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Araştırma

The Effect of Exercise Training on Circulating microRNAs Related to Lipid Metabolism

Egzersizin Dolaşımdaki Lipid Metabolizması ile İlgili mikroRNA'lar Üzerindeki Etkisi

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ABSTRACT

Background: A sedentary lifestyle is associated with cardiometabolic risk factors and obesity. This study aims to investigate the effects of regular exercise training, acute exercise, and a sedentary lifestyle on levels of miRNA-33, miRNA-335, miRNA-370, miRNA-758, which play a regulatory role in lipid metabolism and contribute to the elucidation of the molecular mechanisms involved in the exercise.

Methods: The study included 30 elite taekwondo female athletes and 30 sedentary women who didn't exercise regularly. Plasma miRNA-33, miRNA-335, miRNA-370, miRNA-758 levels were evaluated by using real-time quantitative PCR (RT-PCR) analysis. The levels of selected biochemical parameters were measured by a colorimetric method with an automated Architect C 8000 System (Abbott Laboratories, Abbott Park, IL, USA).

Results: Plasma miRNA-33 levels were found to be significantly higher in the pre-exercise [5.96 (1.48-23.92), $p<0.001$] and post-exercise groups [6.82 (0.72-96.34), $p<0.001$] compared to the sedentary group [2.67 (0.12-8.88)]. miRNA-758 levels were lower in the post-exercise [0.0016 (0.0010-0.02)] group compared to the pre-exercise [0.0099 (0.0009-0.04), $p<0.001$] and sedentary groups [0.0123 (0.0011-0.04), $p<0.001$]. miRNA-370 levels were lower in the pre-exercise [3.11 (0.70-17.15)] group compared to the post-exercise [7.41 (2.81-56.89), $p<0.001$] and sedentary [8.17 (0.32-26.35), $p<0.001$] groups. There was no statistically significant difference between miRNA-335 levels ($p>0.05$).

Conclusions: Our findings suggest that regular exercise has a significant impact on lipid metabolism at the molecular level and miRNA-33, miRNA-370 and miRNA-758 play a key role in regulating the metabolic response to exercise.

Key words: Exercise, microRNA, lipid metabolism, cholesterol

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ÖZET

Amaç: Sedanter yaşam tarzı kardiyometabolik risk faktörleri ve obezite ile ilişkilidir. Bu çalışmanın amacı, düzenli egzersizin, akut egzersizin ve sedanter yaşam tarzının lipid metabolizmasında düzenleyici rol oynayan miRNA-33, miRNA-335, miRNA-370, miRNA-758 serum düzeylerine etkisini araştırmak ve egzersizle ilgili moleküler mekanizmaların aydınlatılmasına katkı sağlamaktır.

Gereç ve Yöntem: Çalışmaya 30 elit tekvando kadın sporcu ve düzenli egzersiz yapmayan 30 sedanter kadın dahil edildi. Plazma miRNA-33, miRNA-335, miRNA-370, miRNA-758 seviyeleri real-time kantitatif PCR (RT-PCR) analizi kullanılarak değerlendirildi. Seçilen biyokimyasal parametrelerin seviyeleri, otomatik Architect C 8000 System (Abbott Laboratories, Abbott Park, IL, ABD) ile kolorimetrik olarak ölçüldü.

Bulgular: Plazma miRNA-33 düzeyleri egzersiz öncesi [5.96 (1.48-23.92)] ve egzersiz sonrası gruplarda [6.82 (0.72-96.34)] sedanter gruba [2.67 (0.12-8.88)] göre istatistiksel olarak anlamlı düzeyde ($p<0.001$) yüksek bulundu. miRNA-758 seviyeleri, egzersiz öncesi [0.0099 (0.0009-0.04)] ve sedanter gruplar [0.0123 (0.0011-0.04) ile karşılaştırıldığında egzersiz sonrası [0.0016 (0.0010-0.02)] grupta istatistiksel olarak anlamlı düzeyde düşüktü ($p <0.001$). miRNA-370 seviyeleri egzersiz öncesi [3.11 (0.70-17.15)] grupta egzersiz sonrası [7.41 (2.81-56.89)] ve sedanter [8.17 (0.32-26.35), $p< 0.001$] gruplara göre istatistiksel olarak anlamlı düzeyde düşüktü ($p<0.001$). miRNA-335 düzeyleri arasında istatistiksel olarak anlamlı fark yoktu ($p>0.05$).

