A Potential Role for Ghrelin in Partial Sleep Deprivation Mediated Immunomodulation

Mary A. Youssef [1], *Mariam Onsy F. Hanna*[2], *Laila A. Rashed* [3].
Departments of Physiology[1], Clinical Pathology[2] and Medical Biochemistry[3], Faculty of Medicine, Cairo University, Cairo, Egypt

ABSTRACT

The mechanisms through which sleep deprivation leads to impairment of the immune system are poorly elucidated. The objectives of the present work were to study the effects of the common, real-life situation - partial sleep deprivation (PSD) on some immune aspects particularly the serum level of interleukin-6 (IL-6) which is implicated to mediate sleep-immune interaction and to investigate the impact of ghrelin, a growth hormone releasing and orexigenic hormone, expressed by the stomach and peripheral blood mononuclear cells (PBMCs) on such interaction. Knowledge gained from basic research into sleep in animals has led to marked advances in the understanding of human sleep, with important diagnostic and therapeutic implications and neurobiological studies of sleep deprivation that require invasive procedures are facilitated by the development of animal models. Short-term PSD (7 days) and long-term PSD (14 days) were associated with an increase in total leukocytes, significant reduction in the proinflammatory cytokine IL-6 and a progressive increase in ghrelin mRNA expression by both the stomach and PBMCs compared to control group. Furthermore, a significant negative correlation was found between the level of IL-6 and the expression of ghrelin by PBMCs and stomach. Interestingly, recovery sleep for 4 days had significantly increased the levels of IL-6 and partially decreased the abnormalities in ghrelin expression of sleep-deprivation although it failed to return these parameters to control group levels. Considering the data presented herein, it seems plausible that sleep is a restorative process that is important for the proper secretion of IL-6 and suggest a functional role of ghrelin as a modulator of cytokine production in sleep restriction.
Key words: *sleep, ghrelin, IL-6*

INTRODUCTION

Sleep is a fundamental physiologic process that is critical for health and life while sleep deprivation is believed to promote disease processes. Therefore, sleep is hypothesized to be a restorative process that is important for the proper functioning of the immune system. Many studies have reported alteration in immune function in response to prolonged and total sleep deprivation. For example, sustained sleep loss in rats lasting about sixteen days produced septicemia and death, an effect that has been attributed to a failure of host defense mechanisms [(1)]. Although the clinical outcome in the total sleep-deprived animals reflected immune suppression, the clinical

immune parameters measured suggested immune activation pointing to competing anti-inflammatory processes or interference with immune effector functions during sleep deprivation[2]. Despite some inconsistencies across studies of total sleep deprivation (i.e, 48–72 hours without sleep) in humans, the findings generally suggested that total sleep deprivation is associated with immune activation, namely increased white blood cell (WBC) count and alteration in granulocyte, monocyte, lymphocyte, and NK cell counts, as well as increased NK cell activity [3,4]. However, the pattern of results of partial sleep deprivation (PSD), that resembles the kind of sleep loss found in many clinical populations on the immune system, has been different in the few partial sleep deprivation experiments conducted [5, 6].

Accumulating data suggest an interaction among sleep, circadian rhythms, and interleukin-6 (IL-6) implicating disordered sleep and sleep loss in alteration of the release of IL-6[5,7], which has been proposed as mediator of an unfavorable metabolic profile, a higher risk of cardiovascular adverse events, and decreased longevity[8,9]. IL-6 and other proinflammatory cytokines are secreted by macrophages and other immune cells in response to infectious challenge and play a key role in the differentiation, maturation, and proliferation of T and B cells [5,10].

Although these data are fairly consistent in demonstrating an effect of sleep deprivation on immunity, the actual physiological underpinnings and the clinical significance of these immune changes are poorly elucidated.

Few recent studies have demonstrated an effect of sleep deprivation on plasma ghrelin level[11,12]. Ghrelin, a recently described endogenous ligand for the growth hormone secretagogue receptor (GHS-R), is produced mainly by stomach cells, however recent studies have demonstrated ghrelin to be widely distributed in the body throughout several major organ systems including the immune system[13]. Interestingly, ghrelin mRNA is expressed in peripheral T cells, B cells, neutrophils [14] as well as monocytes[13]. Major focus of research with ghrelin has been primarily related to the regulation of food intake. Ghrelin plays a central role in the energy balance by increasing food intake and body weight, coupled with a reduction in fat utilization. However, given the capacity of multiple immune subpopulations to produce this orexigenic peptide and the wide distribution of its receptor on various immune cell subsets [15], it was hypothesized that this peptide may exert immunoregulatory effects on immune cell subpopulation; however the functional role of ghrelin in regulation of immune responses in sleep deprivation states remains undefined.

Therefore, the objectives of the current study were to 1) demonstrate the effects of partial sleep deprivation for 7 and 14 days as well as recovery sleep on circulating white blood cell counts, blood leukocyte differentials and serum IL-6. 2) Study the effects of partial sleep deprivation and

recovery sleep on ghrelin mRNA expression by stomach cells as well as by immune cells. 3) Investigate a possible relation of ghrelin expressed by either the stomach or peripheral blood mononuclear cells with any change that might occur in serum IL-6 in response to partial sleep deprivation.

MATERIALS & METHODS

Forty two age matched male Sprague-Dawely rats were included in the study. Mean weight ± SD of the rats was 140±30 g. at the beginning of the study. All animals had free access to commercial rat chow diet and water. Rats were divided into the following groups:

Group 1: control group (n=16). Blood samples were gathered from 8 control rats and the animals were sacrificed to obtain the stomach. Similar 8 control rats were used to follow up their body weight at 7, 14 and 18 days for comparison of body weight with the corresponding groups.

Group 2: (n=8) rats were subjected to partial sleep deprivation (PSD) (4 hours each day from 12:00 a.m. to 04:00 a.m.) for 7 days.

Group 3: (n=9) rats were subjected to partial sleep deprivation (4 hours each day from 12:00 a.m. to 04:00 a.m.) for 14 days.

Group 4: (n=9) rats were subjected to partial sleep deprivation (4 hours each day from 12:00 a.m. to 04:00 a.m.) for 14 days, which was followed by 4 days of recovery sleep.

Partial sleep deprivation was induced by applying audio visual stimuli by a special apparatus[16]. Briefly, the apparatus produced either sound alone, light alone or both of them simultaneously at random intervals.

All animals were weighed before the start of each experimental protocol and the mean percentage change of body weight of each group were assessed at the end of the protocol. Also, retroorbital venous blood samples and smears were collected at the end of the experimental protocol between 09:00 and 10:00 a.m for assessment of total and differential leukocytic counts, IL-6 levels in blood, and gene expression of ghrelin in peripheral blood mononuclear cells. Animals were sacrificed and stomach tissue was obtained and stored at -80ºC in lysis buffer that contained guanidium thiocyanate and β-mercaptoethanol for RNA extraction and subsequent assessment of stomach ghrelin gene expression.

Studies that evaluated the 24-hour secretory pattern of IL-6 in rats had demonstrated that IL-6 levels vary across the light-dark cycle among different tissues[17]. Therefore, blood samples in the current study were gathered between 09:00 and 10:00 a.m. in all groups.

Leukocyte Count and Differential

Immediately following blood collection, 5 ul of blood was used to prepare blood smears, which were stained with Leishman and Giemsa stain for differential leukocytic counts. For the total leukocyte count (TLC), blood was diluted with 3 % acetic acid in saline solution as to lyse all the erythrocytes. Dilutions of 1:20 were prepared and all samples were mixed using a Vortex prior to evaluation. Leukocytes were counted using a hemocytometer. Numbers and

proportions of polymorphonuclear neutrophils including band neutrophils, monocytes and lymphocytes were determined.

