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THE EFFECT OF ENVIRONMENTAL ENRICHMENT AND WESTERN DIET ON THE TRACE ELEMENT STATUS AND OXIDATIVE STRESS IN RATS

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ABSTRACT

Objective: *The Western diet (WD), which is rich in high-fat diet (HFD) and high-sucrose diet (HSD), is related to oxidative stress. Enriched environment (EE) with social interaction, physical exercise and continuous learning tasks has been shown to reduce oxidative stress, the inflammatory response, and increase the anti-oxidative defense. Therefore, the present study has aimed to clarify the effects of the EE and WD-fed rats on marker malondialdehyde (MDA) and trace element (TE) levels (iron [Fe], copper [Cu], zinc [Zn], chromium [Cr], selenium [Se], magnesium [Mg] and molybdenum [Mo]).*

Material and Method: *Male Wistar albino rats were housed in either an enrichment (n=24) or standard environment (n=24) and fed with HFD (35% of energy as fat) (n=8), HSD (100% of carbohydrate as sucrose) (n=8), or standard rat chow(n=8), for 4 weeks. Inductively-coupled plasma mass spectrometry was used for determination of the serum TE levels.*

Results: *The serum levels of MDA ($p<0.05$), Fe, Cu, Mo and Mg increased, while the serum levels of Cr and Se decreased, and additionally, the serum levels of Zn did not changed in the HFD and HSD groups. EE decreased partially the serum levels of MDA, Fe, Mo, and did not affect the serum levels of Cu, while it increased the serum levels of Mg, Cr, Se and Zn; however, there was no significant difference between all of the experimental groups (all; $p>0.05$).*

Conclusions: *Our study demonstrated that HFD and HSD led to oxidative stress and adversely affected the serum level of TE in rats, and that the EE reversed partially this status.*

Keywords: *Trace elements, western diet, malondialdehyde, oxidative stress.*

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RATLARDA ZENGİNLEŞTİRİLMİŞ ÇEVRE VE BATI TİPİ DİYETİN ESER ELEMENT DURUMU VE OKSİDATİF STRESE ETKİSİ

ÖZET

Amaç: Batı tipi diyet, yüksek yağlı diyet (HFD) ve yüksek sukrozlu diyetten (HSD) zengin olup oksidatif stres ile ilgilidir. Sosyal etkileşim, fiziksel egzersiz ve sürekli öğrenme görevleri ile zenginleştirilmiş çevrenin (EE) oksidatif stres ve inflamatuvar cevabı azalttığı buna karşın antioksidatif durumu iyileştirdiği yapılan çalışmalarda gösterilmiştir. Bu çalışmada ratlarda zenginleştirilmiş çevre ve Batı tipi diyetin oksidatif stres göstergesi malondialdehid (MDA) ve eser element seviyelerine (demir [Fe], bakır [Cu], çinko [Zn], krom [Cr], selenyum [Se], magnezyum [Mg] ve molibden [Mo]) etkisini araştırmayı amaçladık.

Materyal ve Metot: Kırk sekiz erkek Wistar suşu sıçan 350±30 g ve yaşları 10-12 hafta ağırlığında olup, ya zenginleştirilmiş (n=24) veya standart çevresi (n=24) olan bir odada yüksek yağlı diyet (toplam enerjinin% 35'i iç yağı) (n=8), HSD (karbohidrat türevi enerjinin %100'ü sukroz) (n=8), ve standart sıçan yemi (n=8), ile 4 hafta beslendi. Serum eser element seviyeleri kütle spektrometresi ile tesbit edildi.

Bulgular: HFD ve HSD ile beslenen grupta, serum MDA (p<0,05), Fe, Cu, Mo ve Mg seviyeleri artmışken, serum Cr ve Se seviyeleri azalmış ve ilaveten, serum Zn seviyesi etkilenmemişti. Zenginleştirilmiş çevre kısmi olarak serum MDA, Fe ve Mo seviyelerini azaltırken Mg, Cr, Se ve Zn seviyelerini artırmış, fakat Cu seviyesini etkilememişti. Bununla birlikte tüm deneysel gruplar arasında anlamlı fark yoktu (p>0,05).

Sonuç: Bizim çalışmamız HFD ve HSD ile beslenme oksidatif strese yol açmış ve serum eser element seviyelerini etkilemiş, zenginleştirilmiş çevre ise bu durumları kısmi olarak tersine çevirdiğini göstermiştir.

Anahtar kelimeler: Batı tipi diyet, eser elementler, malondialdehid, oksidatif stres.

