EFFECTS OF SLEEP DEPRIVATION ON THE EPITHELIAL HEIGHT OF THE PROSTATIC ACINI IN RATS AND THE PROTECTIVE EFFECTS OF OMEGA 3 FATTY ACIDS

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ABSTRACT

Objective: To study the protective role of omega 3 fatty acids (omg 3 FAs) on the histomorphological changes in the height of the prostatic epithelium in rats induced by sleep deprivation.

Study Design: Lab based randomized control trial.

Place and Duration of Study: The study was conducted at Anatomy Department, Army Medical College, Rawalpindi, in collaboration with National Institute of Health (NIH), Rawalpindi for duration of one year, from Nov 2014 to Nov 2015.

Material and Methods: Thirty male Sprague Dawley rats, 3-4 months of age with average weights of 200-300 grams (gm) were divided in three groups each having 10 rats. Group A served as control with standard lab diet and regular sleep -wake cycle. Group B was subjected to sleep deprivation of 16 hours followed by a sleep window of 8 hrs daily for 2 months and group C was administrated with omg 3 fatty acids (FAs) and was sleep deprived as group B for 2 months. At the end of the experimental period rats were anesthetized and their blood sample was drawn for hormonal assay. They were dissected and the prostate gland was removed and fixed in 10 percent formalin. Five micrometer (µm) sections were obtained after tissue processing and stained with haematoxylin and eosin (H&E) for histological study.

Results: Microscopic examination revealed that the epithelium of glandular acini was columnar in group A. Marked decrease in the height of cells was observed in group B whereas the epithelium was nearly cuboidal in group C.

Conclusion: It was concluded that sleep deprivation had deleterious effects on the epithelium of the prostatic acini and that Omega 3 fatty acids had a protective effect on the epithelium of the prostatic acini.

Keywords: Omega 3 fatty acids, Prostate, Rats, Sleep deprivation.

INTRODUCTION

Sleep deprivation has become one of the leading forms of stress causing detrimental effects to the mind and body. Therefore, it is imperative to apprehend the impact of sleep deprivation on the body. Several experiments that have been conducted on rat models have established the physical effects of sleep deprivation such as dermatological findings, weight loss (in spite of regular food intake), decreased immunity followed by death in a couple of weeks proving that sleep is a basic biotic need that has impact on the operation of many organ systems[1]. Sleep deprivation is strongly associated with marked reduction in the levels of androgens[2] but also leads to decrease in the weight of accessory sex glands especially the prostate gland. This indicates reduced steroidogenesis or diminished expression of androgens in the target glands. These effects can be countered by the addition of healthy fats like omg 3 FAs. These diminish the action of 5 alpha-reductase enzyme[3] and have a defensive role on the prostate gland.

Omg 3 FAs, a group of polyunsaturated essential fatty acids that are necessary for human health but cannot be made de novo, so, they have

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to be obtained from exogenous sources, found in large amounts in fish oil[4].Omg 3 FAs greatly influence growth and development. Sleep deprivation is one substantial form of stress. Stress is known to cause inflammation which can damage tissues and organs if not controlled[5]. Fish oil supplements are known to lower inflammation by decreasing the synthesis of pro-inflammatory molecules. Eicosapentanoic Acid (EPA) and Docosahexanoic Acid (DHA) are associated with increased stress resilience[6].The regular use of Omg 3 FAs greatly reduces the risk of developing prostate cancer[7]. It also causes the reduction of oxidative stress and cell apoptosis[8].Chavarro proved that increased blood levels of Omg 3 FAs were linked with a decreased possibility of development of prostate cancer[9]. Investigating the positive effects of Omg 3 FAs on the histomorphology of sleep deprived rat prostates may be of great help. The protocol of current study was to identify the effects of sleep deprivation on the epithelium of prostate gland and to demonstrate the beneficial effects of omg 3 FAs.

Table: Comparison of mean epithelial height (µm) and serum testosterone levels (ng/ml) among the control group A and experimental groups (B) and (C).

