

# Effects of active video gaming on oxidative stress and antioxidant status in university students

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Authors' Contribution: A – Study design; B – Data collection; C – Statistical analysis; D – Manuscript Preparation; E – Funds Collection

## Abstract

**Background and Study Aim** Many findings have demonstrated that several life-threatening diseases, including cardiovascular diseases, obesity-related diseases, and certain types of malignancies, can be prevented by exercise. Reactive oxygen species (ROS) may be the direct or associated factor that causes or prevents these diseases. It is well known that a strenuous and high-intensity acute exercise increases ROS production and intensifies oxidative stress. At this point, the importance of physical activity (PA) and exercise in reducing oxidants and improving antioxidant defense system increasing. The purposes of the current study were to investigate the short term (acute) and long term (chronic) effects of active video gaming (AVG) on oxidative stress indices.

**Material and Methods** Nine healthy male university students participated in the study. The participants played an AVG as vigorous physical activity (MET > 7) for 20 minutes, three days a week, for 4 weeks. Each participant completed twelve game sessions. Blood samples were obtained just before (after 10 min rest in seated position) and immediately after the game session on the first and the last day of the study. Total oxidant and antioxidant status (TOS and TAS) were determined using an automated measurement method, and the oxidative stress index (OSI) was calculated. Total oxidized guanine (TOG) and superoxide dismutase (SOD) activity were analyzed using commercial kits (Cayman Chemical). Data were analyzed with dependent t-tests and significance was accepted as  $p < 0.05$ .

**Results** The SOD activity significantly decreased after the AVG on the first day ( $4.78 \pm 1.26$  vs.  $3.81 \pm 1.80$ ;  $p = 0.026$ ). The TOS ( $6.62 \pm 1.09$  vs.  $7.30 \pm 1.24$ ) and TOG ( $9.26 \pm 2.69$  vs.  $9.78 \pm 2.62$ ) levels increased on the first day, but these alterations were not significant. Additionally, no significant acute changes were observed for the last day of the study

**Conclusions** The 4-week active video gaming practices did not change the oxidative stress status of pre-game, chronically. Four-week-AVG practice did not change oxidative stress indices significantly in rest.

**Keywords:** superoxide dismutase, oxidative stress, antioxidants, preventing diseases, exergames

## Introduction

Many findings have demonstrated that several life-threatening diseases, including cardiovascular diseases, obesity-related diseases, and certain types of malignancies, can be prevented by exercise [1, 2, 3]. Reactive oxygen species (ROS) may be the direct or associated factor that causes or prevents these diseases [4]. Although ROS primarily function as messengers in signal transduction to regulate diverse cellular functions [5], an imbalance between ROS and antioxidants have been shown to increase oxidative stress levels [6, 7]. At this point, the importance of physical activity (PA) and exercise in reducing oxidants and improving antioxidant defense system increasing [8].

It is well known that a strenuous and high-intensity acute exercise increases ROS production and intensifies oxidative stress [9]. On the other hand, when exercise – aerobic or anaerobic – is performed regularly for a long time, the antioxidant defense system, which prevents oxidative stress or reduces its damage, becomes stronger [10, 11].

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Superoxide dismutase (SOD) activity takes place in the first step of the antioxidant defense system and is associated with oxygen consumption ( $VO_2$ ). High SOD values and total antioxidant status (TAS) have been recorded in trained individuals (athletes) with high maximal oxygen consumption ( $VO_2$  max), compared with sedentary ones [12, 13]. Physical activity and exercise are recommended not only for this reason, but also because they positively affect health in many different ways. A dose-response relationship has been observed, with more PA associated with greater health benefits [14].

Unfortunately, reports show that participation of young people (nearly 38.4% of individuals between the ages of 18–24) in PA is not even at the minimum recommended level [15], so that, it is important to incorporate, facilitate, and encourage the use of new tools or forms of exercise that increase PA [16]. On the other hand, with the increase in the use of game consoles, it is observed that people prefer to spend time entertaining themselves by playing video games [17].

