Serum levels of leptin, zinc and tryptophan with obesity: A case-control study

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Abstract: The obesity epidemic has turn into a major health threat worldwide and extensively responsible for the increased incidence of many diseases such as diabetes, cardiovascular disease and certain types of cancer. Excessive food intake along with the insufficient physical exercise is the basic impetus for this development. The aim of the present study is to evaluate the serum levels of leptin, zinc and tryptophan (TRP) in obese and non-obese subjects, which play major role in obesity. With the verbal and written consent eighty men were identified from the 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. Results showed that levels of leptin were increased in obese than non-obese subjects significantly. On the other hand levels of zinc and TRP were significantly decreased in obese than non-obese subjects. Furthermore, there was a positive correlation found among leptin, zinc and TRP with obesity. Based on these facts the involvement of leptin, zinc and TRP with obesity will be discussed.

Keywords: obesity, leptin, zinc, TRP, ELISA, FAAS, HPLC

INTRODUCTION

Obesity has become recognized as a worldwide health threat and a major public health challenge. There is currently a lack of simple and effective therapies or preventive treatments against obesity and the mechanisms involved in the onset of diet-induced obesity remain unknown. Studies have evident that altering the strength or sensitivity to the hedonic attractiveness of food (Meye and Adan, 2014), availability of food (Monteiro *et al*., 2013), learned preferences (Berthoud and Zheng, 2012), or signaling from the gut (Hellstorm, 2013) may be involved in initiating diet induced obesity.

Leptin resistance is a hallmark of obesity (Coppari and Bjorbaek, 2012; 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, 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 (Considine *et al*., 1996; Ostlund *et al*., 1996), and directly linked with body mass index (BMI) (Monti *et al*., 2006; Tsuneyama *et al*., 2016; Wali and Wali, 2016).Individuals with obesity or high BMI have increased concentration of leptin in the circulation, but insensitive to endogenous leptin or leptin resistance (Considine *et al*., 1996; Lissner *et al*., 1999), which may be caused by decrease in the effectiveness of leptin receptors to transport circulating leptin into the central nervous system (CNS) (Caro *et al*., 1996; Bjorbaek *et al*., 1999).Conversely, in individuals with anorexia nervosa percent body fat is low which is correlated with low levels of serum leptin in the circulation when compared to normal individuals (Grinspoon *et al*., 1996; Holtkamp *et al*., 2003; Riccioni *et al*., 2003).

Zinc is an essential micronutrient that functions as an anti-inflammatory and anti-oxidative agent (Bao *et al*., 2010) and is very reproachful for many functions of the brain (Standstead *et al*., 2000; Chi *et al*., 2008). Extensive studies indicate low levels of zinc affect many functions of the brain related to development of the brain and produced neuropsychological symptoms (Keller *et al*., 2001; Takeda and Tamano *et al*., 2009; Azab *et al*., 2014; Lang *et al*., 2015). Studies showed that obese (Marreiro *et al*., 2006; Tungtrongchitr *et al*., 2003) and major depressed (Maes *et al*., 1994; 1997) 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; Parsad *et al*., 2007).

Obesity is considered to be an outcome of increased caloric intake (ingestion of all constituents of food, including carbohydrates, lipids, and proteins) and

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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; Nduhirabandi *et al.*, 2012). This finding may be explained by the conversion of TRP to kynurenine, with tryptophan 2,3-dioxygenase and indolamine 2,3-dioxygenase acting as the rate-limiting enzymes of this pathway (Moffett and Namboodri, 2003).

The present work is aimed to evaluate the serum levels of leptin, zinc and TRP in obese and non-obese subjects.

MATERIALS AND METHODS

Subjects

Forty non-obese and forty obese selected men (from 19 to 28 years) were engaged in the present study, which are resident of various areas of Karachi (North Karachi, Gulshan-e-Iqbal, Gulshan-e-hadeed, Nepa Chorangi, Nazimabad), Pakistan. Obesity was defined as BMI of $\geq$ 25kg/m^2. The study protocol and procedures were approved by institutional ethics committee and, in accordance with the ethical standards of the Helsinki declaration, all participants gave informed consent for their participation.

Individuals engaged in this study have answered a questionnaire during face to face interview. Information was collected on demographic variables, smoking, nutritional supplementation, personal medical history, family history and medication use if any. Exclusion criteria included cigarette smoking, vitamin-mineral supplements and/or any other nutritional supplements, the use of oral contraception in a diet based weight-loss program.

