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# The Levels of C-Reactive Protein, Malondialdehyde and Absolute Lymphocyte Counts in Pre and Post-Acute Exercise

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**Abstract**

This study was designed to determine the level of oxidative stress using the level of circulating C-reactive protein, malondialdehyde and absolute lymphocyte counts in pre and post-acute exercise as studies have shown that acute exercise enhances the immunological responses of stressed individuals. Twenty five (25) healthy young male undergraduate students with an average age of 24.3 ± 3 years and body mass index of 22.7 ± 1.8 (kg/m$^2$) participated fully in the study. The levels of malondialdehyde and C-reactive protein were significantly higher at one hour, four hours and twenty-four hour post exercise when compared with the pre-exercise stage whereas the absolute lymphocyte count and absolute neutrophils count were significantly higher at one hour and four hours post exercise when compared with the pre-exercise stage. Absolute lymphocyte count, absolute neutrophils count, C-reactive protein and malondialdehyde concentrations are increased in acute exercise which is an indication of an acute phase responses during stressful events.

**Keywords:** Immunological responses; Acute exercise; C- reactive protein; Free radicals; Oxidative stress

## Introduction

Physical exercise is important for maintaining physical fitness and can contribute positively to maintaining a healthy weight, muscle strength, promoting physiological well-being and strengthening the immune system [1-3]. Developing research has demonstrated that many of the benefits of exercise are mediated through the release of myokines which promote the growth of new tissue and reduces the risk of developing inflammatory diseases [4]. According to the World Health Organization, lack of physical activity contributes to approximately 17% of heart disease and diabetes, 12% of falls in the elderly and 10% of breast cancer and colon cancer [5].

There is evidence that vigorous exercise significantly increases the production of local and systemic inflammatory cytokines such as interleukin-1(IL-1), IL-6, IL-8 and tumor necrosis factor-α (TNF-α) which modulate the immunological responses of stressed individuals [6]. It has also been reported that prolonged exercise significantly elevates plasma cortisol, epinephrine and nor-epinephrine [7,8]. This study was designed to determine the level of oxidative stress using the level of circulating C-reactive protein, malondialdehyde and absolute lymphocyte counts in pre and post-acute exercise as studies have shown that acute exercise enhances the immunological responses of stressed individuals [6,9]. This will add to the existing level of information on exercise which is beneficial.

## Materials and Methods

### Subjects

The study was carried out in the Faculty of Health Science and Technology, Nnamdi Azikiwe University, Nnewi Campus. Twenty five (25) healthy young male undergraduate students with an average age of 24.3 ± 3 years and body mass index of 22.7 ± 1.8 (kg/m$^2$) participated fully in the study. Patient consent was obtained from the subjects. Ethical approval was obtained from the above named University which followed the declaration of Helsinki. The participants were allowed to rest for at least ten minutes after which the blood pressure and pulse rate were measured from the left arm as described by Musa et al. [10] using an automated digital electronic BP monitor (Omron digital BP monitor, Model 11 EM 403c; Tokyo Japan).

### Inclusion criteria

This study was limited to apparently healthy young male undergraduate students of the Faculty of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus within 18 and 35 years of age who are willing to participate in the study.

### Exclusion criteria

Young male with an underlying history of illness e.g. Hypertension, irregular heart rate, glucose utilization disorders, asthmatics, sickle cell anemia and other forms of anemia were excluded.

## Research design

The research design was an interrupted time series design.

## Study design

The subjects were encouraged to eat balance diet two hours prior to the endurance race and avoid any strenuous activity during the course of the research. The subjects were stressed to exhaustion using the Bruce treadmill protocol for sub maximal exercise. The acute exercise protocol started at 2.7 km/hr and a 10% grade and increased by 2% every 3 minutes in a step-like manner to a final stage at 9.6 km/hr with a 22% grade as described by Vanessa and Elizabeth [11]. The target heart rate on the treadmill was 60-80 percent of the heart rate reserve (HRR).The difference between maximal heart rate (MHR) and resting heart rate (RHR).The HRR was calculated using the formula:

HRR=MHR-RHR

MHR=220-age in years

As described by Ehiaghe et al. [9], the subjects continued this for twenty one minutes or stopped when they are tired. Blood samples and data (blood pressure, pulse rate and body temperature) were collected at four different time points: pre-exercise, one hour, four hours and twenty four hours post exercise stages using standard laboratory techniques.

