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Investigation of Lipid Profile, Malondialdehyde, Sodium, Potassium, Chloride Levels in Rats With Weight Loss

Adem KESKİN[1], Recai ACI[2]

[1]Aydın Gynecology and Pediatrics Hospital, Department of Medicine Biochemistry, Aydın, Turkey
[2]Samsun Training and Educational Hospital, Department of Biochemistry, Samsun, Turkey

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ABSTRACT

Aim: The aim of the fight against obesity is weight loss. Sometimes, the damage caused by weight loss can be ignored rather than its benefits. This situation; sometimes, shock diet or excessive exercise can lead to unwanted consequences such as sudden death. The purpose of our study; to examine changes in lipid profile, malondialdehyde levels in rats in which we have created weight loss. Also, it is to investigate changes in sodium and potassium chloride levels, which are of great importance inside and outside the cell.

Material and Methods: Twenty rats were used in the study. We randomly divided them into two groups. We exercised rats for 10 days. Triglyceride, cholesterol, (high-density-lipoprotein) HDL-cholesterol, (low-density-lipoprotein) LDL-cholesterol, Malondialdehyde, sodium, potassium chloride levels were analyzed. Laboratory findings of the groups were compared with independent samples t-test. Pearson correlation analysis was performed between laboratory findings and weight loss levels.

Results: Sodium(p=0.034), potassium(p=0.018) and LDL-cholesterol(p=0.004) levels of the group with high weight loss were lower than the other group, Malondialdehyde(p=0.005) levels were found to be higher. Weight loss correlates negatively with sodium(p=0.031), potassium(p=0.005), chloride(p=0.036), LDL-cholesterol(p<0.001), HDL-cholesterol(p=0.035) and cholesterol(p=0.001). Weight loss correlates positively with Malondialdehyde(p<0.001).

Conclusion: Weight loss causes the desired decrease in lipid profile and an unwanted increase in Malondialdehyde levels. Also, weight loss correlates negatively with sodium, potassium, and chloride levels. Both oxidative stress and electrolyte balance disorders can be life-threatening.

Keywords: *Cholesterol, Malondialdehyde, Potassium, Sodium, Weight loss*

Kilo Kaybı Olan Ratlarda Lipid Profili, Malondialdehit, Sodyum, Potasyum ve Klor Düzeylerinin Araştırılması

ÖZ

Amaç: Obezite ile mücadelede amaç, kilo kaybıdır. Bazı durumlarda kilo kaybının yararından çok, verdiği zarar göz ardı edilebilmektedir. Bu durum; bazen şok diyet veya aşırı egzersiz yüklenmesi ile ani ölümlere kadar gidebilen istenmeyen sonuçlara yol açabilmektedir. Çalışmamızın amacı; kilo kaybı oluşturduğumuz ratlarda, lipid profilinde ve malondialdehit düzeylerinde meydana gelen değişimleri araştırmaktır. Buna ek olarak hücre içi ve dışında büyük öneme sahip olan sodyum, potasyum ve klor düzeylerindeki değişimleri araştırmaktır.

Gereç ve Yöntemler: Çalışmada yirmi adet erkek rat kullanıldı. Rastgele iki gruba ayrıldı. Ratlara 10 gün egzersiz yaptırıldı. Trigliserit, kolesterol, (yüksek yoğunluklu lipoprotein) HDL-kolesterol, (düşük yoğunluklu lipoprotein) LDL-kolesterol, Malondialdehit, sodyum, potasyum klor düzeylerinin analizi yapıldı. Grupların laboratuvar bulguları, bağımsız örneklem t-testi ile karşılaştırıldı. Laboratuvar bulguları ile kilo kaybı düzeyleri arasında pearson korelasyon analizi yapıldı.