Laboratory-based studies (i.e. highly controlled basic researches) support that active video games

(AVGs) acutely induce light-to moderate PA and enhance health indices such as  $VO_2$  max [18, 19, 20]. The findings indicate that playing AVGs increase  $VO_2$  (ml/kg/min), energy expenditure, heart rate, metabolic equivalent (MET),  $VO_2$  max and PA comparing with resting or sedentary situations (16-18, 21, 22, 23], which suggest that AVGs can be used as an alternative exercise form to improve physical fitness and general health status. Even more importantly, there is a broad spectrum in the definition of AVG's as an exercise. AVGs were defined as low to high-intensity intermittent exercise, and some of them could be accepted as aerobic exercise, while others could be accepted as either aerobic or anaerobic exercise, based on the physiological responses [21, 23]. This shows that the effects of AVGs in terms of PA, physical fitness, and health are still a matter of serious research. Moreover, most of the studies have been conducted acutely and examined the physiological variables, and few studies have examined the chronic effect of AVGs [24, 25, 26]. But strikingly, data on oxidative stress related to a healthy population is almost nonexistent. Hence, the purpose of this study was to investigate the acute and chronic effects of AVGs on indices of oxidative stress in young participants. Based on the findings of the recent studies [21, 27], it was hypothesized that after 4-week AVGs practicing, AVGs would increase antioxidant defense system components (total antioxidant status and SOD activity) and decrease one of the oxidative stress marker – total oxidized guanidine (TOG).

## Materials and Methods

### Participants

Nine healthy, physically active young males who were students in sports science faculty (age:  $21.7 \pm 1.4$  years, height:  $178.5 \pm 5.4$  cm, weight:  $71.3 \pm 8.7$  kg, and Body Mass Index:  $22.4 \pm 2.0$  kg/m<sup>2</sup>) volunteered to participate in the study. Primary exclusion criteria encompassed the utilization of tobacco items, experiencing acute or chronic illnesses, and documented consumption of any medications or supplements with antioxidant properties. Prior to the commencement of the study, all participants were duly notified of the potential experimental hazards and their entitlement to discontinue their involvement at any point without facing adverse outcomes. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki, and written consent forms were obtained.

### Research Design

The active video fighting game was played by participants using the Xbox Kinect console, three days a week, for 20 minutes per day, over a span of four weeks. The previous practices in our laboratory have shown that the active video fighting game provides vigorous physical activity (MET>7) [23]

that was the main reason of selection of this game. Each participant completed twelve game sessions.

### Blood Sampling

Blood samples were obtained just before (after 10 min rest in seated position) and immediately after the game session on the first and the last day of the study. Blood collected without the use of an anticoagulant was centrifuged at 2200xg for 20 minutes at 4°C, then, serum samples were separated, and stored at -80°C until analysis [13].

### Analyses of Oxidative Stress Indices

The automated measurement technique developed by Erel [28] was utilized to ascertain the total antioxidant status (TAS) of serum samples. This method involves the generation of a highly potent biological radical, hydroxyl radical. The assay protocol included adding 500  $\mu$ L of "Reactive 1" and 30  $\mu$ L of each serum sample (or standard/ $H_2O$ ) to duplicate wells on plates. The initial absorbance was then recorded at 660 nm following kit instructions. Subsequently, 75  $\mu$ L of "Reactive 2" was introduced to all rows, and the plates were incubated at 37°C for 5 minutes, after which the second absorbance was measured at 660 nm [13]. The calculation of TAS involved applying Equation 1 to the obtained absorbance values from the two readings [13].

The precision of the assay was maintained below 3%, and the outcomes were expressed as mmol Trolox Equiv./L.

$$\text{TAS Results} = \frac{[(\Delta\text{Abs } H_2O) - (\Delta\text{Abs Sample})]}{[(\Delta\text{Abs } H_2O) - (\Delta\text{Abs Standard})]} \quad 1$$

The serum total oxidant status (TOS) was determined using an automated measurement method developed by Erel [29]. Following this approach, 500  $\mu$ L of "Reactive 1" and 25  $\mu$ L of each serum sample (or standard) were added to duplicate wells on plates. The initial absorbance was recorded at 530 nm in accordance with the kit instructions. Further, 25  $\mu$ L of "Reactive 2" was introduced to all rows, and after incubation at 37°C for 5 minutes, the second absorbance was recorded at 530 nm. Calibration of the assay involved the use of hydrogen peroxide, and the outcomes were reported as micromolar hydrogen peroxide equivalent per liter ( $\mu$ mol  $H_2O_2$  Equiv./L) [13]. The calculation of TOS involved utilizing Equation 2, which relied on the absorbance values from the two readings.

