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Effects of Gender and Aerobic Exercise on Oxidative Stress and Haematological Parameters of Students of Physical Education

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ABSTRACT: The present study examined the effects of sex differences and aerobic exercise on oxidative stress and haematological parameters. The study had participants who were between 19- 20 years and consisted of 15 male and 15 female students of Physical Education in Delta State University, Abraka. The students were made to undergo rigorous aerobic exercise which involved running at an average speed of about 10 Kilometres / hour for about 1 hour and 20 mins. Blood Sample was taken from each student before and after the aerobic exercise and used for analysis. The plasma superoxide dismutase (SOD) activity of all the students (both sexes combined) was significantly ($p < 0.05$) increased after physical exercise. Similarly, the level of lipid peroxidation (LPO) in the plasma of same group of students was significantly ($p<0.05$) increased after physical exercise. When the data obtained were analysed based on gender the activity of SOD was significantly ($p<0.05$) decreased in male students but increased in the females after aerobic exercise. Conversely, there was a significant ($p<0.05$) increase in extent of LPO in male students but a decrease in females after aerobic exercise. Analysis of the haematological parameters showed that only the white blood cell (WBC) count was significantly ($p<0.05$) decreased in all students after aerobic exercises. After aerobic exercise, the Hb concentration and RBC count were significantly ($p<0.05$) decreased in male students only. Similarly, male students had a significant ($p<0.05$) decrease in both HCT and WBC count after aerobic exercise but the females had no significant ($p>0.05$) change in these parameters. In conclusion this study shows that physical exercise can cause changes in oxidative stress and hematological parameters. The study also reveals that male participants were more vulnerable to oxidative stress and variations in hematological indices.

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Physical exercise can induce metabolic reactions in the body, and this can elicit adaptive response leading to establishment of a new dynamic equilibrium (Wiecek *et al.*, 2015). Amongst the most studied metabolic changes caused by exercise is oxidative stress (Bonilla *et al*., 2005; Brunelli, 2014; Accattato *et al*., 2017; Powers *et al*., 2020). Oxidative stress has been described as a physiological condition in which there is disturbance in the prooxidant-antioxidant balance when the antioxidant defense system of the body is overwhelmed by excessive production of reactive oxygen species (ROS) such as superoxide anion radical (O2 •-), hydroxyl radical (•OH), and hydrogen peroxide (H_2O_2) (Brunelli *et al*, 2014; Yavari *et al.*, 2015). When unchecked Oxidative stress can lead to lipid peroxidation (LPO) in cells and this process is involved in the pathogenesis and propagation of metabolic disorders such as anemia cardiovascular disease, diabetes etc. (Tong *et al*., 2013; Thirupathi *et al.*, 2020). The body adapts to oxidative stress by inducing the production of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) and antioxidant compounds such as glutathione (GSH) and ascorbic acid (Balci, 2013; Wiecek, 2018). The induction of these antioxidant enzymes and compounds determine the antioxidant capacity of cells. Anemia is a disease condition in which there is decrease in quantity and quality of circulating erythrocytes and other blood components below the normal levels (Bonilla *et al*. 2005). Available information indicate that physical exercise is a possible cause of anemia as exercise could affect concentrations of haemoglobin (Hb) and other blood components (Çiçek, 2018). Previous reports indicate that oxidative stress is involved in exercise-induced anaemia (Bonilla *et al*., 2005; Hazratian *et al*., 2020).

Very few studies have been done on the effect of gender on exercise induced anaemia. However available reports on the effect of exercise on oxidative stress parameters in men and women are conflicting despite its implication in several exercise induced-metabolic disorders. Studies by Goldfarb *et al*. (2007) indicate that women have enhanced levels of antioxidants than men after intense exercise. Conversely, studies by Wiecek *et al*. (2018) and

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Guerrero *et al* (2021) indicate that plasma antioxidant activity in participants engaged in anaerobic exercise was not influenced by gender. Other published works have shown significantly increased SOD activity and GSH concentration in women after exercise, but not in men (Balci, 2013).

The aim of this study is to determine whether there are any sex-related differences in oxidative stress and haematological parameters after intense exercise.

