired post-exercise strain | Preserved systolic function; favorable strain profile |
| Diastolic function | Reduced E/A ratio; increased passive stiffness | Preserved E/A ratio; lower myocardial stiffness |
| Electrocardiographic features | Higher incidence of sinus bradycardia, incomplete RBBB, QTc prolongation | Predominantly normal ECG; anterior T-wave inversion as physiological variant |
| Arrhythmia and sudden death | Higher incidence of exercise-related SCD; increased atrial fibrillation risk | Rare exercise-related SCD; lower arrhythmic risk |



### 4.2 Sex-specific differences in epigenetic maintenance under ultra-endurance stress

Sex-specific differences in cardiovascular vulnerability to ultra-endurance exercise may be partly related to distinct epigenetic mechanisms governing cardiovascular homeostasis in men and women. The mechanisms discussed below should be interpreted as biologically plausible pathways rather than definitive explanations for sex differences in ultra-endurance-related cardiovascular risk, because direct evidence linking sex hormone–dependent epigenetic regulation to cardiovascular outcomes in human ultra-endurance cohorts remains limited.

Estrogen (E2) may contribute to the maintenance of DNA methylation homeostasis in women. Upon cellular entry, E2 binds to estrogen receptor alpha (ERα) in the nucleus to form an active E2–ERα complex. Experimental studies in estrogen-responsive cell systems have demonstrated that this complex directly recognizes estrogen response elements (EREs) within the promoter region of the DNA methyltransferase 1 (DNMT1) gene[77], hence enhancing DNMT1 transcription and increasing intracellular DNMT1 protein abundance. As the principal maintenance DNA methyltransferase, DNMT1 restores methylation marks lost during DNA replication[78], preventing progressive hypomethylation and preserving global methylation stability.

Through the E2–ERα–ERE–DNMT1 regulatory axis, E2 may enhance DNMT1 expression and function, potentially supporting DNA methylation stability

and reducing susceptibility to hypomethylation or abnormal methylation fluctuations. Although this regulatory pathway has been primarily characterized in experimental models, it provides a biologically plausible basis for more efficient maintenance or recovery of DNA methylation patterns in women under exercise-related stress, which may contribute to cardiovascular epigenetic resilience. Notably, E2-mediated upregulation of DNMT1 may help preserve methylation maintenance during transient post–ultra-endurance declines in E2 levels, although whether DNMT1 activity remains sufficient to prevent epigenetic instability in this setting requires direct validation.

In contrast, testosterone may modulate DNA methylation homeostasis in men through distinct regulatory pathways. Upon cellular entry, testosterone binds to the androgen receptor (AR), forming a functional testosterone–AR complex that translocates to the nucleus and regulates the expression of AR-responsive miRNAs. Cell-based and molecular studies have shown that AR signaling suppresses the transcription of specific AR-targeted miRNAs, including miR-299-3p and miR-30e[79], whereas reductions in testosterone availability attenuate AR signaling and result in upregulation of these miRNAs.

In cell-based studies, AR-targeted miRNAs have been shown to bind the 3′ UTRs of DNA methyltransferase 3A (DNMT3A) and DNA methyltransferase 3B (DNMT3B)[79], thereby reducing their protein abundance and methyltransferase activity by repressing mRNA translation and/or promoting transcript degradation. DNMT3A and DNMT3B function as the principal de novo DNMTs, responsible for recognizing previously unmethylated CpG sites and establishing new methylation marks. In concert with the maintenance methyltransferase DNMT1, these enzymes are essential for preserving DNA methylation homeostasis. Through coordinated regulation of both gene-specific loci, particularly those involved in metabolic and inflammatory pathways, and broader genomic regions, DNMT3A and DNMT3B contribute to the establishment, propagation, and long-term stability of global methylation patterns.

Testosterone signaling has been shown to suppress the expression of AR-regulated miRNAs, and therefore, decreased testosterone levels are associated with increased abundance of these miRNAs, which may alter the regulation of DNA methylation mechanisms. Evidence shows that serum testosterone levels will temporarily be decreased after ultra-endurance exercise[80]. Based on these cell-based regulatory relationships [79], this exercise-related decrease in testosterone may be associated with reduced DNMT3A and DNMT3B expression or activity, potentially contributing to altered DNA methylation regulation. Such epigenetic changes may increase cardiovascular susceptibility to sustained physiological stress, although direct evidence in ultra-endurance cohorts remains limited.

In addition to DNA methylation, sex hormones can also protect

cardiovascular function through histone modifications. For example, experimental evidence from female mouse models suggests that E2 can enhance BHB production, whereas androgens may have a relatively weaker effect on BHB synthesis [81]. As previously mentioned, BHB, as an endogenous HDAC inhibitor[26], can unblock HDAC-mediated inhibition of cardioprotective genes such as KLF4 and disrupt HDAC-transcription factor complexes, including those formed with YY1 and Nkx2.5. These effects may restrain transcriptional programs associated with myocardial fibrosis and vascular endothelial injury, thereby potentially reducing susceptibility to pathological remodeling and vascular dysfunction. This sex-specific epigenetic regulation may represent one mechanistic layer contributing to the lower observed cardiovascular risk in women after ultra-endurance exercise.

