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Comparison of the Effects of a Single Bout of Resistance, Endurance, and Concurrent Exercise on Circulating miRNA-221 Expression in Physically Active Healthy Women

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A B S T R A C T

The present study aimed to compare the effects of a single bout of resistance, endurance, and concurrent exercise on circulating miRNA-221 expression in physically active healthy women. In terms of its objective, this was an applied study, and in terms of its methodology, it employed a quasi-experimental pretest–posttest design with a control group. The statistical population consisted of physically active healthy women. Accordingly, 48 participants were selected through purposive sampling and randomly assigned to four groups: control (n = 12), resistance exercise (n = 12), endurance exercise (n = 12), and concurrent exercise (n = 12). The resistance exercise protocol consisted of front squats, back squats, abdominal crunches, leg presses, and leg extensions performed at 90% of one-repetition maximum for five sets of four to six repetitions. The endurance exercise protocol consisted of running on a treadmill at a 0% incline and a speed of 8 km/h for 30 minutes, followed by cycling on a stationary bicycle at a workload of 100 W for 20 minutes. The concurrent exercise protocol consisted of performing both resistance and endurance exercise within the same session. Blood samples were collected at two time points: before the exercise session and immediately after completion of the exercise protocol. Circulating miRNA-221 expression was quantified using real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR), with cel-miR-39 used as an exogenous control. Data were analyzed using descriptive statistics, mixed-design analysis of variance with repeated measures, and the Bonferroni post hoc test at a significance level of .05. The results showed that a single bout of exercise produced a significant change in circulating miRNA-221 expression in physically active healthy women ($p < .05$). Significant differences in changes in miRNA-221 expression were also observed between the control group and each of the resistance, endurance, and concurrent exercise groups ($p < .05$). Although the mean change in miRNA-221 expression was greater in the concurrent exercise group than in the endurance and resistance exercise groups, the differences among the three exercise groups were not statistically significant ($p > .05$). Overall, the findings indicated that a single bout of exercise, irrespective of exercise modality, significantly alters circulating miRNA-221 expression in physically active healthy women and may contribute to the activation of molecular responses associated with exercise-induced adaptations. However, exercise modality did not produce a significant difference in the magnitude of changes in miRNA-221 expression following a single exercise session.

Keywords: *miRNA-221, resistance exercise, endurance exercise, concurrent exercise, physically active healthy women, gene expression, real-time RT-qPCR.*

1. Introduction

Regular physical activity is widely recognized as a central component of health promotion and disease prevention because it induces coordinated adaptations across the cardiovascular, skeletal-muscle, metabolic, endocrine, immune, and nervous systems. These adaptations extend beyond improvements in physical fitness and include enhanced insulin sensitivity, more favorable lipid metabolism, reduced systemic inflammation, improved vascular function, and greater resistance to cardiometabolic disorders. The health-promoting effects of exercise are therefore not attributable to a single physiological pathway but arise from complex interactions among mechanical loading, energetic stress, hormonal signaling, redox regulation, inflammatory mediators, and gene-expression responses (1). Recent investigations have further demonstrated that the magnitude and nature of these adaptations depend on exercise modality, intensity, duration, training status, and individual biological characteristics. For example, high-intensity interval training may produce distinctive neuromuscular and metabolic adaptations, while moderate-intensity continuous exercise may elicit different behavioral, cognitive, and physiological responses (2, 3). These observations have encouraged researchers to move beyond conventional physiological outcomes and investigate the molecular mechanisms through which different forms of exercise influence health.

Among the molecular regulators implicated in exercise adaptation, microRNAs have attracted considerable attention. MicroRNAs are short, endogenous, noncoding RNA molecules that regulate gene expression primarily through post-transcriptional mechanisms. By binding to complementary sequences in target messenger RNAs, they may promote messenger RNA degradation or inhibit translation, thereby modifying the synthesis of proteins involved in cellular growth, differentiation, apoptosis, inflammation, angiogenesis, metabolism, and tissue remodeling. A single microRNA may regulate numerous target genes, while a particular gene may be influenced by several microRNAs. Consequently, microRNAs form complex regulatory networks capable of coordinating multiple biological processes simultaneously. Their role in vascular biology, metabolic regulation, and disease progression has been documented in several pathological conditions, including diabetes and its vascular complications (4). Accordingly, microRNAs are increasingly viewed not only as intracellular regulators but also as potential biomarkers of physiological adaptation and disease-related molecular dysfunction.

An important development in this field has been the identification of microRNAs in extracellular fluids, including plasma and serum. Circulating microRNAs are relatively stable despite the presence of ribonucleases in the bloodstream because they may be protected through their association with proteins, lipoproteins, extracellular vesicles, or exosome-like particles. This stability makes circulating microRNAs particularly attractive for noninvasive assessment of molecular responses to exercise. Unlike tissue biopsies, blood sampling can be repeated with limited invasiveness, allowing the temporal course of exercise-induced molecular changes to be examined. Research in exercise physiology has therefore increasingly focused on whether circulating microRNAs reflect acute exercise stress, chronic training adaptation, tissue communication, and recovery processes (5). Nevertheless, the interpretation of circulating microRNA responses remains challenging because their measured concentrations may reflect active secretion, passive release from damaged or stressed cells, changes in extracellular-vesicle transport, altered clearance, or shifts in the relative contribution of different tissues.

