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Sleep Deprivation (The Effects of Sleep Deprivation on Salivary Immunoglobulin A

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SENIOR PROJECT - APPROVAL

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I have reviewed this completed senior honors thesis with this student and certify that it is a project commensurate with honors level undergraduate research in this field.

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Running Head: Sleep Deprivation

The Effects of Sleep Deprivation on Salivary Immunoglobulin A

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Abstract

Salivary Immunoglobulin A (sIgA) is one of the many antibodies that form a front line of defense in mucosal immunity. However, previous studies have shown a decrease in the level of sIgA when subjects are exposed to stress. Sleep deprivation, a commonly known stressor, has also been shown by previous research to affect immune functioning. Subjects were selected randomly from a senior seminar. The sample size was 11 (N=11) and consisted of 6 males and 5 females. This study attempted to establish a correlation between subjects having both a restful and restless night's sleep in a 3 week time period and their corresponding levels of sIgA to both of those nights. In addition, subjects completed health and sleep surveys in an attempt to establish a correlation between sIgA levels and the results of the surveys. Saliva samples were collected and assayed using the ELISA procedure. The results held marginal significance and the hypotheses remain unconfirmed.

The Effects of Sleep Deprivation on Salivary Immunoglobulin A

Mucosal immunity is the first line of defense that the body has against pathogens. On average, an adult has 400 square meters of epithelium covering the mucosae, or mucus membranes. Mucosae are favored portals of disease by many allergens and carcinogens. Salivary IgA is one of the many antibodies that form this front line of defense and is considered to be one of the "best-defined" components (Brandtzaeg 1995). Salivary IgA particularly resists invading microorganisms prone to strike the intestines and is therefore known as a surface protector to the gut (Brandtzaeg 1995).

In several studies, stress has been shown to have a significant relationship with various components of the immune system. For example, in one study the blood of 42 college students was analyzed in a period immediately before they were to take an exam and then once again, four weeks later. It was found that interleukin-1 beta (IL-1 beta), IL-6, and IL-10 levels were increased and there was a decrease in the levels of IFN-gammaproduction. Results suggest that examination stress increased Th2 cell-mediated humoral immunity, while decreasing TH1 cell-mediated cellular immunity (Paik, Toh, Lee, Kim, and Lee 2000). In another study, 1,550 men were studied, 12 of which had a past history of posttraumatic stress disorder (PTSD). The men were matched together according to similar stressful life events. Natural killer (NK) cell activity, lymphocyte subset counts, and the production of interferon gamma (IFN-gamma) and interleukin-4 (IL-4) were found to be significantly lower in the twelve men with the PTSD history. These results suggest that PTSD, a form of psychological distress, leads to a lasting immunosuppression (Kawamura, Kim, and Asukai 2000). A third study tested the hypothesis

that stress can inhibit immunoglobulin G (IgG) antibody response to a pneumococcal bacterial vaccine. Previous studies had already shown that caregivers of patients with dementia have poorer antibody and virus specific T cell responses to an influenza vaccine when related to controls. Blood was measured from a group of 52 subjects (11 current caregivers, 13 formal caregivers, and 28 control subjects). Results indicated that the caregivers were in deficit compared to those of the control. That is, IgG was lower after the vaccination in dementia caregivers about 3-6 months after vaccinations were given. These results suggest that chronic stress (in this case induced by caring for patients with dementia) can inhibit the stability of the IgG antibody, an immunity component, with regards to the administration of a vaccine for pneumonia (Glaser, Sheridan, Marlarkey, MacCallum, and Kiecolt-Glaser 2000). A fourth study examined the effects of examination stress on mucosal wound healing. In this study, two "punch biopsy" wounds were placed on the roof of 11 subject's (dental students) mouths. The first wound was placed in the mouth during summer vacation, while the second wound was placed just 3 days before their first major exam of the term. As expected, students took on average 3 days longer for the 2nd wound to heal. Production of interleukin-1 betamessenger RNA declined by 68% during the examination period, suggesting once again that psychological distress can have significant effects on immune functioning, and more specifically in this study, wound healing (Marucha, Kiecolt-Glaser, and Favegehi 1998).