Sample collection and biochemical Estimation

After providing informed consent, fasting blood samples (once for a study) were drawn from patient's arm with intravenous infusion under the hygienic environment to avoid contamination. Serum was collected from blood and then 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 TRP 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 (Haleem *et al*., 2009). A 5 µm Shim-Pack ODS separation column of 4.0 mm internal diameter and 150 mm length was used. Separation was achieved by mobile phase containing methanol (5%), octyl sodium sulfate (0.03%) and EDTA (0.0035%) in 0.1 M phosphate buffer of pH 2.9 at an operating pressure 2,000–3,000 psi on Shimadzu HPLC pump. Electrochemical detection was achieved on Shimadzu L-ECD-6A detector at an operating potential of $\pm$ 0.8 to 1.0 V.

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 and Halaas, 1998) was obtained from BioSource Europe S.A.

STATISTICAL ANALYSIS

Data on levels of leptin, zinc and tryptophan of obese and non-obese groups were analyzed by Paired sample t-test. The association among serum levels of leptin, zinc and tryptophan of non-obese and obese was analyzed by Two way ANOVA. Values of $P \leq 0.05$ were considered as significant.

RESULTS

The demographic characteristics including sex, age group, BMI, education and residence of the 80 men engaged in the present study was collected (table 1).

Table 1: Socio-demographic characteristics of study population (n=80).

Characteristics	Non-Obese	Obese
Sex	Male	Male
Age (Years) 19-28	40 (100)	40 (100)
Body Mass Index (Kg/m^2)	19.11+3.15	35.60+2.31
Education >12 years <12years	 12 (30) 28 (70)	 19 (47.5) 21 (52.5)
Resident	Urban	Urban

Data presented as mean $\pm$ SD or number (%).

The serum levels of leptin (fig1), Zinc (fig 2) and TRP (fig 3)in obese and non-obese subjects analyzed by Paired sample t-test showed that the levels of leptin were significantly (t=42.23 df=39 P<0.05) increased, while levels of zinc(t=19.53 df=39 P<0.05) and TRP(t=24.09 df=39 P<0.05) were significantly decreased in obese than non-obese subjects.

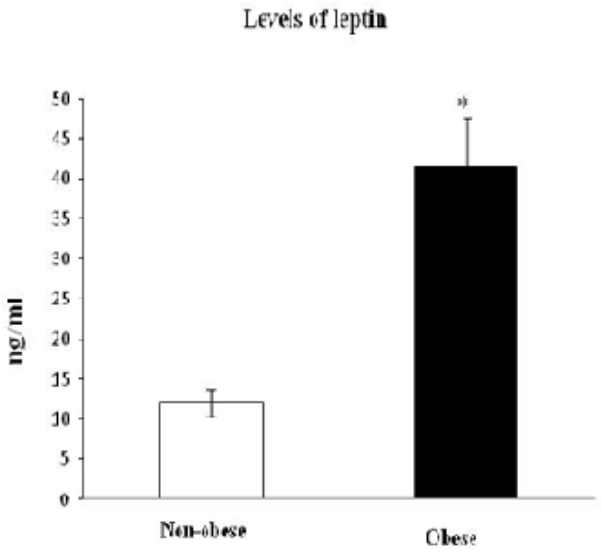

Fig. 1: The serum levels of leptin in obese and non-obese subjects. Values are means ± S.D. (n=40).

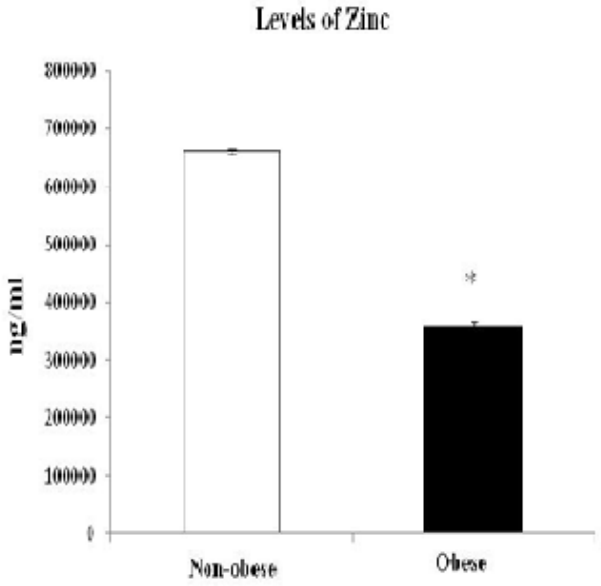

Fig. 2: The serum levels of zinc in obese and non-obese subjects. Values are means ± S.D. (n=40).

The association among serum levels of leptin, zinc and TRP of non-obese and obese was analyzed by Two way ANOVA (table 2)showed that significant effect of obesity ($F=364.73 df=1,234$ $p<0.05$), serum levels of leptin, zinc and TRP ($F=4042.70 df=2,234$ $p<0.05$) and interaction among obesity and serum levels of leptin, zinc and TRP ($F=364.82 df=2,234$ $p<0.05$). Post-hoc analysis by Tukey's test showed that serum levels of zinc were significantly higher than leptin and TRP in both non-obese and obese subjects.