ORCID: Adem Keskin / 0000-0003-1921-2583, Recai Acı / 0000-0002-3332-6619

Correspondence Address / Yazışma Adresi:
Adem KESKİN
Aydın Gynecology and Pediatrics Hospital, Department of Medicine Biochemistry, Aydın, Turkey
Phone: +90 535 372 11 43 • E-mail: adem.keskin2@saglik.gov.tr

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Bulgular: Yüksek kilo kaybı gözlenen grubun sodyum(p=0,034), potasyum(p=0,018) ve LDL-kolesterol(p=0,004) düzeylerinin, diğer gruptan daha düşük, Malondialdehit(p=0,005) düzeylerinin, daha yüksek olduğu saptanmıştır. Kilo kaybı ile sodyum(p=0,031), potasyum(p=0,005), klor(p=0,036), LDL-kolesterol(p<0,001), HDL-kolesterol(p=0,035) ve kolesterol(p=0,011) arasında negatif yönde korelasyon gözlendi. Kilo kaybı ile Malondialdehit(p<0,001) arasında pozitif yönde korelasyon gözlendi.

Sonuç: Kilo kaybı, bir taraftan lipid profilinde istenen düşüklüğe yol açarken diğer taraftan Malondialdehit düzeylerinde istenmeyen yükselmeye neden olmaktadır. Ayrıca, kilo kaybı sodyum, potasyum ve klor düzeyleri ile negatif korelasyon göstermektedir. Hem oksidatif stres hem de elektrolit dengesi bozuklukları yaşamı tehdit edebilir.

Anahtar Sözcükler: *Kilo kaybı, Kolesterol, Malondialdehit, Potasyum, Sodyum*

INTRODUCTION

Obesity is a global health problem, that affects individuals of every sex, age, socioeconomic status, culture, and ethnicity (1). In 2013, The American College of Cardiology/American Heart Association Task Force on Practice Guidelines for managing overweight and obesity in adults state that a sustained 3%-5% loss of initial body weight is recommended to give rise to a clinically meaningful reduction in the risks of developing obesity-related chronic diseases (2). It is a common strategy used to treat obesity, creating a negative energy balance by reducing calorie consumption and increasing physical activity (3).

Regular exercise has long been touted as a strategy for weight loss maintenance, but the lack of clear evidence in clinical trials has caused some to question its effectiveness (4). Although regular mild exercise seems to be useful for oxidative stress and health, acute and tiring workouts of aerobic and anaerobic exercise may trigger reactive oxygen species (ROS) overproduction (5). Strenuous exercise has been shown to increase free radical production and cause oxidative stress, thus causing cellular damage (6).

Plasma sodium (Na^+) concentration is the main determinant of plasma osmolality. Changes in the plasma sodium concentration can cause clinical situations with high morbidity and mortality. Cases of low sodium, thought to be caused by heavy exercise, are increasing. Such cases develop in military services, during a marathon or triathlon, and are often symptomatic (7).

Potassium (K^+) is the most important cation in the cell. It is stated that situations such as heavy exercise cause changes in potassium concentration. This situation is one of the factors that affect the effective operation of the NA/K ATPase pump (8).

Many studies are evaluating different methods to combat obesity. When the studies are examined; many have focused on weight loss. However, in some cases, weight loss can cause harm. This situation can lead to undesirable consequences that can lead to sudden death by shock diet or excessive exercise overload. In line with this information, our study aims to investigate the changes in lipid profile on the one hand and in Malondialdehyde (MDA) levels, a product of lipid peroxidation, observed in cells exposed to oxidative stress on the other hand, in rats on which we lose weight with exercise. Also, to measure the changes in Na^+, K^+, and chloride (Cl^-) levels, which are of great importance inside and outside the cell.

MATERIALS and METHOD

This study is an experimental animal study. Twenty Wistar Albino male rats were used. The reason for choosing the male gender, having more muscle mass than female and because it is more resistant to exercise. The age range of the rats is 11-12 months. Rats weigh approximately 380-465 grams. When the weights of the rats used in the study are evaluated according to their age, the rats are classified as obese. The normal weight of 11-12 month-old Wistar albino rats is between 275 and 350 grams. Two groups were created. Each group consisted of 10 rats. The reason why the groups are of ten is to continue studying against the risk of death during the study. One of these two groups is the control group. This group was named group 1. Only exercise is done. There was no weight loss during the study. The other group is the group with weight loss. Niacin and melatonin supplements were given to this group in addition to exercise. Niacin and melatonin supplements were given to make a difference in weight loss between groups. At the stage of selecting these supplements; the information obtained from the studies of Canto et al. and Mostafavi et al. were used. In the study by Canto et al.; It is stated that niacin supplementation increases the NAD content and energy expenditure (9). In the meta-analysis of Mostafavi et al.; It is hypothesized that melatonin has a role as a buffer in changes in body weight (10).