MATERIALS AND METHODS

Participants: The participants in this study were between 19- 20 years and consisted of 15 male and 15 female students of Physical Education in Delta State University, Abraka. The male and female students were carefully matched in terms of age and weight and were regularly engaged in sports as part of the practical aspects of their training. All the participants submitted voluntarily to the study and were non-smokers with no prior history of cardiovascular disease or other metabolic disorders. They were also not under any medications of any type. Only females with regular menstrual cycle were selected. At all times the method employed was in compliance with the declaration of Helsinki. The height and weight of each participant were measured and the body mass index (BMI) was subsequently calculated for each (Table 1).

Exercise protocol and sample collection: The students were made to undergo rigorous aerobic exercise which involved running at an average speed of about 10 Kilometres / hour for about 1 hour and 20 mins. Blood Sample was taken from the peripheral vein of each student before and after the aerobic exercise. The blood from each student was centrifuged at 3000g for fifteen minutes and Serum was obtained for biochemical analysis. Haematological analysis was done with a portion of blood from each student that was mixed with EDTA to prevent blood clott. Biochemical and haematological analysis were done immediately after collection of samples.

Biochemical Analysis: The assay for SOD activity was by the method of Misra and Fridovich (1972) and the activity of the enzyme is expressed in Unit/ml. One Unit of the enzyme activity is the amount that caused 50% inhibition of the conversion of epinephrine to adenochrome in one minute. CAT activity was determined by the method of Aebi (1984). The principle of the assay is based on the decomposition of hydrogen peroxide. The activity of the enzyme is expressed in Units / ml. One Unit is the amount of the enzyme that decomposes one micromole of H_2O_2 per min. LPO was estimated by the method of Gutteridge and Wilkins (1982) and it involves the measurement of the formation of malondialdehyde (MDA) expressed in terms of MDA Units/ml. One Unit represent one nanomole of MDA.

Haematological Analysis: The heamatological analysis were done with a blood Analyzer (Sysmex, Switzerland) at the General Hospital, Eku, Delta State, Nigeria. The parameters analyzed were White blood cell (WBC) count, Red blood cell (RBC) count, Packed cell volume (PCV) and Hemoglobin concentration (Hb).

Statistical Analysis: All results were expressed as mean ± SD. The means of the physical characteristics of the males and females were compared using unpaired student's t- test. The means of oxidative stress and hematological parameters for all students that volunteered for the study were also compared using student's t-test. The mean values for the oxidative stress and hematological parameters for male and female students before and after running exercise were compared using Analysis of variance (ANOVA) followed by Fisher's LSD post hoc test using SPSS 17 software (SPSS Inc, Chicago). Statistical significance was considered at $p<0.05$.

RESULTS AND DISCUSSION

The present study examined the effect of sex differences on oxidative stress and hematological parameters after acute intense exercise. The students who participated in this study were matched in terms of height and bodyweight in order to have comparable BMI for the male and female participants (Table 1). BMI is a measure of fitness level. The effect of exercise on SOD, CAT and MDA in the plasma of all students of physical education (both sexes combined) is presented in Table 2. The plasma SOD activity of all the students (both sexes combined) was significantly ($p < 0.05$) increased after physical exercise. Similarly, the level of LPO in the plasma of same group of students was significantly ($p<0.05$) increased after engagement in physical exercise, but there was no significant ($p>0.05$) change in the activity of CAT. The increased level of plasma LPO in students who volunteered for the study is an indication of oxidative stress. Physical exercise has been shown to increase ROS production and this in turn can lead to MDA formation an index of LPO (Shadab *et al*., 2014; Accattato *et al*., 2017; Powers *et al*., 2020). The enhancement of SOD activity in the plasma of the students immediately after aerobic exercise (Table 2) is not surprising as physical exercise has been reported to induce SOD synthesis which is an adaptive response to presence of ROS (Yavari *et al*. 2015). This adaptive response to exercise by antioxidant enzymes such as

SOD is occasioned by the need to enhance protection of an organism against ROS (Kawamura and Muraoka, 2018; Thirupathi *et al*., 2020).

Table 1: Physical characteristics of the participants.

