

Serum levels of leptin, zinc and tryptophan in obese subjects with sleep deficits

Noreen Samad¹, Farzana Yasmin², Suad Naheed³, Abu Zeeshan Bari⁴,
M Mazhar Ayaz⁵, Aqal Zaman⁶, Atif Akbar⁷, Saima Saeed⁸ and Najeeb Ullah¹

¹Department of Biochemistry, Bahauddin Zakariya University, Multan, Pakistan

²Department of Food & Biomedical Engineering, NED University of Engineering and Technology, Karachi, Pakistan

³Department of Biotechnology, Jinnah University for Women, 5-C, Nazimabad, Karachi, Pakistan

⁴Department of Biomedical Engineering, NED University of Engineering and Technology, Karachi, Pakistan

⁵Department of Veterinary Sciences, Bahauddin Zakariya University, Multan, Pakistan

⁶Institute of Pure and Applied Sciences, Bahauddin Zakariya University, Multan, Pakistan

⁷Department of Statistics, Bahauddin Zakariya University, Multan, Pakistan

⁸Department of Chemistry, Kohat University of Science and Technology, Kohat, KPK Pakistan

Abstract: Obesity is an important risk factor for sleep disorders. This study aimed to evaluate the association of leptin, zinc and tryptophan (TRP) in obese subjects with sleep deficits [sleep apnea (SA), insomnia (IN)]. In this cross sectional case control, with the verbal and written consent 206, obese with sleep deficits and 30, non-obese/normal identified from various areas of Karachi, Pakistan. The socio-demographic data including; age, body mass index (BMI), education and residence, of participants was collected. After providing informed consent, fasting blood samples were taken and serum was collected. The serum concentration of leptin, zinc and TRP were analyzed by ELISA (Enzyme-linked immunosorbent assay), FAAS (Flame atomic absorption spectrophotometer) and HPLC (High performance liquid chromatography) respectively. A significant correlation was found between BMI (body mass index) and leptin, BMI and zinc, BMI and TRP. The correlation between leptin consecutively was significantly associated with zinc and TRP in obese patients. Sleep deficits elevated circulatory levels of leptin while lower zinc and TRP levels compared to levels seen in non-obese (Normal) subjects with no sleep deficits. Obese subjects exhibited significantly higher levels of leptin with sleep deficits compared with non-obese subjects with normal sleep pattern, while obese subjects with SA had significantly high levels of leptin than obese subjects with IN and IN+SA. Patients with sleep deficits had significantly lower levels of serum TRP and zinc than non-obese subjects with normal sleep pattern. Obese subjects with SA had significantly lower levels of zinc and elevated levels of TRP than obese subjects with IN. Obese patients with IN+SA had significantly lower levels of leptin and zinc than IN and SA, while TRP levels were significantly lower in subjects with IN than obese subjects with IN+SA and IN. These results suggest that elevated levels of leptin which are possibly by adiposity and lessened levels of zinc and TRP have a great impact on progression of obesity and their association can contribute to tempt sleep disorders.

Keywords: Leptin, zinc, tryptophan, sleep deficits, obesity.

INTRODUCTION

Many factor, such as genetics and metabolism, contribute to the development of obesity. Obesity was prevailed doubled since 1980 (Ram *et al.*, 2010) and this outbreak has been coextending by a tendency in diminished sleep duration (Beccuti *et al.*, 2011). Studies (experimental and observational) indicate to short sleep period as a hallmark for obesity and obesity-related disorders including metabolic syndrome, type II diabetes and cardiovascular disorders (Morselli *et al.*, 2010).

Sleep apnea (SA) is characterized by stopover in breathing while asleep is due to hindrance of the airway or by the incapability of the brain to normally order respiration. Inability to fall asleep is termed as insomnia (IN). Both SA and IN are linked to a high risk of

metabolic disorders and commonly arise in overweight individuals (Fredheim *et al.*, 2011).

Leptin resistance is a hallmark of obesity (Mayers *et al.*, 2012). Leptin is a hormone which is derived by gut and adipose tissue, involves in regulation of various biological functions and processes, including energy intake and expenditure, body fat, neuroendocrine system, autonomic function, sleep and insulin and glucose balance (Confavreux *et al.*, 2009). The most well-known effect of leptin is to regulate body weight and energy balance (Wang *et al.*, 1996). Leptin is found to be generally low in the circulation of lean individual and high with adiposity (Ostlund *et al.*, 1996), and directly linked with body mass index (BMI) (Wali & Wali, 2016). Studies also elaborated that low levels of leptin also involved in sleep deficits (Sogut *et al.*, 2016).

*Corresponding author: e-mail: noreen.samad@bzu.edu.pk

It is indicated that low levels of zinc affect many functions of the brain related to development of the brain and produced neuropsychological symptoms (Lang *et al.*, 2015). Studies showed that obese (Marreiro *et al.*, 2006) and major depressed (Maes *et al.*, 1997) and sleep disorder (Luoju *et al.*, 2015) individuals have low serum or plasma zinc concentration. This alteration is associated with redistribution of zinc among various tissues and zinc redistribution in obesity appear to be mediated at least partially by inflammatory cytokines (Gaetke *et al.*, 1997) which may provoke apoptosis and endothelial cell dysfunctioning (Bao *et al.*, 2010).