MDA analysis was studied in Aydın Adnan Menderes University Central Laboratory. Na^+, K^+, Cl^-, total cholesterol, high-density lipoprotein-cholesterol(HDL-C), low-density lipoprotein-cholesterol(LDL-C), triglyceride analysis were studied in Samsun Training and Research Hospital.

The decision of the ethics committee required to study experimental animals was taken at Aydın Adnan Menderes University Animal Experiments Local Ethics Committee Center with the decision number 6453101/2018/039 dated 23/03/2018.

Operations on Rats

Rats were kept in cages made of transparent polyester. Standard rat food and tap water was given to the rats during the study. There was no restriction in nutrition. First, light tempo jogging at a speed of 1 km/hour for 5 min was done to the rats to adapt them to the treadmill and train them, 6 days before studying. Later, rats were moderately exercised for 10 days to induce weight loss. Exercise model applied by Gronowska-Senger et al. was used (11). According to this model, rats do treadmill jogging exercises for 10 days at a speed of 15 minutes/day and 20m/min.

Melatonin was supplied from Sigma-Aldrich (USA) company as a powder preparation of 1 g. While determining the dose and duration of administration; it was determined by considering the study by Reiter et al. on past studies in melatonin applications (12). Each animal was weighed every day 30-45 min before exercise and administered as a dose of 5 mg/kg/day intraperitoneally.

Niacin was supplied from Sigma-Aldrich (USA) company as a 100 g powder preparation. While determining the dose and duration of administration; It was determined by considering the study by Kwon et al. (13). Each animal was weighed every day 30-45 min before exercise and administered a dose of 360 mg/kg/day orally.

Biochemical Analysis

Approximately 7 ml of blood samples were taken by intracardiac anesthesia with ketamine and xylazine, one day after the last exercise application. For biochemical analysis; The blood samples taken were centrifuged at 3000 rpm for 5 min. Serum samples were taken.

Ohkawa method was applied as the measurement method of MDA (14). A color formation close to pink-red is observed by the reaction of the samples under study with thiobarbituric acid after 60 min incubation at 95°C in an environment with optimum pH 3.5. This color formation is based on measuring the absorbance at 532 nm.

Total cholesterol, triglyceride, HDL-Cholesterol analysis was performed on a Beckman Coulter DXC 800 autoanalyzer. LDL-Cholesterol levels were calculated using the Friedewald LDL-Cholesterol formula (Total cholesterol-(Triglyceride/5*HDL-Cholesterol).

Analysis of Na^+, K^+, Cl^- was done using the ion-selective electrode method. They were measured on Beckman Coulter DXC 800 autoanalyzer.

Statistical Analysis

IBM SPSS for Windows 22.0 program was used for statistical analysis. The data obtained from the study were given as mean±standard deviation (X±SD) and P values below 0.05 were considered statistically significant. The normal distribution of the data was evaluated by Shapiro-Wilk normality tests. Groups with a normal distribution of data were compared with the Independent-Sample t-test, which is a parametric test. A Paired-samples t-test was used to evaluate the weight loss in the groups according to body weight. Also, Pearson correlation analysis, which is a parametric correlation test, was performed to investigate the relationship between analyzed parameters and weight loss.

RESULTS

Before exercise, the average body weight of the rats in Group 1 was 440.70±19.03 g, and the average bodyweight of the rats in Group 2 was 418.30±25.82 g. At the end of the 10-day exercise study, an average of 2.30 g (0.53%) weight loss was observed in rats in Group 1, and an average weight loss of 16.00 g (3.98%) in rats in Group 2. When this observed weight loss was evaluated according to the total body weight of the rats, it was found that it was significant in Group 2 ($p<0.001$) and insignificant in Group 1 ($p>0.05$).

