THE EFFECTS OF TWO FITNESS PROGRAMS WITH DIFFERENT METABOLIC DEMANDS ON OXIDATIVE STRESS IN THE BLOOD OF YOUNG FEMALES

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EFEKTI DVA FITNES PROGRAMA SA RAZLIČITIM METABOLIČKIM ZAHTEVIMA NA OKSIDATIVNI STRES U KRVI MLADIH DEVOJAKA

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ABSTRACT

The aim of the present study was to assess the effects of two metabolically different exercise programs on the redox state of women who were physically inactive before the beginning of the study. For this purpose, participants (women 25±5 years old) chose one of two popular fitness programs, Pilates or Tae Bo, and attended it 3 times a week for 12 weeks. At the beginning and end of the study, body composition analysis and venous blood sampling were performed. The levels of superoxide anion radical, hydrogen peroxide, nitric oxide and lipid peroxidation were measured in plasma, and the levels of reduced glutathione and the activity of superoxide dismutase and catalase were measured in erythrocytes. Only the Tae Bo program induced changes (positive) in body composition, whereas both exercise programs induced slight oxidative stress in exercisers. In the Tae Bo group, the levels of hydrogen peroxide were significantly increased, whereas the levels of reduced glutathione were decreased after three months of training. In the Pilates group, hydrogen peroxide and catalase activity were increased, and nitrites decreased. However, at the end of the study, those two groups had no significantly different values for any pro/antioxidant compared with the subjects who served as controls. This finding suggests that moderate physical activity, such as recreational fitness programs, may induce the increased production of reactive oxygen species but do not lead to a serious disturbance of the redox homeostasis of exercisers.

Keywords: *oxidative stress, redox balance, fitness, tae bo, pilates, women*

SAŽETAK

Cilj ove studije je da ispita efekte dva programa vežbanja sa različitim metaboličkim zahtevima na redoks status žena koje su inicijalno bile fizički neaktivne. Žene starosti 25±5 godina samostalno su odabrale jedan od dva ponuđena fitnes programa, Pilates ili Tae bo, i pohađale ga 3 puta nedeljno tokom 12 nedelja. Na početku, i na kraju studije, ispitanicama je procenjen telesni sastav i uzeti uzorci venske krvi. Nivoi superoksid anjon radikala, vodonik peroksida, azot monoksida i lipidne peroksidacije mereni su u plazmi, dok su nivoi redukovanog glutationa, i aktivnost superoksid dismutaze i katalaze mereni u eritrocitima. Samo Tae bo program je doveo do pozitivnih promena telesne kompozicije, dok su oba programa vežbanja dovela do narušavanja redoks homeostaze ispitanica. U Tae bo grupi nivoi vodonik peroksida su bili povećani, a nivoi redukovanog glutationa sniženi nakon 3 meseca treninga. U Pilates grupi, nivoi vodonik peroksida i aktivnost katalaze su bili povećani, a nivoi nitrita smanjeni. Ipak, na kraju studije nije bilo značajnih razlika u nivoima pro-antioksidanata između ove dve grupe vežbačica i osoba koje su činile kontrolnu grupu. Ovi rezultati upućuju na zaključak da umerena fizička aktivnost, kao rekreacioni fitnes programi, može dovesti do povećane produkcije reaktivnih kiseoničnih vrsta, ali ne dovodi do ozbiljnog narušavanja redoks homeostaze vežbača.

Ključne reči: *oksidativni stres, redoks ravnoteža, fitnes, tae bo, pilates, žene*

ABBREVIATIONS

ADS - antioxidative defense system;
CAT - catalase;
GSH - reduced glutathione;
RBCs - red blood cells;
RONS - reactive oxygen and nitrogen species;
SOD - superoxide dismutase;
TBARS - thiobarbituric acid reactive substances

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INTRODUCTION

Hypokinesia represents one of the major risk factors for numerous physical disorders, including cardiovascular diseases, obesity, diabetes, and osteoporosis (1). Recreational physical activities, such as different fitness programs, function in both the prevention and treatment of these abnormalities. Accumulating data indicate that exercise with moderate intensity has systemic and complex health-promoting effects, which undoubtedly involve the regulation of redox homeostasis and signalling (2). The relationship between exercise and oxidative stress has been intensively investigated for decades (3, 4); however, more data are required to address this association and its dependence on various relevant factors (5). For example, few studies have investigated the redox state of females. The reason for the disproportionate number of investigations on exercise-induced oxidative stress in male and female populations likely lies in the complexity of the examination and interpretation of the redox state of females, which occur due to hormonal differences between the sexes and their influence on results. It is believed that women are less susceptible to oxidative stress since because oestrogen is a potent antioxidant (6); however, one study showed that the phase of the menstrual cycle (i.e., estradiol concentration) exerts a minimal influence on the exercise-induced redox changes in young women (7).