Detection of ghrelin gene expression by RT-PCR:

RNA extraction:

RNA was extracted from both stomach tissue after homogenization and whole blood after ficoll separation of peripheral blood mononuclear cells using SV- total RNA isolation kit (Promega, Madison, USA) according to manufacturer's instructions. The concentration of extracted RNA was measured by spectrophotometer at 260 nm.

Reverse Transcription and Polymerase chain reaction (RT-PCR):

For amplification of the targets, reverse transcription and PCR were run in two separate steps. The cDNAs were generated from total RNA using AMV-RT and oligo(dT) primers. Briefly, total RNA (6 µg) were heat denatured and reverse transcribed by incubation at 42ºC for 90 min with 12.5 U avian myeloblastosis virus (AMV) reverse transcriptase (Promega, Madison, USA), 20 U ribonuclease inhibitor RNasin (Promega, USA), 200 nM deoxy-nucleoside 5'-triphosphate mixture, and 1 nM oligo(dT) primer in a final volume of 30 µl of 1x avian myeloblastosis virus reverse transcriptase buffer. The reactions were terminated by heating at 97ºC for 5 min and cooling on ice. The cDNA samples were amplified in 50 µl of 1x PCR buffer in the presence of 2.5 U Taq DNA polymerase (Promega, Madison, USA), 200 nM deoxy-nucleoside 5'-triphosphate mixture, and the appropriate primer pairs (1 nM of each primer). These sets of primers were according to the published cDNA sequences of rat ghrelin [18]; ghrelin sense: 5'-TTG AGC CCA GAG CAC CAG AAA-3' and ghrelin antisense: 5'-AGT TGC AGA GGA GGC AGA AGC T-3'.

PCR consisted in a first denaturing cycle at 97ºC for 5 min, followed by 35 cycles of amplification defined by denaturation at 96ºC for 1.5 min, annealing for 1.5 min, and extension at 72ºC for 3 min. A final extension cycle of 72ºC for 15 min was included. Annealing temperature was adjusted at 55ºC. The PCR product yielded a 347 bp fragment.

Agarose gel electrophoresis:

All PCR products were electrophoresed on 2% agarose stained with ethidium bromide and visualized by UV transilluminator.

Semi-quantitative determination of PCR products:

Semi-quantitation of the PCR products was performed using the gel documentation system (BioDO, Analyser) supplied by Biometra.
According to the following amplification procedure, relative expression of ghrelin gene (R) was calculated following the formula: R= Densitometrical units of ghrelin gene/ Densitometrical units of β-actin gene[19].

PCR detection of β-actin

The "house-keeping" gene β-actin was assessed by PCR for the presence of RNA in all samples and for semi-quantitation of PCR products. cDNA was generated from 1µg of total RNA extracted with AMV reverse transcriptase for 60 min at 37°C. For PCR, 4µl cDNA was

incubated with 30.5 µl water, 4 µl 25 mM $MgCl_2$, 1 µl dNTPs (10 mM), 5 µl 10x PCR buffer, 0.5 µl (2.5U) Taq polymerase and 2.5 µl of each primer containing 10 pmol β-actin primers (forward 5' TGTTGTCCCTGTATGCCTCT 3' and reverse 5' TAATGTCACGCACGATTTCC 3'). The reaction mixture was subjected to 40 cycles of PCR amplification as follows: denaturation at 95 °C for 1 min, annealing at 57 °C for 1 min and extension at 72 °C for 2 min. The PCR product yielded a 206 bp fragment.

Measurement of IL-6:

Serum level of IL-6 was measured by enzyme linked immunosorbent assay (ELISA) using a kit supplied by Biosource Diagnostics, USA according to the manufacturers' instructions.

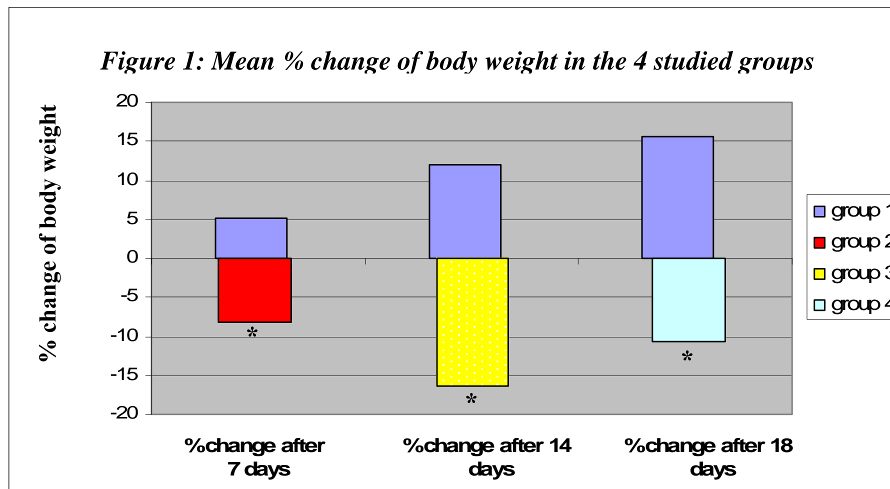
Statistical Analysis:

The results were analyzed using SPSS computer software package, version 10.0 (Chicago-IL, USA) [20]. Data were presented as mean ± S.D. Differences among the parameters of the different groups were compared by one-way analysis of variance (ANOVA). Median was used in attempt to show the differences between band neutrophils among the different studied groups. The results were considered statistically significant at $P \leq 0.05$.

RESULTS

Body weight

Sleep-deprived rats showed significant progressive weight loss compared to the control group. The percentage of body weight loss was only 8.1% in sleep-deprived rats after 7 days of PSD (group 2), and eventually body weight had dropped by 16.4 % after 14 days of PSD (group 3). Four days of recovery sleep led to a decrease in the percentage of body weight loss compared to group 3, however it did not reach statistical significance (Figure 1).



*: Significant compared to group 1.

Total and differential leukocytic counts

PSD for 7 days had significantly increased the total leukocytic counts compared with the control group; however that increase was reduced by extending the PSD for 14 days (group 3) resulting in a non significant difference between group 3 and the control group. On the other hand, recovery sleep after 14 days of PSD led to further reduction of TLC with values comparable to those of group 1 (Figure 2 and table 1).

Neutrophils as a proportion of total leukocytes increased significantly (Table 1) from 16.38 ± 7.6% in the control group to 35.63 ± 9.27% after 7 days of PSD in group 2 (P<0.01). However, group 3, which was subjected to 14 days of PSD, showed a smaller increase in the proportion of neutrophils compared to group 1, as 21.11 ± 2.93% of the total leukocytes, while recovery sleep in group 4 caused a further decrease in the proportion of neutrophils to 14.11 ± 5.73% to become comparable to those of group 1.

