SRI VENKATESWARA INTERNSHIP PROGRAM FOR RESEARCH IN ACADEMICS (SRI-VIPRA)

SRI-VIPRA

Project Report of 2024: SVP-2451

"Salivary Amylase as biomarker for sleep debt"

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SRIVIPRA PROJECT 2024

SVP-2451
Title: Salivary Amylase as biomarker for sleep debt

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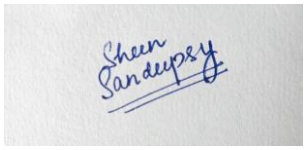
3		Sheen Sandeepsy	1222050	B.Sc. (Hons.) Biochemistry	

Signature of Mentor

Certificate of Originality

This is to certify that the aforementioned students from Sri Venkateswara College have participated in the summer project SVP-2451 titled “Salivary Amylase as biomarker for sleep debt". The participants have carried out the research project work under my guidance and supervision from 1st July, 2024 to 30th September 2024. The work carried out is original and carried out in an online/offline/hybrid mode.

Signature of Mentor

Acknowledgements

With immense pleasure and pride, we share the report of Sri-Vipra Project SVP-2451 titled- '*Salivary Amylase as biomarker for sleep debt'*. We would like to express our deepest gratitude for everyone who worked tirelessly for successful completion of this project. Our deepest gratitude to Sri Venkateswara College for hosting the Sri-Vipra program and encouraging young students like us to gain research experience. We would like to thank our esteemed Principal, Prof. Vajala Ravi for providing us the opportunity to undertake this internship. Our sincere gratitude to our mentor, Dr. Sarika Yadav for her support and guidance throughout the course of this project. This journey has been a valuable learning experience due to her unwavering support, guidance, and mentorship. Her expertise, encouragement, and willingness to share her expertise and knowledge has been instrumental in our growth as a researcher. We would also like to extend our appreciation to co-ordinators of the Sri-Vipra internship program.

Finally, we express our gratitude to the Almighty for countless blessings, knowledge, and opportunities

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Introduction

Insufficient sleep, commonly known as sleep deprivation, occurs when an individual fails to get enough sleep and rest, leading to a range of mental and physical impairments. Sleep deprivation can profoundly affect cognitive abilities, emotional well-being and physical wellness. People with sleep deprivation often struggle with focus, decision-making, memory retention and are more prone to mood swings, anxiety, and depression. At a physical level, it may weaken the immune system causing various conditions like obesity, diabetes, and heart problems increasing. The roots of sleep deprivation are diverse and often intertwined with modern lifestyle choices, irregular sleep patterns, excessive consumption of sleep-disrupting substances like caffeine and alcohol. Work-related factors, medical issues, environmental factors and family obligations can further worsen sleep deprivation. The indicators of sleep deprivation include persistent tiredness, headaches, muscle tension, difficulties with alertness, changes in mood. Cognitive processes may slow down, affecting reaction times and problem-solving abilities. Sleep-deprived may cause increased appetite, particularly for high-calorie foods. Adequate and high-quality sleep at appropriate times, is essential for maintaining mental and physical health and overall well-being.

The concept of sleep debt is closely related to sleep deprivation and is influenced by a multitude of factors. If a person regularly loses sleep or chooses to sleep less than needed, the sleep loss adds up. The total sleep lost is called your sleep debt. Addressing sleep debt requires a comprehensive approach to improving sleep habits like creating a consistent sleep schedule, optimizing the sleep environment, and adopting a good lifestyle. Effective stress management and the cultivation of relaxation techniques can also play a crucial role. By prioritizing sleep and taking steps to address factors that contribute to sleep debt, individuals can significantly enhance their overall well-being.

Importance of sleep and effects of sleep deprivation/sleep debt

Sleep is a fundamental biological process during which the body undergoes critical restorative functions, including tissue repair and muscle growth. Besides, sleep is also crucial for memory consolidation, learning, and cognitive performance. Additionally, sleep has a profound impact on emotional regulation and decision-making, with sleep deprivation leading to increased emotional reactivity and impaired judgment.

