



## VARIATIONS OF CIRCADIAN RHYTHM DURING SLEEP DEPRIVATION AND CHANGES ON CORTISOL IN PLAYERS

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### ABSTRACT

In this study twenty students studied Bachelor of Physical Education and Sports (B.P.E.S) in the Department of Physical Education, Annamalai University were selected on random basis who volunteered to be the subjects. The independent variables used were a) complete sleep deprivation for 48 hours & b) circadian rhythm and the dependent variable selected was cortisol. The chosen 20 subjects were divided into two equal groups of 10 each and they were designated as experimental and control groups. The experimental group deprived their sleep continuously for 48 hours while control group had normal sleeping. Based on purpose of the study, blood was collected from both groups during normal condition and immediately after the treadmill exercise for seven times of a day at the interval of four hours such as : 00:00, 04:00, 08:00,12:00, 16:00, 20:00 and 24:00 hours to have complete biological cycle. The serum cortisol was estimated by ELISA-FOSTER, et. al., equipar kit 74040, Saronno, Italy and it is expressed as ng/ml. The experimental design employed was 2x7 repeated factorial design. In this study 2x7 ANOVA with last factor repeated, Simple Effect test and Scheffe's Post-Hoc tests were computed. It is concluded that a) there is no significant variation between control and sleep deprivation groups on cortisol concentration under normal condition irrespective of times of the day, b) the cortisol concentration between control and sleep deprivation groups differ significantly during all the seven different times of the day, c) the cortisol concentration for control group differ significantly for 14 paired means, d) the cortisol concentration for sleep deprivation group differ significantly for 12 paired means, e) the cortisol concentration after the exercise is significantly higher for control group irrespective of times of the day, f) the cortisol concentration between control and sleep deprivation groups differ significantly during 5 different times of the day, g) the cortisol concentration for control group differ significantly for 11 paired means and h) the cortisol concentration for sleep deprivation group differ significantly for 7 paired means.

**KEYWORDS:** Circadian rhythm – Sleep deprivation – Cortisol.

### INTRODUCTION

The purpose of present study is to explore the changes on cortisol (an endocrine parameter) under normal condition and immediately after the treadmill exercise due to circadian rhythmic variations under normal sleep and sleep deprived conditions.

Biological rhythm in man as well as in other organism can be observed and demonstrated at all lands of organization population group, individuals or their components such as organ system, organ tissues, cells and sub cellular structure (Halberg, et.al., 1997). Rhythm is a regular pattern of changes and bio - rhythm refer the changing pattern of how physical processes happen in the body. Diurnal rhythm denotes the activity during day time and it is opposite of nocturnal rhythm. The activity at night or happening during the night is known as nocturnal rhythm. The circadian rhythm is connected with the changes or the activity occur in the body of people or animals over each period of 24 hours cycle. The PSI derived from three cycles with which it deals namely Physical cycle, 23 days; Sensitivity cycle, 28 days and Intellectual cycle, 23 days (Minoss, 1985).

A cycle of change which occurs in a biologic organism is called as bio-cycle or biological rhythm. It

range extensively in periodically from a fraction of a second to several years, (Wiky Aictiony, 1986). The daily biological variations are not simply a response to 24 hours changes in the environment due to the rotation of the earth on its axis, but rather arise from an internal time- keeping system and persist under constant environmental conditions. The major action of the environment is to synchronize the internal system to a period of 24 hours (Menakar, et. al., 1978 ). In mammals, the supra schismatic nucleus (SCN) was initially supposed by the only master circadian pacemaker. The SCN is a complex structure involving to small bilaterally paid nuclei situated in the anterior hypothalamus and others above the optic chiasm and lateral to the third ventured. The role of the SCN as the circadian clock has been demonstrated by lesion experiments and studies involving transplanted of the SCN. There is evidence demonstrating resynchronization of corticosteroid circadian rhythmicity after CSN destruction. The results may indicate the possibilities of the presence of other circadian clocks in brain are as outside the (SCN), (Krieger, et. al., 1977) . The pituitary is the proximal endocrine gland associated with circadian rhythm.

