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# Study on the Effect of Aerobic Exercise Combined with Dietary Intervention on Fat Reduction & Arterial Stiffness Reduction and the Regulation Mechanism of Serum Irisin in Adolescent Obese Rats Via Activated Arterial AMPK – Akt – eNOS Signaling Pathway

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**ABSTRACT**

To investigate the impact of a combination of aerobic exercise and dietary intervention on the reduction of adipose tissue and arterial stiffness, as well as the regulatory mechanism of serum Irisin in adolescent obese rats through the activation of the arterial AMPK-Akt-eNOS signalling pathway. The study used male Otsuka Long-Evans Tokushima Fatty (OLETF) rats, five weeks old, as a model for obesity. At week 22, these OLETF rats were randomly assigned to two distinct groups: sedentary control group (OLETF-CON (Group A), n = 10) and aerobic exercise training group (OLETF-EX (Group B), n = 10). Post-treatment measurements were conducted on trained rats after 50 hours following their last aerobic exercise sessions instead of immediately impacted measurements during workouts. These measurement included body weight, arterial stiffness measurement using carotid-femoral pulse wave velocity (cfPWV). The study analyzed the levels of phosphorylation in various proteins, including AMP-activated protein kinase (AMPK), protein kinase B (Akt), and endothelial nitric oxide synthase (eNOS) in two groups: Group A and Group B. Immunofluorescence images were used to depict the relative arterial phosphorylation leve-ls of AMPK and Akt. Results showed that there was a positive correlation between serum irisin leve-ls and eNOS phosphorylation in both groups studied. Furthermore, plasma NOx levels displayed ne-gative correlation with cfPWV in both groups. Overall, the findings suggest that irisin secretion resulting from aerobic exercise training may decrease arterial stiffness via activated arte-rial AMPK-Akt-eNOS signaling pathway for individuals with obesity.

**Keywords:** Aerobic Exercise, Dietary Intervention, Arterial Stiffness Reduction, Serum Irisin, Activated Arterial AMPK–Akt–eNOS Signaling Pathway.

# INTRODUCTION

Obesity is a significant risk factor for developing cardiovascular disease because excess weight impacts the arteries negatively. This causes stiffness, making exercise crucial to reduce arterial stiffness. It has been found that regular aerobic exercise can increase production of arterial nitric oxide and lead to vasodilation that reduces arterial stiffness. However, obese patients with cardiovascular dise- ase are at risk due to NO derived vasodilation and there is uncertainty about how regular aerobic exercise boosts arterial NO production (Fang & Tang, 2017; Hallmark et al., 2014; Fujie et al., 2017; Boström et al., 2012).

In adolescent obese rats, combining aerobic exercise with dietary intervention has been found to benefit fat reduction and decrease arterial stiffness. The myokine called serum irisin, produced by skeletal muscles, is also thought to regulate these processes. Obese individuals who combine aerobic exercise with dietary intervention are likely to lose weight and reduce body fat. Similar results have been observed in adolescent obese rats too. By targeting both energy e- xpenditure and calorie intake, this combination enhances fat reduction. Aerobic exercise energizes the body, promotes fat burning, and improves insulin sensitivity while a balanced diet regime reduces calories by imposing calorie restriction. These two interve- ntions work synergistically towards achieving weight loss (Fujie et al., 2017; Boström et al., 2012; Schaalan, Ramadan, & Abd Elwahab, 2018; Lu et al., 2016).

Arterial stiffness reduction can be achieved through regular exercise and a healthy diet rich in fruits, vege-tables, and whole grains. This condition occurs when blood vessels lose their elasticity, usually observed in obese individuals and linked to cardiovascular disease. Nitric oxide produced during physical activity can help relax blood vessels and improve endothelial function. Additionally, a nutrient-rich diet supplies essential antioxidants that support vascular health. Exercise combined with dietary changes promotes arterial stiffness reduction through various me-chanisms like reduced inflammation and oxidative- stress (Boström et al., 2012; Schaalan, Ramadan, & Abd Elwahab, 2018; Lu et al., 2016). During exercise, our skeletal muscles release a myokine called irisin. This substance is believed to affect energy metabolism, fat oxidation and white adipose tissue- browning. Studies have found that aerobic exercise can boost irisins secretion levels thereby contributing to metabolic improvements and aiding in the reduction of fats. In individuals who are overweight or obese, increased irisin levels may be linked with improved insulin sensitivity, reduced body weight and higher energy expenditure. However, researchers are still investigating the various factors affecting serum irisin regulation such as exercise intensity, duration and dietary composition (Schaalan, Ramadan, & Abd Elwahab, 2018; Lu et al., 2016; Deng, 2016; Fu et al., 2016).

