Journal of Environmental Sciences, 2020; Vol. 49, No. 2: 28-36

Journal of Environmental Sciences

JOESE 5

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Reprint

Volume 49, Number 2: 28 - 36

(2020)

http://Joese.mans.edu.eg

P-ISSN 1110-192X

e-ISSN 2090-9233

Journal of Environmental Sciences

JOESE 5

ISSN 2090-9233

Journal homepage http://Joese.mans.edu.eg

Original Article

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Article Info

Article history:

Received 22/ 11 /2019

Received in revised form 17/12/2019

Accepted 21/12/2019

Keywords: *sleep deprivation, oxidative stress, hormonese*

Abstract

In most biological processes in the human body, sufficient sleep time and length during the night is very critical while, the opposite is associated with adverse physical health issues. Forty volunteers 25-35 years old were chosen for participation in this study, 20 has normal sleep at night and 20 exposed to sleep deprivation either voluntary or involuntary, for at least 2 months. Sleep deprived group showed decline in RBCs, Hb, Ht% incorporated with increase in total WBCs and most of their subpopulations especially lymphocytes and neutrophils, glucose concentration, glycated hemoglobin, ghrelin, ACTH, cortisol, melatonin levels, total lipids and triacylglycerol in association with significant decline in leptin and insulin levels. Regarding antioxidant parameters and oxidative stress markers, SOD activity and GSH level declined but, CAT activity, MDA and PC significantly exceeded. Regarding CD4 and CD8 %, they were significantly raised in sleep deprived group. In conclusion, it seems that, sleep deprivation adversely affects metabolic processes, reduces antioxidant status and declines immune system, meanwhile, sleep aid for recuperation from most disease status, improve immune system and restore body activity.

1. Introduction

Loss of sleep caused by increased workload, shift work and numerous other challenges created by modern society may pose a serious health threat. ***Troynikov et al. (2018)*** reported that, after activity the human body can be recovered by sleep for optimal subsequent functions. Exposure to artificial light at night leads to disturbance of the circadian rhythm which is detrimental to health and can increase the incidence of breast cancer in nurses ***Yvan et al., (2017).*** Sleep deprivation or loss is closely linked to hormonal abnormalities that cause heart, brain, gastrointestinal tract and small intestine health problems ***Sallinen and Kecklund, (2010).*** Sleep disturbances can lead to metabolic syndrome that interrelated with risk factors including glucose intolerance, elevated blood pressure, increased plasma triglyceride, decreased HDLc and central obesity ***Einhorn et al. (2003).***

Unlike daytime life, the erratic schedule of work or unusual hours to sleep during night in contrast to daytime work may lead to misalignments between physiological processes such as digestion and metabolism, ***Banks and Dinges (2007) that*** causes shift workers to have poor health and thus exceeds risk factor for type II diabetes and obesity ***Banks et al. (2014).*** The risk of obesity in male shift workers can be associated with a sedentary life style and to a nocturnal consumption of carbohydrates, excessive food intake and decreased physical activity that promotes obesity, ***Haus et al. (2016). Spiegel et al. (2009)*** reported that, stressful situations such as chronic work overload have been accompanied by declining sleep duration and many neuroendocrine effects. Sleep duration and quality were shown by ***Knuston et al. (2006)*** to be predictors of HbA1c, the key marker of glycemic control.

The effect of sleep deprivation on hematological response is conflicted, where ***Boudjeltia et al. (2008)*** and ***Ruiz et al. (2012)*** noted that, partial and total sleep deprivation lead to increased WBCs, neutrophils, lymphocytes and monocytes counts, but no effect on RBCs. Meanwhile, ***Boyum et al. (1996),*** showed a decline in these parameters. In addition, ***Ozturk et al. (1998)*** recorded no effect of sleep deprivation on Ht%.

