ORIGINAL ARTICLE

Enhancement of regeneration and early vascular function as measured by SIRT1 activity, endothelial progenitor cell population, and nitric oxide expression following exercise training in adults

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Abstract

Background: Early endothelial dysfunction can begin in young adulthood and is characterized by reduced Nitric Oxide (NO) bioavailability and diminished endothelial regenerative capacity mediated by Endothelial Progenitor Cells (EPCs). Exercise may enhance vascular repair through Sirtuin-1 (SIRT1)–related pathways, but the optimal training intensity to maximize SIRT1 activity, EPC populations, and NO levels remains unclear. *Aim and Objectives:* To evaluate the effects of moderate- and high-intensity exercise on SIRT1 levels, EPC populations, and NO levels in young adults, compared with a control group, and to determine the optimal exercise intensity for vascular regeneration. *Material and Methods:* This interventional study included 45 young adults. The exercise intervention consisted of 24 sessions over eight weeks. Participants were equally assigned to three groups: moderate-intensity exercise (n = 15), high-intensity exercise (n = 15), and a control group that maintained usual daily activities (n = 15). Peripheral Blood Mononuclear Cells (PBMCs) were measured for SIRT1 activity using flow cytometry. EPCs were identified by the double expression of CD41 and CD62E, while NO expression was identified using fluorescence intensity. *Results*: This study showed a significant increase in SIRT1 activity ($p = 0.017$), followed by an increase in EPC percentage ($p < 0.001$) and NO expression ($p < 0.001$) after exercise training prescription. The study also demonstrated that moderate-intensity exercise increased SIRT1 activity, EPC populations, and NO levels compared with other intensity exercises. *Conclusion*: Exercise training improved endothelial regeneration and vascular function by increasing SIRT1 activity, EPC populations, and NO levels, with moderate-intensity being the best at increasing the three parameters.

Keywords: Aerobic exercise, endothelial progenitor cell, nitric oxide, Sirtuin1, vascular function

Introduction

Recent reports from the World Health Organization indicate that cardiovascular disease is the foremost cause of mortality globally, accounting for an estimated 17.9 million deaths each year [1]. This condition is driven by multiple risk factors, including diabetes, dyslipidemia, obesity, smoking, and physical inactivity [2, 3]. These exposures contribute to endothelial dysfunction, a key process in the

development, progression, and prognosis of cardiovascular disease. Modifiable risk factors are frequently present in adults, particularly when physical activity is lacking. Exercise is a practical, low-cost strategy for cardiovascular prevention; accordingly, structured exercise training is recommended to mitigate the harmful effects of these risk factors [4]. Although various clinical explanations indicate the role of exercise in improving vascular health, few studies have explained the molecular mechanisms of blood vessel regeneration and function. In addition, it is not known which exercise intensity best modulates these three parameters in increasing regeneration and vascular function.

Exercise intensity is a key factor in determining the physiological benefits of physical activity and can be categorized into low-, moderate-, and high-intensity, each corresponding to different heart rate zones. Low-intensity exercise, typically 50-60% of maximum heart rate, or ~ 90-110 beats per minute (bpm) for a 30-year-old, includes activities such as walking or gentle yoga, which slightly elevate heart rate and are ideal for beginners or recovery sessions [5]. Moderate-intensity exercise (60-70% of maximum heart rate, ~114-133 bpm for a 30-year-old), such as brisk walking, cycling, or swimming, noticeably increases heart rate and breathing and improves cardiovascular endurance and endothelial function [6]. High-intensity exercise, 70-85%+ of maximum heart rate, ~ 133-162+ bpm for a 30-year-old, including sprinting or High-Intensity Interval Training (HIIT), pushes the heart rate near its maximum, enhancing vascular adaptation, metabolic efficiency, and cardiorespiratory fitness [7]. Although all intensities provide health benefits, the optimal level for improving vascular regeneration and function remains under investigation, as molecular responses may vary. Tailoring exercise intensity based on the heart rate zones can help maximize cardiovascular protection and overall health [8].

