DOI: https://doi.org/10.53350/pjmhs2023176130

ORIGINAL ARTICLE

Morphometric Study about Effect of Sleep Deprivation on Prostate of Rats with Protective Effect of Omega 3 Fatty Acids

NOMANA MAHMOOD[1], KAUKAB ANJUM[1*], UZMA SHAHID[1], AYESHA SHAHID[2], NOREEN ANWAR[3], MADIHA ALI[4]
[1]Department of Anatomy, Wah Medical College, NUMS, Wah-Cantt, Pakistan.
[2]Department of Anatomy, Dental College HITC-IMS, NUMS, Taxila, Pakistan.
[3]Department of Anatomy, Fauji Foundation University Medical College, Islamabad, Pakistan
[4]Department of Anatomy, Watim Medical College, Islamabad, Pakistan
Correspondence to Dr Kaukab Anjum, E-mail: kanjum@gmail.com, Tel+92-333-5157229

ABSTRACT

Background: Even though sleep makes up nearly a third of a person's lifetime, we do not fully appreciate its significance. The partial or nearly complete loss of sleep from an organism that results in a number of detrimental health issues is known as sleep deprivation.
Aim: To investigate how omega 3 fatty acids affect the size and weight of the prostate gland in sleep-deprived rats.
Study design: Random control trial.
Methodology: Thirty male Sprague Dawley rats (population) aged three to four months and weighing 200 to 300 grammes were utilised. They were kept in a controlled setting at the NIH's animal house in Islamabad. Rats were randomised into three groups by using lottery method, with n = 10 rats in each group. The control group was designated as group A. They experienced a regular cycle of sleep and wakefulness. Rats in group B, which experienced sleep deprivation for 16 hours followed by an 8-hour sleep window each day for 8 weeks, were placed in the sleep-deprived group. The standard lab diet was provided to Groups A and B. For eight weeks, Group C received a typical lab meal combined with omega 3 fatty acids while also being sleep deprived for 16 hours, followed by an 8-hour sleep window each day.
Results: In comparison to groups A and C that received omega 3, the gland weight and volume in group B were significantly lower. Group A: Weight and volume of the prostate: Prostate weight ranged from 1.85 to 0.50 grammes on average. The computed RTBWI was 0.60 + 0.22. The prostate's mean SD volume was 166.81 45.67 mm^3. GROUP B: Weight of the prostate: Prostate weight ranged from 1.30 to 0.22 grammes on average. In the sleep-deprived group B compared to the control group A, the weight of the prostate was considerably lower (p-value=0.008). The computed RTBWI was 0.37 + 0.22. The prostate's mean SD volume was 127.88 67.35 mm^3. GROUP C: Weight of prostate gland: In experimental group C, the prostate's mean SD weight was 2.19 0.42gm. According to the RTBWI calculation, it was 0.64 0.07. The prostate's mean SD volume was 182.89 44.16 mm^3.
Practical Implication: Today, sleep deprivation is a big problem, and this study will assist researchers in examining how Omega 3 fatty acids can help prevent this problem and the harmful effects it has on the prostate glands.
Conclusion: It was concluded that Omega 3 fatty acids played a protective role while sleep deprivation had a negative impact on the weight and volume of the prostate gland.
Keywords: Sleep Deprivation, Omega 3 Fatty Acids, Prostate and Protective Effect.

INTRODUCTION

Even though sleep makes up nearly a third of a person's lifetime, we do not fully appreciate its significance. The partial or nearly complete loss of sleep from an organism that results in a number of detrimental health issues is known as sleep deprivation. Sleep loss or deprivation has been linked to a number of negative effects, including multisystem biological hazards like diabetes, cancer, and mortality[1]. Short sleep duration is linked to an increased risk of death in humans, and chronic sleep deprivation causes death in laboratory animals. Therefore, lack of sleep, a harmful stress, can negatively affect an organism's health by impairing the normal operation of essential organs like the brain, heart, liver, kidneys, and sex organs. Despite this, no research has been published on the effects of sleep deprivation on the prostate gland in human subjects or animal models[2]. Numerous studies have shown that the circadian system can be repeatedly disrupted in ways that increase the risk of cancer, including sleep deprivation, the suppression of the pineal hormone melatonin by exposure to light at night, and the production of proinflammatory reactive oxygen, which has been linked to both cancer and atherosclerosis[3].

