



The Expression Pattern and Role of Interferon Gamma Genes in Exhaustive Exercise

EHIAGHE F. A.^{1, 2, 3*}, ONYENEKWE C.C.³, AKOSILE C. O.⁴, OKOYE C.⁴, MARUF F. A.⁴, AGBONLAHOR D. E.², UWABOR C. I.², EHIAGHE I. J.², OGBU E. C.²

¹ Department of Hematology, College of Health Sciences, Igbinedion University, Okada, Nigeria

² Lahor Research and Medical Centre, 121 Old Benin-Agbor Road, Benin City, Nigeria

³ Department of Medical Laboratory sciences, Nnamdi Azikiwe University, Awka, Nigeria

⁴ Department of Medical Rehabilitation, Nnamdi Azikiwe University, Awka, Nigeria

*Corresponding author. E-mail: fredleo2547@yahoo.com

ABSTRACT

This study is aimed at determining the expression patterns of interferon gamma genes, white blood cell recruitment capacity and the hormonal adaptation process in exercise exhausted young male undergraduate students. Twenty-five young male undergraduate students of the Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus, aged 24.3 ± 3 years participated in this study. The subjects took part in an endurance race using the Bruce treadmill protocol for sub-maximal exercise for a maximum of 21 minutes. Blood samples were collected from the participants before commencement of the study, at 1 hour, 4 hours and 24 hours post exercise. The blood samples were used for follicle stimulating hormone and luteinizing hormone using enzyme-linked immunosorbent assay methods while white blood cell count was estimated using the Sysmex® Automated Hematology analyzer methods. Interferon gamma genes expression patterns were detected using reverse transcriptase polymerase chain reaction method. Interferon gamma genes were expressed in all the participating students as from 4 hours post exercise and the expressions were sustained in all the participants for 24 hours post exercise. There were significant variations between pre and post exercise Mean \pm SD values of absolute lymphocyte counts (cells/ μ l) ($P = 0.000$), absolute neutrophil counts (cells/ μ l) ($P = 0.000$), follicle stimulating hormone (nmol/L) ($P = 0.000$), luteinizing hormone (nmol/L) ($P = 0.000$). Post exercise stress enhances the release of interferon gamma gene which modulates the immune responses of stressed individuals.

Keywords: Exhaustive exercise, immune response, interferon gamma gene, Bruce treadmill protocol

INTRODUCTION

The athlete's reaction to exhaustive exercise is a coordinated response of multiple organ system, which include immune response modulation and generation of reactive oxygen and nitrogen species that, besides their tissue damaging potential, also play a crucial role in cellular signaling (1-2).

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 (3-5). 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 (6).

Studies have shown that exhaustive exercise can cause up-regulation of several genes in the human body including interferon-gamma genes (7-10). However, there is paucity of information on the expression patterns of interferon gamma gene in exercise exhausted young male undergraduate students. This study is aimed at determining the expression patterns of interferon gamma genes, white blood cell recruitment capacity, and the hormonal adaptation process in exercise exhausted young male undergraduate students.

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/m²) participated fully in the study. Patient consent was obtained from the subjects.

Inclusion criteria

This study was delimited 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. Upon arrival at the venue of the research, their height (H) and weight (W) was measured and recorded and they were allowed to rest for at least ten minutes. The subjects were stressed to exhaustion using the Bruce treadmill protocol for sub maximal exercise. The exercise-induced stress 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 maximal reserve (HRR). The difference between maximal heart rate (MHR) and resting heart rate (RHR). The HRR was calculated using the formula:

$$\text{HRR}=\text{MHR}-\text{RHR}.$$

$$\text{MHR}=220\text{-age in years.}$$

As described by Ogwumike et al. (12). The subjects continued this for twenty one minutes or get down when they are tired. Fourteen out twenty five subjects were exhausted before the twenty-one minutes. Blood sample was collected at four different time points: before, one hour, four hours and twenty four hours post exercise stages.

Collection of blood samples

Six milliliters of venous blood sample were collected from the medial cubital vein using vacutainer and needle from each of the subjects shared equally into ethylene diamine tetra acetic acid containers and RNAgard vacutainer for total lymphocyte RNA isolation.

