

Effects of Moderate Exercise on Mild Depressive Mood, Antioxidants and Lipid Peroxidation*

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SUMMARY:

EFFECTS OF MODERATE EXERCISE ON MILD DEPRESSIVE MOOD, ANTIOXIDANTS AND LIPID PEROXIDATION

Objective: Regular exercise has been associated with the maintenance and improvement of physical health and well being. Physical activity causes lipid peroxidation. The purpose of this study was to assess how exercise affects mild depression and enzymatic antioxidant systems as well as lipid peroxidation. **Method:** All of the students who volunteered for this study were chosen. Eight healthy and eight depressive female students volunteered to complete the General Health Questionnaire (GHQ), the Beck Depression Inventory (BDI), the Spielberg State-Trait Anxiety Inventory (STAI) and the Hamilton Depression Rating Scale (HDRS). Psychiatric evaluation was done by a psychiatrist. Subjects of both groups participated in a three-day-per-week 20-25 min. aerobic exercise program for nine weeks. Before and after this nine weeks program, fasting blood samples were obtained and superoxide dismutase (SOD) from plasma and erythrocyte and catalase (CAT) from erythrocyte as antioxidant enzymes, and malondialdehyde (MDA) from plasma and erythrocyte as an index of lipid peroxidation were studied. **Results:** Results indicated that depression and trait anxiety decreased significantly ($p < 0.05$) and state anxiety decreased but not significantly ($p > 0.05$) following exercise. MDA levels in both plasma and erythrocyte increased significantly in both groups ($p < 0.05$) after the exercise program. However, plasma SOD, erythrocyte SOD and erythrocyte CAT levels did not show any significant changes ($p > 0.05$) following exercise. **Conclusions:** In conclusion exercise programs may help psychiatric patients and it may be performed in clinics easily with other treatment programs. A broad based clinical study is needed to find which exercise program is going to be applied to the patients.

Key words: exercise, depression, anxiety, antioxidants, malondialdehyde.

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ÖZET:

ORTA DERECEDE EGZERSİZİN HAFİF DEPREŞİP DUYGUDURUM, ANTIOKSİDANLAR VE LİPİD PEROKSİDASYONU ÜZERİNE ETKİLERİ

Amaç: Düzenli egzersiz ile fiziksel ve ruhsal sağlık arasında yakın bir ilişki vardır. Yapılan araştırmalar düzenli egzersizin bu olumlu etkilerinin yanında moleküler seviyede lipid peroksidasyonuna neden olduğu yönünde kanıtlar ortaya koymuştur. Bu çalışmanın amacı, egzersizin hafif depresyon, enzimatik antioksidan sistemler ve lipid peroksidasyonu üzerine etkisini araştırmaktır. **Yöntem:** Çalışmaya alınan gönüllü bireylerin tümü öğrenciler arasından seçildi. Sekiz sağlıklı ve sekiz depresif gönüllü bayan öğrenci Genel Sağlık Anketi (GSA), Beck Depresyon Ölçeği (BDÖ), Spielberg Durumluk-Süreklilik Kaygı Envanteri (SDSKE) ve Hamilton Depresyon Derecelendirme Ölçeği (HDDÖ) sonuçları, ve bu ölçeklerin uygulanmasını takiben bir psikiyatri uzmanı tarafından yapılan psikiyatrik görüşmeye göre belirlendi. Her iki grup öğrenciyi haftada üç gün 20-25 dakika süren aerobik egzersiz programı dokuz hafta uygulandı. Dokuz haftalık program öncesi ve sonrası açlık kan örnekleri alındı. Plazma ve eritrositte süperoksit dismutaz (SOD) aktivitesi ve malondialdehid (MDA) düzeyi belirlendi. Ayrıca, eritrosit katalaz (CAT) aktiviteleri ölçüldü. **Bulgular:** Depresyon ve süreklilik anksiyetede egzersiz sonrası belirgin azalmanın olduğu ($p < 0.05$) ancak durumluk anksiyetede değişim olmadığı ($p > 0.05$) görüldü. Hem plazma hem de eritrositte MDA seviyeleri her iki grupta da belirgin şekilde artmış olarak bulundu ($p < 0.05$). Ancak plazma ve eritrosit SOD ve CAT aktivitelerinde belirgin bir değişiklik gözlenmedi ($p > 0.05$). **Tartışma:** Egzersiz programlarının kolay uygulanabilir ve ucuz bir tedavi yöntemi olarak psikiyatride kullanılabileceği, fakat bu konuda daha kapsamlı çalışmalara gereksinim olduğu kanısına varılmıştır.