This study postulated that Sirtuin-1 (SIRT1) is an early upstream mediator of exercise-induced vascular regeneration and improved vascular function. SIRT1 belongs to the sirtuin family and functions as a Nicotinamide Adenine Dinucleotide (NAD)-dependent histone deacetylase. It regulates metabolic processes in key vascular cell types, including endothelial cells, perivascular adipose tissue, and vascular smooth muscle cells. This mechanism increases glucose metabolism, mitochondrial activity, and the life span of cells. In contrast, low levels of SIRT1 cause failure of the endothelial cell survival mechanism, leading to cell damage and apoptosis, which can induce atherosclerosis [9]. Studies indicate that acute exercise transiently increases SIRT1 levels, particularly in skeletal muscle and endothelial cells, likely due to elevated NAD^+ availability and metabolic stress. Chronic exercise training, especially moderate to high-intensity aerobic and resistance exercise, has been shown to sustain higher SIRT1 concentrations, enhancing mitochondrial biogenesis, endothelial Nitric Oxide Synthase (eNOS) activation, and antioxidant defense [10]. The maintenance of normal endothelial cells also relies on their capacity for regeneration. Endothelial Progenitor Cells (EPCs) are stem/progenitor cells that can differentiate into endothelial cells and contribute to vascular repair by restoring blood flow and facilitating reperfusion in ischemic regions. EPC populations in the blood can indicate an individuals' ability to repair endothelial cells. [11].

Notably, SIRT1 appears to influence Nitric Oxide (NO) metabolism and EPC activity, which are crucial for vascular homeostasis. Endothelial dysfunction occurs when there is an imbalance

between vasodilator and vasoconstrictive factors. NO, a key vasodilator produced by endothelial cells via eNOS, regulates vascular tone, leukocyte migration, and blood coagulation. SIRT1 enhances eNOS activity by deacetylating and stabilizing the enzyme, thereby promoting NO bioavailability. Additionally, SIRT1 may support EPC function, which is vital for endothelial repair and angiogenesis [12]. These exercise-related alterations in SIRT-1 contribute to improved metabolic health, reduced inflammation, and delayed vascular aging, highlighting its potential role in preventing cardiovascular and metabolic diseases [13].

We hypothesized that moderate-intensity exercise would yield the most pronounced improvements in SIRT1 activity, EPC mobilization, and NO bioavailability because this intensity optimally balances metabolic stimulation with oxidative stress modulation. To investigate this, we examined how different exercise intensities affect SIRT1 expression, circulating EPC populations, and NO levels in young adults. By comparing these physiological markers across intensity groups, we aimed to identify the optimal exercise regimen to enhance vascular regeneration and endothelial function. The focus on young adults allowed us to minimize age-related confounders while elucidating the direct effects of exercise intensity on early vascular adaptation mechanisms.

Material and Methods

Ethical Clearance

The Ethics Committee of the Universitas Brawijaya, Faculty of Medicine, issued letter number No. 7168/UN10.F17.10.4/TU/2023 granting ethical approval. The participants signed an informed consent form to indicate their agreement to participate in the study, following the Helsinki Declaration.

Research design

This study employed an experimental pretest –posttest design with a control group to assess differences in SIRT1 activity, EPC percentage, and NO expression at baseline (pretest) and after the intervention (posttest). Changes in SIRT1, EPC, and NO were evaluated following completion of all training sessions over eight weeks in the moderate-intensity and high-intensity exercise groups, as well as in the control group (daily activities). Participants were allocated into three groups (n = 15 per group). This allocation followed the minimum sample size estimation using G*Power (effect size 0.8, $\alpha = 0.05$, three groups), which indicated a minimum of 10 participants per group.

Participants

The population of this research was students aged 19-30 years who live in the student dormitory of the Universitas Brawijaya. Participants were recruited through voluntary participation after they read the announcement of this research project in the dormitory. Eligibility criteria included nonsmoking status, being clinically healthy without medication use, and having normal body mass index (BMI), blood glucose, blood pressure, and cholesterol levels. A total of 45 eligible individuals were recruited, and participants were randomly allocated using a computer-generated randomization procedure to one of three groups: moderate-intensity exercise (n = 15), high-intensity exercise (n = 15), or control (usual daily activities; n = 15). Written informed consent was obtained from all participants, who indicated their willingness to adhere to the study procedures.

