

## EFFECT OF EXERCISE INDUCED STRESS ON SERUM CORTISOL LEVEL AND CD4 CELL COUNT IN RATS

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### ABSTRACT

**Aim:** To evaluate the effect of exercise induced stress on serum cortisol level and CD4 cells in rats.

**Methods:** A total of 21 male albino rats weighing 100±10g were used for the study. The exercise pre-conditioning was in the form of mere swimming. Serum cortisol was evaluated using Enzyme Linked Immunosorbent Assay. CD4 cell counts were estimated using Partec Cyflow counter, Germany for the quantification of CD4 T lymphocytes.

**Result:** It was observed that, there was a significant decrease ( $P < 0.05$ ) in the serum cortisol level in Group 2 when compared with Group 1 and Group 3, while there was a significant increase ( $P < 0.05$ ) in the CD4 cell count in Group 2 when compared with Group 1 and Group 3.

**Conclusion:** Solitary confinement and strenuous exercise were inhibitory to the proliferation of CD4 cells with the elevation of cortisol being a possible mediator.

**Keywords:** Stress, Cortisol, CD4, Swimming.

### INTRODUCTION

Exercise is any bodily activity that enhances physical fitness. Frequent and regular physical exercises boost the immune system and help prevent diseases such as cardiovascular disease, type 2 Diabetes mellitus and obesity (Stampfer et. al., 2000; Hu et. al., 2001). It improves mental health; helps prevent depression and promote positive self-esteem (Hu et. al., 2001). Exercise reduces the level of cortisol which suppresses the immune response (Cornil et. al., 1965). Cytokines

are soluble communication between components of the immune system and brain. They are involved in stress induced behavioral and cognitive changes known as sickness behavior that resemble the side symptoms of Burnout syndrome associated with chronic form of stress (Maier and Watkin 1998). Cytokines can be divided into pro inflammatory cytokines such as interleukins (IL) – 2, interleukins (IL) – 6, Tumor Necrosis Factor alpha (TNF) and gamma interferon (IFN- $\gamma$ ) and anti-inflammatory

cytokines such as IL – 4 and IL – 10 (Maier and Watkins 1998). Neuroscientists believe that stress should be restricted to conditions where an environmental demand exceeds the natural regulatory capacity of the body (Nakamura et al., 1999). It is widely accepted that chronic stress can lead to down regulation of the immune function (Nakamura et. al., 1999). 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 (Elenkor and Chrousos 2002). 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 digestive and reproductive organs, in other to overcome the challenge at hand (Michael et. al., 2001). The aim of this study is to determine the effect of exercise induced stress on serum cortisol level and CD4 cell count in rats.

## **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. Ethical approval was obtained from the ethical committee of the College of Health Sciences.

### **Study Design**

A total of 21 male albino rats weighing  $100\pm 10$ g was purchased from the Animal production, Federal University of Technology, Akure, Ondo State, Nigeria and housed in the Experimental Animal House, College of Health Science, Department of Hematology, Igbinedion University, Okada, separately in well ventilated wire-bottom steel cage under a hygienic condition with proper aeration at  $25\pm 2^{\circ}\text{C}$  and a relative humidity of 45-50%. The rats were randomly assigned into 3 groups of 7 rats each and fed with standard Rat diet (10g/100g body weight) twice daily and tap water ad libitum. Prior to commencement of exercise training, the rats were allowed to acclimatize in the animal house with standard 12 hours light dark cycle for a period of 14 days. The exercise preconditioning is in the form of mere swimming. The swimming exercise was performed in a 120cm deep and 80cm wide

cylindrical tank with water temperature of  $31\pm 1^{\circ}\text{C}$  as described by (Hashemi et al., 2009). The rats in Group 1 were not conditioned to take part in the swimming exercise and were confined to an enclosed space while Groups 2 and 3 were conditioned to swim for 10 minutes and to exhaustion every day for 6 weeks respectively. All studies on the experimental animals were conducted in accordance with the current Animal Care Regulations and Standards approved by the Institute for Laboratory Animal Research Council (ILARC 1996).

### **Euthanasia**

The animals were starved of food overnight prior to euthanasia and sacrificed by cervical dislocation as described by ILARC (1996). Cardiac blood specimens were obtained from each rat by terminal bleeding from the heart. The first half of the blood collected was transferred into ethylene diamine tetra acetic acid for CD4 cell estimation. While the second portion was transferred into an anticoagulant free test tube and allowed to clot and subsequently centrifuged at  $750\times g$  for 15 minutes to obtain serum. The serum was immediately aliquoted into Eppendorf tubes placed on ice and immediately stored at  $-80^{\circ}\text{C}$  until serum cortisol was evaluated.