1. Cognitive Function and Learning

Sleep enhances the cognitive processes by transferring short-term memories into long-term storage, particularly during deep sleep and REM sleep. Slow-wave sleep (SWS) is particularly important for consolidating declarative memories, while REM sleep supports the consolidation of procedural memories, such as skills and habits. During sleep, the brain reactivates and reorganizes newly acquired information, transferring it from short-term to long-term memory stores. This process strengthens neural connections and helps integrate new knowledge with pre-existing information.

2. Emotional Regulation

Lack of sleep leads to heightened emotional reactivity, including anxiety and stress, due to the increased activity in brain regions like the amygdala. Functional MRI scans show a 60% increase in amygdala activity in response to emotionally negative stimuli compared to well-rested individuals. This heightened emotional

reactivity was associated with a weakened connection between the amygdala and the prefrontal cortex, the brain region responsible for regulating emotions and maintaining rational control.

3. Physical Health
Sleep deficiency has been linked to numerous chronic health issues, including obesity, heart disease, and weakened immune function. Sleep duration directly influences metabolic and cardiovascular health, hormonal imbalances and inflammation caused by inadequate sleep.

4. Mental Health
Sleep problems are associated with various mental health issues, such as depression, anxiety, and mood disorders. Chronic sleep disturbances may disrupt emotional regulation and cognitive function, contributing to mood disorders.

Multidimensional Impact of Sleep Debt:

Sleep debt impacts individuals on multiple levels, disrupting not only physical health but also psychological functioning and social dynamics.

Physiological consequences of sleep debt
Sleep loss in adults typically refers to getting less sleep than the recommended 7 to 8 hours per night. Chronic sleep deprivation is not classified as a syndrome or disorder, it can have significant negative impacts on health, performance, and safety.
The causes of sleep loss are can be grouped into two main categories:

- lifestyle and occupational factors (such as shift work, extended working hours, jet lag, and irregular sleep patterns) and
- sleep disorders (like insomnia, sleep-disordered breathing, restless legs syndrome (RLS), narcolepsy, and circadian rhythm disorders)

Sleep Loss Linked to Obesity
Numerous epidemiological studies have indicated that short sleep duration is linked to higher body mass index (BMI) leading to weight gain and obesity, such as increased food consumption, reduced energy expenditure, and alterations in the levels of appetite-regulating hormones like leptin and ghrelin.

Sleep debt Linked to Diabetes and Impaired Glucose Tolerance
There is a close relationship between sleep debt and impairments in glucose metabolism, which can elevate the risk of type 2 diabetes. Sleep restriction has a negative effect on insulin signalling in human adipocytes and decrease in cellular insulin sensitivity corresponded with a 16% reduction in overall body insulin sensitivity.

Sleep debt and Immune system
Sleep plays a crucial role in supporting the immune system, enhancing the body's resistance against infections and inflammation. Lack of sleep has been linked to disruptions in both innate and adaptive immune responses, contributing to chronic inflammation and raising the risk for various diseases, such as cardiovascular disorders, cancer, autoimmune conditions, and neurodegenerative diseases.

Cancer

Sleep deprivation has become a risk factor for diminished anti-tumor responses. Research indicates a significant association between short sleep duration and an increased risk of various cancers, including breast, colorectal, and prostate cancer.

Sleep Debt and Neurodegenerative Diseases

Neurodegenerative diseases (NDDs) are age-related disorders that selectively impact different neuron groups within the central nervous system (CNS). These include Alzheimer's disease, multiple sclerosis, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis.

Research shows that sleep deprivation and insomnia can result in the accumulation of β-amyloid (Aβ) peptides and tau proteins, which are the two primary hallmarks of Alzheimer's disease (AD)

Sleep deprivation increases the risk for hypertension

Many studies show that short sleep duration and single or combined symptoms of insomnia (excluding difficulty falling asleep) are linked to a higher risk of developing hypertension. It explains the link between short sleep duration and heightened cardiovascular risk observed in epidemiological studies.

