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# Brain in metabolic syndrome model: The effect of exercises and caloric restriction

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**ABSTRACT**: Caloric restriction (CR) and exercise (EX) have impacts on improving metabolic risk factors. This study aimed to investigate the changes in the brain after EX and/or CR in metabolic syndrome (MeS) induced by a high fructose diet in rats. Sprague-Dawley male rats were divided into five groups. Drinking water including 10% fructose solution was given to rats for 12 weeks to develop a MeS rat model. Animals with MeS were submitted to EX and/or CR for 6 weeks. Blood glucose, and brain tissue damage and antioxidant parameters were measured. Brain lipid peroxidation, sialic acid, mucin, fucose levels increased in the MeS group compared to the control (C) group. These parameters reduced significantly in the metabolic syndrome with caloric restriction (MeS+CR) group, and more significantly in the metabolic syndrome with exercise and caloric restriction group (MeS+EXCR), compared to the MeS group. Glutathione levels, superoxide dismutase and catalase activities decreased in the MeS group compared to the C group, increased both in the MeS+CR group, and MeS+EXCR group compared to the MeS group. High fructose diet consumption can lead to brain tissue damage and decreased antioxidant levels were found to be improved best in the MeS+EXCR group.

**KEYWORDS**: Metabolic syndrome; Brain; Antioxidant; Oxidative stress; Exercise; Caloric restriction

## 1. INTRODUCTION

Metabolic syndrome (MeS) is diagnosed clinically when patients have at least three of the following co-morbidities: hypertension, obesity, elevated triglycerides, hyperglycemia, and decreased high-density lipoprotein cholesterol [1]. Although the causes of MeS are multifaceted, high-fructose diets, sedentary lifestyles, and genetic predispositions are all significant risk factors [2]. It is widely recognized that insulin resistance is the primary pathogenic mechanism underlying the first stage of metabolic changes in MeS. In the recent an association between low-level inflammation, and oxidative stress with MeS has been shown [3,4].

The role of brain oxidative stress in metabolic disease development is a relatively recent concept [5]. There are some studies showing that fructose consumption influences brain health adversely by promoting brain mitochondrial dysfunction, insulin resistance, neuroinflammation, and oxidative stress [6-8]. The brain is more vulnerable to oxidative damage than other organs because of its high oxygen intake, its high content of unsaturated fatty acids and its abundance of redox-active transition metal ions [9].

Since underlying causes of MeS are various, there is no single approach to prevent or treat it. Adjusting lifestyle factors such as diet and exercise seems to be the only option for overcoming individual risk factors [10]. Exercise (EX) is an effective method to treat obesity, hypertension, glucose tolerance, dyslipidemia, heart function, and systemic inflammation, which are the components of MeS in both humans and animals [11,12]. Caloric restriction (CR) has become a common recommendation for losing weight, improving metabolism, and possibly extending life expectancy [13], since it reduces free insulin and glucose causing a reduction in

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white adipose tissue mass [14]. Despite the relevance of this issue, no study has been found on the potential effect of isolated and combined intervention of EX and CR on brain oxidative stress in rats with MeS.

The goal of this study was, to determine in MeS model, the effects of EX or CR alone and in combination on rat brain biochemical parameters.

## 2. RESULTS

Glucose levels were measured for the assessment of MeS development. At the beginning of the study (week 0), there were no differences in the blood glucose levels between the groups. In all groups with MeS, blood glucose levels were increased compared to the initial blood glucose levels. Blood glucose levels of the metabolic syndrome with exercise (MeS+EX), metabolic syndrome with caloric restriction (MeS+CR), metabolic syndrome with exercise and caloric restriction (MeS+EXCR) groups decreased compared with the MeS group in week 12 and week 18. Both of interventions seemed to decrease blood glucose levels when compared to the MeS group (Table 1).