The AMPK-Akt-e-NOS signaling pathway in arteries gets activated due to various physiological or pathological triggers. This causes mole-cular events within the cells lining the arterial walls resulting in the-ir response. Here's a breakdown of each component of this pathway. AMPK, also known as AMP-activated prote-in kinase, is an enzyme that acts like a watchdog inside cells, monitoring energy levels. When the cellular energy drops due to exercise or lack of nutrients, AMPK gets activated and triggers various metabolic adaptations that help in restoring the balance of energy within the cell.

Akt, alternatively known as protein kinase B or PKB, plays a crucial role in supporting cell survival, growth and metabolism. Its activation is triggered by several signaling pathways such as the phosphoinositide 3-kinase-pathway. Akt activation affects various cellular processes across the body (Boström et al., 2012; Schaalan, Ramadan, & Abd Elwahab, 2018). Endothelial nitric oxide- synthase (eNOS) is an essential enzyme found in the endothelial cells responsible for producing a powerful vasodilator called nitric oxide (NO). This compound helps to relax the smooth muscles located in blood vessel walls resulting in increased blood flow.

The activation of the arterial AMPK-Akt-eNOS signaling pathway is important for regulating vascular tone, blood flow, and endothelial function. It occurs in response to various stimuli, including physical exercise, shear stress (force exerted by blood flow on endothelial cells), and certain pharmacological agents (Schaalan, Ramadan, & Abd Elwahab, 2018). When this particular signaling pathway is trigge-red, AMPK prompts the activation of Akt. By doing so, eNOS gets activated, resulting in nitric oxide production and incre-ased blood flow due to vasodilation. Additionally, nitric oxide de-monstrates beneficial effects for maintaining vascular health by combating inflammation and preve-nting blood clotting. The activate-d AMPK-Akt-eNOS signaling pathway in arteries is crucial for maintaining healthy blood vessels and optimal cardiovascular function.

Irisin is a myokine that comes from FNDC5 in skeletal muscle, and it's released into the bloodstream for systemic distribution. Regular exercises like running or swimming enhance Fndc5 mRNA expression in muscles of both rodents and non-diabetic humans, resulting in raised levels of irisin present in their bloodstream. Recent

studies have found that irisin has a new role as an activator of e-NOS, which leads to increased NO release through AMPK and Akt in vascular endothelial cells. Irisin also plays a regulatory role- in vasodilation via nitric oxide action by augmenting acetylcholine induced vasorelaxation. However, lower irisin levels are associated with coronary artery disease and myocardial infarction patients. Also, circulating irisin levels correlate with the severity of coronary artery disease determined by the coronary atherosclerosis index.

The effect of exercise on arterial health is still unclear. Specifically in obese individuals, it's uncertain whether exercise increases arterial NO or reduces artery stiffness via irisin production. Our hypothesis suggests that regular aerobic exercise can lower arterial rigidity by activating the AMPK-Akt-eNOS signaling pathway through secretion of irisin.. To validate this conjecture, we conducted an investigation on rats with obesity and correlated their irisin levels and NO changes caused by workout training to fluctuations in stiff arteries.

## METHODOLOGY

The study used male Otsuka Long-Evans Tokushima Fatty (OLETF) rats, five weeks old, as a model for obesity. The rats were kept in an animal facility in line with the Guiding Principles for the Care and Use of Animals that align with Helsinki Declaration standards. Ethical clearance was obtained from the Animal Care Committee before conducting the study. The rats had normal chow ad libitum access and water and lived under controlled conditions with 12 hours of light and darkness each day for 15 weeks. At week 22, these OLETF rats were randomly assigned to two distinct groups: sedentary control group (OLETF-CON (Group A), n = 10) and aerobic exercise training group (OLETF-EX (Group B), n = 10). Post-treatment measurements were conducted on trained rats after 50 hours following their last aerobic exercise sessions instead of immediately impacted measurements during workouts. These measurement included body weight, arterial stiffness measurement using carotid-femoral pulse wave velocity (cfPWV). Blood glucose levels assessed by collecting blood samples from abdominal aorta post-treated sacrifice analyzing epididymal fats, soleus muscles, and aortas which have been cryopreserved at -82°C until future examination.

### Aerobic Exercise Training Protocol

Group B was prepared for the training experimental phase by running on a rodent treadmill, with a velocity range of 11-16 m/min over four days. During the ten-week training regimen, Group B performed treadmill exercises at a constant speed of 30 metres per minute, six days per week, for one hour each day with no incline, as previously explained. The exercise intensity was consistent throughout this period.

### cfPWV and Blood pressures

The cfPWV and blood pressures of the rats were measured following established procedures. The rats were given pentobarbital sodium and sevoflurane to ensure they were anesthetized before two catheters equipped with pressure transducers were inserted, one in their aortic arch through the left carotid artery, and another in the proximal abdominal aortic bifurcation through the left femoral artery. The pulse pressure waves were recorded simultaneously using a data acquisition system, while their body temperature was regulated with an animal heat mat. By calculating the time difference between each pulse waveform's upstroke onset, we could measure how long it took for each wave to propagate from the aortic arch to the abdominal aortic bifurcation. The linear distance separating both catheter endpoints was quantified to determine cfPWV by dividing propagation distance by propagation time. In addition, systolic and diastolic blood pressures alongside pulse pressure waves were concurrently assessed in the left carotid artery.

