ORIGINAL ARTICLE

Özgün Araştırma

The Effects of Sleep Deprivation on Insulin, Resistin and Visfatin Levels in Healthy Humans

Sağlıklı İnsanlarda Uyku Yoksunluğunun İnsülin, Resistin ve Visfatin Düzeylerine Etkileri

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ABSTRACT

Objective:

Sleep deprivation is known to affect circulating insulin and glucose levels which in turn modulate glucose metabolism. However, the mechanism of alterations in glucose homeostasis during sleep deprivation is not known. In this study, we investigated circulating resistin and visfatin levels in response to 40 hours of sleep loss in order to shed light on the above-mentioned mechanism.

Methods:

This study included 12 healthy young adult subjects (aged between 18-32 years). All participants underwent polysomnographic evaluation and oral glucose tolerance test and then fasting venous blood samples were collected in morning hours. Then, subjects remained awake for 40 hours under actigraphic monitorization. At the end of sleep deprivation, blood samples were collected again. Serum insulin, resistin and visfatin levels were measured in all blood samples. Insulin was determined by chemical immune assay method, whereas resistin and visfatin levels were assayed by ELISA.

Results:

Compared to baseline, 40-hour total sleep deprivation resulted in a significant increase in serum insulin levels (10.75±7.75 vs 35.98±27.96 IU; p=0.002) and a significant decrease in resistin levels (21.94±7.65 vs 11.71±5.31 IU; p=0.002). Visfatin levels remained unchanged (6.29±3.31 vs 5.43±5.08 IU; p>0.05).

Conclusion:

These results suggested that short-term total sleep deprivation may lead to insulin resistance which was evidenced by a significant increase in insulin levels independent of resistin. This may contribute to pathophysiology of type 2 diabetes mellitus under conditions of chronic sleep deprivation.

Key Words:

Sleep deprivation, Resistin, Visfatin, Insulin

ÖZ

Giriş:

Uyku yoksunluğunun, dolaşımdaki insülin ve glukoz seviyelerini etkilediği ve bu durumun glukoz metabolizmasını modüle ettiği bilinmektedir. Bununla birlikte, uyku yoksunluğu sırasında glukoz homeostazındaki değişikliklerin mekanizması bilinmemektedir. Bu çalışmada, yukarıda belirtilen mekanizmaya ışık tutmak için dolaşımdaki resistin ve visfatin düzeylerini 40 saatlik uyku kaybına yanıt olarak araştırdık.

Yöntem:

Bu çalışmada 12 sağlıklı genç yetişkin (18-32 yaş arası) yer aldı. Tüm katılımcılara polisomnografik değerlendirme ve oral glukoz tolerans testi uygulandı, ardından sabah saatlerinde açlık venöz kan örnekleri alındı. Daha sonra katılımcılar, aktigrafik izleme altında 40 saat uyanık kaldılar. Uyku yoksunluğunun sonunda tekrar kan örnekleri alındı. Tüm kan örneklerinde serum insülin, resistin ile visfatin düzeyleri ölçüldü. İnsülin kimyasal immün assay metodu ile belirlenirken, resistin ve visfatin seviyeleri ELISA ile tahlil edildi.

Bulgular:

Başlangıç durumuna kıyasla, 40 saatlik toplam uyku yoksunluğu serum insülin seviyelerinde (10,75 ± 7,75 ve 35,98 ± 27,96 IU; p = 0,002) anlamlı artış ve resistin seviyelerinde (21,94 ± 7,65 ve 11,71 ± 5,31 IU; p = 0,002) anlamlı azalma gözlenmiştir. Visfatin seviyelerinde anlamlı değişim gözlenmemiştir (6,29 ± 3,31 ve 5,43 ± 5,08 IU; p> 0,05).

Sonuç:

Bu sonuçlar, kısa süreli toplam uyku yoksunluğunun, resistinden bağımsız şekilde, yüksek insülin düzeyleri ile kanıtlanan, insülin direncine yol açabileceğini düşündürmektedir. Bu durum kronik uyku yoksunluğu koşulları altında tip 2 diabetes mellitusun patofizyolojisine katkıda bulunabilir.