Sonuç: Bulgularımız, düzenli egzersizin moleküler düzeyde lipid metabolizması üzerinde önemli bir etkiye sahip olduğunu ve miRNA-33, miRNA-370 ve miRNA-758'in egzersize metabolik yanıtın düzenlenmesinde önemli bir rol oynadığını göstermektedir.

Anahtar Sözcükler: Egzersiz, mikroRNA, lipid metabolizması, kolesterol

1. INTRODUCTION

The Centers for Disease Control and Prevention (CDC) reports that more than 300,000 individuals die annually from unhealthy diets and low physical activity (1). A sedentary lifestyle is associated with cardiometabolic risk factors and obesity (2). There is strong evidence that regular exercise reduces all-cause mortality and the risk of many diseases, including cancer, metabolic diseases, cardiovascular diseases, neurocognitive disorders, and musculoskeletal system diseases (3). Various studies have shown that exercise reduces blood pressure and glucose concentration, and modulates lipid metabolism and insulin signaling (4). Response to acute exercise and training involves a complex cross-communication between tissues and has profound effects on the gene expression level (5). However, the crucial mechanisms by which gene expression is regulated to orchestrate this response remain partially unknown. Recently, many studies have shown that non-coding transcriptome has a key role in regulating exercise response. Therefore, the need to evaluate the role of miRNAs in the regulation of exercise response has emerged, as new players in the regulation of intracellular communication and gene expression (6).

MicroRNAs (miRNAs) are about 22 nucleotides long, small non-coding RNAs, and they are directly bound to 32 untranslated regions of target messenger RNAs, control important biological events through post-translational modulation of gene expression and RNA silencing (7). miRNAs play a key role in many cellular activities such as growth, differentiation, proliferation, programmed cell death, and immune response, and they have been identified as an important regulator of many cardiometabolic pathologies such as obesity, diabetes, atherosclerosis, heart failure, hypertension (8,9). Moreover, miRNAs are associated with insulin signaling, regulation of blood glucose, lipid and fatty acid metabolism, adipogenesis, and adipocyte differentiation (10). Increasing evidence has shown that several miRNAs, including miRNA-33, miRNA-335, miRNA-370, miRNA-758, are critical regulators of lipid and lipoprotein metabolism, moreover, may be potential therapeutic targets for cardiometabolic

diseases (11). Currently, the molecular effects of exercise on metabolism are unclear. This study aims to investigate the effects of professional sports and a sedentary lifestyle on levels of miR-33, miR-335, miR-370, miR-758, which play a regulatory role in lipid metabolism, and contribute to the elucidation of the molecular mechanisms involved in exercise.

2. MATERIAL AND METHODS

2.1. Study design

2.1.1. Subjects

The study included 30 elite taekwondo female athletes (age: 21.9±4.8 years, weight: 55.1±7.5 kg, height: 162.8±6.1 cm, BMI: 20.7 ± 2.5 kg/m2) and 30 sedentary women (age: 23.4±1.5 years, weight: 60.0 ± 8.5 kg, height: 164.9 ± 6.3 cm, BMI: 21.8 ± 3.4 kg/m2) who didn't exercise regularly. All taekwondo athletes were members of the national team and were black belt owners with at least 5 years of competitive experience. None of the participants were smokers and didn't receive systemic or topical treatments such as corticosteroids, or cyclosporine, or used supplements/vitamins, and they were free of cardiovascular diseases, hypertension, chronic kidney and liver diseases, musculoskeletal diseases, diabetes, and metabolic disorders. Approximately 4 mL of blood samples from athletes were taken into serum separator gel tubes just before and after 2-hour routine training programs. On the other hand, 4 mL of blood samples from sedentary volunteers were taken into serum separator gel tubes after 12 hours of fasting. Blood samples were centrifuged at 2000 g for 10 min, serum samples were separated and stored at -80 °C until analysis. For miRNA, whole blood samples were collected in EDTA tubes, centrifuged at 1300 g for 10 min, and plasma separated and stored at -80°C until analysis. The study was approved by the local ethics committee (Necmettin Erbakan University ethics committee Number: 2017/1009, Date: 22/09/2017).