Obesity is considered to be an outcome of increased caloric intake (ingestion of all constituents of food, including carbohydrates, lipids, and proteins) and decreased substantial activity. Greater protein intake is related with the availability of amino acids such as the essential amino acid tryptophan (TRP), a substrate for the synthesis of serotonin and melatonin, both of which are implicated in the regulation of satiety and caloric intake. Though, morbidly obese persons have been found to have decreased, rather than increased, TRP levels (Brandacher *et al.*, 2006). Recent studies have also shown that obesity (Nduhirabandi *et al.*, 2012), depression as well as impairment of sleeping pattern (Malhotra *et al.*, 2017; Volpe *et al.*, 2017) associated with reduced serum tryptophan levels.

This study is aimed to evaluate the association of serum leptin, zinc and tryptophan (TRP) in obese subjects with a history of SA, IN and IN+SA. However the obesity-related factors are the link between clinical and metabolic manifestation of SA and IN is a point of attention. The relation between metabolic disorders and sleep disorder is multidirectional and complex to define. The complex relationship is due in part to the fact that obesity could be a cause and consequence fact of IN and SA.

METHODS

Subjects and study design

The protocol was approved by institutional ethical committee and, in accordance with the ethical standards of the Helsinki declaration, and the written permission was attained from all participants. All participant included in this study were adult and obese were resident of different areas of Karachi, Pakistan. A history to detect sleep disorder was obtained during the visit in the clinic of university to obtained informed consent to participate in the study. Two-hundred and six (90 male, 116 female) obese with sleep disorder and thirty (15 male, 15 female) non-obese individual with normal sleep pattern were enrolled in this study. Body mass index (BMI) of $>25\text{kg/m}^2$ as documented earlier (Ursav *et al.*, 2010) was described as obesity. With the informed consent all participant called to the laboratory for blood drawn.

Inclusion/exclusion criteria

Participant completed a questionnaire during a face-to-face interview. Information was collected on demographic variables, sleep disorders, socioeconomic status, smoking, nutritional supplementation, personal medical history, family history and medication use if any. Exclusion criteria included cigarette smoking, patients with history of CVD, metabolic disorders and hypertension. Subjects who were taking any supplemental vitamins or herbal products and the use of oral contraception in a diet based weight-loss program are also excluded.

Sample collection and biochemical estimation

Fasting blood samples (5 ml, once for a study) were drawn from patient's arm with intravenous infusion under the hygienic environment to avoid contamination. Serum was extracted from blood and frozen at -40° until the analysis of leptin, zinc and TRP. The techniques were used for research purpose HPLC (High performance liquid chromatography) for finding out tryptophan levels and FAAS (Flame atomic absorption flame spectrophotometry) for zinc level determination and ELISA (Enzyme-linked immunosorbent assay) for leptin levels.

HPLC analysis

Serum samples were extracted and the levels of serum TRP were determined by HPLC-EC (Samad *et al.*, 2007).

FAAS analysis

Serum samples were diluted with 2% nitric acid and the levels of the serum zinc were determined by FAAS (Naureen *et al.*, 2014).

ELISA

Leptin serum samples were determined by ELISA. The kit for serum leptin (Friedman & Halaas, 1998) was obtained from BioSource Europe S.A.

STATISTICAL ANALYSIS

Data on leptin, zinc and TRP with sleep deficits in obese subject was analyzed by using one-way ANOVA. Post hoc analysis was done by Tukey's test. $P < 0.05$ was taken as significant. Bivariate Pearson's correlation was used to determine relationship of BMI and circulating levels of leptin, zinc and TRP. The relationship between circulating levels of leptin, zinc and TRP was also analyzed by Bivariate Pearson's correlation test. All data were analyzed by SPSS software version 20.0.

RESULTS

Table 1 the demographic characteristics including sex, age group, BMI, education and residence of the 236 subjects engaged in the present study was collected.

Fig. 1 shows correlation between BMI and circulating levels of leptin, zinc and TRP in obese subjects. There is a significant positive ($r= 0.524$ $p<0.01$) correlation between BMI and leptin, while, there is a significant negative correlation between BMI and zinc ($r= - 0.558$ $p<0.01$), BMI and TRP ($r= - 0.669$ $p<0.01$).

