

# Exercise and Caloric Restriction Improves Liver Damage in Metabolic Syndrome Model

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## ABSTRACT

**Objective:** Detecting the level of antioxidant and tissue damage that can occur in liver tissue induced metabolic syndrome by a high fructose diet in rats and the changes after exercise and/or caloric restriction.

**Materials and Methods:** Sprague-Dawley male rats were divided into five groups: control (C), metabolic syndrome (M), metabolic syndrome with exercise (ME), metabolic syndrome with caloric restriction (MCR), and metabolic syndrome with exercise and caloric restriction (MECR). To induce metabolic syndrome, a 10% fructose solution was given to rats in drinking water for 3 months. Exercise and caloric restriction were applied to the related groups for 6 weeks after the induction of metabolic syndrome. Glucose in the blood, lipid peroxidation (LPO), sialic acid (SA), hexosamine, mucin, fucose, glutathione (GSH) levels, alkaline phosphatase (ALP), tissue factor (TF), superoxide dismutase (SOD), catalase (CAT), and glutathione-S-transferase (GST) activities were measured in rat liver homogenates.

**Results:** In the liver, LPO levels increased and TF activities decreased in the M group compared to the C group and increased in the MCR and MECR groups compared to M group. GSH levels, SOD, and CAT activities decreased in M compared to C group and increased more significantly in MECR group compared to M group. SA levels increased in MCR and MECR groups compared with M group. Fucose levels also increased in MECR compared with the all others.

**Conclusion:** Liver tissue damage that occurs after a fructose diet and decreased antioxidant levels was shown to improve best in combined exercise and caloric restriction treatment (MECR group).

**Keywords:** Metabolic syndrome, exercise, caloric restriction, antioxidant-oxidant parameters

## INTRODUCTION

Metabolic syndrome is characterized by a group of risk factors including abdominal obesity and/or body mass index, dyslipidemia, elevated blood pressure, insulin resistance, and increased markers of proinflammatory proteins in plasma (1-3). In the European Union, the epidemics of overweight and obesity are associated with

sedentary lifestyle and physical inactivity (4). The etiology of metabolic syndrome is unclear but is associated with a variety of factors such as a modern lifestyle, environmental and hereditary factors, insulin resistance, low-grade inflammation, and oxidative stress (4-6).

The liver is the major tissue of fructose metabolism occurrence. Therefore, most toxic effects are observed



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there after a high-fructose diet. The liver is the initial affected tissue in Type II diabetes by oxidative stress due to metabolic disorder and hepatic insulin resistance (7, 8).

Exercise is an effective method to improve insulin sensitivity. Glucose transport is increased to insulin sensitive tissues through exercise (9). The result of weight loss in the diet was also made with the restriction; a fatal illness that will cause obesity can be seen to decrease 10-40%. Many studies have shown that obese individuals can extend their lives as a result of food restriction or caloric restriction (10-14). When rats only being allowed less than 40% of the average food intake was determined, the average and maximum lifespan increased by 25-40% (15).

The aim of this study is to examine the effects of exercise and/or caloric restriction on rat liver tissue in a metabolic syndrome model induced by a high fructose diet.

## MATERIALS AND METHODS

### Chemicals

All chemical reagents used in this study were of analytical grade and supplied from Merck, Sigma-Aldrich, and Fluka.

### Animals

Male Sprague-Dawley rats were obtained from the Experimental Animal Research Center of Marmara University in Istanbul, Turkey. The animal facilities and protocol were approved by the Laboratory Animal Care and Use Committee of Marmara University in Turkey (101.2013.mar). Fifty-six Sprague-Dawley male rats were kept in cages in a room maintained at  $25 \pm 1^\circ\text{C}$  on a 12 h light/dark cycle and allowed free access to food and water throughout the study. Normal pellet type rat feed produced in Istanbul Çobançeşme Feed Industry Factories was used for feeding. It contained 24% protein, 7% cellulose, 8% crude ash, 2% HCl insoluble ash, 1- 2.8% calcium, 0.9% phosphorus, 0.5-0.7% sodium, and 1% sodium chloride. Drinking water containing 0.6% methionine and 1% lysine and standard rat feed were administered ad libitum. Provided metabolic energy was 2650 kcal/kg.