$$\text{TOS Results} = [(\Delta\text{Abs Sample}) - (\Delta\text{Abs Standard})] * 10 \mu\text{mol/L} \quad 2$$

For the calculation of the oxidative stress index (OSI), the TOS level was divided by the TAS level as a percentage ratio [30], as outlined in Equation 3.

$$\text{OSI (Arbitrary Unit)} = \frac{\text{TOS } (\mu\text{mol } H_2O_2 \text{ Equiv./L})}{\text{TAS (mmol Trolox Equiv./L)}} \quad 3$$

TOG was assessed employing an enzyme-linked

immunosorbent assay kit (Cayman Chemical, DNA/RNA Oxidative damage ELISA Kit). To initiate the process, 8-OHdG standards were prepared at room temperature, following the guidelines provided by the kit. These standards encompassed concentrations of 10.3, 23.1, 52, 117.1, 263.4, 592.6, 1333, and 3000 pg/ml [13]. Subsequently, each well of two rows on the plates received 50 µL of each standard, while duplicate samples of serum, comprising 50 µL each, were also added to the plates. Furthermore, 50 µL of 8-OHdG AChE tracer was introduced to all wells, excluding the Total activity (TA) and Blank (Blk) wells. An additional 50 µL of the primary antibody was applied to a 50 µL aliquot of both the sample and standard on microtiter plates previously coated with 8-OHdG. Following this, the plates were enveloped with a plastic film and left to incubate for 18 hours at 4°C. Subsequent steps involved emptying the plates, rinsing them five times with wash buffer, and then introducing 200 µL of Ellman’s reagent to each well. Additionally, 5 µL of tracer was added to the wells intended for total activity measurement. The plates were then covered with a plastic film and subjected to an orbital shaker in darkness for a duration of 120 minutes. Following this incubation, the plates were read at 420 nm after removing the plastic film, wiping the plate’s bottom with a clean tissue, and adhering to the kit’s instructions [13].

SOD activity was evaluated using a commercial

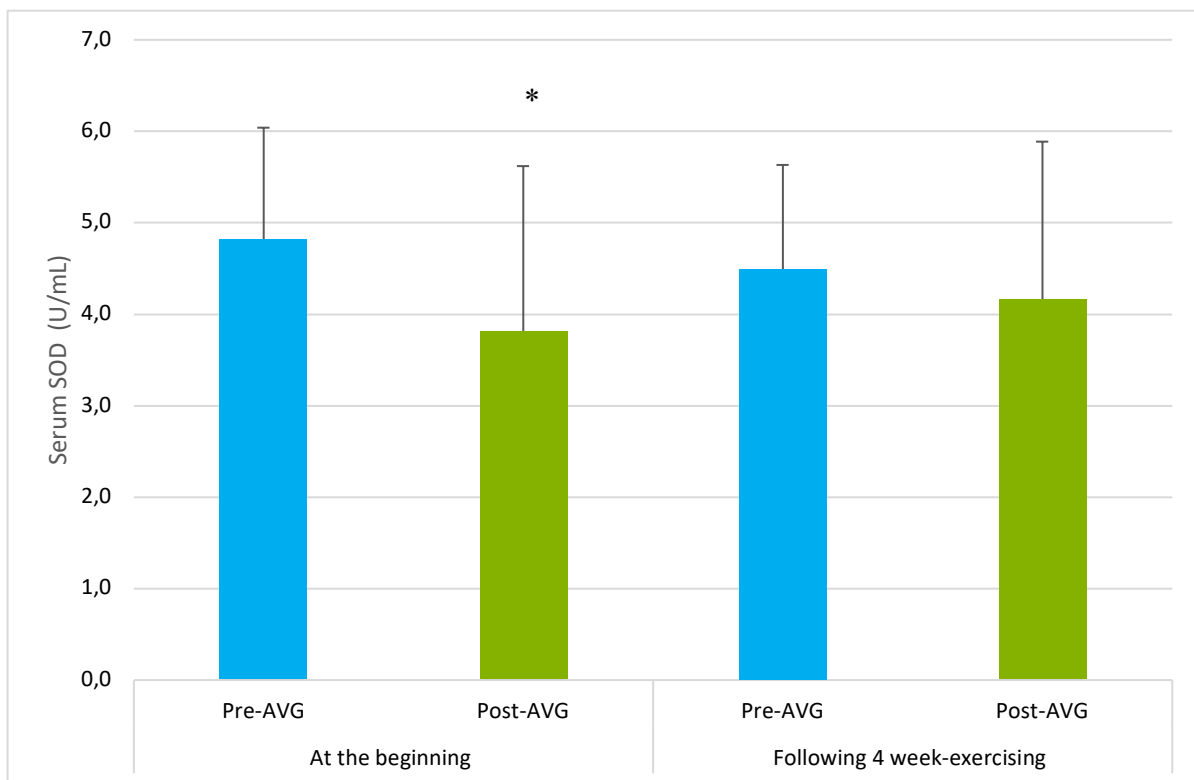
kit from Cayman Chemical, which measures all three SOD types (Cu/Zn-, Mn-, and Fe-SOD). Absorbance was read at 450 nm, and the calculation of serum SOD activity utilized a bovine erythrocyte SOD (Cu/Zn) standard curve (0–0.25 U/ml;  $r^2 > 0.96$ ) [13].