Acute exercise represents a useful experimental model for examining the early molecular signals that precede longer-term physiological adaptation. A single exercise session can rapidly alter intracellular signaling, transcriptional activity, protein phosphorylation, substrate utilization, and the release of regulatory molecules into the circulation. These immediate responses may contribute to the cumulative adaptations that emerge after repeated training sessions. Investigating acute changes is therefore important because chronic adaptations are thought to result partly from the repeated activation of transient molecular events. Studies of circulating microRNAs have shown that even one bout of exercise may modify the plasma microRNA profile, although the direction and magnitude of these responses vary across microRNAs and exercise protocols. In one influential investigation, acute aerobic exercise altered the plasma microRNA signature, whereas

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endurance training produced both overlapping and distinct changes, suggesting that circulating microRNAs respond dynamically to both immediate exercise stress and repeated training exposure (6). A broader review of healthy adults similarly concluded that exercise training modifies circulating microRNAs involved in cardiovascular function, metabolism, inflammation, and skeletal-muscle regulation, although substantial heterogeneity remains among studies (7).

The molecular response to exercise is likely to be modality specific because resistance and endurance exercise impose different mechanical, metabolic, and neuromuscular demands. Resistance exercise is characterized by high levels of muscular tension, recruitment of high-threshold motor units, mechanical deformation of skeletal-muscle fibers, and activation of signaling pathways related to protein synthesis, hypertrophy, neuromuscular adaptation, and tissue remodeling. In contrast, endurance exercise places a greater and more sustained demand on oxidative metabolism, mitochondrial function, cardiovascular transport, and substrate utilization. Concurrent exercise combines resistance and endurance stimuli within the same training session or program and may therefore activate partly overlapping and partly competing molecular pathways. Differences among these exercise modalities have traditionally been examined through outcomes such as muscular strength, aerobic capacity, body composition, and lipid profiles. For example, resistance, endurance, and combined exercise have been compared in healthy adults, with evidence that each modality can modify cardiometabolic outcomes, although the pattern of response may differ across variables (8). However, substantially less is known about whether these exercise modalities produce distinct acute changes in circulating microRNA expression.

Resistance exercise has been shown to alter both skeletal-muscle and circulating microRNA profiles. These alterations may reflect responses associated with muscle contraction, regeneration, protein turnover, inflammation, and extracellular communication. A targeted analysis of tissue and circulating microRNAs following acute resistance exercise demonstrated that a single bout of resistance exercise can modulate microRNA expression, although the changes detected in skeletal muscle do not always correspond directly with those observed in circulation (9). This distinction is important because circulating microRNAs should not automatically be interpreted as direct surrogates of skeletal-muscle expression. Instead, they may represent integrated signals derived from several tissues and transport mechanisms. Age and training status may further influence these responses. Evidence indicates that circulating extracellular-vesicle-associated microRNAs and skeletal-muscle microRNAs differ according to age and may be modified by resistance training, highlighting the role of biological context in shaping microRNA regulation (10). These findings support the need to study relatively homogeneous populations and carefully controlled exercise conditions.

Endurance and aerobic exercise have also been associated with alterations in microRNAs linked to cardiovascular regulation, mitochondrial adaptation, inflammation, and metabolism. The cardiac response to exercise is partly regulated by microRNAs that influence cardiomyocyte growth, angiogenesis, fibrosis, apoptosis, and electrical activity (11). Experimental studies have shown that exercise may modify microRNA expression in cardiac tissue under both healthy and pathological conditions. For instance, high-intensity interval training has been reported to alter cardiac-tissue expression of miRNA-1 and miRNA-21 in healthy animal models (12). Similarly, aerobic training combined with curcumin supplementation influenced cardiomyocyte apoptosis and microRNA expression in rats exposed to arsenic (13), while high-intensity interval training and curcumin supplementation modified left-ventricular miR-133 and miR-1 expression in an experimental model of myocardial infarction (14). Although these studies were conducted in animal models and often included nutritional supplementation or pathological conditions, they demonstrate that exercise-sensitive microRNAs are involved in cardiac and systemic adaptation.

Exercise-related microRNA responses have also been examined in relation to inflammation and metabolic disease. In type 2 diabetic rats, swimming influenced pancreatic inflammatory cytokines, miR-146a expression, and nuclear factor kappa B signaling, suggesting that exercise-induced microRNA regulation may contribute to anti-inflammatory and metabolic effects (15). In obese women, interval and continuous aerobic training combined with calorie restriction produced changes in miR-146a expression and

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circulating inflammatory markers, including tumor necrosis factor, interleukin-6, and C-reactive protein (16). These observations are consistent with the broader proposition that microRNAs function at the interface between exercise, inflammation, metabolism, and tissue adaptation. However, exercise modality, participant characteristics, and co-interventions such as diet or supplementation may strongly affect the findings. A recent review of obesity-related microRNAs in the Iranian population further emphasized that different exercise-training modalities may produce distinct microRNA responses and that additional controlled studies are required to clarify the influence of exercise type (17).

The growing interest in exercise modality is also supported by evidence that different training structures can produce multidimensional effects extending beyond conventional physiological outcomes. High-intensity interval training, for example, has been associated with neuromuscular adaptations relevant to strength and power development (2). Comparisons between moderate-intensity continuous training and high-intensity interval training have also demonstrated that exercise structure may influence behavioral inhibition and clinical symptoms in children, indicating that the effects of exercise modality are not limited to the cardiorespiratory system (3). In young football players, high-intensity interval training and local indigenous games have been compared across physical, technical, and cognitive outcomes, further illustrating the importance of exercise-specific stimulus characteristics (18). Although these studies did not focus on circulating microRNAs, they reinforce the principle that exercise responses are shaped by the qualitative and quantitative characteristics of the imposed workload.