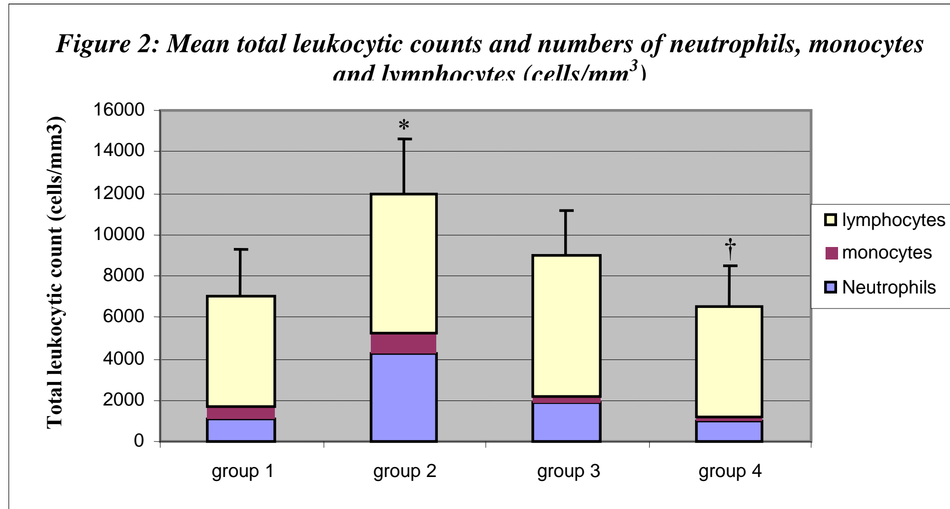
Among neutrophils, the mean proportion and number of bands (immature neutrophils) were significantly increased in group 2 compared with group 1 (P<0.05) (table 1 and figure 3). There was also an increase in the mean proportion and number of band neutrophils in group 3 compared to group 1, although it did not reach statistical significance. Also, the median value of band neutrophils in group 3 (median=2) was higher compared to that of group 1 (median= 0.5, P<0.05).

The proportion of blood leukocytes composed of lymphocytes were significantly decreased in group 2 compared to the control group while they returned to near baseline value in group 3 and 4 (Table 1). The mean number of lymphocytes did not differ

significantly between the control group and either group 2 or 3.

As a proportion of total leukocytes, monocytes did not differ between group 1 and 2, although a significant decrease in monocyte proportion was observed in group 3 and 4 compared to either group 1 or 2. The mean number of monocytes increased significantly in group 2 and decreased significantly in group 3 compared to the control group (P<0.05).

Figure 2: Mean total leukocytic counts and numbers of neutrophils, monocytes and lymphocytes (cells/mm^3)



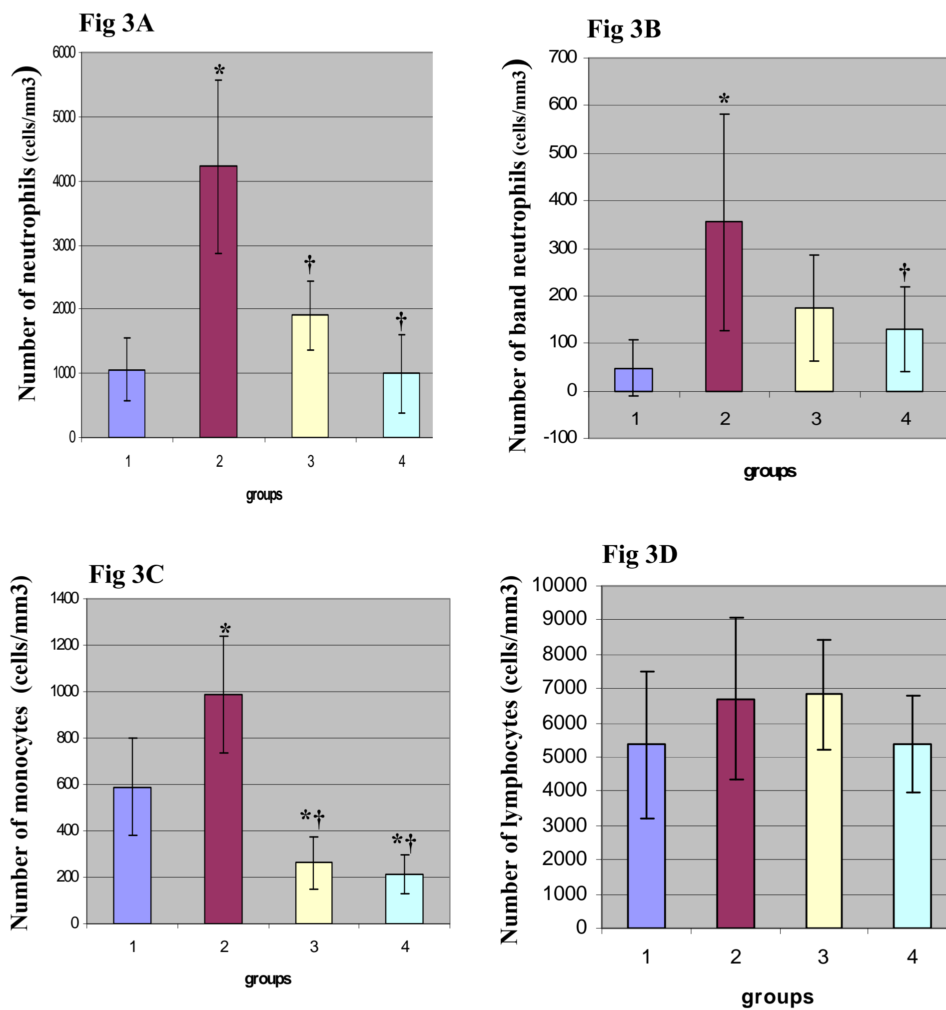
Regarding the mean total leukocytic counts;
**: Significant compared to group 1 at p<0.05.*
†: Significant compared to group 2 at p<0.05.
‡: Significant compared to group 3 at p<0.05.

Table (1): Means and standard deviations (SD) of TLC; neutrophil, monocyte and lymphocyte as proportions of TLC in the 4 studied groups.

	TLC Cells/mm^3	*Neutrophils (%)*	*Band neutrophils (%)*	*Monocytes (%)*	*Lymphocytes (%)*
Group (1)	7000±2267.7	16.38±7.61	0.6±0.7	8.37±1.5	75.25±7.45
Group (2)	11975±2672.4*	35.63±9.27*	3.2±2.4*	8.62±3.06	55.25±9.42*
Group (3)	9000± 2137.7	21.11±2.93†	2.00±1.32	2.89±1.05*†	76±2.82†
Group (4)	6600±1964.6†	14.11±5.73†	2.11±1.45	3.33±1.11*†	82±5.31†

**: Significant compared to group 1 at p<0.05.*
†: Significant compared to group 2 at p<0.05.
‡: Significant compared to group 3 at p<0.05.

Figure 3: Means and SD of neutrophil (3A), band neutrophil (3B) monocytes (3C) and lymphocytes (3D) numbers in the 4 studied groups

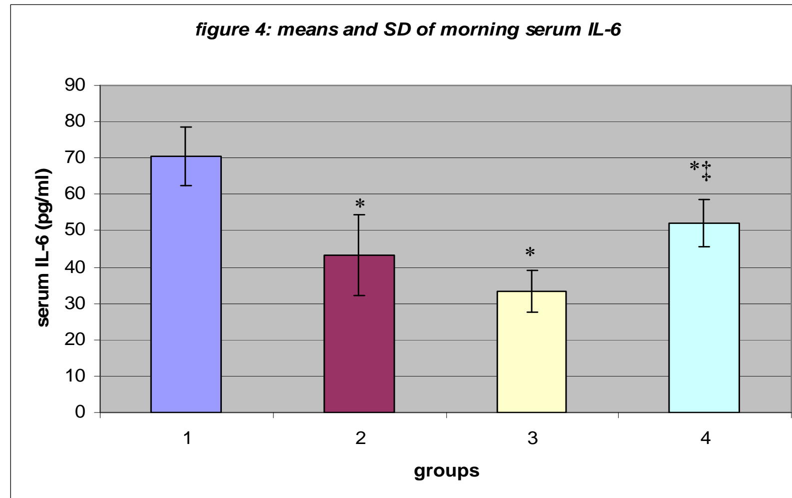


**: Significant compared to group 1 at p<0.05.*
†: Significant compared to group 2 at p<0.05.
‡: Significant compared to group 3 at p<0.05.

