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The Effect of Prolonged Aerobic Exercise on Salivary Secretion of Immunoglobulin A and serum Cortisol Level in Young Male Adult in Okada, Nigeria.

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ABSTRACT

This study was aim at determining the effect of prolonged aerobic exercise on salivary secretion of IgA and serum cortisol in young male adult in Okada, Nigeria. Two hundred and four male volunteers (age 20 ± 2 years, body mass index 22 ± 1.5 kg/m^2) participated in the study. Salivary IgA and serum cortisol level was determined by enzyme linked immuno sorbent assay technique. Blood glucose was determined by glucose oxidase method. We observed that an endurance race using a treadmill ergometer significantly decrease blood glucose concentration and increased serum cortisol ($P < 0.05$). However, there was no significant effect on salivary secretion of IgA ($P > 0.05$). These findings suggested that a single bout of exhaustive aerobic exercise do not appear to acutely affect the mucosal immunity, even with the elevation of serum cortisol.

Keywords: Serum cortisol, Salivary IgA, Exhaustive aerobic exercise, mucosal immunity.

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INTRODUCTION

Exercise is any bodily activity that enhances physical fitness (1–2). It is generally grouped into three types, which include: Flexibility exercise (such as stretching, which improve the range of motion on the muscles and joints), aerobic exercise (such as cycling, swimming, walking, skipping rope, running, biking and playing tennis, which focus on increasing cardiovascular endurance and anaerobic exercise (such as weight training and sprinting, which increase short term strength), (3-5).

Neuroscientists believe that stress should be restricted to conditions where an environmental demand exceeds the natural regulatory capacity of the body, (6) cytokines are soluble communicator between components of the immune system and brain (7). Several stressors have been associated with a shift in cytokine production towards an anti-inflammatory pattern with cortisol as the proposed mediator of the shift (8). Cortisol elevation is one way the brain instructs the body to attempt to regain homeostasis by redistributing energy (glucose) to areas of the body that need it most (Heart and Brain) and away from the digestion and reproductive organs (9).

Secretory immunoglobulin A (sIgA) is the predominant immunoglobulin found in the secretion lining mucosal surfaces and is an important component of saliva (10). Secretory IgA is produced in local plasma cells and seem to function as a multi-layered mucosal defense by preventing antigens and microbes from adhering to and penetrating the epithelium (11). This study was aim at determining the effect of prolonged aerobic exercise on salivary secretion of IgA and serum cortisol in young male adult in Okada, Nigeria.

MATERIALS AND METHODS

Study Area

The study was carried out in the Department of Hematology, College of health Sciences, Igbinedion University, Okada, Ovia- North East Local Government Area of Edo state.

Sample Size Determination

The sample size was determined using the formula (11).

$$N = Z^2 \times P(1 - P)/d^2$$

Where N = Minimum number of sample size

d = Desired level of significance (0.05)

Z = Confidence interval (1.96)

P = Prevalence rate (15.8%) (12).

Using this formula, the minimum number of sample size will be 204 young male adult.

Subjects

Two hundred and four male volunteers (age 20 ± 2 years, body mass index 22 ± 1.5 kg/m2, who were recreationally active, participated in the study. Patient consent and ethical approval was obtained from the subjects and ethical committee of the college of health sciences, Igbinedion University, Okada.

Exclusion Criteria

(1) Subjects diagnosed with systemic diseases were excluded from this study

(2) Those subjects who had performed any strenuous exercise or consume alcohol or medication for 2 weeks before exercise where excluded from the study.

Duration of study:

The study was carried out between march 2011 to april 2012.

Study Design

The subjects will take part in an endurance race using a treadmill ergometer with standard and guideline of the medical device directive (MDD). They will run on the treadmill till they start experiencing symptoms of sickness behavior e.g. tension, headaches, muscles aches, change of mode and exhaustion.

Blood and saliva collected were carried out at three different time points: before, at the end of the race and 24 hours after the race.