### Experimental Design

Animals were randomly divided into five groups; control (C), metabolic syndrome (M), metabolic syndrome with exercise (ME), metabolic syndrome with caloric restriction (MCR), and metabolic syndrome with exercise and caloric restriction (MECR). To induce metabolic syndrome, 10% fructose solution was given to rats in drinking water for 3 months. Exercise (swimming for 30 min 3 times/week) and caloric restriction (40% restriction of daily food) were applied to the related groups for 6 weeks after the induction of metabolic syndrome (1). At the end of the 18 weeks, the rats were sacrificed. Blood samples and liver tissue were taken and examined.

### Blood Glucose Analysis

At the beginning of the experiment (Day 0), in the 3rd month (Week 12), and end of the experiment (Week 18), blood samples

were taken from the orbital veins of rats under light ether anesthesia, and blood glucose levels were measured by glucometer (Accu-Chek, F. Hoffman-La Roche Ltd, Basel, Switzerland).

### Biochemical Analysis

Liver tissue homogenates at 10% (w/v) were prepared. Glutathione (GSH) (16), lipid peroxidation (LPO) (17), sialic acid (SA) (18), hexosamine (19), mucin (19) and fucose (20,21) levels, superoxide dismutase (SOD) (22), catalase (CAT) (23), glutathione-S-transferase (GST) (24), alkaline phosphatase (ALP) (25), and tissue factor (TF) (26) activity in supernatants were measured by spectrophotometric methods.

### Statistics

Statistical analysis was performed using GraphPad Prism 5.0 (GraphPad Software, San Diego, California, USA). All data were expressed as means  $\pm$  standard deviation. An ANOVA analysis of variance was used for comparison of multiple groups; a Tukey test was used for binary comparisons between groups. A  $p$  value  $<0.05$  was regarded as significant.

## RESULTS

The blood glucose values at the beginning of the experiment (Day 0) was found at similar levels in all groups. There was no statistically significant difference between them. In Week 12, the blood glucose levels of the M, ME, MCR, and MECR groups, which were given 10% fructose, increased significantly compared with those at the beginning of the experiment (Day 0). In Week 18, blood glucose levels in the ME, MCR and MECR groups decreased significantly compared with those of Week 12 (Figure 1).

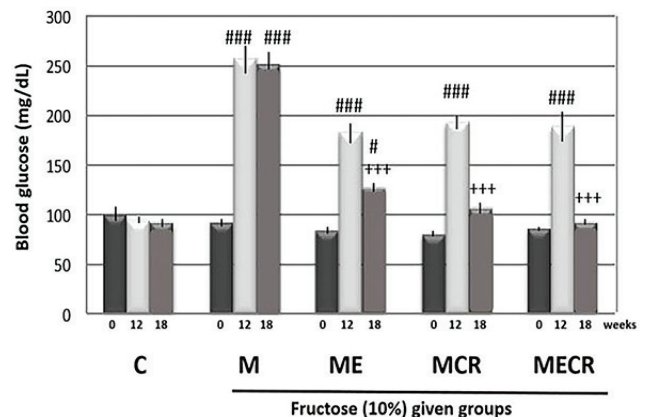


Figure 1. Blood glucose values.

Values were given as mean  $\pm$  SD. C: Control group, M: Metabolic Syndrome, ME: Metabolic syndrome with exercise, MCR: Metabolic syndrome with caloric restriction, MECR: Metabolic syndrome with exercise and caloric restriction. SD: Standard deviation. Dark gray bar: At day 0, White bar: At 12<sup>st</sup> week, Light gray bar: At 18<sup>th</sup> week. # $p < 0.05$ , ### $p < 0.001$ : significantly different from at day 0; +++ $p < 0.001$ : significantly different from at 12<sup>st</sup> week.

In liver tissue, LPO levels increased and TF activities decreased in the M group compared with the C group. Other tissue damage parameters had no significant difference between the C group and the M group. LPO levels decreased in the ME, MCR, and MECR groups compared with the M group. TF activities decreased in the ME group compared with the C group; it increased in the MCR and MECR groups compared with the M and ME groups. Because the clotting time is inversely proportional to the TF activity, the prolonged clotting time is due to lower TF activity. SA levels increased in the MCR and MECR groups compared with the M group. Fucose levels also increased in the MECR group compared to the others (Figure 2).

