# Journal of Vocational Health Studies

# EFFECT OF DIFFERENT TYPES OF SLEEP DEPRIVATION AND SLEEP RECOVERY ON SALIVARY PH

*EFEK BERBAGAI JENIS SLEEP DEPRIVATION DAN SLEEP RECOVERY TERHADAP PH SALIVA*

Fani Tuti Handayani[1]*, Pratiwi Nur Widyaningsih[1], Fitranto Arjadi[2]

[1] Department of Dental Medicine, Faculty of Medicine, Universitas Jenderal Soedirman, Puwokerto-Indonesia
[2] Department of Basic Medical Sciences, Faculty of Medicine, Universitas Jenderal Soedirman, Puwokerto-Indonesia

## A B S T R A C T

*Background: Salivary pH can rise or fall influenced by intrinsic and extrinsic factors. Sleep deprivation is one example of intrinsic factors. Sleep deprivation causes a reduction in sleep time at a certain time. Purpose: Analyze the effect of different types of sleep deprivations and sleep recovery on salivary pH. Method: This study was experimental research with a post-test only with a control group design. Thirty white wistar strain rats were randomly divided into 5 groups: healthy control group (KI), partial sleep deprivation (PSD/KII), total sleep deprivation (TSD/KIII), partial sleep deprivation, and continued sleep recovery (PSD+SR/KIV) and total sleep deprivation and continued sleep recovery (TSD+SR/KV). The treatment is carried out on a single platform method. Salivary pH was measured with the help of color-coded pH strips that were given grading after the completion of sleep deprivation induction. Result: The mean decrease in salivary pH was highest in the TSD group. One Way ANOVA test showed significant differences (p<0.05) in the control group with PSD and TSD, the PSD group with PSD+SR, TSD group with PSD+SR and TSD+SR. Conclusion: Sleep deprivation is proven to reduce the pH of Saliva. Total sleep deprivation is a chronic condition that has the most influence on decreasing salivary pH. The effect of decreasing salivary pH due to sleep deprivation is proven to be overcome by sleep recovery.*

## A B S T R A K

**Latar belakang:** Nilai pH saliva dapat meningkat atau menurun akibat pengaruh faktor intrinsik dan ekstrinsik. *Sleep deprivation* merupakan salah satu faktor intrinsik. *Sleep deprivation* menyebabkan berkurangnya waktu tidur pada periode tertentu. **Tujuan:** Untuk menganalisis efek dari berbagai jenis *sleep deprivation* dan *sleep recovery* terhadap pH saliva. **Metode:** Penelitian ekperimental dengan desain *post test only*. Penelitian ini menggunakan hewan coba sejumlah 30 tikus wistar putih yang terbagi secara *random* pada 5 kelompok: kelompok kontrol sehat (KI), *partial sleep deprivation* (PSD/KII), total *sleep deprivation* (TSD/KIII), *partial sleep deprivation* dilanjutkan *sleep recovery* (PSD+SR/KIV) dan total *sleep deprivation* dilanjutkan *sleep recovery* (TSD+SR/KV). Perlakuan diberikan menggunakan metode *single platform*. Pengukuran nilai pH saliva dilakukan dengan menggunakan panduan warna strip pH. **Hasil:** Penurunan pH saliva dilihat dari nilai rerata ditemukan paling tinggi pada kelompok TSD. Uji *One Way* ANOVA menunjukkan perbedaan signifikan (p <0.05) antara kelompok kontrol dengan PSD maupun TSD, antara kelompok PSD dengan PSD+SR, antara kelompok TSD dengan PSD+SR maupun TSD+SR. **Kesimpulan:** Penelitian ini membuktikan bahwa *sleep deprivation* menurunkan pH saliva. Keadaan kronis atau TSD merupakan keadaan yang menunjukkan penurunan pH saliva paling besar. Efek penurunan pH saliva akibat *sleep deprivation* pada penelitian ini terbukti dapat diatasi dengan *sleep recovery*.

## INTRODUCTION

Normal human sleep patterns include the quantity and quality of sleep. Sleep loss for a certain period of time is also called sleep deprivation (Krishnan and Dennis,2011). A work or lifestyle demand with staying up late can lead to sleep deprivation. The autonomic nervous system and hypothalamic pituitary adrenal (HPA) axis become active when someone experiences sleep deprivation (Hurtado-Alvarado et al.,2013). Activation of the HPA axis will cause an increase in the secretion of corticotropic releasing factor (CRF) by the hypothalamus which can cause the release of adrenocorticotropic hormone (ACTH). ACTH will then activate the biosynthesis process and release glucocorticoids especially cortisol from the adrenal cortex (Krishnan and Dennis,2011). The impact of sleep deprivation can be improved by sleep recovery by returning the HPA axis interaction to normal so that it results in a decrease in corticotropin-releasing hormone (CRH) activity (Machado et al.,2013). The appearance and reduction of the hormone cortisol is very related to the stress experienced by the body and allows for an effect on salivary pH (Jung et al.,2014).