Blood Collection and Analysis

10µl of various bloods were taken from the antecubital vein by venipuncture. It shared equally into sodium fluoride-potassium Oxalate container and an anticoagulant free test tube and allows to cloth and subsequently centrifuge at 750xg from 15minutes to obtain serum. The serum was immediately aliquoted into Eppendorf tubes placed on ice and

immediately stored at -80^{o}C until serum cortisol was evaluated. Serum cortisol level was determined by enzyme linked immuno sorbent assay technique.

This test kit operates on the basis of competition between the hormone conjugate and the cortisol in the sample for a limited number of binding sites on the antibody coated plate. The samples or standard was first added to the microplate. The diluted hormone conjugate was added and the mixture was shaken and incubated for one hour. The plate was then washed to remove all the unbound material. The bound hormone conjugate was then detected by the addition of enzyme chromogen (substrate) which generated an optimal color after 30 minutes. The Quantitative test result was then obtained by measuring and comparing the absorbance reading of the wells of the samples against the standard with a microplate reader at 450nm. The extent of the colour development was inversely proportional to the amount of cortisol in the sample or standard.

Blood Glucose Determination

Reagent Composition

R1a (Buffer)

Phosphate Buffer 0.1 mol/l, pH 7.0

Phenol 11 mmol/l

R1b (GOD-PAP Reagent)

4 aminophenazone 0.77 mmol/l

Glucose Oxidase >1.5 kU/l

Peroxidase >1.5 kU/l

Preparation of working reagent (reagent 1).

Reconstitute the content of one vial of reagent R1b with the entire portion of buffer R1a. The working reagent is stable for 3 months at +2°C to +8°C.

Procedure (Semi Micro Method)

10 µl of the standard or test sample was added to 1000 µl of Reagent 1, mixed and incubated for 25 minutes at 20°C. The absorbance of the standard and the sample was measured against the reagent blank within 60 minutes at a wavelength of 540 mm using a spectrophotometer.

Saliva collection and Analysis of salivary IgA

Participants were seated during all saliva collection with an initial swallow to empty the mouth, unstimulated whole saliva was collected by expectoration into a 7ml capacity plastic bijou tubes with screw top for 2 minutes with eyes open, head tilted slightly forward and making minimal orofacial movement. All saliva samples were stored at -20^{o}C until analysis.

The concentration of salivary IgA were determined by a sandwich – ELISA method Briefly, flat bottomed micro titration plate (costar EIA plate, sigma, Poole, UK) were coated with the primary antibody, rabbit anti-human IgA, at a dilution of 1 in 800 in carbonate buffer, pH 9.6 and kept at 4oC over night. After washing with phosphate buffered saline (PBS, pH 7.2), the plates were coated with blocking protein solution (2g.L-1 bovine serum albumin in PBS). Sample analysis was performed in triplicate using saliva samples diluted 1 in 500 with deionized water. Standards were incorporated into each micro well plate and all samples from a single subject were analyzed on a single plate. The plates were incubated for 90min. at room temperature. Following a washing step, peroxidase – conjugated goat anti-human IgA was added and the plate incubated for a further 90min at room temperature. Following another washing step, the substrate (Boehringer Mannheim, Lewes, Lewes, UK) was added and after 30min, the absorbance was measured at 450nm with a microplate reader.

Statistical Analysis

All results were presented as mean + standard deviation and analyzed using one way analysis of variance (ANOVA) and Turkey – Kramer Multiple comparison test using SPSS – 18.0 statistical program. P values < 0.05 were considered significant.

RESULTS

Table 1 show the mean+ standard deviation of serum Glucose, serum cortisol level and salivary IgA level at stage A, B and C. It was observed that there was a significant decrease in serum Glucose level ($P < 0.05$) and significant increase in serum cortisol level ($P < 0.05$) at stage A when compared with stage B and C. However, there was no significant decrease in the salivary secretion of IgA ($P > 0.05$).

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Table 1: The mean± standard deviation of serum glucose level, serum cortisol and salivary IgA in subjects.