Within this molecular framework, miRNA-221 is of particular interest. miRNA-221 is involved in the regulation of cellular proliferation, angiogenesis, endothelial function, inflammatory signaling, apoptosis, and metabolic processes. Its expression has been investigated in cardiovascular biology, cancer, vascular dysfunction, and metabolic disorders. Because exercise activates vascular, muscular, inflammatory, and metabolic pathways in which miRNA-221 may participate, circulating miRNA-221 could represent a potentially informative marker of the acute molecular response to physical activity. Nevertheless, its exercise-related behavior is not yet fully established. The observed response may depend on the intensity and modality of exercise, the timing of blood sampling, participants' training status, sex, age, and the analytical procedures used for normalization. These methodological factors are particularly important because circulating microRNA studies frequently differ in preanalytical handling, RNA extraction, control selection, and quantitative polymerase chain reaction procedures (5, 7).

The selection of an appropriate exogenous control is especially relevant in plasma microRNA research because plasma lacks a universally accepted endogenous reference microRNA that is stable under all experimental conditions. Synthetic nonhuman microRNAs, such as cel-miR-39, are commonly added during extraction to monitor technical variability and support normalization of laboratory procedures. Likewise, the use of real-time quantitative reverse transcription polymerase chain reaction provides a sensitive method for detecting relatively small changes in circulating microRNA expression. However, technical standardization alone cannot eliminate biological heterogeneity. Differences in menstrual-cycle phase, recent dietary intake, hydration, circadian rhythms, prior exercise, and habitual training may affect circulating molecular markers. Consequently, studies involving healthy active women require careful control of pre-exercise behavior, fasting status, sampling time, and exercise exposure.

Women remain comparatively underrepresented in many areas of exercise molecular biology, and findings derived predominantly from male samples cannot always be generalized to female populations. Sex-related differences in hormonal status, body composition, substrate metabolism, vascular function, inflammatory responses, and recovery may alter both baseline microRNA expression and exercise-induced changes. Studying physically active healthy women is therefore important for understanding molecular responses in a population that is neither sedentary nor clinically diseased. In active individuals, repeated exposure to exercise may modify baseline regulatory networks and influence the responsiveness of circulating microRNAs to an acute exercise stimulus. This may lead to attenuated, enhanced, or qualitatively different responses compared with those observed in sedentary individuals. The interaction

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between habitual activity status and acute exercise modality is therefore a relevant but insufficiently investigated issue.

Another unresolved question concerns concurrent exercise. Because concurrent sessions incorporate both resistance and endurance components, they generate combined mechanical and metabolic stress. This combined stimulus may produce a greater circulating microRNA response than either exercise modality alone, particularly when the total duration or energy expenditure of the session is higher. Conversely, molecular interference between resistance- and endurance-related signaling pathways may reduce or modify the response. Traditional research on concurrent training has often focused on the interference effect in strength and endurance development, but the acute circulating microRNA response to a combined session remains less clearly understood. Comparing resistance, endurance, and concurrent exercise under controlled conditions can therefore help determine whether circulating miRNA-221 responds mainly to the presence of exercise stress or whether it is sensitive to the specific nature of the exercise stimulus.

The available evidence indicates that acute and chronic exercise can alter circulating and tissue microRNAs, but findings vary substantially according to the investigated microRNA, exercise protocol, study population, and sampling procedures (6, 7, 9). Studies in animal models have demonstrated exercise-related changes in microRNAs involved in cardiac function, apoptosis, inflammation, and metabolic regulation (12-15). Human studies have likewise shown that resistance and aerobic exercise can influence circulating microRNA signatures, while age, training status, obesity, and dietary interventions may modify these effects (10, 16). Nevertheless, direct comparisons of acute resistance, endurance, and concurrent exercise within a single controlled study are limited, particularly with regard to circulating miRNA-221 expression in physically active healthy women.

Addressing this gap is important for both theoretical and practical reasons. From a theoretical perspective, identifying whether miRNA-221 responds differently to distinct exercise modalities may improve understanding of the molecular specificity of acute exercise adaptation. From a methodological perspective, comparing several exercise conditions within the same design reduces the difficulties associated with comparing findings across studies that use different populations, laboratory protocols, and sampling time points. From a practical perspective, determining whether one exercise modality produces a greater miRNA-221 response may contribute to the development of exercise prescriptions aimed at promoting vascular, metabolic, or regenerative adaptations. At the same time, an absence of significant differences among exercise modalities would suggest that miRNA-221 responds to the general physiological stress of exercise rather than to a particular type of muscular activity.

Therefore, the aim of the present study was to compare the effects of a single bout of resistance, endurance, and concurrent exercise on circulating miRNA-221 expression in physically active healthy women.

2. Methods and Materials

In terms of its objective, the present study will be applied, and in terms of its methodology, it will employ a quasi-experimental pretest–posttest design with a control group. The statistical population will consist of physically active healthy women. G*Power software and the relevant sample-size estimation equations will be used to determine the required sample size. Assuming a 95% confidence level, 80% statistical power, an effect size of 0.25, and a significance level of .05, a minimum of 10 participants will be estimated for each of the resistance exercise, endurance exercise, concurrent exercise, and control groups. Considering the possibility of participant attrition and to increase the statistical power of the study, a total of 48 participants will be recruited. After completion of the preliminary procedures and confirmation of eligibility, the participants will be randomly assigned to four groups: control (n = 12), resistance exercise (n = 12), endurance exercise (n = 12), and concurrent endurance and resistance exercise (n = 12).