Participant age was verified using official student identification.

Non-smoking status was confirmed by salivary cotinine testing using an immuno-chromatographic assay, with concentrations of 0–10 ng/mL classified as non-smokers, and overall health status was checked via university clinic medical records. BMI was calculated as weight/height² (normal 18.5–24.9 kg/m²), glycemic status was assessed using fasting blood glucose (hexokinase method; normal < 5.55 mmol/L) and HbA1c (HPLC; normal 4.0%–5.6%), blood pressure was measured supine using a digital sphygmomanometer (three readings averaged; normal 110–120/70–80 mmHg), and total cholesterol was determined enzymatically (normal < 200 mg/dL).

Procedure

In this study, there were three groups of participants. Control group participants were students who were not given any exercise training. They carried out routine daily activities while studying on campus and living in dormitories without physical exercise or sports. The second and third groups included participants who underwent moderate- and high-intensity exercise, respectively. Exercises were performed for 30 min daily, three times a week for eight weeks. They received aerobic exercise training from a certified trainer and were supervised by sports medicine specialists. The moderate-intensity group performed 100 steps/min. Meanwhile, the high-intensity exercise group did more than 100 steps per minute. The trainer tracked the participant's steps using motion sensors. The participant's heart rates determined the intensity of the exercise. The formula 220 minus age was used to calculate the maximum heart rate. Moderate-intensity exercise was estimated at 65-75% of the maximum heart rate, and high-intensity was 76-96% of the maximum heart rate [14]. The participants used a smartwatch to calculate their maximum heart rate in beats per minute (bpm).

SIRT1 activity, EPC populations, and NO levels measurement: Flow cytometry

A 5 mL blood sample was drawn from the median cubital vein, and Peripheral Blood Mononuclear Cells (PBMCs) were isolated for SIRT1 activity assessment by flow cytometry after incubation with NAD and specific substrates at room temperature. EPC percentage was quantified by flow cytometry (Biology Laboratory, Universitas Brawijaya) using PerCP-conjugated anti-human vWF/CD41 (Bio-Legend; 303720) and PerCP-conjugated anti-human CD62E (BioLegend; 322606) after Ficoll-based PBMCs separation, washing, fixation/staining, and a 20-minute dark incubation before acquisition.

NO expression was quantified using an NO Assay Kit (Abcam Flow Cytometry – Red, ab219934). Whole blood samples were stained with a NO-sensitive red fluorescent dye and incubated at 37°C for 30 min. For experimental samples, 1 mM DA/NONOate in assay buffer was added prior to incubation, whereas control samples were processed without buffer addition. Flow cytometry analysis was performed using a flow cytometer with Ex/Em 610/620 nm excitation. Fluorescence intensity was measured in the PE/red channel emission, with positive events gated based on fluorescence intensity compared to the unstained controls. Data were collected for a minimum of 10,000 cellular events per sample, and the percentage of NO-positive cells was determined from the gated population. The 104 events threshold was established to ensure statistical reliability while maintaining analysis efficiency, as preliminary studies showed that this provided a <5% coefficient of variation between technical replicates.

Data analysis

The Statistical Package for the Social Sciences (SPSS) version 23 was used to analyze the data normality distribution of SIRT1 activity, EPC populations, and NO levels, which were tested using Shapiro-Wilk, with p-values of 0.607, 0.745, and 0.770, respectively. These results indicated that the data were normally distributed. All participants' characteristics were examined by One-way Analysis of Variance (ANOVA), except for sex, which was analyzed using Chi-Square. ANOVA was also used to determine the mean differences in SIRT1 activity, EPC populations, and NO levels during pre-exercise and post-exercise training. Finally, post-hoc analysis was performed using Least Significant Difference (LSD) to determine differences in mean SIRT1 activity, EPC population, and NO levels between groups.