Anahtar Sözcükler:

Uyku yoksunluğu, Resistin, Visfatin, İnsülin

INTRODUCTION

As a result of round-the-clock modern city life, voluntary sleep curtailment profoundly disturbed biological rhythms and metabolic processes. The mean sleep duration of adult population decreased by almost 2 hours a night over the last 50 years (1). Sleep durations 6 hours or less were found to be related with glucose tolerance deterioration and development of type 2 diabetes (2,3). Sleep has crucial effects on glucose metabolism and recent data suggest association between short sleep duration and impaired glucose tolerance which in turn may lead to type 2 diabetes (4). Spiegel et al. studied the effects of sleep duration on 11 healthy young men. They first restricted and then extended the time in bed. In the sleep restriction period of 6 hour/night bedtime, the blood glucose response to a carbohydrate breakfast was higher when compared to extended sleep period of 12 hour/night, despite the same insulin secretory response. They reported that sleep restriction shorter than 1 week led to endocrine and metabolic alterations in healthy volunteers and increased sympathetic tone and decreased carbohydrate tolerance which are well-known risk factors of insulin resistance (5). The effects of sleep deprivation on glucose metabolism is well-known. But the underlying mechanism remains to be elucidated.

Adipokines, secretory products of adipocytes, are linked to body energy homeostasis. They may play important role in development of insulin resistance and type 2 diabetes mellitus, but it has been debated lately whether this relationship is causative or not (6). Resistin shows prohyperglycemic whereas visfatin has antihyperglycemic effects.

Resistin, an adipose tissue hormone, was discovered in 2001 (7). It is released from adipocytes and macrophages in humans while it has also been detected in other primates and animals. When injected to mice, it led to insulin resistance which is the reason for the name. Resistin and visfatin has been linked to energy homeostasis, obesity and insulin resistance (7,8).

Visfatin is a proinflammatory adipokine which has similar functions to the immune modulating pre-B-cell colony enhancing factor (8). Visfatin plays a role in various functions such as the maturation of vascular smooth muscle cells and inhibition of neutrophil apoptosis. Visfatin has insulin mimetic effects. It activates the insulin receptor and has been shown to increase insulin sensitivity to lower blood sugar in mice (8). Visfatin is expressed in high levels in the visceral fat of both humans and mice. The cells perception of insulin levels lower than what is and producing more visfatin in response may indicate insulin resistance. Accordingly, visfatin levels may play a role of type 2 diabetes mellitus (9). In addition, it was reported that visfatin plasma levels increase during the development of obesity (6). In healthy, young adult humans, the relationship of sleep deprivation and circulating resistin and visfatin levels has not yet been studied. The aim of this study was to investigate the effects of 40 hours acute total sleep deprivation on insulin, resistin and visfatin levels in healthy, young adult humans.

METHODS

Subjects

The study protocol was approved by local committee of ethics (Trakya University Ethical Committee, approval number: 2009/08 Date: 27.10.2008) The study was conducted in accordance with the principles of the Declaration of Helsinki. Written consent was obtained from all participants for the study. Before starting the study, we consulted a statistician and have made a power analysis in order to determine the number of participants. Twelve participants were sufficient for 90% power. We decided to end the study when we reached 12 volunteers who completed the entire study protocol. In total, twenty-three healthy young adult volunteers (aged between 18-32 years) with a regular sleep-wake

schedule were included to initial examinations. Exclusion criteria were any drug use and daytime napping. All participants completed a face-to-face administered questionnaire that included personal and family medical history and informed consents were taken. Before all participants underwent 40 hours acute total sleep deprivation, polysomnographic evaluations were performed in order to eliminate any sleep disorder. Oral glucose tolerance test was performed to exclude glucose tolerance disorders. Healthy 12 participants were included to final investigations in this study.

Sleep deprivation protocol

Twelve volunteers underwent 40 hours of sleep deprivation following the polysomnography (PSG) night and venous blood samples were collected from antecubital vein. The first blood samples were taken at 07.00 a.m. at the beginning of sleep deprivation period. The last blood samples were taken at the 40th hour of study at 23:00 p.m. Meals were provided ad libitum. Participants were both under supervision of an investigator and used a wrist accelerometer (Actiwatch 2 Respironics, U.S.) to monitor the whole period. Figure 1 shows the study protocol.

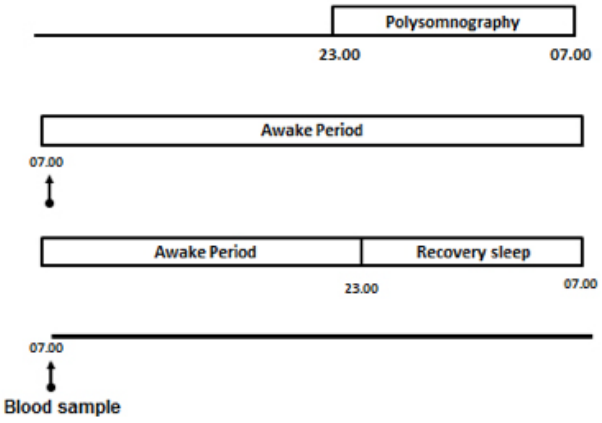

Figure 1: Study protocol.

