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Effects of Aerobic Exercise on Leukocyte-Mediated Liver Destruction in a Rat Model of Metabolic Syndrome

Metabolik Sendromlu Sıçan Modelinde Aerobik Egzersizin Lökosit Aracılı Karaciğer Tahribatı Üzerindeki Etkileri

Fatmanur Er[1], Leyla Çimen[2], Ceren Suveren[3], Canan Yılmaz[4], Nurten Türközkan*

[1]Department of Action and Training, Atatürk University Faculty of Sports Sciences, Erzurum, Türkiye
[2]Department of Medical Biochemistry, Faculty of Medicine, Gaziantep Islam Science and Technology University, Gaziantep, Türkiye
[3]Department of Training Sciences, Gazi University Faculty of Sports Sciences, Ankara, Türkiye
[4]Department of Biochemistry, Faculty of Medicine, Gazi University, Ankara, Türkiye
*Retired

ABSTRACT

Objective: In this study, the levels of malondialdehyde (MDA), myeloperoxidase (MPO), and 3-nitrotyrosine (3-NT), which are known as oxidative/nitrosative stress markers, were investigated in the liver tissues of rats with metabolic syndrome model induced by a high fructose diet, and the possible protective effects of aerobic exercise in fructose-fed rats were determined.

Methods: Rats were divided into four groups: control, fructose, exercise, and fructose plus exercise. Metabolic syndrome was induced in rats using 20% (w/v) fructose solution in tap water, and exercise was administered every day at the same hour for an experimental period of 8 weeks in total, 30 min a day, five days a week. After eight weeks, systolic blood pressure (SBP), serum lipid, glucose, insulin, MDA MPO, and 3-NT levels were quantified.

Results: The metabolic syndrome model was successfully demonstrated by fructose administration. Compared with the C group, F caused a significant increase in SBP, serum insulin, and triglyceride levels and liver MDA, MPO, and 3-NT levels. Exercise counteracted and healed the changes in SBP, serum insulin, triglyceride, and liver MDA, MPO, and 3-NT levels in fructose-fed rats ($p<0.05$).

Conclusion: These results indicate that high fructose consumption causes metabolic syndrome in rats, and aerobic exercise has beneficial effects on the components of metabolic syndrome. Exercise not only reduces the known risk factors of the disease, but also protects the liver while preventing oxidative and nitrosative damage caused by the MPO-H_2O_2 system in the liver, which increases with the effect of

ÖZ

Amaç: Bu çalışmada, yüksek fruktozlu diyetle metabolik sendrom modeli oluşturulan sıçanların karaciğer dokularında oksidatif/nitrosatif stres belirteçleri olarak bilinen malondialdehit (MDA), miyeloperoksidaz (MPO) ve 3-nitrotirozin (3-NT) düzeyleri araştırılmış ve fruktozla beslenen sıçanlarda aerobik egzersizin olası koruyucu etkileri belirlenmiştir.

Yöntemler: Sıçanlar dört gruba ayrıldı: kontrol, fruktoz, egzersiz ve fruktoz artı egzersiz. Sıçanlarda metabolik sendrom musluk suyunda %20 (ağırlık/hacim) fruktoz çözeltisi kullanılarak oluşturuldu ve egzersiz, toplamda 8 haftalık deneysel bir süre boyunca her gün aynı saatte, haftada beş gün, günde 30 dakika uygulandı. Sekiz hafta sonra sistolik kan basıncı (SBP), serum lipid, glikoz, insülin, MDA, MPO ve 3-NT düzeyleri ölçüldü.

Bulgular: Metabolik sendrom modeli fruktoz uygulamasıyla başarılı bir şekilde gösterildi. Fruktoz grubu kontrol grubuyla karşılaştırıldığında, SBP, serum insülin ve trigliserit düzeylerinde ve karaciğer MDA, MPO ve 3-NT düzeylerinde önemli bir artışa neden oldu. Egzersiz, fruktozla beslenen sıçanlarda SBP, serum insülin, trigliserit ve karaciğer MDA, MPO ve 3-NT düzeylerindeki değişiklikleri dengeledi ve iyileştirdi ($p<0,05$).