The inclusion criteria will comprise general good health, being physically active, no history of cardiovascular, metabolic, respiratory, renal, or neurological diseases, no injury or medical condition affecting the ability to perform the exercises, no history of using medications that influence metabolic or inflammatory responses, and the ability to perform the prescribed exercise protocols. Participants who develop an illness, injury, or other condition during the study

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that may affect their ability to perform the exercise protocol or influence the study variables will be excluded. Before the beginning of the study, the objectives and research procedures will be fully explained to the participants. After agreeing to participate, they will complete a written informed consent form. Participants will also be instructed to refrain from strenuous physical activity, alcohol consumption, sports supplement use, and any other behavior that may affect the study variables during the period preceding testing and blood sampling.

Before implementation of the selected exercise protocols, participants' demographic information and anthropometric characteristics, including age, height, body weight, and body mass index, will be recorded. Body weight will be measured using a Seca digital scale manufactured in Germany, with an accuracy of 0.01 kg, and height will be measured using a Seca stadiometer with an accuracy of 0.1 cm. Body mass index will be calculated by dividing body weight in kilograms by height in meters squared. To minimize the effects of diurnal fluctuations, anthropometric measurements will be conducted under standardized conditions and at approximately the same time of day.

To assess circulating miRNA-221 expression, 5 mL of venous blood will be collected from an antecubital vein by a nurse or trained phlebotomist before exercise, following a 12-hour overnight fast. Baseline blood sampling will be performed under identical conditions and at a standardized time of day to control, as far as possible, for the effects of circadian variation on the study variables. Following the initial blood collection, participants in the intervention groups will perform their assigned exercise protocol during a single session. During the exercise protocols, the control group will not engage in any structured physical exercise and will maintain its usual resting and daily activity conditions.

Before implementation of the exercise protocols, the maximal strength of participants in the resistance and concurrent exercise groups will be determined using a one-repetition maximum test. To prevent fatigue and avoid any influence of strength testing on the primary study variable, one-repetition maximum testing will be performed during a separate session, with an adequate interval before the main exercise session. Resistance exercise intensity will be prescribed as a percentage of each participant's one-repetition maximum. All exercise sessions will be conducted under the supervision of an exercise science specialist and a qualified instructor. Participants will receive instruction regarding correct exercise technique, appropriate breathing patterns, and adherence to safety procedures.

The resistance exercise protocol will consist of front squats, back squats, abdominal crunches, leg presses, and leg extensions. Participants in the resistance exercise group will perform each exercise at 90% of one-repetition maximum for five sets of four to six repetitions. The exercises will be performed in a circuit format under the direct supervision of an exercise science specialist and a qualified resistance-training instructor. The total duration of the resistance exercise session, including general and specific warm-up phases, the main exercise component, rest intervals, and cool-down, will be approximately 90 minutes. At the beginning of the session, participants will complete a 10-minute general warm-up consisting of walking and light jogging on a treadmill at a 0% incline. This will be followed by five minutes of dynamic stretching, light calisthenics, and general preparatory movements. For the specific warm-up, participants will then perform two sets of each exercise at 50% and 60% of one-repetition maximum, respectively, with 10 repetitions per exercise. After completing the main exercise component, participants will perform a 10-minute cool-down consisting of five minutes of stretching and light calisthenics, followed by five minutes of walking or very light jogging.

The endurance exercise protocol will be designed as a single session of aerobic exercise. Participants in the endurance exercise group will initially run on a treadmill at a 0% incline and a speed of 8 km/h for 30 minutes. They will then cycle on a stationary bicycle at a workload of 100 W for 20 minutes. The endurance exercise session will be performed under the supervision of an exercise science specialist and a qualified aerobic exercise instructor. The total duration of the session, including the warm-up, main exercise component, transition time between activities, and cool-down, will be approximately 90 minutes. At the beginning of the session, participants will complete a 10-minute general warm-up consisting of walking and light jogging on a treadmill at a 0% incline. This will be followed by five minutes of dynamic stretching, light calisthenics, and general preparatory movements. After the main endurance exercise component, participants will perform a 10-minute

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cool-down consisting of five minutes of stretching and light calisthenics, followed by five minutes of walking or light jogging.

The concurrent exercise protocol will consist of resistance and endurance exercise performed within the same session. Participants in the concurrent exercise group will first perform the resistance exercise component and then complete the endurance exercise component. During the resistance component, front squats, back squats, abdominal crunches, leg presses, and leg extensions will be performed in a circuit format for three sets at 90% of one-repetition maximum, with four to six repetitions of each exercise. Following completion of the resistance component and after an appropriate rest and transition interval, the endurance exercise component will be performed. The endurance component will consist of treadmill exercise at a 0% incline and a speed of 8 km/h for 30 minutes. The total duration of the concurrent exercise session, including the general and specific warm-up phases, resistance and endurance components, rest intervals, and cool-down, will be approximately 90 minutes.

At the beginning of the concurrent exercise session, participants will complete a 10-minute general warm-up consisting of walking and light jogging on a treadmill at a 0% incline, followed by five minutes of dynamic stretching, light calisthenics, and general preparatory movements. For the specific warm-up, participants will perform two sets of each exercise at 50% and 60% of one-repetition maximum, respectively, with 10 repetitions per exercise. After completing the resistance and endurance components, participants will perform a 10-minute cool-down consisting of five minutes of stretching and light calisthenics, followed by five minutes of walking or light jogging (Author, Year). The order of the resistance and endurance components in the concurrent exercise group will be kept constant to control for the potential effect of exercise sequence on the molecular responses under investigation.