Hematological parameter estimation

Hematological parameter was determined using Sysmex® Automated Hematology analyzer as described by Samuel et al. (13).

Follicle stimulating hormone estimation

Enzyme-linked immunosorbent assay was used in the determination of the level of Follicle stimulating hormone in the serum according to manufacturer's instruction. In brief, 50 µl of standards or samples was added into their respective wells. A 100 µl volume of the enzyme conjugate was added into each well and mixed for 30 seconds. The wells were covered with the foil supplied with the kit and incubated at room temperature for 45 minutes. The wells were washed five times with a 300 µl volume of wash solution. A 100 µl volume of TMB reagents was added into each well and incubated at room temperature for 20 minutes in the dark with gentle shaking. The reaction was stopped by adding 100 µl volume of stop solution to each well. The intensity of the color produced was directly proportional to the amount of follicle stimulating hormone present in the sample(s) and the intensity was measured at 450 nm.

Luteinizing hormone estimation

Enzyme-linked immunosorbent assay was used in the determination of the level of Luteinizing hormone in the serum according to manufacturer's instruction. In brief, 50 µl of standards or samples was added into their respective wells. A 100 µl volume of the enzyme conjugate was added into each well and mixed for 30 seconds. The wells were covered with the foil supplied with the kit and incubated at room temperature for 45 minutes. The wells were washed five times with a 300 µl volume of wash solution. A 100 µl volume of TMB reagents was added into each well and incubated at room temperature for 20 minutes in the dark with gentle shaking. The reaction was stopped by adding 100 µl volume of stop solution to each well. The intensity of the color produced was directly proportional to the amount of follicle stimulating hormone present in the sample(s) and the intensity was measured at 450 nm.

Polymerase chain reaction methods

Total RNA Extraction using the ZYMO RESEARCH Whole-Blood RNA MiniPrep

Total RNA was extracted using the ZR Whole –Blood RNA MiniPrep with catalog number R1020 and R1021 by ZYMO RESEARCH CORPORATION according to manufacturer's specification at the Lahor Research Laboratory and Medical centre, 121 Old Benin- Agbor Road, Benin City, Edo State, Nigeria. 70 µl of the Total RNA extracted was transferred into an RNA stable tube supplied by Biomatrix with catalog number 93221-001 for storage of Total RNA at room temperature after proper drying.

One Taq One-Step reverse transcriptase polymerase chain reaction

The extracted Total RNA was retro-transcribed and amplified using One Taq One Step RT-PCR kit with catalog number NEB E5315S by NEW ENGLAND BioLabs incorporation according to the manufacturer's specification. Interferon gamma genes forward and reverse primers (TCTGCATCGTTTTGGGCT; GCAGGAGACAACCATTACT) were used to target lymphocyte template using Peltier thermal cycler polymerase chain reaction machine at the Lahor Research Laboratory and Medical Centre, 121, Old Benin-Agbor Road, Benin City, Edo state, Nigeria. The system components were thaw and mixed by inverting ten times. The PCR was performed in a 50 µl reaction mixture containing 25 µl One Taq one-step reaction master mix (2x), 2µl One Taq one- step enzyme mix (2x), 2µl of each gene-specific forward primer (10 µM),



2 μ of each gene-specific reverse primer (10 μ M), 9 μ l of nuclease-free water and 10 μ l of the RNA template(s) was added last. The PCR was started immediately as follows: Reverse transcriptase at 48 $^{\circ}$ C for 30 minutes, initial denaturation at 94 $^{\circ}$ C for 1 minute, denaturation at 94 $^{\circ}$ C for 15 seconds, annealing at 53 $^{\circ}$ C for 30 seconds, extension at 68 $^{\circ}$ C for 1 minute, Go to the denaturation step for 39 cycles, final extension at 68 $^{\circ}$ C for 5 minutes and final holding at 4 $^{\circ}$ C forever. Five micro liters of the amplified PCR products were analyzed on 1% agarose gel containing ethidium bromide in 1X Tris EDTA buffer. Electrophoresis was performed at 90 V for 30 minutes with the EDVOTEK tetra source electrophoresis machine, Bethesda, USA. The targeted genes were visualized by Wealtec Dolphin-Doc UV transilluminator and photographed. Molecular weights were calculated using molecular weight standard of the marker. The graphical analysis was done on a bar chart.