It is well known that as prostate volume rises, the incidence of prostate cancer declines. Omega-3 fatty acids (FA) are one of two kinds of essential fatty acids (EFA) (the other being Omega-6)[4] and patients who had been diagnosed with cancer had prostates that were much smaller than those of people who had a negative sextant biopsy. They are both regarded as essential since the human body is unable to convert them into one another, and because the diet must provide the precursors, linoleic acid and linolenic acid for omega-6 and omega-3 FA, respectively[5]. A compelling case is made for the possibility of omega-3 FA in the prevention of cancer based on the impact of dietary lipids on the COX and LOX pathways and their various bioactive intermediates. Omega 3 fatty acids decrease carcinogenic expression with regard to tumor latency and multiplicity, according to experimental animal research[6].

One similar study 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. 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.[7]

Due to the lack of local data regarding the protective effect of Omega 3 fatty acids on epithelium of sleep deprived rats' prostate. Thus we planned current project in-order to check the proposed hypothesis that sleep deprivation might have a negative impact on rats' prostate glands. Thus our results will signify the role of omega 3 fatty acids in stopping histological changes due to sleep deprivation, although more studies will be required in future to confirm its affectivity.

METHODOLOGY

In partnership with the National Institute of Health (NIH), Islamabad, and the Armed Force Institute of Pathology (AFIP), Rawalpindi, the study was conducted in the Department of Anatomy at the Army Medical College of Rawalpindi. It was a two-month long randomised control experiment that took place in a lab.

Received on 07-12-2022
Accepted on 17-05-2023

The Army Medical College in Rawalpindi's ethical committee on animal research gave its clearance for the experiment's conduct. Thirty male, in the experiment, Sprague Dawley rats aged three to four months and weighing 200 to 300 grammes were utilised. They were kept in a controlled setting at the NIH's animal house in Islamabad. With the aid of a central temperature control system, they were kept in a well-ventilated space at a temperature between 20 and 26 °C[8]. For two months, rats were fed NIH laboratory diet. Water was available at all times. Rats were randomized into three groups at random, with n = 10 rats in each group.

Group A, (control group) number = 10

In this group, rats were used as controls. They experienced a regular cycle of sleep and wakefulness. They were fed a routine laboratory diet.

Group B, (sleep deprived) number = 10

For eight weeks, rats in this group underwent a 16-hour period of sleep deprivation followed by an 8-hour sleep window each day. They were fed a routine laboratory diet.

Group C, (sleep deprived +omega 3 administrated) number = 10

This group of rats underwent a 16-hour period of sleep deprivation followed by an 8-hour sleep window each day for 8 weeks. They received an oral gavage of 260 mg/kg body weight of omega 3 administered as part of a routine lab diet[8,9].

Modified Pendulum Technique: The equipment for this approach was a cage divided into two sections and outfitted with two distinct trays that were connected to a mechanical mechanism. To induce imbalance and keep the rats from falling asleep, the trays were shifted back and forth at regular intervals of about 2 minutes. To provide the rats a window of time to sleep, the machine was left on for 16 hours and then turned off for 8 hours during the day. In the event of load shedding, the gadget received an uninterrupted power supply (UPS).

RESULTS

Before the animals were sacrificed at the beginning and end of the trial, the total body weight of all the animals (gm) was noted. With the aid of a triple beam balance, the weight was measured. Route 2 Health Pvt Ltd purchased omega 3 fatty acid gel capsules from Good 'N' Natural imported. (Product no CP95). The chemical that was obtained was pure and suitable for analysis. Through an oral gavage tube, omega-3 fatty acids were administered. The calculated dose per rat per day was 52 mg. After the rats were put down, five millilitres of blood were drawn through a heart puncture[10], for biochemical analysis and the quantitative assessment of testosterone levels in all of the animals. The samples were given labels based on the groupings.