In liver tissue, GSH levels decreased in the M group compared to the C group and increased in the ME, MCR, and MECR groups compared to the M group. SOD activities decreased in the M, ME, and MCR groups compared to the C group and increased in MECR compared to the M, ME, and MCR groups. CAT activities decreased in the M and ME groups compared to the C group and increased in the MCR and MECR groups compared to the M and ME groups. GST activities decreased in the MECR group compared to the C, M, and ME groups (Figure 3).

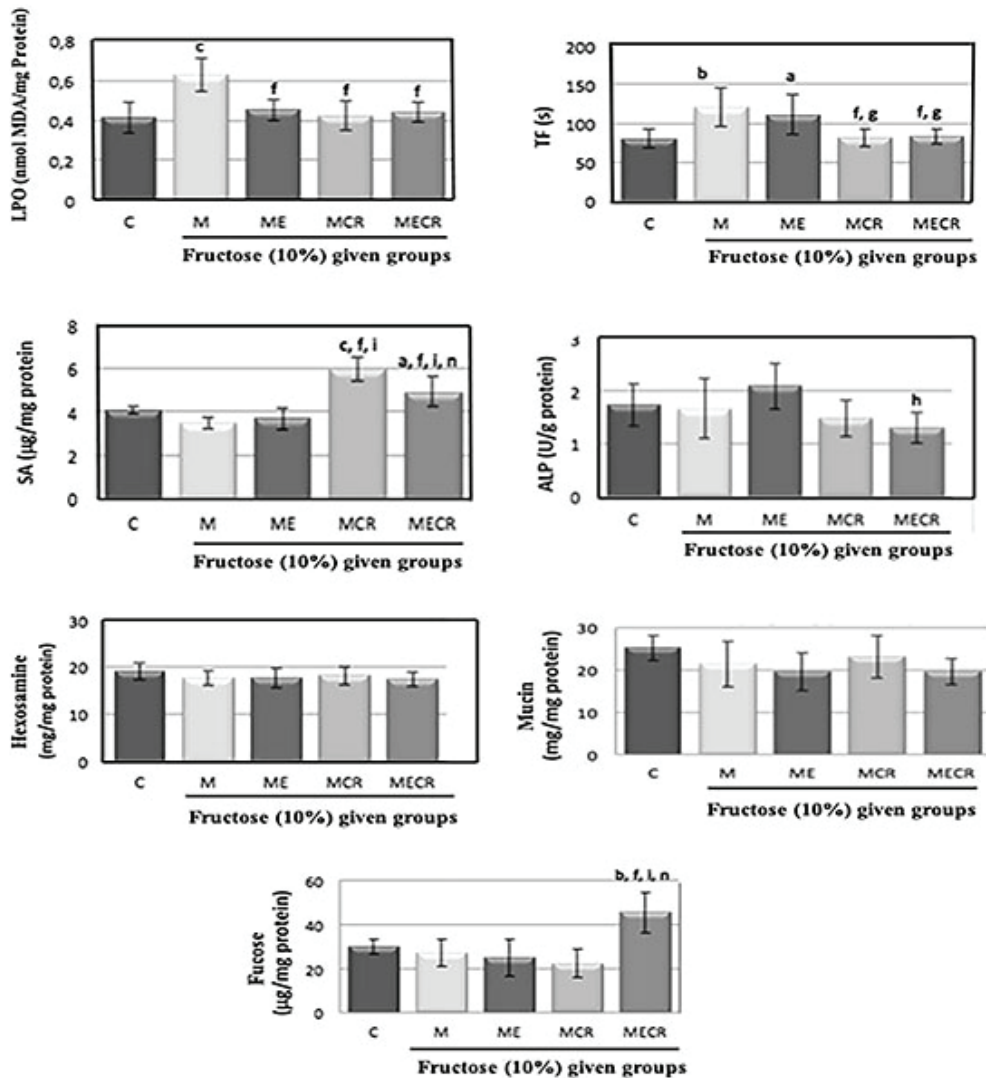


Figure 2. Liver tissue damage parameters.