#### Statistical Analysis

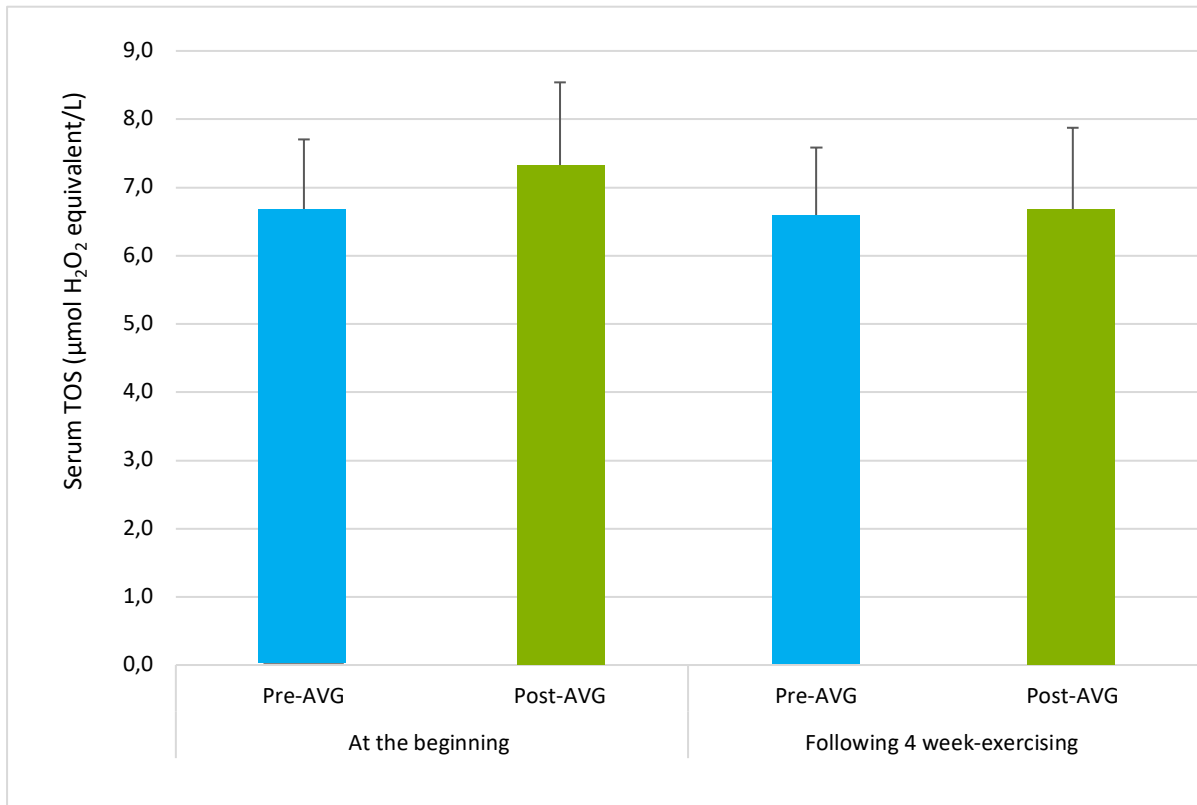
All values were presented as mean ± standard deviation (SD). Before parametric analyses were done, the normality of distribution of the data was assessed with Kolmogorov-Smirnov test. Data were analyzed with dependent t-tests and significance was accepted as  $p < 0.05$ . Statistical analyses were performed using the SPSS software version 23.0 for Windows.