As stated above, a number of studies have established a relationship between reduction in cellular immune functioning and individuals whom are undergoing psychological distress (Cover

and Irwin, 1994). For example, sleep deprivation (a known stressor) has been shown to affect the functioning of several different organs, including those involved in immunity (Palmblad, Petrini, Wasserman, and Akerstedt, 1979). It has been found that DNA synthesis of blood lymphocytes was reduced after 48 hours of sleep deprivation. However, 5 days afterwards at the post-experimental checkup, levels had returned to the same pre-exposure levels. Granulocyte adherence and stainable activity was unaltered, although, there was a depression of granulocyte phagocytosis (Palmblad, Petrini, Wasserman, and Akerstedt, 1979). In another study, When blood samples after partial sleep deprivation (from 3 a.m. to 7 a.m.) were analyzed, it was found that reduction in NK cell activity occurred. After the recovery sleep, however, activity was found to be comparable to that of baseline levels. This data suggested that partial sleep deprivation did produce a significant reduction in NK cell activity, and therefore immunity in general. (Irwin, Mascovich, Gillin, Willoughby, Pike, and Smith, 1994). Another study also shows that insomnia as a side effect of depression reduces NK activity. For instance, it was found that two specific depressive symptoms: retardation and sleep disturbance were negatively correlated with NK cell activity. No association was found between NK cell activity and anxiety/somatization, diurnal variation, weight loss, or cognitive disturbance (Cover, and Irwin, 1994).

Because sIgA is an antibody that has been found to be affected by stressful events, this study attempted to establish a positive correlation between sleep deprivation and a decrease in sIgA levels. It also attempted to establish a relationship between the scores from the Pittsburgh Sleep Quality Index and the Cohen-Hoberman Inventory of Physical Symptoms.

Method

Participants

Eleven students were randomly selected from a senior level seminar class. (N = 11) All subjects were unmarried seniors and the subject pool consisted of 6 males and 5 females. All subjects were also Euro-American.

Materials

Subjects were asked to fill out a health survey (Cohen & Hoberman, 1983), sleep survey (Buysse, Reynolds, Monk, Berman, and Kupfer 1989) and a demographic survey. The Cohen-Hoberman Inventory of Physical Symptoms listed 33 experiences, which most people have had at one time, or another. Participants indicated how much each experience had been a part of their life over the past month. The choices were based on a 4 point Likert scale. (Cohen and Hoberman, 1983).

The Pittsburgh Sleep Quality Index assessed more information concerning the subjects' level of sleep deprivation. Choices were once again based on a 4 point Likert scale and the top score was 21, with anything over "5" indicating sleep deprivation. (Buysse, et al., 1989).

A basic demographics survey was also administered to subjects.

Saliva sampling. Subjects provided samples of their saliva after a restful night's sleep and a restless night's sleep using the unstimulated method. (Aufricht, Tenner, Salzer, Khoss, Wurst,

and Herkner 1992). A restful night's sleep was one in which the subject reported feeling alert and energetic upon awaking. A restless night's sleep was one in which the subject reported feeling "groggy" or lethargic upon awaking. For the collection method, subjects rinsed their mouths with water and swallowed all water until their mouths felt dry. While relaxing in the sitting position, subjects tilted their heads slightly to the side allowing their saliva to flow spontaneously into the tube. Subjects then expectorated every minute for three minutes. Samples were collected by subjects during the first four hours after they had awakened, before they ate or smoked. They were stored in the freezer until the date of analysis. The Salimetrics kit, a commercial assay was used to analyze salivary IgA.

Procedure

After saliva samples were collected, they were analyzed using the ELISA method. Five microcentrifuge test tubes were labeled 2-6. The standard was then serially diluted by adding 15 μL of sIgA diluent from tube 2 to tube 3 and onward to tube 6. The sIgA diluent was a 5X phosphate buffered solution containing a non-mercury preservative. Fifty mL was added to 200 mL of deionized water to create diluent. The standard at 600 μL /mL was located in tube 1. Thus the final concentrations for tubes 1 through 6 respectively were 600 μg/mL, 200 μg/mL, 66.7 μg/mL, 22.2 μg/mL, 7.4 μg/mL, and 2.5 μg/mL. Two small tubes were then labeled for each saliva sample totaling 22 tubes. 100 μL of the sIgA diluent was then added to each tube. Twenty-five μL of saliva was pipetted into each tube. Next, tubes were labeled for the control, sample

