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Moderate intensity continuous and interval training increased VEGF and decreased cholesterol levels in female rats high calorie diet
El entrenamiento continuo y por intervalos de intensidad moderada aumentó el VEGF y disminuyó los niveles de colesterol en ratas hembras con dieta alta en calorías

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Abstract This study investigated the impact of moderate-intensity continuous training and moderate-intensity interval training on VEGF and cholesterol levels in female rats given high-calorie diet. The research was a randomized post-test with only a control group design. Thirty-two female rats were randomly assigned to four groups, P_1 (n=8, standard diet group), P_2 (n=8, high-calorie diet group), P_3 (n=8, combination high-calorie diet and moderate intensity continuous training), P_4 (n=8, combination high-calorie diet and moderate intensity interval training). The P_2,P_3,P_4 consisted of an ad libitum standard diet plus a dextrose solution for 4 weeks. The intervention group of P_3 underwent swimming plus 6% load of body weight for 10 minutes in first week, 20 minutes in second week, and 30 minutes in third and fourth week. The intervention group of P_4 underwent swimming with a ratio of 2:1 between swimming and rest time, plus 6% load of body weight, progressively increased each week. Cholesterol and VEGF levels were measured post-intervention. The mean cholesterol levels in both high-calorie diet group combined with MICT and MIIT were significantly lower ($p < 0.001$) compared to high-calorie diet group. The mean VEGF of high-calorie diet group combined with MICT was significantly higher ($p = 0.025$) compared to standard and high-calorie diet group, and high-calorie diet group combined with MIIT was significantly higher ($p = 0.004$) compared to high-calorie diet group. It can be concluded that both moderate-intensity continuous and interval training significantly increased VEGF and reduced cholesterol levels.
Key Words: obesity, interval training, continuous training, cholesterol, VEGF.

Resumen Este estudio investigó el impacto del entrenamiento continuo de intensidad moderada y el entrenamiento en intervalos de intensidad moderada sobre los niveles de VEGF y colesterol en ratas hembras que recibieron una dieta alta en calorías. La investigación fue un post-test aleatorizado con un diseño de grupo control. Se asignaron aleatoriamente treinta y dos ratas hembra a cuatro grupos, P1 (n=8, grupo de dieta estándar), P2 (n=8, grupo de dieta alta en calorías), P3 (n=8, dieta combinada alta en calorías y dieta de intensidad moderada). entrenamiento continuo), P4 (n=8, combinación de dieta hipercalórica y entrenamiento interválico de intensidad moderada). Los grupos P2, P3, P4 recibieron una dieta estándar ad libitum más una solución de dextrosa durante 4 semanas. El grupo de intervención de P3 se sometió a natación más un 6% de carga de peso corporal durante 10 minutos en la primera semana, 20 minutos en la segunda semana y 30 minutos en la tercera y cuarta semana. El grupo de intervención de P4 se sometió a natación con una proporción de 2:1 entre tiempo de natación y descanso, más un 6% de carga de peso corporal, incrementada progresivamente cada semana. Los niveles de colesterol y VEGF se midieron después de la intervención. Los niveles medios de colesterol en el grupo de dieta alta en calorías combinada con MICT y MIIT fueron significativamente más bajos ($p < 0.001$) en comparación con el grupo de dieta alta en calorías. El VEGF medio del grupo de dieta alta en calorías combinado con MICT fue significativamente más alto ($p = 0.025$) en comparación con el grupo de dieta estándar y el grupo de dieta alta en calorías, y el grupo de dieta alta en calorías combinado con MIIT fue significativamente más alto ($p = 0.004$) en comparación con el grupo de dieta alta en calorías. Se puede concluir que tanto el entrenamiento continuo de intensidad moderada como el entrenamiento por intervalos aumentaron significativamente el VEGF y redujeron los niveles de colesterol.
Palabras clave: obesidad, entrenamiento interválico, entrenamiento continuo, colesterol, VEGF.