INTRODUCTION

Obesity, mainly triggered by a diet rich in high-fat and/or refined sugar (commonly known as Western diet), has been a pandemic disorder in Western countries.[1] A high-fat diet (HFD) and high-sucrose diet (HSD) rise the oxidative stress and drop the antioxidative enzyme activity, and have been linked by many complications, including metabolic syndrome (MS), type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD), cancer and non-alcoholic fatty liver disease.[2,3] HFD and HSD give rise to hypertriglyceridemia, hyperglycemia, hyperinsulinemia and hyperleptinemia.[2,4] Enhanced plasma glucose, free fatty acids (FFA) and triglycerides (TG) also lead to increased reactive oxygen species (ROS) production, as well as startle insulin resistance (IR).[4] HSD and HFD feeding would lead to increased adiposity and visceral fat mass causing inflammation and cytokine secretion.[4,5] In several chronic human diseases such as atherosclerosis, cancer, neurodegeneration and the aging process, a significant contributing element is oxidative stress.[6] Oxidative stress can be described as an increase in oxidants and/or a decrease in antioxidants, and harms to biomolecules, which contribute to cellular changes in the pathogenesis of many diseases including diabetes, CVD, and ischemia reperfusion injury.[4,7]

Pro-oxidative processes are also attenuated and anti-oxidative defense mechanisms in the Alzheimer's disease model are triggered by environmental enrichment (EE) with social interaction, physical exercise and continuous learning tasks.[8] Cechetti *et al.* [8] demonstrated that hypoperfusion give rise to an increase in free radical level and basal TBARS in hippocampus and that these values decline to normal levels with exposure to an EE. However, animals kept under EE have also been indicated to have lowered aggression, stress and excitability, as well as better learning skills than those maintained under standard situations.[9]

Trace elements (TE) are basic nutrients with regulatory, immunological and antioxidant functions.[10] TE have greater importance in multitude of biological processes involving signal transduction (zinc [Zn]), enzymology (copper [Cu], Zn, selenium [Se]) and structural integrity (Zn, iron[Fe]).[11] TE activate or preclude enzymatic reactions, modulate cell membrane permeability, take part in electron transport and the synthesis of hormones and vitamins.[7] TE are involved in the protection against inflammation and peroxidation, comprising major elements in the development of the metabolic complications of obesity such as arterial hypertension, dyslipidemia, IR and diabetes.[10] The relationship between the EE and the TE has not yet been studied.

TE are present in the structure of some enzymes that prevent oxidative stress. As noted literature, the EE may have reduced the use of antioxidant defense system including TE due to reducing oxidative stress.[8] The roles of a HFD, HSD and EE on serum levels of malondialdehyde (MDA) and TE (Fe, Cu, Se, magnesium [Mg], chromium [Cr], molybdenum [Mo]

and Zn) in rats have not been studied yet. In this study, our aim is to predict the effects of a HFD, a HSD and EE on serum levels of MDA and TE (Fe, Se, Mg, Cr, Mo, Cu and Zn) in rats.

Materials and Methods

Experimental Groups and Diets

Forty-eight Wistar rats weighing 350±30 g, and aged 10-12 weeks, were purchased from the Necmettin Erbakan University Experimental Medical Research and Application Center (Konya, Turkey). The rats were accommodated in a chamber under climate control (temperature of 22±2° C and humidity of %50±5), with a 12/12 dark/light cycle (light was provided between 07:00 and 19:00 hours), in which sufficient food and water were provided. The animals were divided randomly into EE (n=24) and "standard condition" (SC; n=24) groups and nutrition was provided either with the standard rat chow, HFD or HSU (for each, n=8) for a period of 4 weeks. In HFD, %35 of the total energy was provided with the internal fat, whereas in HSD, %100 of the carbohydrate type energy was provided by sucrose. The proportionally adjusted foods were purchased from the local supplier in ready-to-use packages (Nukleon Ltd., Ankara, Turkey). All of the procedures (1996 revision no.: 86-23) were carried out abiding by the National Institutes Health Guide for the Care and Use of Laboratory Animals, and the study was approved by the Necmettin Erbakan University Institutional Board of Animal Care and Use (Konya, Turkey) (date: 12.02.2014/ number: 2014-017).

Accommodation Conditions

The animals in the EE group were accommodated in specially designed cages measuring 90x75x45 cm in dimensions. These cages contained wooden, plastic and metal objects such as turning wheels, toys, platforms, tunnels, balls and stairs. Rising in socializing was supplied with group housing (8 rats in each cage). In order to provide environmental renovation, the locatins of the objects were changed in the cages twice a week. The SC groups were accommodated in Eurostandard Type IV polycarbonate rat cages measuring 60x38x20 cm in dimensions, each cage accommodating 4 rats, with no exciting objects inside. The rats were stayed in these experimental conditions for 4 weeks. During the period of the experiment, food and fresh water (standard or specific to the group of rats) were used for both groups as much as it was needed. Anesthesia was given to the rats with a single inraperitoneal injection of the combination of Ketamine and Xylazine (60 and 10 mg/g, respectively). Blood samples were drawn through a cardiac puncture, and transferred to tubes with EDTA. Cold centrifugation was performed at 4° C for 10 minutes at 3000 rpm and the sera were separated. The serum samples were then stored at -80° C until the time for the serum trace elements measurements and biochemical analyses.