The analyzed parameter results of the groups were compared. According to the comparison result; in group 2 Na^+ ($p=0.034$), K^+ ($p=0.018$), and LDL-C ($p=0.004$) levels were lower than in group 1 levels, in group 2 MDA ($p=0.005$) levels were found to be higher than group 1 levels (Table 1).

Correlation analysis was performed to see the relationship between the weight loss observed in the groups and parameters analyzed. According to the correlation analysis made; A negative correlation was observed between Na^+ ($p=0.031$), K^+ ($p=0.005$), Cl^- ($p=0.036$), LDL-C ($p<0.001$), HDL-C ($p=0.035$) and total cholesterol ($p=0.001$) with weight loss. A positive correlation was observed between weight loss and MDA ($p<0.001$), (Table 2).

DISCUSSION

It is known that moderate and heavy exercise is a strategy for weight loss, increases oxidant activity, and is harmful to the organism, and can threaten health. In our study, Wistar Albino male rats exercised moderately. We continued this exercise workout for 10 days. We observed 0.53% weight loss in one group and 3.98% weight loss in the other group, after 10 days of exercise. When this observed weight loss

Table 1: Group averages, standard deviation values, and independent samples t-test results of the parameters analyzed in rats.

Parameters	Group 1 (n = 10)	Group 2 (n = 10)	p
Sodium (mmol/L)	146.75±6.43	141.12±2.10	p=0.034
Potassium (mmol/L)	7.80±1.10	6.72±0.29	p=0.018
Chloride (mmol/L)	106.38±5.04	102.62±0.84	p>0.05
LDL-C (mg/dL)	21.63±4.47	14.13±4.19	p=0.004
HDL-C (mg/dL)	33.38±9.00	29.75±3.20	p>0.05
Total C (mg/dL)	54.50±13.87	46.63±5.15	p>0.05
Trigliserit (mg/dL)	84.25±23.64	103.13±34.45	p>0.05
MDA (µmol/L)	3.01±0.52	3.77±0.53	p=0.005

LDL-C: Low-density-lipoprotein -cholesterol, **HDL-C:** High-density-lipoprotein-cholesterol, **Total C:** Total cholesterol, **MDA:** Malondialdehyde.

Table 2: Pearson correlation analysis between weight loss and analyzed parameters.

Parameters	Correlation coefficient	p
Sodium	-0.540	p=0.031
Potassium	-0.660	p=0.005
Chloride	-0.527	p=0.036
LDL-C	-0.785	p<0.001
HDL-C	-0.529	p=0.035
Total-C	-0.616	p=0.001
Triglyceride	0.102	p>0.05
MDA	0.929	p<0.001

LDL-C: Low-density-lipoprotein -cholesterol, **HDL-C:** High-density-lipoprotein-cholesterol, **Total C:** Total cholesterol,
MDA: Malondialdehyde.

was evaluated according to the total body weight of the rats, it was found that it was significant in Group 2 and insignificant in Group 1. We examined the effects of weight loss on lipid profile, MDA, Na^+, K^+, and Cl^- levels over 10 days.

In Oktem's study with obese children; It has been reported that blood pressure and total cholesterol and LDL levels are higher in obese patients. According to this result, the risk of high blood pressure is increased in obese patients. It has been found to be considered as a very important risk factor in the development of important problems such as hypertension and hyperlipidemia (15). Kurtuncu et al. conducted a study on the relationship between serum lipid levels, hypertension and obesity. They concluded that follow-up of LDL and HDL should be initiated in childhood (16). In the study of Altundag and Tayfur, it was concluded that body weight loss in obese individuals may decrease blood lipids (17).

In this study, weight loss showed a negative correlation with HDL-C, LDL-C, and total cholesterol levels. Also, there was no significant change in HDL-C, total cholesterol, and triglyceride levels between the group, while LDL-C levels of the group with weight loss were lower than the other group because of the exercise model applied.