Anahtar sözcükler: egzersiz, depresyon, anksiyete, antioksidanlar, malondialdehid

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INTRODUCTION

It has been suggested that exercise enhances mood as well as physical well-being. Many studies show the psychological benefits of exercise such as anxiety reduction and depression reduction (1-8).

Some ideas have been proposed to explain mechanisms of the effects of exercise mentioned above. Exercise decreases pain by increasing the levels of natural opiates, endorphins, and increases feelings of well-being and euphoria. It may be associated with psychological well-being through psy-

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chosocial mechanisms such as improved self-esteem, sense of achievement, feelings of control, confidence, improved body image and stress reduction (6).

Physical exercise triggers the body's neuroendocrine arousal. By acting as a stressor, physical exercise increases catecholamine production. As a result of changed neuroendocrine capacities, toughness and a heightened energy occurs (9). Reactive oxygen species released causes the peroxidation of polyunsaturated fatty acids in the biological membranes and blood resulting in alterations of cell functions (10). Malondialdehyde (MDA), by-product of lipid peroxide, is the most frequently studied marker of oxidative tissue damage during exercise (10;11). Some oxidative reactions occur and oxygen-centred radicals are produced during exercise. Depending on the exercise intensity, oxidative reactions and antioxidant capacity may be perturbed, and MDA increase as a result of subsequent lipid peroxidation. The physiological mechanisms responsible for increasing the defence capacity against free radicals are conflicting. Chronic aerobic training has been claimed to reduce the exercise-increased lipid peroxidation by inducing antioxidant enzymatic activities, however, strenuous and long duration exercise overwhelm body's capacity to detoxify reactive oxygen species that severely hampers the antioxidant defences with resulting changes in cellular homeostasis (10).

Many studies show that aerobic activity continuing at least 20 minutes is necessary for obtaining psychological well-being. Either high or low intensity exercise lead to positive changes in mood (12). However, intense exercise such as long-distance running and swimming increases mood disturbance (13). On the other hand some studies indicated that moderate-intensity endurance exercise may enhance the physiological defence (10;11;14). Habitual physical activity has been suggested to maintain and promote antioxidant capacity to defend against oxidative tissue damage (11).

The aim of this study, thus, was to examine how exercise affects mild depressive mood, some antioxidant enzyme activities such as superoxide dismutase (SOD), catalase (CAT) and MDA levels, as an index of lipid peroxidation.

MATERIAL AND METHOD

The study design was reviewed by Inonu

University Medical School Ethical Committee and their permission was granted. The study was explained to the participants and their permission was granted, too.

The Scales Used in This Study:

1. General Health Questionnaire (GHQ) is a scale with 12 items and measures well-being (15).
2. Beck Depression Inventory (BDI) is a scale with 21 items of self rating scale and measures affective, cognitive and motivational signs of depression (16).
3. Spielberger State-Trait Anxiety Inventory (STAI) is a scale which measures state-trait anxieties (17).
4. Hamilton Depression Rating Scale (HDRS) has 17 items and is applied by a physician (18). HDRS scores which are equal to 14 points or more are accepted as sign of depression.
5. Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM IV), criteria for major depressive disorder (19).

Subjects And Psychiatric Measurements:

In this study, the samples have been chosen from the female students of The Nursing and Midwifery Department of İnönü University Health Services Higher Education School. We visited the school between 05 / 04 / 1999 and 09 / 04 / 1999. We applied GHQ with 12 items to 140 students who were at the school on those days and then BDI and STAI-S and STAI-T were applied to the 126 Students who had 1 or more points from the assessment of GHQ. Twenty-eight students who got 21 or more points from assessment from BDI were invited to attempt personal interview at Department of Psychiatry, Medical school of İnönü University. Four of 28 students rejected to attempt such a study and didn't come to the interview. One student was not accepted for this study because of being a severe smoker. Three students didn't come to interview although they were determined to attempt to this study.

Twenty students who agreed to attempt this interview were applied a psychiatric assessment by a psychiatrist. Seven student who didn't meet the diagnostic criteria of adjustment disorder by depressive signs and got 13 or less points on HDRS were kept out of this study. Five of subjects dropped out the

study without any definition. Eight subjects who fulfilled DSM-IV criteria for depression and done regularly exercise program were kept in this study as depression group. The mean age of depression group was 20.3 ± 0.6 (n=8).