Results

Baseline characteristics of participants

Study participants were assessed for age, sex, cotinine levels, weight, height, BMI, fasting blood glucose, blood pressure, and total cholesterol. All participants resided in the same dormitory facility to ensure similar daily activity patterns. Prior to the exercise intervention, participants commuted primarily by walking. During the study period, participants performed exercises according to their assigned intensity groups. As shown in Table 1, the baseline characteristics were homogeneous across all study groups. While minor age variations existed between the intensity groups (range: 19-30 years), all participants fell within the predetermined age range selected to account for optimal vascular regeneration capacity. This age range was specifically chosen because young adults typically demonstrate robust vascular repair mechanisms, which minimizes age-related variations in endothelial function and allows for a more precise assessment of exercise effects without significant confounding from age-related vascular changes. Table 1 confirms no significant differences in body weight (p = 0.165), BMI (p = 0.075), or other metabolic parameters between the groups, strengthening the validity of our exercise intervention results by reducing the likelihood of observed effects stemming from pre-existing group differences rather than the exercise protocols themselves.

Most participants were females (73.3%), and the number of male and female participants was distributed equally within the intensity groups (p = 0.376). Participants were also screened for a history

Table 1: Baseline characteristics of study participants in each group

Variable	Daily Activities (n=15)		Moderate Intensity (n=15)		High Intensity (n=15)		*p*
	Pre-test	Post-test	Pre-test	Post-test	Pre-test	Post-test	
Age (years)	22.8 ± 1.7	22.8 ± 1.7	24.2 ± 1.5	24.2 ± 1.5	25.0 ± 1.2	25.0 ± 1.2	<0.001* S
Gender (Male/Female)	3/12	3/12	3/12	3/12	6/9	6/9	0.376** NS

Continued...

Weight (kg)	56.8 ± 8.3	56.6 ± 10.5	54.3 ± 11.6	52.8 ± 12.2	58.6 ± 11.2	58.9 ± 12.1	0.165* NS
Height (m)	1.56 ± 0.05	1.56 ± 0.05	1.58 ± 0.05	1.58 ± 0.05	1.61 ± 0.06	1.61 ± 0.06	0.165* NS
Body Mass Index (kg/m²)	24.8 ± 4.2	24.8 ± 4.6	21.3 ± 3.4	21.3 ± 3.6	22.8 ± 3.8	22.8 ± 4.0	0.075* NS
Fasting Blood Glucose (mmol/L)	4.57 ± 0.45	4.57 ± 0.46	4.84 ± 0.51	4.79 ± 0.57	4.76 ± 0.49	4.65 ± 0.53	0.468* NS
Haemoglobin A1c (%)	5.0 ± 0.6	5.0 ± 0.6	4.9 ± 0.5	4.9 ± 0.5	4.8 ± 0.6	4.8 ± 0.6	0.711* NS
Blood Pressure (mmHg) Systolic	113.7 ± 1.8	113.7 ± 2.0	125 ± 2.9	115.6 ± 3.1	126 ± 2.3	114.6 ± 2.7	0.168* NS
Diastolic	80.7 ± 2.2	80.7 ± 2.4	80.2 ± 2.5	80.4 ± 2.7	80.3 ± 2.6	80.4 ± 2.6	0.689* NS
Total Cholesterol (mmol/L)	4.69 ± 0.11	4.74 ± 0.10	4.80 ± 0.10	4.72 ± 0.11	4.71 ± 0.12	4.76 ± 0.12	0.645* NS

S = Significant ($p < 0.05$)
NS = Not significant
**One-Way ANOVA test with the level of significance 5%*

of illnesses such as cardiovascular disease, metabolic disease or chronic disease. There were no significant differences in body weight ($p = 0.165$) and BMI ($p = 0.075$) across the intensity groups. To rule out diseases that could affect the results of this study, fasting blood glucose testing, haemoglobin A1c measurement, blood pressure measurement, and total cholesterol testing were conducted. These parameters were within the normal range before and after exercise training. There were no significant differences between the intensity groups, indicating that the division of the participant groups was homogenous. Statistically significant baseline characteristics between groups were needed to ensure that these factors do not contribute to differences in SIRT1 activity, EPC population, and NO levels in the study.