## Collection of blood samples and analysis

Six milliliters volume of venous blood sample were collected from the ante-cubital vein of the subjects using standard laboratory collection technique and shared equally into ethylene diamine tetra acetic acid (EDTA) vacutainers and an anticoagulant free vacutainers, subsequently centrifuged at 750 × g for 15 minutes to obtain serum. The blood samples collected in EDTA were used for absolute white blood cell count and total white blood cell count using Sysmex® Automated Hematology Analyzer as previously described by Ehiaghe et al. [9] whereas the serum obtained in the anticoagulant free vacutainers was used for malondialdehyde estimation using spectrophotometric method. The reaction was performed in 18 × 150 mm Pyrex test tube labeled as: test and blank, into which 1 ml each of reagent 1 (Trichloroacetic acid 17.5%), reagent 2 (Trichloroacetic acid 70%) and reagent 3 (Thiobarbituric acid 70%) was added into the test tubes respectively. One (1 ml) of the serum was added to the test tube(s) labeled test while 1 ml of distilled water was added to the test tube labeled blank. The tubes were mixed and incubated in boiling bath for 15 minutes. The tubes were centrifuged at 2000 rpm for 15 minutes after cooling. The supernatant layer was read at 534 nm with a spectrophotometer. The concentration of MDA (µmol/L) was calculated using the formula: Concentration of the test=Absorbance (test) – Absorbance (blank) / 1.56 × 1000000 as previously described by Ehiaghe et al. [12]. Serum C-reactive protein was estimated using enzyme-linked immunosorbent assay method. The assay employs an antibody specific for C-reactive protein coated on a 96 well plate. Briefly, 100 µl of assay diluents was added to each well. 50 µl of standard or sample(s) was added per well and the mixture was incubated for 2 hours. The solution was discarded and microplates washed four times with 300 µl of 1X wash solution. 200 µl C-reactive protein conjugate was added to the standard or sample(s) and covered with a sealing tape and incubated at 250°C for 2 hours. The mixture was discarded and microplates washed four times with 300 µl of 1X wash solution. 200 µl of tetramethyl benzidine substrate was added to each well and incubated in the dark with gentle shaking. 50 µl of stop solution was added to each microplate. The intensity of the color developed was measured at 450 nm wavelength using stat fax® 4700 micro strip reader as previously described by Ehiaghe et al. [12].

## Statistical analysis

All numerical results were analyzed with one-way ANOVA with post hoc multiple comparisons test using SPSS version 20.0 statistical program. P values<0.05 were considered significant.

## Results

The systolic blood pressure, diastolic blood pressure and body temperature at one hour post exercise, four hours post exercise and twenty-four hour post exercise shows no significant difference when compared with the pre-exercise stage whereas, the pulse rate was significantly higher at one hour, four hours and twenty-four hour post exercise when compared with the pre-exercise stage (Figures 1-3). In Figures 4 and 5, the levels of malondialdehyde and C-reactive protein were significantly higher at one hour, four hours and twenty-four hour post exercise when compared with the pre-exercise stage. The absolute lymphocyte count and absolute neutrophils count were significantly higher at one hour and four hours post exercise when compared with the pre-exercise stage (Figures 6 and 7).