Measurement of Behavioral and Cognitive Findings

Rats were put the left quadrant of a 40x40x40-cm open-field produced white wood. Black lines were drawn on the floor to divide it into 10 equal quadrants. For locomotor activity, crossing the quadrant lines were measured over a period of 5 min. Rats were first practised individually in the diveces and left to freely investigate it for 10 min during four sequential days before the training period. In the training period, two different objects (A and B) were put in the divece, and rats were let to freely investigate for 5 min. After 3 h one of the diveces was randomly replaced for a novel diveces (C and D), the rats were reintroduced into the apparatus for 5 min. The time spent exploring the familiar and the novel diveces was recorded.[12]

Measurement of Serum Trace Element Levels

Measurement of the serum TE (Fe, Cu, Zn, Cr, Se, Mg and Mo) was carried out using the ELAN DRC-e (Perkin Elmer SCIEX Inc., Ontario, Canada) inductively-coupled plasma mass spectrometry (ICP-MS) system. Furthermore, multiple element standard solution was used for IV and ICP (PerkinElmer Pure Plus, PerkinElmer Life and Analytical Sciences, USA). The serum samples were then mineralized using the MARS microwave digestion system (CEM Corporation, 3100 Smith Farm Road, Matthews, NC 28105–5044, USA). The operating conditions for the ICP-MS were: Rf power (watts), 1,100.00; plasma gas flow (litres per minute), 15.00; nebulizer gas flow (litres per minute), 0.82; auxiliary gas flow (litres per minute), 1.20; scanning mode: peak hopping, dwelling time (milliseconds), 50; sweeps/reading, 20; number of replicates, 3; read delay time (seconds), 15; cell gas, 0.05; DRC gas flow, 0; rejection parameter q, 0.25; sampling depth, 0.5 cm and sample volume per microlitre, 2.5 mL.

Malondialdehyde Measurements

Evaluation of the oxidative status of the rats was made through determination of the MDA levels. MDA measurement is considered a standard marker in determination of the oxidative lipid damage and lipid peroxidation. The serum MDA level was measured using the Draper and Hadley method.[13] The results were calculated as micromoles per liter.

Table 1. Comparison of parameters of the all groups

Parameter	Control	HSD	HFD	EE- Control	EE- HSD	EE- HFD	*p*
TC (mg/dl)	48.63 ± 6.61	58.75 ± 7.91	65.88 ± 11.80	45.63 ± 5.26	48.25 ± 8.65	65.00 ± 10.80	<0.001
TG (mg/dl)	57.75 ± 9.68	109.29 ± 16.41	66.88 ± 5.36	53.38 ± 8.09	71.50 ± 17.81	52.75 ± 7.36	<0.001
LDL-C (mg/dl)	10.31 ± 2.68	14.03 ± 2.38	20.55± 6.84	10.15 ± 3.87	12.18 ± 2.76	19.15 ± 5.52	<0.001
HDL-C (mg/dl)	38.63 ± 5.29	28.00 ± 3.12	31.13 ± 2.59	39.38 ± 4.50	30.75 ± 3.37	33.25 ± 3.99	<0.001
AST (U/L)	87.00 ± 12.32	96.00 ± 10.35	93.88 ± 13.78	87.13 ± 8.10	85.38 ± 10.74	87.88 ± 16.33	0.432
ALT (U/L)	66.13 ± 12.04	67.00 ± 6.60	69.38 ± 14.44	65.75 ± 10.14	68.13 ± 33.19	60.75 ± 8.40	0.935
MDA (nmol/µL)	2.60 ± 0.34	3.89 ± 0.23	3.77 ± 0.71	2.44 ± 1.26	2.17 ± 0.67	2.24 ± 0.87	<0.001

[1]All values are mean ± standart deviation.
HSD: High-sucrose diet, **HFD:** high-fat diet, **EE:** enriched environment, **TC:** total cholesterol, **TG:** total trigliserid, **LDL-C:** low density lipoprotein-cholesterol, **HDL-C:** high density lipoprotein-cholesterol, **AST:** aspartate aminotransferase, **ALT:** Alanine aminotransferase, **MDA:** malondialdehyde

Biochemical analyses

Measurements of the levels of serum glucose, triglycerides (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), aspartate-amino-transferase (AST) and alanin amino transferase (ALT) were made using the commercial kits in accordance with the routine method in the Architect C 8000 System (Abbott Laboratories, Abbott Park, Illinois, USA).

Table 2. Comparison of parameters of the groups

Parameter	Controls	HSD	HFD
TC (mg/dl)	48.63 ± 6.61	58.75±7.91	65.88 ± 11.80*
TG (mg/dl)	57.75 ± 9.68	109.29 ± 16.41**	66.88 ± 5.36
LDL-C (mg/dl)	10.31 ± 2.68	14.03 ± 2.38	20.55± 6.84*
HDL-C (mg/dl)	38.63 ± 5.29	28.00 ± 3.12***	31.13 ± 2.59***
AST (U/L)	87.00 ± 12.32	96.00 ± 10.35	93.88 ± 13.78
ALT (U/L)	66.13 ± 12.04	67.00 ± 6.60	69.38 ± 14.44
MDA (nmol/µL)	2.60 ± 0.34	3.89 ± 0.23****	3.77 ± 0.71****