Sleep is a rest in lying position with eyes closed

in which body and mind are not active. Sleep is a state of unconsciousness from which a person can be aroused appropriate sensory or other stimuli. Meirh views that sleep is very complex amalgam physiological behavioural processes. Biological clock is a natural system in living things, that controls regular sleeping activities. All living creatures, people, animal, fish and even the tiniest insects have a daily need for sleep and complete rest. Work and rest are closely associated and how far work is important in the same way rest is also significant. Sleep is the finest form of rest for the entire human body. The duration of sleep should be in proportion to the work performed. Approximately one third of our life is spent in sleep and it is dependent upon several factors including age. A sleeping person is unconscious and unaware of what is going on around him. The ECG recording shows the typical features of sleep and it is divided into two different phases a) Rapid eye movement sleep (REM sleep) or desynchronized sleep or active sleep or paradoxical sleep, b) Non REM sleep (NREM) or synchronized sleep (Hiraj, 1992). During deep sleep arterial blood pressure falls, pulse rate decreases, muscles fall into mainly relaxed state and the overall basal metabolic rate of the body falls to 30 per cent.

Sleep has a special significance in neurophysiology. Research in the area of neurophysiology of sleep began in the 1930s (Loomis Harvey & Hobart, 1937). They have invented new techniques for measuring the physiological functions of sleep. Mechanisms that control the sleep waking cycle apparently lie in the tegmentum of the brain stem, especially the pons, mesencephalon and lateral hypothalamus. Like other control systems of brain stem and hypothalamus two distinct portions can be identified. One neural complex is responsible for waking the body and keeping it awake and the other initiates sleep and determines its depth or stage. To a degree these subsystems are characterized by different transmitter substances, (Best, 1985).

Sleep deprivation is deprived of sleep or avoiding sleep or to prevent from having sleep. Sleep deprivation is the term used to describe the acute and chronic condition of insufficient or lack of sleep. Ten to thirty per cent of the populations are having the insomnia problem. Women reporting a higher incidence of sleep disturbances than men. Experimental deprivation of sleep is an important method to find out the functions of sleep. After a few days of a sleep deprivation, the ECG recordings show a gradual diminishing of an activity with increase in lower frequency activity (Ahuja, 1992). After four to five days of sleep deprivation, psychological symptoms become prominent. In predisposed individuals, psychotic symptoms may appear on the first night. Sleep deprivation for prolonged period sometimes lead to disorganization, hallucinations and delusions (Kalpan, 1994). Further, automatic subcortical functions begin to deteriorate and tremors, hystagnus, ptosis dysarthria and defective disorders

appear (West, 1990).

The sleep cycles are regulated by a biological clock. Circadian rhythm has a powerful effect on sleep patterns. The circadian variation of the HPA axis changes with the manipulation of rhythm by phase shifting a synchronizer such as the light dark, sleep-wake and rest activity cycles, (Gycyton, 1986). Research has shown that lack of sleep certainly does affect the functions of the central nervous system, malfunction of the mind, experience restless and irritation and unable to concentrate on tasks. Sleep deprivation also affects sports performance. The performance of players mainly depends upon the proper combination among training load, diet and rest. In Indian condition, during previous day night players travel in bus to participate in sports competition and it leads to disturbed sleep and sleep deprivation. It will affect the fine & gross motor skills and performance of players. For better performance, players require adequate rest in the form of sleep before competition.

The influence of circadian rhythm and sleep deprivation can be assessed accurately by biochemical and endocrine variables. Cortisol is an endocrine parameter and a relatively slow acting hormone that alters metabolism and stimulated by variety of stress like anxiety, fear, pain, hemorrhage, infections and low blood glucose, which are stimulates release of the corticosteroid hormone cortisol from adrenal cortex, (Foster, 1947).

## METHODOLOGY

In the present study an effort was made to select the volunteers as subjects among the students (boys) studied Bachelor of Physical Education and Sports Sciences in the Department of Physical Education, Annamalai University. The subjects regularly practicing and participating in competitions and represented for Annamalai University in cricket, football and hockey games. The investigator explained to them the purpose, nature of experiment, the procedure to be employed to collect the blood, role of the subjects during experimentation and testing periods. Twenty seven players volunteered to be the subjects and finally twenty subjects were selected at random by lot sampling technique. Written consent form to participate in the study was received from each subject separately. Their age ranged between 18 and 21 years. The independent variables used in the present study were a) complete sleep deprivation for 48 hours & b) circadian rhythm and the dependent variable selected was cortisol.