2.1.2. Laboratory tests

The levels of serum urea, creatinine, total cholesterol, triglycerides, high-density lipoprotein-cholesterol (HDL-Chol), very low-density lipoprotein (VLDL-Chol), glucose, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured by a colorimetric method with automated Architect C 8000 System (Abbott Laboratories, Abbott Park, IL, USA). Low-density lipoprotein (LDL-Chol) was calculated according to Friedewald's formula.

2.1.3. miRNA expression profiing

Total RNA was isolated from plasma samples by using a High Pure miRNA Isolation Kit (Roche Life Science, Mannheim, Germany). Total RNA was reversely transcribed to cDNA by using miScript II RT Kit (Qiagen, Hilden, Germany). cDNA samples are PreAmplified by using miScript Microfluidics PreAMP Kit (Qiagen, Hilden, Germany). Quantitative real-time PCR (qRT-PCR) analysis was performed by using miScript miRNA Assays (Qiagen, Hilden, Germany) with Dynamic Array (Fluidigm, South San Francisco, CA, USA) on BioMark System (Fluidigm, South San Francisco, CA, USA) according to manufacturer's protocol. The relative gene expression was calculated with the comparison of cycle times for target PCR using this equation: relative gene expression $= 2-(\Delta Ctsample - \Delta Ctcontrol)$.

2.1.4. Statistical analysis

Statistical analysis was performed with SPSS statistical software package version 21.0. One-Sample Kolmogorov-Smirnov test was performed to find out the distribution. Student's t and Mann-Whitney U tests were used to compare the mean and median values between the two groups, respectively. Kruskal – Wallis test (post-hoc analysis Mann-Whitney U) was also performed in comparison of multiple groups. Correlations between the variables were determined using Spearman's correlation tests. $p < 0.05$ was considered to be significant.

3. RESULTS

The clinical and demographic characteristics of the participants were summarized in Table 1.

In our study, miRNA-33 levels were found to be significantly higher in the pre-exercise ($p<0.001$) and post-exercise groups ($p<0.001$) compared to the sedentary group, but no statistically significant difference was found between the pre-exercise and post-exercise groups ($p=0.481$). miRNA-758 levels were lower in the post-exercise group compared to the pre-exercise ($p<0.001$) and sedentary groups ($p<0.001$), but no significant difference was found between the pre-exercise and sedentary groups ($p=0.673$). miRNA-370 levels were lower in the pre-exercise group compared to the post-exercise ($p<0.001$) and sedentary ($p<0.001$) groups. There was no statistically significant difference between miRNA-335 levels of sedentary, pre-exercise, and post-exercise groups ($p>0.05$). Plasma miRNA levels of the participants are given in Table 2 and serum miRNA levels are expressed as median (min-max).

Table 1. Clinical and demographic characteristics of the participants.

Parameters	Sedentary group	Taekwondo group	p
Age (years)	23.44 ± 1.48	21.96 ± 4.84	0.121
Weight (kg)	60.0 ± 8.5	55.1 ± 7.5	0.020
Height (cm)	164.94 ± 6.3	162.86 ± 6.1	0.122
BMI (kg/m2)	21.8 ± 3.4	20.7 ± 2.5	0.143
Glucose (mg/dL)	92.4 ± 7.8	83.4 ± 14.8	0.011
Cholesterol (mg/dL)	166.6 ± 28.4	145.8 ± 22.3	<0.001
LDL-Chol (mg/dL)	88.8 ± 23.8	77.5 ± 15.7	0.032
HDL-Chol (mg/dL)	56.6 ± 9.5	52.1 ± 11.1	0.093
VLDL-Chol (mg/dL)	15.9 ± 6.4	20.1 ± 9.7	0.052
Triglyceride (mg/dL)	83.2 ± 1.2	94.1 ± 32.6	0.194
Urea (mg/dL)	22.3 ± 5.1	23.2 ± 5.7	0.496
Creatinine (mg/dL)	0.71 ± 0.08	0.68 ± 0.07	0.135
ALT (U/L)	14.8 ± 7.2	7.7 ± 2.5	<0.001
AST (U/L)	16.6 ± 4.07	17.5 ± 9.7	0.636

All values were expressed as mean±standard deviation.

Table 2. Plasma miRNAs of pre-, post-exercise and sedantary groups.