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Introduction

Obesity has become a global pandemic due to its increasing prevalence in both adults and children. The global rise in obesity rates can be attributed to high caloric intake coupled with inadequate physical activity, with women being more affected than men (Pranoto et al., 2024). Exercise is one of the factors that can increase energy requirements, so when body movement is low, the likelihood of obesity increases, while moderate and high levels of exercise routine reduce the likelihood of obesity (Oemiati and Rustika, 2014). Lifestyle modifications, including dietary adjustments and consistent physical exertion, can help reduce the likelihood of health issues associated with a sedentary lifestyle while improving overall quality of life (dos Santos et al., 2023).

Obesity is associated with the development of metabolic syndrome, including type 2 diabetes mellitus, atherosclerotic disease, hypercholesterolemia, and vascular endothelial damage. The present study indicated that gaining weight also significantly increased the risk of hypercholesterolemia, which can trigger damage to the endothelial lining of blood vessels (Ishikawa-Takata et al., 2002). Physical activity is essential for improving the quality of life for individuals with type 2 diabetes mellitus, offering diverse benefits. Lowering levels of low-density lipoprotein (LDL) cholesterol and triglycerides, and increasing high-density lipoprotein (HDL) cholesterol, significantly reduces cardiovascular risk, a critical concern in type 2 diabetes mellitus (Franco Gallegos et al., 2024). The infiltration of adipose tissue macrophages (ATMs) causes inflammation in adipose tissue. In addition, endogenous and exogenous factors mediated by Toll-like receptor (TLR) antigens cause local metabolic endotoxemia and insulin resistance, contributing to type 2 diabetes mellitus, and atherosclerotic disease (Shah et al., 2008). In one study, it was shown that inducing experimental animals to develop diabetes led to a decrease in VEGF in the heart. This indicates a downregulation of VEGF in the heart due to insulin resistance. Decreased VEGF levels disrupt collateral revascularization in diabetic cardiac tissue, which is likely considered an increased risk factor for morbidity and mortality in patients with diabetes mellitus. Furthermore, exercise may help improve the downregulation of myocardial VEGF levels in cardiac dysfunction in diabetic patients (Erekat et al., 2014).

Physical exercise has been consistently linked to a reduction in several cardiometabolic risk factors, including body weight, BMI, waist circumference, body fat percentage, blood pressure, fasting glucose, insulin, HOMA-IR, total cholesterol, HDL cholesterol, and LDL cholesterol (Sanhueza-morales & Hermosilla-palma, 2024). Exercise offers numerous health benefits in preventing and managing noncommunicable diseases such as hypertension, diabetes, and dyslipidemia. It also helps improve obesity and insulin resistance. The optimal dose of exercise should be implemented to achieve the best results, as it plays a significant role in regulating body weight and preventing and treating diseases (D. R. Sari et al., 2024). Low to moderate-intensity exercise can enhance the circulation of catecholamines and hormone-sensitive lipase (HSL) required for carrying out lipolysis activity through the β3 adrenergic receptor (β3AR)-Gs protein pathway (Liu et al., 2020). Low to moderate-intensity exercise demonstrates that moderate intensity provokes an increase in lipolysis in white adipocytes (Ogasawara et al., 2015). Several studies have found that regular physical activity can have a positive effect on cholesterol metabolism. Exercise activity can enhance the production of certain enzymes that reverse the cholesterol transport system. However, the mechanisms explaining the details are still unclear (Woods, 2010). Physical exercise affects blood cholesterol levels through an increase in LCAT (Lecithin Cholesterol Acyltransferase) via a mechanism that is still not clear (Mann et al., 2014). Existing research indicates that a high-calorie diet leads to an increase in energy intake, which is a crucial factor in the development of obesity. This occurs due to the stimulation of endogenous CCK. This effect also leads to elevated serum levels of free fatty acids, total cholesterol, and triglycerides (Akiyama et al., 1996). Physical exercise can significantly and specifically reduce blood lipid levels, including total cholesterol, triglycerides, medium VLDL, and medium HDL (Fisher et al., 2015).