Control group was established by 14 students who got 0 point from GHQ-12 item and did not have any sign of psychiatric disorder in the psychiatric examination performed by a psychiatrist. Even all of these 14 students agreed to attempt this study, only 8 students started the exercise program and finished whole program and were included in the result assessments as healthy control group. The mean age of control group was 20.8 ± 0.4 (n=8).

Subjects of both groups participated in a three-day-per-week aerobic exercise program for nine weeks (20) and 20-25 min. exercise was performed (21). These scales were applied to the depression group at the beginning and after 9 weeks exercise program. In the control group, firstly GHQ-12 item were applied. After exercise program BDI, STAI-S and STAI-T were applied to 3 students who got one or more points in GHQ. Their points from BDI were less than 21 points and did not demonstrate any symptoms and signs as obtained from psychiatric interview.

Enzymes, Chemicals And Instruments:

Xanthine oxidase, xanthine, nitrobluetetrazolium (NBT), thiobarbituric acid, 1,1,3,3 tetraethoxypropane were supplied from Sigma Chemical Company, USA and CuCl_2 , bovine serum albumin, H_2O_2 , EDTA, Na_2O_3 , $(\text{NH}_4)_2\text{SO}_4 \cdot 2\text{H}_2\text{O}$ were supplied from Merck, Germany. LKB Biochrom Ultraspec Plus model uv/visible spectrophotometer (Cambridge, England) and fluorescence spectrophotometer (Hitachi model F-4010, Japan) were used to measure SOD and CAT activities, and MDA levels, respectively.

Blood Samples:

Fasting blood samples from forearm vein were taken into heparinized tubes in the morning to prepare erythrocyte sediments before and after 9 weeks exercise program. At that stage, some haematological parameters were carried out by routine laboratory techniques. The samples were centrifuged at $1500 \times g$ for 10 min at $+4^\circ\text{C}$. Aliquots of the both

samples were taken into other tubes to be used in the assays of plasma and erythrocyte antioxidant enzymes and plasma MDA levels and stored at -75°C until analyses. After thawing, erythrocytes were treated with 4-fold ice-cold deionized water to obtain hemolyzate.

CAT Activity Determination

CAT (EC 1.11.1.6) activity was determined according to Aebi (22). An aliquot of the lysate was diluted 10-fold in deionized water. A mixture of 10 J of this diluted lysate and 2.99 ml of 50 mM phosphate buffer (pH 7) plus hydrogen peroxide solution (0.34 μl H_2O_2 /dL phosphate buffer gives approximately 0.500 absorbency at 240 nm) was incubated at 25 $^\circ\text{C}$. By measuring the absorbance change in every minute, rate constant of the enzyme can be determined. The principle of the CAT activity assay is based on the determination of the rate constant (s^{-1} , k) of the hydrogen peroxide decomposition rate.

Rate constant, $k = 1 / (t_2 - t_1) \cdot 2.3 \log A_1 / A_2$ where A_1 and A_2 are the absorbance values of hydrogen peroxide at t_1 and t_2 times where absorbance diminishing was linear. Activities were expressed as k/gr Hb.

SOD Activity Determination

SOD (EC 1.15.1.1) activity was determined according to the method of Sun et al (23). An aliquot of the lysate was diluted 4-fold in deionized water. After 1.0 ml ethanol/chloroform mixture (v/v, 5/3) was added to the 10 ml diluted lysate to remove the haemoglobin and centrifuged at $3000 \times g$ at $+4^\circ\text{C}$ for 20 min, the upper supernatant was taken and used in the enzyme assays (24). The principle of SOD activity assay is based on the inhibition of NBT reduction with the xanthine-xanthine oxidase system as a superoxide generator. Superoxide radicals, thus, produced reduced NBT and formed formazone. SOD prevents this reaction, and its activity is inversely proportional to the absorbency value of formazone at 560 nm. By using a blank study in which all reagents are present except erythrocyte lysate and blank, activity was calculated as given below: Percent inhibition = $(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}} \times 100 \times f$ Where A_{blank} and A_{sample} are the absorbance values of the blank and sample, and f is the dilution factor. One SOD unit was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate,

and SOD activity was also expressed as units per gr haemoglobin. Each plasma sample was also used to measure total SOD activity and the results were expressed as units per milliliter plasma.