The mean SIRT1, EPC, and NO levels pre- and post-exercise training are shown in Table 2 and Figure 1-3. The data analysis showed a significant increase in SIRT1 activity in moderate and high exercise intensity groups but a slight decrease (0.5 ± 13.1) in the daily activity group. EPC populations significantly increased after exercise training ($p < 0.001$), showing that moderate-intensity exercise training had the highest average increase in EPC populations. Similar results were also observed for NO levels after the exercise training prescription. This study showed a significant increase in NO levels ($p < 0.001$), with the highest increase

Table 2: Difference in the number of Sirtuin 1 activity, EPC Populations, and NO Levels Measurement pre and post exercise training

Variable	Daily Activities (n=15)		Moderate (n=15)		High (n=15)		*p**	
	Pre-test	Post-test	Pre-test	Post-test	Pre-test	Post-test		
SIRT1 Activity	23.9 ± 5.6	23.4 ± 17.1	10.8 ± 5.8	17.7 ± 7.5	15.5 ± 5.3	21.3 ± 16.8	0.017	S
Δ (Post–Pre)	0.5 ± 13.1[a]		7.9 ± 7.3[b]		5.8 ± 20.3[c]			
EPC Populations	2.7 ± 0.7	3.7 ± 1.9	2.6 ± 1.6	6.4 ± 4.7	1.8 ± 0.9	3.0 ± 1.1	<0.001	S
Δ (Post–Pre)	0.9 ± 2.7[a]		3.9 ± 3.4[b]		1.3 ± 0.9[c]			
NO Levels	2.8 ± 0.5	5.2 ± 2.4	3.3 ± 0.9	8.7 ± 4.8	3.4 ± 2.2	6.3 ± 1.7	<0.001	S
Δ (Post–Pre)	2.4 ± 2.7[a]		5.4 ± 4.1[b]		2.9 ± 2.5[c]			

S = Significant (p < 0.05)
NS = Not significant
**One-Way ANOVA test with the level of significance 5%*

observed in moderate-intensity exercise. Table 2 shows moderate-intensity exercise consistently had more significant positive effects than daily or high-intensity exercise. In addition, the highest average increase in SIRT1 activity (7.9 ± 7.3), EPC populations (3.9 ± 3.4), and NO levels (5.4 ± 4.1) occurred in the moderate-intensity exercise group, indicating an optimal regeneration process and vascular function in the moderate-intensity exercise group.

Discussion

Roles of SIRT1 in improving regeneration and vascular function measured by EPCs and NO after exercise training in adults. Our research indicates that moderate-intensity exercise training significantly enhances SIRT1 activity (7.9 ± 7.3), EPC counts (3.9 ± 3.4), and increases NO levels (5.4 ± 4.1) ($p < 0.001$), outperforming both high-intensity and regular activity regimens. This corroborates previous findings in animal studies which have shown that exercise-induced activation of SIRT1 boosts defense against oxidative stress and extends these observations to human subjects by underscoring the importance of exercise intensity [14, 15]. The superior results in the moderate-intensity group indicate an optimal balance between metabolic activation and the mitigation of oxidative stress, suggesting that overly intense activities, such as high-intensity exercise, could hinder EPCs mobilization (2.1 ± 2.8 vs. 3.9 ± 3.4; p = 0.011) via ROS-mediated pathways [16]. The mechanistic role of SIRT1, particularly through the deacetylation of eNOS, reflected in elevated NO levels (Table 2), is likely central to its vascular benefits, which enhance vasodilation and promote endothelial cell survival. These findings resonate with prior research connecting SIRT1 to the upregulation of antioxidants driven by FOXO3 [17]. However, our study links these pathways to exercise intensity, providing a foundation for