Psychological Impact of Sleep Debt

Sleep debt is said to heighten emotional instability, leading to increased anxiety and confusion, along with sleepiness and impaired psychomotor function.

The amygdala is a brain region mainly linked to emotional processes. The findings of a study indicate that ongoing and cumulative sleep debt, commonly experienced in daily life, can lead to reduced functional suppression of the amygdala by the ventral anterior cingulate cortex (vACC).

Sleep debt is also associated with significant sociological effects.

Sleep deprivation can affect mental well-being and disrupt social activities. It may cause problems with learning, focus, and reaction times, while also hindering the ability to understand others' emotions and responses. Moreover, lack of sleep can result in frustration, irritability, or anxiety during social interactions. Data from a study shows that sleep deprivation—whether total or more moderate, real-world reductions in sleep quality—leads to social withdrawal and feelings of loneliness. Prioritizing sleep can foster better mental health, increase productivity, and contribute to healthier communities.

<u>Bio-markers of sleep debt</u>

Biomarkers of sleep debt are biological markers that can be used to quantify sleep debt or otherwise insufficient sleep.

• *Blood mRNA Biomarkers*: Some of the blood mRNA molecules that have been identified as indicators of both acute and chronic sleep loss, in studies. These are stress-related biomarkers, including heat shock protein activity and proteolysis.

• *Metabolic Biomarkers*: Reducing the sleep time of subjects changes their lipid metabolism and increases oxidative stress throughout the body.

• *Inflammatory Biomarkers*: Circulating levels of CRP (C-reactive protein), IL-6 (Interleukin-6) and IL-8 (Interleukin-8) have been associated with sleep debt. Both saliva and serum can measure these markers.

Sleep debt can indeed be reflected in various biomarkers found in saliva. Some Salivary biomarkers are:

1. **Stress Hormones**
   • Cortisol: High cortisol levels, can interfere with sleep by making you more alert and pushing back bedtime.

• Melatonin: Helps control sleep-wake cycles. Sleeping disorder is due to imbalance of secretion of melatonin.

2. Inflammatory Cytokines

• Interleukin-6 (IL-6): High concentrations in saliva of IL-6 correlated with a bad sleep quality and different intra-sleep disorders.
• Tumour Necrosis Factor Alpha (TNF-α): involved in systemic inflammation and known to play a role in sleep disturbances.
• IL-1β (Interleukin-1β): IL-1β is a second anti-sleep cytokine.

Mechanism of Action of these Biomarkers

• Circadian Rhythm: Salivary biomarkers help maintain the circadian rhythm, which is crucial for sleep regulation. Disruptions in these biomarkers can lead to circadian misalignment, affecting sleep quality.
• Inflammatory Response: Chronic inflammation can interfere with sleep by affecting the hypothalamic-pituitary-adrenal (HPA) axis and increasing the production of stress hormones.
• Feedback Loops: The interaction between stress hormones and inflammatory cytokines creates feedback loops that can either promote or disrupt sleep. For example, high cortisol levels can increase IL-6 production, which in turn can further elevate cortisol levels, creating a cycle that disrupts sleep.

Salivary Alpha-Amylase

Among salivary markers, a growing body of literature suggests that salivary α-amylase (sAA) may be a cross-species marker of sleep debt. sAA levels in saliva can indicate sleep debt in both humans and other species. Assaying the sAA is a non-invasive method to study sleep debt and its effects across different species.