In recent decades, a number of popular programmed group exercises for women have emerged, such as Pilates and Tae Bo. Those two fitness programs differ significantly in their metabolic and motoric demands. Tae Bo is high-intensity aerobic training that uses different movements from martial arts, dance and aerobics that are combined in choreography with fast music. In contrast, Pilates is a specific form of training that is based on breathing and uses untypical initial positions and exercises to develop muscular strength and flexibility (8). Keeping in mind the deficiency of data on exercise-induced oxidative stress in females as well as the popularity of the above-mentioned fitness programs, the aim of our research was to assess the changes in the redox state of young, previously sedentary females after three months of programmed exercise. The secondary aim of the research was to compare the effects of two metabolically different exercise programs because different mechanisms and the quantity of reactive oxygen species production may be expected from a highly aerobically demanding and static, strength-oriented physical activity.

MATERIAL AND METHODS

Subjects

The sample consisted of 59 sedentary women (25±5 years old). The control group consisted of 10 sedentary women, and there were two experimental groups: the Pilates (n=19) and Tae Bo (n=20) groups. The Pilates and Tae Bo groups took part in the experiment at will and were obligated to attend these programmed activities in addition to maintaining their usual everyday activities and nutrition. All participants were healthy, did not use medications or supplements before the beginning of the study, and were non-smokers. The study was performed in accordance with the Declaration of Helsinki and was approved by the ethical committee of The Faculty of Medical Sciences, University of Kragujevac.

Protocol

Venous blood samples were taken from all participants before the beginning of the study and 3 months later. The characteristics of the two fitness programs are presented in Table 1.

The intensity of both programs was monitored by the Polar Team² System (Polar Electro Oy, Finland) for heart rate monitoring, which was worn by every second exerciser. Warming up in Pilates and Tae Bo consisted of exercises that included large muscle groups and lasted approximately 10 minutes. The main part lasted 45 minutes and consisted of specific exercises depending on the program (Pilates or Tae Bo). Relaxing and stretching included exercises that provided body cool down and relaxation and lasted approximately 5 minutes.

Body composition was measured using the *Tanita BC-418* apparatus for bioelectrical impedance analysis.

Table 1. Characteristics of Pilates and Tae Bo programs.

Characteristic	Pilates	Tae Bo
Duration of class	60 minutes	60 minutes
Duration of program	3 months	3 months
Number of classes per week	3 classes per week	3 classes per week
Intensity	50-65 % HRmax	60-85 % HRmax
Structure of class	1) Warm up 2) Main portionRelaxation (breathing exercises)	1) Warm up 2) Main portionRelaxation and stretching
Musical tempo of main portion of class	100-110 bpm	130-150 bpm
Equipment	Resistance bands	Weights

Biochemical assays

Blood samples were taken from an antecubital vein into a Vacutainer test tube containing sodium citrate anticoagulant. Blood samples were analyzed immediately. Blood was centrifuged to separate plasma and red blood cells (RBCs). Biochemical parameters were measured spectrophotometrically.

Superoxide anion radical determination

The level of superoxide anion radical (O_2^-) was measured using nitro blue tetrazolium reaction in TRIS-buffer combined with plasma samples and read at 530 nm (9). The levels of O_2^- are presented in nmol/ml of plasma.

Hydrogen peroxide determination

The protocol for measuring hydrogen peroxide (H_2O_2) is based on the oxidation of phenol red in the presence of horseradish peroxidase (10). A 200 µl sample with 800 µl phenol red solution) and 10 µl horseradish peroxidase were combined (1:20). The level of H_2O_2 in plasma was measured at 610 nm. The levels of H_2O_2 are presented in nmol/ml of plasma.

Nitric oxide determination

Nitric oxide (NO) decomposes rapidly to form stable metabolite nitrite/nitrate products. Nitrite (NO_2^-) was determined as an index of nitric oxide production with Griess reagent (11). Approximately 0.1 ml 3N perchloride acid, 0.4 ml 20 mM ethylenediaminetetraacetic acid and 0.2 ml plasma were put on ice for 15 min and were then centrifuged for 15 min at 6000 rpm. After pouring off the supernatant, 220 µl K_2CO_3 was added. Nitrites were measured at 550 nm. Distilled water was used as a blank probe. The levels of NO_2^- are presented in nmol/ml of plasma.