Statistical analysis

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

Ethics

Ethical approval were obtained from the Ethics Committee of the Faculty of Health Science and Technology, Nnamdi Azikiwe University, Nnewi Campus and Lahor Research Laboratory and Medical centre in Benin City, Edo State, Nigeria with reference number LRL/ 005/014.

RESULTS

The study revealed that mean \pm S.D of the absolute lymphocyte count of subjects who were exhausted before the end of the exercise bout were 1.5 \pm 0.34 cells/ μ l \times 10 3 pre-exercise, 2.0 \pm 0.44 cells/ μ l \times 10 3 one hour post exercise, 2.0 \pm 0.47 cells/ μ l \times 10 3 four hours post exercise and 1.6 \pm 0.36 cells/ μ l twenty-four hours post exercise. The mean \pm S.D of the absolute lymphocyte count of subjects who were exhausted at the end of the exercise bout were 1.1 \pm 0.29 cells/ μ l \times 10 3 pre-exercise, 1.6 \pm 0.43 cells/ μ l \times 10 3 one hour post exercise, 1.5 \pm 0.37 cells/ μ l \times 10 3 four hours post exercise and 1.2 \pm 0.24 cells/ μ l twenty-four hours post exercise. The absolute lymphocyte count were significantly higher at one hour and four hours post exercise when compared with the pre-exercise stage (F = 9.394, P = 0.000) (Table 1).

Furthermore, the mean \pm S.D of the absolute neutrophils count of subjects who were exhausted before the end of the exercise bout were 2.4 \pm 0.53 cells/ μ l \times 10 3 pre-exercise, 3.0 \pm 0.59 cells/ μ l \times 10 3 one hour post exercise, 3.3 \pm 0.73 cells/ μ l \times 10 3 four hours post exercise and 2.7 \pm 0.59 cells/ μ l twenty-four hours post exercise. The mean \pm S.D of the absolute neutrophils count of subjects who were exhausted at the end of the exercise bout were 1.7 \pm 0.36 cells/ μ l \times 10 3 pre-exercise, 2.3 \pm 0.43 cells/ μ l \times 10 3 one hour post exercise, 2.5 \pm 0.63 cells/ μ l \times 10 3 four hours post exercise and 1.9 \pm 0.38 cells/ μ l twenty-four hours post exercise. The absolute lymphocyte count were significantly higher at one hour and four hours post exercise when compared with pre-exercise stage (F = 10.037, P = 0.000) (Table 1).

The mean \pm S.D of luteinizing hormone level of subjects who were exhausted before the end of the exercise bout were 6.1 \pm 1.84 mIU/ml pre-exercise, 2.7 \pm 1.6 mIU/ml one hour post exercise, 5.1 \pm 2.02 mIU/ml four hours post exercise and 6.6 \pm 2.0 mIU/ml twenty-four hours post exercise. The luteinizing hormone level were significantly lower at one hour post exercise when compared with pre-exercise (F = 9.153, P = 0.000). However, there was no significant difference when compared with the four hours post and twenty-four hours post exercise (P = 0.121 and P = 0.490) respectively. The mean \pm S.D of luteinizing hormone level of subjects who were exhausted at the end of the exercise bout were 6.0 \pm 1.49 mIU/ml pre-exercise, 3.0 \pm 2.10 mIU/ml one hour after exercise, 4.5 \pm 1.52 mIU /ml four hours post exercise and 6.2 \pm 1.34 mIU/ml twenty-four hours post exercise. The luteinizing hormone level were significantly lower at one hour post exercise and four hours post exercise when compared with pre-exercise (F = 9.153; P = 0.000). However, there was no significant difference when compared with the twenty-four hours post exercise (P = 0.971). It was also observed that there were no significant difference between subjects who were exhausted before the end of the exercise bout and those who were exhausted at the end of the exercise bout (Table 2).

