ORIGINAL ARTICLE

Effects of Sleep Deprivation on the Area 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 on the histomorphological changes in the area of the prostatic acini 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 a duration of one year from 11th Nov 2014 to 11th Nov 2015.

Materials 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 omega 3 fatty acids 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 sections were obtained after tissue processing and stained with haematoxylin and eosin (H&E) for histological study.

Results: Microscopic examination revealed that the percentage area of the prostatic acini was decreased by 100% in group B and 30% in group C. This revealed that the area of the prostatic acini was decreased in the group B as compared to the control group A. Decrease in the acinar area in the experimental group C was not that marked as compared to experimental group B.

Conclusion: It is concluded that sleep deprivation has deleterious effects on the area of the prostatic acini and that omega 3 fatty acids has an ameliorative effect on the area of the prostatic acini.

Key Words: *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. Prolonged sleep deprivation usually takes place in extreme situations or under experimental conditions.[1] The most common causes of sleep deprivation are those related to contemporary lifestyle and work-related factors; thus the condition affects a considerable number of people. Sleep deficiency (insomnia) accompanies certain pathological states and may require treatment[2] It has become one of the greatest health risk factor that contributes to several disease processes. It leads to biochemical, hormonal, behavioral and neurological alterations. 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.[3] As sleep deprivation has been proven to be a form of stress it exerts allostatic load i.e wear and tear of the systems in the body, circadian disruption including increase in the cortisol levels.[4] The increase in cortisol levels due to stress is associated with decrease in testosterone levels as sleep deprivation results in many alterations in the morphology of various organs and hormonal abnormalities.[5] It is strongly associated with alteration in the male sex hormone levels causing detrimental effects on the prostate gland.[6] Testosterone plays a pivotal role in the development

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and functioning of the prostate gland.[7] According to a latest study men with sleep problems have been linked to the development of prostate cancers.[8] Sleep deprivation is strongly concomitant with noticeable drop in the levels of androgens.[9] This shows reduced steroidogenesis or lessened expression of androgens in the target glands. These effects can be counteracted by the addition of healthy fats like omega 3 fatty acids. These lessen the action of 5 alpha- reductase enzyme and have a protective role on the prostate gland.

Omega 3 fatty acids (Omg 3 FA), an assembly of polyunsaturated essential fatty acids that are compulsory for human health but cannot be made de novo, so, they have to be attained from exogenous sources, found in enormous quantities in fish oil . The consistent use of omega 3 fatty acids greatly diminishes the risk of developing prostate cancer.[10] Eicosapentaenoic acid (EPA) a form of omega 3 FAs is linked with reduction in the progression of prostate cancer.[11] It also results in the decline of oxidative stress and cell apoptosis.[12] Chavarro proved that augmented blood levels of omega 3 FAs were concomitant with a decreased likelihood of development of prostate cancer.[13] Stress is known to cause inflammation which can impair tissues and organs if not controlled. Exploring the positive effects of omega 3 fatty acids on the histomorphology of sleep deprived rat prostates may be of great help. The modus operandi of current study was to ascertain the effects of sleep deprivation on the area of acini in the prostate gland of rats and to establish the beneficial effects of omega 3 fatty acids.

The effects of sleep deprivation on the secondary sex glands especially in males e.g prostate gland, have not been focused upon. Therefore, this study was aiming at the histological effects of sleep deprivation on the prostate gland in rats and the protective effects that omega 3 fatty acids brought about to minimize these detrimental changes.