Keremi et al. (2017) states that protein in saliva is closely related to sympathetic nerve control so that changes in saliva can be used as indicators of stress states. Regulation of salivary pH is regulated by the sympathetic and parasympathetic nervous system. Cohen and Khalaila's research (2014) proves that stress can trigger changes in salivary pH by increasing the work of the sympathetic nerves causing salivary secretion to become thick and decrease thereby affecting the degree of acidity and salivary buffer capacities (Cohen and Khalaila,2014). Stress is a biological response when the body receives an intrinsic or extrinsic stimulus. The influence of stress on the body can vary greatly, in the form of deviations from the body's homeostasis to life-threatening conditions (Han et al.,2012).

Saliva has a very important role to maintain oral health. Salivary composition is a collection of secretions from the salivary glands, gingival sulcus fluid, desquamated epithelial cells, microorganisms and leukocytes. Salivary secretions vary between 800-1500 ml per day or about 1 to 3 ml per minute with a pH range of 6-7 in unstimulated saliva (Pedersen et al.,2018; Dawes and Wong,2019). Since 1930-1940, experts have studied the factors that cause changes in saliva. Salivary abnormalities can occur due to changes in composition, pH, concentration and flow of saliva (Ruhl,2012; Tothova et al.,2015). Changes in salivary pH are widely used as indicators of oral health status or as a marker diagnosis because changes can be detected quickly, accurately and the examination is relatively easy and non-invasive (Manikandan et al.,2018; Baliga et al.,2013). The pattern of sleep deprivation and sleep recovery on salivary pH has not been studied, therefore researchers are interested in studying the effect of sleep deprivation and sleep recovery on salivary pH.

## MATERIAL AND METHOD

This study is a true experimental with a post test only with control group design of white rats (Rattus norvegicus) male Wistar strains aged 3-4 months with a body weight of 200-300 grams given induction of several types of sleep deprivation, namely induction with partial sleep deprivation (PSD) model, total sleep deprivation (TSD), partial sleep deprivation with sleep recovery (PSD+SR), and total sleep deprivation with sleep recovery (TSD+SR). The treatment is carried out on a single platform method (SPM) using tank measuring 23x23x35 cm with 1 mouse in 1 flowerpot equipped with atonia muscle that automatically provides a shock effect every 10 minutes in experimental animals (Jung et al.,2014).

Samples of this research were obtained from the Department of Pharmacology and Therapy, Faculty of Medicine, Gadjah Mada University. All experiments conducted in this study were approved by the Ethics Commission of the Jenderal Soedirman University No. Ref. 4704 / KEPK / X / 2018. Experimental animals were 30, divided into 5 groups which were previously randomly divided into the healthy control group (KI), PSD group (KII), TSD group (KIII), PSD+SR group (KIV), and TSD+SR group (KV). Each group consists of 6 white rats, determined according to the minimum sample calculation according to (Federer,1967).

All experimental animal samples were acclimatized for 7 days before sleep deprivation was induced. After acclimatization, groups II and IV were given PSD treatment for 120 hours, and groups III and V were given TSD treatment for 120 hours, while group I was not given treatment. Groups I, II, III then returned to their original state while groups IV and V continued with sleep recovery for 120 hours and then returned to their original health.

This research was conducted in the Research Laboratory, Pharmacology Laboratory, and Experimental Animal Laboratory Faculty of Medicine Jenderal Soedirman University on November to December 2018. Salivary pH was measured with the help of colour coded pH strips or litmus paper that was given grading after the completion of sleep deprivation induction. Salivary pH was analyzed with the pH guide with colour indicators. Salivary pH uptake was adjusted to the rat circadian rhythm, which was 7:00 to 10:00 am.

Calculation of the mean pH was carried out in all five groups. Normality and homogeneity tests were performed for each group, then a statistical analysis was carried out in the form of One Way ANOVA parametric

test (p<0.05) and post hoc LSD test to assess significant differences between groups (p<0.05).