The mean \pm S.D of follicle stimulating hormone level of subjects who were exhausted before the end of the exercise bout were 4.1 \pm 2.27 mIU/ml pre-exercise, 2.9 \pm 2.30 mIU/ml one hour post exercise, 3.6 \pm 2.27 mIU/ml four hours post exercise and 4.0 \pm 2.21 mIU/ml twenty-four hours post exercise. The follicle stimulating hormone level were significantly lower at one hour post exercise when compared with pre-exercise (F = 2.344, P= 0.000). The mean \pm S.D of luteinizing hormone level of subjects who were exhausted at the end of the exercise bout were 4.1 \pm 1.29 mIU/ml pre-exercise, 2.0 \pm 1.14 mIU/ml one hour post exercise, 2.0 \pm 1.19 mIU /ml four hours post exercise and 3.2 \pm 1.22 mIU/ml twenty-four hours post exercise. The follicle stimulating hormone level were significantly lower at one hour post exercise when compared with pre exercise (F = 2.344, P = 0.002) (Table 2).

Moreover, the reverse transcriptase PCR results for interferon gamma genes revealed that the gene were up-regulated at 4 hours post exercise and sustained till 24 hours post exercise at 375bp on a 1% agarose gel electrophoresis stained with ethidium bromide in 20(75%) of the subjects when compared with the pre-exercise stage (χ^2 = 39, P = 0.000; χ^2 = 50, P = 0.000) while an up-regulation were also observed at 1 hour post exercise and sustained till 24 hours post exercise in 5(25%) of the subjects (χ^2 = 5.6, P = 0.000; χ^2 = 50, P = 0.000) (Plate 1) and (Figures 1).

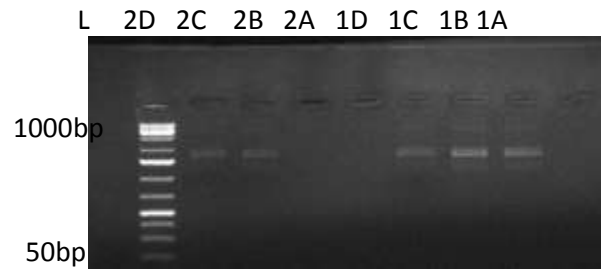


Plate 1: Sample of reverse transcriptase PCR results for interferon gamma genes analyzed on a 1.0 % agarose gel electrophoresis stained with ethidium bromide. L is a 50bp-1000bp DNA ladder (molecular marker). Lanes 1B, 1C, 1D, 2C and 2D are positive bands for the expressed interferon gamma genes at 375bp from the exercised subjects. Lanes 1A, 2A and 2B are negative bands from the pre stage and 1 hour post exercise respectively.

Keys:

- A = pre-exercise
- B = 1-hour post exercise
- C = 4-hours post exercise
- D = 24-hours post exercise