ROS increase in the skeletal muscle, during exercise. However, if the production of ROS is not balanced by the endogen antioxidant system, it can lead to oxidative stress and damage cells (18). According to the principle of hormesis, exercise-induced ROS production in skeletal muscle, low to moderate-intensity exercise can cause ROS-mediated adaptive responses, which protect cells from oxidative stress and maintains oxidant-antioxidant balance during exercise (19). Although regular mild exercise seems to be useful for oxidative stress and health, acute and tiring workouts of aerobic and anaerobic exercise may trigger ROS overproduction (4). Mohn et al, study that compared obese children and healthy subjects, the oxidant-antioxidant state was examined in association with weight loss (20). When compared with the control group, fat mass, body mass index (BMI), and waist-hip ratio (whr), decreased significantly, whereas MDA increased significantly. It was found that MDA was correlated with fat mass, BMI, and whr, and antioxidant state was found to return to normal after six months of dietary restriction (20).

In this study, weight loss showed a positive correlation with MDA levels. Also, MDA levels of the group with weight loss were lower than the other group because of the exercise model applied.

Sodium is the main cation of the extracellular fluid and accounts for about half of the plasma osmotic power. Therefore, it plays the most important role in the regula-

tion of osmotic pressure and normal water distribution in the extracellular fluid compartment. Potassium is the main cation inside the cell. Intracellular concentration is kept high. Because while K^+ leaves the cell membrane very slowly, the Na/K-ATPase pump fed with oxidative energy continuously takes in potassium against the concentration gradient/difference. Chloride is the main anion of extracellular fluids. Chloride, along with sodium, constitutes the bulk of the osmotically active components of plasma. Hence, chloride plays a prominent role in osmotic pressure, anion-cation balance, and maintaining water distribution in the extracellular fluid compartments (21).

In this study, weight loss showed a negative correlation with Na^+, K^+, and Cl^- levels. Also, there was no significant change in Cl^- levels between the group, while Na^+, and K^+ levels of the group with weight loss were lower than the other group because of the exercise model applied.

In our study, melatonin and niacin supplements were given to a group to buffer weight loss. There is a lot of literature showing the effect of lowering MDA levels and the antioxidant properties of melatonin. Despite this property of melatonin, a positive correlation was observed between weight loss and increased MDA in this group. On the other hand, in the study by Canto et al.; It is stated that niacin supplementation increases the NAD content and energy expenditure (9). Because of this feature, it is used for rapid weight loss in overweight individuals and obese people. However, there is no study investigating the effect of niacin supplementation on MDA, sodium, potassium, and chloride levels.

We examined the effects of weight loss on lipid profile, MDA, Na^+, K^+, and Cl^- levels over 10 days. Weight loss, on the one hand, leads to a desired low lipid profile, on the other hand, it raises lipid peroxidation product MDA levels with the increase in excess ROS. When these rising MDA levels cannot be sufficiently reduced by the antioxidant system, the oxidative balance is disrupted. This situation causes exposure to unwanted oxidative stress. Also, weight loss leads to low blood Na^+ and K^+ levels. Both exposures to oxidative stress and electrolyte balance disorders can be life-threatening. For healthy weight loss, we recommend that these parameters be followed while exercising and dieting. Besides, we recommend exercising and dieting over a long period. It supports our suggestion in the study by Mohn et al. It was found that MDA was correlated with fat mass, BMI, and whr, and antioxidant state was found to return to normal after six months of dietary restriction (20).

Weight loss, on the one hand, leads to the desired decrease in the lipid profile, on the other hand, it leads to an undesirable increase in malondialdehyde levels. In addition, weight loss was negatively correlated with cholesterol levels and positively correlated with malondialdehyde levels. Also, weight loss is negatively correlated with sodium, potassium, and chloride levels. When planning for weight loss, it should be kept in mind that both oxidative stress and electrolyte balance disorders can be life-threatening.

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Author Contributions

Adem Keskin conducted design of project, ethical and project processes, modelling experimental animals, laboratory experiments, statistical analysis, translation and constitution of full text. **Recai Acı** conducted laboratory experiments and constitution of full text.

Conflict of Interest

There is no conflict of interest among the authors.

Financial Disclosure

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Ethical Approval

The decision of the ethics committee required to study experimental animals was taken at Aydın Adnan Menderes University Animal Experiments Local Ethics Committee Center with the decision number 6453101/2018/039 dated 23/03/2018.

Peer Review Process

Extremely peer-reviewed and accepted.

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