Sonuç: Bu çalışma yüksek fruktoz tüketiminin sıçanlarda metabolik sendroma neden olduğunu ve aerobik egzersizin metabolik sendromun bileşenleri üzerinde yararlı etkileri olduğunu göstermektedir. Egzersiz sadece hastalığın bilinen risk faktörlerini azaltmakla kalmaz, aynı zamanda karaciğeri korurken karaciğerde fruktozun etkisiyle artan ve alkolsüz yağlı karaciğer hastalığının oluşumu için gerekli olan MPO-

Address for Correspondence/Yazışma Adresi: Fatmanur Er, MD, Department of Action and Training, Atatürk University Faculty of Sports Sciences, Erzurum, Türkiye
E-mail / E-posta: fatmanur.er@atauni.edu.tr
ORCID ID: orcid.org/0000-0002-9203-4974

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fructose and is necessary for the formation of non-alcoholic fatty liver disease.

Keywords: Metabolic syndrome, non-alcoholic fatty liver disease, malondialdehyde, myeloperoxidase, 3-nitrotyrosine

H_2O_2 sisteminin neden olduğu oksidatif ve nitrozatif hasarı önlediği söylenebilir.

Anahtar Sözcükler: Metabolik sendrom, non-alkolik yağlı karaciğer hastalığı, malondialdehit, miyeloperoksidaz, 3-nitrotirozin

INTRODUCTION

Metabolic syndrome, also known as syndrome X, is a disease characterized by abdominal obesity, hypertension, hypertriglyceridemia, and insulin resistance. Metabolic syndrome is not a single disease; it is a significant risk factor for other conditions, such as cardiovascular disease, obesity, and type II diabetes. In addition, the critical risk factor for non-alcoholic fatty liver disease (NAFLD) is metabolic syndrome. One of the essential causes of metabolic syndrome is excessive intake of F and high fructose corn syrup (1-3). Humans and animals that are administered fructose develop the criteria of metabolic syndrome. There is much evidence that excess fructose intake has defective effects on liver function (3-5). Fructose consumption is considered a risk factor in metabolic syndrome as a lipogenic compound and accumulates in the liver; the critical marker of NAFLD is hepatic triglyceride accumulation (6,7).

NAFLD is known for hepatic and systemic insulin resistance and dyslipidemia. Accumulating evidence supports that NAFLD is often referred to as a hepatic manifestation of metabolic syndrome. The first step in NAFLD is hepatic steatosis, which can then progress to steatohepatitis, hepatic fibrosis, and cirrhosis (6,8-10). It has been reported that insulin resistance may be a significant risk factor, and it leads to defects in fatty acid accumulation. As a result of high amounts of lipid in hepatocytes, steatosis and inflammation may be observed in the liver (11,12). Although the pathogenesis of NAFLD is not well understood, it has been suggested that inflammatory mediators play a central role in inflammation and fibrosis (13). On the other hand, neutrophils are the critical and first type of immune cells that respond to liver inflammatory changes. It has been reported that neutrophils are implicated in metabolic dysregulation, inflammation, and fibrosis of NAFLD development (14).

Myeloperoxidase (MPO) is a heme peroxidase found in leukocytes. It is stored in cytoplasmic granules and, due to phagocytic activation, is released into the extracellular compartment. It has been reported that MPO is defined-as an early marker of inflammation and oxidative stress. There is a relationship between chronic inflammation, insulin resistance, and increased MPO activity (15-18). To date, it has been reported that there is no safe and effective pharmacotherapy for the treatment of NAFLD (7). Investigators recommend lifestyle modification such as dietary modification and regular physical activity, for the management of NAFLD. It has been reported that physical exercise may be effective against NAFDL through insulin resistance, reduces excess delivery synthesis to the liver, and increases fatty acid oxidation (19-21).