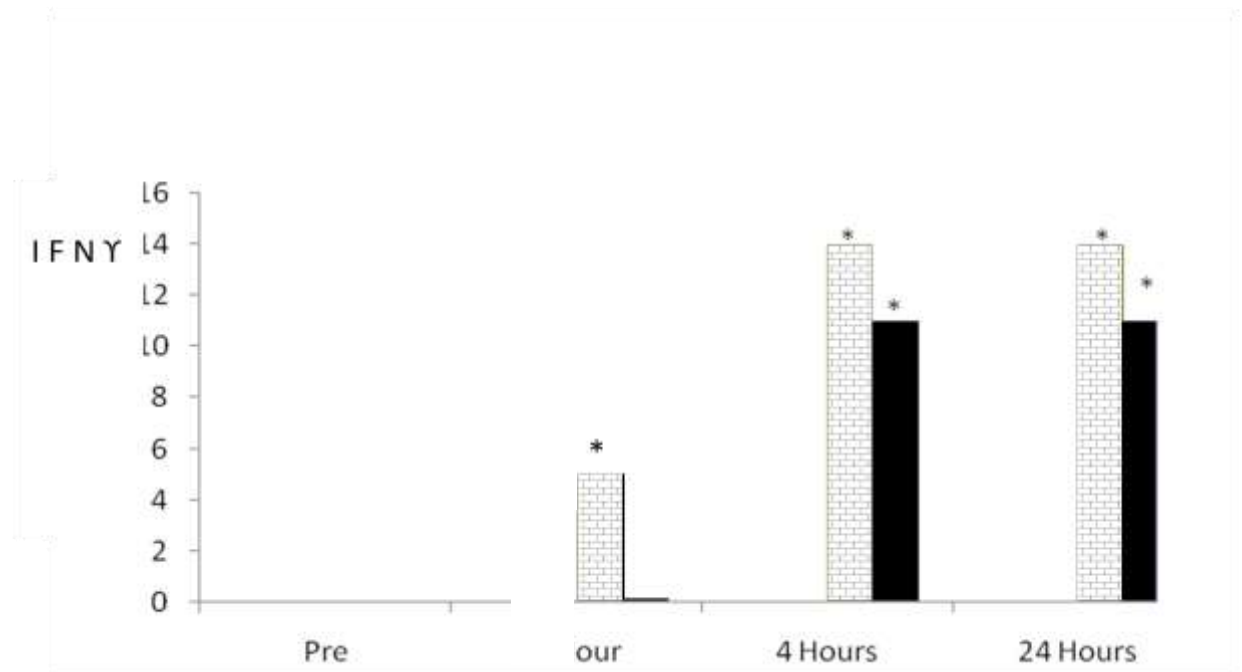


Figure 1: An overall multiple bar chart representation of the up-regulation of IFN Y gene detected in exercised subjects at different time intervals. The expression patterns were up-regulated at 4 hour post exercise and sustained till 24 hours post exercise in 20 (75%) of the subjects when compared with the pre-exercise stage ($\chi^2 = 39$, $P = 0.000$; $\chi^2 = 50$, $P = 0.000$) while an up-regulation were observed at 1 hour post exercise and sustained till 24 hours post exercise in 5 (25%) of the subjects ($\chi^2 = 5.6$, $P = 0.000$; $\chi^2 = 50$, $P = 0.000$) respectively.

Keys:

- Pre = Gene not expressed in exercised participants
- Bricks = Gene expression in subjects who were exhausted before 21 minutes at different time intervals
- Black = Gene expression in subjects who were exhausted at 21 minutes at different time intervals
- *= Significant $P < 0.05$



Table 1: Mean (\pm SD) values of the lymphocyte count (%), neutrophils count (%), total white blood cell count (Cells/ul), absolute lymphocyte count (cells/ul) and absolute neutrophils count (cells/ul) of the exercised participants.

Time intervals	Lymphocyte count		Neutrophils count		Total while cell count x 10 ³		Absolute lymphocyte count x 10 ³		Absolute neutrophils count x 10 ³	
	Subjects exhausted before 21 minutes (n = 14)	Subjects exhausted at 21 minutes (n = 11)	Subjects exhausted before 21 minutes (n = 14)	Subjects exhausted at 21 minutes (n = 11)	Subjects exhausted before 21 minutes (n = 14)	Subjects exhausted at 21 minutes (n = 11)	Subjects exhausted before 21 minutes (n = 14)	Subjects exhausted at 21 minutes (n = 11)	Subjects exhausted before 21 minutes (n = 14)	Subjects exhausted at 21 minutes (n = 11)
Pre-exercise(A)	30.9 \pm 1.23	30.9 \pm 1.64	51.1 \pm 1.21	50.5 \pm 1.04	4.71 \pm 1.08	3.3 \pm 0.72	1.5 \pm 0.34	1.1 \pm 0.29	2.4 \pm 0.53	1.7 \pm 0.36
1hour post exercise(B)	36.7 \pm 1.90	36.6 \pm 1.63	54.7 \pm 1.77	54.2 \pm 1.66	5.4 \pm 1.14	4.3 \pm 0.99	2.0 \pm 0.44	1.6 \pm 0.43	3.0 \pm 0.59	2.3 \pm 0.6
4 hours post exercise(C)	34.4 \pm 1.15	34.2 \pm 1.40	57.4 \pm 1.07	57.4 \pm 1.57	5.7 \pm 1.30	4.3 \pm 1.05	2.0 \pm 0.47	1.5 \pm 0.37	3.3 \pm 0.73	2.5 \pm 0.63
24 hours post exercise(D)	33.0 \pm 1.18	33.0 \pm 1.55	53.2 \pm 0.89	52.6 \pm 1.21	5.0 \pm 1.09	3.6 \pm 0.71	1.6 \pm 0.36	1.2 \pm 2.24	2.7 \pm 0.59	1.9 \pm 0.38
F Value	29.191		41.424		7.663		9.394		10.037	
P Value	0.000		0.000		0.000		0.000		0.000	
A vs. B	0.000*	0.000*	0.000*	0.000*	0.071(ns)	0.035*	0.000*	0.002*	0.012*	0.010*
A vs. C	0.000*	0.000*	0.000*	0.000*	0.015*	0.025*	0.001*	0.011*	0.000*	0.001*
A vs. D	0.000*	0.001*	0.000*	0.001*	0.428(ns)	0.503(ns)	0.181(ns)	0.401(ns)	0.363(ns)	0.392(ns)
A ₂₁ Vs. A _{<21}	0.930(ns)		0.283(ns)		0.002*		0.015*		0.003*	
B ₂₁ Vs. B _{<21}	0.000*		0.354(ns)		0.008*		0.006*		0.008*	
C ₂₁ Vs. C _{<21}	0.769(ns)		0.910(ns)		0.002*		0.003*		0.001*	
D ₂₁ Vs. D _{<21}	1.000(ns)		0.314(ns)		0.001*		0.006*		0.001*	