**Table 1.** Blood glucose levels of rats in different experimental groups

| Groups | Days | | |
|---|---|---|---|
| | **Week 0 (mg/dL)** | **Week 12 (mg/dL)** | **Week 18 (mg/dL)** |
| C | 100±6.9 | 94±3.2 | 91±4.1 |
| MeS | 92±4.1 | 255±13.4*** | 249±15.2*** |
| MeS+EX | 84±3.9 | 181±9.6*** | 125±6.4*### |
| MeS+CR | 80±3.3 | 192±6.5*** | 105±6.7### |
| MeS+EXCR | 85±2.6 | 188±15.6*** | 92±3.6### |

Values were given as mean ± SD. C: Control group (n=5), MeS: Metabolic Syndrome (n=11), MeS+EX: Metabolic syndrome with exercise (n=9), MeS+CR: Metabolic syndrome with caloric restriction (n=8), MeS+EXCR: Metabolic syndrome with exercise and caloric restriction (n=12) SD: Standard deviation. *p<0.05, ***p<0.001: significantly different from in week 0; ###p<0.001: significantly different from in week 12

In our study, the brain oxidative damage was assessed by the tissue lipid peroxidation (LPO), sialic acid (SA), hexosamine, mucin, fucose levels and tissue factor (TF) activity. Also, cells' ability to counteract reactive oxygen species (ROS) for cellular protection was evaluated by measuring superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST) activities and glutathione (GSH) level.

In the brain tissues LPO, SA, mucin and fucose levels were increased in the MeS group compared to the C group. They were decreased in the MeS+CR and MeS+EXCR groups compared to the MeS group. Although hexosamine values had no significant difference between the C and MeS groups, a significant reduction was observed in the MeS+CR and MeS+EXCR groups compared with the C, MeS and MeS+EX groups. TF activity was increased in the MeS+EX group compared with the control group (C) (Figure 1).

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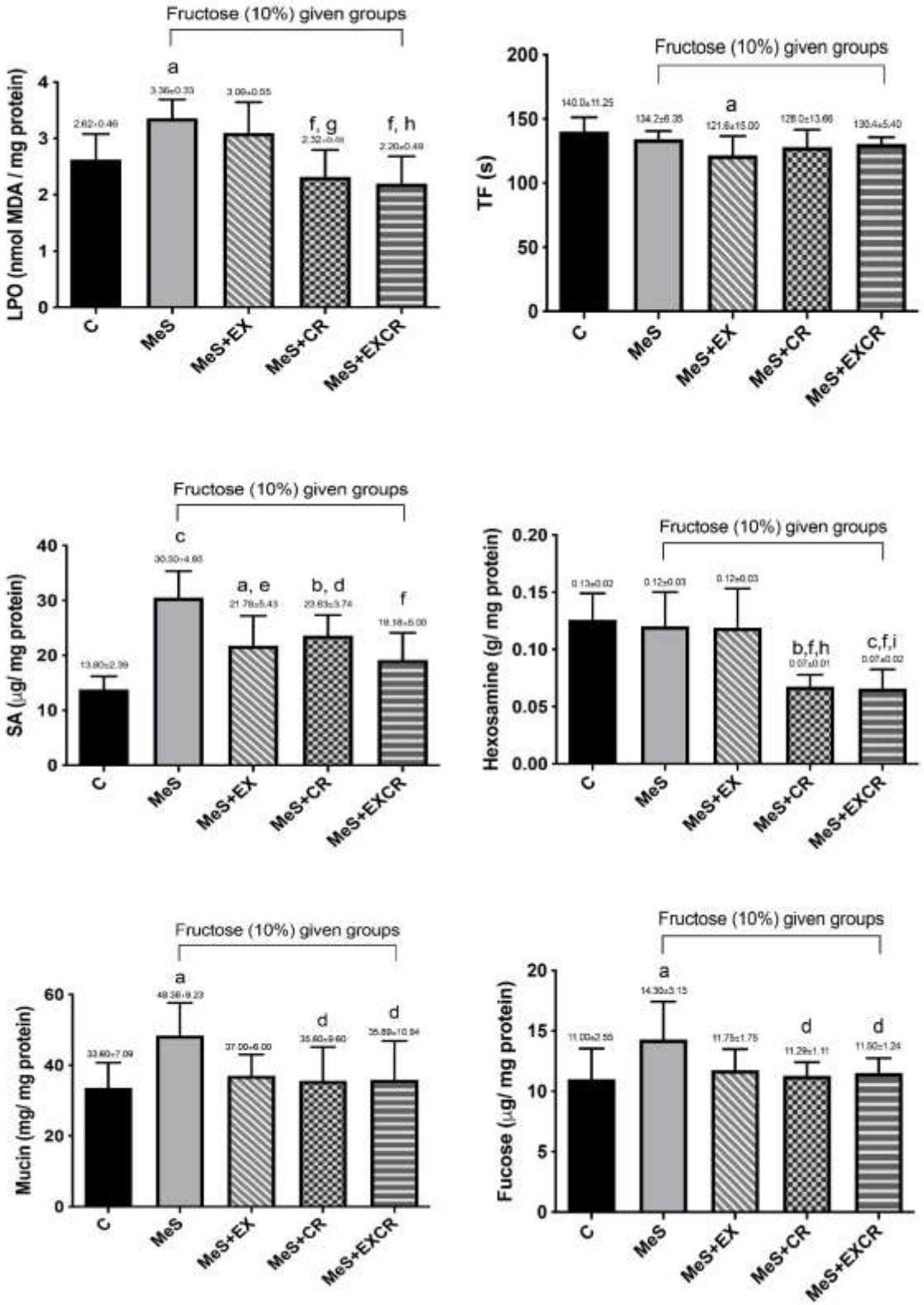