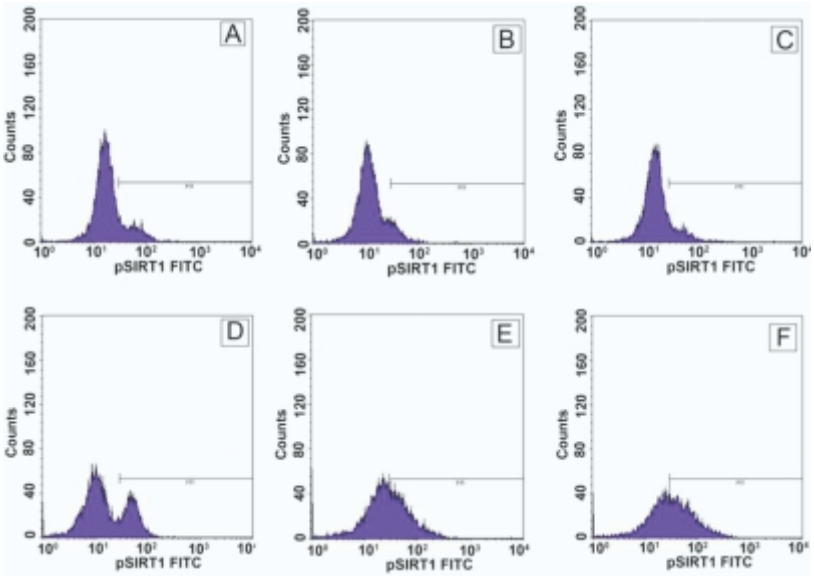

Figure 1: Gate view of Sirtuin 1 activity pre and post exercise training analysed using flow cytometry.

(A) Daily activity group pre-test, (B) moderate-intensity pre-test, (C) high-intensity pre-test, (D) daily activity post-test, (E) moderate-intensity post-test, and (F) high-intensity post-test.

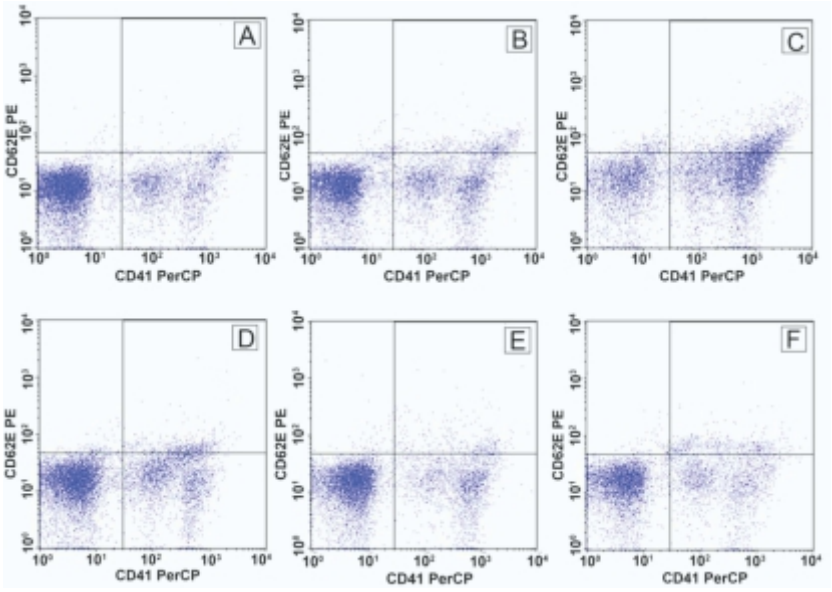

Figure 2: Gate view of endothelial precursor cell population pre and post exercise training analysed using flow cytometry.

(A) Daily activity group pre-test, (B) moderate-intensity pre-test, (C) high-intensity pre-test, (D) daily activity post-test, (E) moderate-intensity post-test, and (F) high-intensity post-test.