ns = non significant * = significant



Table 2: Mean (\pm SD) values of the luteinizing hormone (mIU/ml) and follicle stimulating hormone (mIU/ml) of the exercised participants

Time intervals	Luteinizing hormone		Follicle stimulating hormone	
	Subject that were exhausted before 21 minutes (n =14)	Subject that were exhausted at 21 minutes (n =11)	Subject that were exhausted before 21 minutes (n =14)	Subject that were exhausted at 21 minutes (n =11)
Pre-exercise(A)	6.1 \pm 1.84	6.0 \pm 1.49	4.1 \pm 2.27	4.2 \pm 1.29
1hour exercise(B) post	2.7 \pm 1.60	3.0 \pm 2.10	2.9 \pm 2.30	2.0 \pm 1.14
4 hours exercise(C) post	5.1 \pm 2.02	4.5 \pm 1.52	3.6 \pm 2.27	2.0 \pm 1.19
24 hours exercise(D) post	6.6 \pm 2.00	6.2 \pm 1.34	4.0 \pm 2.21	3.2 \pm 1.22
F Value	9.153		2.344	
P Value	0.000*		0.000*	
A vs. B	0.000*	0.000*	0.000*	0.002*
A vs. C	0.121 (ns)	0.120(ns)	0.515 (ns)	0.166 (ns)
A vs. D	0.490 (ns)	0.971 (ns)	0.928 (ns)	0.946 (ns)
A ₂₁ Vs. A _{<21}	0.887 (ns)		0.754 (ns)	
B ₂₁ Vs. B _{<21}	0.637(ns)		0.248 (ns)	
C ₂₁ Vs. C _{<21}	0.416 (ns)		0.052(ns)	
D ₂₁ Vs. D _{<21}	0.587(ns)		0.249 (ns)	

ns = non significant

* = significant



DISCUSSION

In response to stressors some genes are known to be either up-regulated while others are down-regulated (9). The duration of expression of such genes may be an indication of possible role in mediating response to stress. Some group of lymphocyte genes have been shown to be either up-regulated or down regulated in different physiologic or pathologic conditions (9).

In the present study, lymphocyte interferon gamma genes were not expressed before 4 hours post exercise in about 75% of the participating students while about 25% of the participants had the gene expressed as from 1 hour post exercise. However, the lymphocyte interferon gamma genes were expressed in all the participating students as from 4 hours post exercise and the expressions were sustained in all the participants for 24 hours post exercise. The delayed expression of the lymphocyte interferon gamma genes in about ¼ of the studied population calls for concern and might be an indication of individual susceptibility or resistance to stress. It further indicates that there may be other regulatory factors that may be protecting the expression of these genes. Intense studies are required in this direction to unravel the reason behind these bivalent observations at early state of post induction of stress.