Biochemical tests

Serum insulin and plasma blood glucose levels were determined. Glucose levels were studied by glucose oxidase method shortly after the blood samples were taken (glucose analyzer, Advia 1800/Siemens, Germany; glucose kit, Siemens/Advia Chemistry Gluo R1, Lot No: 264827 REF: 10492319, Germany). Then, samples were stored at -80°C until assay. Insulin analyses were conducted by chemical immunoassay method using insulin analyzer (Immulite 2000/Siemens, Germany) and insulin kit (Immulite 2000, Catalog No: LINC1-2, Lot No: 407, Germany). Resistin and visfatin levels were determined by ELISA assays (Biotek µQuant-MQX2ØØ, Switzerland). Resistin ELISA kit (Product Name: Human Resistin Elisa kit Catalog No: ER1001-1, Lot No: 11571106 Formulation: 96T /ASSAYPRO, US) and visfatin ELISA kit (Product Name: Human Visfatin Elisa kit Catalog No: SK00121-01, Lot No: 20110780 Formulation: 96T/ADIPOBIOSCIENCE, US) were used for resistin and visfatin measurements. HOMA-IR was calculated by the following formula by Gökçel et al. fasting insulin concentration (IU/ml) × fasting glucose concentration (mmol/L)/22.5 (10).

Statistical analysis

Descriptive data were given as means and standard deviations in tables. Comparison of the two conditions (40 hours sleep deprivation before and after) was performed by non-parametric Wilcoxon signed ranks test. Significance was granted for $p \leq 0.05$.

RESULTS

A total of 23 subjects underwent PSG protocol in the sleep laboratory. Eleven subjects were excluded due to premature drop out and 12 subjects remained for further analysis. General characteristics, HOMA-IR and OGTT values of the study group were given in Table I. The mean age of the study group was 24.5 years and all participants were Caucasians. All volunteers were within normal limits for BMI and HOMA-IR, OGTT values and total sleep time and time in different sleep stages. The oxygen saturation values during PSG study ranged between 90% to 98% which were considered normal.

Table I: General characteristics, HOMA-IR, OGTT values of the study group.

Parameter	Mean	SD
Age, year	24.5	4.4
Height, cm	167.5	6.4
Weight, kg	66.8	12.9
BMI, kg/cm²	23.7	4.1
HOMA-IR	2.47	1.80
OGTT 0. hour	91.8	9.0
OGTT 1. hour	111.2	21.1
OGTT 2. hour	92.7	17.0
Total sleep time, min	269.9	103.2
Sleep Stage 1, min	10.3	2.8
Sleep Stage 2, min	165.0	47.5
Sleep Stage 3, min	94.3	34.4
REM, min	45.2	15.2
SaO_2, %	95.9	2.0

Abbreviation: BMI, Body mass index; **HOMA-IR,** Homeostasis Model of Assessment; **OGTT,** Oral glucose tolerance test; **SaO2,** Oxygen saturation; **SD,** standard deviation.

Insulin, resistin and visfatin levels before and after 40 hours of sleep deprivation were given in Table II. Accordingly, insulin levels were significantly increased and resistin levels were significantly decreased. Whereas, there was no change in visfatin levels.

Table II: Insulin, resistin and visfatin values are given as mean, ±SD and median before and after 40 hours of sleep deprivation.

	Before	After
Insulin (IU)	10.75±7.75	35.98±27.96*
Resistin (IU)	21.94±7.65	11.71±5.31*
Visfatin (IU)	6.29±3.31	5.43±5.08

*p<0.002

Forty hours sleep deprivation increased insulin levels (Figure 2a). Insulin levels increased almost 3.5-fold at the end of sleep loss period with respect to baseline. Resistin levels were shown in Figure 2b. After 40 hours of deprivation, significantly decreased levels of resistin was observed compared to the first measurement.