It has been reported that physical exercise may be effective on NAFLD through various pathways. It improves peripheral insulin resistance, reduces the excess delivery of fatty acids and glucose for free acids synthesis to the liver, and increases fatty acid oxidation (19-21). Little is known about the role of leucocyte sequestration in fructose-induced metabolic syndrome model in both mechanisms of MPO-mediated tyrosine nitration and lipid peroxidation in NAFLD. In addition, we did not find evidence that regular exercise improves MPO-mediated tissue damage in NAFLD in rat' fructose-induced metabolic syndrome model. Therefore, our study to evaluate the effect of regular aerobic exercise on leukocyte-mediated liver tissue damage in metabolic syndrome-induced NAFLD.

MATERIALS AND METHODS

Adult Sprague-Dawley male rats were purchased from the Gazi University Laboratory Animals Raising and Experimental Research Center. Ethical approval was received from Gazi University Animal Experiments Local Ethics Committee (approval number: E-66332047-604.01.02-786048, date: 01.11.2023). Four groups were created with six experimental animals in each group. During the eight weeks, animals that were fed a standard rat diet, were housed on a 12:12 h light: Dark cycle and free access to food and drinking.

Control (C): Untreated normal control group.

Fructose (F): This group, until the end of the eighth weeks, were fed fresh, prepared 20% fructose in tap water. There were also no restrictions on drinking water either (22,23).

Exercise (E): Running exercise was administered to the animals every day using a treadmill at the same hour for a study period of 8 weeks, 30 min a day, five days a week (24).

Fructose + exercise (F + E): Animals in this group, during the study, both fructose was given and treadmill running exercise was applied (24).

All rats' weights were recorded weekly, and systolic blood pressures (SBP) were measured with the tail-cuff sphygmomanometer method at the beginning of the study, at the end of week 4, and at the end of week 8 (24). At the end of eight weeks, the animals were sacrificed under ketamine-xylazine anesthesia. Intracardiac blood samples were collected, serum was separated, and rat liver tissue were taken and stored at -80 °C until analysis.

Biochemical Measurement

Serum glucose and triglyceride concentrations were measured using standard enzymatic methods with AU5800 clinical chemistry autoanalyzer (Beckman Coulter, USA). Serum insulin concentrations were measured by using an ELISA kit (Millipore, Billerica, MA). Insulin resistance calculated by Homeostasis Model Assessment of Insulin Resistance Index [(HOMA-IR):Fasting insulin (mU/L)*fasting glucose (mmol/L)/22.5]. Liver tissue MPO activity was determined by Schierwagen et al. (25) method. The liver MDA amount was measured with the HPLC method (26). For 3-nitrotyrosine (3-NT) measurements in the liver, tissue homogenates were prepared according to the method described by Kamisaki et al. (27). Liver tissue 3-NT levels were detected on HPLC with a UV detector set at 274 nm as (28).

Statistical Analysis

All statistical analyses were performed using "IBM SPSS Statistics 24" statistical package software (SPSS Inc., Chicago, IL). The Kolmogorov-Smirnov test was used to determine whether continuous variable distributions were normal. Since study groups did not show a normal distribution were used Kruskal-Wallis analysis and Comparisons between groups were performed using the Mann-Whitney U test. More than two measurements in a single group the Friedman Variance analysis is used to determine changes over time. Probability values of less than 0.05 were accepted as significant.

RESULTS

The body weights and SBP are given in Table 1. The body weights of F group were higher compared with the E and F + E groups (p<0.05, p=0.004; p=0.032). The SBF value of the F group was higher than that of the C group (p<0.05, p=0.004). Furthermore, the SBF of the F group was higher compared with the E and the F + E groups (p<0.05, p=0.001; p=0.014).