*†

Serum IL-6

To further evaluate the effects of PSD on immune system, morning serum level of the pro-inflammatory cytokine IL-6 was measured in the 4 groups. Figure 4 shows significant reduction of serum IL-6 in group 2 with further reduction in group 3 compared to that of group 1. Serum IL-6 levels were significantly higher after recovery sleep for 4 days compared to those of group 3, although significantly lower than group 1.



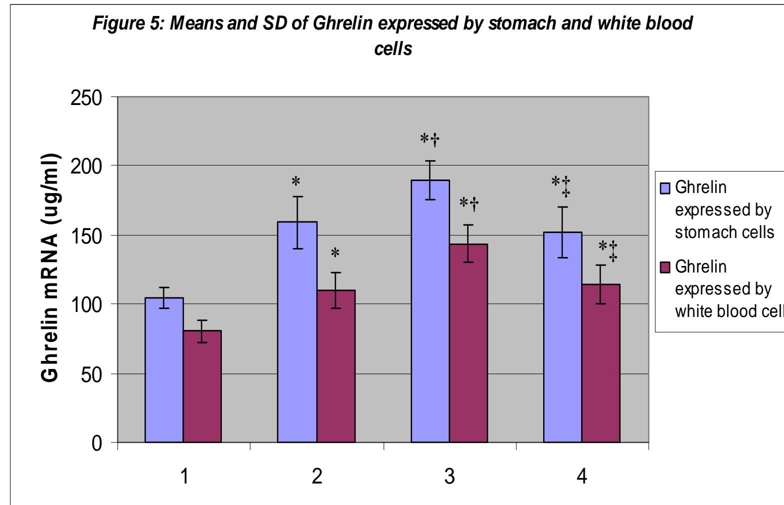
**: Significant compared to group 1 at p<0.05.*
†: Significant compared to group 2 at p<0.05.
‡: Significant compared to group 3 at p<0.05.

Expression of ghrelin

To study the effects of PSD on ghrelin expression and the relation between ghrelin and the immune function, ghrelin mRNA was measured in both the PBMCs and stomach tissues. As figure 5 reveals, ghrelin expression by the stomach and PBMCs were highly responsive to PSD, displaying statistically significant increase after 7 days of PSD (group 2) with further increase in group 3 (14 days of PSD) compared to group 1. Although ghrelin expression by stomach and white blood cells decreased significantly in response to recovery sleep when compared to group 3, it was still significantly overexpressed compared to the control group.

Considering the recent notion that ghrelin is a peptide endogenously produced by immune cells that is affected by sleep deprivation, and plays a role in controlling immune functions, the relation between the expression of ghrelin and serum IL-6 was studied in the present work (Figure 6). A negative correlation was found between the expression of

ghrelin by PBMCs and morning level of serum IL-6 (r = -0.740, P<0.01) in the studied groups. A similar correlation was also detected between the expression of ghrelin in the stomach and level of serum IL-6 (r = -0.729, P<0.01).



**: Significant compared to group 1 at p<0.05.*
†: Significant compared to group 2 at p<0.05.
‡: Significant compared to group 3 at p<0.05.

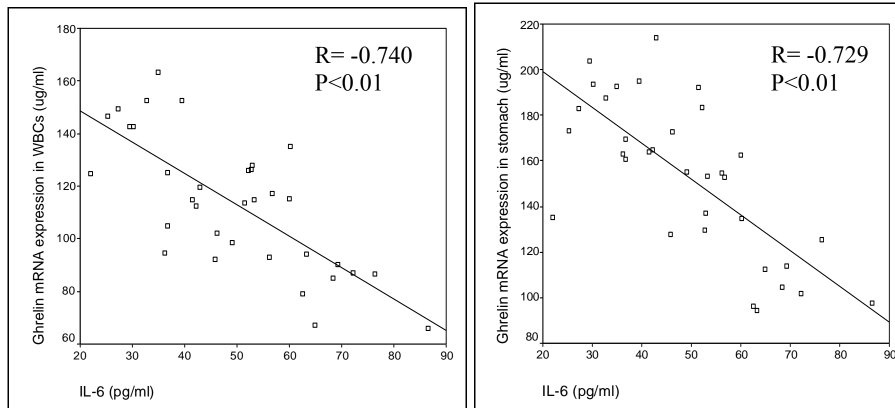


Figure 6: Relationship between serum IL-6 and Ghrelin mRNA expression

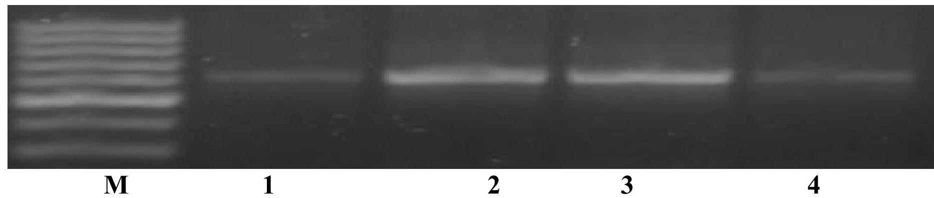


Figure 7: An agarose gel electrophoresis showing PCR products of gherlin gene expression (347 bp): *Lane M: PCR marker (50, 150, 300, 500, 700, 1000 bp), Lane 1: PCR product in control group, Lane 2: PCR product in group 2 (PSD for 7 days), Lane 3: PCR product in group 3 (PSD for 14 days), Lane 4: PCR product in group 4 (Recovery sleep for 4 days).*

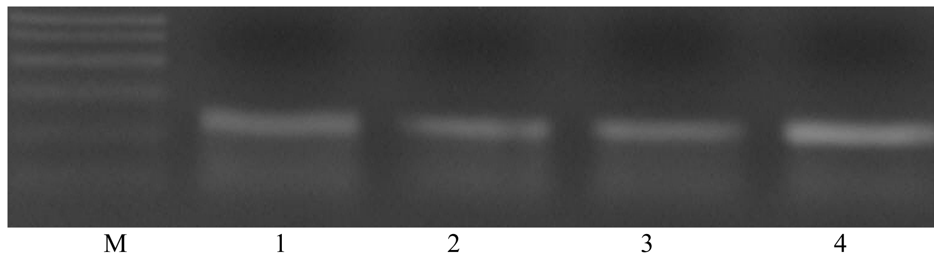


Figure 8: An agarose gel electrophoresis showing PCR products of beta actin gene expression: product size (206 bp). *Lane M: PCR marker (50, 150, 300, 500, 700, 1000 bp), Lane 1: PCR product in control group, Lane 2: PCR product in group 2 (PSD for 7 days), Lane 3: PCR product in group 3 (PSD for 14 days), Lane 4: PCR product in group 4 (Recovery sleep for 4 days).*

DISCUSSION

Sleep loss is endemic in society, and loss of sleep during only part of the night is one of the most common complaints of persons who experience environmental or psychological stress, travel across time meridians, engage in shift work, or suffer from a psychiatric disorder. The specific systems and mechanisms affected by sleep deprivation that may perpetuate disease processes still are speculative. Knowledge gained from basic research into sleep in animals has led to marked advances in the understanding of human sleep, with important diagnostic and therapeutic implications[(21)]. Neurobiological studies of sleep deprivation that require invasive procedures are facilitated by the development of animal models[(22)].