During implementation of the exercise protocols, the control group will not engage in any planned exercise and will be instructed to continue its usual daily activities without modification. To ensure comparability in terms of elapsed time, participants in the control group will remain at the research facility and rest for a period equivalent to the duration of the exercise protocols. At the end of the designated period, a second blood sample will be collected from the control-group participants.

Following completion of the single exercise session, a second blood sample will be collected from participants in the intervention groups and, at the corresponding time point, from participants in the control group. The timing of posttest blood collection will be standardized for all participants. Blood samples will be collected in tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant and transported to the laboratory as rapidly as possible. The samples will then be centrifuged for 15 minutes at 2,000–3,000 rpm to separate the plasma fraction from the cellular components of the blood. Following plasma separation, the samples will be stored under appropriate temperature conditions to preserve their molecular stability until RNA extraction and assessment of miRNA-221 expression are performed.

Total RNA will be extracted from plasma samples using the mirVana PARIS Kit manufactured by Ambion, United States. The samples will first be homogenized using the kit-specific cell lysis buffer. Subsequently, 200 µL of each plasma sample will be used for RNA extraction. To control the RNA-extraction process and standardize the laboratory procedures, synthetic exogenous cel-miR-39, manufactured by RiboBio, Guangzhou, will be added to the samples at a final concentration of 50 pmol/L. The cel-miR-39 will be used as an exogenous control during reverse transcription and real-time polymerase chain reaction procedures. The miRNA-221-specific primer and the other materials required for the laboratory analyses will be obtained from RiboBio.

The iScript cDNA Synthesis Kit manufactured by Bio-Rad, United States, will be used for complementary DNA synthesis. Reverse transcription of the extracted miRNAs will be performed according to the manufacturer's instructions, with a final cDNA synthesis reaction volume of 10 µL. Following synthesis, the cDNA samples will be stored at −20 °C. Real-time quantitative polymerase chain reaction will be used to quantify miRNA-221 expression. SYBR Green fluorescent dye manufactured by Bio-Rad, United States, will be used for amplification and detection of the PCR products. The final volume of each reaction will be 10 µL, and the assays will be performed using a Roche LightCycler 480 Real-Time PCR System. To increase the precision and reliability of the findings, all samples will be

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analyzed using appropriate technical replicates. Finally, relative changes in miRNA-221 expression will be calculated using the 2^−ΔΔCt method.

Following data collection, the obtained data will be analyzed using appropriate statistical software. Descriptive statistics, including the mean and standard deviation, will first be calculated to describe the participants' characteristics and the study variables. The Shapiro–Wilk test will be used to assess the normality of the data distribution, and Levene's test will be used to evaluate the homogeneity of variances. Given the presence of four groups and two measurement time points, namely pretest and posttest, a mixed-design analysis of variance with repeated measures will be used to examine the effects of time, group, and the time-by-group interaction on circulating miRNA-221 expression. When a significant difference is identified, the Bonferroni post hoc test will be used to conduct pairwise comparisons and determine the source of the differences. The significance level for all statistical tests will be set at .05.

3. Findings and Results

A total of 48 physically active healthy women participated in the study and were equally assigned to the control, resistance exercise, endurance exercise, and concurrent exercise groups, with 12 participants in each group. Analysis of covariance (ANCOVA) was used to compare posttest circulating miRNA-221 expression among the four groups while controlling for baseline values.

Table 1

Descriptive Statistics for Posttest Circulating miRNA-221 Expression by Group

Variable	Group	*n*	Mean	Standard Deviation
miRNA-221	Control	12	0.89	0.11
	Resistance exercise	12	2.26	0.21
	Endurance exercise	12	2.25	0.34
	Concurrent exercise	12	3.06	0.21

As presented in Table 1, the concurrent exercise group had the highest mean posttest circulating miRNA-221 expression (M = 3.06, SD = 0.21), followed by the resistance exercise group (M = 2.26, SD = 0.21) and the endurance exercise group (M = 2.25, SD = 0.34). The control group demonstrated the lowest mean expression level (M = 0.89, SD = 0.11). These descriptive findings indicate that circulating miRNA-221 expression was higher following all three exercise protocols than in the control condition, whereas the mean values of the resistance and endurance exercise groups were nearly identical.

Before conducting ANCOVA, its statistical assumptions were examined. The Shapiro–Wilk test indicated that circulating miRNA-221 values were normally distributed within all groups at both the pretest and posttest stages, as all significance values exceeded .05. Levene's test was nonsignificant, $F(2, 46) = 0.783$, $p = .464$, supporting the assumption of homogeneity of variances. A significant positive correlation was found between pretest and posttest miRNA-221 expression, $r = .880$, $p = .001$, confirming an adequate linear relationship between the covariate and the dependent variable. Furthermore, the group-by-pretest interaction was not statistically significant, $F = 0.669$, $p = .418$, indicating that the assumption of homogeneity of regression slopes was satisfied. Therefore, the data were considered appropriate for ANCOVA.

Table 2

Analysis of Covariance for Posttest Circulating miRNA-221 Expression

Source	Type III Sum of Squares	*df*	Mean Square	*F*	*p*
Corrected model	1027.639	4	342.546	106.710	.001
Intercept	2.149	1	2.149	0.669	.418
Group	130.774	3	65.387	20.369	.001
Pretest miRNA-221	992.070	1	992.070	309.050	.001

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The ANCOVA results presented in Table 2 showed that, after controlling for pretest miRNA-221 expression, the effect of group on posttest circulating miRNA-221 expression was statistically significant, $F = 20.369$, $p = .001$. This result indicates that at least one group differed significantly from the other groups in adjusted posttest miRNA-221 expression. The effect of the pretest covariate was also statistically significant, $F = 309.050$, $p = .001$, demonstrating a strong association between baseline and post-intervention miRNA-221 expression and confirming the importance of controlling for baseline values. In contrast, the intercept was not statistically significant, $F = 0.669$, $p = .418$.