## EXPERIMENTATION

The chosen 20 subjects were divided into two equal groups of 10 each on random basis and they were designated as experimental and control groups. The experimental group deprived (avoided) their sleep continuously for 48 hours and control group had normal sleeping. Blood was collected from both groups for seven times of a day at the interval of four hours. Each

time, blood was collected twice that is during basal (normal) and after treadmill exercise.

#### **EXPERIMENTATION OF SLEEP DEPRIVATION**

During day and night the subjects of experimental group were along with the investigator in a hall which had bathroom and toilet facilities. Whenever they felt sleeping they engaged themselves in reading, writing, record work, academic activities, listening television with mild sound, playing carrom & chess, walking within the hall, conversation among themselves etc based on their interest. During this period they had normal diet with in the hall. Throughout the experimentation period the investigator was along with the subjects of experimental group and avoided their sleep continuously for 48 hours and there by the sleep deprivation is confirmed.

#### **TREADMILL EXERCISE FOR TESTING PURPOSE**

Pilot study was conducted with the help of five players (men) at the age group of 18 to 21 years to decide the load of treadmill exercise. They were directed to perform treadmill exercise at different intensity and duration. Based on the pilot study the load was fixed. The speed was 12km/h, the slope was 5 per cent and the duration was 5 minutes. After the exercise the players had pulse rate between 150 and 160 per minute and with the help of pulse rate the sub-maximal load was confirmed. After finalizing the load, both groups performed the treadmill exercise.

#### **COLLECTION OF BLOOD SAMPLE**

Five milliliter of venous blood was collected from each subject through venus puncture by using standard disposal syring. The serum was separated by 3000 rpm for 10 minutes. Based on the purpose of the study, blood sample were collected from the subjects of both control and experimental groups at seven different times of a day with four hours interval starting from 12.00 O clock of midnight. The seven different times of a day are 00:00, 04.00, 08.00, 12.00, 16:00, 20:00 and 24.00 hours to have complete biological cycle. During each of the said period, blood sample were collected from both groups during normal condition and

immediately after the treadmill exercise.

#### **ASSAY OF CORTISOL**

The serum cortisol was measured by ELISA equipar kit 74040/saronno, Italy. 200µl of diluted marker added to 10µl of serum standard and distilled water (bank) an incubated at 37°C for 1 our in a covered ELISA wells. 100 of TMB- substrate were added to all the wells and incubated at room temperature (20-25°C) for 15 minutes in dark. Finally 100 µl of stop solution was added to all the wells and the O.D was taken at 450 nm against blank and the cortisol is expressed as ng/ml (Fosten and Dunn, 1974).

#### **EXPERIMENT DESIGN AND STATISTICAL TECHNIQUE**

The experimental design employed was 2x7 repeated factorial design. In this study 2x7 ANOVA with last factor repeated was calculated to explore the changes on cortisol due to circadian rhythmic variations under normal sleep (control group) and sleep deprived conditions (experimental group). The same statistics was computed separately for normal condition and immediately after the treadmill exercise.

When the interaction (group X times of testing) was found to be significant, Simple Effect test was used as Post - Hoc test (Rothstein, 1985). Further, whenever Simple Effect test showed significant differences, Scheffe's test was applied to find out which of the period means differ significantly. Further, when the interaction is not significant, as suggested by Thomas and Nelson, (1985) the Main effect (Rows and Columns) are evaluated by applying Scheffe's Post- Hoc test.

#### **ANALYSIS OF CORTISOL UNDER NORMAL CONDITION**

The mean and standard deviation for control and sleep deprivation groups on cortisol concentration under normal condition at seven different time of the day is given in table I.