Scientific evidence indicates that physical activity regulates learning, neurogenesis, and angiogenesis by activating a cascade of growth factors such as BDNF, IGF-1, and VEGF. These factors work together to mediate the beneficial effects of exercise (Pulido & Ramírez Ortega, 2020). Exercise improves blood vessel function by reducing reactive oxygen species (ROS) and pro-inflammatory cytokines. This helps activate the VEGF pathway, promoting the growth of new blood vessels in hypoxic tissues. Both interval and continuous physical training can increase myocardial VEGF protein levels, followed by an increase in capillary density and attenuation of interstitial fibrosis. Treadmill exercise increases VEGF expression in the heart muscles of type 1 diabetic mice (Yazdani et al., 2020). In research conducted on experimentally induced diabetic animals, a decrease in the expression of miR-126, VEGF, and Raf-1 protein, along with an increase in the expression of Spred-1 protein, was observed. Physical exercise can improve glucose homeostasis, affecting VEGF and angiogenesis in the heart. In a study by Swimming exercise for 8 weeks increased VEGF levels in the animal model. This increase was mediated through the AKT and eNOS signaling pathways. The upregulation of AKT activity resulting from physical exercise influences VEGF signaling (Sabzevari Rad et al., 2020). Moderate-intensity continuous training (MICT) offers benefits in repairing body composition, cardiorespiratory health, insulin resistance, and lipid profile. Exercise improves whole-body energy metabolism and affects the liver and adipose tissue, including individuals with obesity and dyslipidaemia, by regulating gene expression of molecules connected to fat oxidation and lipogenesis (Coswig et al., 2020).

Despite the wealth of research on the impact of exercise on health, there remains a noticeable gap in our understanding of the distinct effects of moderate-intensity continuous training (MICT) and moderate-intensity interval training (MIIT) on key biomarkers such as VEGF and cholesterol levels in rats subjected to a high-calorie diet. Addressing this knowledge gap and unraveling the potential benefits of these regimens for both rats and potentially humans is of paramount importance. This study aimed to explore how MICT and MIIT affect female rats on a high-calorie diet. The hypothesis was that both types of training could increase VEGF levels and lower cholesterol, enhancing our understanding of exercise's impact on these important physiological markers.

Material and methods

This study was approved by the Komisi Etik Penelitian Kesehatan (KEPK) of the Faculty of Medicine, Universitas Airlangga. The research was a true experimental research design with a randomized post-test-only control group design.

Animals

Eight-week-old healthy female rats (*Wistar norvegicus*), 100-200 gram. The animal subjects were obtained from Faculty of Medicine, Universitas Airlangga (Indonesia) and observed at the biochemistry laboratory of the same faculty. The experimental animals were placed in a room with a temperature of 26±2°C and humidity of 50-60%. The lighting was set to a light-dark cycle with a regulation of 12 hours each with food and water ad libitum. Total sample size used in this research was 32. This research used female rats because women constitute the majority of obesity cases, with their prevalence increasing more rapidly than that of men (A. R. Sari et al., 2024).

This research was a true experimental research design. Thirty-two rats were randomly divided into four groups, P_1 as standard diet group, P_2 as high-calorie diet group, P_3 as combination high-calorie diet and MICT group, and P_4 combination high-calorie diet and MIIT group. Rats were habituated to the conditions for a week before interventions. Before the intervention began, body weight, body mass index, and fasting blood glucose were measured in the morning after overnight fast of at least 12 hours as starting point. Body weight was measured using a digital scale that ranged from 0 to 600 grams. Fasting blood glucose was measured using tail blood, which was dripped onto the multiparametric strip reader of EasyTouch® GCU Bioptik Technology. Body mass index was calculated using Lee index [LI = body weight (g) $^{1/3}$ x 1000/body length (cm)]. Body length was defined as the distance from nose to anus of rats (Wu et al., 2014).

High calorie treatment

In the standard diet group, rats were fed with standard or pellet diet. The intervention groups (P_2, P_3, P_4) were given a high-calorie diet consisting of a normal amount of food *ad libitum* per day added with oral gavage of glucose solution with D40 in the amount of dose 3-5% calories (0.013 gBB) or 0.0325 ml/gBB (Herawati et al., 2020). The dextrose solution was given at noon 7 times/week for 4 weeks.