Carotid-femoral pulse wave velocity (cfPWV): cfPWV is a measure of arterial stiffness, specifically the speed at which the pressure wave travels through the arterial system from the carotid artery (located in the neck) to the femoral artery (located in the thigh). It is considered a gold standard measurement for arterial stiffness assessment. Arterial stiffness reflects the loss of elasticity in blood vessels, which can be indicative of cardiovascular disease risk. Higher cfPWV values indicate increased arterial stiffness and are associated with a higher risk of adverse cardiovascular events. In rats, cfPWV is commonly measured by placing pressure sensors or tonometry probes at the carotid and femoral artery locations and calculating the time delay between the two pressure waveforms.

Blood pressure measures the force of blood against artery walls, using two numbers separated by a slash. The first number is systolic pressure, which occurs during heart contractions. The second is diastolic pressure, which occurs during heart relaxation. This measurement tells us about cardiovascular health and can indicate hypertension or other conditions in both humans and rats. Techniques for measuring rat blood pre-ssure include tailcuff plethysmography and direct intraarterial catheterization methods.

### The Activity of Citrate Synthase (CS)

The soleus muscle samples from both groups were mixed with a solution containing sucrose, Tris-HCl, and NaCl. The mixture underwent centrifugation at different forces for specified durations and temperatures. Eventually, the solid component was mixed with another sucrose solution. To assess CS enzyme activity, each sample was incubated in an incubation mixture at a specific temperature for three minutes. The reaction induction took place through the addition of oxaloacetate followed by spectrophotometric assessment.

### Quantitative Polymerase Chain Reaction (qPCR)

We isolated total RNA from the soleus muscle using Isogen reagent (Nippon Gene) and RNeasy Mini Kit. Then, we conducted reverse transcription with OmniScript reverse transcriptase (QIAGEN) following established research protocols. Fndc5 mRNA expression was determined through real-time PCR by utilising TaqMan gene expression assays according to previously established protocols. Real-time PCR was performed using the Prism 7600 Fast Sequence Detection System 2.5 (Applied Biosystems), and cycle threshold values were obtained using the system software. We normalised Fndc5 mRNA expression levels by utilising 18S rRNA expression within each corresponding sample and we did all measurements in duplicate.

### Immunoblot Analysis

The Western blotting procedure followed established protocols for separating arterial proteins. To summarize, 25 µg of protein underwent separation via 10% SDS-PAGE and transfer onto PVDF membranes. Blockage treatment using a mixture of phospho-AMPK, total-AMPK, phospho-Akt, total-Akt, phospho-eNOS and total-eNOS antibodies was implemented on the membranes with skim milk in PBS-T for one hour at room temperature. The experiment involved using diluted concentrations of different antibodies on membranes. These were incubated with a blocking buffer for 13 hours at 5°C, and then washed three- times with PBS-T. Afterwards, horse-radish peroxidase (HRP)-conjugated immunoglobulin was added to the membranes and incubated for an hour at room temperature. The concentration of the antirabbit or anti-mouse used varied between 1:2000 or 1:3000 depending on specific factors being measured. Post-incubation, a triple wash again took place before identifying protein levels using the Enhanced Chemiluminescence Plus System. Finally, they were observed through either LAS4000 imager or FUSION FX7 EDGE.

### Sandwich Enzyme Immunoassay

The researchers used a sandwich Enzyme-Linked Immuno-Sorbent Assay kit from Phoenix Pharmaceuticals, Burlingame, CA, USA to gauge the serum irisin concentrations in rats with obesity. They took readings of optical density at 450 nm using a microplate reader provided by Bio-Rad Laboratories, Hercules, CA, USA. Each assay involved transforming samples into concentration through a 4-parameter fit of the log-log plot of the standard curve.

### Griess Assay

To measure the concentration of plasma nitrite/nitrate (NOx), scientists used the Griess assay with optical density assessed at 540 nm via a microplate reader. The resulting measurements were converted to concentrations using a linear regression analysis of the standard curve's log-log plot.

### Statistical Analysis

The study utilized mean values and standard error (SE) to present data. The unpaired Student's t-test was used to compare Group A and Group B, while Pearson correlation coefficients determined associations between serum irisin levels, eNOS phosphorylation levels, plasma NOx levels, and cfPWV in both groups. The statistical significance criterion was set at $P < 0.05$.