Overall, the ANCOVA findings indicated that a single bout of exercise significantly affected circulating miRNA-221 expression in physically active healthy women. Nevertheless, the significant omnibus group effect does not by itself identify which groups differed; therefore, Bonferroni-adjusted pairwise comparisons were conducted.

Table 3

Bonferroni-Adjusted Pairwise Comparisons of Circulating miRNA-221 Expression Across Groups

Variable	Group (I)	Group (J)	Mean Difference (I − J)	Standard Error	*p*
miRNA-221	Control	Resistance exercise	−1.37	0.23	.001
	Control	Endurance exercise	−1.36	0.18	.005
	Control	Concurrent exercise	−2.17	0.65	.001
	Endurance exercise	Control	1.36	0.18	.005
	Endurance exercise	Resistance exercise	−0.01	0.254	.121
	Endurance exercise	Concurrent exercise	−0.81	0.210	.090
	Resistance exercise	Control	1.37	0.24	.001
	Resistance exercise	Endurance exercise	0.01	0.255	.121
	Resistance exercise	Concurrent exercise	−0.80	0.212	.089
	Concurrent exercise	Control	2.17	0.72	.001
	Concurrent exercise	Resistance exercise	0.80	0.209	.089
	Concurrent exercise	Endurance exercise	0.81	0.215	.090

As shown in Table 3, the control group had significantly lower circulating miRNA-221 expression than the resistance exercise group (mean difference = −1.37, $p = .001$), the endurance exercise group (mean difference = −1.36, $p = .005$), and the concurrent exercise group (mean difference = −2.17, $p = .001$). However, no statistically significant difference was observed between the resistance and endurance exercise groups (mean difference = 0.01, $p = .121$), between the resistance and concurrent exercise groups (mean difference = −0.80, $p = .089$), or between the endurance and concurrent exercise groups (mean difference = −0.81, $p = .090$). Therefore, although the concurrent exercise group exhibited the highest mean miRNA-221 expression, the three exercise modalities did not differ significantly from one another. These findings suggest that a single bout of resistance, endurance, or concurrent exercise increases circulating miRNA-221 expression relative to the control condition, but the magnitude of this response does not significantly depend on exercise modality.

4. Discussion and Conclusion

The present study compared the effects of a single bout of resistance, endurance, and concurrent exercise on circulating miRNA-221 expression in physically active healthy women. The principal finding was that, after controlling for baseline values, circulating miRNA-221 expression differed significantly among the four study groups. Each exercise group demonstrated significantly greater post-exercise miRNA-221 expression than the control group. However, although the concurrent exercise group showed the highest mean posttest value, followed by the resistance and endurance exercise groups, the differences among the three exercise modalities were not statistically significant. Therefore, the findings suggest that a single exercise bout is sufficient to alter circulating miRNA-221 expression in active healthy women, but the magnitude of this acute response does not appear to depend significantly on whether the exercise stimulus is resistance-based, endurance-based, or concurrent.

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The significant increase in circulating miRNA-221 expression following all three exercise protocols supports the proposition that microRNAs are sensitive molecular mediators of acute exercise stress. Exercise rapidly activates mechanical, metabolic, endocrine, inflammatory, and oxidative signaling pathways, and these responses can modify the transcription, processing, release, and transport of microRNAs. Acute exercise may also influence the packaging of microRNAs into extracellular vesicles or their association with protein and lipoprotein carriers, thereby changing their detectable concentration in the circulation. Circulating microRNAs have increasingly been recognized as dynamic biomarkers of exercise-induced molecular responses because they can reflect cellular communication among skeletal muscle, vascular tissue, cardiac tissue, adipose tissue, and immune cells (5). The present findings are consistent with this framework and suggest that miRNA-221 may be among the circulating regulatory molecules responsive to acute physical stress.

The observed exercise-induced response is also aligned with evidence that a single bout of aerobic exercise can alter the circulating microRNA profile. Nielsen et al. showed that acute aerobic exercise modified several plasma microRNAs and that the acute response differed partly from the molecular signature associated with endurance training (6). Similarly, a review of exercise studies in healthy adults concluded that both acute and chronic exercise can alter circulating microRNAs involved in vascular regulation, inflammation, energy metabolism, skeletal-muscle adaptation, and cardiac function (7). These findings indicate that circulating microRNAs do not merely reflect long-term training adaptations but can respond within a short period after a single exercise session. The significant post-exercise elevation of miRNA-221 in the present study may therefore represent an early molecular event that contributes to, or signals, subsequent physiological adaptation.

The significant difference between the resistance exercise and control groups is consistent with previous evidence that acute resistance exercise modifies tissue and circulating microRNA expression. D'Souza et al. reported that a single resistance exercise session altered selected microRNAs in skeletal muscle and circulation, although tissue and plasma responses were not always parallel (9). This finding is important for interpreting the current results because circulating miRNA-221 may not originate exclusively from skeletal muscle. Resistance exercise imposes substantial mechanical tension, activates high-threshold motor units, and induces acute changes in muscle perfusion, cellular stress, inflammatory signaling, and vascular shear forces. These events may stimulate the release of miRNA-221 from skeletal muscle, endothelial cells, circulating blood cells, or extracellular vesicles. The increase observed after resistance exercise may consequently represent an integrated systemic response rather than a direct reflection of intramuscular miRNA-221 expression.