Mechanism of Salivary Alpha-Amylase

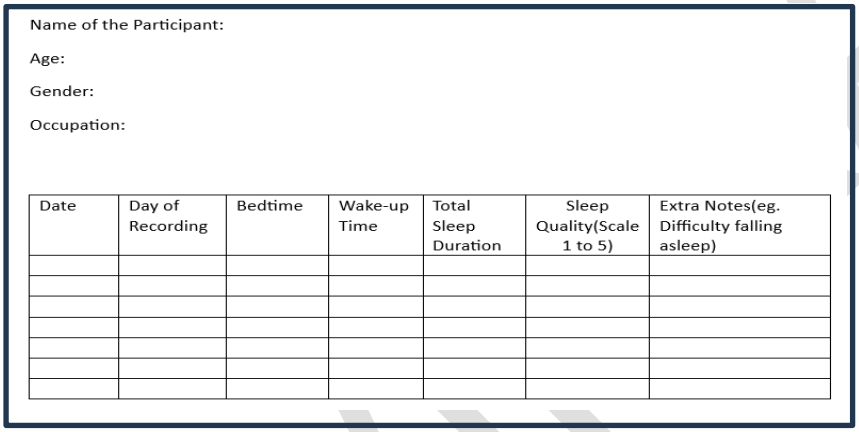
- Activation of the Stress Response: In response to stress, the autonomic nervous system (ANS) becomes engaged. This system comprises two primary components: the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS). The SNS triggers the "fight or flight" mechanism which involves the secretion of stress hormones such as adrenaline and cortisol, along with an elevation in salivary alpha-amylase (sAA) levels.
- Function of Salivary Alpha-Amylase: Salivary alpha-amylase synthesis by the salivary glands increases in response to SNS activation and is a non-invasive indicator of SNS activity.
- Implications of Increased sAA: Prolonged elevations in sAA levels due to chronic stress and sleep deprivation can cause heightened heart rate, increased blood pressure, and metabolic alterations, all of which can further disturb sleep patterns and create a detrimental cycle of stress and sleep loss.
- Feedback Mechanism: The ongoing interplay of stress and sleep deprivation can result in additional rises in sAA levels, sustaining the stress response and complicating the attainment of restorative sleep.

Objectives of the Study-

- **Primary Objective**: Investigate and compare salivary alpha-amylase (a biomarker for sleep debt and autonomic nervous system activity) levels between individuals aged 16 to 25 with normal sleep patterns and those experiencing sleep debt.
- **Biomarker Reliability**: Assess whether salivary alpha-amylase is a reliable biomarker for sleep debt.
- **Physiological Stress Response**: Evaluate if chronic sleep deprivation significantly impacts physiological stress responses in individuals within the 16 to 25 age group.
- **Health Implications**: Provide insights into the potential long-term health effects of inadequate sleep on young adults.

Materials and Methods

1. *Recording and maintenance of Sleep log*
   Participants were provided with sleep logs and asked to record their bedtimes and wake-up times for seven days (a week). Additionally, they noted any sleep disorders, difficulties in sleeping, and whether they were taking any medications or stimulants (such as coffee or energy drinks).



Name of the Participant:

Age:

Gender:

Occupation:

Date	Day of Recording	Bedtime	Wake-up Time	Total Sleep Duration	Sleep Quality(Scale 1 to 5)	Extra Notes(eg. Difficulty falling asleep)

2. *Preparation of Reagents*:
   - 1% starch solution (substrate for amylase activity)
   - 1% NaCl solution (to maintain ionic strength)
   - Phosphate-buffered saline (PBS)- 0.05M; to maintain pH stability
   - DNSA (3,5-dinitrosalicylic acid) reagent for colorimetric determination of reducing sugars (maltose) produced during the salivary amylase reaction

3. *Maltose standard Solutions*:
   Standard solutions of maltose were prepared using a stock solution of maltose to plot a standard curve. The range of maltose standard solution concentrations used was 0 to 1000 ug/ml. It was used to determine the concentrations of maltose produced by the enzyme activity in test samples.

Test tubes	Concentration of maltose (ug/ml)	Volume of maltose (ml)	Volume of Distilled water (ml)	DNSA (ml)		Absorbance at 570nm
B	-			0.5	Boil at 100 degrees for 10 minutes	
1	50	0.05	0.95			
2	100	0.1	0.9			
3	200	0.2	0.8			
4	300	0.3	0.7			
5	400	0.4	0.6			
6	500	0.5	0.5			
7	600	0.6	0.4			
8	700	0.7	0.3			
9	800	0.8	0.2			
10	900	0.9	0.1			
11	1000	1.0	0			

3. *Experimental Procedure*:

- The saliva samples were collected from the subjects/volunteers after recording their sleep pattern for 7-8 days. Two types of subjects were considered- with normal sleep pattern and with sleep dept. Samples collected were suitably diluted and the salivary amylase assay was set-up in control and test tube with following details.