[1]All values are mean ± standart deviation.
HSD: High-sucrose diet, **HFD:** high-fat diet, **TC:** total cholesterol, **TG:** total trigliserid, **LDL-C:** low density lipoprotein-cholesterol, **HDL-C:** high density lipoprotein-cholesterol, **AST:** aspartate aminotransferase, **ALT:** Alanine aminotransferase, **MDA:** malondialdehyde
**p*<0.005 compared with controls. ***p*<0.001 compared with control and HFD groups.
****p*<0.005 compared with controls. *****p*<0.05 compared with controls

Table 3. Comparison of parameters of the EE groups

Parameter	EE- Controls	EE- HSD	EE- HFD
TC (mg/dl)	45.63 ± 5.26	48.25 ± 8.65	65.00 ± 10.80*
TG (mg/dl)	53.38 ± 8.09	71.50 ± 17.81**	52.75 ± 7.36
LDL-C (mg/dl)	10.15 ± 3.87	12.18 ± 2.76	19.15± 5.52*
HDL-C (mg/dl)	39.38 ± 4.50	30.75 ± 3.37***	33.25 ± 3.99***
AST (U/L)	87.13 ± 8.10	85.38 ± 10.74	87.88 ± 16.33
ALT (U/L)	65.75 ± 10.14	68.13 ± 33.19	60.75 ± 8.40
MDA (nmol/µL)	2.44 ± 1.26	2.17 ± 0.67	2.24 ± 0.87

[1]All values are mean ± standart deviation.
EE: Enriched environment, **HSD:** high-sucrose diet, **HFD:** high-fat diet, **TC:** total cholesterol, **TG:** total trigliserid, **LDL-C:** low density lipoprotein-cholesterol, **HDL-C:** high density lipoprotein-cholesterol, **AST:** aspartate aminotransferase, **ALT:** Alanine aminotransferase, **MDA:** malondialdehyde
**p*<0.01 compared with EE-control and EE-HSD groups. ***p*<0.05 compared with EE-control and EE-HFD groups. ****p*<0.01 compared with EE-controls

Statistical Analysis

All data were calculated as mean±standard deviations. The statiscal analyses were carried out with the SPSS v. 16.0 (SPSS Inc., IL, USA). Comparison of the group data was made using the analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. A p value of < 0.05 was considered as a significant difference.

RESULTS

Neither HFD nor HSD didn't change the total distance movement (i.e. swimming path length) and swimming swiftness, and in the training period they were not so significant amidst all groups (data not shown; $p>0.05$). The EE improved HFD-induced cognitive disorders ($p<0.05$). There was no difference between the groups for the total distance movement and the swimming swiftness in the probe experiment ($p>0.05$).

The weights of the animals were reported weekly. At the end of 4 weeks, the body weights of the animals were increasingly rose, but there was no significant difference in body weight alterations in all experimental groups when they were compared with the control rats at the end of the study (all; $p>0.05$). There wasn't significant difference in the serum levels of glucose in all experimental groups compared with the control rats at the end of the study (all; $p>0.05$).

The serum lipids, glucose and MDA levels of the rats have been illustrated in Table 1. The ANOVA test indicated that there were significant differences in the serum levels of TG, TC, LDL-C and HDL-C ($p<0.001$) and MDA ($p<0.005$) between the experimental groups, while there were no differences in serum AST and ALT levels between the experimental groups (p=0.432 and p=0.935, respectively).

As seen as Table 2 and Table 3, we found that the serum levels of TG significantly increased in HSD groups compared with control and HFD groups ($p<0.001$),

Table 4. Serum trace elements levels of the all groups

Parameter	Control	HSD	HFD	EE- Control	EE- HSD	EE- HFD	*p*
Fe (ppb)	6684.10 ± 1486.28	7797.74 ± 3942.98	7929.86 ± 1970.62	6052.88 ± 1928.40	6803.49 ± 2265.90	6448.53 ± 2378.74	0.590
Cu (ppb)	1975.71 ± 160.11	2034.62 ± 297.40	2285.54 ± 556.40	2064.31 ± 338.21	2293.99 ± 188.65	2159.89 ± 558.37	0.450
Zn (ppb)	1577.48 ± 103.37	1699.36 ± 212.63	1587.38 ± 286.18	1723.12 ± 864.04	2175.70 ± 440.27	1824.01 ± 552.88	0.156
Cr (ppb)	480.94 ± 36.97	456.55 ± 20.88	468.64 ± 68.72	531.71 ± 132.37	551.95 ± 55.83	537.33 ± 96.68	0.077
Se (ppb)	678.84 ± 39.25	652.54 ± 26.92	627.77 ± 71.87	742.04 ± 189.84	701.56 ± 73.61	638.19 ± 113.63	0.229
Mg (ppb)	14362.23 ± 1290.49	14427.66 ± 529.44	15529.72 ± 3049.55	18181.36 ± 4666.64	17821.04 ± 2077.53	16254.39 ± 4329.99	0.062
Mo (ppb)	29.82 ± 4.64	32.94 ± 7.49	36.12 ± 12.46	22.20 ± 4.98	27.05 ± 11.82	31.45 ± 11.70	0.082