Index of lipid peroxidation (thiobarbituric acid reactive substances, TBARS)

The degree of lipid peroxidation in plasma was estimated by measuring the thiobarbituric acid reactive substances (TBARS) using 1 % thiobarbituric acid in 0.05 NaOH, whch were incubated with plasma at 100 °C for 15 min and read at 530 nm. Distilled water was used as a blank probe. Thiobarbituric acid extract was obtained by combining 0.8 ml plasma and 0.4 ml trichloroacetic acid; the samples were then put on ice for 10 minutes and centrifuged for 15 min at 6000 rpm. This method was described previously (12). The levels of TBARS are presented in µmol/ml of plasma.

Determination of antioxidant enzymes

Isolated RBCs were washed three times with 3 volumes of ice-cold 0.9 mmol/l NaCl and hemolysates containing approximately 50 g Hb/l (prepared according to McCord and Fridovich (13)) were used for the determination of catalase (CAT) activity. CAT activity was determined according to Beutler (14). Lysates were diluted with distilled water (1:7 v/v) and treated with chloroform-ethanol (0.6:1 v/v) to remove haemoglobin (15). Then, 50 µl catalase buffer, 100 µl sample and 1 ml 10 mM H_2O_2 were added to the samples. Detection was performed at 360 nm. Distilled water was used as a blank probe. Superoxide dismutase (SOD) activity was determined using the epinephrine method of Misra and Fridovich (16). Approximately 100 µl lysate and 1 ml carbonate buffer were mixed, and 100 µl of epinephrine was added. Detection was performed at 470 nm. The activities of SOD and CAT in red blood cells (RBCs) are presented in units per gram of haemoglobin x 10^3 (U/g Hb x 10^3)

Determination of glutathione

The level of reduced glutathione (GSH) was determined based on GSH oxidation with 5.5- dithio-bis-6.2-nitrobenzoic acid using the Beutler method (17); the concentration is expressed as nanomoles per millilitre of RBCs.

Statistics

The distribution of the data was checked with the Shapiro-Wilk test, and depending on its result, the appropriate parametric or nonparametric test was used. The differences between the values of means from two related samples (before and after the exercise period) were assessed by a paired t-test or Wilcoxon's test. The difference among three unrelated samples (between groups on initial and on final examination) was assessed by ANOVA or the Kruskal Wallis test, followed by the T test or Mann-Whitney U test. The alpha level for significance was set to $P < 0.05$.

Table 2. Anthropometric characteristics of the investigated group (*P<0.05 when compared to the initial value).

Characteristic	Beginning of the study (X±SD)	End of the study (X±SD)
Height (cm)		
Controls	171.50±4.08	
Pilates	169.15±6.36	
Tae Bo	168.70±6.20	
Weight (kg)		
Controls	61.85±7.29	62.16±7.84
Pilates	63.14±7.93	62.49±6.98
Tae Bo	67.35±12.80	65.23±12.04*
Body mass index (kg/m²)		
Controls	21.36±2.21	21.47±2.30
Pilates	22.17±3.28	21.89±3.03
Tae Bo	23.64±4.04	23.03±3.79*
Fat (%)		
Controls	19.91±5.11	20.20±5.35*
Pilates	21.69±7.59	21.30±6.80
Tae Bo	25.23±9.40	24.02±8.88*
Muscle (%)		
Controls	13.76±0.97	13.84±1.07
Pilates	13.71±1.37	13.67±1.18
Tae Bo	12.98±1.62	13.19±1.55*

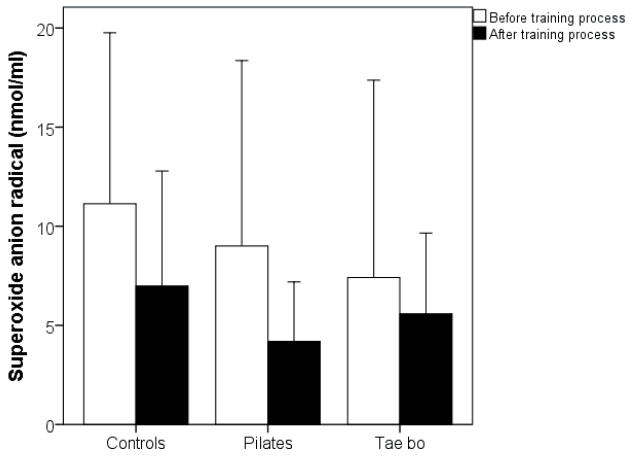

Figure 1. Levels of superoxide anion radical (X±SD) in the investigated groups before and after the training period.