	Control group A(n = 10)	Experimental group B(n = 10)	Experimental group C(n = 10)	*p*-value
Epithelial height (µm)	14.67 ± 1.17	4.61 ± 1.26	9.41±1.32	<0.001*
Serum testosterone levels (ng/ml)	1.32±0.25	0.53±0.16	1.18±0.41	<0.001*

p value ≤0.05 is statistically significant
*= highly significant

MATERIAL AND METHODS

The study was a laboratory based randomized control trial carried out in the Department of Anatomy, Army Medical College Rawalpindi, in collaboration with National Institute of Health (NIH) Islamabad and Armed Force Institute of Pathology (AFIP), Rawalpindi. It was spanned from November 2014 to November 2015 with the approval of ethical committee on animal experiments, of the Army Medical College, Rawalpindi. A total of thirty, 3-4 months old rats, grouped by using random number table method, selected by non-probability convenient sampling, weighing 200-300 grams (gm) were studied. They were kept in a well ventilated room and under a temperature range of 20-26°C. Rats were fed with NIH laboratory diet for two months. Water was provided ad libitum. Rats were randomly divided into three groups (10 animals in each group). The rats of group A served as controls, they were given standard lab diet and were subjected to normal sleep wake cycle. The rats in group B were given standard lab diet and were subjected to sleep deprivation for a period of 16 hours daily followed by a sleep window of 8 hours daily, for 8 weeks. Rats in group C were also subjected to sleep deprivation as group B and were administrated with Omg 3 FAs at a dose of 260 milligram/kilogram/day (mg/kg/day), through oral gavage in addition to the regular lab diet. The dose of Omg 3 FAs was set based on previous studies[10] and it was obtained from Good`N`Natural, imported by Route 2 Health Pvt Ltd. The sleep deprivation apparatus was based on a modified pendulum technique and it consisted of a cage partitioned into 2 for each of group B and group C. It was fitted with an electrical device that caused to and fro jerky movements every 2 minutes set by a timer. This brought unrest in the rats causing sleep deprivation[11].

At the end of 8 weeks the rats were dissected under chloroform anesthesia before which 5 milliliter (ml) blood was drawn from each rat via intracardiac puncture, for assessing serum testosterone. The prostate glands were removed

and fixed in 10 percent formalin and processed in automatic tissue processor. Paraffin was used for infiltration and embedding. Cross sections of 5 micrometer (µm) thickness were obtained from the tissue blocks. All processing and staining procedures were done in histopathology lab at AFIP, Rawalpindi. Haematoxylin and eosin (H&E) stains were used for routine histological study. Epithelial height in the prostatic acini was measured by observing 3 random fields per slide. Three acini were randomly selected from each field. In each acinus 20 cells were randomly measured for epithelial height.The height of each cell was measured from the basement membrane till the apex of the cell facing the lumen at X40 and a mean height was calculated[12]. A morphometric computer software "Motic Images plus 2.0", was used for the calculation of height with the help of a measurement tool for straight lines.

IBM-SPSS version 21 was used for data analysis. ANOVA test was applied followed by Post Hoc Tukey`s test, for intergroup comparison of quantitative variables which was taken as means and standard deviations (mean ±SD). A *p* value <0.05 was considered significant.

RESULTS

Thirty sprague dawley rats with an average age of 3-4 months and a mean weight of 203.16 ± 12.80 grams were used in the experiment. After dissection and tissue processing, examination of the acinar epithelium in group A showed that the acini were lined by simple columnar epithelium with a mean height of 14.60 µm ±1.17 and the mean testosterone level was measured to be 1.32 nano gram/ milliliter (ng/ml) ± 0.25.The epithelium of the prostatic acini in group B was markedly decreased in height as compared to groups A with cells having a mean height of 4.61 ± 1.26 µm and serum testosterone level was 0.53 ± .16 ng/ml. The epithelial height in group C was observed to be 9.41 µm ±1.32 and the serum testosterone level was 1.18±0.41 ng/ml. Intergroup comparison of the epithelial height, after the application of Post Hoc Tukey`s test, revealed a *p* value of 0.001, when group A was compared to group B which was statistically very significant. On comparison of groups A and C *p* value was found to be 0.001 and when group B was compared to group C the *p* value 0.001 which was also statistically highly significant (table). Intergroup comparison of serum testosterone