Significant detrimental effects on immune functioning have been reported after a few days of total sleep deprivation. However, most studies applied short-lasting total sleep deprivation and not restriction of sleep over a longer period of time, as often occurs in human society. One of the

goals of the present study was to evaluate some of the effects of partial sleep deprivation (PSD) on the immune system. To this end, circulating white blood cell counts, differential counts as well as the level of serum IL-6 were assessed in an animal model in response to PSD for 7 days (group 2) and 14 days (group 3) as well as recovery sleep for 4 days (group 4) .

In the present study, PSD for 7 days showed a significant increase in total leukocytes compared with control values. With more prolonged PSD for 14 days the TLC was still higher than the controls, although did not reach statistical significance. After 4 days of recovery sleep, TLC returned to baseline levels. Leukocytosis induced by sleep deprivation has been a consistent finding in most animal and human sleep deprivation studies[2,23], although few studies had reported absence of any effect of PSD on total WBC count in humans[24]. However, the study from which that notion was drawn examined the effects of only 2 nights of PSD.

The findings of the present study showed that PSD altered some classes of leukocytes. PSD for 7 days is associated with a significant rise in neutrophils and monocytes as well as a significant decrease in lymphocytes compared to controls. This was associated with the appearance of a significant increase in circulating band neutrophils. These findings are compatible with the assumption that sleep deprivation is commonly associated with bacterial infection of internal tissues[25] and increased vulnerability to opportunistic pathogens[26]. These possible bacterial infections have been commonly attributed to suppression of host defense mechanisms against pathogens[1,27] particularly that suppression of different types of immunity after PSD has been pronounced in many studies [24,28,29]. This is consistent with what was reported in clinical populations who show disordered sleep due to stress or a variety of diseases, including depression, alcohol dependence, and human immunodeficiency virus (HIV) infection, in whom decrements in natural and cellular immunity coincide with disturbances of sleep architecture and loss of sleep [30, 31].

The present findings of altered leukocyte populations in PSD are consistent with those reported by Everson[2] in rats and Kerkhofs et al.[32] in postmenopausal women. Also, Ruiz et al.[33] observed that loss of sleep induced lymphopenia in a type 1 diabetes model and suggested that sleep deprivation should be considered a risk factor in the onset of autoimmune disorders. Other studies had reported absence of an effect of sleep deprivation[34,35]. These different outcomes in terms of leukocyte numbers could be attributed to different deprivation schedules.

Moreover, in the present study more prolonged PSD (14 days) led to a trend towards increased TLC and increased neutrophils associated with rise in immature granulocytes compared to control group which may suggest persistence of infection while monocytes were significantly decreased compared to control. Although the stress induced by PSD appears as one of the putative mechanisms involved in the present findings, it has been observed that serum corticosteroids are not increased by sleep deprivation[36,37]

suggesting that glucocorticoid excess is not a leading mediator. These data implicate sleep in the modulation of leukocyte populations and demonstrate that even a modest disturbance of sleep for 7 days alters the immune outcome particularly in terms of leukocyte numbers and types. This is further supported by the finding that after 4 days of recovery sleep, TLC and neutrophils returned to baseline levels.

White blood cells are known to produce the inflammatory biomediator IL-6 [(38)] and recently, Dimitrov et al [(39)] have suggested that the underlying sleep-immune interaction appears to rely critically on cytokines, like IL-6 that combine effects on immune and neuronal functions. Although, PSD was associated with an increase in total leukocytes, the results of the present study showed a significant decrease in the level of serum IL-6 in both short and long-term PSD compared to the control group. Recovery sleep had significantly increased the levels of IL-6 compared to long term PSD although it failed to return to control group levels.

There is considerable heterogeneity in studies linking sleep deprivation and the secretion of IL-6. In agreement with our results and consistent with the idea that sleep has a restorative function, normal sleep was found to be associated with enhancement of IL-6 release[(3)] while loss of sleep served to decrease IL-6 levels with effects on the integrity of immune system function[(5)]. Dimitrov et al.[(39)] demonstrated that sleep enhances serum IL-6 and induces IL-6 trans-signaling, potentiating greatly its effect on the immune system. Therefore, loss of sleep during part of the night may be one factor exacerbating immunological alterations in persons who experience psychological stress or suffer from a psychiatric disorder and commonly complain of insomnia [(40)].

On the other hand, other studies were apparently at odds with the present work. For example, Irwin et al.,[(6)] reported an increase in monocyte production of IL-6 after one night of PSD. Such discrepant results may be explained by the fact that IL-6 is produced by numerous types of immune and non immune cells [(41)] and only about 20% of circulating IL-6 is derived from peripheral blood cells [(42)]. In another study, increased serum IL-6 has been reported in subjects whose sleep was reduced to 6 hours for 1 week [(7)] and some studies have failed to find effects of sleep loss on IL-6 [(3,4)]. Although these discrepant results may be explained in part by differences in experimental design, it should be noted that findings of increased serum IL-6 have been called into question on the basis of evidence that local cytokine production in response to indwelling venous catheters and implanted cortical electrodes used in most of the previously mentioned studies, may create false-positive findings [(43)].

Among the mechanisms underlying the reverse relation between sleep loss and IL-6 secretion, the body weight and melatonin secretion need to be considered. Regarding the body weight, it was found that IL-6 correlates positively with body mass index [(44)] and up to 30% could be derived from adipose tissue[(45)]. Therefore, in the present study it may seem that the weight loss

observed with short and long PSD may be one of the factors contributing to decreased circulating IL-6. Previous studies have shown that, during sleep deprivation, rats are hyperphagic but, paradoxically, losing weight which has been attributed to increased energy expenditure and loss of fat content [46] or as a response to infectious processes[47]. However, recovery sleep for 4 days did not significantly reduce the percentage change of body weight in comparison to that of prolonged PSD, but it had significantly raised IL-6 levels. Thus, in view of previously reported data that melatonin enhances IL-6 production by PBMCs[48] and that melatonin secretion decreases with sleep disruption[49], it can be postulated that the sleep deprived rats may have a low melatonin level and subsequently low levels of serum IL-6.

Finally, the sleep loss associated modulation in the proinflammatory cytokine IL-6 levels may be mediated by other endocrine parameters. It has been reported that the recently discovered hormone, ghrelin has potent anti-inflammatory effect[50]. Ghrelin is a peptide hormone originally identified as the endogenous ligand of the growth hormone secretagogue receptor. Given the potent effect of ghrelin on cytokine expression[13] and its strong link to sleep deprivation[11], it can be hypothesized that ghrelin may function as a key signal coupling the neural axis to the immune system. Ghrelin which is predominantly produced by the stomach was recently detected in lymphoid organs[51] and various leukocyte subsets[13,14], suggesting a possible immunoregulatory effect of ghrelin produced by immune cells in sleep deprived situations. Therefore, in the present study, we attempted to study ghrelin expression in PBMCs and stomach in response to PSD and find a possible correlation between ghrelin expression and serum IL-6.

The results of the present study demonstrated that PSD for 7 or 14 days resulted in a significant increase in the expression of ghrelin in both PBMCs and stomach tissue compared to controls with a significantly higher level in long term compared to short term PSD. Recovery sleep had partially decreased the abnormalities in ghrelin expression of PSD. The current findings are in accord with studies which showed that short sleep duration leads to higher levels of serum ghrelin[11,52,53]. Also, Taheri and colleagues' study [54] is congruent with the idea that inadequate sleep enhances ghrelin secretion, which in turn acts as an endogenous sleep factor. There is evidence that administration of ghrelin can promote sleep[55,56], although these studies did not examine the expression of ghrelin in different tissues in response to sleep restriction.