[1]All values are mean ± standart deviation.
HSD: High-sucrose diet, **HFD:** high-fat diet, **EE:** enriched environment
Fe: Demir; Cu: Bakır; **Zn:** çinko; Cr: krom; **Se:** selenyum; **Mg:** magnezyum; **Mo:** molibden

and in EE- HSD groups compared with EE-control and EE-HFD groups ($p<0.05$). On the other hand, the serum levels of TC and LDL-C significantly increased in the HFD group compared with the control groups ($p<0.005$), and in EE-HFD groups compared with the EE-control and EE-HSD groups ($p<0.01$). We also noticed a significant decrease in serum levels of HDL-C in the HSD and HFD groups compared with the control groups ($p<0.005$), and in the EE-HFD and EE-HSD groups compared with EE-control groups ($p<0.01$).

Similarly, the serum levels of MDA significantly increased in the HFD and HSF groups compared with the control groups ($p<0.05$), and decreased in the EE-HFD and EE-HSD groups compared with EE-control groups; however, there were no significant differences ($p>0.05$). As a result, EE decreased the serum levels of MDA, TG, TC and LDL-C, and increased the serum levels of HDL-C.

The serum TE levels of the rats have been demonstrated in Table 4. We found that the serum levels of Fe, Cu, Mo and Mg increased not significant in the HFD and HSF groups, but there were no significant differences in the serum levels of Fe, Cu, Mo and Mg compared with the control rats. EE decreased the serum levels of Fe and Mo, but there was no significant difference among the experimental groups (all; $p=0.590$ and $p=0.082$, respectively). EE did not affect the serum levels of Cu, and there was no significant difference amidst the experimental groups (all; $p=0.450$). EE increased the serum levels of Mg, but there was no significant difference among the experimental groups (all; $p=0.062$). HFD and HSD did not affect the serum levels of Zn; moreover, EE increased the serum levels of Zn, but there was no significant difference among the experimental groups (all; $p=0.156$). While HFD and HSD decreased the serum levels of Cr and Se, EE increased the serum levels of Cr and Se; however, there was no significant difference among the experimental groups (all; $p=0.077$ and $p=0.229$, respectively).

As seen as Table 5, we found that the serum levels of Zn, Cr, Cu, Se and Mg increased and Fe and Mo decreased not significant in the EE-HSD groups compared with HSD groups. On the other hand, the serum levels of MDA significantly decreased in the EE-HSD groups compared with HSD groups ($p=0.002$).

In Table 6, we found that the serum levels of Zn, Cr, Se and Mg increased and MDA, Fe, Cu and Mo decreased not significant in the EE-HFD groups compared with HFD groups.

Table 5. Comparison of parameters of the HSD and EE- HSD groups

Parameter	HSD	EE- HSD	*p*
MDA (nmol/µL)	3.89 ± 0.23	2.17 ± 0.67	0.002
Fe (ppb)	7797.74 ± 3942.98	6803.49 ± 2265.90	0.964
Cu (ppb)	2034.62 ± 297.40	2293.99 ± 188.65	0.755
Zn (ppb)	1699.36 ± 212.63	2175.70 ± 440.27	0.369
Cr (ppb)	456.55 ± 20.88	551.95 ± 55.83	0.164
Se (ppb)	652.54 ± 26.92	701.56 ± 73.61	0.926
Mg (ppb)	14427.66 ± 529.44	17821.04 ± 2077.53	0.251
Mo (ppb)	32.94 ± 7.49	27.05 ± 11.82	0.811

[1]All values are mean ± standart deviation.
HSD: High-sucrose diet, **HFD:** high-fat diet, **EE:** enriched environment
MDA: Malondialdehyde; **Fe:** Demir; Cu: Bakır; **Zn:** çinko; **Cr:** krom; **Se:** selenyum; **Mg:** magnezyum; **Mo:** molibden

Table 6. Comparison of parameters of the HFD and EE- HFD groups

Parameter	HFD	EE- HFD	*p*
MDA (nmol/µL)	7929.86 ± 1970.62	6448.53 ± 2378.74	0.445
Fe (ppb)	2285.54 ± 556.40	2159.89 ± 558.37	0.831
Cu (ppb)	1587.38 ± 286.18	1824.01 ± 552.88	0.986
Zn (ppb)	468.64 ± 68.72	537.33 ± 96.68	0.920
Cr (ppb)	627.77 ± 71.87	638.19 ± 113.63	0.501
Se (ppb)	15529.72 ± 3049.55	16254.39 ± 4329.99	1.000
Mg (ppb)	36.12 ± 12.46	31.45 ± 11.70	0.970
Mo (ppb)	HFD	EE- HFD	0.919

[1]All values are mean ± standart deviation.
HFD: High-fat diet, **EE:** enriched environment
MDA: Malondialdehyde; **Fe:** Demir; **Cu:** Bakır; **Zn:** çinko; **Cr:** krom; **Se:** selenyum; **Mg:** magnezyum; **Mo:** molibden

DISCUSSION

The effect of EE and diet on serum TE level is not obvious yet. To the best of our knowledge, this was the first study on the effects of EE and diet on serum MDA, lipid profiles and the TE status. In the present study, we investigated the effects of EE and Western diet (HFD and HSD-fed rats) on the serum MDA, lipid profiles and TE levels.