**TABLE –I**  
**MEAN AND STANDARD DEVIATION FOR CONTROL AND SLEEP DEPRIVATION GROUPS ON CORTISOL (NG/ML) UNDER NORMAL CONDITION AT SEVEN DIFFERENT TIMES OF THE DAY**

Groups	Mean ± SD of Testing Times							Mx
	00:00	04:00	08:00	12:00	16:00	20:00	24:00	
Control	88.30 ± 21.91	65.92 ±17.604	462.27 ±52.46	345.65 ±142.11	313.50 ±155.46	159.70 ±73.89	387.33 ±117.92	260.38
Sleep	162.83 ±47.98	415.04 ±42.64	290.37 ±97.29	123.00 ±18.78	237.90 ±78.86	121.04 ±23.66	240.22 ±32.29	227.2
My	125.57	240.48	376.32	234.325	275.7	140.37	313.78	

**Mx:** Combined mean of control and sleep deprivation groups irrespective of different times of the day

**My :** Combined mean of different time of the day irrespective of control and sleep deprivation groups

The analysis of variance is presented in table II.

**TABLE –II**  
**ANALYSIS OF VARIANCE FOR CONTROL AND SLEEP DEPRIVATION GROUPS ON CORTISOL (NG/ML) UNDER NORMAL CONDITION AT SEVEN DIFFERENT TIMES OF THE DAY**

Source of Variance	Sum of Squares	df	Mean Squares	F-ratio	Level of Significance
Between Groups	38512.66	1	38518.66	2.75	NS
Error	252440.6	18	14024.47		
Times of the Day	965134.26	6	160855.10	31.65	0.01
Times of the x Groups	1138556.7	6	189759.46	37.33	0.01
Error	54886.65	108	5082.11		

Tabulated F –ratio for : 0.05      0.01 level

df 1 & 18                    : 4.41    8.28

df 6 & 108                 : 2.19    2.99

NS: Not Significant

It is clear from the table II that the obtained F ratio 2.75 for the group is not significant. It is inferred that there is no statically significant variation between control and sleep deprivation groups on cortisol concentration under normal condition irrespective of different times of the day. With regard to cortisol concentration at seven different times of the day, the F ratio arrived at by the statistical calculation 31.65 is

greater than the table value and significant at 0.01 level. It reveals that the cortisol concentration differs significantly at seven different times of the day under normal condition irrespective of groups. From the table II it is further observed that the obtained F ratio 37.33 for interaction between times of the day and group is significant at 0.01 level. The results of simple effect test is given in table III.

**TABLE –III**  
**SIMPLE EFFECT TEST FOR CONTROL AND SLEEP DEPRIVATION GROUPS ON CORTISOL (NG/ML) UNDER NORMAL CONDITION AT SEVEN DIFFERENT TIMES OF THE DAY**

Source of variance Hours	Sum of Square	df	Mean Square	F	Level of Significance
00.00	27773.60	1	2773.60	5.47	0.01
04.00	609428.80	1	60948.80	120.00	0.01
08.00	147748.05	1	147748.05	29.07	0.01
12.00	247865.11	1	247865.11	27.77	0.01
16.00	28576.80	1	28576.80	5.62	0.01
20.00	3620456.00	1	3620546.00	712.41	0.01
24.00	108192.05	1	108192.05	21.29	0.01
Control	342996672.00	6	5766112.62	11248.50	0.01
Sleep deprivation	3950562.42	6	658427.07	129.56	0.01
Error	548868.65	108	5082.11		

The F ratio required for : 0.05      0.01 level df 1 & 108                    =      3.92    6.85  
 df 6 & 108                    =      2.19    2.99

Table III shows that the cortisol concentration under normal condition between control and sleep

deprivation groups differ significantly during all the seven different times of the day in favour of control

group. From the table III it is also clear that the cortisol concentration under normal condition at seven different times of the day differ significantly at 0.01 level for both control and sleep deprivation groups. As the simple effect

test is significant, Scheffe's test was calculated separately for control and sleep deprivation groups, which is presented in tables IV and V respectively.

**TABLE-IV**  
**SCHEFFE'S TEST OF SIGNIFICANCE FOR CONTROL GROUP ON CORTISOL (NG/ML) UNDER NORMAL CONDITION AT DIFFERENT TIMES OF THE DAY**