At the conclusion of the eighth week, the rats were killed by being put in glass jars with cotton that had been soaked in ether[11]. The animals' extremities were fixed with their abdomens facing up on a clean dissection board. With a pair of scissors, the midline was cut through the skin and muscles of the abdomen and pelvis from the xiphoid process to the pubic symphysis[12]. Skin flaps were pulled back to reveal the organs. The bladder of the kidneys was recognised. Two lobes of the prostate gland with encircling connective tissue were visible wrapped around the bladder's neck[13]. The seminal vesicles were properly separated from the prostatic lobes. Seminal vesicles were reflected medially to disclose the lateral lobes, whereas seminal vesicles were retracted distally to reveal the dorsal lobes. The dorsal lobes of the prostate were then visible to be separated in the midline after the surrounding tissue and covering fascia were removed. The two club-shaped swellings on the gland's front side were the ventral lobes[14]. The prostate gland was then taken out, cleaned with regular saline, patted dry, and put on a scale. Using an analytical balance, prostates from each of the three groups were weighed independently.

Weight of the prostate gland: Weight of prostate glands was recorded in grams using digital weighing scale. Relative tissue body weight index (RTBWI) was calculated by using the following formula: RTBWI= weight of organ (gm)/ body weight (gm) x100.

Volume of the prostate gland: Volume of the prostate gland was recorded in mm^3 using a digital Vernier`s Caliper (Appendix IV, Fig-4b, c and d). The following formula was used for calculating the volume of prostate: $V = 4/3 \times \pi \times L/2 \times W/2 \times T/2$.

Here, V stands for the prostate gland's volume, L for its length, W for its width, and T for its thickness, all expressed in millimetres. Pi () has a value of 3.141 and is a constant. The Setchell and Waites equation was utilised in this investigation[11]. The moment at which all prostate glands reach their greatest length was taken into account when measuring length. The prostate glands' front and posterior thicknesses were measured to determine thickness. In all prostate specimens, the combined breadth of the two ventral lobes was measured to determine the prostate's width.

Group A: Weight and volume of the prostate. Prostate weight ranged from 1.85 to 0.50 grammes on average. The computed RTBWI was 0.60 + 0.22. The prostate's mean SD volume was 166.81 45.67 mm^3.

Group B: Weight of the prostate: Prostate weight ranged from 1.30 to 0.22 grammes on average. In the sleep-deprived group B compared to the control group A, the weight of the prostate was considerably lower (p-value=0.008). The computed RTBWI was 0.37+0.22. The prostate's mean SD volume was 127.88 67.35 mm^3.

Group C: Weight of prostate gland. In experimental group C, the prostate's mean SD weight was 2.19 0.42 gm. According to the RTBWI calculation, it was 0.64 0.07. The prostate's mean SD volume was 182.89 44.16 mm^3.

Figure-1: Photograph showing measurement of weight of prostate with a digital analytical balance and volume of the prostate gland with Vernier Caliper.

Table-1: Mean values of initial and final weight of animals, weight gain, weight of prostate glands and RTBWI in control group A, sleep deprived (SD) group B and SD+ Omega 3 administrated group C

	Group A Mean±SD (n = 10)	Group B Mean±SD (n = 10)	Group C Mean±SD (n = 10)	P-value
Initial Animal Weight (gm)	201.93±10.86	208.2±15.86	200±10.52	0.351
Final Animal Weight (gm)	291.6±16	356.18±39	331±39	0.012*
Weight gain(gm)	100.7±38	147.90±32.13	131.25±32.9	0.016*
Weight of prostate glands (gm)	1.85±0.5	1.30±0.22	2.19± 0.42	0.008*
Volume of prostate glands(mm^3)	166.81±45.67	127.88±67.35	182.89±44.16	0.079
RTBWI	0.60±0.22	0.37±0.22	0.64±0.07	0.008*

p- value ≤ 0.05 is statistically significant

DISCUSSION

This study's goals were to analyse the beneficial effects of omega 3 fatty acids and the effects of sleep deprivation on the histomorphology of the rat prostate. All of the animals in the current investigation were active and in good health throughout the experiment. The body weight difference between the experimental and control groups was statistically significant.

Sleep deprivation is one of the pressures of the modern social order that is most inevitable. Numerous experimental studies are carried out to determine the detrimental effects of sleep deprivation on the body's many organ systems since sleep is crucial for cognitive performance and physical health[13]. Anxiety, depression, a busy schedule, sleep apnea, working nights or weekends, jet lag, and other circumstances are only a few of the many that contribute to this terrible tension. Sleep deprivation is believed to have a negative effect on weight and health and causes allostatic load throughout the body in order to prevent the risks of these outcomes. The anti-inflammatory and anti-stress characteristics of omega 3 fatty acids have received a lot of attention in recent scientific studies. It is advised to use it for a variety of ailments, including rheumatoid arthritis, CVS issues, etc.