MDA Determination

MDA was estimated according to the method of Wasowicz et al (25) with minor changes. The method is based on the coupling of MDA with thiobarbituric acid (TBA) at +95°C. All the measurements (standards and samples) were made at upper n-butanol phase. Results were expressed as mol per liter plasma. In sample storage, only plasma aliquots in which MDA measurements will be done were added GSH and EDTA to final concentrations of 0.65 and 1.34 mmol/L, respectively to avoid any newly produced malondialdehyde by oxidation.

Statistical Analysis

For statistical evaluation, Wilcoxon matched pair analyses was used to compare the dependent groups. Non-parametric tests were used due to small study sample. The changes in the groups were compared with the Mann-Whitney U test. All the results were expressed as mean \pm standard error of mean (SEM). Differences were considered significant at $p < 0.05$.

RESULTS Biochemical

Results

Before exercise program, the plasma MDA levels in healthy and mild depressive groups were 1.37 ± 0.05 and 1.15 ± 0.22 mmol/mL, respectively. After 9 weeks exercise program, the plasma MDA levels in healthy and mild depressive groups were found to be 4.63 ± 0.67 and 4.97 ± 0.66 mmol/mL respectively (Figure 1).

Before exercise program, the erythrocyte MDA levels in healthy and minimal depressive groups were 55.17 ± 3.29 and 62.90 ± 6.06 nmol/gr Hb, respectively. After 9 weeks exercise program, the erythrocyte MDA levels in healthy and minimal depressive groups increased to 163.36 ± 25.34 and 122.25 ± 9.38 nmol/gr Hb, respectively (Figure 2).

MDA levels of plasma and erythrocyte were significantly different before and after the exercise program ($p < 0.05$).

Figure 1. Mean plasma MDA levels before and after exercise program (* $p < 0.05$).

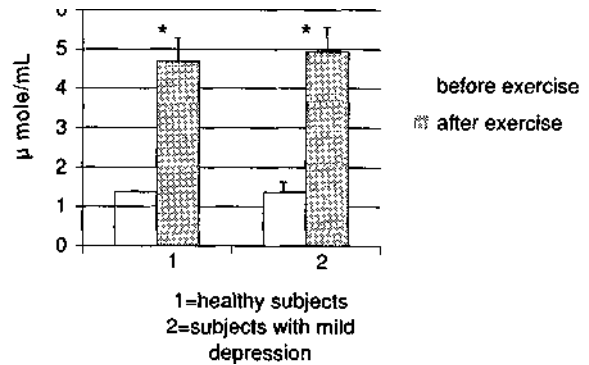
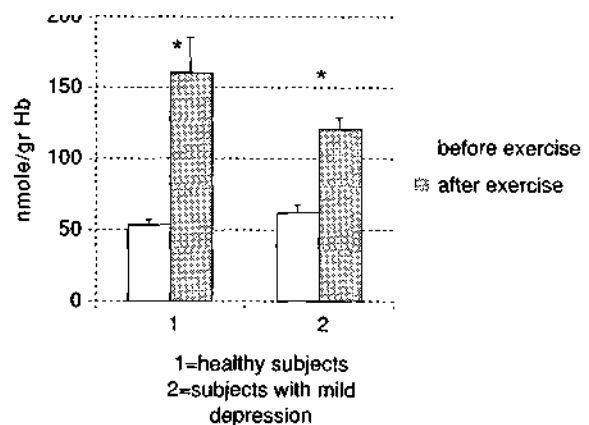


Figure 2. Mean erythrocyte MDA levels before and after exercise program (* $p < 0.05$).



There was no significant difference before and after exercise program in plasma and erythrocyte SOD levels in both groups ($p > 0.05$) (Table 1). There was no significant difference of erythrocyte CAT levels before and after exercise program in the healthy and the minimal depression groups ($p > 0.05$) (Table 1).

Psychological Results

BDI scores of mild depression group were 25.25 ± 1.06 and 13.38 ± 3.08 respectively before and after exercise program (Table 2). BDI decreased significantly in mild depression group following the exercise program ($p < 0.05$).

Table 1. Erythrocyte superoxide dismutase (SOD), plasma SOD and erythrocyte catalase (CAT) activities (mean \pm SEM) in healthy and depression groups .