Exercise procedure

The rats in the P_1 and P_2 group were fed at room temperature without swimming training. The exercise groups swam in a plastic pool measuring 60 cm in diameter, with a water depth of 55 cm and a temperature ranging from 28 to 32°C. For the moderate-intensity continuous training, the rats swam for 10 minutes in the first week, 20 minutes in the second week, and 30 minutes in the third and fourth weeks (Lee et al., 2010; Lutfi et al., 2021). For the moderate-intensity interval training, subjects performed interval/intermittent swimming carried out in a ratio of 2:1 between swimming and rest time. In the first week, the rats swam for 5 minutes and rested for 2.5 minutes with 2 repetitions. In the second week, the rats swam for 5 minutes and rested for 2.5 minutes with 4 repetitions. In the third and fourth weeks, the rats swam for 5 minutes and rested for 2.5 minutes with 6 repetitions (Foss *et al*., 1998; Lilik, 2004; Putri *et al*., 2018; Riyono *et al*., 2022). The moderate-intensity continuous training and interval training were carried out by providing the rat with a load equal to 6% of its body weight. The load was placed in the middle of the rat's tail (Poole et al., 2020). To prevent subject fatigue, swimming interventions were given 5 times per week for 4 weeks, conducted at 15:00 p.m. On the same days and time as the intervention group, the P1 and P2 groups were allowed to play in water, with water up to their feet, to minimize any influence of water. A high-calorie diet and physical exercise were also administered on the same days (Rliliiyono *et al*., 2022).

Data collection

After 4 weeks of training, the rats were retested and fasted for 12 hours. Their total cholesterol and VEGF levels were measured. Subsequently, the rats were anesthetized, and 5 ml of blood was drawn from the left ventricle of each rat. To isolate the serum, the blood was centrifuged for 10 minutes at 3000 rpm. The serum was then stored at -80°C for analysis of VEGF levels the following day. Total cholesterol levels were measured using tail blood, which was dripped onto the strip reader of the multiparametric EasyTouch® GCU Bioptik Technology. VEGF serum levels were measured using an Enzyme–Linked Immunosorbent Assay (ELISA) kit with a concentration unit of ng/L (Catalogue Number: E0659Ra).

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Science (SPSS) software version 21 (Chicago, IL, USA). The comparison test among each group was conducted using parametric statistics, specifically One-Way ANOVA. If there were significant differences, the analysis continued with the Least Significant Difference (LSD) post hoc test. The data were presented as means, and the statistical analyses used a significance value (sig.) of less than 0.05 ($p < 0.05$).

Results

Body weight, body mass index, and fasting blood glucose of 32 rats were measured as the baseline data show in Table 1. They were randomly divided into four groups, the standard diet group (P1), high-calorie diet group (P2), high-calorie diet combined with MICT group (P3), and high-calorie diet combined with MIIT group (P4). Each group consisted of eight rats. The body mass index of subjects was categorized as obese if the Lee index showed > 300 (Fabiyi-Edebor, 2020). Lee index shows that none of the subjects are obese. The result of body weight, BMI, and fasting blood glucose pre-intervention in each group had a normal distribution ($p > 0.05$).

Table 1.
Characteristic of the subject and the baseline data (before intervention) among groups

Group	Body weight (gram)	Body Mass Index (g/m^2)	Fasting blood glucose (md/dL)
P_1 (n=8)	150.50 ± 15.45	295.25 ± 19.37	82.50 ± 5.26
P_2 (n=8)	156.50 ± 10.81	289.38 ± 14.39	75.50 ± 10.23
P_3 (n=8)	150.25 ± 9.60	259.50 ± 11.52	88.00 ± 9.43
P_4 (n=8)	170.38 ± 20.52	271.75 ± 5.60	79.38 ± 12.96

Data = mean±SD; P_1= standard diet group, P_2=high-calorie diet group, P_3= high-calorie diet combined with MICT group, P_4= high-calorie diet combined with MIIT group.