## RESULT

The mean salivary pH in each group is shown in Table 1. The group of experimental animals with the highest pH was the control group with the mean pH=10, while the lowest pH was found in the TSD group, which was 8.8.

Normality test is done by Saphiro Wilk test and homogeneity test is done by Levene test. The test results showed that the data were normally distributed and homogeneous, then the statistical analysis continued using the One Way ANOVA parametric test and obtained significant results (p<0.05). These results indicate there is an influence of sleep deprivation treatment on salivary pH. Post hoc LSD test was performed to determine the existence of significant differences between each treatment in the sample group.

Table 2 shows that there are two groups with significant results (p<0.05) in the control group, namely the PSD and TSD groups. Other intergroup results that showed a significant value (p<0.05) were the PSD group with PSD+SR, and the TSD group with PSD+SR and TSD+SR.

**Table 1.** Mean and standard deviation of pH

| Group | Average | Standard Deviation |
|---|---|---|
| Control | 10.00 | 0.00 |
| PSD | 9.20 | 0.45 |
| TSD | 8.80 | 0.45 |
| PSD+SR | 9.80 | 0.45 |
| TSD+SR | 9.80 | 0.45 |

**Table 2.** Post hoc test mean salivary pH of sleep deprivation treatment in each group

| Group | Value p value | | | | |
|---|---|---|---|---|---|
| | Control | PSD | TSD | PSD+SR | TSD+SR |
| Control | | 0.005 * | 0.001 * | 0.806 | 0.907 |
| PSD | | | 0.394 | 0.008 * | 0.168 |
| TSD | | | | 0.001 * | 0.032 * |
| PSD+SR | | | | | 0.151 |
| TSD+SR | | | | | |

Note: * shows a significant difference (p<0.05)

## DISCUSSION

This study shows that sleep deprivation causes a decrease in salivary pH. Sleep deprivation can occur both acute and chronic (Krishnan et al.,2016). Acute sleep deprivation is a long period of wakefulness, whereas chronic sleep deprivation is an accumulation of sleep deprivation that occurs for several days. Partial sleep deprivation (PSD) is acute, whereas total sleep deprivation (TSD) is a chronic condition (Landolt et al.,2014). Based on the results of the study, the PSD and TSD group experienced a significant decrease in salivary pH compared to the control group (p <0.05) and the TSD state caused a significant decrease the biggest. The greater effect shown on the TSD state indicates the impact of chronic sleep deprivation disorders that is greater than the acute state.

Sleep deprivation is a condition that is difficult for the body to adapt, so that the impact will be even greater in an increasingly chronic condition (Krishnan et al.,2016). Chronic sleep deprivation can cause a decrease in sensitivity to glucocorticoids which serves as the main stress measurement index, which will cause interference with the HPA axis (Jung et al.,2014). An acute state of sleep deprivation will show that glucocorticoid levels continue to increase because of exposure to stress that can not be predicted, while chronic conditions indicate the body's response that has been able to control the emergence of stress and glucocorticoid response will disappear and then cause permanent corticosterone levels (Hurtado-Alvarado et al.,2013). Cortisol levels accumulation will continue to activate sympathetic nerves and causes a decrease in salivary pH. Therefore, salivary pH can serve as a biomarker of stress. Same study results were obtained by (Cohen and Khalaila,2014) where they conclude that pH levels may serve as a reliable, accessible and inexpensive means by which to assess the degree of physiological reactions to exams and other naturalistic stressors. Sleep deprivation is one of the naturalistic stressors.

This study considers that maintaining normal salivary pH can be done by avoiding sleep deprivation. Maintaining the normal salivary pH is necessary to avoid the negative effects on oral cavity. Saliva secretion derived from major and minor salivary glands. Important components of saliva include electrolytes such as bicarbonate, calcium, fluoride and phosphate; enzymes such as α-amylase, invertase and mucins; immunoglobulins (Igs) including IgA, IgG and IgM; lipids including neutral lipids, glycolipids and phospholipids; non-Igs such as histidine-rich proteins, lactoferrin and lysozyme; and proteins such as peroxidase, proline-rich proteins, agglutinins and statherin. Alkaline phosphatase (ALP) is an intracellular enzyme present in the saliva. Salivary pH is a key factor in enzyme functionality. Various studies have shown that altered salivary pH, and ALP levels are associated with the formation or development of dental caries, gingivitis and periodontitis. Changes in salivary pH can also result in pathological changes to the teeth and oral cavity (Pedersen et al.,2018; Pachori et al.,2018; Bhat et al.,2016). The salivary buffer capacity has the ability to keep the pH within a neutral range (Pedersen et al., 2018; Pedersen and Belstrom,2019).