Values were given as mean±SD. LPO: Lipidperoxidation, MDA: Malondialdehyde, TF: Tissue factor, SA: Sialic acid, ALP: Alkalen phosphatase, C: Control group, M: Metabolic Syndrome, ME: Metabolic syndrome with exercise, MCR: Metabolic syndrome with caloric restriction, MECR: Metabolic syndrome with exercise and caloric restriction. s: Second. SD: Standard deviation. <sup>a</sup>*p*<0.05, <sup>b</sup>*p*<0.01, <sup>c</sup>*p*<0.001 significantly different from group C; <sup>d</sup>*p*<0.05, <sup>e</sup>*p*<0.01, <sup>f</sup>*p*<0.001 significantly different from group M; <sup>g</sup>*p*<0.05, <sup>h</sup>*p*<0.01, <sup>i</sup>*p*<0.001 significantly different from group ME; <sup>j</sup>*p*<0.05, <sup>k</sup>*p*<0.01, <sup>l</sup>*p*<0.001 significantly different from group MCR; <sup>m</sup>*p*<0.05, <sup>n</sup>*p*<0.001 significantly different from group MECR.

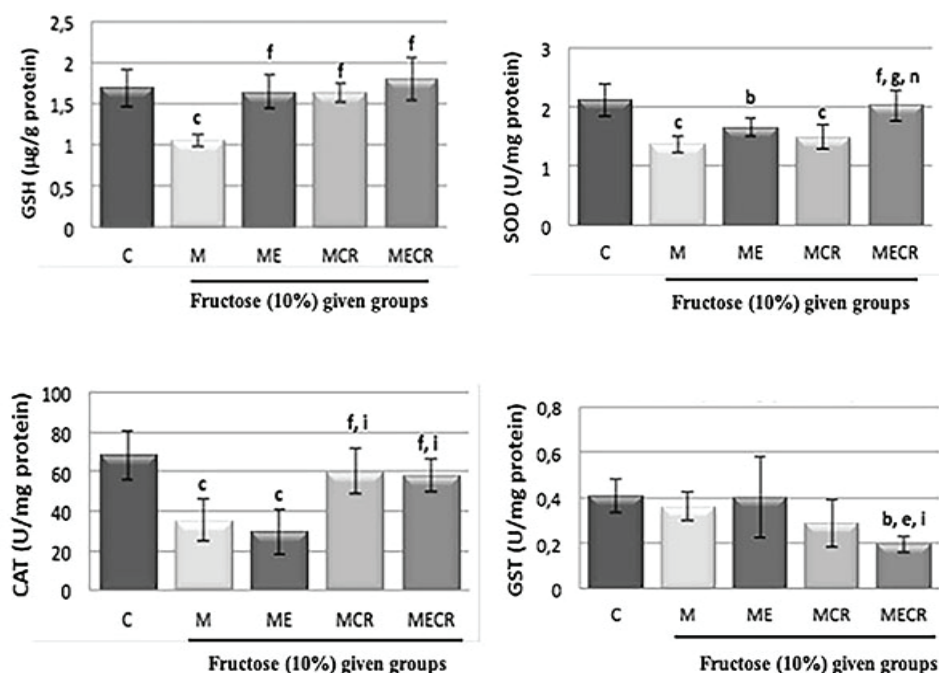


Figure 3. Liver antioxidant parameters.

Values were given as mean  $\pm$  SD. GSH: Glutathione, SOD: Superoxide dismutase, CAT: Catalase, GST: Glutathione-S-transferase, C: Control group, M: Metabolic Syndrome, ME: Metabolic syndrome with exercise, MCR: Metabolic syndrome with caloric restriction, MECR: Metabolic syndrome with exercise and caloric restriction. SD: Standard deviation. <sup>a</sup> $p < 0.05$ , <sup>b</sup> $p < 0.01$ , <sup>c</sup> $p < 0.001$  significantly different from group C; <sup>d</sup> $p < 0.05$ , <sup>e</sup> $p < 0.01$ , <sup>f</sup> $p < 0.001$  significantly different from group M; <sup>g</sup> $p < 0.05$ , <sup>h</sup> $p < 0.01$ , <sup>i</sup> $p < 0.001$  significantly different from group ME; <sup>j</sup> $p < 0.05$ , <sup>k</sup> $p < 0.01$ , <sup>l</sup> $p < 0.001$  significantly different from group MECR.