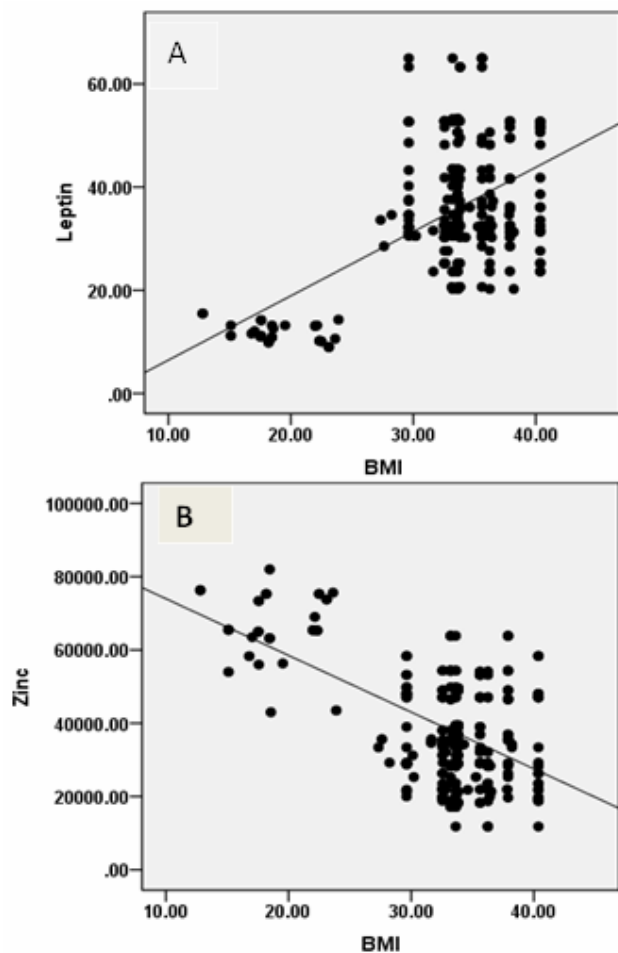


Fig. 1: The relationship between BMI and leptin (A) BMI and zinc (B), BMI and TRP (C). Values are mean \pm S.D.

Fig. 2 shows correlation between leptin and zinc, leptin and TRP in obese subjects. There is a significant negative correlation between the leptin and zinc ($r=-0.492$ $p<0.01$), leptin and TRP ($r=-0.698$ $p<0.01$).

Fig. 3 shows levels of leptin (2A) zinc (2B) and tryptophan (2C) in obese with IN, SA, IN+SA. Data on leptin levels analyzed by one way ANOVA ($F(3,232)=430.51$ $P<0.05$) showed significant difference between sleep deficits and leptin in obese subjects. Tukey's test exhibited that serum levels of leptin were increased in obese subjects with history of IN, SA, IN+SA than non-obese with no history of sleep deficits. Levels of leptin were significantly increased in SA than IN+SA and IN. Similarly levels of leptin were also increased in IN than IN+SA.

Data on zinc levels analyzed by one way ANOVA ($F(3,232)=218.87$ $p<0.05$) showed significant difference between sleep deficits and zinc in obese subjects. Tukey's test exhibited that serum levels of zinc were decreased in obese subjects with history of IN, SA, IN+SA than non-obese with no history of sleep deficits. Levels of zinc were higher in IN than IN+SA, while lower in SA than IN.

Data on TRP levels analyzed by one way ANOVA ($F(3,232)= 430.51$ $P<0.05$) showed significant difference between sleep deficits and TRP. Tukey's test exhibited that serum levels of tryptophan were decreased in obese subjects with history of IN, SA and IN+SA than non-obese with no history of sleep deficits. The levels of TRP were increased in SA than IN and IN+SA, while decreased in IN than IN+SA.

DISCUSSION

Obesity produces a great affect on circulating levels of leptin (Nimptsch & Pischon, 2016), zinc (Rathnayake *et al.*, 2016) and TRP subjects (Gatti *et al.*, 1993), which is also consistent with our present study (fig. 2A, 2B, 2C). Extensive research data have revealed that obesity may cause sleep disorders in which SA and IN have got major attention (Sateia & Nowell, 2004). In this cross-sectional study of obese subjects, we found that sleep deficits (IN, SA, IN+SA) were associated with hyperleptinemia (fig. 3A), hypozyneaemia (fig. 3B) and hypotryptophanemia (fig. 3C). Novelty of the present study is that it compared circulating levels of leptin, tryptophan, zinc and BMI (table 1) in obese subjects with the history of sleep disorders such as IN, SA and IN+SA. It is appear that SA in obese subjects elevates leptin and lower tryptophan levels than IN while IN increased the levels of zinc than SA. The data also shows that IN+SA in obese subjects lower the levels of leptin, and zinc while comparable levels of TRP than IN and SA in obese subjects.

Epidemiological studies suggest that short sleep duration is correlated with risk of developing obesity (Donga *et al.*, 2010). SA is the most common category of sleep disordered breathing. At the level of throat, the human airway is composed of collapsible walls of soft tissue that can obstruct breathing during sleep. Individuals with low muscle tone and soft tissue around the airway (e.g. those with obesity) or those with structural features that give rise to narrow airway are at high risk for obstructive sleep apnea (OSA). The elderly are more likely to have OSA than young people, and men are more likely to suffer SA than women. The risk of OSA rises with increasing body weight and age. IN is not a natural part of aging and is often reversible with prompt and appropriate treatment (Sateia & Nowell, 2004).