**Figure 1.** Brain tissue damage parameters
Values were given as mean ± SD. LPO: Lipid peroxidation, MDA: Malondialdehyde, TF: Tissue factor, SA: Sialic acid, C: Control group (n=5), MeS: Metabolic Syndrome (n=11), MeS+EX: Metabolic syndrome with exercise (n=9), MeS+CR: Metabolic syndrome with caloric restriction (n=8), MeS+EXCR: Metabolic syndrome with exercise and caloric restriction

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(n=12). s: Second. SD: Standard deviation. [a]p<0,05, [b]p<0,01, [c]p<0,001 significantly different from group C; [d]p<0.05, [e]p<0.01, [f]p<0.001 significantly different from group MeS; [g]p<0.05, [h]p<0.01, [i]p<0.001 significantly different from group MeS+EX

In the brain tissues SOD, CAT and GST activities and GSH levels, decreased in the MeS group compared to the C group. GSH levels, and SOD and CAT activities increased in the MeS+CR and MeS+EXCR groups compared to the MeS group. Also, GSH levels and CAT activities increased in the MeS+EX group compared to the MeS group. GST activities only increased in the MeS+EXCR group compared to the MeS group (Figure 2).

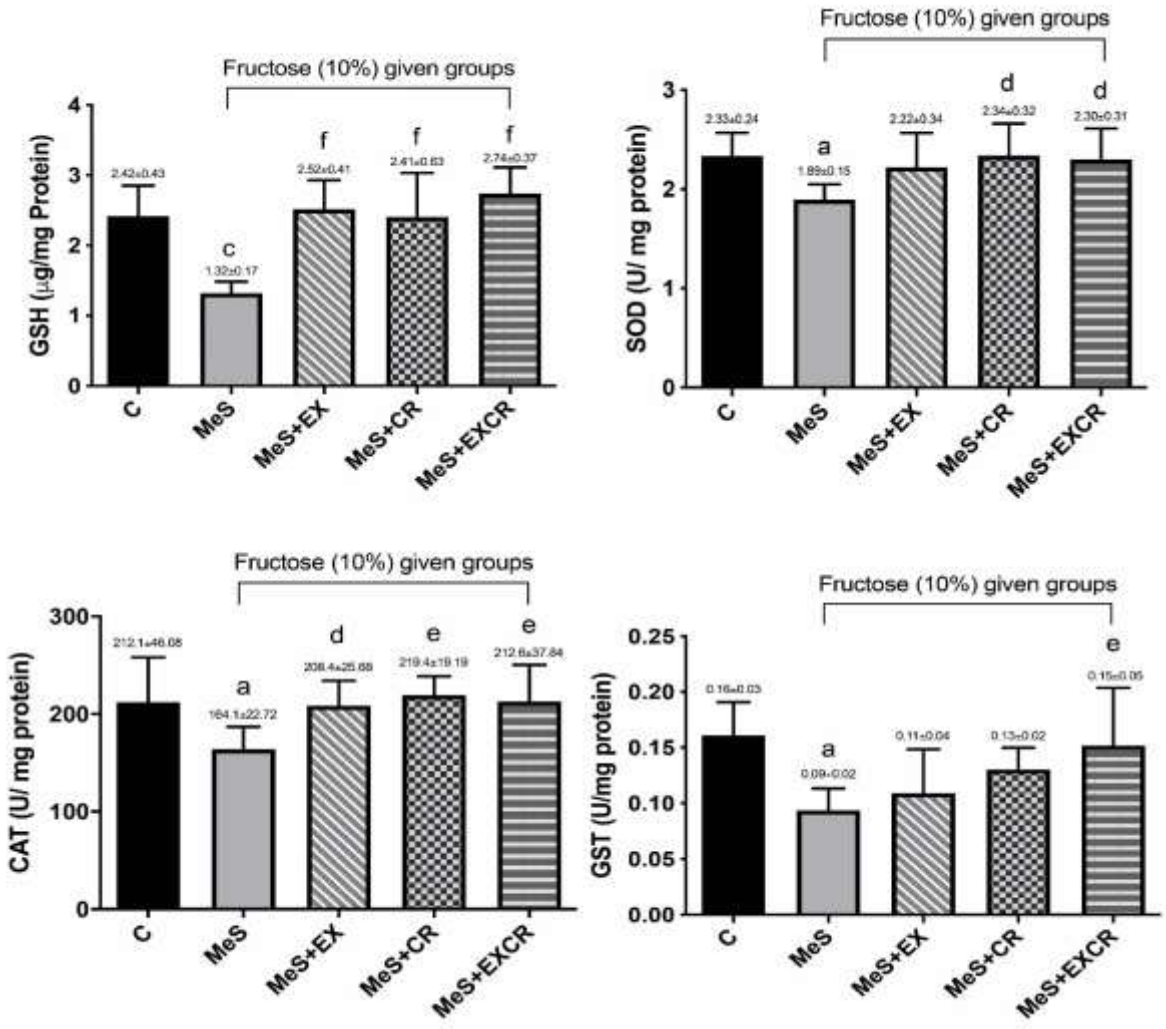