Ghrelin seems to stimulate appetite and food intake, ***Cummings and Shanon (2003). Taheri et al. (2004)*** reported that, poor or restricted sleep is accompanied by altered level of ghrelin and leptin. The pineal hormone, melatonin involved in the circadian regulation and facilitation of sleep as well as the enhancement of immune functions, ***Blask, (2009).*** It acts also as an antioxidant and free radicals scavenger ***Tan et al. (1993).*** Dark environments affects the secretion of melatonin, where its serum levels begin to rise shortly after nightfall and its peak is at midnight, ***Brzezinski (1997).*** Acute sleep deprivation -which

accompanied by higher cortisol level, physiological stress and metabolic response during the night- is associated with chronic circadian misalignment which reduces cortisol levels/ 24h, ***Kenneth et al. (2015).*** ACTH has been shown by ***Dibner et al. (2012)*** to be an important component of the hypothalamic pituitary-adrenal (HPA) axis and is produced mainly as a response to biological stress. Alterations of HPA axis plays a role in sleep disorders, ***Roth (2007).***

Sumida, (1991) noted negative regression between the CD4/CD8 ratio with HbA1c and diabetic duration, mean CD4 and CD8 lymphocytes counts were higher in diabetic patients than normal persons.

The present study was design to examine the relationship between sleep disorders and individuals physiological functions, oxidative stress markers as well as immune functions.

2. Subjects and Methods

Twenty normal healthy volunteers 25-35 years old from both sexes and other twenty sleep deprived participants due to several factors were chosen. They didn't drink alcohol, taking any medications and always within the same normal social life.

The first group (control) does not have history of sleep disorders and they had regular sleeping during night at least over two months period before the experimentation.

The second group (sleep deprived) was chosen from people whom sleep by day and awake during night either selectively or due to their shift work.

Before starting the research, these subjects were informed about the experimental protocol and the possible risk associated with the study results. Each participant signed a written informed consent prior to participation. The study was approved by Mansoura University Ethical committee number DZ 18005, before starting.

In EDTA containing tubes, blood samples were obtained at 7.0 ±30 a.m. with an antecubital venous puncture for determination of hematological parameters and then allowed to centrifuge immediately at 800 xg in cooling centrifuge for 15 min. The obtained plasma was stored at -80 °C for subsequent biochemical estimations.

Hematological parameters were assessed as described in Dacie and Lewis (1991).

HbA1c in blood was estimated using ELSIA technique: Cat. No. CSB-E08139h.

Glucose, total lipid, triacylglycerol were estimated according to the methods of Josef (1955), Frimgs et al. (1972) and Fossati and Prencipe (1982), respectively.

Insulin, was assessed as recommended by Yallow and Berson (1959), while leptin, ghrelin, and melatonin were analyzed using an enzyme linked immunosorbent assay (ELSIA) (Cusabio, California, USA).

ACTH and cortisol were analyzed by electrochemiluminescence assay technique (ECLIA). ROCHE, Germany: Ref. No 03255751 for ACTH and Ref. No 11875116 for Cortisol).

The oxidative stress parameters GSH, MDA and PC were assessed by the method of Beutler et al. (1963), Ohkawa et al. (1982) and Davis and Delsignore (1987), respectivly.

Antioxidant enzymes activity SOD and CAT were determined as showed by Nishikimi et al. (1972) and Bock et al. (1980), respectively.

CD4 and CD8% were assessed using immunofluorescence staining immunofluorescence positive cells were determined by flow cytometry applying the method of Bleavins et al. (1993).

Statistical analysis:

The difference between data in both groups were examined using student-t-test. A P value $\leq$ 0.05 was considered significant. All statistical analyses were conducted using SPSS software ***(Senedecor and Cochran, 1982)***.

3. Results

Obtained data in Fig1 and Fig 2 (a, b, c) revealed that, sleep deprivation significantly decline Ht%, RBCs and WBCs count, Hb concentration and neutrophils as well as basophils percent.

As shown in table 1, there was significant increase in blood glucose, total lipids, triaceylglycerol, ACTH, cortisol and ghrelin levels. In concomitant with lower leptin level, hypoinsulinaemia, and melatonin level.

Data listed in table 2, shows decline in SOD activity and GSH level, this was accompanied by an increase in CAT activity, MDA and PC levels as well as the percentage of natural killer cells (CD4 and CD8 subsets) relative to normal central daytime wakeup participants.