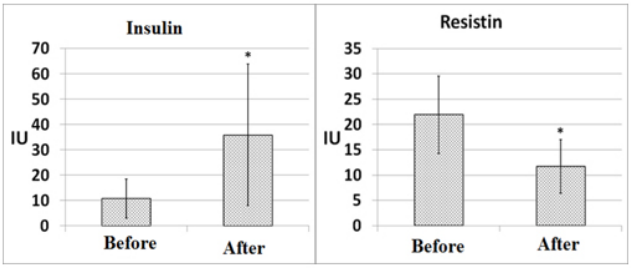

Figure 2a. Before and after 40 hours of sleep deprivation period of insulin. After 40 hours of sleep deprivation compared to first measurement. After 40 hours of deprivation, significantly increased levels of insulin was observed compared to the first measurement.

Figure 2b. Before and after 40 hours of sleep deprivation period of resistin. After 40 hours of deprivation, significantly decreased levels of resistin was observed compared to the first measurement.

According to results of Pearson correlation analysis, there was no correlation between insulin, resistin and visfatin levels before and after 40 hours of sleep deprivation (before 40 hours of sleep deprivation respectively insulin-resistin, insulin-visfatin, resistin-visfatin: 0.99; 0.387; 0.280 and after 40 hours of sleep deprivation respectively 0.242; 0.084; 0.154).

DISCUSSION

The main finding of this study was that one night of sleep deprivation (40 hours) led to almost 3.5-fold increase in systemic insulin levels and significant decrease in resistin levels whereas visfatin levels remained unchanged. To our knowledge, this is the first study in which insulin, resistin and visfatin levels were measured after a total sleep deprivation protocol.

Stress conditions are well-known to induce insulin resistance and reduce insulin levels (11,12). It may be suggested that sleep deprivation leads to stress response which in turn elevates serum cortisol levels and reduce insulin levels. On the contrary, we found a bold increase in serum insulin. In addition, we previously showed that short-term sleep deprivation may not necessarily increase cortisol secretion (13). Taken together, it can be concluded that sleep deprivation does not reveal a non specific stress response. Rather it has specific consequences.

Visfatin is an adipocytokine which was initially reported to have insulin-mimetic effects. Visfatin expression substantially takes places in adipose tissue followed by skeletal muscle, bone marrow and hepatocytes (14). Visfatin has been studied extensively in conjunction with the hormone insulin. It alters insulin sensitivity of hepatocytes in autocrine fashion (15). It may also regulate insulin release from pancreatic beta cells (16). More recent studies demonstrated that visfatin is positively correlated with insulin and HOMA-IR (17). In this study, systemic visfatin levels remained unchanged following a 40-hour sleep deprivation. This result suggested that sleep loss-induced insulin increase was independent of serum visfatin alterations.

Resistin is a cysteine-rich polypeptide hormone which was initially believed to play an important role in the development of insulin resistance (7). Despite controversial studies, later systematic reviews and meta-analyses revealed individuals with insulin resistance showed higher resistin levels than subjects without insulin resistance (18). The link between resistin and insulin resistance may involve inflammatory pathways as in vitro studies demonstrated that production of pro-inflammatory cytokines resulted in resistin expression (19). In our study, 40-hour sleep deprivation led to a significant decrease in resistin level. This result suggested that insulin increase was not related with resistin.

The study has several limitations. First, the study group comprised of young adult volunteers. Thus, one should be careful when extrapolating these results to older adults. Geriatric group may show different response to sleep deprivation. Second, daily caloric intake was not standardized. Yet, feeding habits of volunteers were maintained. And finally, adding glucose measurements would enable us to calculate insulin resistance.

CONCLUSION

In conclusion, the effects of short-term total sleep deprivation on glucose homeostasis were investigated for the first time in terms of plasma resistin, visfatin, and insulin levels. Sleep deprivation-induced insulin increase seemed unrelated to resistin and visfatin alterations during the same period. These findings contribute to the mechanism of the relationship between sleep duration, sleep deprivation, insulin resistance and diabetes.

Ethics Committee Approval:
This research complies with all the relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration and has been approved by the Trakya University Medical Faculty Ethical Committee, Trakya University (approval number 2009/08).

Informed Consent:
All the participants rights were protected and written informed consents were obtained before the procedures according to the Helsinki Declaration.

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Author Contributions:
Concept- E.E.G.; Design- L.Ö.; Supervision- L.Ö.; Resources- E.E.G.; Materials- E.E.G.; Data Collection and/or Processing- E.E.G.; Analysis and/or Interpretation- E.E.G., L. A., L.Ö.; Literature Search- E.E.G., L.A.; Writing Manuscript- E.E.G., L.Ö.; Critical Review- E.E.G., L.A.; Others: L.A.

Conflict of Interest:
The authors have no conflict of interest to declare. This study was produced from the thesis named “The effects of sleep deprivation on glucose homeostasis”.
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