**Figure 2.** Brain antioxidant parameters
Values were given as mean ± SD. GSH: Glutathione, SOD: Superoxide dismutase, CAT: Catalase, GST: Glutathione-S-transferase, C: Control group (n=5), MeS: Metabolic Syndrome (n=11), MeS+EX: Metabolic syndrome with exercise (n=9), MeS+CR: Metabolic syndrome with caloric restriction (n=8), MeS+EXCR: Metabolic syndrome with exercise and caloric restriction (n=12). SD: Standard deviation. ap<0,05, bp<0,01, cp<0,001 significantly different from group C; dp<0.05, ep<0.01, fp<0.001 significantly different from group MeS; gp<0.05, hp<0.01, ip<0.001 significantly different from group MeS+EX

## 3. DISCUSSION

In this study, we demonstrated that MeS increases tissue damage and decreases antioxidant parameters in the brain. The findings of the presented study showed that caloric restriction with exercise might protect brain tissue against fructose-induced MeS damage by ameloriating these changes.

MeS, which is contributed by excessive calorie intake and sedentary lifestyle, has become a serious public health problem. Since fructose consumption has greatly increased in recent years, the presented study was designed to examine the effects of high fructose diet on oxidative stress in the brain, and the ability of EX and/or CR to counteract MeS.