Biochemical parameters, triglyceride, glucose, insulin and HOMA-IR values are given in Table 2. The serum triglyceride levels of the F group were significantly higher compared with the C, E and F + E groups (p<0.05, p=0.045; p=0.008; p=0.004). Compared with the F group, the serum glucose and insulin levels and HOMA-IR indexes were significantly higher compared with the C and E groups (p<0.05, p=0.004; p=0.008). In addition, as shown in Table 2, a statistically significantly higher in the HOMA-IR values of the F group was compared with the F + E group levels (p<0.05, p=0.004); however, C and E groups were significantly lower compared with the F + E group (p<0.05, p=0.002; p=0.004).

The liver MDA, MPO, and 3-NT levels of the four groups were indicated in Table 3. Compared with the F group, the MDA, MPO, and 3-NT levels in the F group were higher compared with the C and E groups (p<0.05, p=0.001). The MDA levels of the F + E group was higher compared with the C group (p<0.05, p=0.020). As can be seen in Table 3, 3-NT levels were detected clearly in the liver tissue of animals exposed to fructose, whereas 3-NT was not detected in the liver tissue of control and exercise animals.

DISCUSSION

In our study, hypertension, hypertriglyceridemia, insulin resistance, and blood insulin values, which are accepted as metabolic syndrome criteria, increased in rats after fructose administration. Thus, the metabolic syndrome model was successfully realized. In addition to being associated with cardiovascular diseases and type II diabetes, metabolic syndrome has been considered an essential risk factor for NAFLD in recent years (9,11). However, the sedentary life of our age increases the frequency of metabolic syndrome and its hepatic component, NAFLD, day by day.

Table 1. Body weights and systolic blood pressures of groups

Group	Body weight (g)		Systolic blood pressure (mmHg)		
	Beginning	End	Beginning	4th week	8th week
Control	208.66±8.213	251.83±8.232*	128.33±3.204	124.30±7.437	121.81±6.635
Fructose	217.4±8.734	279.2±20.166*	126.6±6.066	139.75±3.869	176.57±4.280*[a]
Exercise	215.2±4.266	234.6±3.646*[b]	124.2±4.658	118.26±6.753[b]	117.13±5.907[b]
Fructose + exercise	200.83±4.266[b]	240.5±3.646*[b]	121.83±7.277	122.62±6.593	133.17±8.443[#]

[a]p<0.05 compare with the control group, [b]p<0.05 compere with the fructose group, [c]p<0.05 compare with the exercise group, [d]p<0.05 compare with the fructose plus exercise group, [*]p<0.05 systolic blood pressures compared with initial values, [#]p<0.05 systolic blood pressures compared with 4th week values.

Table 2. Biochemical parameters of groups

Group	Glucose (mmol/L)	Triglyceride, (mg/dL)	Insulin, (mU/L)	HOMA-IR
Control	9,293±0.172	35.66±5,278	4,518±0.561	1.86±0.215
Fructose	13.41±1,064[a]	62.74±7,483[a]	12.36±1,236[a]	7.34±0.618[a]
Exercise	9.46±0.478[b]	35.64±4,375[b]	4.71±0.723[b]	1.97±0.287[b]
Fructose + exercise	10.62±1,040	34.16±7,359[b]	8.25±1,459	3.94±1,045[a,b,c]

[a]p<0.05 compare with the control group, [b]p<0.05 compere with the fructose group, [c]p<0.05 compare with the exercise group, [d]p<0.05 compare with the fructose plus exercise group, HOMA-IR: Homeostasis Model Assessment of Insulin Resistance.