00:00	04:00	08:00	12:00	16:00	20:00	24:00	Mean	Level of Significance
88.30	65.92						022.38	NS
88.30		462.27					373.97	0.01
88.30			345.65				257.35	0.01
88.30				313.50			225.20	0.01
88.30					159.70		071.40	NS
88.30						387.33	299.03	0.01
	65.92	467.27					396.35	0.01
	65.92		345.65				279.73	0.01
	65.92			313.50			247.58	0.01
	65.92				159.70		093.78	NS
	65.92					387.33	321.41	0.01
		462.27	345.65				116.62	0.05
		462.27		313.50			148.77	0.01
		462.27			159.70		302.57	0.01
		462.27				387.33	074.94	NS
			345.65	313.50			032.15	NS
			345.65		159.70		185.95	0.01
			345.65			387.33	041.68	NS
				313.50	0.347		153.8	0.01
				313.50		387.33	73.83	NS
					0.374	387.33	227.63	0.01

The confidence interval required at 0.05 and 0.01 level for significance are 115.41 and 135.18 respectively

NS= Not significant

Table IV shows that the cortisol concentration under normal condition for control group differ significantly for 14 paired means out of 21.

**TABLE- V**  
**SCHEFFE'S TEST OF SIGNIFICANCE FOR SLEEP DEPRIVATION GROUP ON CORTISOL (NG/ML) UNDER NORMAL CONDITION AT DIFFERENT TIMES OF THE DAY**

00:00	04:00	08:00	12:00	16:00	20:00	24:00	Mean	Level of Significance
162.83	415.04						252.21	0.01
162.83		290.37					127.54	0.05
162.83			123.00				39.83	NS
162.83				237.90			075.07	NS
162.83					121.04		041.79	NS
162.83						240.22	077.39	NS
	415.04	290.37					041.79	0.05
	415.04		123.00				124.67	0.01
	415.04			237.90			177.14	0.01
	415.04				121.04		294	0.01
	415.04					240.22	174.82	0.01
		290.37	123.00				167.37	0.01
		290.37		237.90			52.47	NS
		290.37			121.04		169.33	0.01
		290.37				240.22	050.15	NS
			123.00	237.90			114.9	NS
			123.00		121.04		01.96	NS
			123.00			240.22	117.22	0.05
				237.90	121.04		116.86	0.05
				237.90		240.22	02.32	NS
					121.04	240.22	119.18	0.05

The confidence interval required at 0.05 and 0.01 level for significance are 115.41 and 135.18 respectively  
 NS= Not significant

Table V shows that the cortisol concentration under normal condition for sleep deprivation group differ significantly for 12 paired means out of 21.

**AFTER THE TREADMILL EXERCISE**

The mean and standard deviation for control and sleep deprivation groups on cortisol after the treadmill exercise at seven different times of the day is given in table VI.

**TABLE –VI  
 MEAN AND STANDARD DEVIATION FOR CONTROL AND SLEEP DEPRIVATION GROUPS ON CORTISOL (NG/ML) AFTER THE EXERCISE AT SEVEN DIFFERENT TIMES OF THE DAY**

Groups	Means ±SD of Testing Times							Mx
	00:00	04:00	08:00	12:00	16:00	20:00	24:00	
Control	364.71 ±147.71	433.06 ±62.45	287.54 ±69.60	410.34 ±148.67	252.85 ±143.53	271.15 ±106.93	55.93 ±31.87	282.22
Sleep Deprivation	359.49 ±85.23	288.70 ±136.22	130.79 ±22.12	228.96 ±80.80	171.50 ±56.70	140.0 ±25.97	97.71 ±13.93	202.45
My	312.1	360.88	209.17	319.65	212.17	205.58	76.82	

**Mx:** Combined mean of control and sleep deprivation groups irrespective of different times of the day

**My :** Combined mean of different times of the day irrespective of control and sleep deprivation groups

The analysis of variance is presented in table VII.

**TABLE - VII  
 ANALYSIS OF VARIANCE FOR CONTROL AND SLEEP DEPRIVATION GROUPS ON CORTISOL (NG/ML) AFTER THE EXERCISE AT SEVEN DIFFERENT TIMES OF THE DAY**

Source if Variance	Sum of Squares	df	Mean Squares	F-ratio	Level of Significance
Between Groups	222748.95	1	222748.95	14.82	0.01
Error	270599.57	18	15033.31		
Times of the Day	1113087.8	6	185514.63	23.89	0.01
Times of the Day x Groups	341537.01	6	56922.84	7.33	0.01
Error	838603.31	108	7764.85		