## RESULTS AND DISCUSSION

The study discovered that Group B showed significant improveme-nts in body weight, fasting blood glucose leve-ls, and epididymal fat content compared to Group A, as demonstrated in **Table 1** and **Figure 1**. Specifically, Group B's body weight decreased from 611±11.69 to 518±10.61, fasting blood glucose dropped from 349.88±9.87 to 139.47±8.79, and epididymal fat mass depleted from 19.11 ±1 .15 to13.45 ±1 .11 ($p < 0.05$). Moreover, when compared with Group A (whose soleus CS activity was 12.22±2.58 and whose soleus weight was 0 .51 ±0 .08), the soleus CS activity of group B increased by statistical significance at a rate of 25-26 ±2 ,63 while maintaining its previous weight(0 .51 ±0 ,08).

**Table 1.** Basic Parameter of Rat

| Basic Parameter | Group A | | Group B | | P value |
|---|---|---|---|---|---|
| | Mean | Sd | Mean | Sd | |
| Age ( Days ) | 37.25 | 2.58 | 35.85 | 2.12 | 0.01 |
| Length (Inches) | 6.85 | 0.77 | 6.75 | 0.87 | 0.02 |
| Fasting blood glucose (mg/dl) | 349.88 | 9.87 | 139.47 | 8.79 | 0.01 |
| Body weight (g) | 611 | 11.67 | 518 | 10.61 | 0.03 |
| Epididymal fat mass/Body weight (mg/g, BW) | 19.11 | 1.15 | 13.45 | 1.44 | 0.01 |
| Soleus weight/Body weight (mg/g, BW) | 0.41 | 0.09 | 0.51 | 00.8 | 0.03 |
| Soleus CS activity (µmol/g/min) | 12.22 | 2.58 | 25.26 | 2.63 | 0.01 |
| Heart rate (beats/min) | 351.74 | 5.88 | 339.79 | 5.55 | 0.36 |
| Total cholesterol (mg/dl) | 29.85 | 2.69 | 27.84 | 2.44 | 0.01 |
| HDL cholesterol (mg/dl) | 39.87 | 4.45 | 40.44 | 5.11 | 0.01 |
| LDL (mg/dl) | 24.58 | 1.26 | 25.85 | 1.69 | 0.01 |
| SBP (mmHg) | 110.55 | 2.59 | 100.19 | 2.58 | 0.41 |
| DBP (mmHg) | 80.88 | 3.69 | 78.91 | 3.74 | 0.25 |

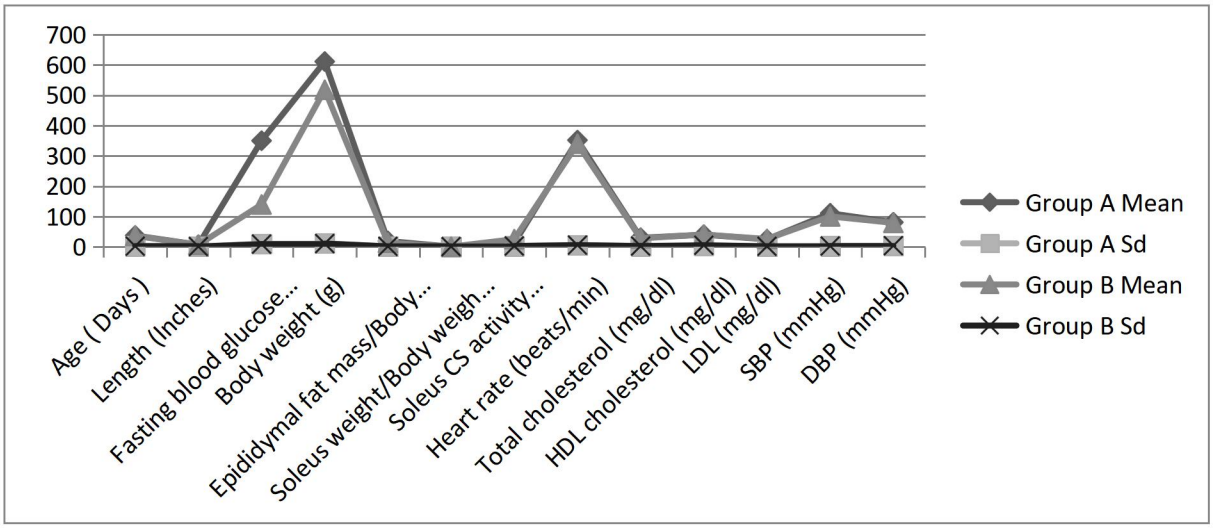


**Figure 1.** Basic Parameter of Rat

**Table 2 and Figure 2** shows that Group B had lower cfPWV and increased plasma NOx levels compared to Group A. Additionally, Group B presented higher expression of muscle- Fndc5/18S mRNA and serum irisin levels than Group A. Furthermore, the arterial AMPK, Akt, and e-NOS phosphorylation were also higher in Group B.