	Pre Exercise	Immediately after exercise	24hours after exercise
Parameters			
Serum glucose level (mg/dl)	95 ±0.04	72 ± 0.01^{S}	80± 0.02^{P,X}
Serum Cortisol (µg/dl)	20 ± 0.06	100 ±0.02^{S}	140 + 0.04^{P,X}
Salivary IgA (µg/ml)	134± 0.04	133.5 ± 0.04^{M}	133.4 ±0.05^{N,O}

Keys:

S = Significant (P < 0.05) Comparison between stage A and B

P = Significant (P < 0.05) comparison between stage A and C

X = Significant (P < 0.05) Comparison between stage B and C

M = Insignificant (P > 0.05) comparison between stage A and B

N= Insignificant (P > 0.05) comparison between stage A and C

O= Insignificant (P > 0.05) comparison between stage B and C

A = Pre-exercise stage

B= immediately after exercise

C = 24hours after exercise

DISCUSSION

We observed that an endurance race using a treadmill ergometer significantly decrease blood glucose concentration and increased serum cortisol (P < 0.05), Table 1. However, there were no significant effect on salivary secretion of IgA (P > 0.05), Table 1. This could be a normal physiological findings associated with stress response to the endurance race. This is in line with these findings. The reduction of glucose availability is an the important factors in activating hypothalamic pituitary-adrenal (HPA) activity (13) and glucose regulatory hormone secretion (14) several stressor have been associated with a shift in cytokine production towards an anti-inflammatory pattern with cortisol as the proposed mediator of the shift (8). Cortisol elevation is one way the brain instructs the body to attempt to regain homeostasis by redistributing glucose to areas of the body that need it most (Heart and Brain) and away from the digestive and reproductive organs in other to overcome the challenge at hand(9). Strenuous exercises were inhibitory to the proliferation of CD4 cells with the elevation of cortisol being a possible mediator (15). Cortisol elevation prevents the proliferation of T cells by rendering the interleukin 2 producer T cells unresponsive to interleukin – 1 and unable to produce the T cell growth factor (16).

Salivary glands are innervated by both parasympathetic cholinergic nerve and sympathetic adrenergic nerve. During exercise, the sympathetic is increased and induces vasoconstriction, which limits saliva secretion rate (17). Salivary IgA secretion has been shown to be stimulated by 2 – adrenoceptors (18). The stimulation of β-adrenoreceptors increased IgA secretion in a dose independent manner above a certain threshold, however, prolonged β adrenergic stimulation appeared to reduce the replenished of IgA into the glandular pool (19). Acute decrease in sIgA secretion rate during exercise is mediated by α 1-adrenegic mechanism, the interaction between different types of stimulation and their receptors are seen during exercise; when α 1 adrenergic stimulation is stronger than other types, such as β-adrenergic activity, and is above a certain threshold, sIgA output may be decreased. Conversely, when β- adrenergic stimulation is stronger than α 1 adrenergic stimulation, sIgA output increases (20-25).

CONCLUSION

These findings suggested that a single bout of exhaustive aerobic exercise do not appear to acutely affect the mucosal immunity, even with the elevation of serum cortisol. The molecular mechanism behind is findings needs further investigation.

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REFERENCES

1. 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.

2. 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.

3. O' Connor, O., Crowe, M., Spinks, W. (2005). Effects of static stretching on leg capacity during cycling. *Turin* 46(1): 52-56.

4. Wilmore, J. and Knittgen, H. (2003). Aerobic Exercise and Endurance Improving Fitness for Health Benefits. *The Physician and Sports Medicine* 31(5): 45.

5. Devos, N., Singh, N., Ross, D., Stavrines, T. (2005). Optimal load for Increasing Muscle power. During Explosive Resistance Training on Older Adults. *The journals of Gerontology* 60A(5): 638-647.

6. Nakamura, H. Nagase, H., Yoshida, M., Ogino, K. (1999). Natural killer (NK) Cell activity and NK cell subsets in workers with a tendency of burnout. *Journal of Psychosomal Review* 46.569-578.

7. Maier, S.F. and Watkins, L.R. (1998). Cytokines for Psychologists: Implications of bidirectional immune to brain communication for understanding behaviour, mood and cognition. *Psychology Review* 105:83-107.