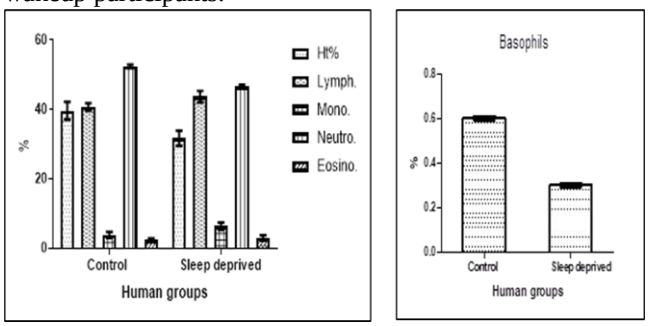
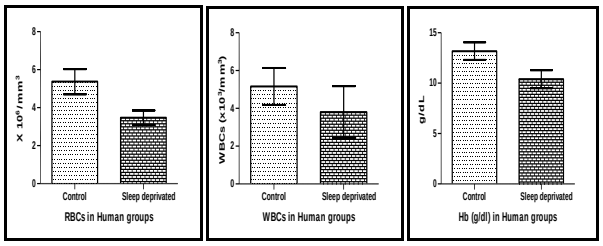


Fig 1: Hematocrit and differential count percent in estimated human groups.

4. Discussion

The normal health and safety require adequate sleeping time and duration are necessary for. It is controlled by both circadian processes and homeostasis. Sleep helps the human body to recover after activity to achieve his optimal functioning, ***Luyster et al. (2012).***

Unlike daytime normal human life which generally sleep by night, many people do the reverse irregularly where they sleep by day and wake up by night, willingly or mandatory. These individuals suffer mostly from cardiovascular and metabolic problems, neurological and endocrine abnormalities, ***Buxton et al. (2012).***



(a) (b) (c)

Fig 2: RBCs (a), WBCs count (b), and Hb (c) concentration in estimated human groups.

The obtained decrease in RBCs number and Ht% in sleep deprived group is in accordance with the results obtained by ***Dinges et al. (1994)*** whom attributed these data to exhaust in bone marrow that decrease RBCs synthesis or due to exceeded RBCs destruction as a response to the effect of physical stress as mentioned by ***Hammouda et al. (2012).*** The drop in sleep disturbed haemoglobin concentration is consistent with ***Jackowska et al. (2013)*** who recorded that, sleep disturbance was associated with lower haemoglobin levels and those individuals suffer from anemia and iron deficiency due to ferritin level disruption after sleep deprivation ***Song et al. (2012).*** In addition, WBCs and lymphocytes showed significant increase after sleep deprivation, these results are not unexpected and run parallel with that of ***Ruiz et al. (2012)***. In addition, ***Burgueno et al. (2010)*** indicated that, shift workers have excess number of WBCs compared to daytime workers and may be an attempt to overcome the stressors caused by sleep deprivation and/or infections caused by unhealthy food with low metabolic activity ***Banks and Dings (2007)*** who have reported that, irregular work schedule leads to misalignment between physiological processes such as digestion and metabolism; irregular eating habits due to temporary food reorganization may play a role in these results ***Lennernas et al. (1995).*** The capability of lymphocytes proliferation was decline in shift workers relative to day time workers, ***Nakano et al. (1982).***

Table 1: Some metabolic products and hormonal concentrations in control and sleep deprived human volunteers

Parameters / Groups	Glucose (mg/dl)	HbA1c %	Insulin (ng/ml)	Total lipid (mg/dl)	Triacevl glycerol (mg/dl)	ACTH (Pg/ml)	Cortisol (ng/ml)	Leptin (ng/ml)	Ghrelin (Pg/ml)	Melatonin (ng/ml)
Control	102 ± 9.93	5.43 ± 0.51	6.99 ± 0.52	615 ± 3.47	45.7 ± 2.21	19.85 ± 2.80	9.46 ± 1.95	0.44 ± 0.071	0.36 ± 0.08	0.39 ± 0.013
Sleep deprived	152.8 ± 8.90	7.51 ± 0.74	2.53 ± 1.08	723 [a] ± 17.18	60.6 ± 2.06	30.3 [a] ± 5.0	16.72 [a] ± 1.57	0.19 [a] ± 0.02	0.42 ± 0.06	0.23 [a] ± 0.018