After 4 weeks of training, the rats underwent a retesting phase following a 12-hour fasting period. During this phase, their total cholesterol and VEGF levels were quantified. It

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is evident that the mean total cholesterol levels show in Figure 1 that group P4 were notably lower than those observed in groups P1 and P2. To delve into the statistical significance of these findings, we performed a one-way ANOVA and subsequently conducted a post hoc LSD test. The post hoc analysis unveiled significant distinctions in mean cholesterol levels, with a p-value of < 0.001, within both the high-calorie diet group combined with MICT (p-value = 0.0000001) and the high-calorie diet group combined with MIIT (p-value = 0.000000001) when compared to the high-calorie diet group alone. These findings were robust, as they displayed a post hoc value of $p < 0.001$ ($p < 0.05$), underscoring the statistical significance of the differences observed.

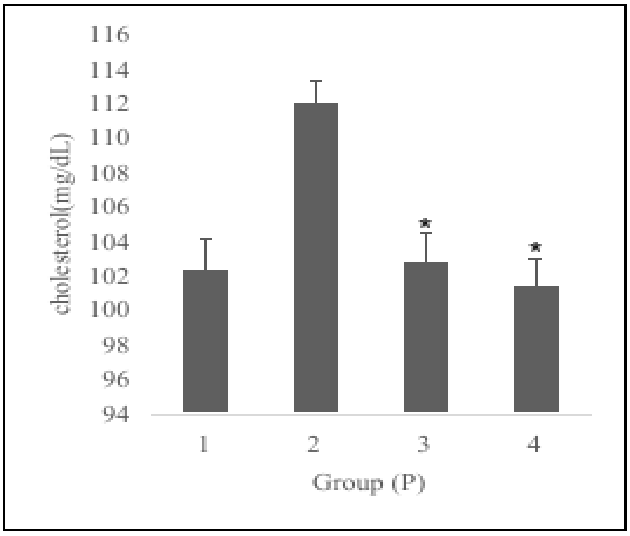

Figure 1. Total cholesterol levels post intervention from each group. P_1= standard diet group, P_2=high-calorie diet group, P_3= high-calorie diet combined MICT group, P_4= high-calorie diet combined MIIT group. Total cholesterol analyzed with one-way ANOVA (p<0,05), post hoc test. (*sig. diff. with high-calorie diet group)

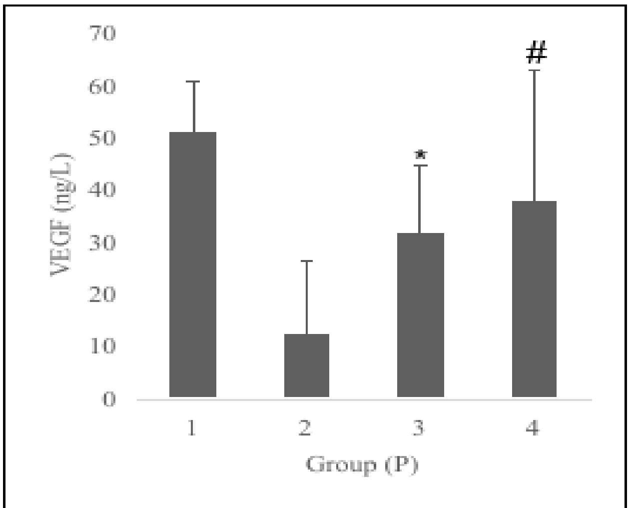

Figure 2. VEGF levels post intervention from each group. P_1= standard diet group, P_2=high-calorie diet group, P_3= high-calorie diet combined MICT group, P_4= high-calorie diet combined MIIT group. VEGF analyzed with One-way ANOVA, post hoc. (*sig. diff with standard group and high-calorie diet group; #sig. diff with high-calorie diet group)