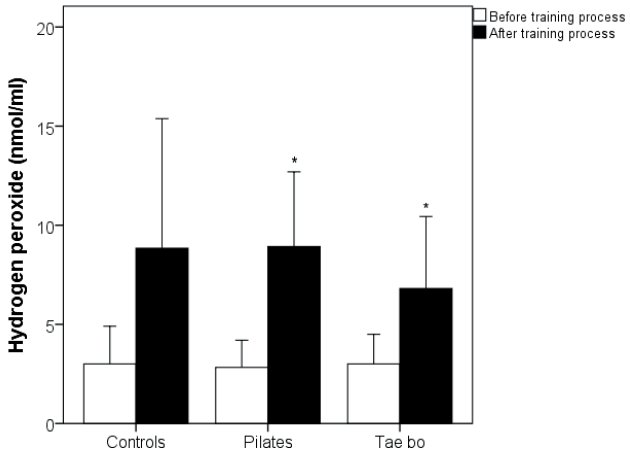

Figure 2. Levels of hydrogen peroxide (X±SD) in the investigated groups before and after the training period (*P<0.05 compared with the beginning of the study).

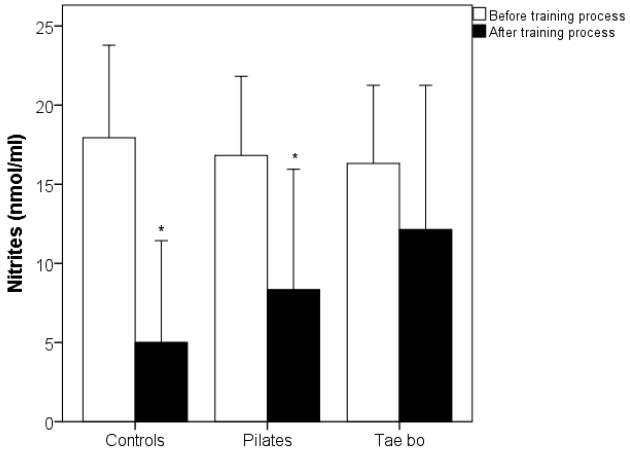

Figure 3. Levels of nitrites (X±SD) in the investigated groups before and after the training period (*P<0.05 compared with the beginning of the study).

RESULTS

The anthropometric characteristics of the investigated groups at the beginning and at the end of the study are presented in Table 2. After three months of training, all parameters of body composition changed significantly in subjects who practiced Tae Bo (body weight: P=0.002, % fat: P=0.005, % muscle: P=0.008, BMI: P=0.002), and the body fat increased in the control group (P=0.038). However, there was no significant difference between groups in the investigated anthropometric parameters at either the beginning or the end of the study.

Changes in levels of pro/antioxidants in plasma and red blood cells of subjects are presented in Figures 1 to 7. The levels of NO_2^- were significantly lower in the Control (P=0.043) and Pilates (P=0.007) groups compared with the levels measured at the beginning of the study. The Pilates group also had higher levels of H_2O_2 (P=0.001) and CAT activity (P=0.011) at the end of the study. In the Tae Bo group, H_2O_2 increased (P=0.002), but GSH decreased (P=0.006) after three months of training.

The differences between groups in the levels of investigated redox parameters are also presented in Figures 1 to 7. At the beginning of the study, subjects from the Control group had significantly lower SOD activity compared with both the Tae Bo (P=0.040) and Pilates groups (P=0.037). At the end of the study, the groups did not significantly differ in any redox parameter.

DISCUSSION

The aim of the present study was to assess the effects of two metabolically different exercise programs on the redox state of women who were physically inactive before the beginning of the study. For this purpose, participants chose one of two popular fitness programs, Pilates or Tae Bo, and attended it three times a week for 12 weeks. At the beginning and end of the study, body composition analysis and blood sampling were performed. Only the Tae Bo program induced (positive) changes in body composition, although both exercise programs disturbed the redox homeostasis of subjects.