Reagents	control	Test
Buffer	0.9 ml	0.85 ml
Starch	0.05 ml	0.05 ml
NaCl	0.05 ml	0.05 ml
Test sample	-	0.05 ml
Mix and incubate at 37 degrees for 15 min		
DNSA	0.5 ml	0.5 ml
Boil at 100 degrees for 10 minutes		

  - Control tube: The control reaction was set up using the starch, NaCl, and PBS buffer without any salivary sample, serving as a baseline for comparison.
  - Test/experiment tube: The test reactions were carried out using the starch, NaCl, PBS buffer with the addition of a salivary sample from both normal sleep and sleep-debt individuals.

- The starch solution was used as the substrate in both control and test reactions. The salivary sample was added to the test reaction to initiate the enzymatic breakdown of starch by salivary amylase. The reaction was allowed to proceed for a fixed period of 15 minutes at 37°C.

- Colour Development with DNSA: After the incubation time for enzyme assay, DNSA reagent was added to the reaction mixtures to detect the amount of maltose produced by salivary amylase activity. The mixtures were then boiled to facilitate colour development. A reddish-brown colour was produced as a result of the reduction of DNSA by maltose.

- Dilution and Measurement: The samples were diluted 1000- and 2500-times using PBS buffer before the enzyme assay was set-up to ensure the absorbance readings fall within the detectable range of the spectrophotometer. The absorbance of each sample was measured at 570 nm using a spectrophotometer to detect the reduced DNSA and thus concentrations of maltose

- Data Analysis: The absorbance in test samples were compared to the standard curve prepared using the maltose solutions. Salivary amylase activity in the control and test samples was calculated based on the amount of maltose produced. The results from normal sleep and sleep-debt individuals were analysed and compared to assess differences in salivary amylase levels under normal and sleep debt conditions.

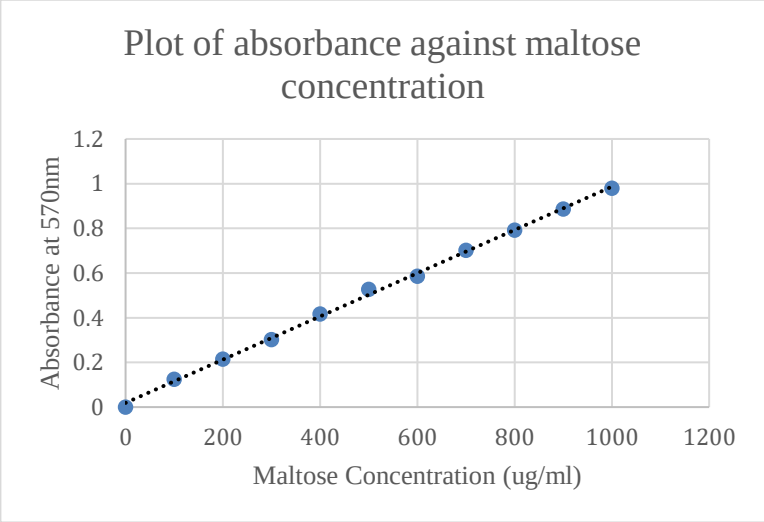


Fig.2 – Standard curve for maltose

Observation Table:

Serial number	Sample	Accumulated sleep debt	Enzyme activity (mmol/min/ml)	
			1000 folds dilution	2500 folds dilution
1	A1	No sleep debt	0.156	Not performed
2	A2	No sleep debt	0.156	Not performed
3	A3	No sleep debt	0.351	Not performed
4	A4	No sleep debt	0.273	Not performed
5	B1	No sleep debt	0.585	0.487
6	B2	No sleep debt	0.702	1.072
7	B3	No sleep debt	0.390	0.292
8	B4	No sleep debt	0.117	0.390
9	B5	4 hours	0.780	0.877
10	C1	1 hour	0.273	0.585
11	C2	No sleep debt	0.273	0.292
12	C3	1 hour	0.234	0.975
13	C4	No sleep debt	0.312	0.097