Parameters	Males	Females
Age (yr)	20.00±2.00[a]	19.50±1.50[a]
Weight (Kg)	65.80±4.80[a]	64.60±3.60[a]
Height (Cm)	170.00±6.50[a]	162.00±5.00[a]
BMI	23.2±2.40[a]	23.75±3.20[a]

Results are expressed as Mean±SD. Means of the same row with different letters as superscript differ significantly (p<0.05)

Table 2: Effect of aerobic exercise on levels of SOD, CAT and MDA in plasma of participants (both sexes combined).

Parameters	Before Exercise	After Exercise
SOD (U/ml)	66.05±11.82[a]	88.5±12.50[b]
CAT (U/ml)	4.62±1.32[a]	4.68±1.80[a]
MDA (U/ml)	10.60±1.80[a]	16.70±2.30[b]

Results are expressed as Mean±SD. Means of the same row with different letters as superscript differ significantly (p<0.05)

The effects of gender differences and aerobic exercise on SOD, CAT and LPO in plasma of all the students is presented in Table 3. No significant (p>0.05) difference was observed in the level of SOD in the plasma of male and female physical education students before aerobic exercise. However, the activity of the enzyme was significantly (p<0.05) decreased in male students but increased in the females after aerobic exercise. Irrespective of sex no significant (p>0.05) difference was observed in CAT activity before and after aerobic exercise. Prior reports in literature indicate that women have more capacity for SOD gene expression than men after physical exercise (Wiecek et al., 2015; Brunelli *et al*., 2014; Balci, 2013). This is also not unconnected with the levels of estradiol or estrogen in the blood which have been reported to be increased in women after physical exercise (Wiecek *et al*., 2015; Brunelli *et al*., 2014; Balci, 2013). These hormones are involved in the expression of genes that encode antioxidant enzymes (Wiecek *et al*., 2015; Brunelli *et al*., 2014). H_2O_2 is one of the free radicals produced during physical exercise and is product of the action of SOD (Wiecek *et al*., 2018) and is further catabolized by CAT or gluthathione peroxidase (GPx) (Wiecek *et al*., 2018). However CAT requires higher H_2O_2 concentration, than GPx (Makino *et al*., 1994; Wiecek *et al*., 2018). The activity of GPx was not measured in this study however the lack of significant difference observed in the activity of CAT in the plasma of students of both sexes is a likely indication that this enzyme was not involved in H_2O_2 catabolism probably due to low concentration of this substrate.

Table 3: Effects of gender differences and aerobic exercise on levels of plasma SOD, CAT and MDA of participants.

	Pre exercise		**Post exercise**	
parameters	**Male**	**Female**	**Male**	**Female**
SOD (U/ml)	65.30±10.42[a]	66.72±12.5[a]	58.65±13.0[b]	105.60±10.4[c]
CAT (U/ml)	4.60±1.32[a]	4.68±1.60[a]	4.70±1.80[a]	4.65±1.32[a]
MDA (U/ml)	10.51±1.80[a]	10.65±1.2[a]	23.70±2.0[b]	8.40±1.82[c]

Results are expressed as Mean±SD. Means of the same row with different letters as superscript differ significantly (p<0.05)

Table 4: Effect of aerobic exercise on blood Hb concentration, HCT, RBC count and WBC count of participants (both sexes combined).

Parameters	**Before Exercise**	**After Exercise**
Hb (g/dL)	15.20±6.20[a]	14.80±4.62[a]
HCT (%)	43.60±5.50[a]	42.68±6.70[a]
RBC (10^6/µL)	4.90±0.60[a]	4.80±1.70[a]
WBC (10^3/µL)	6.13±0.60[a]	5.75±0.70[b]

Results are expressed as Mean±SD. Means of the same row with different letters as superscript differ significantly (p<0.05)

Table 5: Effects of gender differences and aerobic exercise on blood Hb concentration, HCT, RBC counts and WBC counts of participants.