High fructose diets are thought to be one of the key factors that contribute to the development of MeS by inducing oxidative stress, which leads to hypertriglyceridemia, insulin resistance, and obesity [15]. Fructose-fed rats have been reported as a rat model of MeS [16]. The meta-analysis studies in the literature confirms that, fructose consumption results in an increase in rodent body weight, systolic blood pressure and blood glucose, insulin and triglyceride levels [17]. Earlier studies showed the metabolic effect induced by consumption of fructose drinking water 10% for 8-12 weeks [18-20]. In this study follows on from our previous study, in which hepatic oxidative stress in MeS investigated [21], administration of drinking water with 10% fructose concentration was found to cause significant increase in blood glucose in week 12 and week 18 in the MeS group.

High fructose concentrations have been reported to reach the brain and affect brain homeostasis. The effects of such diets on the brain are poorly understood but it is possible that fructose enhanced insulin resistance in the brain by upregulating fructose-sensitive glucose transporters 5 expression [5]. Insulin resistance affects the brain's energy and neuroprotective mechanisms [22].

Furthermore, it is well known that a high-fat, high-fructose diet leads to the increased free radicals and the decreased antioxidant levels, resulting in a redox imbalance and oxidative stress [9]. These resultant oxidative stresses accelerate oxidative DNA damage by lipid peroxidation products and deterioration of the structure and function of the intracellular biomolecules, organelles, enzymes and cell membranes [7]. The increased insulin resistance together with hepatic mitochondrial oxidative damage has been found in rats fed a high-fructose diet [23].

Oxidative stress has a significant impact on brain integrity [24]. Fructose leads to overeating and therefore increased production of reactive oxygen species in the brain [25]. It was thought that high glucose levels, which increase free radical production from glucose autoxidation, might be linked to increased brain lipid peroxidation in high fructose fed rats [5]. In studies that evaluated brain antioxidant enzymes, SOD, CAT, glutathione peroxidase (GPx) activities and GSH levels have been shown to decrease and LPO and protein carbonylation levels to increase in the brain tissue [5,26,27]. In agreement with these studies, we reported significant increases in LPO levels, and decreases in GSH, CAT, SOD and GST levels in the brain tissues of fructose fed rats. The decreased efficiency of enzymatic and non-enzymatic brain antioxidants may be a response to increased production of $H_2O_2$ by the autoxidation of excess glucose and non-enzymatic glycation of proteins [5]. The increase in lipid peroxidation also suggests increased oxidative stress in the brain of MeS group rats.

EX is an effective intervention for maintaining a healthy body and normal brain activity. It can help to reduce inflammation and oxidative stress. This modulation may be one of the pathways responsible for improving a variety of clinical conditions, such as decreasing cellular aging and increasing insulin sensitivity [28]. An EX regimen of four weeks, has been shown to reduce certain cardiovascular risk factors such as hyperglycemia, obesity, and hypertension [29]. Furthermore, exercise reduced ROS and normalized its components, such as thiobarbituric acid reactive substances, SOD, CAT, and GPx in the brain of hyperphenylalaninemic rats [30]. It has also been suggested that the frequency and duration of EX are determinants of the reversal of metabolic disorders in the experimental MeS model induced by the fructose diet [31]. Alipour et al. have designed a running exercise for 8 weeks in a group of rats which they have induced diabetes [32]. They have found that LPO levels increased in the the diabetic groups in the hippocampus zone of the rats compared to the control group. Enzymatic activities of GPx, CAT and SOD were found to be reduced in the diabetic group, and raised in the EX group compared with the control. Activities of SOD and GPx were found to be increased significantly in the diabetes + exercise group compared to the diabetic group [32]. In our study, blood glucose levels in week 18 were decreased compared to week 12 in the MeS+EX group, however, at the end of the experiment, blood glucose levels were still higher than the C group. Brain GSH levels and CAT activities were significantly increased in the MeS+EX group compared to the MeS group. No significant differences were observed comparing the MeS+EX group to the MeS group for LPO levels, and SOD and GST activities. EX did not alter the brain antioxidant enzymes activities in rats with MeS. This may suggest that antioxidant enzymes keep the consumption capacity of reactive oxygen species. SOD and GST activities increased slightly in the MeS+EX group, yet not significantly.

