The effect of regular exercise and massage on oxidant and antioxidant parameters

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Abstract

This experimental study aimed to determine the effects of the combined application of regular exercises and massage on the values of Malondialdehyde (MDA), Nitric Oxide (NOx), Glutathione (GSH), Adenosine Deaminase (ADA) and Superoxide Dismutase (SOD). Twenty five sedentary women (32–50 years) who did not have the habit of getting regularly massages or exercising and participated voluntarily in the study. The subjects were randomly separated into three groups: control group (CG, n=9), exercise group (EG, n=8), and massage and exercise group (MEG, n=8). The basic result of this study was that a statistically significant decrease was observed in the post-test MDA values of both EG and MEG subjects. Moreover, when the GSH and SOD values are compared to CG, a statistically significant increase was determined in the values of both EG and MEG. As a result, the findings show that regular physical activities and massage manipulations significantly decrease MDA, increase SOD and GSH activities, and result in no change in NOx and ADA activities supports the assumption that regular physical activity has positive health effects.

Introduction

Oxidative stress have been represented an imbalance between the pro-oxidant (free radical) and antioxidant defense system, which is positive for free radicals. Free radicals include one or more unpaired electrons (1). There have been many free radical types; however, oxygen or nitrogen radicals produced in living systems are quite important for humans (2). The antioxidant defense system has both endogenous (superoxide dismutase, catalase, glutathione etc.) and exogenous (carotenoid, tocopherol, etc.) defense agents (3). Exogenous antioxidants have been segregated by fruits and vegetables (4), while endogenous antioxidants have segregated by ultra-free radicals (5). Over-production of free radicals depends on many factors, such as environmental pollution (6), excessive food intake (7) and physical activity (8).

Oxidative stress has been known to cause some diseases as well as cardiovascular, DNA and cell damage (9-11). There were a lot of studies related to the effect of acute exercises on the oxidative stress (12-14). The researches stated that the acute exercise causes an statistically significant differences of the serum MDA values of the human participants (9, 15, 16). Shin et al. (2008) also found that submaximal exercise caused a decrease in SOD
enzyme level (17). In addition to some studies reported that the obesity increases the oxidative stress (12, 13). The adenosine deaminaz rate-limiting enzyme in purine nucleotide cycle to decrease the activity of adenosine increased activity has been performed. It was also reported that adenosine had a cardioprotective effect (18). There was no study in the literature that ADA activity on the oxidative stress parameters. Therefore the ADA activity is important as regard to showing the effect of the exercise.

Massage is one of the most popular treatment methods used for the prevention of fatigue after intensive muscular activity and of muscle damage. In the literature there were many studies examining the effect of massage on biochemical parameters and the effects of acute exercises on oxidative stress (19-23). However, there was no study in the literature examining the effects of combined application of regular exercises and massage manipulations on oxidant and antioxidant activity. Therefore, the present experimental study aimed to determine the effects of the combined application of regular exercises and massage on the values of Malondialdehyde (MDA), Nitric Oxide (NOx), Glutathione (GSH), Adenosine Deaminase (ADA) and Superoxide Dismutase (SOD).

Material and Method

Experimental subjects

Thirty overweight sedentary women (32–50 years) who did not have the habit of getting regularly massages or exercising and participated voluntarily in the study. The present study was experimental study. The subjects were informed of the experimental risks prior to the research, and were asked to sign a voluntary participation form. Approval was obtained from Inonu University Ethics Committee in order to use human subjects in the study. The subjects chosen for the study had neither performed any kind of physical exercise nor undergone any massage treatment to improve health and fitness. The general health of the subjects and the presence of a chronic disease or medical treatment were determined via routine biochemical and ECG tests, questionnaires and face-to-face interviews. Five of subjects were excluded, as they did not follow the exercise and massage program regularly and were considered to negatively affect the results. No specific advice on dietary habits was given any of the participants during the study period. All women were wished to not alter their dietary habits during of the study.