The increase glucose concentration after sleep deprivation in concomitant with low insulin level is consistent with ***Zimberg et al. (2012).*** Also ***Vorona et al. (2005)*** found an association between short sleep deprivation and higher risk for type II diabetes. These results agree also with ***Tsujimura et al. (2009)*** who recorded hyperglycemia and higher glycated hemoglobin (HbA1c) level after sleep rhythm disturbances. This association may be attributed to the hyperactivation of HPA axis. Obtained hyperglycemia may be due to increased cortisol level that contributes to insulin resistance ***Buxton et al. (2012)*** and declined in insulin or its sensitivity leading to exhaust pancreatic islets after sleep deprivation, ***Van Helder et al. (1993).***

Table 2: Some plasma oxidative stress and antioxidant markers, cytokines in control and sleep deprived human volunteers

Parameters / Groups	SOD (U/ml)	CAT (U/l)	GSH (mg/dl)	MDA (nmol/ml)	PC (nmol/ml)	CD4 %	CD8 %
Control	3.65 ± 0.32	247.1± 1.59	27.99 ± 0.95	11.79 ± 0.73	0.24 ± 0.03	22.76 ± 3.87	13.31 ± 3.18
Sleep deprived	2.82 [a] ± 0.21	521.7 ± 2.98	11.60 [a] ± 0.85	29.71 [a] ± 1.27	0.41 ± 0.01	41.35 ± 3.05	24.60 ± 2.11

Night sleep restriction may decrease glucose effectiveness and acute insulin response ***Spiegel et al. (1999).*** Also due to impaired glucose tolerance that associated with poor sleep quality, ***Hung et al. (2013).***The decline in insulin secretion from islets of Langerhans after sleep restriction may lead to these results, ***Rakshit et al. (2015).***In addition, reduced sleep duration or quality decline insulin sensitivity, and glucose tolerance where diabetic risk exceeds which may be due to elevation of sympathetic versus parasympathetic activity, as reported by ***Esler et al. (2001).***

Bjorvatn et al. (2007) found an association between shorter sleep duration and lipids concentration. Also, ***Zhan et al. (2014)*** showed abnormal serum lipid profile in consistent with short sleep duration. The obtained hyperlipidemia and triglycerdemia in sleep deprivation group run parallel with the result of ***Taheri, (2006)*** who found a link between short sleep duration and obesity. These results may be due to chronic circadian

rhythm sleep-wake disorders, ***Sack et al. (2007)*** which is accompanied change in leptin level, after sleep restriction leading to obesity ***Cassidy and Tong (2017).***

Excess plasma cortisol level in sleep deprived group is in agreement with that obtained by ***Chapotot et al. (2001).*** The resulted excess cortisol level in sleep deprived group is also in consistent with ***Crispim et al. (2011)*** which reflect the impairment of HPA axis regulation resulting in glucocorticoid overload. ***Hirotsu et al. (2015)*** showed that, sleep and stress interact in sharing to affect CNS. Increased cortisol level may be also due to physiological stress and/or metabolic challenges caused by sleep deprivation as mentioned by ***Minkel et al. (2012).***

The increased cortisol level may be also as a result of regeneration of active cortisol from its inactive form by the expression of active β-hydroxy steroid dehydrogenase in liver and adipose tissue which modulate HPA axis activity, an explanation which is in agreement with reported by***Tomlinson et al. (2004).*** This increase in cortisol level is in parallel with the results of ***Kenneth et al. (2015),*** who mentioned that, sleep deprivation increased plasma cortisol level, where stress rating increased during sleep deprivation.