8. Elenkor, I.J. and Chrousos E. (2002). Stress hormones, proinflammatory and anti-inflammatory Cytokines and autoimmunity. *Annals of Academic Science*. 966:290-303.

9. Michael, D.D., George, P.C., Lorah, D.D., Lillian, B., Karin, H., Mitchel, A., Penelope, K., Frank, W.P. (2001). Hypothalamic-Pituitary-Adrenal Axis Dysregulation in Sexually Abused girls. *Journal of Clinical Psychiatry* 62. (11): 22-27.

10. Brandtzaeg, P. (1998). Synthesis and Secretion of human salivary immunoglobulines, In J.R. Garrett, J. Ekstrom and L.C. Anderson (Eds). Glandular Mechanisms of Salivary secretion (Pp 167 – 199).Basel, Karger: London.

11. Naing, L., Winn, T., Rush, B.N. (2006). Practical issues in calculating the sample size for prevalence studies. *Archives of Orofacial Sciences* 1: 9 -14.

12. Hood, S. and Northcote, R.J. (1999). Cardiac assessment of veteran endurance athletes: 12 year follow up study. *British Journal Sport Medical* 33:239-243.

13. Mitchell, J.B., Costill, D.L., Houmard, J.A., Flynn, M.G. Fink, W.J., Beltz, J.D. (1990). Influence of Carbohydrate ingestion on Counter regulatory hormones during prolonged exercise. *International journal of sports Medicine*. 11: 33-36.

14. Schwartz, N.S., Clutter, W.E. Shah, S.D., Cryer, P.E. (1987). Glycemic thresholds for activation of glucose counter regulatory system are higher than the threshold for symptoms. *Journal of clinical investigation*. 79: 777 – 781.

15. Ehiaghe A.F, Ehiaghe I.J., Igere E.B and Iyen I R., (2013). The effects of aqueous extracts of *Acalypha Wilkesiana* supplementation and Exercise Training on Hematopoietic system in Rats. *American Journal of Plant Science*. 4: 1834 – 1838.

16. Palacious R., Sugawara, I. (1982). Hydrocortisone abrogates proliferation of T cells in autologous mixed lymphocyte reaction by rendering the interleukin 2 – producer T cells unresponsive to interleukin – 1 and Unable to synthesize the T cell growth factor. *Scandinavian Journal of immunology* 15(1): 25-31.

17. Tharp, G.D. and Barnes, M.W. (1990). Reduction of saliva immunoglobulin level by swim training. *European Journal of Applied Physiology*. 60: 61 – 64.

18. Tomasi, T.B., Trudeau, F.B., Czerwinski, D., Erredge, S. (1982). Immune parameters in athletes before and after strenuous exercise. *Journal of clinical immunology*. 2: 173-178.

19. Proctor, G.B., Garret, J.R., Carpenter, G.H., Ebersole, L.E. (2003). Salivary secretion of immunoglobulin A by submandibular glands in response to autonomimetic infusions in anaesthetized rats. *Journal of Neuroimmuno logy*. 136: 17-24.

20. Ring, C., Harrison, L., Winzer, A., Carrou, D., Drayson, M., Kendau, M. (2000). Secretory immunoglobulin A and cardiovascular reactions to mental arithmetic, cold pressor and exercise. Effects of alpha-adrenergic blockade. *Psychophysiology* – 37:634 -643.

21. Walsh, N.P., Bishop, N.C., Blackwell, J., Wierzbicki, S.G., Montague, J.C. (2002). Salivary IgA response to prolong exercise a cold environment in trained cyclist. *Medicine and Science in sports and Exercise*. 34:1632 – 1637.

22. Bishop, N.C., Blannin, A.K., Robson, P.J., Walsh, N.P., Gleeson, M. (1999). The effects of carbohydrate supplementation on immune response to a soccer-specific exercise protocol .*journal of sports science*. 17: 787-796.

23. Li, T.L. and Glesson, M. (2005). The effects of carbohydrate supplementation during repeated bouts of prolonged exercise on saliva flow rate and immunoglobulin A. *Journal of Sports Science*. 23(7) 713-722.

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