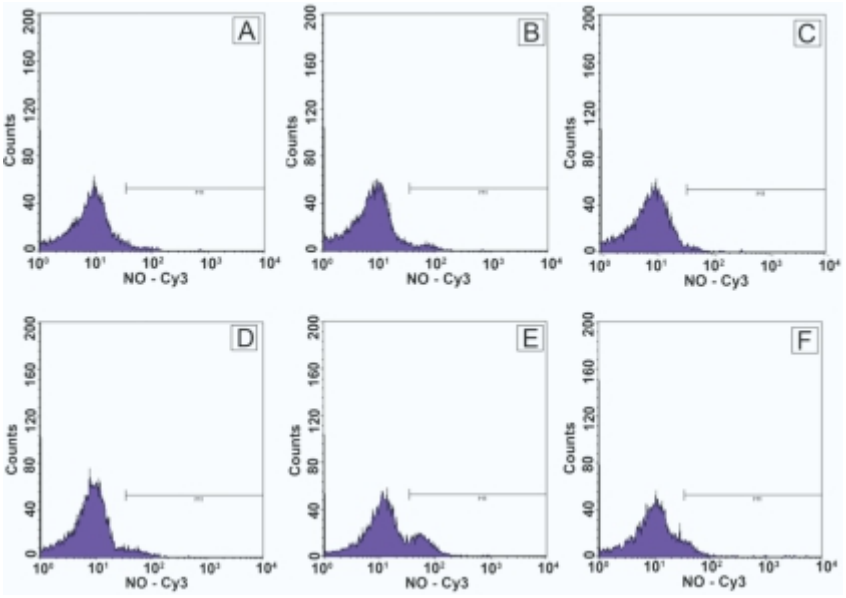

Figure 3: Gate view of nitric oxide levels pre and post exercise training analysed using flow cytometry.

(A) Daily activity group pre-test, (B) moderate-intensity pre-test, (C) high-intensity pre-test, (D) daily activity post-test, (E) moderate-intensity post-test, and (F) high-intensity post-test.

tailoring clinical exercise recommendations. Additionally, the detected SIRT1 activity in PBMCs underscores its systemic importance in vascular adaptation. The association of increased SIRT1 with reduced oxidative stress and lower inflammatory gene expression aligns with its function in deacetylating p53, which inhibits apoptosis and boosting FOXO3-mediated antioxidant responses. Although similar mechanisms have been observed in models of coronary artery disease [18], our study uniquely associated these beneficial effects with exercise-induced improvements in vascular health among healthy young adults. The interaction between SIRT1 and eNOS [19], which is crucial for NO production, may explain the maintained endothelial function in the moderate-intensity group. However, unlike previous studies focusing on aging or disease [35, 39], our results highlight SIRT1's sensitivity to regulated exercise stimuli, emphasizing its viability as a target for primary cardiovascular prevention.

EPC percentage improvement by exercise training

EPCs, originating from the bone marrow, play a pivotal role in vascular repair and prevention of atherosclerosis in response to conditions such as hypoxia, shear stress, and growth factors such as VEGF and SDF-1α [20, 21]. Heiss *et al*. demonstrated that EPC-mediated vascular homeostasis is age-dependent, with significantly reduced EPC survival, migration, and proliferation in older adults (61 ± 2 years) compared with younger individuals (25 ± 1 years) [21]. Vascular regeneration capacity varies based on multiple factors, particularly the extent and site of vascular damage [23]. In adult populations, this regenerative process primarily involves bone marrow-derived progenitor cells, specifically EPCs, which play a crucial role in endothelial repair and maintenance [23].

EPCs progress through distinct developmental stages: early EPCs (E-EPCs) are characterized by the expression of CD34, CD45, and CD31, whereas later stages (L-EPCs) exhibit increased levels of CD41, CD62E, and NO production, which facilitates their direct incorporation into the developing vasculature [24]. This transition from a signalling role to a structural function in endothelial repair is facilitated by L-EPCs promoting angiogenesis via HIF-1-driven pathways [21, 24]. Our research supports these processes, revealing that exercise prompts EPC mobilization ($p < 0.001$), with moderate-intensity exercise showing the most significant rise (3.9 ± 3.4 versus high-intensity: 2.1 ± 2.8; $p = 0.011$), likely due to optimal activation of HIF-1/VEGF without excessive oxidative stress [25].