Exercise protocol

All subjects performed the Bruce Protocol exhaustion exercise, in which the grade and speed are increased every three minutes in order to determine their capacities. Target pulse rates of participants were calculated using the Karvonen method: Target Heart Rate (THR) = Maximum Heart Rate (MHR) – Resting Heart Rate (RHR) x Overloading Density +RHR (24). Besides, the max VO\textsubscript{2} values of the subjects were determined via the Bruce Protocol, and the data were calculated and recorded using the formula (mL.kg/min) developed by Pollock et al. (1984) for sedentary women (25). The subjects were randomly separated into three groups:

Control group (CG, n=9): CG participants were told to abstain from to perform any kind of exercise or food supplements that could affect their oxidant – antioxidant status during 3 months.

Exercise group (EG, n=8): EG, exercises were performed on a treadmill for 45 minutes with a 50% overloading rate. During all cardio exercises, participants were monitored and motivated by 2 trainers for the efficiency of the study.

Massage and exercise group (MEG, n=8): MEG, exercises were performed on a treadmill for 45 minutes over 3 days in a week (Monday, Wednesday, and Friday) with a 50% overloading rate. Also, MEG subjects had an effleurage and petrissage massage manipulation with apricot kernel oil during 20 minutes after the exercise. During the study, participants were monitored and motivated by 2 trainers for the efficiency of the study. All exercise and massage sessions were performed at the same time of the day (08.30-11.30 AM). During 12 weeks study period EG and MEG subjects had exercised and massage manipulations three times a week (Monday, Wednesday, and Friday).
Massage program

The five main techniques of classical massage are effleurage, petrissage, friction, tapotement and vibration. The most important are these techniques for sport atmosphere Effleurage (or stroking) and Petrissage (or kneading). Effleurage is a deep stroking that should be in the direction of venous or lymph flow. Effleurage massage characterized by long, slow, gliding strokes (26), is a technique that used to over the centuries in various cultures as a traditional, non-pharmacological means of promoting rest and relaxation (27). Petrissage is a deeper technique than effleurage and is directed towards the muscles. The fingers and closed thumbs work like tongs grasping the tissues gently (28). This sort of massage can have a stimulating or relaxing effect on a muscle rely on the rate and pressure of massage as well as the amount of stretch applied to tissue. In the literature there were studies about massage time of its average time is 20 min (29). We have not yet established only massage group. So, we investigated to effect of exercise along with massage treatments.

Blood collection and biochemical analysis

Prior to- and one day after the twelve-week exercise and massage program, venous blood samples of 10 cc were taken from the subjects with a plastic syringe in a sitting position; the samples were centrifuged and stored at −80° Celsius in a deep-freezer until the final analyses were conducted. Plasma MDA level was measured via the Uchiyama and Mihara method (30). Sample absorbencies were first multiplied by the dilution factor 10, and then by the factor obtained through the standard graph, thus calculating the MDA amount as nmol/L. Plasma Total Nitric Oxide was measured via the Cortas and Wakid method. The result was calculated as μmol/L (31). ADA was calculated via the method developed by Ellis and Goldberg (32), and ADA activity was expressed as μmol/l. For GSH, the absorbance of the yellow product obtained through the reaction of Elman reagent with sulfhydryl groups was evaluated at 410 nm using spectrophotometric analysis, using the method developed by Fairbanks and Klee (33). Sample absorbencies were then multiplied by the factor obtained through the standard graph, thus calculating GSH activity as μmol/l. This method includes converting the superoxide radicals produced via xanthine oxidase by total (Cu-Zn and Mn) SOD (EC 1.15.1.1) into H₂O₂, and regressing nitroblue tetrazolium. Regressed NBT was converted into a blue formazan whose absorbance was at maximum at 560 nm (34), and the data was calculated to be U/l.

Data analysis

All statistical analyses were conducted using SPSS Program (version 17.0; Statistical Package for Social Science, SPSS Inc.). The analysis of the data began with a homogeneity test. As the data was not distributed homogeneously, the Wilcoxon Signed Ranks test was used to determine the significance level of the differences between the pre- and post-tests, and the Kruskal-Wallis H (KWH) and Mann-Whitney U (MWU) tests were used to determine the significance levels of the differences between the groups. Descriptive statistics were presented as mean±SD. A significance level p<0.05 was used in all statistical analyses.