Parameters	Pre-exercise (n=30)	Post-exercise (n=30)	Sedentary group (n=30)	p
miR-33	5.96 (1.48-23.92)	6.82 (0.72-96.34)	2.67 (0.12-8.88)	**a:<0.001** **b:<0.001** c:0.481
miR-335	0.65 (0.18-15.67)	0.53 (0.24-3.81)	0.58 (0.01-7.31)	a:0.690 b:0.252 c:0.347
miR-370	3.11 (0.70-17.15)	7.41 (2.81-56.89)	8.17 (0.32-26.35)	**a:<0.001** b:0.888 **c:<0.001**
miR-758	0.0099 (0.001-0.04)	0.0016 (0.001-0.02)	0.0123 (0.001-0.04)	a:0.673 **b:<0.001** **c:<0.001**

miRNAs were not normally distributed. All values were expressed as median (Minimum-Maximum).
a: pre-exercise and sedentary groups; b: post-exercise and sedentary groups; c:pre- and post exercise groups.

Correlation analyses were performed using the Spermans' correlation test. In the exercise group, miRNA-370 was positively correlated with LDL- Chol (r = 0.410, p = 0.024), but negatively correlated with HDL-Chol (r = -0.367, p = 0.046). In the sedentary group, miR-758 was negatively correlated with HDL- Chol (r = -0.366; p <0.05), and positively correlated with VLDL- Chol (r = 0.319; p <0.05) and triglyceride (r = 0.387; p <0.05). miRNA-370 was positively correlated with triglyceride (r = 0.348, p = 0.047), VLDL- Chol (r = 0.359, p = 0.032), while negatively correlated with HDL- Chol (r = -0.338, p = 0.044).

4. DISCUSSION

Under physiological conditions, macrophages transfer intracellular cholesterol to HDL-Chol by the reverse cholesterol transport process. The adenosine triphosphate (ATP) -binding cassette A1 (ABCA1) and G1 (ABCG1) are two main proteins that mediate cholesterol efflux from macrophages, reverse cholesterol transport and prevent the formation of foam cells by cholesterol (12, 13). miRNA-33a and miRNA-33b are miRNAs of the miRNA-33 family and are encoded in the introns of the SREBF2 and SREBF1 genes, respectively (14). Rayner et al. (15) and Najafi-Shoushtari et al. (16) demonstrated that miRNA-33 modulates the expression of ABCA-1 and ABCG-1 in both mouse and human cells. Marquart et al. (17) showed that increased expression of miRNA-33 in mouse liver decreased ABCA-1 and ABCG-1 expression and reduced plasma HDL-Chol concentrations, while they increased after hepatic miRNA-33 silencing. Inhibition of miRNA-33 has been shown to increase plasma HDL-Chol levels in non-human primates and contribute to the regression of atherosclerosis in mice (18, 19). However, Horie et al. reported that miRNA-33 knock-out (KO) mice developed obesity and increased circulating triacylglycerols over time (20). Furthermore, Price et al. showed that the whole-animal genetic ablation of miR-33 is associated with obesity, enlarged white adipose tissue, fat storage, inflammation, lipid metabolism abnormalities, insulin resistance, and impaired glucose tolerance (21, 22). In our study, miRNA33 levels were statistically significantly higher in the regular exercise group compared to sedentary individuals. Moreover, miRNA-33 levels were found to be significantly higher in the pre-exercise and post-exercise groups compared to the sedentary group, but no statistically significant difference was found between the pre-exercise and post-exercise groups.

It has been demonstrated that miRNA-758 downregulates ABCA-1 expression in mouse and human cells, and the inhibition of this miRNA using anti-miRNA-758 increases ABCA-1 expression levels. miRNA-758 reduced cholesterol transport to apoA1 23 in mouse cells (24). In macrophages and liver cells of mice fed a high-fat diet, miRNA-758 was downregulated by cellular cholesterol content. These data support that miRNA-758 downregulation in cholesterol-rich cells promotes to up-regulation of ABCA1 expression to prevent excessive cholesterol accumulation (25). In our study, miRNA758 levels were statistically significantly lower in the regular exercise group compared to sedentary individuals. Also, our findings showed that miRNA-758 levels were lower in the post-exercise group compared to the pre-exercise and sedentary groups, but no significant difference was found between the pre-exercise and sedentary groups.