Resistance exercise can also alter extracellular-vesicle-associated microRNAs, and this response may be influenced by age and training status. Xhuti et al. demonstrated that circulating exosome-like vesicle microRNAs and skeletal-muscle microRNAs were modified in association with resistance training and differed across age groups (10). Although their study examined training-related and age-related responses rather than the exact acute protocol used in the present investigation, it supports the concept that resistance loading affects microRNA-mediated intercellular communication. Because the women in the present study were physically active, their prior adaptation to exercise may have influenced both baseline miRNA-221 expression and the magnitude of the acute response. Nevertheless, the clear difference from the control group indicates that the resistance stimulus remained sufficiently strong to induce a measurable molecular change.

The significantly higher miRNA-221 expression in the endurance exercise group than in the control group is likewise consistent with research showing that aerobic exercise alters circulating and tissue-specific microRNAs. Endurance activity produces sustained increases in oxygen consumption, cardiac output, blood flow, endothelial shear stress, and oxidative metabolism. These physiological demands activate signaling pathways related to mitochondrial function, angiogenesis, substrate utilization, vascular adaptation, and cardiac remodeling. MicroRNAs are involved in many of these processes, particularly in the cardiac response to exercise (11). Accordingly, the increase in circulating miRNA-221 following treadmill running and stationary cycling may reflect a coordinated vascular and metabolic response to prolonged aerobic work.

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Evidence from both human and animal studies supports the responsiveness of microRNAs to aerobic exercise. Continuous and interval aerobic exercise have been associated with alterations in microRNAs and inflammatory markers in obese women (16). Swimming exercise has also been shown to modify miR-146a, inflammatory cytokines, and nuclear factor kappa B signaling in diabetic rats (15). Although these studies examined different microRNAs and populations, they demonstrate that aerobic exercise can influence microRNA networks linked to inflammation and metabolism. Moreover, aerobic training has altered cardiac microRNA expression and apoptosis-related pathways in animal models exposed to pathological stressors (13). Thus, the endurance-related increase in miRNA-221 observed in the present study is compatible with the broader evidence that aerobic exercise activates microRNA-mediated molecular regulation.

The concurrent exercise group displayed the highest mean circulating miRNA-221 expression. This descriptive pattern may be explained by the broader physiological demands of combining resistance and endurance exercise within the same session. Concurrent exercise exposes the body to both high mechanical loading and sustained metabolic stress, potentially activating a wider range of molecular pathways than either modality alone. The resistance component may stimulate muscle-remodeling and mechanotransduction pathways, whereas the endurance component may increase oxidative demand, cardiovascular strain, endothelial shear stress, and metabolic signaling. The cumulative effect of these stimuli may have contributed to the numerically greater miRNA-221 response in the concurrent group. Previous comparisons of resistance, endurance, and combined exercise have demonstrated that combined exercise can produce substantial changes in cardiometabolic variables, although the relative superiority of one modality depends on the specific outcome examined (8).

Nevertheless, the difference between concurrent exercise and the other exercise modalities did not reach statistical significance. One explanation is that circulating miRNA-221 may respond primarily to the presence and overall intensity of acute exercise rather than to the specific mode of muscle activity. All three protocols were sufficiently demanding to activate systemic molecular signaling, and a threshold response may have occurred once the physiological stimulus exceeded a certain level. If miRNA-221 release is triggered by common exercise-related factors such as catecholamine elevation, changes in blood flow, oxidative stress, inflammatory signaling, or extracellular-vesicle secretion, then different exercise modalities may produce broadly similar acute responses despite their distinct mechanical and metabolic characteristics.

The absence of significant differences among the three exercise groups may also be related to the sample size and variability of circulating microRNA responses. Although the concurrent exercise group showed a noticeably higher mean value, the standard error and interindividual variation may have reduced the statistical power to detect pairwise differences. Circulating microRNA measurements are influenced by numerous biological and technical factors, including baseline expression, training history, nutritional status, hormonal conditions, plasma-processing procedures, extraction efficiency, and normalization strategy (5, 7). Consequently, a true but modest modality-specific effect may not have been detectable in the present sample. The nonsignificant pairwise findings should therefore be interpreted as evidence that no statistically reliable difference was established, rather than proof that the three exercise modalities produce identical molecular responses.

The results are also consistent with the broader observation that exercise adaptations vary according to the biological outcome under investigation. Different exercise modalities may produce distinct effects on strength, power, aerobic capacity, cognitive performance, or clinical symptoms, but these differences may not necessarily be reflected in every molecular marker. High-intensity interval training can generate neuromuscular adaptations related to strength and power development (2), and different training structures have been associated with varying behavioral and cognitive outcomes (3, 18). However, miRNA-221 may represent a more generalized molecular response to acute physical activity. Thus, the lack of significant differences among resistance, endurance, and concurrent exercise does not imply that the three modalities are physiologically equivalent; rather, it suggests that circulating miRNA-221 alone may not discriminate clearly among their immediate effects.

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The significant influence of the pretest covariate further indicates that baseline miRNA-221 expression was strongly associated with posttest expression. This finding emphasizes the importance of controlling for initial values in studies of circulating microRNAs, because participants may differ considerably in their basal molecular profiles. Habitual physical activity, genetic background, body composition, recent exercise exposure, diet, sleep, and hormonal status may all contribute to baseline variation. The strong pretest–posttest association supports the appropriateness of ANCOVA and suggests that individual molecular characteristics remain influential even after an acute exercise stimulus. It also reinforces the need for repeated-measures designs and standardized preanalytical conditions in exercise-microRNA research.