Table 3. Biochemical parameters of groups

Group	MDA, nmol/g tissue	MPO, U/min/g tissue	3-NT, nmol/g tissue
Control	0.03±0.002	7.1±0.296	Not detectable
Fructose	1.22±0.081a	19.03±0.280a	4.01±0.067
Exercise	0.03±0.003b	7.26±0.233b	Not detectable
Fructose + exercise	0.52±0.085a	11.9±0.352	1.94±0.100

[a]p<0.05 compare with the control group, [b]p<0.05 compere with the fructose group, [c]p<0.05 compare with the exercise group, [d]p<0.05 compare with the fructose plus exercise group, MDA: Malondialdehyde, MPO: Myeloperoxidase, 3-NT: 3-nitrotyrosine.

NAFLD is a liver disease characterized by excessive fat storage in liver cells. In some cases, NAFLD is a pathological condition that progresses from steatosis to steatohepatitis, fibrosis, and end-stage liver disease (cirrhosis). Triglycerides accumulated in the liver cause hepatocyte stress, resulting in inflammation and cell death (7,12). Chemokines and cytokines released from dying cells activate immune cells such as neutrophils and macrophages. Inactivated neutrophils trigger a respiratory burst event, and free oxygen radicals that cause liver tissue destruction are formed (29,30). Although there are many factors in the development of NAFLD, oxidative stress and insulin resistance are considered the main problems. Although there have been various studies on the pathogenesis of NAFLD, it is still among the subjects that still need to be fully elucidated. The first step in the pathogenesis is the formation of steatosis, followed by the addition of inflammation. The neutrophil count has been reported to be important in the development and progression of NAFLD, and it has been suggested that these cells are essential markers of chronic inflammation. A relationship exists between NAFLD, insulin resistance, and neutrophil count (31,32).

In this study, to determine the oxidative/nitrosative stress that may be caused by a fructose diet, the levels of MDA, MPO, which is an indicator of lipid peroxidation, and 3-NT, which is a marker of protein nitration, and the effect of exercise on these parameters in liver tissue were investigated.

MPO is a cytoplasmic heme peroxidase found in neutrophil granules. The enzyme released into the extracellular compartment during phagocytic activation has been accepted as the earliest marker of inflammation and oxidative stress (15-18). Researchers have emphasized a close relationship between ROS production by MPO and inflammation and tissue destruction observed in chronic inflammatory diseases (29,33). Rensen et al. (33) revealed that the number of neutrophils increased in NAFLD and MPO activity and expression in the detected inflammation. Our study observed a significant increase in MPO activity in fructose-mediated metabolic syndrome rat livers. Our results follow those of other investigators working on this subject (29,33).

In various pathologies, measuring MDA in biological samples is a reliable indicator of radical production (34). It is known that fructose administration generally produces a prooxidant environment and renders cell membranes vulnerable to peroxidative damage. Lipid peroxidation is a critical process for atherogenesis, and the development of hypertension, and the products formed from that place may also contribute to tissue damage through direct cytotoxic effects (35). It has been reported that MDA levels, an indicator of lipid peroxidation, are significantly increased in various tissues of rodents administered a high fructose diet (34,36,37). Our model observed that the amount of MDA, a lipid peroxidation product, increased in parallel with an increase in hepatic MPO by a fructose diet. da Fonseca et al. (38) found lipid peroxidation increased in the plasma of patients diagnosed with metabolic syndrome due to an increase in MPO. Hendriks and Bunder reported that fatty acids are enzymatically subjected to lipid peroxidation. Enzymes such as MPO and lipoxygenase are responsible for this and the increase in MDA in the tissue. Thus, they stated that lipids lose their properties and cause liver tissue destruction in NAFLD (30).