MiRNA-370 reduces fatty acid oxidation by suppressing the expression of carnitine palmitoyl transferase (Cpt1a), a mitochondrial enzyme. Transfection of the human hepatic cell line with miRNA-370 resulted in up-regulation of miRNA-122 expression by increased lipogenic gene expressions such as sterol regulatory element-binding protein 1 (SREBP1c) and diacylglycerol acyltransferase 2 (DGAT2). Therefore, miRNA-370 is indirectly involved in lipid metabolism by increasing the expression of SREBP-1c, DGAT-2, fatty acid synthase (FAS), and acetyl-CoA carboxylase

(ACC1) genes (26). Gao et al. (27) compared the plasma levels of miRNA-122, miRNA-370, miRNA-33a, and miRNA-33b in 225 hyperlipidemic patients and 100 healthy individuals with normal lipid levels without any coronary artery disease. This study showed that miRNA-122 and miRNA-370 levels were higher in hyperlipidemic patients than controls, moreover, miR-122 and miR-370 were positively correlated with total cholesterol, triglyceride, LDL- Chol and the severity of coronary artery disease quantified by the Gensini score levels in both hyperlipidemia patients and controls. Motawae et al. (28) reported that miRNA-370 positively correlated with body mass index (BMI), LDL- Chol, and LDL- Chol / HDL- Chol ratio, moreover, it was higher in patients with coronary artery disease and type 2 diabetic patients with coronary artery disease compared to the control group. Our findings showed that miRNA 370 levels were significantly lower in the exercise group than in the sedentary group. When subgroups were compared, miRNA-370 levels were lower in the pre-exercise group compared to the post-exercise and sedentary groups. There was no statistically significant difference between the post-exercise and sedentary groups.

MiRNA-335 is involved in lipid metabolism and adipocyte differentiation. However, the exact role of miRNA-335 in lipid metabolism and obesity is unknown (29). Nakanishi et al. (30) showed that miRNA-335 was upregulated in white adipose tissue and liver cells of ob/ob, db/db, and KKAy mice. This study reported that increased miRNA-335 expression levels were associated with the increased body, liver, and white adipose tissue weight, hepatic triglycerides, and cholesterol. Moreover, miRNA-335 levels were shown to correlate with adipocyte differentiation markers such as peroxisome proliferator-activated receptor gamma (PPAR-gamma), adipocyte fatty acid-binding protein (aP2), and FAS in 3T3-L1 adipocytes. Salunkhe et al. (31) suggested that overexpression of miRNA-335 had negative effects on insulin secretion through a reduction in multiple exocytosis protein targets and impaired priming of insulin granules. In our study, there was no statistically significant difference between miRNA-335 levels.

In summary, when previous studies were evaluated, it was stated that high levels of miRNA 33 may play a protective role in disorders associated with lipid metabolism, while high levels of miRNA 370, miRNA 758, and miRNA 335 may be associated with impaired lipid metabolism. Similarly, our findings showed a positive correlation between miRNA 370 and miRNA 758 levels and VLDL, LDL, and triglyceride levels, while a negative correlation with HDL. Our findings show that miRNA 33 levels increase in those who exercise regularly, while miRNA 370 and miRNA 758 levels decrease significantly. Therefore, it has been shown that regular exercise has positive effects on lipid metabolism at the level of miRNAs. The secondary subject of our study is the evaluation of the effect of acute exercise on lipid metabolism-related miRNA levels. It was determined that while miRNA 370 levels increased after acute exercise, miRNA 758 levels decreased. Our findings showed that acute exercise has an effect on lipid metabolism at the miRNA level, but the available findings are insufficient to fully evaluate the effect of acute exercise. Our study is superior in that it is a unique study investigating the relationship of lipid metabolism-related miRNAs with regular exercise for the first time. However, the limited number of patients and the low diversity of miRNAs analyzed are the main disadvantages of the study.

CONCLUSION

Our best knowledge is that this is the first study to investigate the effect of regular exercise training on miRNAs associated with lipid metabolism. Our findings suggest that regular exercise has a significant impact on lipid metabolism at the molecular level and circulating miRNAs play a key role in

regulating the metabolic response to exercise. Understanding the molecular basis of adipogenesis and lipid metabolism is very important for the identification of new biomarkers and therapeutic targets that play a role in the development of many diseases such as obesity, cardiovascular diseases, and dyslipidemias. Anti-miRNA therapy may be useful in the treatment of these diseases. The relationship between miRNAs and energy balance, regulation of exercise response, metabolic diseases and inflammation is still unclear. Further studies are needed to elucidate the role of miRNAs in exercise response and diseases associated with lipid metabolism.

Declaration of Interest statement

The authors declare that they have no conflict of interest relevant to the content of this manuscript.

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