Materials and Methods

The study was a laboratory based randomized control trial carried out in the Department of Anatomy, Army Medical College Rawalpindi, in alliance with National Institute of Health (NIH) Islamabad and Armed Force Institute of Pathology (AFIP), Rawalpindi. It was spanned from 11th November 2014 to 11th November 2015 with the approval of ethical committee on animal experiments, of the Army Medical College, Rawalpindi. A total of thirty rats were used by random number table method, selected by non-probability convenient sampling. The rats were 3-4 months of age and weighing 200-300 grams (gm). They were kept in a well ventilated room and under a temperature range of 22-26°C. Rats were given NIH laboratory diet for two months. Water was provided ad libitum. Rats were indiscriminately divided into three groups (10 animals in each group). The rats of group A served as controls, they were fed with standard lab diet and subjected to normal sleep wake cycle. The rats in group B were fed with regular lab diet and 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 FA at a dose of 260 milligram/kilogram/day (mg/kg/day), through oral gavage in addition to the regular lab diet. The dose of Omega 3 fatty acids was established based on prior studies [14] 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.[15]

At the completion of 8 weeks, 5 milliliter (ml) blood was drawn from each rat via intracardiac puncture, for evaluating serum testosterone then rats were dissected under chloroform anesthesia. The prostate glands were excised and fixed in 10% formalin and processed in automatic tissue processor. The tissue was infiltrated and embedded with paraffin wax. 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. H&E stains were used for routine histological study. Area of the acinar lumen was calculated by using a morphometric computer software “Motic Image Plus 2.0” a software for calculating area and other user defined morphometric parameters.[16] Images of three

selected fields were taken from each slide with the help of Olympus digital camera (10 mega pixel), Stylus 1010 were used through the ocular of the Olympus DP21 light microscope. The images were then transferred in the computer. Each image was opened in morphometric computer software "Motic Image plus 2.0". A scale was set to measure the area in micrometer square at 10X. Measurement tool for irregular shapes was selected and the area to be measured was outlined, the measurement was then analyzed and recorded. The final reading was recorded as the mean of area of 3 acini per three fields/slide per specimen.

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 220.16±10.80 grams were used in the experiment. After dissection and tissue processing, histological examination of the prostatic acini in group A showed that the mean area of the acini was 471.48±101.66 µm.² The mean area of the prostatic acini in group B was markedly decreased as compared to control group A, it was measured to be 246.69±17.08 µm.² The area of the prostatic acini in group C was observed to be 341.70±68.60 µm.² Intergroup comparison of the area of the prostatic acini, 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. When group B was compared to group C the p value= <0.016 which was also statistically highly significant. Intergroup comparison of serum testosterone 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.

Discussion

The difference in area among all the three groups was statistically significant (p<0.001). the group that was exposed to sleep deprivation showed marked changes in the histomorphology when the area of the prostatic acini was studied. This is because 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 prostatic acini. The decrease in area of the prostatic acini is a result of decrease in the testosterone levels in blood due to sleep deprivation. 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 including sleep deprivation were inflicted on rats with resultant decrease in the testosterone levels. Sleep deprivation causes alterations in hypothalamo-hypophyseal axis leading to decreased circulating androgens in healthy individuals.[17] Omega 3 fatty acids 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 area of the prostatic acini is not markedly decreased in rats of omega 3 administrated group C as compared to those of group B ,hence, proving the objective of the study.[18] Sleep deprivation causes low levels of androgens and results in activation of apoptosis, hence, atrophy or decrease in size of the acini due to cell death.[19] The male sex hormone levels i.e testosterone levels were estimated which specified a noteworthy diminution in the hormonal level of the rats in the experimental group B when it was related with the control group A (p <0.001). Nevertheless, the variance in the serum testosterone level in the experimental group C was not statistically significant when it was equated with the control group A. This is in agreement with the previous study which proved that the lack of sleep is associated with decrease in testosterone levels in rats. Omg 3 FAs are known to cause rise in luteinizing hormone (LH), especially in animals. This leads to the making of testosterone inside the Leydig cells .This establishes why the levels of testosterone were increased in rats that were given Omg 3 FAs i.e group C as compared to those of group B.[20]

Conclusion

It is concluded that sleep deprivation has harmful effects on the prostatic acini and that omega 3 fatty