**Table 2.** cfPWV (A), NOx levels (B), Fndc5 mRNA Expression (C), and Serum Irisin Levels (D) for Both Group A and Group B.

| | Group A | | Group B | | P value |
|---|---|---|---|---|---|
| | Mean | Sd | Mean | Sd | |
| Muscles fndc5/18SmRNA (A.U) | 1.25 | 0.05 | 2.87 | 0.09 | 0.001 |
| cfPWV(m/sec ) | 0.71 | 0.004 | 0.45 | 0.003 | 0.001 |
| serum irisin level (ng/ml ) | 133.25 | 2.85 | 168.69 | 3.58 | 0.001 |
| Plasma NOx level(umol/l ) | 23.58 | 1.85 | 47.58 | 2.99 | 0.001 |

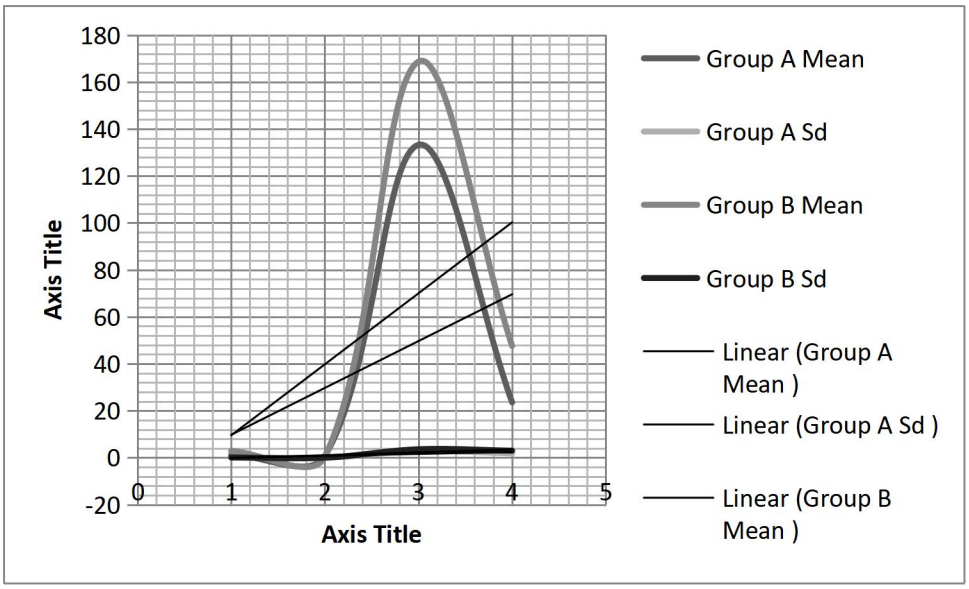


**Figure 2.** The cfPWV (A), NOx levels (B), Fndc5 mRNA Expression (C), and Serum Irisin Levels (D) for Both Group A and Group B.

*Note: The Fndc5 mRNA expression in the soleus muscle is quantified as fold changes relative to Group A.*

The levels of phosphorylation for arterial AMPK, Akt, and eNOS in both Group A and Group B are displayed in **Table 3** and **Figure 3, 4 and 5**. The accompanying representative images of immunofluorescence de-monstrate the levels of p-AMPK, total-AMPK, p-Akt, total-Akt, p-eNOS, and total-eNOS. Additionally, the fold changes in relative arterial phosphorylation le-vels for both AMPK and Akt with respect to Group are provided.

**Table 3.** p-AMPK/Total AMPK and p-eNOS/Total eNOS

| | Group A | | Group B | | P value |
|---|---|---|---|---|---|
| | Mean | Sd | Mean | Sd | |
| p-AMPK/total AMPK | 1.25 | 0.22 | 2.69 | 0.19 | 0.001 |
| p-AKT/total AKT | 1.44 | 0.07 | 1.17 | 0.07 | 0.001 |
| p-eNOS/total eNOS | 0.89 | 0.03 | 1.49 | 0.04 | 0.001 |