## DISCUSSION

Metabolic syndrome is an endocrinopathy characterized by insulin resistance, abdominal obesity, hyperinsulinemia, hypertension, and dyslipidemia (1). This disease emerges with a modern lifestyle, characterized with overeating and physical inactivity. Specifically, fructose rich diets are known to trigger other diseases with obesity (27).

Either a high fructose containing diet or drinking water is the most commonly used method to create a metabolic syndrome model with experimental animals (28-31). Fructose, depending on the dose and duration of use, is associated with a broad spectrum of pathologies such as glucose intolerance, insulin resistance, dyslipidemia, hypertension, and fatty liver, as well as indirectly clogging of heart and brain vessels (31, 32).

Recent studies have shown that being fed water containing 10% fructose for 6 or 8 weeks has led to an increase of triglyceride (TG), insulin, and glucose levels in plasma/serum (33-35). There was a study where a fructose diet caused an increase of a homeostatic model assessment (HOMA) value, endothelial dysfunction, and smooth muscle proliferation (36). It has also been shown that high fructose increased oxidative stress in the serum in rats (8, 29, 37, 38). In agreement with these studies, we reported that after being fed 10% fructose for 18 weeks, Week 12 and 18 blood glucose values were found to be significantly

higher compared to the beginning of the experiment. The end of the experiment blood glucose of the rats in the MECR group decreased compared with Week 12 blood glucose.

Reactive oxygen species (ROS) cause oxidative stress and lead to oxidative damage in various tissues. This case leads to the depletion of the antioxidant system elements (SOD, CAT, vitamins C and E, reduced GSH), the development of cell injury induced by various inflammatory mediator molecules, organ dysfunction, and death. Kannappan et al. reported an increase in plasma glucose, insulin levels, and HOMA values as well as an increase in MDA, LPO, and PCO levels of livers and a decrease in GSH, SOD and vitamin E and C levels, CAT, GPx (glutathione peroxidase), glutathione reductase, and GST activity in rats fed with a diet containing 60% fructose for 2 months (8). In our study, in accordance with these studies, we found an increase in LPO levels and a decrease in GSH levels and CAT and SOD activity in the livers of rats given drinking water containing 10% fructose. We found no significant difference in GST activity.

There have been several studies that have examined the beneficial effects of exercise on human health (39-41). Energy production and consumption increase parallel to muscle activity. Oxygen consumption increases directly proportional to the intensity of exercise. Also, during exercise, production of ROS increases because of increased oxygen consumption. It has been pointed out that acute heavy exercise may cause tissue damage



and the rise of LPO, but regular physical activity can increase antioxidant capacity and can decrease LPO (40-42).

Alipour et al. have designed a running exercise for 8 weeks in a group of rats in which they had induced diabetes. They found increased values of LPO in the hippocampus zone of the rats for the diabetes and diabetes+exercise groups compared to the control group of rats. SOD, CAT, and GPx activities were detected to have decreased in the diabetic group and to have increased in the exercise group compared with the control. SOD and GPx activities increased significantly in the diabetes + exercise group compared with the diabetic group (43). In our study, the blood glucose levels of the exercise group of Week 18 decreased compared to those of Week 12. Although this decrease with exercise was significant, blood glucose levels remained significantly higher in the exercise group compared with the control group.

Lima et al. have designed a running exercise for 9 weeks in one group of rats, which they have created a model of diabetes. Increased TG levels in the livers of diabetic rats decreased in the diabetic rats that exercised. It was reported that SOD and CAT activities increased in the exercise group compared with the control and also in the diabetes+exercise group compared with the diabetic group (44). In our study, only increased significantly compared with the level of GSH in the ME group while the LPO levels were significantly decreased in the liver. Our study has shown that in liver tissue, the levels of GSH of the exercise group increased significantly and that the levels of LPO decreased significantly compared to the M group. There is no significant difference in the SOD, CAT, and GST activities. Moreover, SOD and CAT activities decreased compared to the control. Exercise was not enough to improve the activities of antioxidant enzymes in the livers of rats with metabolic syndrome. The exercise protocol may be heavy to rats with metabolic syndrome, and antioxidant enzymes in the liver may be caused by the loss of activity.