### Results

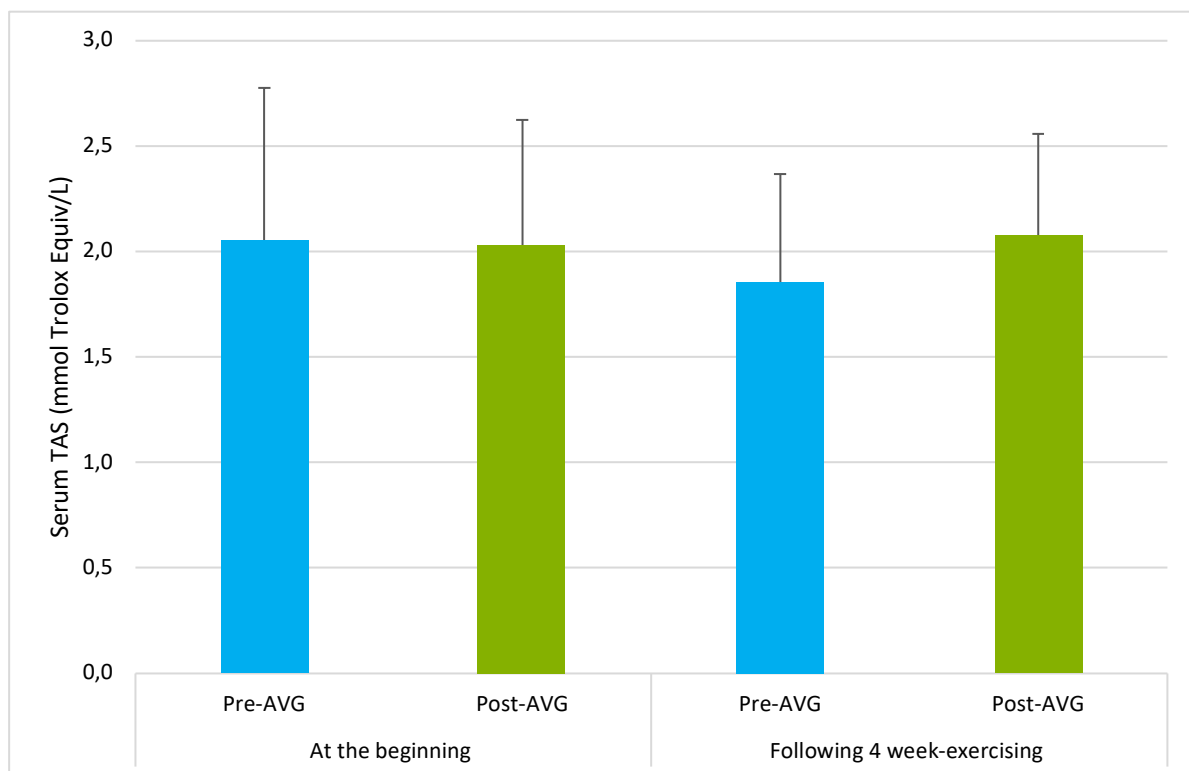
The alterations in SOD, TOS, TAS, OSI, and TOG values, selected as oxidative stress indices, are presented in Figures 1, 2, 3, 4, and 5, respectively. The findings of the current study showed that the SOD activity (U/ml) decreases immediately after the game, but only the decrement on the first day was statistically significant ( $4.78 \pm 1.26$  vs.  $3.81 \pm 1.80$ ;  $p = 0.026$ ; Figure 1). The TOS ( $6.62 \pm 1.09$  vs.  $7.30 \pm 1.24$ ) and TOG ( $9.26 \pm 2.69$  vs.  $9.78 \pm 2.62$ ) values increased on the first day, but these alterations were not significant (Figure 2, 3, 4, 5). Additionally, no significant acute changes were observed for the last day of the study. For the long term effects the