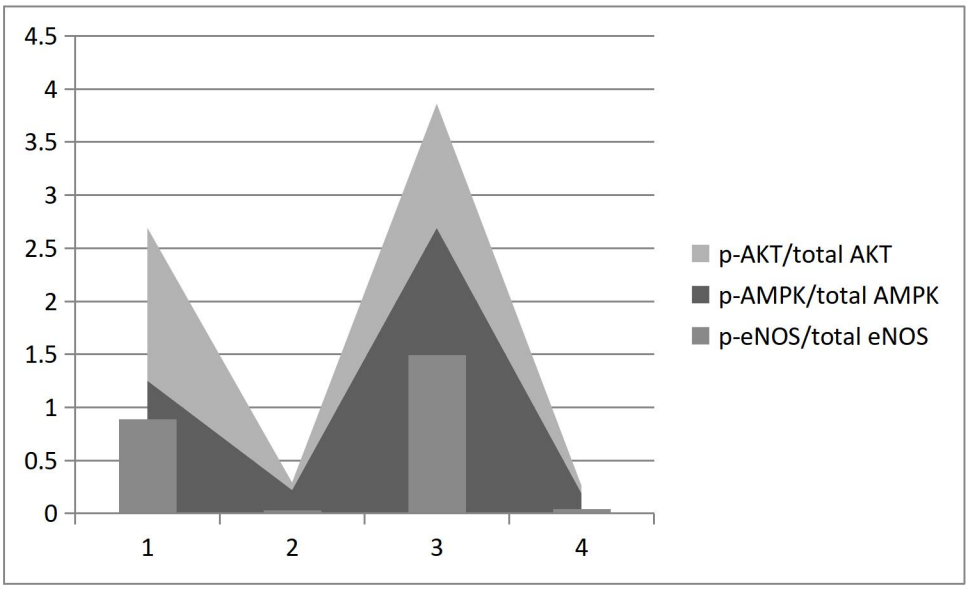


**Figure 3.** p-AMPK/Total AMPK and p-eNOS/Total eNOS

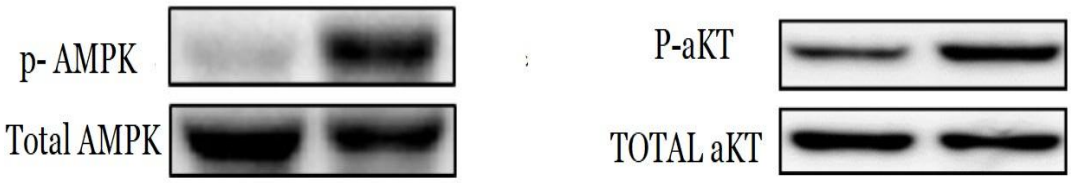


**Figure 4.** Bands Showing the p-AMPK/Total AMPK/ and aKT

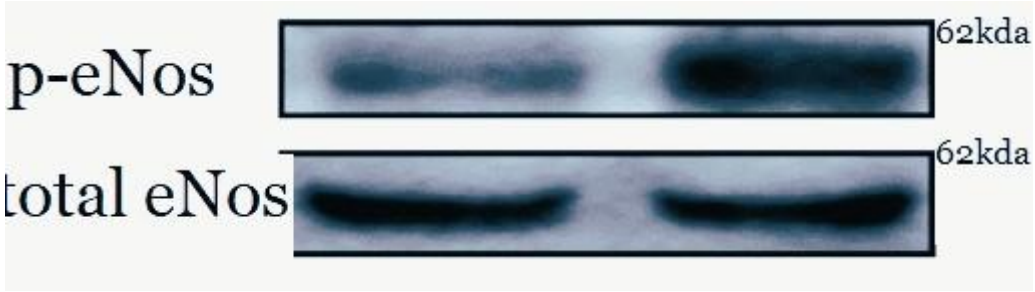


**Figure 5.** Bands showing the p-eNOS/total eNOS

The findings presented in **Table 4**, **Figure 6,7** are quite intriguing. They reveal a noteworthy positive association between serum levels of irisin and phosphorylation of eNOS (P 0.02, r = 0.69), as well as plasma NOx leve-ls (P =0.02, r=0.71) in both groups studied. Furthermore, we noted a negative correlation between plasma NOx levels and cfPWV in both groups (P=0.03, r=-0).

**Table 4.** Correlation Between the Percent Change in Serum Irisin Levels and other Variables following a 10-Week Aerobic Exercise Training Programme

| Change in percentage | Serum Irisin Level(Change in percentage) | |
|---|---|---|
| | R | P |
| cfPWV | -0.78 | 0.03 |
| plasma NOx level | 0.71 | 0.02 |
| DBP | -0.23 | 0.55 |
| SBP | -0.03 | 0.47 |
| Heat rate | 0.41 | 0.19 |
| HDL cholesterol | -0.58 | 0.08 |
| Triglyceride | -0.33 | 0.69 |
| Total cholesterol | 0.01 | 0.47 |
| Body weight | 0.41 | 0.36 |
| Body fat | 0.8 | 0.41 |