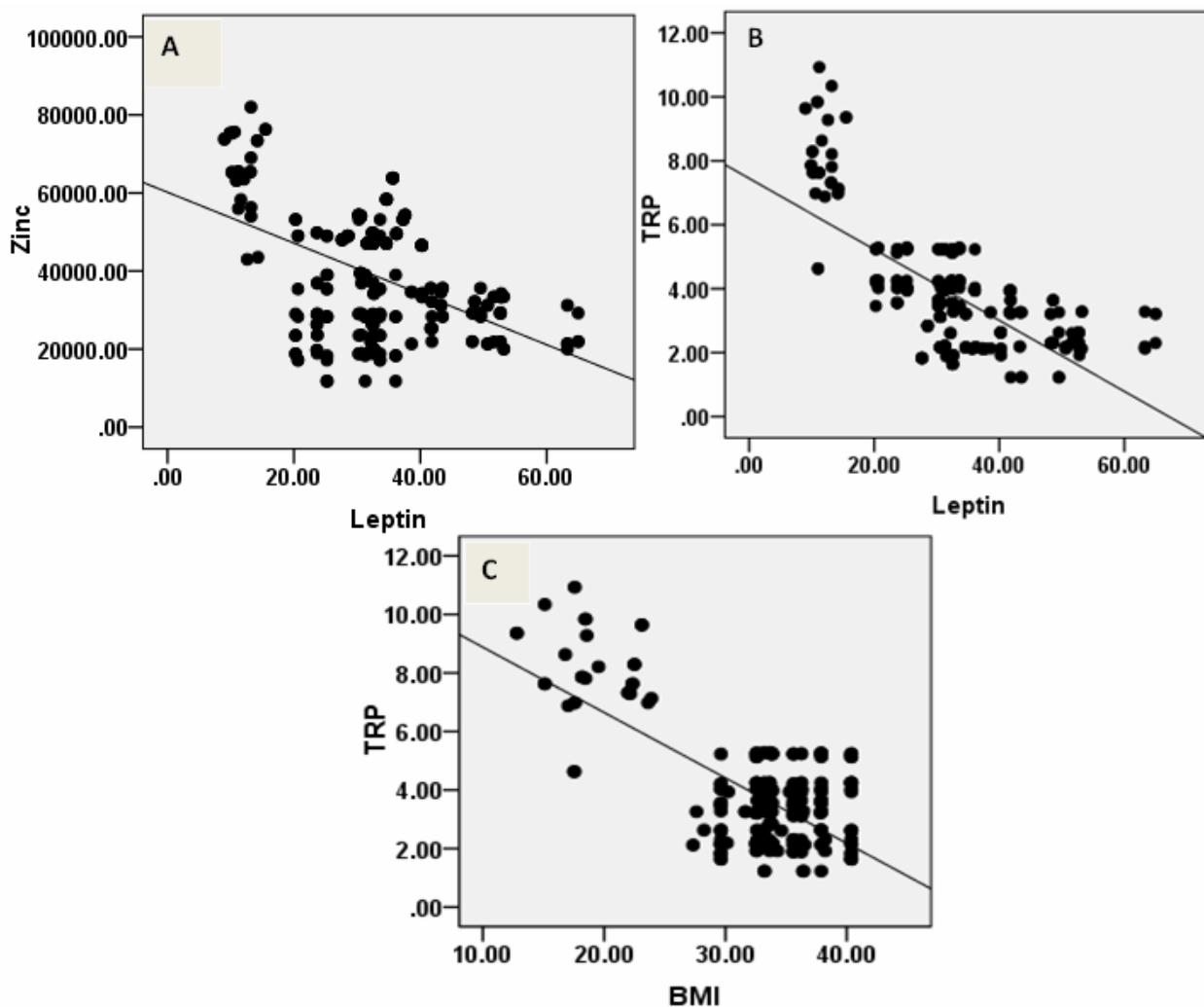


Fig. 2: The relationship between leptin and zinc (A), leptin and TRP (B). Values are mean \pm S.D.

A role of leptin in the regulation of BMI is well documented. A number of studies show that information on adipocyte metabolism and body weight to the appetite centers in the hypothalamic regions of the brain is communicated by leptin. These studies reported that treatment of leptin-deficient ob/ob mice with recombinant leptin decreases food intake and increases energy expenditure leptin (Campfield *et al.*, 1995). The present data (fig. 1A) and previous studies (Motivala *et al.*, 2004) showed that circulating levels of leptin are elevated with increased BMI (table 1).

The association between sleep deficits and leptin is still unclear. Our finding, however, are in general agreement with results from some (Pejovic *et al.*, 2010), though not all (Knutson, 2010), studies that have assessed leptin concentration in relation to habitual sleep duration or acute sleep deprivation among men and women. The association between leptin and sleep disorder remained significant after adjusting for BMI and age (fig. 1A, table 1, AARC-APT., 1995).

Leptin is produced by adipocytes and is believed to contribute to the central regulation of food intake (Mantzoros *et al.*, 2011). Its reduction may contribute to the development of obesity. Except for very rare genetic syndrome, obesity is associated with highly elevated serum levels of leptin due to leptin resistance. Human studies with normal subjects observed an increase in serum leptin with sleep restriction but not in controls (Van Leeuwen *et al.*, 2010). Previous study has shown that circulating levels of leptin were higher in SA than IN and IN+SA in diabetic patients (Jain *et al.*, 2012), other studies also showed comparable increase of circulating levels of leptin in chronic IN than control (Motivala *et al.*, 2009). The present study is also consistent with previous studies (Jain *et al.*, 2012) that SA is increased circulating levels of leptin than IN and IN+SA in obese subjects (fig. 3C).

Dietary precursor can influence the rate of synthesis and function of small number of neurotransmitters, including serotonin (Grimmett & Sillence, 2000). Evidences have

revealed that diet may influence central nervous system through the production of serotonin and melatonin (Van Cauter & Tasali, 2011). Synthesis of serotonin is dependent on its precursor availability in the brain, the amino acid L-tryptophan. Tryptophan is transported across the blood-brain barrier by a system that shares other transporters including a number of large neutral amino acid. Melatonin is a hormone released from the pineal gland that transmits information regarding the light-dark cycle and retinal light exposure result in a suppression of melatonin (Halson, 2014). Melatonin can influence the sleep-wake cycle, by a sleep promoting effects, and therefore a number of nutritional interventions aim to increased melatonin by the manipulation of tryptophan (Pijl *et al.*, 1993). Several other studies examined plasma tryptophan concentration and plasma tryptophan ratios in obese and lean subjects. Almost all such studies measured a single time point. Although plasma tryptophan levels are consistently lower in obese (fig. 3C) than in lean subjects (Ashley *et al.*, 1985) [although not always (Breum *et al.*, 1996), and to remain low after weight reduction (Yunus *et al.*, 1992).