There is a common acceptance that Pilates and other forms of low-intensity exercises, when they are performed slowly and with proper breathing, will do far more to improve health status than will a vigorous cardiovascular or strength workout (18). Those types of exercise may be classified as parasympathetic exercise: they do not raise the heart rate and breathing rate significantly, but they significantly reduce stress levels (18). In contrast, intensive aerobic exercise, such as Tae Bo, is characterized by increased oxygen consumption and a possible disturbance of intracellular pro/antioxidant homeostasis. In general, the body has adequate antioxidant reserves to cope with the production of reactive oxygen species (ROS) under physiological conditions and perhaps during low- to moderate-

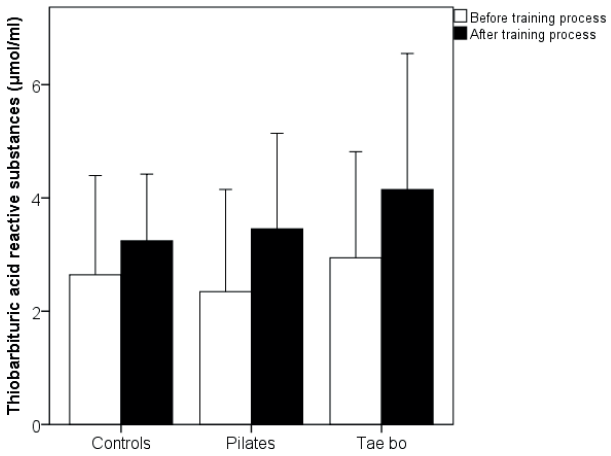

Figure 4. Levels of thiobarbituric acid reactive substances (X±SD) in the investigated groups before and after the training period.

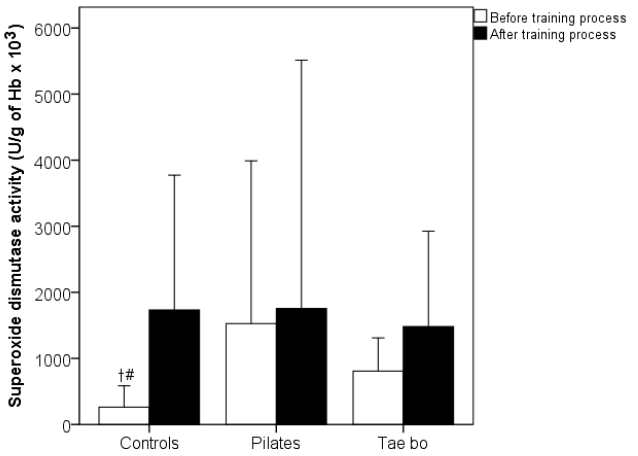

Figure 5. Superoxide dismutase activity (X±SD) in the investigated groups before and after the training period (†P<0.05 compared with the Pilates group at the beginning of the study; #P<0.05 compared with the Tae Bo group at the beginning of the study).

intensity exercise, but when ROS production is excessive, as occurs during intensive physical efforts, an imbalance between prooxidants and antioxidants in favour of the prooxidants may occur and may lead to a disruption of redox signalling and control and/or molecular damage (19). The effect of exercise on the redox state of an individual depends on many factors, such as the type of training, training load, and individual reaction of an athlete depending on age, gender, and the coexisting factors of risk and physical condition (20). In our study, subjects from both the Pilates and Tae Bo groups had increased levels of H_2O_2 compared with the levels measured before engagement in exercise training, although the levels of this prooxidant all other measured redox parameters were not significantly different among the investigated groups (Control, Pilates and Tae Bo) at the end of the study. In addition to the increase in H_2O_2 levels, at the end of the study, the Pilates group had increased CAT activity and decreased levels of NO_2^- (as a marker of NO) in blood, whereas the Tae Bo group experienced a decrease in the levels of GSH. In the Control group, a decrease in the NO_2^- levels was observed at the end of the study. Those results are consistent with the results of the only similar previously published study (18). In that study, Radovanovic and colleagues reported increased values of the activity of malondialdehyde, protein carbonyls and total sulphydryls in both Pilates- and Tae Bo-trained subjects, but there were no significant differences between the groups (18). After 12 weeks of training, the CAT activity increased in both groups, but the increase was significant only in the Pilates group, whereas Tae Bo subjects had significantly increased total serum antioxidant activity (18).