Furthermore, studies have reported that ultra-endurance exercise is associated with selective upregulation of potentially cardioprotective miRNAs in female subjects, including miR-22-3p and miR-100-5p[82]. Specifically, miR-22-3p has been reported to promote myocardial repair and maintain metabolic homeostasis in rodent models of cardiac injury [83], potentially reducing myocardial damage and vascular dysfunction associated with metabolic disorders. In human endothelial cell systems, miR-100-5p has been shown to protect endothelial function under hypoxia–reoxygenation conditions [84], suggesting a potential role in limiting endothelial injury and vascular dysfunction during ischemic stress. This female-specific exercise-associated miRNA response may represent an additional epigenetic regulatory layer underlying sex differences in cardiovascular risk after ultra-endurance exercise. Overall, these sex hormone–dependent and miRNA-mediated pathways should be viewed as hypothesis-generating mechanisms that may contribute to sex-specific cardiovascular responses to ultra-endurance exercise, rather than as established causal explanations.

## 5.Conclusion

Increasing evidence suggests that exercise can induce a series of epigenetic alterations that influence cardiovascular health through molecular, metabolic and inflammatory signaling pathways. Aerobic exercise and resistance training are generally associated with cardiovascular benefits, whereas ultra-endurance exercise may increase cardiovascular vulnerability in susceptible individuals, particularly under repeated extreme exposure or insufficient recovery. Notably, cardiovascular responses to ultra-endurance exercise vary across populations and may differ between men and women, with available evidence suggesting greater susceptibility to adverse cardiovascular remodeling in men.

The biological significance of exercise-induced epigenetic remodeling likely depends on its temporal trajectory, reversibility and cumulative exposure context. Evidence from human skeletal muscle suggests that DNA methylation at exercise-responsive genes can change rapidly after acute

exercise and can also be reshaped by repeated training[16,85], while detraining and retraining studies indicate that some methylation marks may return toward baseline whereas others may persist as an epigenetic memory of prior exercise exposure[86]. These findings suggest that exercise-induced epigenetic changes should be interpreted as dynamic regulatory responses rather than fixed molecular outcomes. In moderate aerobic exercise and resistance training, repeated but recoverable epigenetic responses may contribute to adaptive remodeling, whereas repeated extreme exposure may become maladaptive if these responses fail to resolve, particularly when insufficient recovery prolongs redox and inflammatory stress.

A precise exposure threshold for this transition remains undefined. Large dose-response studies support the overall cardiovascular benefits of leisure-time physical activity, but they do not identify a specific intensity, duration or cumulative exposure level at which adaptive epigenetic remodeling shifts toward maladaptive remodeling[24,87]. Conversely, studies in master or lifelong endurance athletes suggest that high cumulative endurance exposure may be associated with atrial fibrillation, coronary atherosclerotic features or myocardial fibrosis in a subset of individuals, although these findings are heterogeneous and should not be interpreted as uniform harm[88–91]. Oxidative stress responses after endurance exercise also vary according to exercise duration, training status, sampling time and biomarker selection, further complicating efforts to define a universal redox or epigenetic threshold[92–94].

Although research on ultra-endurance exercise is increasing, current findings remain insufficient to support specific practical interventions or evidence-based exposure thresholds. Furthermore, studies vary substantially in exercise intensity, duration and cumulative load, making it difficult for researchers and clinicians to determine which exercise dose, duration or cumulative exposure profile may increase cardiovascular risk.

Moreover, the current evidence mainly comes from cellular systems, animal models, peripheral tissues and small, highly selective athlete cohorts, and lacks large-scale longitudinal human studies. In particular, few studies have simultaneously assessed exercise dose, cumulative lifetime exposure, recovery status, tissue-specific epigenetic profiles and cardiovascular phenotypes within the same individuals. Evidence for temporal dynamics is currently strongest for DNA methylation in human skeletal muscle, whereas comparable longitudinal data for histone modifications, non-coding RNA networks and cardiovascular tissues remain limited. Direct evidence linking recovery intervals to cardiovascular epigenetic remodeling is also sparse; recovery should therefore be considered an important but underexplored modifier rather than an established determinant of persistent epigenetic dysregulation. Future studies should incorporate repeated sampling at baseline, immediately after exercise, during recovery and after long-term training or detraining, together with detailed assessment of training load,

recovery status, tissue source, circulating biomarkers, cardiac imaging and electrophysiological phenotypes. Such designs will be essential to determine whether exercise-induced epigenetic signatures represent transient adaptive responses, persistent molecular memories, causal mediators or secondary correlates of cardiovascular remodeling.

## Conflict of interest

The authors declare that they have no conflict of interest.

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## Author contributions

Yuetong Huang, Ziyu Yang and Zhiyi Chu contributed equally to this work. Yifan Chen and Jun Pu proposed the central concept of the study and designed its overall framework. Yuetong Huang, Ziyu Yang and Zhiyi Chu collected, reviewed, and organized the literature. Zheng Zhang, Xinyue Huang, Chengze Zheng provided suggestions and assisted with manuscript polishing. Yuetong Huang and Ziyu Yang created all illustrations for the article. Jun Pu and Yifan Chen guided and supervised the writing process, reviewed and edited the manuscript, and provided valuable insights and expertise. All authors have reviewed and approved the final version of the article.

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