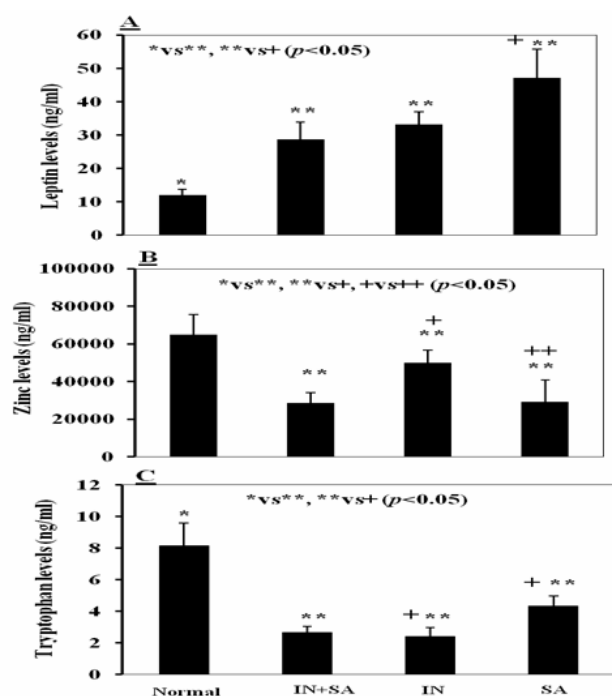


Fig. 3: Serum levels of leptin (A), zinc (B), and TRP (C) in obese subject with insomnia and sleep apnea (IN+SA), insomnia (IN), and sleep apnea (SA). Values are mean \pm S.D.

Previous work has been shown that both low levels of TRP (Yunus *et al.*, 1992) and serotonin (Hrycaj *et al.*, 1993) in the serum and low levels of TRP and 5-hydroxytryptophan (5HTP) in the cerebrospinal fluid (CSF) were found in fibromyalgia syndrome, which is also characterized by non-restorative sleep, such as IN.

Studies also examined that serotonin substrate supplementation via L-TRP (Hudson *et al.*, 2005) or serotonin (Juhl, 1998) has been shown to improve depression, anxiety and sleep deficits. The relationship of leptin, TRP and serotonin has reported in various studies (Haque *et al.*, 2002). Present study indicates that sleep deficits increased leptin (fig. 3A) and decreased TRP (fig. 3C) in obese subjects. Elevated leptin or leptin resistance and lower TRP levels may play crucial role in accelerating the development of physiological problem in obese subjects (fig. 2C). The present data also shows that IN causes a greater decrease of serum TRP levels than SA and IN+SA in obese subjects (fig. 3C). A decreased in circulating levels of TRP was also found in depressed patients (Lindseth *et al.*, 2015). Previously it has also been examined that patients with chronic IN have decreased levels of tryptophan (Sarris & Byrne 2011). The present study is also in agreement with the previous report and suggests that IN exhibit greater decrease in TRP levels in obese subjects (fig. 3C), with increased BMI (fig. 3A, table 1) which may also contribute in severe depression than IN+SA and SA in obese subjects.

Zinc ions are important modulators and signaling molecules in the central nervous system influencing monoaminergic, endocrine and immune system. In human low level of zinc is associated with dysphoria, lethargy, anxiety, maladaptive effects of regulation, anhedonic, anorexia, impairment of taste and smell and impaired cognitive functions (Lustberg & Reynolds, 2000). However, little is known on the impact of plasma zinc concentration on clinical features of depression.

Epidemiological studies have indicated that people with sleep disturbance are much more likely to require health care, which imposes a substantial economic burden on individuals and health care system. Sleep disorder impairs normal day time functioning as a result of sleep insufficiency. These impairments generally include fatigue, irritability, poorer memory and concentration and malaise (Morselli *et al.*, 2010). A greater incidence of depression symptoms correlates with poor sleep quality or chronic insomnia, disturbances that appear to be major risk factors for depression (Swardfager *et al.*, 2013). Zinc not only exhibits an antidepressant-like activity, as stated in preclinical (Lustberg & Reynolds, 2000) and clinical (Rondanelli *et al.*, 2011) studies, and also improves the deficits of sleep (Carpenter *et al.*, 2013). It is stated previously (Chen & Lin, 2000) that deficiency of zinc is found in obese subjects that are consistent with our study (fig. 2B). Cabala *et al.* (2014), studied that there is no association found between IN and zinc, while the present data showed that obese subjects with IN have greater levels of zinc than SA and IN+SA. It is suggested that, although decreased levels of zinc exhibit depression with obesity, its persistent decrease may also contribute to sleep deficits.

Table 1: Characteristics of non-obese (Normal), and obese patients with Insomnia (IN), sleep apnea (SA), and insomnia + sleep apnea (IN+SA).