In this study, 3-NT levels, which is an indicator of protein nitration in tissues, were examined, and it was observed that 3NT, a marker of nitrosative damage in tissues, could not be detected in the liver tissue of the control group. In contrast, it showed a significant increase after fructose administration. 3NT occurs as a result of the nitration of protein-bound and free tyrosine residues with reactive peroxynitrite and causes protein modifications (39). Nitrosative modifications of proteins cause fragmentation, increased crosslinking, and aggregation and may lead to irreversible loss of function in enzyme and receptor proteins due to nitration (40). As in our results, it was determined by immunohistochemical methods that 3-NT staining was significantly increased in various tissues of rodents administered a fructose diet in previous studies (40-42). In a survey by Ahsan (39), he emphasized that 3NT is formed by catalysis of a class of peroxidases using H_2O_2 and nitrite as substrates and may be an essential marker in tissue destruction. It has been reported that activated macrophages form superoxide and NO, then the two combine to form peroxynitrite, and this peroxynitrite nitrifies the amino acid tyrosine to form 3NT (43).

3-NT is a peroxynitrite-mediated pathological marker. Again, Rensen et al. (33) demonstrated the presence of hypocrisy-modified proteins in the liver of patients with NAFLD and showed that this was mediated through the MPO-H_2O_2 system and ultimately led to nitrite accumulation in the tissue.

All of these results support our findings. The main aim of our study was to administer fructose only to one group of rats. In contrast, in a fructose-mediated metabolic syndrome model, the other group received exercise training with fructose for eight weeks to examine its effect on MPO-mediated oxidative and nitrosative stress in the liver.

In a previous study, we applied the same exercise in a metabolic syndrome model that we created under the same conditions and observed that fructose-mediated increased hypertension decreased, blood triglyceride and insulin levels decreased, and insulin resistance, which is very important in NAFLD, was regulated (44). In this study, in our fructose-mediated metabolic syndrome model, it was observed that the increased liver MPO activity after treatment running exercise decreased significantly compared to the values in the fructose group, even if it did not decrease to the level of the control group. In the literature, researchers have reported that physical exercise prevents hepatic stethocin development and does this by stimulating lipid oxidation and inhibiting lipid synthesis (20). In another similar study, researchers emphasized that regular moderate aerobic exercise increases the killing capacity of neutrophils and makes the organism resistant to infection. In contrast, long periods of heavy exercise may significantly reduce neutrophil activation and decrease the resistance of organisms to infections (45).

As seen from all these results, the treatment running aerobic exercise we applied in our study was appropriate regarding dose and period. On the one hand, it prevented the oxidative and nitrosative damage caused by the MPO-H_2O_2 system, which increases with the effect of fructose in the liver and is vital for forming NAFLD. On the other hand, it protects cell resistance mechanisms against infections by keeping this system balanced.

Study Limitations

Finally, there are some limitations to this study. First, It may not be possible for the animal models used to create complex models such as MetS to fully resemble human physiology. The second limitation is that the duration sufficient in animal models for the MetS model may not fully reflect the chronic processes seen in humans. In addition, another limitation of the study is that practices such as diet and exercise applied to animals may affect the validity as they create an environment different from the animals' natural environment.

CONCLUSION

More human and animal studies with larger sample sizes and longer follow-up periods are needed to compare the long-term effects of exercise therapy in patients with MetS.

Ethics

Ethics Committee Approval: Adult Sprague-Dawley male rats were purchased from the Gazi University Laboratory Animals Raising and Experimental Research Center. Ethical approval was received from Gazi University Animal Experiments Local Ethics Committee (approval number: E-66332047-604.01.02-786048, date: 01.11.2023).

Informed Consent: Patient approval has not been obtained as it is performed on animals.

Authorship Contributions

Concept: F.E., L.Ç., C.S., C.Y., N.T., Design: F.E., C.Y., N.T., Supervision: F.E., L.Ç., C.S., C.Y., N.T., Resources: F.E., C.Y., Materials: F.E., C.Y., N.T., Data Collection or Processing: F.E., C.Y., N.T., Analysis or Interpretation: F.E., L.Ç., C.S., C.Y., N.T., Literature Search: F.E., C.Y., Writing: F.E., N.T., Critical Review: F.E., L.Ç., C.S., C.Y., N.T.

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