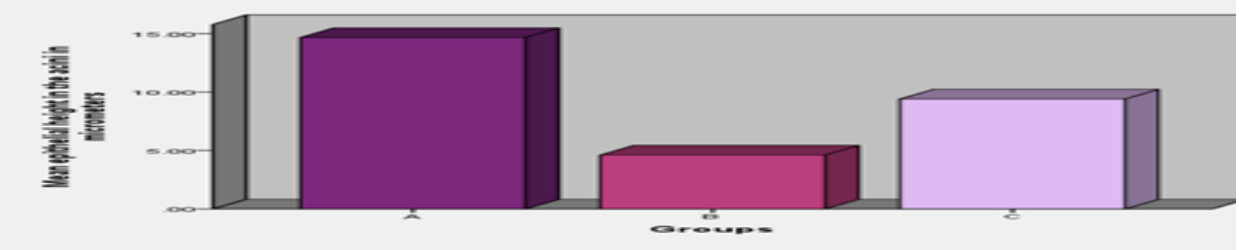

Figure-1: Comparison of mean values of height of the acinar epithelium among the control group A, experimental group B and experimental group C.

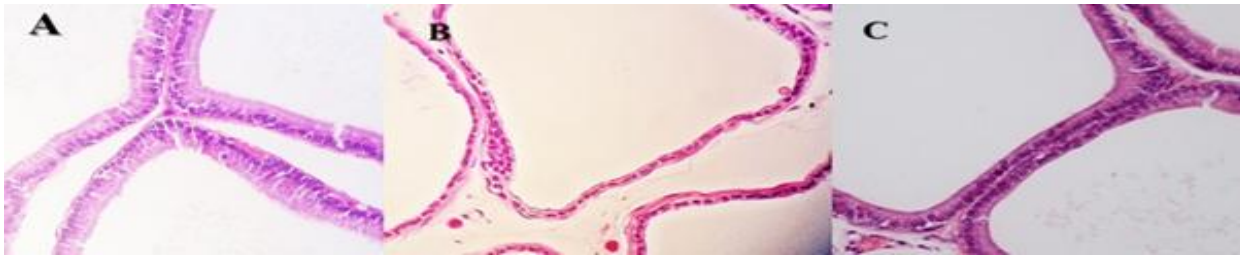

Figure-2: Change in the epithelial height in control group (A), experimental group (B) and experimental group (C).

levels revealed a *p* value of 0.000 when group A was compared to group B and when group B was compared to group C, which was statistically significant. However, on comparison of group A with group C, *p* value= 0.526 which was statistically insignificant. The epithelial height in the acini of all the three groups were compared with each other and it was found that the epithelial height in the experimental group B was significantly lesser as compared to the control group (*p*=<0.001) (fig-I & II).

DISCUSSION

It has been found that the prostate is an androgen dependent organ. Sleep deprivation results in decreased androgen levels in the blood which also effects the growth of prostate and hence, affects the epithelial height. Mean height of the epithelium in the experimental group C was more as compared to group B. The difference was, however, statistically significant with a *p* value of = 0.001 indicating the decrease in height was prevented by Omg 3 FAs. On comparison of the height of epithelium between the control group A and group C the difference was statistically significant (*p*=<0.001). This is in agreement with a study conducted on seminal vesicles of rats where epithelial height decreased due to application of stress[13]. It has also been proven that under stressful conditions the epithelial height decreases[14].The serum testosterone levels were measured which indicated a significant decrease in the hormonal level of the rats in the experimental group B when it was compared with the control group A (*p*=0.001). However, the difference in the serum testosterone level in the experimental group C was not statistically significant when it was compared with the control group A. This is consistent with the previous study[15] which proved that the lack of sleep is associated with decrease in testosterone levels in rats. Sleep deprivation is a form of stress and its effect on the sex hormones of rats was also proved in a study conducted where different stress modalities were inflicted on rats with resultant decrease in the testosterone levels[16].Omg 3 FAs are known to be responsible for the upsurge in luteinizing hormone (LH) formation, especially in animals. This leads to the production of testosterone inside the Leydig cells.This is one of the basic reasons why the levels of testosterone were more in rats of Omg 3 FAs administrated group C as compared to those of group B[17].