CR is a dietary intervention that reduces caloric intake while avoiding malnutrition. In rodents, reduced calorie intake intervention by 10-30% extends lifespan [33]. It has been suggested that CR may extend life span by reducing cellular oxidative stress. CR has been shown to reduce mitochondrial DNA, protein, and lipid oxidative damage [34]. Therefore, CR is the one of the most suitable interventions to prevent age-related diseases such as cancer, diabetes, cardiovascular disease [35]. Xia et al. reported decreased levels of LPO; increased activities of SOD and CAT in cerebral cortex, heart and kidney tissue of rats that have been applied

CR of 40% [36]. In another study, Ramkumar and Anuradha found decreased liver LPO levels, increased liver SOD, CAT, GPx activities and GSH levels in food-restricted rats compared with ad libitum control groups after carbon tetrachloride exposure [37]. In our study, at the end of the experiment blood glucose levels were decreased significantly compared to week 12 in the MeS+CR group. In agreement with these studies mentioned in the literature, in our study, GSH levels, and SOD and CAT activities were increased in the MeS+CR group compared to the MeS group and LPO levels were decreased compared to the MeS and MeS+EX groups. The reduced levels of LPO in the MeS+CR group can be related to the better defense offered by increased levels of antioxidant enzymes. Also increased GSH levels in the MeS+CR group indicate a positive change in the brain's redox status.

CR and EX interventions in MeS patients lead to weight loss, decrease in visceral fat and inflammatory markers [12]. In addition, physical exercise and diet have beneficial effects on neuronal function, mitochondrial health, and redox homeostasis. It is also thought to be protective against Alzheimer's disease [28]. In our study, at the end of the experiment blood glucose levels were decreased significantly compared to week 12 in the MeS+EXCR group. Moreover, GSH levels, and SOD, CAT and GST activities were increased significantly in the MeS+EXCR group compared to the MeS group and LPO levels were decreased significantly compared to both the MeS and MeS+EX groups. EX accompanied with CR was found to be a better approach for improving antioxidant-oxidant balance compared to only EX or only CR intervention.

TF is a transmembrane protein known as primary cellular initiator of blood coagulation [38]. It plays an essential role in thrombosis and thrombogenesis [39]. Diet and systemic diseases are known to affect TF, which has different activity levels in various tissues and body fluids. [40-42]. Weight loss from diet, exercise, and gastric bypass surgery increases insulin sensitivity while also lowering circulating levels of prothrombotic markers like TF [43]. However, it has been shown that exercise activates the hemostatic system depending on the type, duration and intensity of physical exercise [44]. It should be remembered that when determining TF activity, the clotting time is inversely proportional to the TF activity. In our study, there were no significant diferences in the brain TF activities between the C and MeS groups, but the activities of TF in the MeS+EX group increased significantly compared to the C group. This may be indicating the increased tendency to coagulation in the brain.

SA is an acetylated derivative of neuraminic acid with a nine-carbon backbone. It is presented in the terminal sugar chains in glycoproteins and glycolipids. The increase in free SA in body fluids reflects increased secretion of SA from the cell membrane due to elevated of sialidase activity, which is linked to oxidative stress [45]. Following inflammation or tissue damage, SA levels were found increased [46,47]. Also, in MeS patients blood SA levels were reported to be significantly higher [48]. In this study, we detected that SA levels increased significantly in the MeS group compared to the C group in the brain tissue. Inflammation may be a potential reason of high SA levels in MeS. It is well acknowledged that inflammation represents one of the underlying mechanisms in MeS [49]. In the brain tissues of the MeS+EX, MeS+CR and MeS+EXCR groups, a significant decrease in SA levels compared to the MeS group was also observed.