The state of acute sleep deprivation was demonstrated in this study through the PSD group. Differences in mean salivary pH in the PSD group with the control group and the PSD+SR group showed a significant difference, while the other groups showed no significant differences. This shows that the administration of sleep recovery after acute sleep deprivation can restore salivary pH under normal conditions even though it is not in a maximal state. Providing acute sleep recovery after sleep deprivation will reduce the secretion of the CRH hormone so that it will reduce the activation of the HPA axis. This causes sleep to become normal, thus causing an increase in parasympathetic nerve impulses which can increase salivary pH (Rasch and Born,2013; Yaribeygi et al.,2017).

Toker and Melamed (2017) stated that sufficient recovery may prevent some negative consequences caused by stress. This research proves that sleep recovery can reduce the effects of sleep deprivation. Significant differences were found between the TSD group with PSD+SR and TSD+SR. This indicates that sleep recovery has a role in controlling changes in salivary pH. Some research state that sleep can function as an antistress through the mechanism of HPA axis inhibition (Faraut et al.,2011). The state of sleep deprivation will make the body respond defense by influencing the adrenal medulla in the sympathetic nervous system to secrete the hormones epineprin and nor epinephrine, thereby stimulating the spinal cord to release impulses. sympathetic nerves and produces lower salivary volume. This will affect the rate of salivary flow and cause a decrease in bicarbonate ions which results in a decrease in salivary pH (Vinayak et al.,2013). (Mokoginta et al.,2017) states that one of the functions of bicarbonate ions is the main buffer in maintaining salivary pH stability.

Changes in mean salivary pH in the PSD and TSD groups with sleep recovery showed insignificant results in the control group. This shows that changes in salivary pH that occur after experiencing sleep recovery close to normal conditions. (Loke et al.,2016) states that salivary pH can rise or fall influenced by intrinsic and extrinsic factors. Sleep deprivation is one example of intrinsic factors (Nollet et al.,2020). Each individual has different variations in salivary composition and buffer ability, this will cause differences in the time of salivary pH adjustment and response to stimulus. Stimulus of the parasympathetic nerve will cause an increase in salivary secretion, while the stimulus to the sympathetic nerve will result in consistency of saliva becoming thicker and having less volume, causing the mouth to feel drier and metabolism to take place more quickly. The accumulation of residual metabolic products will cause a decrease in salivary pH level (Loke et al.,2016).

## CONCLUSION

Sleep deprivation has an influence on salivary pH. Sleep deprivation is proven to reduce the pH of Saliva. Total sleep deprivation (TSD) is a chronic condition that has the most influence on decreasing salivary pH. The effect of decreasing salivary pH due to sleep deprivation is proven to be overcome by sleep recovery.

## ACKNOWLEDGEMENTS

## REFERENCES

Baliga, S., Muglikar, S., Kale, R., 2013. Salivary pH: A Diagnostic Biomarker. J. Indian Acad. Periodontol. 17, 461–465.

Bhat, S.S., Hegde, S.K., Bhat, V.S., Ramya, K., Jodalli, P.S., 2016. Acidogenic Potential of Plain Milk, Milk with Sugar, Milk with Cornflakes and Milk Cornflakes with Sugar: A Comparative Study. Int. J. Clin. Pediatr. Dent. 9, 218–221.

Cohen, M., Khalaila, R., 2014. Saliva pH as A Biomarker of Exam Stress and A Predictor of Exam Performance. J. Psychosom. Res. 77, 420–425.

Dawes, C., Wong, D.T.W., 2019. Role of Saliva and Salivary Diagnostics in The Advancement of Oral Health. J. Dent. Res. 98, 133–141.

Faraut, B., Boudjeltia, K.Z., Rousseau, A., David, E., Stenuit, P., Franck, T., Antwerpen, P. Van, Vanhaeverbeek, M., Kerkhofs, M., 2011. Benefits of Napping and An Extended Duration of Recovery Sleep on Alertness and Immune Cells after Acute Sleep Restriction. Brain, Behav. Immun. 25, 16–24.

Federer, W.T., 1967. Experimental Design Theory and Application, Journal of the Royal Statistical Society. Calcutta Oxford & IBH.

Han, K.S., Kim, L., Shim, I., 2012. Stress and Sleep Disorder. Exp. Neurobiol. 21, 141–150.