Caloric restriction is the reduction of food intake lower than the level of ad libitum, without becoming malnutrition. The generation of free radicals and oxidative damage is reduced by caloric restriction (45). Therefore, caloric restriction is a method used in research in order to prevent or delay the onset of diseases such as diabetes, cancer, and cardiovascular disease (46). Caloric restriction preserves  $\beta$ -cell function, prevents the onset of diabetes caused by the excessive production of free radicals, and provides treatment, all of which makes it clinically important (47). It is suggested that caloric restriction can improve the inflammatory state of the liver in mice with mild fibrotic livers and that of aged mice (48).

Mohammadi et al. reported that SOD and GPx activity increased significantly and LPO levels decreased in the livers of rats to which caloric restriction was applied (49). In this study we found an increase in GSH levels and CAT activities and a decrease in LPO levels of the MCR group compared with the M group in liver tissues. However, we found no difference in SOD and GST

activities. Caloric restriction and exercise applied separately is not enough to increase liver antioxidant enzyme levels of rats with metabolic syndrome.

TF is a transmembrane receptor and cellular initiator of coagulation extrinsic pathways (50). TF plays a main role in thrombosis and thrombogenesis (51). TF is known to have different activities in various tissues and bodily fluids and is affected by diet and systemic diseases (52-55). In the method of determination of TF activity, it should be noted that the clotting time is inversely proportional to the TF activity (52-55). Otherwise, it may cause a misunderstanding of the results. In the liver, the TF activity of the M and ME groups decreased compared with the C group. The TF activity increased in the MCR and MECR groups compared with the M and ME groups.

Another parameter studied in the liver tissue is ALP. There was no significant difference between the M and C group. ALP is one of the tests used as indicators of cholestasis rather than the destruction of liver cells (56). We found an increase in the livers of the ME group, but it was not significant. The reason for this could be a physiologic increase of ALP activity after exercise (57). ALP values decreased in the MECR group compared to the ME group. Exercise with caloric restriction seems to be the most effective way to reduce the level of ALP.

SA is a nine-carbon sugar derived from neuraminic acid, and it forms the terminal sugar component of glycoproteins and glycolipids. Sialidase activity increases as a result of oxidative stress, and it cleaves SA from end portions of glycoproteins and glycolipids and leads to the increase in free SA in body fluids (58). Serum SA levels were found to be increased in many other diseases associated with inflammation, such as Behcet's disease, central nervous system diseases, cardiovascular diseases, bacterial infections, and rheumatoid arthritis (59). The SA concentrations in the blood of patients with metabolic syndrome have been reported to be significantly higher (60). In this study, in liver tissue, there was no significant difference between the C and M groups in the levels of SA. Liver tissue SA values were detected to be significantly increased in the MCR and MECR groups compared with the M and ME groups.

Carbohydrates play a central role in the development of chronic diabetic complications. Glycoproteins, which are carbohydrate-linked protein macromolecules found on the cell surface, are one of the principal components of animal cells. Hexose, hexosamine, fucose, and sialic acid are the basic sugar components found in glycoproteins and glycosaminoglycans. Glycoprotein metabolism plays a major role in the pathogenesis of diabetes mellitus. Glycoproteins have multiple and complex functions and are found as hormones, enzymes, and blood group substances and as constituents of extracellular membranes (61, 62). They play an important role in functions such as cell differentiation and recognition, membrane transport, and the absorption of macromolecules (63). In a hyperglycemic state, high blood glucose levels accelerate the synthesis of basement membrane components, such as glycoproteins (64). In the liver,

we found no differences in hexosamine and mucin parameters, but fucose increased significantly in the MECR group compared with all other groups. Exercise plus caloric restriction did not reduce the fucose value, rather it increased even further.

## CONCLUSION

Liver tissue damage that occurs after a fructose diet and decreased antioxidant levels were shown to be improved best in combined exercise and caloric restriction treatment (MECR group).

**Peer-review:** Externally peer-reviewed.

**Conflict of Interest:** The authors declare that they have no conflicts of interest to disclose.

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