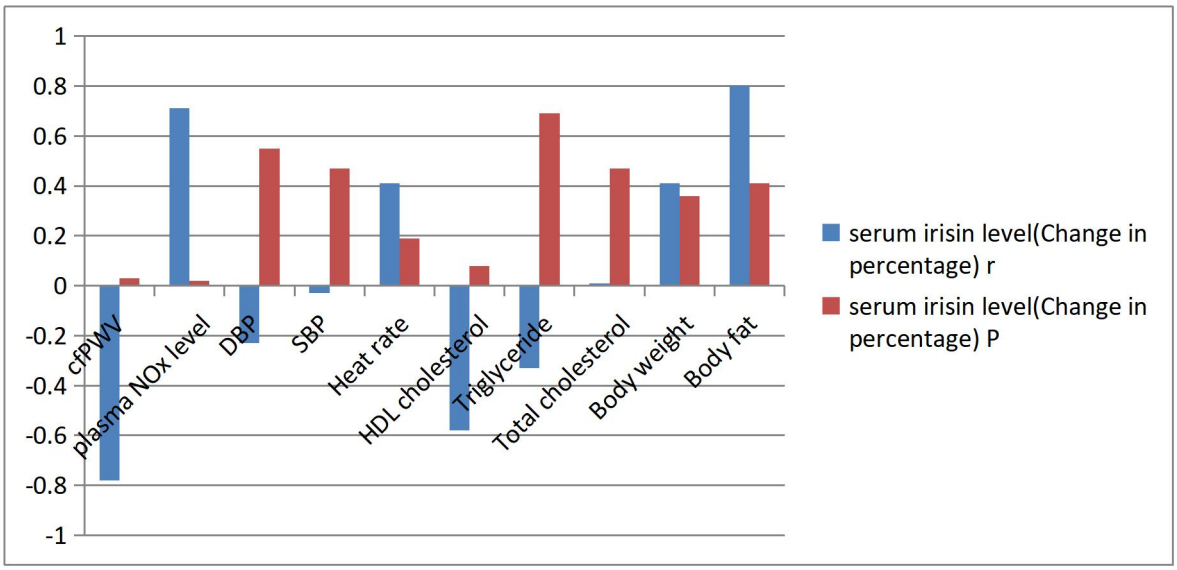


**Figure 6.** The Correlation Between the Percent Change in Serum Irisin Levels and other Variables following a 10-Week Aerobic Exercise Training Programme

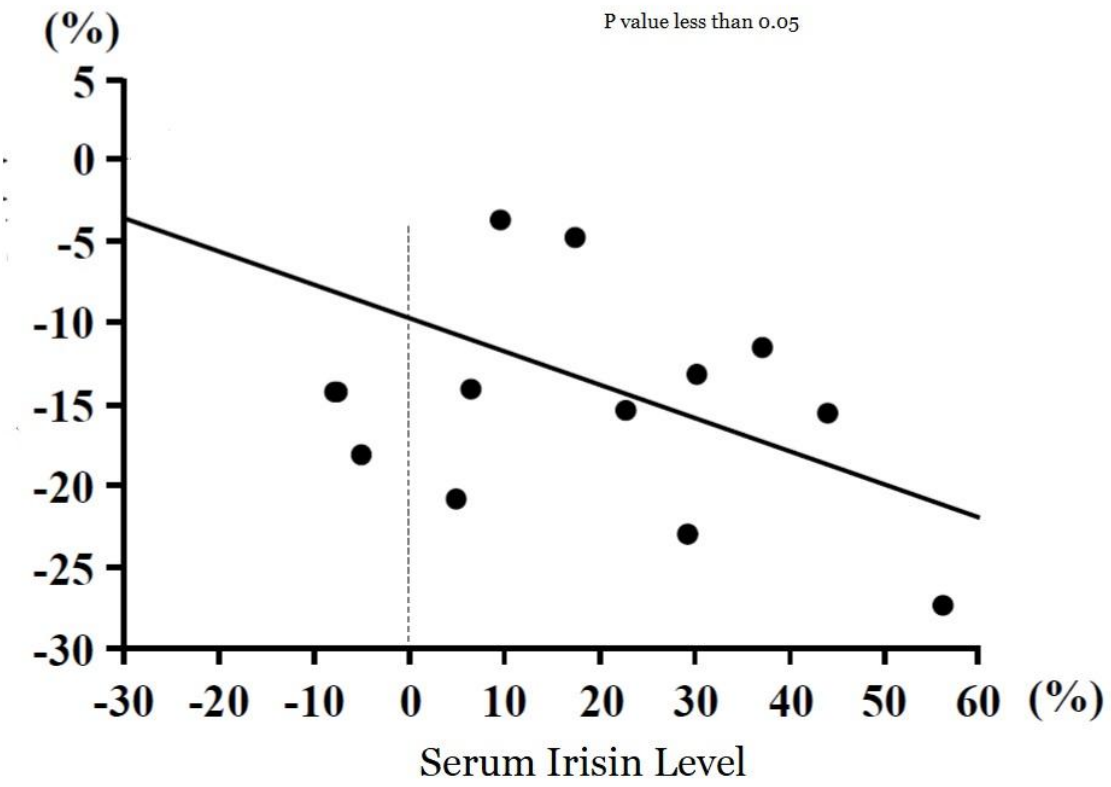


**Figure 7.** The Correlation Between the Percent Change in Serum Irisin Levels with cfPWV

## Discussion

The animal component of this investigation revealed that rats with obesity who underwent 8-weeks of aerobic exercise experienced a significant boost in irisin and NOx levels in the bloodstream, as well as a reduction in cfPWV. The study also showed that serum irisin levels were positively associated with plasma NOx levels. Furthermore, muscle tissue exhibited an increase in Fndc5 mRNA expression while arterial tissue showcased a

rise in AMPK, Akt, and eNOS phosphorylation levels. Additionally, our intervention study on obese adults demonstrated that an 8-week aerobic exercise program resulted in higher circulating levels of irisin and NOx alongside lower cfPWV. We also found that increased circulating irisin is related to higher circulating NOx and lower cfPWV. Regular aerobic exercises for people facing obesity allow muscles to secrete more irisin which eventually leads to better eNOS signalling pathway hence less stiffness in arteries (Anastasilakis et al., 2017; Miyamoto-Mikami et al., 2015; Han et al., 2015; Hou et al., 2017; Du et al., 2016; Sahin-Efe et al., 2018).