14	C5	No sleep debt	0.234	0.097
15	C6	No sleep debt	0.078	0.00
16	C7	No sleep debt	0.019	0.049
17	D1	No sleep debt	0,546	1.072
18	D2	No sleep debt	0.390	0.780
19	D3	No sleep debt	1.805	0.585
20	D4	No sleep debt	0.429	0.877
21	D5	No sleep debt	0.351	0.975
22	E1	No sleep debt	0.604	0.634
23	E2	11 hours	0.292	0.487
24	F1	2 hours	0.234	0.828
25	F2	1 hour 20 mins	0.156	0.292
26	G1	19 hours 25mins	0.183	0.146
27	G2	27 hours 50 min	0.487	0.097
28	G3	26 hours 35 mins	0.682	0.078
29	G4	25 hours 40 mins	0.780	0.195
30	G5	20 hours 15 min	0.858	0.926
31	G6	27 hours 50 mins	0.850	0.780
32	H1	12 hours 10 min	0.585	0.390
33	H2	No sleep debt	0.117	1.949
34	H3	9 hours 15 mins	1.676	2.534
35	H4	12 hours 15 mins	0.429	0.975
36	H5	12 hours	2.105	3.119
37	H6	14 hours	1.589	2.632
38	H7	7 hours	1.404	2.730
39	I1	23 hours 10 mins	2.144	4.581
40	I2	25 hours 35 mins	2.144	4.581
41	I3	14 hours 45 min	1.949	3.314
42	I4	21 hours	2.846	4.678
43	I5	3 hours 30 mins	2.183	3.996
44	I6	8 hours 45 mins	1.676	2.924
45	I7	3 hours 30 mins	1.637	2.827
46	I8	19 hours 30 min	1.637	3.021
47	I9	1 hour	0.741	2.242

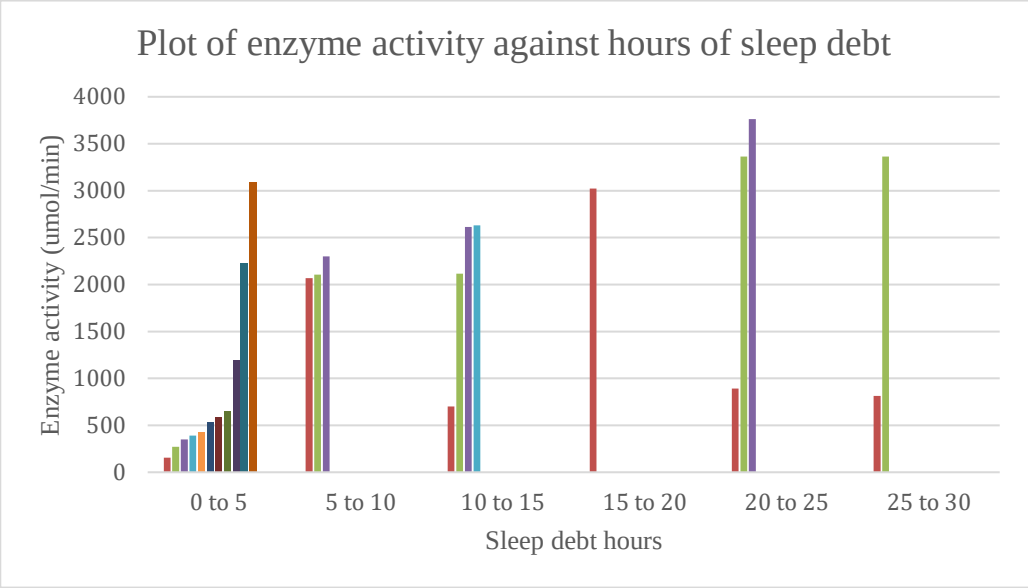


Fig.3 - A bar graph illustrating the comparison of enzyme activity between samples with little to no sleep debt and those with significant sleep debt hours.