Furthermore, we observed that the mean VEGF levels show in Figure 2 that group P2 were notably lower than those in groups P1, P3, and P4. To rigorously assess the statistical significance of these findings, we conducted a one-way ANOVA and subsequently carried out an LSD test. The statistical analysis revealed a significant difference in mean VEGF levels, with a p-value of 0.001, within the high-calorie diet group combined with MICT when compared to the standard group (post hoc p-value = 0.025) and the high-calorie diet group (post hoc p-value = 0.025). Furthermore, the high-calorie diet group combined with MIIT displayed a significant difference from the high-calorie diet group, with a post hoc value of $p = 0.004$ ($p < 0.05$). These results underscore the statistical significance of the observed distinctions in VEGF levels among the groups.

Discussion

Exercising, whether acutely or chronically, offers potential for treating cardiovascular diseases, type 2 diabetes mellitus, and obesity, by secreting exerkines (dos Santos et al., 2023). The scientific literature underscores that managing obesity relies heavily on multidisciplinary interventions such as nutritional therapy, physical exercise, psychological treatment, and pharmacological intervention (Regina de Sousa et al., 2024). This research is aimed to analyze the impacts of moderate-intensity continuous training (MICT) and moderate-intensity interval training (MIIT) on cholesterol and VEGF levels in female Wistar rats fed a high-calorie diet. The results of this study have demonstrated that exercise, whether in the form of MICT or MIIT, can effectively reduce cholesterol levels and elevating VEGF levels. These findings provide valuable insights into the potential benefits of exercise in mitigating the health risks associated with metabolic conditions and CVD, underlining the importance of physical activity as a component of a comprehensive health management strategy. Given that 65% of patients with type 2 diabetes mellitus experience mortality due to cardiovascular disease, with coronary artery disease being the primary cause of death, understanding how exercise interventions can influence key biomarkers is of paramount importance. Notably, VEGF plays a crucial role in revascularization (angiogenesis) by promoting proliferation, growth, migration, and tube formation in endothelial cells, encompassing both physiological and pathophysiological conditions, as well as compensatory angiogenesis in cardiac tissues (Mohammadi & Chodari, 2016). In these experimental results, significant differences were demonstrated in the repaired cholesterol levels of both the MICT and MIIT groups compared to the high-calorie diet group. The reduction in total cholesterol levels is attributed to the improvement of adipose tissue sensitivity related to catecholamine action and increased secretion during exercise. This phenomenon leads to a tremendous lipolytic adaptation at rest and during the training response (Costa *et al.*, 2020). In this research, a high-calorie diet was administered to rats, aiming to investigate the impact of high energy intake on obesity. High energy intake is known to be a significant factor in the development of obesity, even in genetically predisposed obese animals. It has the capacity to stimulate the release of endogenous catecholamines and, as a consequence, can lead to an automatic reduction in meal consumption. This, in turn, results in increased levels of free fatty acids, total cholesterol, and triglycerides (Akiyama et al., 1996). These findings underscore the intricate relationship between dietary factors, hormonal responses, and the metabolic changes that contribute to obesity in animal models, shedding light on the complexities of obesity development.