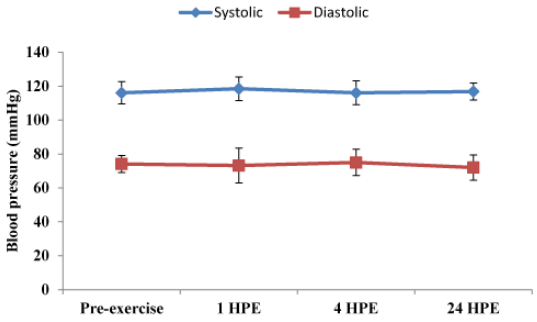


**Figure 1:** Blood pressure of the exercised subjects at different intervals.

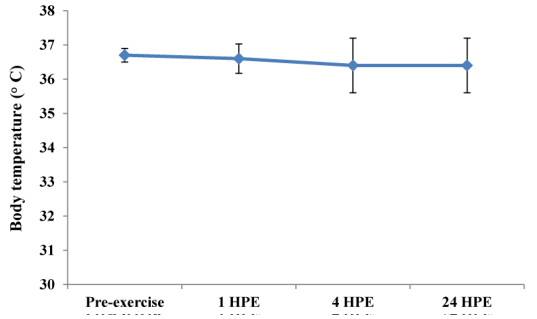


**Figure 2:** Body temperature of the exercised subjects at different intervals.

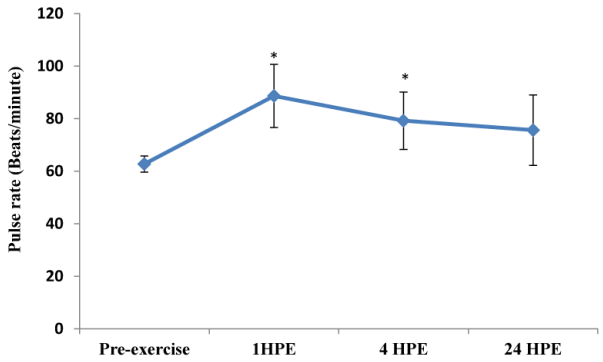


**Figure 3:** The pulse rate of the exercised subjects at different intervals.

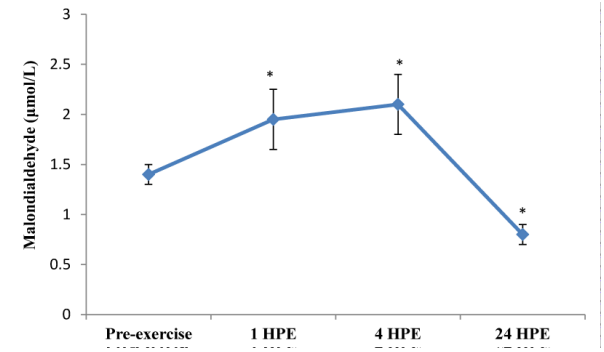


**Figure 4:** Malondialdehyde of the exercised subjects at different intervals.

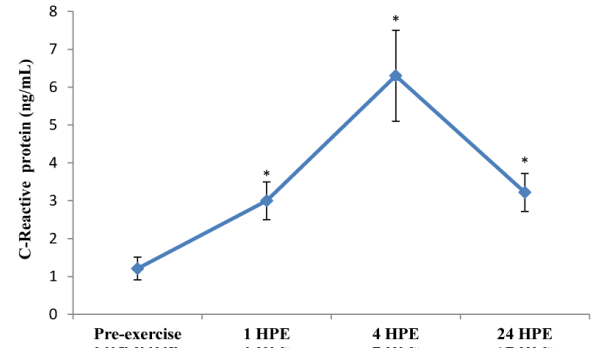


**Figure 5:** Blood pressure of the exercised subjects at different intervals.

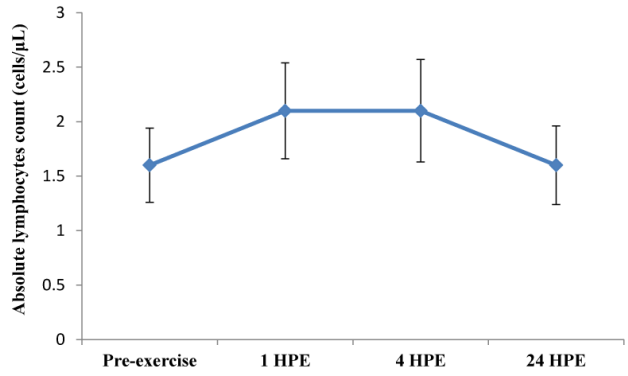


**Figure 6:** Absolute lymphocyte counts of the exercised subjects at different intervals.

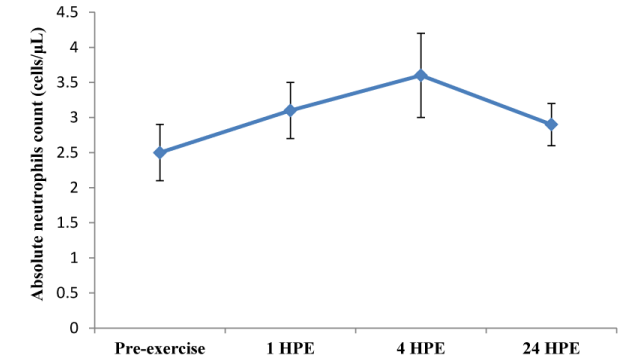


**Figure 7:** Absolute neutrophils count of the exercised subjects at different intervals.

## Discussion

The study revealed a significant higher CRP at one hour, four hour and twenty-four post exercises when compared with the pre exercise stage, suggesting that level of CRP contributes to the acute phase responses that are associated with acute exercise. Strachan et al. [13] showed that CRP concentrations were significantly elevated in long distance runners competing in events ranging from 15 to 88 km. Akinwande et al. [14] suggested that increased level of CRP after prolonged exercise is an indication of an acute phase response to stressful exercise. Several studies have showed that CRP is elevated in strenuous exercise [14-17].

The study also demonstrated a significantly higher malondialdehyde concentration at one hour, four hours and twenty-four hour post exercise. Thus, indicating an increased oxidative stress during and after the acute exercise bout. Santos-Silva et al. [18] also reported elevated resting malondialdehyde levels in trained adolescent swimmers when compared with control subjects. In contrast, Niess et al. [19] reported higher plasma malondialdehyde in untrained subjects when compared with trained subjects, and Miyazaki et al. [20] observed no change in erythrocyte malondialdehyde after a 12-week training program. Thus, it is possible that the acute exercise bout significantly increases the level of lipid peroxidation in the exercising skeletal muscles which