The findings may have particular relevance to vascular and metabolic adaptation because miRNA-221 has been implicated in endothelial function, cellular proliferation, angiogenesis, apoptosis, and inflammatory signaling. Exercise-induced changes in this microRNA may participate in the regulation of vascular remodeling or cellular responses to mechanical and metabolic stress. MicroRNAs are also implicated in the vascular complications of diabetes and other metabolic disorders (4). Therefore, the acute elevation of circulating miRNA-221 in healthy women may represent a physiological regulatory response rather than a disease-related abnormality. However, because circulating levels do not directly indicate whether miRNA-221 activity increased or decreased within a specific tissue, the biological meaning of the observed elevation should be interpreted cautiously.

Animal studies examining cardiac microRNAs further support the possibility that acute or repeated exercise activates molecular pathways associated with cardiovascular remodeling. High-intensity interval training has altered myocardial expression of miRNA-1 and miRNA-21 in healthy rats (12), while exercise and curcumin interventions have influenced miR-133, miR-1, apoptosis, and cardiac injury-related responses in experimental models (13, 14). Although these findings cannot be directly generalized to circulating miRNA-221 in healthy women, they demonstrate that exercise-responsive microRNAs are involved in cardiovascular adaptation. The current findings extend this general line of evidence by showing that miRNA-221 in the circulation responds acutely to three distinct exercise modalities.

The present results reinforce the broader health significance of exercise as a stimulus capable of producing rapid biological regulation. The benefits of exercise extend well beyond visible changes in fitness or body composition and involve extensive communication among tissues through cytokines, metabolites, hormones, extracellular vesicles, and noncoding RNAs (1). The significant miRNA-221 response after only one session suggests that molecular adaptation begins immediately and may be repeatedly reinforced through regular training. Reviews of exercise-related miRNAs in human populations have similarly emphasized that exercise modality can alter microRNA profiles associated with obesity and metabolic regulation, although findings remain heterogeneous and require further confirmation (17). Overall, the present study contributes to this literature by demonstrating that acute resistance, endurance, and concurrent exercise all produce a significant circulating miRNA-221 response in active healthy women, while no one modality appears statistically superior after a single session.

Several limitations should be considered when interpreting the findings. First, the relatively small number of participants in each group may have limited the ability to detect modest differences among the exercise modalities. Second, the study included only physically active healthy women, which limits the generalizability of the findings to men, sedentary individuals, older adults, athletes, or clinical populations. Third, circulating miRNA-221 was assessed only immediately after exercise, and no additional blood samples were collected during recovery; therefore, the peak response and duration of the observed change remain unknown. Fourth, circulating miRNA-221 was measured without simultaneous assessment of its expression in skeletal muscle, vascular tissue, or extracellular-vesicle subfractions, preventing identification of its tissue of origin. Fifth, although major pre-exercise behaviors were controlled, factors such as menstrual-cycle phase, hormonal fluctuations, nutritional intake, sleep quality, and recent training load may have contributed to interindividual variability. Finally, differences in total exercise volume and energy expenditure among the protocols may have influenced the results.

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Future studies should recruit larger samples and include both women and men to determine whether sex moderates the acute miRNA-221 response to different exercise modalities. Multiple post-exercise sampling points should be used to characterize the temporal pattern of miRNA-221 expression during early and late recovery. Researchers should also compare trained, recreationally active, and sedentary participants to evaluate the influence of training status. Simultaneous measurement of circulating and skeletal-muscle miRNA-221, extracellular-vesicle-associated miRNA-221, inflammatory markers, angiogenic factors, and metabolic variables would provide a more comprehensive explanation of the underlying mechanisms. Future investigations should match exercise protocols for total work, duration, or energy expenditure and examine different exercise intensities and exercise-order arrangements. Longitudinal training studies are also needed to determine whether repeated acute changes in miRNA-221 contribute to chronic cardiovascular, metabolic, or muscular adaptations.

Exercise professionals may use resistance, endurance, or concurrent exercise to stimulate acute molecular responses in physically active healthy women, because all three modalities were associated with increased circulating miRNA-221 expression relative to rest. The choice of exercise modality should therefore be based primarily on the individual's fitness goals, preferences, health status, available equipment, and tolerance rather than on an assumed superiority of one modality in modifying miRNA-221. Concurrent exercise may be appropriate when simultaneous development of muscular and cardiorespiratory fitness is desired, whereas resistance or endurance exercise may be selected when more specialized adaptations are prioritized. Exercise sessions should be supervised, progressively prescribed, and accompanied by standardized warm-up and cool-down procedures, particularly when high-intensity resistance exercise is used.

Authors' Contributions

All authors contributed substantially to the study and to manuscript development, and all approved the final version.

Declaration

The authors declare that artificial intelligence tools were used only to assist with language editing, translation, and improvement of the manuscript's readability. All conceptualization, study design, data collection, data analysis, interpretation of findings, and final approval of the manuscript were performed by the authors. The authors take full responsibility for the accuracy, integrity, and originality of the content.

Transparency Statement

Data are available for research purposes upon reasonable request to the corresponding author.

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Declaration of Interest

The authors report no conflict of interest.

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Ethics Considerations

The study placed a high emphasis on ethical considerations. Informed consent obtained from all participants, ensuring they are fully aware of the nature of the study and their role in it. Confidentiality strictly maintained, with data anonymized to protect individual privacy. The study adhered to the ethical guidelines for research with human subjects as outlined in the Declaration of Helsinki.

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