As anticipated, HFD induced dyslipidemia, which is appraised by the increased TC, TG, and phospholipids in the serum and also promoted a oxidative stress status and an increase in MDA.[14] In our study, we found that while the serum levels of TG significantly increased in the HSD groups compared with the other groups, TC and LDL-C significantly increased in the HFD group. Besides, the serum levels of HDL significantly decreased in both HSD and the HFD groups compared with the control rats. Our finding is in conformity with the findings of prior experimental studies. In a study by Amin *et al.*, the serum levels of MDA, TC, TG and LDL-C significantly increased in the HFD and HSD groups compared with those in the controls.[15] Kang *et al.* figured out that the serum levels of TG and TC increased significantly with HFD feeding compared to the control animals.[16] A significant decrease in serum Se levels was observed in animals fed on HFD in comparison to the controls. In another study, the serum LDL-C concentrations were significantly higher in both the HSD and the HFD groups than in the controls, and the HSD fed animals had significantly higher serum TG and TC levels than the other groups. The serum levels of HDL-C in the HFD group were importantly lower than those of the control and HSD groups.[17]

EE increases social communication, physical exertion and permanent exposure to learning processes, making beneficial effects including increase in learning and angiogenesis, while it diminishes oxidative stress and the inflammatory response.[8] Zeeni *et al.* found that the stress-induced increase in the corticosterone and adrenocorticotropic hormone decreased in the presence of EE. [9] In this study, we found that MDA also significantly elevated in HSD fed rats compared to the controls. It also elevated in HFD, but there was no significant difference between the HFD fed rats, controls and the HSD fed rats. EE caused a decrease in the serum MDA levels of controls, HFD and HSD fed rats, but there was no significant difference between the groups. EE decreased the serum levels of MDA, TG, TC and LDL-C, and increased the serum levels of HDL-C. Sweazea *et al.* have reported that the levels of plasma thiobarbituric acid reactive substances also significantly elevated in both HSD and HFD fed rats.[4]

Trace elements lead to peroxidation and inflammation in the pathogenesis of obesity and its complications.[18]

Functional cofactors of different antioxidant enzymes are TE.[7] The HFD treatment also influenced the decrease in catalase (CAT), Glutathione peroxidase (GPx), and Cu/Zn-superoxide dismutase (Cu/Zn-SOD) activities, and leading to ROS accumulation. Above all, HFD cure unexpectedly increased the Fe-SOD isoform, which acts as a pro-oxidative enzyme.[19] In our study, while the serum levels of Fe, Cu, Mo and Mg increased, the serum levels of Cr and Se decreased, and the serum levels of Zn were not affected in the HFD and HSD groups. Se and Zn has essential anti-inflammatory, anti-hyperlipidemic, anti-atherogenic and antioxidant actions to keep against varied oxidative and nitrosative stress.[20,21] Cu and Zn are cofactors of the antioxidant CuZn-SOD enzyme to catalyze the $O_2^{\cdot}$- to oxygen and H_2O_2.[21] Gursel *et al.* found that the serum Mg, Cu and Fe levels were significantly higher in rats fed with HFD than in the control group on day 45.[20] Additionally, serum Zn levels were not significantly different between the two groups. Dietary lipids have remarkable effects on mineral absorption and bioavailability.[20] In another study, HFD increased the plasma Zn by 133%, Cu by 733%, $O_2^{\cdot}$- by 66%, OH by 63% and MDA by 28%, and decreased the free Fe by 15%. HFD also confined the plasma antioxidant enzyme activities such as GPx by 25% and SOD by 17%.[22] Charradi *et al.* found that HFD decreased liver Zn by 92%, GPx by 17 % and SOD by 40%, and had only a minimal (not significant) effect on Cu, while increased the MDA by 37%.[23] As far as this result is concerned, HFD induced a obvious oxidative stress characterized by high MDA, and depressed the GPx and Cu/Zn-SOD activities and decreased Zn in the liver. Cu deficiency significantly increases the vulnerability of lipoproteins and cardiovascular tissues to lipid peroxidation.[24] CAT is an Fe-binding enzyme that catalyzes the conversion of H_2O_2 to water and oxygen. Cu deficiency can bring about a reduction in CAT activity in tissues, such as the heart and the liver.[24] As antioxidants, Se may dwindle oxidative stress, ROS production and inflammation.[25] Se which is a part of selenoproteins (such as GPx, thioredoxin reductase and selenoprotein P), and protects lipids from oxidation.[10] Azab *et al.* found that obese and overweight children had lower serum Se, Fe and Zn levels compared to controls, which can contribute to low antioxidant protection.[10] Additionally, serum Cu levels were significantly higher than those of controls. Fe is an intrinsic component of hemoglobin, myoglobin and cytochromes, and is a prosthetic group of CAT.[7] According to the study of Pinhas-Hamiel *et al.* overweight and obese children revealed an increased prevalence of Fe deficiency.[26] That Fe interacts with minerals such as Zn and Cu is well known fact. Among