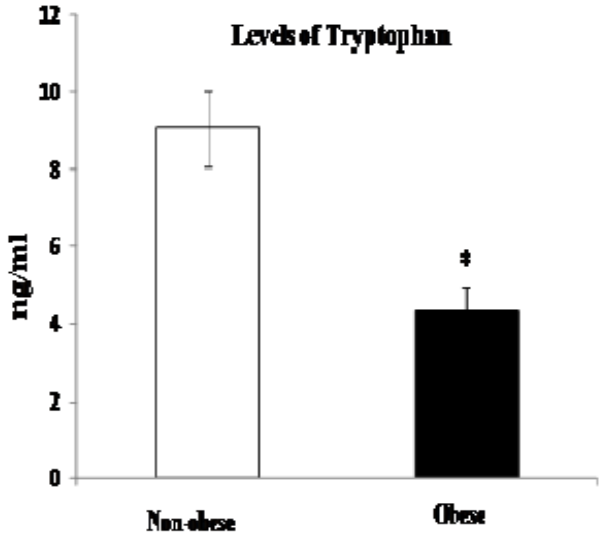

Fig. 3: The serum levels of tryptophan in obese and non-obese subjects. Values are means ± S.D. (n=40).

Table 2: Serum levels of leptin, zinc and tryptophan in non-obese and obese subjects.

	Leptin (ng/ml)	Zinc (ng/ml)	Tryptophan (ng/ml)
Non-obese	12.09^{*} ±1.79	664625^{**} ±48437.47	9.063^{+} ±0.97
Obese	40.39^{*} ±3.98	357575^{**} ±89396.23	4.31^{+} ±0.60

Values are ± SD. Differences in *vs**, **vs+ are significant ($P<0.05$).

DISCUSSION

Recent evidence suggests that sufficiency and deficiency of some biomolecules are related to obesity. Human obesity is associated with higher circulating leptin (Haque *et al*., 2003, 2012) resulting in leptin resistance. A role of leptin in the regulation of BMI is well documented (Tsuneyama *et al*., 2016; Wali&Wali, 2016). A number of studies show that information on adipocytes metabolism and body weight to the appetite center in hypothalamic region of the brain is communicated by leptin. A decrease in appetite and increase in energy expenditure was observed in leptin deficient ob/ob mice when treated with recombinant leptin. (Campfield *et al*., 1995; Weigle *et al*., 1995). The present studyshows that higher BMI (table 1) is directly proportional to higher leptin levels (fig 1 and table 1) suggests the relationship between leptin and BMI is significant and is associated with the relationship of leptin to total body weight as BMI and fatness.

Zinc, an essential trace element is found to be closely in relation with BMI. Studies have shown that low intake of zinc and low serum levels of zinc are associated with an increase prevalence of obesity (Considine *et al*., 1996).

Studies have explored the relationship between leptin and zinc and demonstrated that leptin secretion critically affect zinc (Singh *et al*., 1998). The hippocampus which is enriched in glucocorticoid receptors, in this region deficiency of zinc elicits the neurochemical and metabolical changes (Takeda *et al*., 2008; Tamano *et al*., 2009).

A role of leptin in hypothalamic-pituitary-adrenal (HPA)-axis functioning emerged because leptin-deficient (ob/ob) mice was found to hyper-secrete corticosterone (DeVos *et al*., 1995; Slieker *et al*., 1996) and chronic administration of leptin corrected the hypercorticosteronemia in these rats (Stephens *et al*., 1995; Arvaniti *et al*., 2001). The HPA axis was also found to be hyperactive in db/db mice (Chen *et al*., 1996) with mutation in the leptin receptor but hypersecretion of corticosterone in db/db mice was not corrected by administration of leptin, suggesting that normal functioning of leptin receptors is essential for the expression of its effect on the HPA-axis (Haleem, 2014). In our present findings, we observed, that low levels of zinc substantially increased leptin production (fig1 and 2; table 2), suggesting obesity has inverse feed back on HPA-axis to decreases the serum concentration of zinc and increases concentration of leptin. These results suggest that increased levels of leptin and decreased levels of zinc are also linked to neuropsychological symptoms such as increase in depressive like behavior (Keller *et al*., 2001; Jin *et al*, 2008; Takeda and Tamano *et al*., 2009; Azab *et al*., 2014; Lang *et al*., 2015).

In the biosynthesis of serotonin, 5-hydroxytryptophan (5-HTP) is the intermediate metabolite of the essential amino acid L-TRP (Timothy and Birdsall, 1998). Serotonin (Jans *et al*., 2007) and TRP (Ashley *et al*., 1985; Gatti *et al*., 1993) deficiency is well-known to be associated with onset and development of depression, indicating that the deficiency of serotonin and TRP in obese mice might explain the depressive moods associated with obesity. Kim *et al*.(2013) have proposed that circulating serotonin levels can be lowered by obesity. However plasma levels may be better surrogate biomarkers for mood change in obesity than brain serotonin levels in terms of tissue accessibility. The present study (fig 3, table2) is consistent with the previous studies (Ashley *et al*., 1985; Gatti *et al*., 1993) that showed low levels of TRP in obese individuals.