The F ratio required for :     0.05     0.01 level  
 df 1 & 18                     =     4.41     8.28  
 df 6 & 108                    =     2.19     2.99

It is clear from the table VIII that the obtained F ratio 14.82 for the group is higher than the table value and significant at 0.01 level. It is inferred that there is statistically significant variation between control and sleep deprivation groups on cortisol concentration after the exercise irrespective of different times of the day. The cortisol concentration after the exercise is significantly higher for control group. With regard to cortisol concentration at seven different times of the day,

the F ratio arrived at by statistical calculation 23.89 is greater than the table value and significant at 0.01 level. It reveals that the cortisol concentration differ significantly at seven different times of the day after the exercise condition irrespective of groups. From the table VII it is further observed the obtained that F ratio 7.33 for interaction between times of the day and group is significant at 0.01 level. The result of simple effect test is given in table VIII.



**TABLE –X**  
**SCHEFFE’S TEST OF SIGNIFICANCE FOR SLEEP DEPRIVATION GROUP ON CORTISOL (NG/ML) AFTER THE EXERCISE AT DIFFERENT TIMES OF THE DAY**

00:00	04:00	08:00	12:00	16:00	20:00	24:00	Mean	Level of Significance
359.49	288.70						70.79	NS
359.49		130.79					228.7	0.01
359.49			228.96				130.7	NS
359.49				171.50			187.99	0.01
359.49					140.0		219.49	0.01
359.49						97.71	261.78	0.01
	288.70	130.79					157.78	0.05
	288.70		228.96				059.74	NS
	288.70			171.50			117.2	NS
	288.70				140.0		148.7	0.05
	288.70					97.71	190.99	0.01
		130.79	228.96				098.17	NS
		130.79		171.50			040.71	NS
		130.79			140.0		090.21	NS
		130.79				97.71	033.08	NS
			228.96	171.50			057.46	NS
			228.96		140.0		0131.25	NS
			228.96			97.71	031.5	NS
				171.50	140.0		031.5	NS
				171.50		97.71	073.79	NS
					140.0	97.71	042.29	NS

The confidence interval require at 0.05 and 0.01 level for significance are 142.64 and 167.10 respectively  
 NS= Not significant

Table X shows that the cortisol concentration after the exercise for sleep deprivation group differ significantly for 7 paired means out of 21.

**DISCUSSION ON FINDINGS**

The cortisol and insulin shows a 24 hours variation in normal individuals (Herpertz, et. al., 1998). Cortisol is the major regulator adaptive response to the stress (Best and Taylor, 1985), Von Treuer, Norman & Armstrong (1996) have conducted a study to examine the overnight human cortisol and prolactin under the conditions of normal sleep, sleep deprivation and sleep recovery. It was found that cortisol was significantly higher on the sleep deprivation night presumably reflecting the aroused state accompanying being awake however, there were several time points on control night when it was normal in comparison. Cortisol and prolactin levels vary with states and therefore useful indices of arousal. In another study, the relative significance of physical exercise energy and sleep deprivation for the morning levels of hormones and the endocrine response to short term cycle exercise were investigated among players. Cortisol and GH increased, whereas glucose and insulin decreased due to energy deficiency. The cortisol response to the bicycle test was increased in all groups and energy deficiency caused slower post exercise recovery. The results suggested that the endocrine responses during long-lasting exhausting strain were mainly due to physical exertion and energy deficiency (Opstad & Falch, 1991).

**RESULTS OF CORTISOL**

In the present study it is concluded that :

**UNDER NORMAL CONDITION**

1. There is no significant variation between control and sleep deprivation groups on cortisol concentration under normal condition irrespective of times of the day.
2. The cortisol concentration between control and sleep deprivation groups differ significantly during all the seven different times of the day.
3. The cortisol concentration for control group differ significantly for 14 paired means.
4. The cortisol concentration for sleep deprivation group differ significantly for 12 paired means.

**AFTER THE TREADMILL EXERCISE**

5. The cortisol concentration after the exercise is significantly higher for control group irrespective of times of the day.
6. The cortisol concentration between control and sleep deprivation groups differ significantly during 5 different times of the day.
7. The cortisol concentration for control group differ significantly for 11 paired means.
8. The cortisol concentration for sleep deprivation group differ significantly for 7 paired means.

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