Sleep restriction may lead to metabolic disturbance and disorders leading to energy expenditure which cause fatigue, ***Buman et al. (2011).***

Oxidative stress occurs due to an imbalance between formation of oxidants and the strength of antioxidant defense leading to oxidation of lipid, protein, and nucleic acid ***Gopalakrishnan et al. (2004).*** Sleep deprivation accompanied with oxidative stress in this study was also noticed by ***Alzoubi et al. (2012)*** lead to theses result and probably due to sympathetic activation.

In addition excessive production of ROS due to exposure to oxidative stress after sleep disturbance may lead to these results, ***Kanabrioki et al. (2002).*** The significant decrease in SOD activity and increase in CAT activity is suggestion to oxidative stress. The obtained disturbance in SOD, CAT, MDA and PC as oxidative stress markers after sleep deprivation may be attributed to sympathetic activation as mentioned by ***Cohen et al. (2012).*** The resulted abnormalities in antioxidant parameters in sleep deprived group may be related to the suggestion that during sleep glutathione and uridine may aid the brain oxidative detoxification by facilitating γ-amino butyric acid related transmission and inhibiting glutamatergic transmission, ***Onaolapo et al. (2016).***

The obtained decline in melatonin levels in sleep deprived group, are in agreement with ***Vinogradova et al. (2010)*** who noticed that, sleep deprivation is accompanied by disruption of circadian rhythm of melatonin, cortisol and cytokine formation. For melatonin levels, ***Wright et al. (2013)*** noticed that, circadian melatonin occur at different phase angles relative to sleep. Melatonin decreased in peoples suffer from sleep restriction, may be as a consequence of decrease of tryptophan an essential amino acid responsible for melatonin synthesis, where these persons depend mostly on unhealthy food filled with carbohydrates and snakes ***Cipolla-Neto et al. (2014).***

Ghrelin and leptin affecting the brain to promote body satiety or hunger, ***Muccioli et al. (2002).*** The obtained declines in leptin in concomitant with raised ghrelin are in agreement with ***Spiegel et al. (2004)*** who showed decreased leptin in sleep restricted group. They also reported that, ghrelin level were higher at sleep restricted persons. In addition, ***Mullington et al. (2003)*** showed that, sleep deprivation showed decrease leptin secretion and increase that of ghrelin that may be attributed to increase in food intake due to increase hunger and appetite for carbohydrate food as energy supplement ***Bodosi et al. (2004).*** The resulted increase in ghrelin level may be through activation of ACTH at night, where ghrelin level stimulates ACTH secretion as reported by ***Weikel et al. (2003)***. Also, ***Taheri et al. (2004)*** clarified that, shorter sleep duration in human is accompanied with higher ghrelin level.

Alterations in immune parameters CD4 and CD8 in sleep deprivation were showed by ***Irwin et al. (2002). Fondell et al. (2011)*** showed variation in sleep duration which is associated with modulation of immune cell number and decrease activity of natural killer (NK) cells which produce interferon gamma (IFN-γ). Alteration in inflammatory proteins in sleep and circadian disruption run parallel with the observation of ***Chennaoui et al. (2011)***. In addition, circadian misalignment increased the pro and anti-inflammatory protein, tumor necrosis factor-α , interleukin 10 and C-Reactive protein; ***Kenneth et al. (2015).*** The alteration in CD4 and CD8 in sleep deprived may be as a result of alteration of inflammatory proteins due to the effect of glucocorticoids, ***Yeager et al. (2011).*** Sleep disturbance might cause T-helper 2 dominance ***Sakami et al. (2002)*** and increased catecholamines ***Peled et al. (1998).*** The high levels of CD8 may be as a result of some depression disorders which impaired immunity after sleep disturbance ***Suzuki et al. (2017).*** The alteration observed in immune functions may be as a result of fatigue in shift

workers and reduced natural killer cells ***Nagai et al. (2011)***.

In conclusion, the present work showed that, there are a great interrelation regarding sleep and physiological activity, oxidative stress balance, and immune systems in human body which are adversely affected by sleep deprivation.

We recommended that if possible; please don't eat during night shifts to reduce the risk of metabolic disturbance for night- workers.

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