Characteristics	Non-obese	IN+SA	IN	SA
Sex (M/F)	15/15	25/46	31/40	29/35
Age (Years) 20-35	100 (100)	100 (100)	100 (100)	100 (100)
Body Mass Index (Kg/m ²)	19.05±3.20	34.01±3.00	34.79±2.85	34.79±2.91
Education >25 years	52 (52)	49 (49)	35(35)	43(43)
<25years	48 (48)	51 (51)	65(65)	57(57)
Resident	Urban	Urban	Urban	Urban

Data presented as mean ± SD or number (%).

Zinc deficiency is associated with reduced serum leptin concentration in healthy human and rats (Cabala *et al.*, 2014). However, obese human and mice exhibit higher leptin in tandem with lower zinc concentration in blood or adipose tissues, suggesting of an interrelationship between each factor in obesity (Chen & Lin, 2000).

Taken altogether, we report that obesity is a one of the risk factor of sleep disorders. A circulating level of leptin is elevated with increased BMI and has a significant negative association with zinc and tryptophan. Similarly obesity induced sleep curtailment also exhibit the same effects on serum levels of leptin, zinc and tryptophan.

CONCLUSION

In conclusion hyperleptinemia, hypozincemia and hypotryptophanemia are significantly associated with sleep deficits (IN, SA, IN+SA) in obese subjects. Since curtailment of sleep is highly prevalent in modern societies, and its association with BMI, hyperleptinemia, hypozincemia and hypotryptophanemia, and in turn, the potential adverse impact on metabolic and endocrine processes can have important implications. Our findings underscores the importance of zinc and tryptophan nutrition and metabolism in obesity and sleep disorders and pledge future mechanism studies that examine whether zinc and tryptophan supplementation can reduce sleep curtailment and the related co-morbidities that occur in obesity. Further studies in a large patient population are needed to establish the role of altered levels of leptin, zinc and TRP in the pathophysiology of IN, SA and IN+SA in obese subjects.

REFERENCES

AARC-APT (American Association of Polysomnography Technologists) (1995). Clinical Practice Guidelines, "Polysomnography", *Respiratory care*, **40**: 1336-1343.
 Ashley DV, Fleury MO, Golay A, Maeder E and Leathwood PD (1985). Evidence for diminished brain 5-hydroxytryptamine biosynthesis in obese diabetic and non-diabetic humans. *Am. J. Clin. Nutr.*, **42**: 1240-1245.

Baltaci AK, Mogulkoc R and Halifeoglu I (2005). Effects of zinc deficiency and supplementation on plasma leptin levels in rats. *Biol. Trace. Elem. Res.*, **104**: 41-46.
 Bao B, Prasad AS, Beck FW, Fitzgerald JT, Snell D, Bao GW, Singh T and Cardozo LJ (2010). Zinc decreases C-reactive protein, lipid per oxidation and inflammatory cytokines in elderly subjects: A Potential implication of zinc as an atheroprotective agent. *Am. J. Clin. Nutr.*, **91**: 1634-1641.
 Beccuti G and Pannain S (2011). Sleep and obesity. *Curr. Opin. Clin. Nutr. Metab. Care.*, **14**: 402-12.
 Brandacher G, Winkler C, Aigner F, Schwelberger H, Schroecksnadel K, Margreiter R, Fuchs D and Weiss HG (2006). Bariatric surgery cannot prevent tryptophan depletion due to chronic immune activation in morbidly obese patients. *Obese. Surg.*, **16**: 514-548.
 Breum L, Møller SE, Andersen T and Astrup A (1996). Long-term effect of dexfenfluramine on amino acid profiles and food selection in obese patients during weight loss. *Int. J. Obes. Relat. Metab. Disord.*, **20**: 147-153.
 Campfield LA, Smith FJ, Guisez Y, Devos R and Burn P (1995). Recombinant mouse OB protein: Evidence for a peripheral signal linking adiposity and central neural networks. *Science*, **269**: 546-549.
 Carpenter WE, Lam D and Toney GM and Weintraub NL (2013). Weintraub NL. Qin Z. Zinc, copper, and blood pressure: Human population studies. *Med. Sci. Monit.*, **19**: 1-8.
 Chen MD and Lin PY (2000). Zinc-induced hyperleptinemia relates to the amelioration of sucrose-induced obesity with zinc repletion. *Obes. Res.*, **8**: 525-529.
 Cabala WJ, Landowski J, Szyszko M and Wielgomas B (2014). Zinc in drug-naïve patients with short-illness-duration first episode major depressive disorder: impact on psychopathological features. *Neuro. Endocrinol. Lett.*, **35**: 741-745.
 Confavreux CB, Levine RL and Karsenty G (2009). A paradigm of integrative physiology, the crosstalk between bone and energy metabolisms. *Mol. and Cell. Endocrinol.*, **310**: 21-29.
 Donga E, van Dijk M, van Dijk JG, Biermasz NR, Lammers GJ, van Kralingen K, Hoogma RP, Corssmit