Result

47 saliva samples were collected from subjects of age group, 16-24 years. After measuring the enzyme activity for each sample, they were classified into four groups: samples with a normal sleep pattern, sleep-debt samples, samples with minimal sleep debt, and those with unexpected results. From the observation table, an increase in the enzyme activity was observed in the sleep debt samples as compared with normal sleep samples.

Bar Graph Description:

The bar graph comparing enzyme activity between samples with minimal or no sleep debt and those with extensive sleep debt hours (Fig.3) reveals distinct trends. The average enzyme activity values at 1000 and 2500 dilutions were calculated and then plotted against the sleep debt hours.

The lowest enzyme activity is observed in the group with 0 to 5 hours of sleep debt, while the highest activity is seen in the 20 to 25 hours sleep debt group. Interestingly, the group with 0 to 5 hours of sleep debt also shows relatively high enzyme activity, while even the 25 to 30 hours sleep debt group displays notably low enzyme activity.

However, overall, it is evident that as sleep debt hours increase, there is a significant rise in salivary amylase enzyme activity.

The bar graph comparing average enzyme activities between samples without sleep debt and those with significant sleep debt (Fig.4) shows a clear upward trend. As sleep debt hours increase, enzyme activity also rises.

Discussion

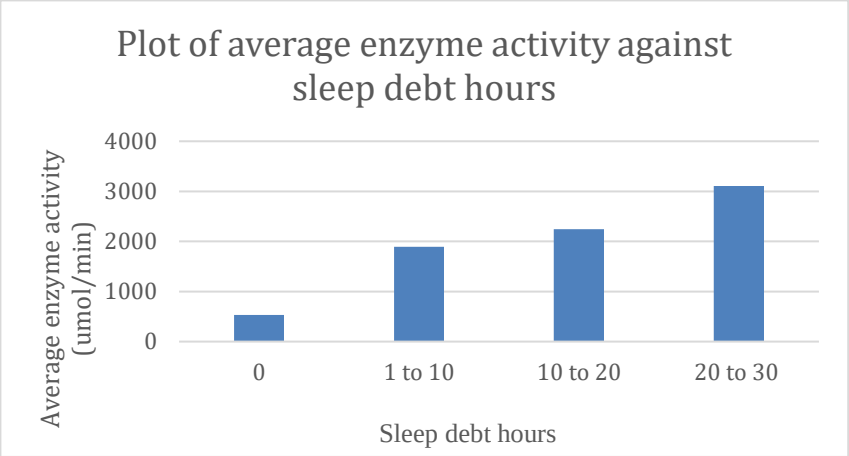


Fig.4 – A bar graph showing a comparison of the average enzyme activities between samples without sleep debt and those with significant sleep debt.

Salivary alpha-amylase (SAA) is increasingly explored as a non-invasive biomarker due to its association with the autonomic nervous system, particularly in relation to the stress response. However, its relationship with sleep deprivation remains underexplored. This study aimed to assess the reliability of SAA levels as an indicator of sleep debt by comparing participants who experienced varying degrees of sleep deprivation over time.

Subjects were identified and categorized into either normal or sleep debt groups based on their sleep patterns. They were instructed to maintain detailed sleep records for at least seven days prior to sample collection, with saliva samples collected on the eighth day. Each subject also completed a specially designed questionnaire to provide a rough analysis of lifestyle factors and physiological parameters that could influence SAA levels through activation of the sympathetic nervous system (SNS). In addition, subjects were required to provide detailed accounts of their sleep patterns throughout the week, recording any sleep loss—specifically quantified in hours and minutes—as well as additional notes on sleep quality, difficulty falling asleep, and feelings of tiredness or fatigue during the day.

Sample collection followed strict protocols: subjects were instructed to collect saliva samples at least one hour after breakfast, between 9:00 AM and 11:00 AM. The samples were carefully transported under temperature-controlled conditions, surrounded by ice packs, to prevent enzyme denaturation from temperature fluctuations or sample contamination.