The objectives of this study were to examine how sleep deprivation and omega 3 fatty acids affect the histo-morphology of the rat prostate. The animals used in the current study were all awake and healthy during the trial. There was a statistically significant difference in body weight between the experimental and control groups. The mean animal weight gain was statistically significant (p-value=0.016) in group B which was 147.90±32.13 gm when it was compared with group A and C. There are studies which report that sleep deprivation may cause an increase in hunger and craving for food intake, due to disruption in neuroendocrine control of appetite which causes increased vulnerability to gain weight and decreased energy expenditure. Leptin and ghrelin are the two hormones that influence hunger and calorie expenditure. Leptin impedes appetite and escalates energy expenditure while ghrelin has reverse affects. Lack of sleep is associated with lower levels of leptin and higher levels of ghrelin, which increases appetite and hunger. Rats in group A had lower mean final weights than those in group B. When the mean weights of the rats in groups C and A were compared, the same finding was made. This was in line with another research which demonstrated that using omega 3 fatty acids did not contribute to weight loss.[13] When compared to group A, the weight gain in group B rats was statistically significant (p-value = 0.014). However, it was statistically insignificant when group A was compared to group C (p-value=0.138) or when group B was compared to group C (p-value=0.536).

When the mean weights of the prostate glands were compared, it was shown to be statistically significant. The mean prostate weight of group B was significantly lower than that of control group A and group C that received omega 3 treatment. A statistically significant p-value of 0.009 was found for the intergroup comparison. Although the prostate gland's mean volume was lower in the sleep-deprived group than in the control group, the difference was statistically insignificant. In the experimental group C, the mean volume of the prostate glands was higher than in the experimental group B, although this difference was statistically insignificant. The current research's findings corroborated with another study which demonstrated that sleep deprivation causes the prostate gland to shrink and atrophy[15]. The decrease in serum testosterone levels is thought to be responsible for the prostate gland's weight loss. Androgens have a significant impact on the prostate gland's development and maintenance. Lack of sleep is a sort of stress that raises stress hormone levels, which are adversely correlated with sex hormone levels in the body. This is also consistent with Anderson's research, which shown that low amounts of sleep cause testosterone levels to drop. In comparison to control group A, the sleep-deprived group's prostates had lower mean weights and volumes[16]. The mean weight and volume of prostates were similarly larger in group C receiving omega-3 supplementation than in group B. The lipid peroxidation process that omega 3 fatty acids undergo helps to explain this. Lipid peroxides, one of the related enzymes, increase the activity of the enzyme 5-reductase, which leads to the production of DHT which is more effective than testosterone at promoting prostate growth and cellular proliferation in the gland's stroma and epithelium, which results in a rise in the weight and volume of the gland.

The experimental rats were kept in a 123 x 44 x 44 cm acrylic tank that included 14 circular platforms with 6.5 cm in diameter. Water was added to the tank until it was roughly 1 cm below the platform's surface. In a tank, the rats can move by leaping from one platform to another. The reduction of muscular tone that happens during rapid eye movement (REM) sleep enables the rats to make contact with the water, which causes them to be abruptly awoken. This method has been shown to fragment slow wave sleep in addition to eliminating REM sleep. The current study intended to assess sperm quality, hormone levels, and the histology of the testis in sleep-deprived male rats to ascertain whether sleep deprivation has an effect on the male reproductive system. By keeping the rats on the platforms for an hour every day for three days in a row, the rats became accustomed to their new surroundings. The current study's findings suggest that sleep deprivation may have an impact on rats' male reproductive systems. This is the first study that, as far as we are aware, identifies the negative consequences of sleep deprivation on the male reproductive system, including sperm and testicles, in rats. Loss of sleep can cause significant alterations in the male reproductive system in rats, especially reducing spermatic function and interfering with the testicular nitric oxide pathway[14].

Volume of the prostate gland: An electronic Vernier's Calliper was used to measure the prostate's volume in millimetres cubic. The volume of the prostate was determined using the formula

$$V = 4/3 \times \pi \times L/2 \times W/2 \times T/2.$$

Here, the prostate gland's volume (V), length (L), width (W), and thickness (T) are all expressed in millimetres (mm). Pi () has a value of 3.141 and is a constant. The moment at which all prostate glands reach their greatest length was taken into account when measuring length. The thickness of the ventral lobes of each prostate gland was measured in order to determine thickness. In all prostate specimens, the combined breadth of the two ventral lobes was measured to determine the prostate's width.