- EP and Romijn JA (2010). Partial sleep restriction decreases insulin sensitivity in type 1 diabetes. *Diabetes Care.*, **33**: 1573-1577.
- Fredheim JM, Rollheim J, Omland T, Hofsø D, Røislien J, Vegsgaard K and Hjelmæsæthm J (2011). Type 2 diabetes and pre-diabetes are associated with obstructive sleep apnea in extremely obese subjects: a cross-sectional study. *Cardiovasc. Diabetol.*, **25**: 10-84.
- Friedman JM and Halaas JL (1988). Leptin and regulation of body weight in mammals. *Nature*, **1969**: 763-770.
- Gaetke LM, McClain CJ, Talwalkar RT and Shedlofsky SI (1997). Effects of endotoxin on zinc metabolism in human volunteers. *Am. J. Physiol.*, **272**: 952-956.
- Gatti E, Porrini M and Neo D (1993). Plasma amino acid changes in obese patients on very low-calorie diets. *Int. J. Vitam. Nutr. Res.*, **63**: 81-85.
- García OP, Long KZ and Rosado JL (2009). Impact of micronutrient deficiencies on obesity. *Nutr. Rev.*, **67**:559-72.
- Grimmett A and Sillence MN (2005). Calmatives for the excitable horse: A review of L-tryptophan. *Vet. J.*, **170**:24-32.
- Halson SL (2014). Sleep in elite athletes and nutritional interventions to enhance sleep. *Sport. Med.* 44: 13-23
- Haque Z, Yasmeen F and Haleem DJ (2012). A G15719C leptin gene polymorphism is associated with lesser perception of stress and other physiological reactions in adult working Pakistani women. *Int. J. Collab. Res. Internal. Med. Pub. Health.*, **4**:296-307.
- Hrycaj P, Stratz T and Müller W (1993). Platelet 3H-imipramine uptake receptor density and serum serotonin levels in patients with fibromyalgia/fibrositis syndrome. *J. Rheumatol.*, **20**:1986-8.
- Hudson C, Hudson SP and Hecht T (2005). Protein source tryptophan versus pharmaceutical grade tryptophan as an efficacious treatment for chronic insomnia. *Nutr. Neurosci.*, **8**:121-7.
- Jain SK, Kahlon G and Morehead L (2012). The effect of sleep apnea and insomnia on blood levels of leptin, insulin resistance, IP-10, and hydrogen sulfide in type 2 diabetic patients. *Metab. Syndr. Relat. Disord.*, **10**:331-6.
- Juhl JH (1998). Fibromyalgia and the serotonin pathway. *Altern. Med. Rev.*, **3**:367-75.
- Knutson KL (2010). Sleep duration and cardiometabolic risk: a review of the epidemiologic evidence. *Best. Pract. Res. Clin. Endocrinol. Metab.* **24**:731-43
- Lang UE, Beglinger C, Schweinfurth N, Walter M and Borgwardt S (2015). Nutritional aspects of depression. *Cell. Physiol. Biochem.* **37**: 1029-43.
- Lindseth G, Helland B and Caspers J (2015). The effects of dietary tryptophan on affective disorders. *Arch. Psychiatr. Nurs.*, **29**:102-7.
- Luojus MK, Lehto SM, Tolmunen T, Elomaa AP and Kauhanen J (2015). Serum copper, zinc and high-sensitivity C-reactive protein in short and long sleep duration in ageing men. *J. Trace. Elem. Med. Biol.* 32:177-82.
- Lustberg L and Reynolds CF (2000). Depression and insomnia: Questions of cause and effect. *Sleep Med Rev.*, **4**:253-262.
- Malhotra R, Persic V, Zhang W, Brown J, Tao X, Rosales L, Thijssen S, Finkelstein FO, Unruh ML, Ikizler A, Garimella PS, Ix JH, Kooman J, Levin NW, Handelman GJ and Kotanko P (2017). Tryptophan and Kynurenine Levels and Its Association With Sleep, Nonphysical Fatigue, and Depression in Chronic Hemodialysis Patients. *J. Ren. Nutr.* **27**: 260-66.
- Maes M, Vandoolaeghe E, Neels H, Demedts P, Wauters A, Meltzer HY, Carlo A and Roger D (1997). Low serum zinc in major depression is a sensitive marker of treatment resistance and of the immune/inflammatory response in that illness. *Biol. Psychiat.*, **42**: 349-358.
- Mantzoros CS, Prasad AS and Beck FW (1988). Zinc may regulate serum leptin concentrations in humans. *J. Am. Coll. Nutr.*, **17**: 270-275.
- Marreiro DN, Geloneze B, Tambascia MA, Lerario AC, Halpern A and Cozzoline SM (2006). Effect of zinc supplementation on serum leptin levels and insulin resistance of obese woman. *Biol. Trace. Elem. Res.*, **112**: 109-18.
- Morselli L, Leproult R, Balbo M and Spiegel K (2011). Spiegel K. Role of sleep duration in the regulation of glucose metabolism and appetite. *Best. Pract. Res. Clin. Endocrinol. Metab.* **24**: 687-702.
- Motivala SJ, Tomiyama AJ, Ziegler M, Khandrika S and Irwin MR (2009). Nocturnal levels of ghrelin and leptin and sleep in chronic insomnia. *Psychoneuroendocrinology.*, **34**:540-545.
- Myers Jr, M.G, Heymsfield SB, Haft C, Kahn BB, Laughlin M and Leibel RL (2012). Challenges and opportunities of defining clinical leptin resistance. *Cell Metabol.*, **15**: 150-156.
- Naureen F, Masroor K, Khatoon F, Ayub S, Ahmed MI, Hasnat A and Samad N (2014). Zinc and tryptophan levels in anorexia nervosa; a co-relational. *Int. J. Biomed. Engg. Sci.*, **1**: 27-33.
- Nduhirabandi F, du Toit EF and Lochner A (2012). A melatonin and the metabolic syndrome: A tool for effective therapy in obesity-associated abnormalities? *Acta. Physiol.*, **205**: 209-223.
- Nimptsch K and Pischon T (2016). Obesity Biomarkers, Metabolism and Risk of Cancer: An Epidemiological Perspective. *Recent. Results Cancer. Res.*, **208**: 199-217.
- Nowak G, Szewczyk B, Sadlik K, Piekoszewski W, Trela F, Florek E and Pilc A (2003). Reduced potency of zinc to interact with NMDA receptors in hippocampal tissue of suicide victims. *Pol. J. Pharmacol.*, **55**: 455-459.
- Ostlund RE Jr, Yang JW, Klein S and Gingerich R (1996). Relation between plasma leptin concentration and body