and the zero value. 4 mL of the sIgA diluent was then added to each tube. 25 μL of the antibody-enzyme conjugate (goat anti-human sIgA antibody conjugated to horseradish peroxidase) was added to 3 mL of the sIgA diluent. Fifty μL of the diluted conjugate was added to all tubes using a repeater pipette. Each tube was then vortexed and allowed to incubate for 90 minutes. Afterwards, each tube was vortexed again and 50 μL of the above solution was added to each microtitre plate according to a plate templated created beforehand. Fifty μL of sIgA diluent was then added to the NSB wells. The plate was then covered with an adhesive plate sealer and allowed to incubate at room temperature for 90 minutes with continual mixing at 500 rpm. Afterwards, the plate was washed 6X with a wash buffer (A 10X phosphate buffered solution containing detergents and a non-mercury preservative that had been diluted 10X with deionized water). 50 μL of TMB solution (tetramethylbenzidine) was added to each well. The plate was then mixed on a rotator for 5 minutes and incubated in the dark at room temperature for an additional 40 minutes. 50 μL of stop solution was then added to each well and the plate was then placed on the rotator for an additional 3 minutes at 500 rpm. The samples were read using a plate reader spectrophotometer which measures optical density (OD). The wavelength was set at 450 nm. The spectrophotometer was connected to a computer and using the SoftMax Pro Windows application, OD measurements were converted into measurements of μg/mL. (Concentrations of IgA were measured in OD.)

Results

Data was analyzed using SPSS program for Windows. Optical density (OD) of sIgA was then calculated for the restful night's sleep and the restless night's sleep, with a higher OD indicating less sIgA. It was hypothesized that levels of salivary IgA would decrease after a restless night's sleep, compared to that of a restful night's sleep. However, a mean of 397.46 μg/mL sIgA was found after a restful night's sleep and a mean total of 400. 61 μg/mL of sIgA was found after a restless night's sleep. These results held a 0.079 significance indicating only a marginally significant difference between a restful and restless night's sleep and sIgA levels. It was also hypothesized that salivary IgA levels would be correlated with the sleep and health surveys. Using a Pearson correlation analysis, there was no significant relationship between the sleep survey and IgA levels. Likewise, there was no significant relationship between IgA levels and the health index. Six was the mean score of the Pittsburgh Sleep Quality surveys, indicating slight sleep deprivation of subjects overall. 46 was the mean score of the Cohen-Hoberman out of a possible score of 132. Using a standard t-test relationships between the two levels of sIgA and the sleep and health surveys were analyzed. There appeared to be no correlation between among them.

Table 1. Descriptive Statistics

Variables	**Frequency**	**Percent**
Gender		
Males	6	54.5
Females	5	45.5
Smokers	0	0.0
Exercise		
Daily	2	18.2
Weekly	6	54.5
Never	3	27.3
Classification	11	100.00

Table 2. Descriptive Statistics for Dependent Measures

Variables	**Mean**	**Standard Deviation**
G-IgA	397.46	58.71
B-IgA	400.61	94.11
Sleep Inventory	6.00	3.06
Health Inventory	46.00	9.09

(G-IgA = restful sleep, B-IgA = restless sleep)

Hypothesis 1: The restless night's sleep would reduce levels of salivary IgA compared with the restful night's sleep IgA levels.

Test: This hypothesis was tested using a paired t-test.

Resutls: $t(10) = -.133$, $p > .05$ ($p = .079$) These results were not significant.

Hypothesis 2: There will be a significant relationship between levels of salivary IgA and scores on the sleep survey.

Test: Pearson Correlation

Hypothesis 3: There will be a significant relationship between levels of salivary IgA and scores on the health inventory

Test: Pearson Correlation

Table 3. Pearson Correlation

Variables	Restful sleep (Optical Density)	Restless sleep (Optical Density)	Sleep inventory	Health inventory
Restful sleep (Optical Density)	-----	.551 .079	.524 .098	-.218 .520
Restless sleep (Optical Density)	-----	-----	.475 .140	.164 .630
Sleep Inventory	-----	-----	-----	.068 .842
Health Inventory	-----	-----	-----	-----

Conclusion

This study attempted to establish a correlation between three entities: 1) restful and restless night's sleep and levels of salivary IGA. 2) Scores on the Pittsburgh Sleep Quality Index and levels of salivary IGA. 3) Score on the Cohen-Hoberman Health Inventory and levels of salivary IGA. To date these hypotheses remain unconfirmed. These results are inconsistent with previous studies indicating a reduction in immunity due to sleep deprivation. However, these studies investigated systemic immunity, while this one investigated mucosal immunity. One confounding effect of this study lies within the small sample size (N). Another possible confounder could be the result of human error.

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