Interferon-gamma is known to induce the release of interleukin-1 from monocytes to amplify immune responses (14). Thus, the expression of the interferon gamma genes for 24 hours post exhaustive exercise might be an indication of enhanced immune responses to stress within these study periods. Ambarish et al. (16) in their study observed that post exercise elevation of interferon gamma acts as a positive feedback mechanism on the leukocytes by enhancing their ability to produce additional cytokines such as tumor necrosis factor alpha, and interleukin-10 during muscle contractions. Liburt et al. (17), in their study reported that the immunological responses to post exercise stress are characterized by a significant increase in the expression of interferon-gamma, tumor necrosis factor alpha, and interleukin-10 genes within the blood and contracting muscle cells. The possible mechanism of the up-regulation of the lymphocytic gene expression patterns observed in this study could be linked to adenosine triphosphate depletion, accumulation of adenosine diphosphate, and adenosine monophosphate due to consumption of adenosine triphosphate by the exercising muscles (18).

The findings with absolute lymphocytes and neutrophils counts showed that they were significantly higher at 1 hour and 4 hours post exhaustive exercises when compared with pre-exercise. This is suggestive that post exercise stress enhances lymphocyte and neutrophil cells recruitment from tissue pools such as spleen, lymph nodes and the gastrointestinal tract, thus, suggesting a reduced risk of infection for those who engages in regular exercise. Ortega (19) 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 (20). It has been reported that elevated cortisol level during exhaustive exercise enhances white blood cells recruitment capacity (20). However, there was no significant difference at 24 hours post exhaustive exercise stage when compared with the pre exercise stage. This is indicative of a restored homeostasis during the recovery period of the exhaustive exercise bout.

In this study, the serum luteinizing hormone level and follicle stimulating hormone were significantly lower at 1 hour post exercise when compared with the pre exercise stage. The observed decrease is suggestive that luteinizing hormone level and follicle stimulating hormone production are inhibited when the body is faced with stress associated with exhaustive exercise. Warren and Perlroth, (21), in their research reported an inhibitory effect of elevated cortisol secretion on serum luteinizing hormone level and follicle stimulating hormone post exercise. The post exercise-induced reduction of luteinizing hormone level and follicle stimulating hormone are stimulated by the inhibitory effect of the elevated cortisol during exhaustive exercise (22). However, there was no significant difference at 4 hour post exercise stage and 24 hours post exercise stage when compared with the pre exercise stage. This is suggestive of a restored homeostasis.

Conclusion

The findings with absolute lymphocytes and neutrophils counts showed that post exercise stress enhances lymphocyte and neutrophil cells recruitment. It was also revealed that post exercise stress enhances the release of interferon gamma gene which modulates the immune responses of stressed individuals. Furthermore, post exercise stress inhibits the production of luteinizing hormone and follicle stimulating hormone level of the exercised subjects.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

This work was carried out in collaboration between all authors. Authors FAE, CCO, COA, FAM and CO designed the study, conducted the exercise protocol and performed the statistical analysis. Authors FAE, CIU, IJE, DEA and ECO conducted and managed the Laboratory analysis. All authors read and approved the final manuscript.