Category 1: Samples with normal sleep patterns

In participants with normal sleep patterns, we observed that salivary amylase activity remained within the lower range. This is consistent showing that individuals who receive 7-9 hours of sleep per night typically exhibit balanced autonomic nervous system activity, which regulates amylase secretion. In well-rested individuals, the autonomic nervous system (ANS) functions normally. This means that the sympathetic nervous system (SNS), which controls the stress response and amylase secretion, is not fully activated. As a result, normal levels of amylase appear, indicating low stress and optimal function.

Good sleep ensures the proper regulation of the circadian rhythm, which helps to maintain hormone levels, including those related to enzyme secretion. This balance keeps the salivary amylase level within the normal

range. People who get enough sleep experience less stress, which is known to increase levels of salivary amylase. Their results show natural stress management as measured by this biomarker.

Category 2: Sleep debt samples

Participants in this category, experiencing chronic sleep debt, showed significantly elevated salivary amylase levels. Enzyme activity exceeded indicating prolonged stress and physiological imbalance. In individuals with prolonged sleep debt, the sympathetic nervous system is continuously activated due to insufficient rest. This leads to chronic stress, which directly correlates with increased production of salivary amylase as a part of the fight-or-flight response. Lack of sleep for a long time disrupts the circadian rhythm in the body. This leads to abnormal regulation of hormones and enzymes and increases the level of salivary amylase. The inability of the body to recover from stress causes the increase of this enzyme to continue. Long-term sleep deprivation is associated with inflammation, body disease and an increased risk of diseases such as in heart disease. Elevated salivary amylase is a marker of this chronic stress and a long-term indicator of health. Increased amylase activity reflects long-term activation of the hypothalamic-pituitary-adrenal (HPA) axis, leading to increased stress hormone production.

Category 3: Samples with negligible sleep debt

Among participants who had light sleep hours. The level of salivary amylase activity increased compared to the samples with normal sleep patterns. Enzyme activity in this group often remains at the upper end of the normal range but does not show a sharp increase.

These people experience Sleep on weekdays, but try to get enough sleep more on weekends instead. This pattern results in a mild disturbance in their circadian rhythm, which increases the sympathetic response during the week of hibernation. However, a week's sleep probably moderates the ANS to some extent, preventing chronic stress and significant increases in amylase levels.

Autonomic Nervous System Differences: Moderate SNS Activation due to differences in sleep patterns. Although salivary amylase increased during the week, it remained stable after sleep deprivation, indicating that the body is adapting to periodic sleep deprivation

Category 4: samples with unexpected results

Among the subjects studied, a positive correlation was observed between salivary alpha-amylase (SAA) levels and accumulated sleep debt. However, certain subjects presented unexpected results. Some subjects (G1 to G6) were identified as highly sleep-deprived individuals with significant accumulated sleep debt, but their samples did not exhibit the high SAA levels expected.

Sample B2 showed unexpectedly high SAA activity, despite the subject having no sleep debt over the past seven days. The subject reported high stress levels due to academic pressure, which highlights the interplay between the body’s stress response and sleep loss. Elevated stress triggers the sympathetic nervous system (SNS) and increases cortisol levels, which promotes wakefulness. This explains the elevated SAA activity, likely driven by SNS activation. A similar result was observed in sample C4, where the subject reported negligible sleep loss but had stress and anxiety issues. The higher enzyme activity in this case can also be attributed to the body's heightened stress response via SNS activation.

Sample I9 exhibited elevated amylase activity, although the subject reported normal sleep patterns and negligible sleep debt. This is likely due to poor sleep quality, as the subject reported unsatisfactory sleep, waking during the night, and daytime fatigue. Poor sleep quality can activate the stress response, leading to SNS activation and elevated SAA levels.

Bar Graph Description:

The bar graph comparing enzyme activity between samples with minimal or no sleep debt and those with extensive sleep debt hours (Fig.3) reveals distinct trends. The average enzyme activity values at 1000 and 2500 dilutions were calculated and then plotted against the sleep debt hours.

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