The mechanisms through which sleep restriction achieves such a rapid effect on ghrelin mRNA expression are not known. Loss of body weight with PSD observed in the present work could be one factor contributing to the significant elevation in ghrelin expression in both the stomach and PBMCs. However, it could not be the sole factor because recovery sleep for 4 days did not significantly reduce the percentage change of body weight in comparison to that of prolonged PSD, but it had significantly lowered the expression of ghrelin in both types of

cells. Reduction in BMI was reported to be associated with an increase in ghrelin and a decrease in leptin[57]. Based on current knowledge, ghrelin released during negative energy balance situations plays an important role in maintaining GH release and stimulating appetite[46]. Therefore, it can be hypothesized that high ghrelin level could contribute for increased appetite but not for weight loss found with sleep restriction. Similarly, it has been established that circulating ghrelin concentrations under conditions presenting with low body fat mass, as for example in cancer cachexia [58] and anorexia nervosa [59] are significantly elevated, suggesting a compensatory rather than contributory effect of ghrelin in these situations. In addition, Taheri et al.[54] reported that experimental curtailment of sleep increases serum ghrelin independent of body mass index, age, sex and other possible confounding factors.

Based on current published data demonstrating the presence of systemic infection and inflammation in sleep deprived rats [25,60], high ghrelin levels might be part of a complex immuno-neuro-endocrine response activated by systemic infection and inflammation [61]. Recent studies have shown that sleep deprivation induces an elevation of some inflammatory proteins such as; E-selectin, ICAM-1, IL-1beta, and IL-1ra[60]. Therefore, the other possible role of ghrelin in sleep restriction might be its novel anti-inflammatory properties[62]. This is of particular interest in light of recent findings revealing that treatment with ghrelin significantly down-regulates circulating levels of pro-inflammatory cytokines in rat models of endotoxemia and sepsis [63,64] and the potential therapeutic use of ghrelin in the management of inflammation.

Interestingly, a significant negative correlation was found in the present work between the expression of ghrelin by PBMCs and the level of serum IL-6, possibly implicating ghrelin produced by WBCs in reducing IL-6 production and suggesting a functional role of ghrelin as a modulator of cytokine production. A similar correlation was also detected between the expression of ghrelin in stomach cells and level of serum IL-6.

The wide distribution of ghrelin expression and its receptor in PBMCs may suggest multiple paracrine and autocrine roles of the endogenously produced ghrelin, where it can inhibit cytokine activation, including interleukins[13]. Xia et al.[65] showed that ghrelin dose-dependently suppresses T helper 1 (IL-2 and IFN-γ) and T helper 2 (IL-4 and IL-10) cytokine mRNA expression. This is consistent with the present findings of a negative correlation between ghrelin expression and IL-6, a cytokine produced by T helper 2 lymphocytes [66]. In one study, plasma IL-6 concentration at rest in fed subjects was negatively correlated with plasma ghrelin concentration [67].

In conclusion, short and long term PSD is associated with alteration in some immune parameters in the form of altered leukocyte numbers and types and reduction in serum IL-6 levels. The present work shows that ghrelin expression increases with PSD. The significant negative correlation observed between the expression of ghrelin and the

proinflammatory cytokine IL-6 in PSD suggests that ghrelin may function as a signal coupling the neural axis to the immune system and may modulate inflammatory processes in PSD.

REFERENCES

1. **Everson CA (1993):** Sustained sleep deprivation impairs host defense. *Am. J. Physiol.* 265: R1148–54.
2. **Everson CA (2005):** Clinical assessment of blood leukocytes, serum cytokines, and serum immunoglobulins as responses to sleep deprivation in laboratory rats. *J. Physiol. Regul. Integr. Comp. Physiol.* 289: R1054-R1063.
3. **Born J, Lange T, Hansen K, Mölle M, Fehm HL (1997):** Effects of sleep and circadian rhythm on human circulating immune cells. *J. Immunol.* 158: 4454–64.
4. **Dinges DF, Douglas SD, Hamarman S, Zaugg L, Kapoor S (1995):** Sleep deprivation and human immune function. *Adv. Neuroimmunol.* 5: 97–110.
5. **Redwine L, Hauger RL, Gillin JC, Irwin M (2000):** Effects of sleep and sleep deprivation on interleukin-6, growth hormone, cortisol, and melatonin levels in humans. *J. Clin. Endo. & Metabo.* 85:10 3597-3603.
6. **Irwin MR, Wang M, Campomayor CO, Collado-Hidalgo A, Cole S (2006):** Sleep Deprivation and Activation of Morning Levels of Cellular and Genomic Markers of Inflammation. *Arch. Intern. Med.* 166:1756-1762.
7. **Vgontzas AN, Zoumakis E, Bixler EO, Lin HM, Follett H, Kales A, Chrousos GP (2004):** Adverse effects of modest sleep restriction on sleepiness, performance, and inflammatory cytokines. *J. Clin. Endocrinol. Metab.* 89: 2119–2126.
8. **Harris TM, Ferrucci L, Tracy RP, Corti MC, Wacholder S, Ettinger WH Jr, Heimovitz H, Cohen HJ, Wallace R (2000):** Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. *Am. J. Med.* 106: 506–512.
9. **Ridker PM, Rifai N, Stampfer MJ, Hennekens CH (2000):** Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation* 101: 1767–1772.
10. **Papanicolaou DA, Wilder RL, Manolagas SC, Chrousos GP (1998):** The pathophysiologic roles of interleukin-6 in human disease. *Ann. Intern. Med.* 128: 127–137.
11. **Spiegel K, Tasali E, Penev P, Cauter EV (2004):** Brief Communication: Sleep curtailment in healthy young men is associated with decreased leptin levels, elevated ghrelin levels, and increased hunger and appetite. *Annals of internal medicine* 141: 846-850.
12. **Dzaja A, Dalal MA, Himmerich H, Uhr M, Pollmächer T, Schuld A (2004):** Sleep enhances nocturnal plasma ghrelin levels in

healthy subjects. *Am. J. Physiol. Endocrinol. Metab.* 286: E963-E967.