might be mediated via the post-exercise malondialdehyde and C-reactive protein elevations.

The findings with absolute lymphocytes and neutrophils counts showed that they were significantly higher at one hour and four hours post exhaustive exercises when compared with pre-exercise stage. This is suggestive that post exercise stress enhances lymphocyte and neutrophils cell recruitment from tissue pools such as spleen and lymph nodes. Ortega [21] in his study observed that post exercise stress enhances white blood cells phagocytic capacity. Post exercise stress is associated with increased leukocytes migration from the lymphoid organs to the circulating blood [22].

On the other hand, the pulse rate was significantly higher at one hour, four hours and twenty-four hours post exercise when compared with the pre-exercise. This is indicative of an increased sympathetic nerve activity induced by the acute exercise. Kavey et al. [23] reported that post exercise stress induces hyperactivity of the sympathetic nervous system during and after the acute exercise. The systolic, diastolic blood pressure and body temperature at one hour, four hours and twenty-four hour post exercise showed no significant differences when compared with the pre-exercise stage. These could be attributed to a restored homeostasis as the subjects feel relieved of the stress induced by the exhaustive exercise bout.

## Conclusion

Absolute lymphocyte count, absolute neutrophils count, C-reactive protein and malondialdehyde concentrations are increased in acute exercise which is an indication of an acute phase responses during stressful events. Acute exercise should be undertaken with caution to prevent acute phase protein associated cardiovascular complications during and after exercise.

## Competing Interests

The authors declare that they have no competing interests.

## Authors' Contributions

This work was carried out in collaboration between all authors. Author EFA designed the study, conducted the exercise protocol and performed the statistical analysis. Authors AED, EFA, ABO, EIO, JS and ES conducted and managed the Laboratory analysis. All authors read and approved the final manuscript.

## Acknowledgements

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