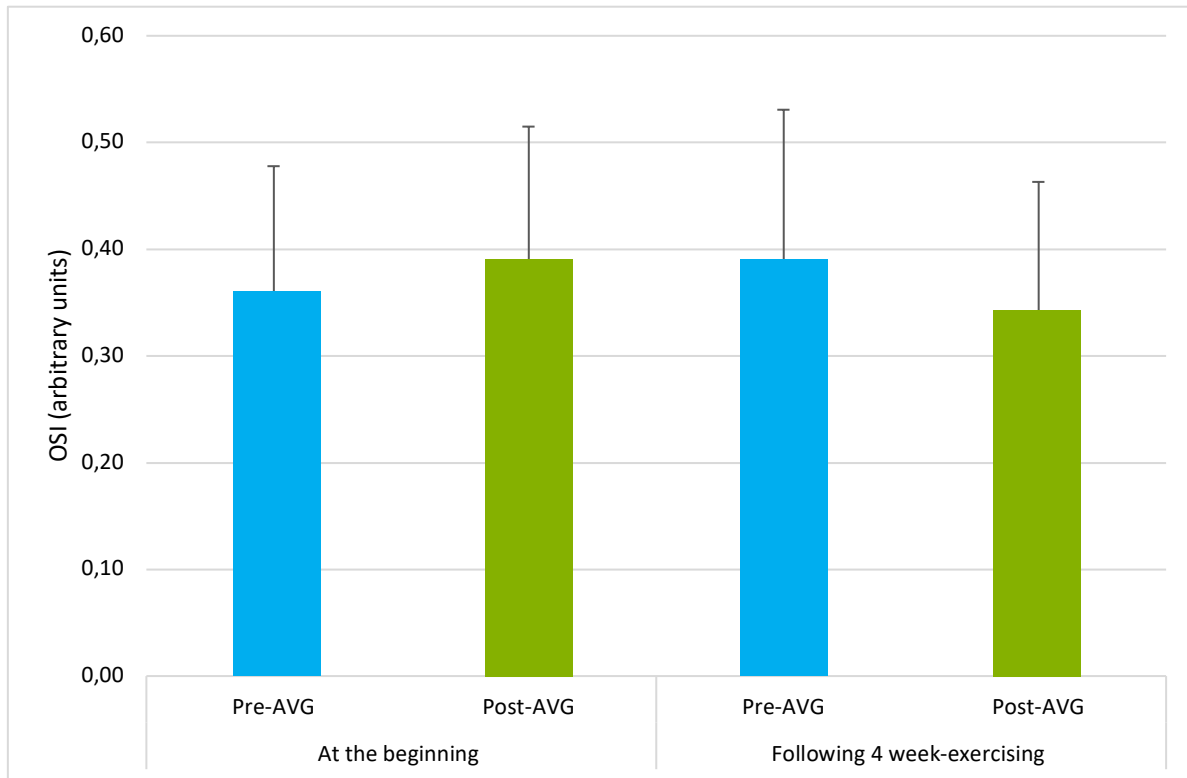
**Figure 1.** Changes in the serum superoxide dismutase activity. SOD: superoxide dismutase; Pre: before the active video fighting game; Post: immediately after the active video fighting game; \*  $p < 0.05$ : significantly different from Pre-AVG value.