Results

Table I shows the average age and height of the subjects significant differences between the subjects' body weights, BMI and MaxVO₂ values calculated before and after the study was determined to be positive for the values calculated after the exercise.

Twenty five overweight women participated in study, with a mean age of 40.3±6.1 years, body mass of 84.7±3.8 kg, heigh of 158.2±5.3 cm, BMI of 34.4±2.7 and MaxVO₂ 32.6±3.3 mL.kg/dk. Table I has been showed that a significant difference was found between the pre-test vs. post-test values of Body Mass, BMI and MaxVO₂ (p<0.05).

Table II shows the results of the Wilcoxon Signed Ranks Analysis, which was used to determine whether the MDA, NOx, GSH, ADA and SOD values differed significantly before and after the exercise.

There were significant differences of the pre and post-test MDA, GSH, and SOD (p<0.05) within groups. In
The effect of regular exercise and massage on oxidant levels.

Discussion

The main result of this study was that a statistically significant decrease was observed in the post-test MDA values of both EG and MEG participants. In addition to, when the GSH and SOD values are compared to CG, a statistically significant increase was determined in the values of both EG and MEG. These findings about MDA supported previous results in relevant literature in which Leaf et al., and Miyazaki noted that regular exercise no changed MDA, GSH, NOx and SOD enzyme activities. However, there were statistical differences between EG vs. MEG, ADA values in favour of MEG.

### Table I: Wilcoxon signed ranks test for pre-exercise and 12-week post-exercise physical and physiological parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CG</th>
<th>EG</th>
<th>MEG</th>
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<tbody>
<tr>
<td></td>
<td>X±Sd</td>
<td>X±Sd</td>
<td>X±Sd</td>
</tr>
<tr>
<td>N</td>
<td>9</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Age (year)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MaxVO₂ (mL.kg/ dk)</td>
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</table>

### Table II: Analysis of pre- and post test MDA, NOx, GSH, ADA and SOD values.

<table>
<thead>
<tr>
<th>Parameters</th>
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<th>EG</th>
<th>MEG</th>
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<tr>
<td></td>
<td>X±Sd</td>
<td>X±Sd</td>
<td>X±Sd</td>
</tr>
<tr>
<td>N</td>
<td>9</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOx (μmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSH (μmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADA (μmol/l)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>SOD (U/l)</td>
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### Table III: KWH and MWU analyses of pre- and post-test values of MDA, NOx, GSH, ADA and SOD.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group</th>
<th>X±Sd</th>
<th>KWH (X²)</th>
<th>MWU</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>p</td>
<td></td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td>CG</td>
<td>0.70±0.05</td>
<td>4.630</td>
<td>0.843</td>
</tr>
<tr>
<td></td>
<td>EG</td>
<td>0.64±0.04</td>
<td>1=3</td>
<td>1-3</td>
</tr>
<tr>
<td></td>
<td>MEG</td>
<td>0.66±0.03</td>
<td>2=3</td>
<td>2-3</td>
</tr>
<tr>
<td>NOx (μmol/l)</td>
<td>CG</td>
<td>0.19±0.06</td>
<td>6.507</td>
<td>0.672</td>
</tr>
<tr>
<td></td>
<td>EG</td>
<td>0.16±0.02</td>
<td>1=3</td>
<td>1-3</td>
</tr>
<tr>
<td></td>
<td>MEG</td>
<td>0.10±0.03</td>
<td>2=3</td>
<td>2-3</td>
</tr>
<tr>
<td>GSH (μmol/l)</td>
<td>CG</td>
<td>0.05±0.01</td>
<td>18.199</td>
<td>0.000*</td>
</tr>
<tr>
<td></td>
<td>EG</td>
<td>0.30±0.00</td>
<td>1=3</td>
<td>1-3</td>
</tr>
<tr>
<td></td>
<td>MEG</td>
<td>0.30±0.01</td>
<td>2=3</td>
<td>2-3</td>
</tr>
<tr>
<td>ADA (μmol/l)</td>
<td>CG</td>
<td>0.10±0.01</td>
<td>17.085</td>
<td>0.000*</td>
</tr>
<tr>
<td></td>
<td>EG</td>
<td>0.99±0.04</td>
<td>1=3</td>
<td>1-3</td>
</tr>
<tr>
<td></td>
<td>MEG</td>
<td>0.99±0.01</td>
<td>2=3</td>
<td>2-3</td>
</tr>
<tr>
<td>SOD (U/l)</td>
<td>CG</td>
<td>0.02±0.00</td>
<td>17.993</td>
<td>0.000*</td>
</tr>
<tr>
<td></td>
<td>EG</td>
<td>0.21±0.03</td>
<td>1=3</td>
<td>1-3</td>
</tr>
<tr>
<td></td>
<td>MEG</td>
<td>0.20±0.02</td>
<td>2=3</td>
<td>2-3</td>
</tr>
</tbody>
</table>