One similar study 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. 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.[7]

Limitations: Financial constrains with limited resources and single centre study added to limitations.

Conclusion: It was concluded that Omega 3 fatty acids played a protective role while sleep deprivation had a negative impact on the weight and volume of the prostate gland.

Author's contribution: NM&KA: Overall supervision and Write up and literature review. **US&AS:** Statistics application, analysis literature review, help in write up. **NA&MA:** Literature review help in write-up.

Conflict of interest: None

Funding: None

REFERENCES

1. Carroll JE, Irwin MR, Merkin SS, Seeman TE. Sleep and multisystem biological risk: a population-based study. PloS one. 2015;10(2):e0118467.

2. Choi JH, Lee SH, Bae JH, Shim JS, Park HS, Kim YS, et al. Effect of sleep deprivation on the male reproductive system in rats. Journal of Korean medical science. 2016;31(10):1624-30.
3. Patel R, Rinker L, Peng J, Chilian WM. Reactive oxygen species: The good and the bad. Reactive Oxygen Species (ROS) in Living Cells. 2018;7(8).
4. Doluoğlu ÖG, Öztekin ÇV, Karabakan M, Akdemir AÖ, Çetinkaya M. The importance of prostate volume in prostate needle biopsy. Turkish journal of urology. 2013;39(2):74.
5. Uzzo RG, Wei JT, Waldbaum RS, Perlmutter AP, Byrne JC, Vaughan Jr D. The influence of prostate size on cancer detection. Urology. 1995;46(6):831-6.
6. Hessler J, Lehner N. Planning and Designing Research Animal Facilities: Elsevier Science; 2011.
7. Van Hulzen Z, Coenen A. Paradoxical sleep deprivation and locomotor activity in rats. Physiology & behavior. 1981;27(4):741-4.
8. Mahmood N, Butt SA, Hamid S. Effects of sleep deprivation on the epithelial height of the prostatic acini in rats and the protective effects of omega 3 fatty acids. Pakistan Armed Forces Medical Journal. 2017;67(1):93-7.
9. Beeton C, Garcia A, Chandy KG. Drawing blood from rats through the saphenous vein and by cardiac puncture. Journal of visualized experiments. 2007;226(7).
10. Takehara H, Makita M, Tanaka R, Tsuchiya M, Naya M, Hayashi M. Lung toxicity assessment using bronchoalveolar lavage fluid and pleural lavage fluid cytology by intratracheal treatment in rats. The Journal of toxicological sciences. 2014;39(1):141-5.
11. Lovegrove C, Ahmed K, Challacombe B, Khan M, Popert R, Dasgupta P. Systematic review of prostate cancer risk and association with consumption of fish and fish-oils: analysis of 495,321 participants. International journal of clinical practice. 2015;69(1):87-105.
12. Everson CA, Szabo A. Repeated exposure to severely limited sleep results in distinctive and persistent physiological imbalances in rats. PLoS One. 2011;6(8):e22987.
Alvarenga TA, Hirotsu C, Mazaro-Costa R, Tufik S, Andersen ML. Impairment of male reproductive function after sleep deprivation. Fertility and sterility. 2015;103(5):1355-62. e1
13. Oyibo EU, Umar MA, Abdullahi K, Muhammad SA, Agwu PN. Clinicopathological Correlates of Patients with Prostate Cancer in a Tertiary Hospital in Northwestern Nigeria: Patients with Prostate Cancer. Orient Journal of Medicine. 2023;35(1-2):1-0.
14. Maison PO, Arthur D, Asante-Asamani A, Akakpo PK. An Analysis of Prostate Biopsy Results at the Cape Coast Teaching Hospital, Ghana. International Journal of Medical Science and Clinical Invention. 2022 Oct 23;9(10):6281-5.
15. Yunusa B, Abdullahi M, Mashi SA, Aji SA, Alhassan SU. Determination of the sensitivity and specificity of serum prostate-specific antigen in the diagnosis of prostrate cancer in Kano, Northwestern Nigeria. Nigerian Journal of Basic and Clinical Sciences. 2017 Jul 1;14(2):88.