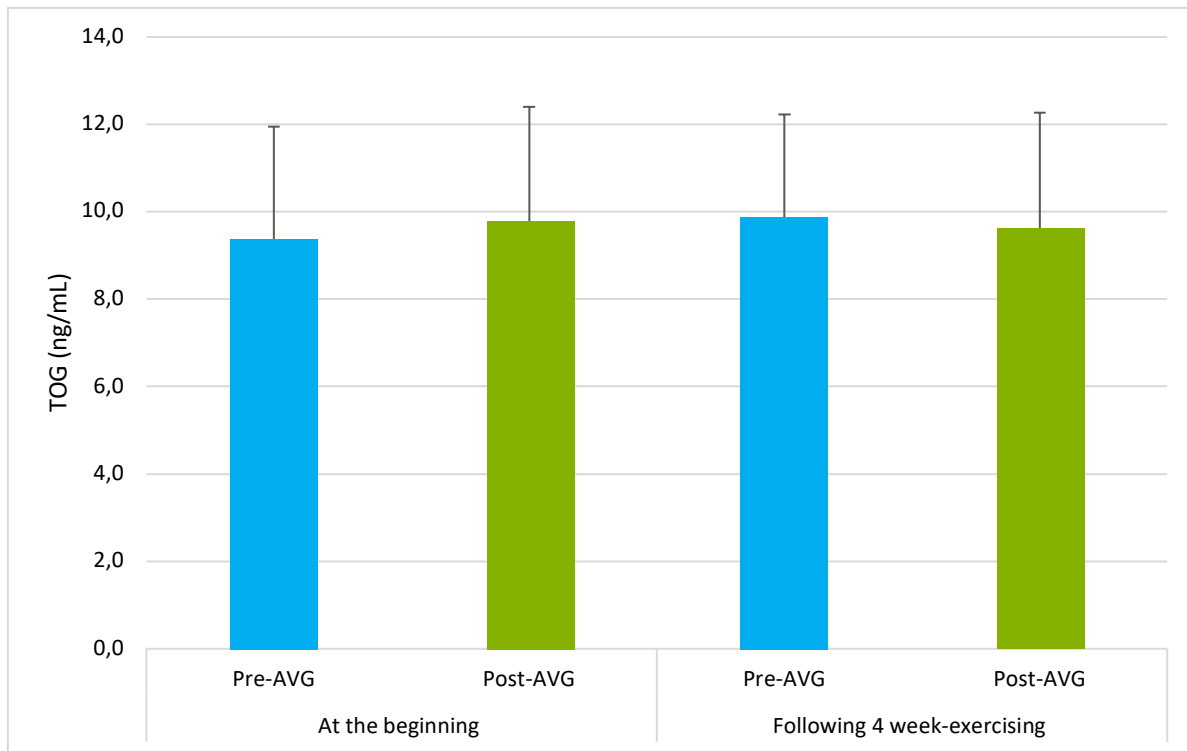
**Figure 2.** Changes in the serum total oxidant status. TOS: total oxidant status; Pre: before the active video fighting game; Post: immediately after the active video fighting game; No significant alterations were observed.



**Figure 3.** Changes in the serum total antioxidant status. TAS: total antioxidant status; Pre: before the active video fighting game; Post: immediately after the active video fighting game; No significant alterations were observed.



**Figure 4.** Changes in the serum oxidative stress index values. OSI: oxidative stress index; Pre: before the active video fighting game; Post: immediately after the active video fighting game; No significant alterations were observed



**Figure 5.** Changes in the serum total oxidized guanine values. TOG: total oxidized guanine; Pre: before the active video fighting game; Post: immediately after the active video fighting game; No significant alterations were observed.

pre-game (Pre-AVG) measurements were compared; as a result, the 4-week active video fighting game practices did not change the oxidative stress status.

## Discussion

This research aimed to investigate the acute and chronic effects of 4-week active video fighting game exercising on selected oxidative stress indices. Findings of the current study show that except the SOD activity on the first day of the study, no significant acute effects were observed. Also, contrary to our hypothesis, following the 4-week practices there was no significant alteration for rest (pre) measurements, which reflect the chronic effects.