Glycoproteins are carbohydrate-linked protein macromolecules that are found on the cell surface. The oligosaccharide moieties of glycoproteins such as fucose, hexose, hexosamine, and sialic acid, are well known for their importance in protein function, stability, and turnover [50]. They also participate in various biological events such as membrane transport, cell differentiation and recognition [51]. Many pathological conditions, including diabetes, change the carbohydrate structure of glycoprotein components. Chandramohan et al. found a significant increase in the levels of plasma hexose, hexosamine, SA, and fucose in STZ-induced diabetic control rats compared to normal control rats. In the study, it was stated that insulin deficiency and high plasma glucose levels in diabetic condition might cause an increase in the synthesis of glycoprotein components. In our study, we found that mucin and fucose levels increased in the MeS group compared to the C group and decreased in the MeS+EXCR group compared to the MeS group. However, we found no differences in hexosamine levels between the MeS and C groups, however it was decreased in MeS+CR and MeS+EXCR groups compared to the C, MeS and MeS+EX groups.

## 4. CONCLUSION

In the present study, CR with EX is an effective option to ameliorate tissue damage in brain in many different aspects of MeS, such as by decreasing LPO, SA, hexosamine, mucin, fucose levels and increasing antioxidants such as SOD, CAT, GST and GSH.

## 5. MATERIALS AND METHODS

### 5.1. Chemicals

In the study, all chemicals were of reagent grade and used without further purification. They were supplied by Merck, Sigma-Aldrich, Fluka and were as follows: D-fructose (Merck 5321), albumin (Merck 112018), 5,5'-dithiobis (2-nitrobenzoic acid) (Sigma D8130), 2-thiobarbituric acid (Fluka 88481), riboflavine (Sigma 47861), hydrogen peroxide (Merck 108600), 1-chloro-2,4-dinitrobenzene (Fluka 24440), sodium meta-periodate (Fluka 71860), glucosamine (Fluka 49130), fucose (Sigma-Aldrich F8150), orcinol monohydrate (Sigma-Aldrich O1875) and L-cysteine (Merck 2839).

### 5.2. Animals, Diets and Experimental Design

Forty-five Sprague-Dawley male rats were used and all animal procedures were approved by the Marmara University Animal Care and Use Committee (101.2013.mar- Ethics Committee Decision Number). The animals were kept under controlled environmental conditions with free access to food and water. The standard pellet diet consisted of 24% protein, 8% crude ash, 7% cellulose, 1- 2.8% calcium, 2% HCl insoluble ash, 1% sodium chloride, 0.9% phosphorus, 0.5-0.7% sodium (Çobançeşme Feed Industry Factories, Istanbul). Drinking water containing lysine (1%) and methionine (0.6%). Provided metabolic energy was 2650 kcal/kg.

Rats were randomly divided into five groups (n=5-12 per group) Group I: C, Group II: MeS, Group III: MeS+EX, Group IV: MeS+CR, Group V: MeS+EXCR. Animals of the experimental groups received drinking water containing 10% fructose for 12 weeks in order to induce MeS, and the control group only received drinking water. For 6 weeks after the induction of MeS, the rats of related groups were submitted to swimming exercise for 30 min 3 times/week and/or caloric intake was reduced by 40% when compared to control animals. The diets and interventions allocated to groups are given in the Table 2. At the end of 18 weeks, the rats were sacrificed, blood samples and brain tissues were collected (Figure 3).

**Table 2.** Experimental groups and their corresponding diets and interventions

| Group | Diet | Intervention |
|---|---|---|
| C | Drinking water + Standard pellet feed | - |
| MeS | 10% Fructose containing drinking water + Standard pellet feed | - |
| MeS+EX | | EX |
| MeS+CR | | CR |
| MeS+EXCR | | EX+CR |

C: Control group (n=5), MeS: Metabolic Syndrome (n=11), MeS+EX: Metabolic syndrome with exercise (n=9), MeS+CR: Metabolic syndrome with caloric restriction (n=8), MeS+EXCR: Metabolic syndrome with exercise and caloric restriction (n=12), EX: Swimming for 3 days/week for 30 min each day CR: 40% restricted calorie intake