Groups		Erythrocyte SOD	Plasma SOD	Erythrocyte CAT
		U/gr Hg	U/mL	k/gr Hg
Healthy (n=8)	b.e	221138 \pm 702.71	5.09 \pm 0.67	1077.75 \pm 95.41
	a.e.	2197.50 \pm 176.09	3.90 \pm 0.43	1072.13 \pm 92.34
Depression (n=8)	b.e.	1940.63 \pm 104.29	3.60 \pm 0.78	1063.88 \pm 62.17
	a.e.	2251.63 \pm 154.37	2.54 \pm 0.58	1061.25 \pm 49.88

b.e.: before exercise, a.e.: after exercise

Table 2. Beck Depression Inventory (BDI), Spielberger State-Trait Anxiety Inventory (STAI-S and STAI-T) and Hamilton Depression Rating Scale (HDRS) scores of depression group before and after exercise program, results are presented as mean \pm SEM.

Scales	Before exercise program	After exercise program
BDI scores	25.25 \pm 1.06	13.38 \pm 3.08
STAI-S scores	65.43 \pm 3.36	57.00 \pm 3.24
STAI-T scores	56.00 \pm 4.16	48.88 \pm 1.88
HDR scores	16.25 \pm 1.19	8.25 \pm 2.03

STAI-S scores of mild depression group were not changed significantly ($p > 0.05$) and the results were 65.43 ± 3.36 and 57.00 ± 3.24 respectively before and after exercise program (Table 2). STAI-T scores of mild depression group were 56.00 ± 4.16 and 48.88 ± 1.88 before and after exercise (Table 2) and the scores decreased after program significantly ($p < 0.05$).

HDRS of mild depression group also changed after exercise program. There was a statistically significant decrease in scores ($p < 0.05$). HDRS scores before and after exercise respectively (Table 2).

Three of healthy subjects showed scores equal to one point or more (mean = 1) from GHQ after exercise program. But their BDI scores were less than 21 points (mean = 2.5) and their health status did not change during the program.

DISCUSSION

The type of activity and the amount of exertion during exercise are important for psychological effects. Running, swimming, aerobic dancing, cycling, and walking are suitable for producing these effects (26). The aerobic metabolic rate may increase up to 10-fold during physical exercise, enhancing leakage of O_2 from the mitochondria to the cytosol. This increase in oxygen free radical concentrations

could exceed the protective capacity of cell antioxidant defence systems. Animal studies have shown that strenuous exercise promotes free radical formation and lipid peroxidation in skeletal muscle (14). Aerobic, non-competitive, predictable, and rhythmical activities produce greater psychological benefit than those that are not (27). Although early research has suggested that aerobic exercise is necessary to produce antidepressant and anxiolytic effects (3), later studies have suggested enhancing psychological well-being in non-aerobic passive exercise (28).

Exercise has beneficial effects on mild depression according to our results. Two of eight patients had more than 21 points of BDI scores after exercise program, but one of them showed more than 14 points according to their HDR scores. Exercise is positively associated with psychological well-being (6,29) and it is similar to our results. Brown et al (20) performed nine week exercise program to hospitalised patients with dysthymia and conduct disorder. Main effects on the BDI and the POMS Tension-Anxiety for condition tests showed decrease in depression and anxiety (20). In the present study the exercise program was performed to non-hospitalised subjects and clinical examination with HDRS as well as BDI was applied to the subjects. STAI-S scores decreased after exercise program, but it was not significant. Chronic exercise training programs have been associ-

ated with significant reduction in trait anxiety (29). In the study STAI-T scores decreased significantly with exercise in mild depression group as Koltyn study.

Marzatico et al found that athletes' basal MDA plasma levels were higher than control groups. On the other hand, marathon runners and sprint-trained athletes had a progressive increase in MDA levels at 48 hrs post exercise in their results. But MDA levels increased significantly after performance and decreased after 24 and 48 hrs (10). In our study we examined MDA levels on plasma and erythrocytes after nine weeks exercise program. They also found increased SOD and CAT activities after exercise (10) contrary to our results. It might depend on their heavier exercise program than our exercise program, although our results showed increased MDA levels in both plasma and erythrocyte. Increased muscle, heart and plasma MDA levels have been usually reported after exhausting exercise (11). We aimed to perform non-destructive exercise. It seems to be done according to study-results. The exercise program may have a successful end for mild depression patients. A non-destructive exercise may positively affect mood without oxidative tissue damage. Physiological defence without unexpected adverse effects may be gained with moderate exercise (10,11,14).

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