the rats fed the High Fat, Fructose, and Salt Diets, distinctly higher serum level of Fe and lower levels of Zn and Cu were noticed in the tissues.[27] In another study, compared to the normal groups, all groups with obesity, MS or T2DM elevated levels of Cu, but Mg concentrations were paltry. Furthermore, participants with overweightedness/obesity, MS and T2DM have higher levels of Fe, Cr and Se, or Cr and Se as well.[11] Mg is a constituent of or an activator of some enzymes, mainly antioxidants, thereby it has conservative effects against the appearence of MS. Lower serum Mg, Cu and Zn may be connected with MS, diabetes and growth of diabetic complications.[28] Se deficiency is relevant to an increase in plasma TC levels, CVD and lipid metabolic disorders, along with a higher prevalence of T2DM.[29] In a study by Kaur*et al.* the serum Se levels diminished by 31% and the ROS levels in the liver increased by 2-fold with a HFD.[29] Nevertheless, high doses of Cr triggers oxidative stress, including promoted production of $O_2^{\cdot-}$-$^{\cdot}OH$, increased lipid peroxidation and apoptotic cell death.[30] Rats were fed with a HFD, which increased Cu levels by 29% and 16%, respectively, yet it did not influence the Zn and Cr levels in tissues as opposed to the controls.[31] In a study by Sahin *et al.* compared with the controls, HFD dropped the serum and brain tissue Cr levels by 22.2% and 42.3%, respectively.[32] In another study, the levels of Cr, Zn, Se, and Mn in serum, liver, and kidney of diabetic rats were remarkably lower than in the control rats. Conversely, higher Fe and Cu levels were noticed in serum and tissues from diabetic in opposition to the non-diabetic rats. In diabetic and non-diabetic rats alike, supplying Cr caused increases in Cr, Zn, Se, and Mn, but diminished Cu levels in the serum, liver, and the kidney, and did not influence the Fe levels in all groups.[33] Mo plays an effective role as antihyperglycaemic way in obese-diabetic animals with serious IR.[34]

CONCLUSION

So far as we know, this is the first study which has investigated and measured the levels of serum MDA and TE levels in EE and Western diet (HFD and HSD)-treated rats. This study revealed that the serum levels of MDA, Fe, Cu, Mo and Mg increased, and that the serum levels of Cr and Se decreased in the HFD and HSFgroups. EE decreased the serum levels of MDA, Fe, Mo, and did not affect the serum levels of Cu, whereas it increased the serum levels of Mg, Cr, Se and Zn; even so, no significant difference was observed amidst all experimental groups. Our study has indicated that the HFD leads to oxidative stress and adversely affects the serum TE in rats, the EE reverses partially this situation. Nevertheless, the underlying mechanism of this finding needs to be investigated further.

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*The authors declare that there are no conflicts of interest.

REFERENCES

1. Grant WB, Campbell A, Itzhaki RF, Savory J. The significance of environmental factors in the etiology of Alzheimer's disease. J Alzheimer's Dis 2002; 4: 179-189.
2. Sreekumar R, Unnikrishnan J, Fu A et al. Impact of high-fat diet and antioxidant supplement on mitochondrial functions and gene transcripts in rat muscle. Am J Physiol Endocrinol Metab 2002; 282: E1055-1061.
3. Ragab SM, Abd Elghaffar SKh, El-Metwally TH et al. Effect of a high fat, high sucrose diet on the promotion of non-alcoholic fatty liver disease in male rats: the ameliorative role of three natural compounds. Lipids Health Dis 2015; 14: 83.
4. Sweazea KL, Lekic M, Walker BR. Comparison of mechanisms involved in impaired vascular reactivity between high sucrose and high fat diets in rats. Nutr Metab (Lond) 2010; 7: 48.
5. Morrison CD, Huypens P, Stewart LK, Gettys TW. Implications of crosstalk between leptin and insulin signaling during the development of diet-induced obesity. Biochim Biophys Acta 2010; 1792: 409-416.
6. Prasad AS. Zinc in human health: effect of zinc on immune cells. Mol Med 2008; 14: 353-357.
7. Feng JF, Lu L, Zeng P et al. Serum total oxidant/anti oxidant status and trace element levels in breast cancer patients. Int J Clin Oncol 2012; 17: 575-583.
8. Cechetti F, Worm PV, Lovatel G et al. Environmental enrichment prevents behavioral deficits and oxidative stress caused by chronic cerebral hypoperfusion in the rat. Life Sci 2012; 91: 29-36.
9. Zeeni N, Bassil M, Fromentin G et al. Environmental enrichment and cafeteria diet attenuate the response to chronic variable stress in rats. Physiol Behav 2015; 139: 41-49.
10. Azab SF, Saleh SH, Elsaeed WF et al. Serum trace elements in obese Egyptian children: a case-control study. Ital J Pediatr 2014; 40: 20.

11. Sun L, Yu Y, Huang T et al. Associations between ionomic profile and metabolic abnormalities in human population. PLoS One 2012; 7: e38845.