13. **Dixit VD, Schaffer EM, Pyle RS, Collins GD, Sakthivel SK, Palaniappan R, Lillard JW Jr, Taub DD (2004):** Ghrelin inhibits leptin and activation induced proinflammatory cytokine expression by human monocytes and T cells. *J. Clin. Invest.* 114(1): 57–66.
14. **Hattori N, Saito T, Yagyu T, Jiang BH, Kitagawa K, Inagaki C (2001):** GH, GH receptor, GH secretagogue receptor, and ghrelin expression in human T cells, B cells, and neutrophils. *J. Clin. Endocrinol. Metab.* 86: 4284–4291.
15. **Dixit VD, Taub DD (2005):** Ghrelin and immunity: A young player in an old field. *Exp. Gero.* 40: 900-910.
16. **Persengiev SP, Kanchev LN (1991):** Melatonin and adrenal cortex steroid production: in vivo and in vitro studies. *Folia. Histochem. Cytobiol.* 29(1):15-8.
17. **Guan Z, Vgontzas AN, Omori T, Peng X, Bixler EO, Fang J (2005):** Interleukin-6 levels fluctuate with the light-dark cycle in the brain and peripheral tissues in rats. *Brain. Behav. Immun.* 19(6):526-9.
18. **Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K (1999):** Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 402: 656–660.
19. **Zhang B, Rao Q, Zheng G, Cao Z, Ma X, Li G, Lin Y, Geng Y, Wu K (2003):** Expression and significance of IL-18 in the bone marrow of patients with hematological diseases. *Chin. Med. J. (Engl.)* 116(2):218-21.
20. **Norusis M (1997):** SPSS 7.5 Guide to data analysis, Simon and Schuster Company, Upper Saddle River. New Jersey.
21. Mahowald MW, Schenck CH (2005): Insights from studying human sleep disorders. *Nature* 437(7063):1279-85.
22. **Lopez-Rodriguez F, Kim J, Poland RE (2004):** Total sleep deprivation decreases immobility in the forced-swim test. *Neuropsychopharmacology* 29(6):1105-11.
23. **Dinges DF, Douglas SD, Zaugg L, Campbell DE, McMann JM, Whitehouse WG, Orne EC, Kapoor SC, Icaza E, and Orne MT (1994):** Leukocytosis and natural killer cell function parallel neurobehavioral fatigue induced by 64 hours of sleep deprivation. *J. Clin. Invest.* 93: 1930–1939.
24. **Irwin M, Mccuntick J, Costlow C, Fortner M, Wihte J and Gillin C (1996):** Partial night sleep deprivation reduces natural killer and cellular immune responses in humans. *Faseb. J.* 10: 643-653.
25. **Everson CA, Toth LA (2000):** Systemic bacterial invasion induced by sleep deprivation. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 278: R905-R916.
26. **Benca RM, Quintans J (1997):** Sleep and host defenses: a review. *Sleep* 20: 1027–37.
27. **Horohov DW, Pourciau SS, Mistric L, Chapman A, Ryan DH (2001):** Increased dietary fat

prevents sleep deprivation-induced immune suppression in rats. *Comp. Med.* 51(3): 230-3.

28. **Irwin M, Mascovich A, Gillin JC, Willoughby R, Pike J, Smith TL (1994):** Partial sleep deprivation reduces natural killer cell activity in humans. *Psychosom. Med.* 56: 493–498.
29. **Zager A, Tufik S, Antunes IB, Ruiz FS and Anderson ML (2007):** Effects of acute and chronic sleep loss on immune modulation of rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol*. 293(1): R504-9.
30. **Irwin M. (1999):** Immune correlates of depression. *Adv. Exp. Med. Biol.* 461:1–24.
31. **Darko DF, Miller JC, Gallen C, White J, Koziol J, Brown SJ, Hayduk R, Atkinson JH, Assmus J, Munnell DT, Naitoh P, McCutchan JA, Mitler MM (1995):** Sleep electro-encephalogram δ-frequency amplitude, night plasma levels of tumor necrosis factor α, and human immunodeficiency virus infection. *Proc. Natl. Acad. Sci.* 92:12080–12084.
32. **Kerkhofs M, Boudjeltia KZ, Stenuit P, Brohée D, Cauchie P, Vanhaeverbeek M (2007):** Sleep restriction increases blood neutrophils, total cholesterol and low density lipoprotein cholesterol in postmenopausal women: A preliminary study. *Maturitas* 56(2):212-5.
33. **Ruiz FS, Andersen ML, Zager A, Martins RC, Tufik S (2007):** Sleep deprivation reduces the lymphocyte count in a non-obese mouse model of type 1 diabetes mellitus. *Braz. J. Med. Biol. Res*. 40(5):633-7.
34. **Renegar KB, Floyd RA, Krueger JM (1998):** Effects of short-term sleep deprivation on murine immunity to influenza virus in young adult and senescent mice. *Sleep* 21: 241–8.
35. **Haack M, Schuld A, Kraus T, Pollmächer T (2001):** Effects of sleep on endotoxin-induced host responses in healthy men. *Psychosomatic. Medicine.* 63: 568-578.
36. **Everson CA, Crowley WR (2004):** Reductions in circulating anabolic hormones induced by sustained sleep deprivation in rats. *Am. J. Physiol. Endocrinol. Metab.* 286: E1060–E1070.
37. **Altemus M, Rao B, Dhabhar FS, Ding W, Granstein RD (2001):** Stress-induced changes in skin barrier function in healthy women. *J. Invest. Dermatol.* 117: 309–317.
38. **Leng S, Xue QL, Huang Y, Semba R, Chaves P, Bandeen-Roche K, Fried L, Walston J (2005):** Total and differential white blood cell counts and their associations with circulating interleukin-6 levels in community-dwelling older women. *J. Gerontol. A. Biol. Sci. Med. Sci*. 60(2):195-9.
39. **Dimitrov S, Lange T, Benedict C, Nowell MA, Jones SA, Scheller J, Rose-John S and Born J (2006):** Sleep enhances IL-6 trans-signaling in humans. *FASEB Journal* 20:2174-2176.
40. **Hall M, Baum A, Buysse DJ, Prigerson HG, Kupfer DJ, Reynolds CF (1998):** Sleep as a

mediator of the stress-immune relationship. *Psychosom. Med.* 60:48–51.

41. **Vgontzas AN, Bixler EO, Lin HM, Prolo P, Trakada G, Chrousos GP (2005):** IL-6 and its circadian secretion in humans. *Neuroimmunomodulation* 12(3):131-40.
42. **Vgontzas AN, Papanicolaou DA, Bixler EO, Kales A, Tyson K, Chrousos GP (1997):** Elevation of plasma cytokines in disorders of excessive daytime sleepiness: role of sleep disturbance and obesity. *J. Clin. Endocrinol. Metab.* 82: 1311–1312.
43. **Haack M, Kraus T, Schuld A, Dalal M, Koethe D, and Pollmacher T (2002):** Diurnal variations of interleukin-6 plasma levels are confounded by blood drawing procedures. *Psychoneuroendocrinology* 27: 921–931.
44. **Vozarova B, Weyer C, Hanson K, Tataranni PA, Bogardus C, Pratley RE (2001):** Circulating interleukin-6 in relation to adiposity, insulin action, and insulin secretion. *Obes. Res.* 9:414–417.
45. **Mohamed-Ali V, Goodrick S, Rawesh A, Katz DR, Miles JM, Yudkin JS, Klein S, Coppack SW (1997):** Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-α, in vivo. *J. Clin. Endocrinol. Metab.* 82: 4196–4200.
46. **Martins PJ, D'Almeida V, Nobrega JN, Tufik S (2006):** A reassessment of the hyperphagia/ weight-loss paradox during sleep deprivation. *Sleep* 29(9): 1233-8.
47. **Hipólide DC, Suchecki D, Pimentel de Carvalho Pinto A, Chiconelli Faria E, Tufik S, Luz J (2006):** Paradoxical sleep deprivation and sleep recovery: effects on the hypothalamic-pituitary-adrenal axis activity, energy balance and body composition of rats. *J. Neuroendocrinol.* 18(4): 231-8.
48. **García-Mauriño S, González-Haba MG, Calvo JR., Rafii-El-Idrissi, M, Sánchez-Margalet V, Goberna, R, Guerrero JM (1997):** Melatonin enhances IL-2, IL-6 and IFNγ production by human circulating CD4+ cells: A posible nuclear receptor-mediated mechanism involving T helper type 1 lymphocytes and monocytes. *J. Immunol.* 159: 574-581.
49. **Haimov I, Laudon M, Zisapel N, Souroujon M, Nof D, Schlitner A, and Lavie P (1994):** The relationship between urinary 6-sulphatoxymelatonin rhythm and insomnia in old age. In: Advances in Pineal Research, edited by Møller M., and Pévet P. London: Libbey, p. 433-438.
50. **Guneli E, Kazikdas KC, Kolatan E (2007):** Ghrelin may attenuate proinflammatory cytokine-mediated neuropathic pain. *Med. Hypotheses.* 69 (2): 356-60.
51. **Gnanapavan S, Kola B, Bustin SA, Morris DG, McGee P, Fairclough P, Bhattacharya S, Carpenter R, Grossman AB, Korbonits M (2002):** The tissue distribution of the mRNA of

ghrelin and subtypes of its receptor, GHS-R, in humans. *J. Clin. Endo. & Metab.* 87: 2988.