	Pre exercise		**Post exercise**	
parameters	**Male**	**Female**	**Male**	**Female**
Hb (g/dL)	16.25±0.45[a]	14.51±0.7[b]	15.38±0.60[c]	14.12±0.5[b]
HCT (%)	46.5±3.40[a]	42.2±5.34[b]	41.7±5.00[b]	43.6±4.82[b]
RBC (10^6/µL)	5.60±0.42[a]	4.50±0.53[b]	5.20±1.24[c]	4.40±1.30[b]
WBC (10^3/µL)	5.70±0.53[a]	6.80±1.20[b]	4.80±0.40[c]	6.78±2.10[b]

Results are expressed as Mean±SD. Means of the same row with different letters as superscript differ significantly (p<0.05)

Further analysis of the data showed no significant (p>0.05) difference in the level of LPO (MDA) in male and female students before aerobic exercise. However, the MDA level was significantly (p<0.05) increased in male students but decreased in females after aerobic exercise. This finding agrees with that of Baghaiee et al (2016) who reported that men have higher levels of MDA after physical activity. According to the authors men have an effective cardiovascular system and highly developed capillary and mitochondrial density, which makes the production of O_2 and the duration of its presence in tissues very high and this causes increase in free radicals in men. The enhanced activity of SOD in females after aerobic exercise would have contributed to the lower concentration of MDA observed in their plasma (Table 3).

Available reports indicate that physical exercise can influence hematological parameters but this depends on gender, age, environment or nutrition (Çiçek, 2018). Thus, the effect of aerobic exercises on the haematological parameters of all students irrespective of sex was examined in the present study (Table 4). No significant ($p>0.05$) change was observed in the Hb concentration, HCT and RBC count, but the WBC count was significantly ($p<0.05$) decreased in all students after aerobic exercises. WBC is part of the immune system of the body as it protects against pathogenic microorganisms and other foreign materials entering the body as well as infections and damaged cells that are capable of disturbing the normal function of the body (Belviranli *et al*., 2017). Thus, the decrease in WBC counts observed in the blood of all students is a likely indication that the immune system is compromised as result of the exercise undertaken by the students.

Table 5 presents the effect of gender differences and aerobic exercise on blood Hb concentration, HCT, RBC count and WBC count of students who volunteered for the study. The Hb concentration and RBC counts of the female students were significantly ($p<0.05$) lower than the values for the males before aerobic exercise. These parameters were significantly ($p<0.05$) decreased in male students after aerobic exercise, while no significant change was observed in the females relative to their respective values before aerobic exercise. Evaluation of the data indicate an inverse relationship between HCT and WBC counts of male and female students before aerobic exercise. The HCT was significantly ($p<0.05$) lower, while WBC count was significantly ($p<0.05$) higher in females relative to males. However, after the aerobic exercise both HCT and WBC counts of the male students were significantly ($p<0.05$) decreased but no significant ($p>0.05$) change was observed in these parameters in female students. Many researchers have reported decreases in haematological parameters in male subjects after physical exercise (Hazratian *et al.*, 2020; Nwoke *et al*., 2020; Tayebi et al., 2010) while others had contrary findings (Belviranli *et al*., 2017; Shivalingaiah *et al*., 2015) and attrib;uted their findings on the need for the body to adapt during exercise by stimulating erythropoiesis. However, it is important to note that changes in hematological parameters depend on severity and type of activity performed. The enhancement of LPO in the male participants after aerobic exercise (Table 3) could have contributed to the observed decrease in Hb concentration and RBC counts. Available evidence indicates that erythrocyte damage after physical exercise could be due to oxidative stress occasioned by increased LPO (Hazratian *et al*., 2020; Bonilla *et al*., 2005). Other mechanisms have been postulated to account for the decrease in Hb concentration after physical exercise amongst which are intravascular hemolysis caused by mechanical trauma, gastrointestinal bleeding and body water loss induced by sweating (Hazratian *et al*., 2020). It is noteworthy that despite the significant difference observed in some of the hematological values of the participants after physical exercise, all values were within normal range for humans. In addition, in many studies where measured blood parameters increased immediately after acute physical exercise it was observed that these parameters returned to the baseline levels some hours after the exercise (Belviranli *et al*., 2017; Heidari *et al*., 2016).

*Conclusion***:** In conclusion this work shows that physical exercise can cause changes in oxidative stress and hematological parameters. The work also reveals that male participants were more vulnerable to oxidative stress and variations in hematological indices. Thus, the findings of the work suggest that sex differences influence the effects of physical exercise on oxidative stress and hematological parameters.

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