It was observed in the present study that sleep deprivation caused significant changes in the histomorphology of the prostate gland in rats as regards the epithelium and it was also shown that Omg 3 FAs had ameliorative effect on the histomorphology of the rat prostate gland. The prostate of sleep deprived group showed decrease in epithelial height. Moreover, there was a significant decrease in serum testosterone levels.

CONCLUSION

It was concluded that sleep deprivation had deleterious effects on the epithelium of the prostatic acini and that omg 3 FAs had a protective effect on the epithelium of the prostatic acini.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

REFERENCES

1. Bianchi MT. Sleep Deprivation and Disease: Effects on the Body, Brain and Behavior. Springer Science & Business Media; 2013.
2. Wittert G.The relationship between sleep disorders and testosterone in men. Asian J Androl. 2014; 16(2): 262-65.
3. Liang T, Liao S. Inhibition of steroid 5x-reductase by specific aliphatic unsaturated fatty acids. Biochem. J. 1992; 285: 557-562.
4. Kumar A S, Deepthi K B, Prasad M D V, Mary PG, Kumar SS. Swathi M. Evaluation of the Protective Effects of Omega-3 Fatty Acids against Methotrexate Induced Testicular Toxicity in Male Albino Mice. IJP. 2011; 2(2): 48-52.
5. Yates C M, Calder PC, Ed, Rainger G. Pharmacology and therapeutics of omega-3 polyunsaturated fatty acids in chronic inflammatory disease. Pharmacol Ther 2014; 141(3):272-82.
6. Bahadori B, Uitz E, Thonhofer R, Trummer M, Pastemer Leech I, McCarty M et al. Omega-3 Fatty acids infusions as adjuvant therapy in rheumatoid arthritis. J Parenter Enteral Nutr 2010; 34(2):151-5.
7. Freeman VL, Meydani M, Yong S, Pyle J, Flanigan RC, Waters WB et al. Prostatic levels of fatty acids and the histopathology of localized prostate cancer. J Urol 2000; 164(6):2168- 72.
8. Suphioglu C, Mel D. D, Kumar L, Sadli N, Freestone D, Michalczyk et al. The omega-3 fatty acid, DHA, decreases neuronal cell death in association with altered zinc transport. Febslet 2010; 584(3): 612-618.

9. Chavarro JE, Stampfer MJ, Li H, Campos H, Kurth T, Ma J. A prospective study of polyunsaturated fatty acid levels in blood and prostate cancer risk. Cancer Epidemiology Biomarkers Prev 2007;16(7):1364–70.
10. Venâncio DP, Andersen ML, Vilamaior PS, Santos FC, Zager A, Tufik S, et al. Sleep deprivation alters rat ventral prostate morphology, leading to glandular atrophy: a microscopic study contrasted with the hormonal assays. BioMed Research International. 2012;2012.
11. Van Hulzen ZJ, CoenenAM .The pendulum technique for paradoxical sleep deprivation in rats. Physiol Behav. 1980; 25 (6): 807–11.
12. Gonzales GF, Miranda S, Neito J,Fernandez G,Yucra S, Rubio J et al. Red Mecca (Lipidium Meyenii) reduced prostate size in rats . Reprod Biol Endocrinol.2005; 20: 3-5.
13. Mukherjee B, Rajan. Morphometric study of rat prostate in normal and under stressed condition. J Anat. Soc India. 2004; 53 (1): 53-8.
14. Mukherjee, Rajan T. Morphometric study of seminal vesicles of rat in normal health and stress conditions. 2006: 55(1):31-36.
15. Wu J L, Wu R S, Yang J G, Huang CC, Chen KB, Fang KH et al.Effect of sleep deprivation on serum testosterone concentration in the rats. Neurosci lett 2011; 494:124-29.
16. Yan L, Bai XL, Fang ZF, Che LQ, Xu XY, Wu D. Effect of different dietary omega-3/omega-6 fatty acid ratios on reproduction in male rats. Lipids Health Dis. 2013; 12(13): 33.
17. Andersen M L, Bignotto M, Machado RB, Tufik S. Different stress modalities result in distinct steroid hormone responses by male rats. Braz J Med Biol Res 2004; 37(6): 791-97.