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REFERENCES

1. Connolly, P. H., Caiozzo, V.J., Zaldiver, F., Nemet, D., Larson, J., Hung, S.P., Heck, J.O., Hatfield, G.W., Cooper, D.M. (2004). Effect of exercise on Gene expression in human peripheral blood mononuclear cells. *Journal of Applied Physiology*. 97:1461-1469.
2. Zicker, D., Fehrenbach, E., Dietzsch, J., Fliegner, J., Waidmann, M., Nieselt, K., Gebicke-Haerter, P., Spanagel, R., Simon, P., Niess, A.M., Northoff, H. (2005). cDNA microarray analysis reveals novel candidate Genes expressed in human peripheral blood following exhaustive exercise. *Physiological Genomics*. 23:287-294.
3. Stampfer, M.J., Hu, F.B., Manson, J.E., Rimm, E. B., Willet, W.C. (2000). Primary prevention of Coronary Heart Disease in Women through Diet and Lifestyle. *New England Journal of Medicine*. 343(1): 16-22.
4. Hu, F.B., Manson, J., Stampfer, M., Graham, C. (2001). Diet, Lifestyle and risk of type 2 diabetes mellitus in women. *The New England journal of Medicine*. 345 (11): 790 – 797.
5. Gosker, H.R., Scholes, A.M. (2008). Fatigued muscles in COPD but no finishing line in sight. *European Respiratory Journal*. 31:693-694.
6. Pedersen, B.K. (2013). American Physiological Society. *Comprehensive Physiology*. 3:1337-1362.
7. Steinberg, J.G., Delliaux, S., Jammes, T. (2006). Reliability of different blood indices to explore the oxidative stress in response to maximal cycling and static exercise. *Clinical Physiological Functional Imaging*. 26: 106-112.
8. Cappelli, K., Verini-Supplizi, A., Capomaccio, S., Silvestrelli, M. (2007). Analysis of peripheral blood mononuclear cells gene expression in endurance horses by cDNA-AFLP technique. *Research in Veterinary Science*. 82:335–343.
9. Bermudez, D.M., Canning, D.A., Liechty, K.N. (2011). Age and pro-inflammatory cytokine production: Wound-healing implications for scar-formation and the timing of genital surgery in boys. *Journal of Pediatric Urology*. 7: 324- 331.
10. Josef, F. (2012). Biomarkers of peripheral muscle fatigue during exercise. *Musculoskeletal Disorders*. 13: 213-218.
11. Vanessa, N., Elizabeth, D. (2000). Submaximal exercise testing: Clinical application and interpretation. *Journal of the American Physical Therapy Association*. 80: 782- 807.
12. Ogwumike O.O., Arowojolu A.O., Sanya A.O. (2011). Effects of a 12- week endurance exercise program on adiposity and flexibility of Nigerian perimenopausal and postmenopausal women. *Nigerian Journal of Physiology Sciences*. 26: 199- 206.
13. Samuel, O.I., Thomas, N., Ernest, O.U., Imelda, N.N., Elvis, N.S., Ifeyinwa, E. (2010). Comparison of Hematological parameters determined by the sysmex KX-2IN automated hematology analyzer and the manual count. *British Medical Council Clinical Pathology*. Pp 12-14.
14. Kate, S., Paul, J.H., Timothy, R., David, A.H. (2004). Interferon gamma: an overview of signal, mechanisms and function. *Journal of Leukocyte Biology*. 75: 163-189.
15. Rastogi, S.C. (2008). Elements of Immunology. 5th Edition. Satish Kumar, New Delhi. Pp 151- 154.
16. Ambarish, V., Radhika, K. (2014). Alteration of interferon gamma in Human plasma with graded physical activity. *Journal of Clinical Diagnosis*. 8: 5- 7.
17. Liburt, N.R., Adam, A.A., Betancourt, A., Horohov, D.W., Mckeevor, K.H. (2010). Exercise-induced increase in inflammatory cytokines in muscle and blood of horses. *Equine Veterinary Journal Supplement*. 38: 280- 288.
18. Vamshi, K.R.G. (2012). Biological scavengers of free radical-antioxidant: *International Journal of Pharmaceutical Biology and Chemical Science*. 1: 13-19.
19. Ortega, E. (2003). Neuroendocrine mediator in the modulation of phagocytosis by exercise: physiological implications: *Exercise Immunological Review*. 9: 70- 93.
20. Clinton, K.O.F., Jonato, P., Felipe, F.D., Rozangela, V., James, W.N., Claudia, R.C. (2010). Phagocytic responses of peritoneal macrophages neutrophils are different in rats following exercise. *Clinics*. 65: 1167- 1173.
21. Warren, M., Perlroth, N. (2001).The effects of intense exercise on the female reproductive system. *Journal of Endocrinology*.170, 3-11.
22. Vaamonde, D., Silva, M.E., Poblader, M.S., Lancho, J.L. (2005). Reproduction profile of physically active men after exhaustive endurance exercise. *International Journal of Sport Medicine*. 26: 1- 10