12. Prado Lima MG, Schimidt HL, Garcia A et al. Environmental enrichment and exercise are better than social enrichment to reduce memory deficits in amyloid beta neurotoxicity. Proc Natl Acad Sci U S A. 2018; 115: E2403-E2409.

13. Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol 1990; 186: 421-431.

14. Coelho DF, Pereira-Lancha LO, Chaves DS et al. Effect of high-fat diets on body composition, lipid metabolism and insulin sensitivity, and the role of exercise on these parameters. Braz J Med Biol Res 2011; 44: 966-972.

15. Amin KA, Kamel HH, Abd Eltawab MA. Protective effect of Garcinia against renal oxidative stress and biomarkers induced by high fat and sucrose diet. Lipids Health Dis 2011; 10: 6.

16. Kang BP, Bansal MP, Mehta U. Hyperlipidemia and type I 5'-monodeiodinase activity: regulation by selenium supplementation in rabbits. Biol Trace Elem Res 2000; 77: 231-239.

17. Ryu MH, Cha YS. The effects of a high-fat or high-sucrose diet on serum lipid profiles, hepatic acyl-CoA synthetase, carnitine palmitoyltransferase-I, and the acetyl-CoA carboxylase mRNA levels in rats. J Biochem Mol Biol 2003; 36: 312-318.

18. Yerlikaya H, Toker A. Obezite ve eser elementler. Journal of Dialog in Endocrinology / Endokrinolide Diyalog Dergisi 2012; 9: 64-70.

19. Charradi K, Sebai H, Elkahoui S et al. Grape seed extract alleviates high-fat diet-induced obesity and heart dysfunction by preventing cardiac siderosis. Cardiovasc Toxicol 2011; 11: 28-37.

20. Gürsel FE, Ateş A, Bilal T, Altiner A. Effect of dietary Garcinia cambogia extract on serum essential minerals (calcium, phosphorus, magnesium) and trace elements (iron, copper, zinc) in rats fed with high-lipid diet. Biol Trace Elem Res 2012; 148: 378-382.

21. Błażewicz A, Klatka M, Astel A, Partyka M, Kocjan R. Differences in trace metal concentrations (Co, Cu, Fe, Mn, Zn, Cd, And Ni) in whole blood, plasma, and urine of obese and nonobese children. Biol Trace Elem Res 2013; 155: 190-200.

22. Charradi K, Elkahoui S, Limam F, Aouani E. High-fat diet induced an oxidative stress in white adipose tissue and disturbed plasma transition metals in rat: prevention by grape seed and skin extract. J Physiol Sci 2013; 63: 445-455.

23. Charradi K, Elkahoui S, Karkouch I et al. Protective effect of grape seed and skin extract against high-fat diet-induced liver steatosis and zinc depletion in rat. Dig Dis Sci 2014; 59: 1768-1778.

24. Hordyjewska A, Popiołek Ł, Kocot J. The many "faces" of copper in medicine and treatment. Biometals 2014; 27: 611-621.

25. Horn D, Barrientos A. Mitochondrial copper metabolism and delivery to cytochrome c oxidase. IUBMB Life 2008; 60: 421-429.

26. Pinhas-Hamiel O, Newfield RS, Koren I et al. Greater prevalence of iron deficiency in overweight and obese children and adolescents. Int J Obes (Lond) 2003; 27: 416-441.

27. Sulibursk J, Bogdański P, Szulińska M. Iron excess disturbs metabolic status and relative gonad mass in rats on high fat, fructose, and salt diets. Biol Trace Elem Res 2013; 151: 263-268.

28. Wang X, Li H, Fan Z, Liu Y. Effect of zinc supplementation on type 2 diabetes parameters and liver metallothionein expressions in Wistar rats. J Physiol Biochem 2012; 68: 563-572.

29. Kaur HD, Bansal MP. Studies on HDL associated enzymes under experimental hypercholesterolemia: possible modulation on selenium supplementation. Lipids Health Dis 2009; 8: 55.

30. Shrivastava R, Upreti RK, Seth PK, Chaturvedi UC. Effects of chromium on the immune system. FEMS Immunol Med Microbiol 2002; 34: 1-7.

31. Król E, Krejpcio Z, Iwanik K. Supplementary chromium (III) propionate complex does not protect against insulin resistance in high-fat-fed rats. Biol Trace Elem Res 2014; 157: 147-155.

32. Sahin K, Tuzcu M, Orhan C et al. The effects of chromium complex and level on glucose metabolism and memory acquisition in rats fed high-fat diet. Biol Trace Elem Res 2011; 143: 1018-1030.

33. Dogukan A, Sahin N, Tuzcu M et al. The effects of chromium histidinate on mineral status of serum and tissue in fat-fed and streptozotocin-treated type II diabetic rats. Biol Trace Elem Res 2009; 131: 124-132.

34. Reul BA, Becker DJ, Ongemba LN et al. Improvement of glucose homeostasis and hepatic insulin resistance in ob/ob mice given oral molybdate. J Endocrinol 1997; 155: 55-64.