Exercise is beneficial for neovascularization because it stimulates the synthesis of EPCs and their release from the bone marrow [21, 25]. Moderate-intensity exercise optimally recruits EPCs by balancing metabolic demands and oxidative stability. In contrast, high-intensity routines, such as marathon running, can compromise EPC survival through excessive production of free radicals, thereby reducing their migratory effectiveness [16]. In contrast, routine daily activities did not provide sufficient stimuli for significant EPC responses. These findings on the impact of exercise intensity underscore the need for well-adjusted exercise prescriptions to optimize EPC-mediated vascular repair, especially in individuals prone to endothelial dysfunction.

NO expression improvement by exercise training

Exercise training enhances endothelial function by augmenting shear stress, which stimulates NO production via eNOS activation. Regular exercise also upregulates antioxidant enzymes (e.g., superoxide dismutase), reducing free radicals that degrade NO into peroxynitrite ($ONOO^-$) [26, 27]. This dual mechanism, eNOS stabilization and oxidative stress mitigation, preserves NO bioavailability, as evidenced by our findings: a significant post-intervention NO increase ($p < 0.001$), particularly in the moderate-intensity group (5.4 ± 4.1 vs. other groups, $p = 0.003$). SIRT1 further amplifies this process by deacetylating eNOS [26], linking its activity to NO elevation. However, the excessive intensity may offset benefits through oxidative eNOS uncoupling [26], underscoring the need for balanced exercise regimens that optimize metabolic adaptation without overwhelming redox defense.

Endothelial dysfunction, marked by NO deficiency and proinflammatory factor dominance, drives atherosclerosis and cardiovascular disease [28, 29]. Our study demonstrated that exercise counteracts this by synergistically elevating EPCs and NO, key mediators of vascular repair. EPCs, mobilized by VEGF and SDF-1α during hypoxia [20, 21], integrate into the damaged endothelium, while NO promotes vasodilation and stem cell regulation [30]. Notably, moderate-intensity exercise maximized both EPCs (3.9 ± 3.4) and NO, highlighting its superiority in sustaining vascular health. These adaptations occur independently of traditional risk factors (e.g., lipids and BMI), emphasizing the direct role of exercise in preserving endothelial integrity and preventing cardiovascular pathology.

Limitations of the study

This study had several limitations that should be acknowledged. As a pilot investigation with a modest sample size, the findings may lack sufficient statistical power to detect subtle physiological effects. They may not be fully representative of broader population responses. The exclusive focus on young adults (19–30 years) further limits

generalizability, as age-related changes in vascular biology and the potential presence of comorbidities in older populations could substantially alter the observed relationships between exercise intensity and biomarkers of vascular regeneration (SIRT1, EPCs, and NO).

Methodologically, the absence of a non-exercise control group prevented definitive attribution of observed effects solely to the intervention, as natural biological fluctuations and unmeasured lifestyle factors could contribute to the outcomes. Although baseline anthropometric and metabolic parameters (e.g., BMI and blood glucose) were well balanced, the lack of standardized cardiorespiratory fitness assessments (e.g., VO_2 max) both before and after the intervention represents an important oversight, as baseline fitness levels may modulate individual responses to different exercise intensities.

Furthermore, potential confounding variables, including dietary patterns, sleep quality, and psychosocial stress, were not systematically controlled or measured, any of which could independently influence the biomarkers under investigation. Future research should benefit from larger-scale, longitudinal designs incorporating diverse age cohorts, comprehensive fitness profiling, and stricter control of lifestyle variables to enhance the validity and clinical applicability of the findings.

Conclusion

Moderate-intensity exercise significantly enhanced SIRT1 activity, EPC population, and NO levels, confirming its superiority in promoting vascular regeneration. High-intensity exercise showed lesser gains, likely due to oxidative stress, whereas daily activity had negligible effects. These findings suggest that moderate-intensity exercise is a non-pharmacological strategy for optimizing endothelial health in young adults.

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