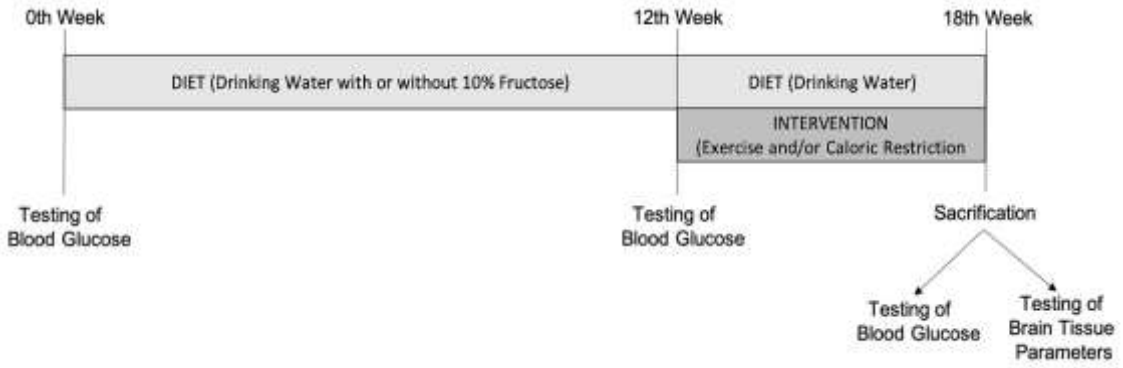


**Figure 3.** Experimental timeline

### 5.3. Blood Glucose Analysis

Blood samples were collected from animals at the 0, 12 and 18 weeks and glucose levels were measured (Accu-Chek Glucometer, Switzerland).

### 5.4. Brain Biochemical Analysis

The brain tissue samples were homogenized using saline solution (0.9% NaCl) to make up to a 10% (w/v) homogenate. Then the homogenates were centrifuged and the supernatants were used for biochemical analysis. In brain homogenates, GSH levels were determined by the Ellman's method [52], LPO and SA levels were measured by the methods of Ledwozyw et al. [53] and Warren [54], respectively. Fucose levels of the brain were evaluated according to the method of Dische and Shettles [55] and hexosamine and mucin contents were estimated by the method of Winzler [56]. CAT activity was described by the methods of Aebi [57]. The activities of GST and SOD were measured by the Habig and Jakoby [58], and Mylorie et al. [59] methods, respectively. TF activities of the brain tissues were performed according to Quick's one-step methods using healthy plasma [60]. TF activities were expressed in seconds. The prolonged clotting time was due to lower TF activity.

### 5.5. Statistics

The power analysis was performed to determine the minimum sample size before starting the study. Kolmogorov-Smirnov test and Levene's test were employed to check for normality and homogeneity of variances, respectively. Due to the normal distribution ANOVA analysis of variance was used for the comparison of multiple groups, a Tukey test was used for the binary comparisons between groups. All data were expressed as means ± standard deviation (SD), and $p<0.05$ indicates statistical significance. Statistical analysis was performed using GraphPad Prism 5.0 (GraphPad Software, San Diego, California, USA). In addition, IBM SPSS version 24.0 (IBM Corp., Armonk, N.Y., USA) was also utilized. Experimenters were blinded to the experimental groups.

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**Author contributions:** Concept – G.S., A.Y.; Design – G.S., A.Y.; Supervision – A.Y.; Resources – A.Y.; Materials –G.S., E.E.A., T.T.A., A.Y; Data Collection and/or Processing B.A.T., N.G.K., H.I., U.V.U.; Analysis and/or Interpretation – G.S., E.E.A., T.T.A., A.Y; Literature Search – B.A.T., A.Y; Writing – B.A.T., A.Y.; Critical Reviews B.A.T., N.G.K., H.I., U.V.U., G.S., E.E.A., T.T.A., A.Y.

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