The outcomes of this experiment have provided noteworthy insights, indicating that both MIIT and MICT interventions exerted a positive influence on lowering cholesterol levels, in stark contrast to the group of rats that did not engage in exercise and were subjected to a high-calorie diet. Lipid metabolism disorder often occurs in individuals with obesity, increasing their risk of cardiovascular disease. Exercise reduces fat accumulation, but comparisons between interval and continuous training interventions indicate no significant differences in their effects on triglycerides, total cholesterol, LDL, and HDL. Several studies have shown that the decomposition and metabolism of fat impact hormones and training. This includes not only increasing epinephrine, norepinephrine, and growth hormone but also stimulating fat decomposition (Su et al., 2019). Norepinephrine, secreted from sympathetic nerve endings, plays an important role in increasing lipolysis and promoting lipoprotein lipase synthesis. It enhances the uptake of triacylglycerol highly consistent lipoproteins from the circulation. The sympathetic nervous system plays a crucial role in the mobilization of free fatty acids. The effect of growth hormone in promoting lipolysis depends on the synthesis of proteins that play a role in the formation of cAMP. Glucocorticoids increase lipolysis through the synthesis of protein lipases by the cAMP-dependent pathway, which can be inhibited by insulin, and by increasing the transcription of genes involved in the signaling of the cAMP cascade. These facts explain the role of the pituitary gland and adrenal cortex in improving fat mobilization (Murray et al., 2006). In a prior study, exercise was shown to result in a reduction in body weight, albeit without a statistically significant difference. Nevertheless, this decrease in body weight was observed to improve autonomic function and activate cardiovascular markers, such as fasting blood glucose, insulin resistance, hypertension, and hypercholesterolemia. These findings highlight the potential benefits of exercise on various aspects of cardiovascular health, even in cases where the reduction in body weight may not be statistically significant (Dimkpa & Ugwu, 2010). These findings underscore the remarkable effectiveness of both exercise modalities in tackling cholesterol-related health issues, underscoring the potential benefits of integrating them into fitness and healthcare routines. This research contributes valuable knowledge to the realm of preventive and therapeutic strategies for managing cholesterol levels and associated health risks.

The result of the study unveiled remarkable differences in the elevated levels of VEGF (Vascular Endothelial Growth Factor) within both the MICT and MIIT groups when compared to the high-calorie diet group. These findings signify the substantial impact of exercise on VEGF levels, suggesting that both training methods have the potential to positively influence angiogenesis and vascular health, which are vital factors in various physiological processes, including tissue repair and cardiovascular well-being. The observed disparities underscore the significance of incorporating exercise, whether continuous or interval-based, in mitigating the adverse effects of a high-calorie diet on VEGF levels and, consequently, on overall health. Exercise training is a crucial aspect of cardiac rehabilitation, but it remains unclear which exercise characteristics are most effective for individuals at high risk of coronary artery disease. Both continuous and interval training showed similar effects on cardiovascular response (Conraads et al., 2015). Previous study has demonstrated significant variations in Vascular Endothelial Growth Factor (VEGF) activity between the training and control groups. Sylviana *et al.*, (2022) noted that VEGF activity reached its peak in the high-intensity exercise group, followed by the moderate-intensity group, and then the low-intensity group in sequential order. These findings emphasize the influence of exercise intensity on VEGF levels, suggesting that higher-intensity exercise may trigger a more pronounced response in promoting angiogenesis and potentially contributing to enhanced vascular health. In a prior study, Sprague-Dawley rats were engaged in treadmill running for 60 minutes, five days a week, over a span of six weeks, categorized into light-, moderate-, and high-intensity groups. This investigation revealed an increase in myocardial VEGF protein expression in both the moderate- and high-intensity exercise groups when compared to the control group. However, notably, there was no statistically significant disparity observed in VEGF protein expression levels between the moderate- and high-intensity exercise groups. These findings highlight the potential for moderate-intensity exercise to induce similar VEGF responses as high-intensity exercise, shedding light on the nuanced relationship between exercise intensity and VEGF expression in the myocardium. The enhancement of VEGF activity plays a crucial role in the differentiation and migration of Endothelial Progenitor Cells (EPCs) to the endothelium, facilitating the angiogenesis process (Sylviana et al., 2022). This orchestrated response is pivotal in the development of new blood vessels, which can contribute to tissue repair and regeneration. The intricate interplay between VEGF and EPCs highlights the significance of VEGF in orchestrating physiological processes essential for vascular health and tissue restoration.