### **Evaluation of Parameters**

CD4 cell count was estimated using Partec Cyflow counter, Germany for the quantification of CD4 T lymphocytes as described (PCC 2010). 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 and standard were 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.

### Statistical Analysis

All numerical data were obtained from the three (3) groups (control and treated). Data were represented as mean  $\pm$  standard deviation and analyzed using one way analysis of variance (ANOVA) and Tukey – Kramer Multiple comparisons Test using SPSS – 18.0 statistical program. P values  $\leq$  0.05 were considered significant.

### RESULTS

Table 1 shows the mean  $\pm$  standard deviation of serum cortisol level and CD4 cell counts of rats in Groups 1, 2 and 3. It was observed that, there was a significant decrease ( $P < 0.05$ ) in the serum cortisol level in Group 2 when compared with Group 1 and Group 3, while there was a significant increase ( $P < 0.05$ ) in the CD4 cell count in Group 2 when compared with Group 1 and Group 3.

Table 1. CD4 cell count and cortisol level in Groups 1, 2 and 3

Parameters	G 1(n=7)	G 2 (n=7)	G 3(n=7)
Cortisol (ug/dl)	280 $\pm$ 0.06	28 $\pm$ 0.02 <sup>S</sup>	480 $\pm$ 0.03 <sup>P,X</sup>
CD4 cells count (cells/ul)	600 $\pm$ 0.02	1200 $\pm$ 0.03 <sup>S</sup>	300 $\pm$ 0.06 <sup>P,X</sup>

S= Statistically Significant ( $P < 0.05$ ) comparison between Group 1 and 2.

P= Statistically Significant ( $P < 0.05$ ) comparison between Group 1 and 3.

X= Statistically Significant ( $P < 0.05$ ) comparison between Group 2 and 3.

### DISCUSSION

The significant reduction in serum cortisol level in Group 2 as compared with Group 1 and Group 3 seen in Table 1 could be attributed to the effect of moderate exercise, which is known to reduce the serum cortisol level in contrast to solitary confinement and strenuous exercise which tends to build up serum cortisol level. This is in accordance with the findings of Cornil et al., (1965), where moderate exercise reduced the serum cortisol level which suppresses the immune response. Several stressors including strenuous exercise have been associated with a shift in cytokine production towards the anti-inflammatory pattern with cortisol evaluation being the proposed mediator of the shift (Elenkor and Chrousos 2002). The significant reduction in the CD4 cell count seen in Group 1 and Group 3 as compared with Group 2, (Table 1), could be linked to the prolong serum cortisol secretion from the adrenal cortex which could lead to chronic stress, resulting in significant physiological change and cellular composition of blood. Cortisol is released in response to stress, sparing available glucose for the brain and heart, generating new energy from stored reserves and diverting energy from low priority organs in order to survive the immediate threats. However, prolong cortisol secretion which may be due to

chronic stress results in significant physiological changes (Scott 2011). Cortisol prevents the proliferation of T cells by rendering the interleukins 2 producer T – cells unresponsive to interleukin – 1 and unable to produce the T cell growth factor (Palacios and Sugawara 1982) and inhibits Immunoglobulin A and Immunoglobulin M in serum (Posey et. al., 1978). Long-term exposure to cortisol damages cells in the brain (Hippocampus) which results in learning impairment and inability to retrieve already stored information, (De Quervain et al., 2000). It shuts down the reproductive system, resulting in an increased chance of miscarriages and temporary infertility, (Nelson 2011). Cortisol secretion reduces bone formation, favoring long-term development of osteoporosis. Elevated level of cortisol, if prolonged, can lead to proteolysis and muscle wasting (Simmons et al., 1984; Knight et. al., 1955; Djurhuus et al., 2002). The immune system is merely responding to the damage done by the exercise bout, during which most of the adaptation leads to greater fitness, if balance diet and proper resting are observed after exercises (Ehiaghe et. al., 2013).

### Conclusion

It was observed that solitary confinement and strenuous exercise were inhibitory to the proliferation of CD4 cells with the elevation of cortisol been a possible mediator. Regular and frequent moderate exercise should be encouraged because it suppresses the elevation of serum cortisol.

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