Table I: Showing Mean Area of the Prostatic Acini (µm2) 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
Area of the prostatic acini (µm²)	471.48±101.66	246.69±17.08	341.70±68.60	<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

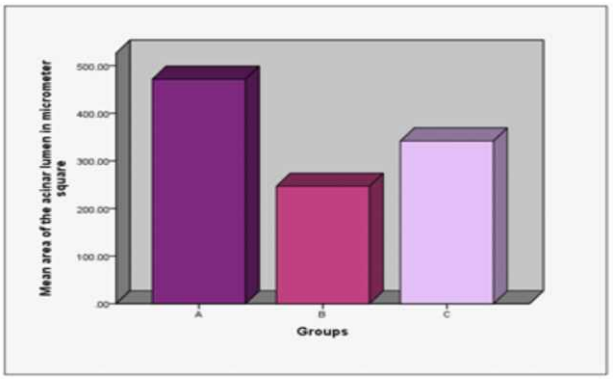
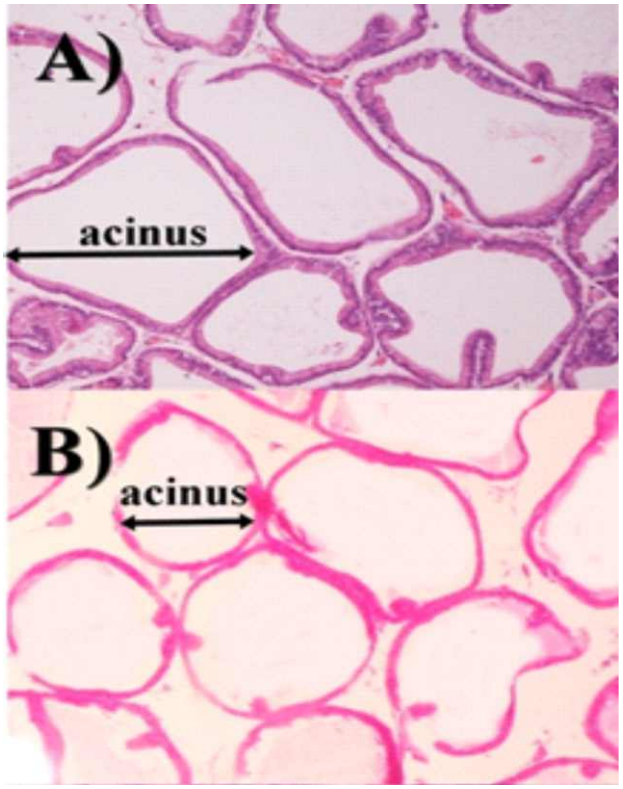


Fig 1: Bar Chart Showing Comparison of Mean Values of Area of the Prostatic Acini among the Control Group A, Experimental Group B and Experimental Group C



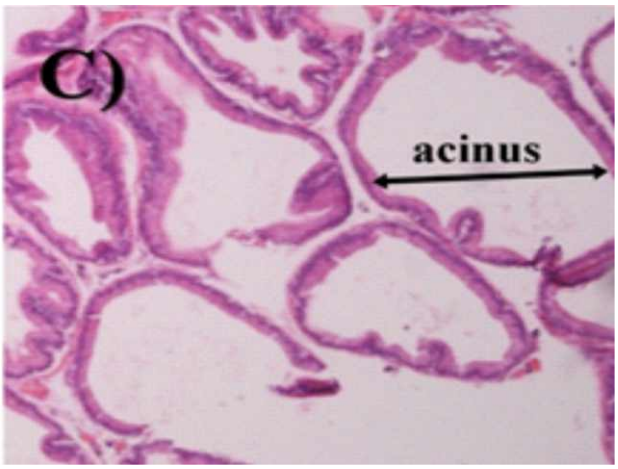


Fig 2: Photomicrograph Showing Comparison of the Area of the Acinar Lumen in Control Group (A), Experimental Group (B) and Experimental Group (C).

acids had a protective effect on the prostate gland in rats.

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