52. **Tander B, Atmaca A, Aliyazicioglu Y, Canturk F (2007):** Serum ghrelin levels but not GH, IGF-1 and IGFBP-3 levels are altered in patients with fibromyalgia syndrome. *Joint Bone Spine* 74(5): 477-81.
53. **Copinschi G (2005):** Metabolic and endocrine effects of sleep deprivation. *Essent Psycho-pharmacol*. 6(6):341-7.
54. **Taheri S, Lin L, Austin D, Young T, Mignot E (2004):** Short sleep duration is associated with reduced leptin, elevated ghrelin, and increased body mass index (BMI). Proc 18th Annual Meeting of the Associated Professional Sleep Societies, Philadelphia, PA, p 172.
55. **Oba΄l F Jr, Alt J, Taishi P, Gardi J, Krueger JM (2003):** Sleep in mice with non-functional growth hormone-releasing hormone receptors. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 284: R131–R139.
56. **Weikel JC, Wichniak A, Ising M, Brunner H, Friess E, Held K, Mathias S, Schmid DA, Uhr M, Steiger A (2003):** Ghrelin promotes slow-wave sleep in humans. *Am. J. Physiol. Endocrinol. Metab.* 284: E407–E415.
57. **Hansen TK, Dall R, Hosoda H, Kojima M, Kangawa K, Christiansen JS, Jorgensen JQ (2002):** Weight loss increases circulating levels of ghrelin in human obesity. *Clin. Endocrinol. (Oxf.)* 56: 203–206.
58. **Wisse BE, Frayo RS, Schwartz MW, Cummings DE (2001):** Reversal of cancer anorexia by blockade of central melanocortin receptors in rats. *Endocr.* 142: 3292–3301.
59. **Otto B, Cuntz U, Fruehauf E, Wawarta R, Folwaczny C, Riepl RL, Heiman ML, Lehnert P, Fichter M, Tschop M (2001):** Weight gain decreases elevated plasma ghrelin concentrations of patients with anorexia nervosa. *Eur. J. Endocrinol.* 145: 669–673.
60. **Frey DJ, Fleshner M, Wright KP Jr (2007):** The effects of 40 hours of total sleep deprivation on inflammatory markers in healthy young adults. *Brain. Behav. Immun*. 21(8): 1050-7.
61. **Vila G, Maier C, Riedl M, Nowotny P, Ludvik B, Luger A, Clodi M (2007):** Bacterial endotoxin induces biphasic changes in plasma ghrelin in healthy humans. *J. Clin. Endocrin. Metab.* 92(10): 3930-4.
62. **Granado M, Priego T, Martin AI, Villanּa MA, Lֹpez-Calderֹn A (2005):** Antiinflammatory effect of the ghrelin agonist growth hormone-releasing peptide-2 (GHRP-2) in arthritic rats. *Am. J. Physiol. Endocrinol. Metab.* 288: E486-E492.
63. **Li WG, Gavrila D, Liu X, Wang L, Gunnlaugsson S, Stoll LL, McCormick ML, Sigmund CD, Tang C, Weintraub NL (2004):** Ghrelin inhibits proinflammatory responses and nuclear factor-B activation in

human endothelial cells. *Circ.* 109: 2221-2226.

64. **Wu R, Dong W, Cui X, Zhou M, Simms H, Ravikumar TS, Wang P (2007):** Ghrelin down-regulates proinflammatory cytokines in sepsis through activation of the vagus nerve. *Ann. Surg*. 245: 480–486.
65. **Xia Q, Pang W, Pan H, Zheng Y, Kang JS, Zhu SG (2004):** Effects of ghrelin on the proliferation and secretion of splenic T lymphocytes in mice. *Regul. Pept.* 122(3):173-8.
66. **Yanagida M, Fukamachi H, Ohgami K, Kuwaki T, Ishii H, Uzumaki H, Amano K, Tokiwa T, Mitsui H, Saito H, Iikura Y, Ishizaka T, Nakahata T (1995):** Effects of T-helper 2-type cytokines, interleukin-3 (IL-3), IL-4, IL-5, and IL-6 on the survival of cultured human mast cells. *Blood* 86(10):3705-14.
67. **Zoladz JA, Konturek SJ, Duda K, Majerczak J, Sliwowski Z, Grandys M, Bielanski W (2005):** Effect of moderate incremental exercise, performed in fed and fasted state on cardio-respiratory variables and leptin and ghrelin concentrations in young healthy men. *J. Physiol. Pharmacol.* 56(1):63-85.

دور الجريلين فى تطويع مستوى الإستجابة المناعية الناتج عن فقدان النوم الجزئى

مارى يوسف[1] – مريم أنسى[2] - ليلى راشد[3]
اقسام الفسيولوجى[1] - الباثولوجيا الإكلينيكية[2] - الكيمياء الحيوية[3]

الملخص العربي

تعتبر آليات تأدية فقدان النوم إلى خلل فى الجهاز المناعى غير معروفه. وبالتالي كانت اهداف هذا العمل دراسة تأثيرات الحالة الحياتية الحقيقية من فقدان النوم الجزئى على بعض اوجه المناعة خاصة مستوى انترلوكين ٦ فى الدم حيث انه وجد ان له دور فى التفاعل بين النوم والمناعة وبحث دور الجريلين على هذا التفاعل وهو هرمون مفرز لهرمون النمو ومنبه الإشتهاء معبر من المعدة وخلايا الدم أحادية النواه . وقد أدت المعرفة التى تكونت من الأبحاث المبدئيه فى الحيوانات فى مجال النوم إلى تحسين فى فهم عملية النوم فى الإنسان وبالتالى إلى فوائد تشخيصيه وعلاجيه مهمة. وقد تسهلت الدراسات العصبية والبيولوجية فى مجال فقدان النوم والتى تحتوى على أساليب غزوية بوجود النموذج الحيوانى. أظهرت نتائج الدراسة أن فقدان النوم الجزئى لمدة ٧ أيام و١٤ يوم أرتبط بزيادة فى خلايا الدم البيضاء ونقص فى انترلوكين ٦ وزيادة مضطردة فى تعبير الجريلين فى المعدة وخلايا الدم أحادية النواه مقارنة مع المجموعه الضابطة. وأيضاً وجد تلازماً سلبياً ذو دلالة احصائيه بين مستوى انترلوكين ٦ وتعبير الجزيلين فى خلايا الدم احادية النواه والمعده. غير أن عودة النوم للحالة الطبيعيه لمدة ٤ أيام ادت إلى زيادة فى مستوى انترلوكين ٦ ونقص جزئى فى التعبير الغير طبيعي للجريلين فى حالة فقدان النوم الجزئى لكنها لم ترجع لمعدلات المجموعة الضابطة. وبالتالى يبدو أن النوم هو عملية ترميمية مهمة فى افراز انترلوكين ٦ وأنه يوجد دور وظيفي للجريلين فى تطويع مستوى افرازه فى حالات فقدان النوم.