In other words the exhaustive exercise has decreased the MDA values and has increased the GSH and SOD enzyme activities. However for massage and exercise group there were no statistical difference between ADA and NOx values.

There were not significant differences of the pre and post-test differ EG vs. MEG values of MDA, GSH, NOx and SOD (p<0.05) within groups. However, there were statistical differences between EG vs. MEG, ADA values in favour of MEG.

**Discussion**

The main result of this study was that a statistically significant decrease was observed in the post-test MDA values of both EG and MEG participants. In addition to, when the GSH and SOD values are compared to CG, a statistically significant increase was determined in the values of both EG and MEG. These findings about MDA supported previous results in relevant literature in which Leaf et al., and Miyazaki
et al., reported that decreased MDA after regular exercise (35, 36). In the present study, the MDA values of the EG and MEG subjects were found to decrease after regular exercise. Therefore, regular exercise has a positive effect on the MDA value. Moreover, the combined application of exercise and massage was determined to decrease MDA. Thus, regular exercise combined with massage manipulations may have a positive effect on oxidative stress.

Regular physical activity was found to result in no statistical change in the NOx level. However, some studies suggested that acute exercises increased NOx value. Those studies of NOx were conducted with both animals and humans, and indicated that increases in the NOx level had negative effects on human health (37-39). Moreover, increased NOx level in skeletal muscles may cause muscular damage (40). Therefore, both regular exercises and massage manipulations may be beneficial in terms of changes in the NOx level. In the present study, the ADA parameter was also examined, which was addressed in only a few studies in the literature. ADA is related to the regulation of myocardial and coronary circulation functions (41-42). Therefore, the present research is unique in terms of its findings in this regard. ADA activity was found to decrease significantly in terms of both EG and MEG compared to the CG. However, further research should be conducted regarding the ADA activity.

The antioxidant defense system produces antioxidants in order to eliminate or prevent the negative effects of increased production of free radicals. Enzymatic antioxidants GSH and SOD have an important role in the antioxidant defense system (43, 44). Scholars have routinely investigated GSH and SOD status as a marker of oxidative stress because this marker to be one of the most confidential indices of exercise induced oxidant production (45). The present study determined a statistically significant increase in the SOD activity at the end of twelve weeks, which was positive for EG and MEG. When the pre- and post-test GSH and SOD parameters of the EG and MEG subjects were compared, they were found to increase compared to the CG subjects. Previous studies reported that antioxidant enzyme activities increased after regular exercise (46). In addition, Atabek et al., (47) reported that after regular exercise significantly increased SOD enzyme activities. The present study determined an increase in GSH level as a result of regular exercises and massage manipulations. Our result was paralleled with findings of Aslan et al., (48) and Atabek et al., (47) and found that increased GSH after regular exercise. It can be suggested that regular exercises resulted in an increase in the antioxidant activity parameters of GSH and SOD levels, thus having a positive effect on the oxidant and antioxidant balance.

Conclusions

As a result, the findings that regular physical activities and massage manipulations significantly decrease MDA, increase SOD and GSH activities, and result in no change in NOx and ADA activities supports the assumption that regular physical activity has positive health effects. However, the findings of the present study should be supported by further studies, specifically on the effect of massage manipulations on the oxidant and antioxidant balance.

References