Hurtado-Alvarado, G., Pavon, L., Castillo-Garcia, S.A., Hernandez, M.E., Dominguez-Salazar, E., Velazquez-Moctezuma, J., Gomez-Gonzalez, B., 2013. Sleep Loss as A Factor to Induce Cellular and Molecular Inflammatory Variations. Clin. Exp. Immunomodulation 266–271.

Jung, C., Greco, S., Nguyen, H.H., Ho, J.T., Lewis, J.G., Torpy, D.J., Inder, W.J., 2014. Plasma, Salivary and Urinary Cortisol Levels Following Physiological and Stress Doses of Hydrocortisone in Normal Volunteers. BMC Endocr. Disord. 14, 1–10.

Keremi, B., Beck, A., Fabian, T.K., Fabian, G., Szabo, G., Nagy, A., Varga, G., 2017. Stress and Salivary Glands. Curr. Pharm. Des. 23.

Krishnan, C.H., Noakes, E.J., Lyons, L.C., 2016. Chronic Sleep Deprivation Differentially Affects Short and Long-term Operant Memory in Aplysia. Neurobiol. Learn. Mem. 134, 349–359.

Krishnan, V., Dennis, A., 2011. Sleep Deprivation: Pathophysiology, Sleep & Sa. ed. Bentham e-Books, USA.

Landolt, H., Holst, S.C., Sousek, A., 2014. Effect of Acute and Chronic Sleep Deprivation. In: Bassetti, C., Dogaz, Z., Peigneux, P. (Eds.), Sleep Medicine Textbook. European Sleep Research Society, Regensburg, pp. 49–61.

Loke, C., Lee, J., Sander, S., Mei, L., Farella, M., 2016. Factors Affecting Intra-oral pH - A Review. J. Oral Rehabil. 43, 778–785.

Machado, B.R., Tufik, S., Suchecki, D., 2013. Role of Corticosterone on Sleep Homeostasis Induced by REM Sleep Deprivation in Rats. PLoS One 8, 1–10.

Manikandan, S., Ayyappan, Ilanchezhian, T., Saimahitha, V., 2018. Salivary pH as A Diagnostic Marker of Oral Helath Status. Int. J. Pharm. Sci. 51, 41–44.

Mokoginta, Z.P., Wowor, V.N.S., Juliatri, 2017. Effect of Gargling of Young Coconut Water on Saliva pH of Elementary School Children in Batua Village, Makasar City. Pharmacon 6, 24–30.

Nollet, M., Wisden, W., Franks, N.P., 2020. Sleep Deprivation and Stress: A Reciprocal Relationship. Interface Focus 10, 1–11.

Pachori, A., Kambalimath, H., Maran, S., Niranjan, B., 2018. Evaluation of Changes in Salivary pH after Intake of Different Eatables and Beverages in Children at Different Time Intervals. Int. J. Clin. Pediatr. Dent. 11, 177–182.

Pedersen, A.M.L., Belstrom, D., 2019. The Role of Natural Salivary Defences in Maintaining a Healthy Oral Microbiota. J. Dent. 80, S3–S12.

Pedersen, C.C.E., Hardy, M., Blankholm, A.D., 2018. An Evaluation of Image Acquisition Techniques, Radiographic Practice and Technical Quality in Neonatal Chest Radiography. Med. Imaging Radiat. Sci. 49, 257–264.

Rasch, B., Born, J., 2013. About Sleep;s Role in Memory. Physiol. Rev. 93, 681–766.

Ruhl, S., 2012. The Scientific Exploration of Saliva in The Post-proteomic Era: from Database Back to Basic Function. Expert Rev. Proteomics 9, 85–96.

Toker, S., Melamed, S., 2017. Stress, Recovery, Sleep and Burnout. In: Cooper, C.L., Quick, J.C. (Eds.), The Handbook of Stress and Health: A Guide to Research and Practice. John Wiley and Sons Ltd, pp. 168–185.

Tothova, L., Kamodyova, N., Cervenka, T., Celec, P., 2015. Salivary Markers of Oxidative Stress in Oral Diseases. Front. Cell. Infect. Mirobiology 5, 1–23.

Vinayak, V., Annigeri, R.G., Mittal, S., Patel, H.A., 2013. Adverse Affects of Drugs on Saliva and Salivary Glands. J. Orofac. Sci. 5, 15–20.

Yaribeygi, H., Panahi, Y., Sahraei, H., Johnston, T.P., Sahebkar, A., 2017. The Impact of Stress on Body Function: A Review. EXCLI J. 21, 1057–1072.