The results that we obtained suggest that both of the exercise programs induced changes in the redox state of exercisers in the direction of oxidative stress. At the end of the study, the Tae Bo subjects had significantly higher levels of H_2O_2 and lower levels of GSH, which is an element of the endogenous antioxidant system that is directly involved in H_2O_2 elimination. In contrast, in the Pilates group, the

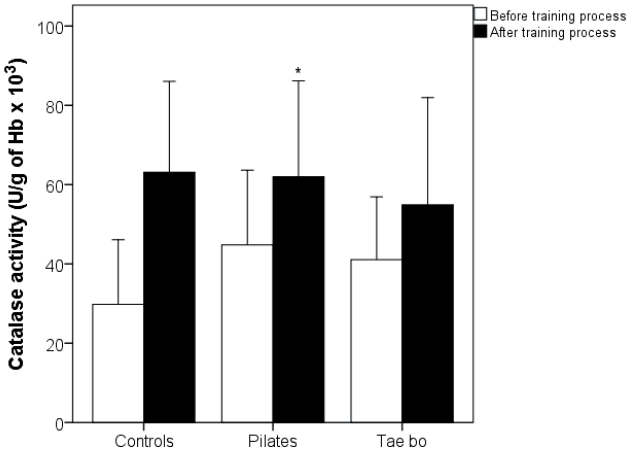

Figure 6. Catalase activity (X±SD) in the investigated groups before and after the training period (*P<0.05 compared with the beginning of the study).

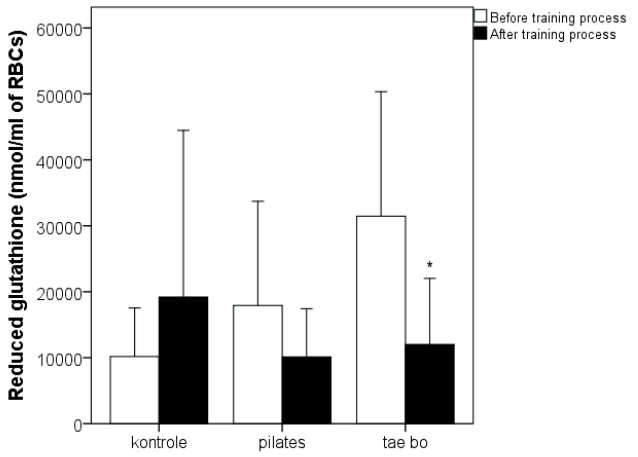

Figure 7. Levels of reduced glutathione (X±SD) in the investigated groups before and after the training period (*P<0.05 compared with the beginning of the study).

increased H_2O_2 levels were followed by increased CAT activity, with no significant change in the levels of GSH. GSH both directly and indirectly (as a cofactor for glutathione peroxidase - GPx) eliminates free radicals (21). CAT and GPx are both engaged in H_2O_2 elimination, but their affinity for it is different and dose-dependent. The affinity of GPx for H_2O_2 is higher at low H_2O_2 levels, whereas CAT affinity increases with an increase in the H_2O_2 levels. Although the difference was not found to be statistically significant, the Pilates group had higher levels of H_2O_2 than did the Tae Bo group, which increased the CAT activation to higher (significant) levels. Interestingly, no group had increased levels of lipid peroxidation (measured through TBARS), which would be expected, especially in the Tae Bo group, whose levels of GSH were exhausted. It was previously shown that GSH blood levels are a determinant of plasma TBARS at rest (22). Subjects with a favourable blood glutathione redox status at rest maintain a more favourable redox status in response to exercise-induced oxidative stress, and vice versa (22). Furthermore, the levels of NO_2^- were also found to be decreased in the Control and Pilates groups, though that was not the case in subjects who practiced Tae Bo. The absence of change in the Tae Bo group may be explained by the effect of increased shear stress on endothelium, characteristic for high-intensity aerobic exercise. Numerous previous studies have shown that regular endurance activity increases the bioavailibity of NO (23-25) and that physically active people have greater basal NO production compared with a sedentary population (26, 27). Finally, the observed differences between the groups in the levels of SOD at the beginning of the study may be explained by the more active lifestyle of subjects who chose to be engaged in training, and they likely do not represent the control group. The absence of significant changes in SOD activity due to the three-month training program is surprising because the majority of previously published studies showed that it is the enzyme that is most susceptible to change due to acute or chronic exercise (28).

CONCLUSION

Although both exercise programs induced an increase in the production of reactive oxygen species, the fact that exercisers did not have significantly different values for any pro/antioxidant relative to controls suggests that moderate physical activity, such as participation in recreational fitness programs, does not lead to a serious disturbance of the redox homeostasis of exercisers. Furthermore, as we previously reported (29), both Pilates and Tae Bo induced significant improvements of the motoric status of women, especially balance and leg and abdominal strength. However, because only Tae Bo induced significant changes in the body composition of exercisers, this high-intensity aerobic activity should be the activity of choice for those who wish to lose and control their weight.

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