- fat, gender diet, age and metabolic co-variables. *J. Clin. Endocrinol. Metab.*, **81**: 3909-13.
- Pejovic S, Vgontzas AN, Basta M, Tsaoussoglou M, Zoumakis E, Vgontzas A, Bixler EO and Chrousos GP (2010). Leptin and hunger levels in young healthy adults after one night of sleep loss. *J. Sleep. Res.*, Pejovic S, Vgontzas AN, Basta M, Tsaoussoglou M, Zoumakis E, Vgontzas A, Bixler EO. Chrousos GP:552-558.
- Pijl H, Koppeschaar HP, Cohen AF, Iestra JA, Schoemaker HC, Frölich M, Onkenhout W and Meinders AE (1993). Evidence for brain serotonin-mediated control of carbohydrate consumption in normal weight and obese humans. *Int. J. Obes. Relat. Metab. Disord.*, **17**: 513-520.
- Ram S, Seirawan H and Kumar SK (2010). Prevalence and impact of sleep disorders and sleep habits in the United States. *Sleep. Breath.*, **14**: 63-70.
- Rathnayake KM, Silva K and Jayawardena R (2016). Effects of zinc supplementation on obesity: Study protocol for a randomized controlled clinical trial. *Trials.*, **17**: 534.
- Rondanelli M, Opizzi A and Monteferrario F (2011). The effect of melatonin, magnesium, and zinc on primary insomnia in long-term care facility residents in Italy: A double-blind, placebo-controlled clinical trial. *J. Am. Geriatr. Soc.*, **59**: 82-90.
- Samad N, Haleem MA and Haleem DJ (2016). Protective effects of rice bran oil in haloperidol-induced tardive dyskinesia and serotonergic responses in rats. *Pak. J. Pharm. Sci.*, **29**:1467-1471.
- Sateia MJ and Nowell PD. (2004). Insomnia. *Lancet.*, **364**:1959-73.
- Sarris J and Byrne GJA (2011). Systematic review of insomnia and complementary medicine. *Sleep. Med. Rev.*, **15**: 99-106.
- Siwek M, Dudek D, Paul IA, Sowa-Kućma M, Zieba A, Popik P, Pilc A and Nowak G (2009). Zinc supplementation augments efficacy of imipramine in treatment resistant patients: A double blind, placebo-controlled study. *J. Affect. Disord.*, **118**:187-95.
- Söğüt A, Açıkgöz Ş, Uzun L, Uğur MB, Altın R, Dağlı E, Kaditis A and Ersu R (2016). Leptin levels in children with obstructive sleep apnea syndrome. *Tuberk. Toraks.*, **64**: 283-288.
- Swardfager W, Herrmann N, McIntyre RS, Mazereeuw G, Goldberger K, Cha DS, Schwartz Y and Lanctôt KL (2013). Potential roles of zinc in the pathophysiology and treatment of major depressive disorder. *Neurosci. Biobehav. Rev.*, **37**: 911-29.
- Wali V and Wali VV (2016). Assessment of Various Biochemical Parameters and BMI in Patients with Skin Tags. *J. Clin. Diagn. Res.*, **10**: 09-11.
- Wang MY, Zhou YT, Newgard CB and Unger RH (1996). A novel leptin receptor isoform in rat. *F.E.B.S. Letters.*, **392**: 87-90.
- Van Cauter E and Tasali E (2011) Endocrine physiology in relation to sleep and sleep disturbances. In: Kryger MH, Roth T, Dement WC, editors. Principles and practice of sleep medicine. 5th ed. St. Louis: Elsevier pp.291-311.
- Van Leeuwen WM, Hublin C, Sallinen M, Härmä M, Hrvonen A and Porkka-Heiskanen T (2010). Prolonged sleep restriction affects glucose metabolism in healthy young men. *Int. J. Endocrinol.*, **2010**: 108641.
- Volpe Ad, Lucia A, Pirozzi C and Pastore V (2017). Comparative study between the use of melatonin and a solution with melatonin, tryptophan and vitamin b6 as an inducer of spontaneous sleep in children during an auditory response test: an alternative to commonly used sedative drugs. *J. Int. Adv. Otol.*, **13**: 69-73.
- Yunus MB, Dailey JW and Aldag JC (1992). Plasma and urinary catecholamines in primary fibromyalgia: A controlled study. *J. Rheumatol.*, **19**: 95-97.