Boström et al. (2012) conducted a study that showed targeting the elimination of PGC1α in muscles, which regulates Fndc5 gene transcription, significantly reduced irisin levels in mice by about 72%. This suggests that skeletal muscle is the primary source of irisin release. Moreover, rats with obesity showed an increase in muscle Fndc5 mRNA expre-ssion after aerobic exercise training based on rece-nt investigations (Sahin-Efe et al., 2018; Shoukry et al., 2016;Jang et al., 2017; Roca-Rivada et al., 2013; Polyzos et al., 2018).

The intervention study on adults with obesity found that consistent aerobic exercise increased irisin levels in the body. Similarly, studies on rats with high-fat diets showed that aerobic exercise resulted in elevated levels of circulating irisin and muscle Fndc5 mRNA expression. In healthy middle-aged and elderly individuals, participating in regular aerobic exercise can lead to higher serum irisin levels and a decrease in overall body fat. Consistent aerobic exercise may achieve this by boosting Fndc5 gene expression in muscle tissue amongst obese individuals (Polyzos et al., 2018; Shadia, Kundu, & Hossain, 2021; Sunuwar et al., 2021).

The study shows that aerobic exercise training in obese rats increases the levels of phosphorylation in AMPK, Akt, and eNOS. Furthermore, it also boosts the circulating irisin. Several human cell studies have also indicated that irisin is linked to increased nitric oxide production through the activation of AMPK-Akt-eNOS signaling pathways. In a model using spontaneously hypertensive rats, irisin promptly reduced blood pressure and improved vasodilation response. This happened due to faster nitric oxide production, activated by the aortic AMPK-Akt-eNOS signaling pathway (Fu et al., 2016). Chronic administration of irisin has been shown to elevate its basal level in circulation and enhance vasorelaxation. Our study builds on previous research and suggests that increased levels of irisin resulting from aerobic exercise training may improve AMPK-Akt-eNOS signalling pathway, leading to faster NO production in individuals with obesity. We found a positive link between serum irisin levels, eNOS phosphorylation in arteries, as well as plasma NOx levels, in rats with obesity. Moreover, we discovered the impact of aerobic exercise training for changing circulating irisin levels favorably affects NOx in adults affected by obesity.

In a study conducted on mice with high fat-induced obesity, it was found that administering irisin for eight weeks increase-d their circulating irisin level. This increase in turn raised the levels of adiponectin by binding with adiponectin receptor 1 in endothelial cells. As a result, the AMPK-Akt-eNOS signaling pathway was activated, leading to a boost in NO production in endothelial cells. The indirect pathway behind this mechanism is attributed to how iris promotes increased adiponectin secretion.

Administering irisin for an extended period has also been observed to upregulate hemeoxygenase-1 expression in obese mice's perivascular adipose tissue, boosting the production of nitric oxide and adiponectin. When we engage in aerobic exercise, our body's endothelial cells produce more nitric oxide and experience better vasodilation. What's fascinating is that both humans and animals demonstrate an increase in the irisin hormone and NOx levels following exercise, highlighting a meaningful link between these two molecules present in the bloodstream.

Aerobic exercise training's effect on the irisin hormone secretion, which regulates metabolism and muscle function, is still being studied. Interestingly, valuable insights may be gained from obese individuals who haven't had formal training. Keep in mind that the study doesn't prove a causal connection between aerobic exercise-induced irisin secretion and arterial stiffness reduction. Indepth research involving muscle-specific FNDC5 knockout mice is needed to determine these aspects comprehensively (Sunuwar et al., 2021; Leonard & Williams, 2021).

During a study with obese rats, it was discovered that aerobic exercise increased levels of irisin and muscle expression of Fndc5 mRNA. This increase is related to an activation in the AMPK-Akt-eNOS signalling pathway, which helps to restore NO production and potentially decrease arterial stiffness associated with obesity. There is a clear correlation between higher NO and irisin levels in the bloodstream resulting from regular aerobic exercise and reduced arterial stiffness. The rise in irisin secretion due to aerobic exercise training also appears to be effective for reducing arterial stiffness through the activation of the AMPK-Akt-eNOS signalling pathway and increasing NO production, particularly for individuals affected by obesity.

## CONCLUSION

The study's findings suggest that the rise in irisin secretion resulting from aerobic exercise training may be associated with the decrease in arterial stiffness attained through the production of NO via the activated arterial AMPK-Akt-eNOS signalling pathway in individuals with obesity.

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