Diabetes improves *TGF-β,* but interval and continuous training reduces *TGF-β*. In addition, after eight weeks of interval and continuous training, VEGF and MMP9 levels were decreased in diabetic condition but increased significantly. Both interval and continuous training improve myocardium VEGF protein levels, supported by enhanced capillary attenuation and density of interstitial fibrosis (Yazdani et al., 2020). Although diabetes can reduce *miR-126* expression, *Raf-1* proteins, while increasing *Spred-1* expression, six weeks of interval training led to an improvement in the expression of *miR-126*, VEGF, *Raf-1* proteins, with a decrease in *Spred-1*. Hyperglycemia affects the signaling of VEGFR2 cascade in endothelial cells. High blood glucose due to diabetes suppresses the angiogenic markers in cardiac tissue, leading to a decline in cardiac and skeletal muscle angiogenesis in both human and animal models. Exercise helps repair glucose homeostasis, which affects VEGF and angiogenesis in the cardiac tissue. Exercise improves VEGF levels through the AKT and *eNOS* signaling pathways. Furthermore, exercise enhances angiogenic factors and increases capillary density in the cardiac tissue, thus postponing apoptosis and fibrosis of the cardiac tissue (Sabzevari Rad et al., 2020). Li *et al.* (2022) noted that current studies indicate interval training is more beneficial than continuous training for elevating serum Brain-Derived Neurotrophic Factor (BDNF) and VEGF-A levels in healthy young men. This finding underscores the potential of interval training as an effective strategy to enhance angiogenic factors, which play crucial roles in vascular function. Although both MIIT and MICT operate on the principle of enhancing vascular endothelial growth factor (VEGF) levels within the body, they do so through distinct pathways. MIIT has been found to elevate levels of both Fibroblast Growth Factor 21 (FGF-21) and VEGF through the activation of Akt1. Akt1, a serine/threonine-specific protein kinase, plays a crucial role in various cellular processes, including cell growth, survival, and metabolism. Through the activation of Akt1, MIIT triggers signaling pathways that lead to the upregulation of FGF-21 and VEGF (Roohbakhsh et al., 2021). MICT appears to modulate levels of Tissue Inhibitors of Metalloproteinase 2 (TIMP2) and cleaved Matrix Metalloproteinase 2 (MMP2) in diabetic rats, suggesting intricate interactions within the VEGF-MMPs-TIMPs pathways that merit deeper exploration in the context of exercise-induced myocardial angiogenesis (Ramos et al., 2015).

Consistent with the findings of this study, we have observed that both forms of exercise, MICT and MIIT, result in a decrease in total cholesterol levels (Figure 1) and an increase in Vascular Endothelial Growth Factor (VEGF) levels (Figure 2). These results emphasize the positive impact of physical activity on lipid profiles and angiogenic factors, highlighting the potential health benefits of these exercise modalities. These exercise approaches leverage the body's capacity to improve cardiovascular health and lipid profiles, demonstrating their potential to significantly contribute to overall well-being. Importantly, these exercise regimens are applicable to human beings. These two training methods offer different approaches to achieving fitness goals, catering to a wide range of preferences and fitness levels. MIIT, with its moderate-intensity intervals, provides a balanced workout that is less strenuous than high-intensity options, making it suitable for those looking for a more sustainable and gentler exercise routine. On the other hand, MICT, with its steady, continuous pace, offers a more consistent and less demanding alternative for individuals who may prefer a more conventional exercise approach. The versatility of these options ensures that individuals can select the training method that aligns best with their personal fitness goals and overall well-being.

However, this study has limitations. It would be important to consider the duration, intensity, and type of physical exercise in the subjects to enhance the findings. Additionally, further research is needed to expand the variables studied, including VLDL, LDL, HDL, insulin receptors, PKC, GLUT, endothelial inflammatory mediators other than VEGF, as well as organ histopathology (heart, muscle, liver).

Conclusions

This study aimed to investigate the influence of moderate-intensity continuous training and moderate-intensity interval training in female rats exposed to a high-calorie diet. The overarching hypothesis was that both MICT and MIIT have the potential to positively impact VEGF levels while simultaneously reducing cholesterol levels. This study contributes to our understanding of the complex relationship between exercise and these vital physiological markers. This study conclude that, both moderate-intensity continuous training (MICT) and moderate-intensity interval training (MIIT) significantly improved VEGF and reduced cholesterol levels. In summary, there is a relationship between exercise and these vital physiological markers. However, further research is still needed to clarify the underlying mechanisms, specifically focusing on specific signaling pathways, gene expression patterns, and cellular responses.

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