Oxidative stress has been shown to increase in active muscle during exercise [31]. Additionally, studies show that high-intensity endurance exercise raises oxidative stress levels in the blood of untrained subjects, and in addition to the intensity and duration of exercise, exercise mode also contributes to the amount of oxidant production [32]. The current findings support this; the SOD activity significantly decreased after the AVG trial on the first day of the study. On the other hand, an increase in TOS level was observed, although this enhancement was not significant. As can be seen in our findings, the decrease in the SOD level is often explained by the increase of oxidant level. Oxidative stress markers responses seem to depend on the intensity of exercise as well as the duration of exercise and may help in the prescription of more effective and less harmful effects of exercise-induced oxidative stress [32]. At this point, AVGs with different alternatives in terms of exercise intensity may create new physical activity and exercise application areas for individuals.

Adaptation to exercise is related to the intensity, frequency and extent (volume) of exercise applied. The current study was design according to the ACSM [33] criteria, which recommend to be physically active by exercising at moderate intensity at least 30 min a day, 5 days per week, or at vigorous intensity at least 20 min a day, 3 times per week. In the current study the active video fighting game was performed as the vigorous exercise (MET > 6). The intensity of the game was evaluated in two different studies [22, 23] where the MET was reported higher than 9 in young healthy males. So that, there has been no doubt about the intensity of the game. During the study, the participation schedule was followed, and individuals who completed 12 game sessions were included in the statistical analysis. Additionally, the gaming time was tightly controlled. All game sessions were carried out under the supervision of a one of the researchers. Finally, and perhaps one of the most important points, the 4-week exercise program may have not been enough to observe the expected results. Previously, it was believed that

increased intensity of exercise would overwhelm antioxidant defenses, resulting in oxidative stress [34]. Recent reports, however, indicate that even low or moderate intensity can induce oxidative stress, indicating that exercise volume (duration intensities) and a compromised antioxidant defense system are the primary mediators of exercise-induced oxidative stress [35]. The type of exercise plays a crucial role in inducing oxidative damage, as high-intensity cycling has been found to decrease oxidative damage and increase enzymatic antioxidants [36]. On the other hand, performing sprint exercises at the same intensity level actually increases oxidative damage [37]. Additionally, acute eccentric exercise increases muscle damage and oxidative stress [38]. On the other hand, circuit resistance training, has been found to decrease oxidative damage and improve antioxidant levels compared to traditional resistance training [39]. This suggests that both the type of exercise and the overall volume of exercise are important factors in exercise-induced oxidative stress. Some studies have indicated that exercise volume alone is insufficient to deplete antioxidants and increase oxidative stress [39]. The discrepancies among these studies can be attributed to variations in exercise protocols, duration, exercise modes, and the specific oxidative damage markers analyzed [35]. It is known that the active video fighting game tested in this study has a high-intensity interval training characteristic [23]. However, it is noteworthy that this specific exercise mode acutely significantly decreased the SOD value in the findings of this study and that there was no statistically significant difference in the 4-week chronic effect in the pre- and post-tests. In the literature, it is important to emphasize that oxidative damage and improving the antioxidant level are affected by the characteristics of the exercise, even if the intensity of the exercise is similar. This result, which may be related to the characteristics of the active video fighting game, is important to the literature's results. Also, in addition to this, we acknowledge that certain aspects of the study design and implementation may limit the interpretation of our findings. First, on the last day of exercising the rest blood samples (Pre-AVG following 4 week-exercising) were obtained after the participants seated for 10 minutes. As the exercise days of the participants were arranged according to their own schedules depending on their availability, the blood samples were taken one day after the previous workout for some participants and two days after the previous workout for other participants. Therefore, the complete and sufficient rest condition might have not been provided for everyone. Second, 4-week exercise program might have not been enough to observe significant changes in selected oxidative stress indices.

## Conclusions

In conclusion, although the SOD value decreased significantly after the play of the active video fighting game as an acute effect, 4 weeks of AVG exercise did not significantly increase the SOD activity in the current study. To draw comprehensive conclusions about reducing oxidative stress damage and improving the antioxidant level, it is necessary to conduct studies involving a wide range of different types of AVGs that employ various modes and durations. These studies should be supplemented with follow-up programs and involve a substantial

number of participants. Only through such extensive research can the effectiveness of AVGs in reducing oxidative stress damage and improving antioxidant levels be determined.

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## Conflict of interest

The authors declare no conflict of interest.

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