An impaired CNS action of leptin (Yamada *et al*., 2011) and zinc (Watanabe *et al*., 2010) is also reported in depression with obesity. The circulating serotonin reduces leptin secretion via its receptors on the adipocytes (Stunes *et al*., 2011) indicating a role of peripheral serotonin in leptin expression/secretion. It has been reported that in the brain stem and hypothalamus of obese mice serotonin metabolism is increased following administration of leptin when compared to lean mice (Harris *et al*., 1998), suggesting that serum levels of leptin and TRP (fig 1 and 3; table 2) are inversely proportional in obesity and depression as well. Apart from that, zinc plays a vital role in TRP production (Pearson *et al*., 2006).

In conclusion, the present study suggests, that interdependency of serum levels of leptin, zinc and TRP on one another and their disturb levels may cause obesity.

REFERENCES

Ashley DV, Fleury MO, Golay A, Maeder E and Leathwood PD (1985). Evidence for diminished brain 5-HT biosynthesis in obese diabetic and no-diabetic humans. *Am. J. Clin. Nutr.,* **42**: 1240-1245.

Arvaniti K, Richard d, Picard F and Deshaies Y (2001). Lipid deposition in rats centrally infused with leptin in the presence or absence of corticosterone. *Am. J. Physiol. Endocrinol. Metab.,* **28**: 809-816.

Azab FAS, Saleh HS, Elsaad FW, Mona AM, Sherief ML and Esh MHA (2014). Serum trace elements in obese Egyptian children: a case-control study. *J. Pediatr.,* **40**: 20-27.

Bano F, Ahmed A, Ahmed M & Parveen T (2014). Anthumgraveolens seeds aqueous extract stimulates whole brain 5-HT metabolism and reduces feeding behavior and body weight in obese rats. *Pak. J. Pharm. Sci.,* **28**: 221-225.

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.

Berthoud HR & Zheng H (2012). Modulation of taste responsiveness and food preference by obesity and weight loss. *Physio. Behav.,* **107**: 527-532.

BjorbeakC, El-Haschimi K, Frantz JD and Filier JS (1999). The role of SOCS-3 in leptin signaling and leptin resistance. *J. Biol. Chem.,* **274**: 30059-30065.

BlundellJE(1986). Serotonin and appetite. Neuropharmacol., **23**: 1537-1551.

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.

Caro JF, Kolaczynski JW, Nyce MR, Ohannesian JP, Opentanova I, Goldman WH, Lynn RB, Zhang PL, Sinha MK and Considine RV (1996). Decreased cerebrospinal fluid/serum leptin ration in obesity: A possible mechanism for leptin resistance. *Lancet.,* **348**: 159-161.

CampfieldLA, Smith FJ, Guisez Y, Devos R and Burn P (1995). Recombinant mouse OB protein: Evidence for a peripheral signal linking adiposity and central neural network. *Science,* **169**: 546-549.

Chen H, Chariat O, Tartaglia LA, Woolf EA, Weng X, Ellis SJ, Lakey ND, Culpepper J, Moore KJ, Breitbart RE, Duyk GM, Tepper RI and Morgenstern JP (1995). Evidence that the diabetes gene encodes in the leptin receptor: identification of a mutation in the leptin receptors gene in db/db mice. *Cell.,* **84**: 491-495.

Chi Z, Wang X, Cai JQ, Stoltenberg M, Danscher G & Wang ZY (2008). Zinc transporter 3 immunohistochemical tracing of sprouting mossy fibers. *Neurochem Int.,* **52**: 1305-1309.

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.

Considine RV, Sinha M., Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ and Bauer TL (1996). Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N. Engl. J. Med.,* **334**: 292-295.

Coppar R and Bjorbeak C (2012). Leptin revisited: its mechanism of action and potential for treating diabetes. *Nature. Reviews. Drug. Discove.,* **11**: 629-708.

DeVos P, Saladin R, Auxerx J and Stael B (1995). Induction of ob gene expression by corticosteroids is accompanied by body weight loss and reduced food intake. *J. Biochem.,* **270**: 15958-15961.

Ferstrom JD and Wurtman R (1971). Brain serotonin content: Physiological dependence on plasma TRP level. *Science,* **197**: 114-152.

Friedman JM and Halaas JL (1998).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.

GattiE, Porrini M, Neo D, Crovetti R and Testolin G (1993). Plasma amino acid changes in obese patients on very low-calorie-diet. *Int. J. Vitam. Nutr. Res.,* **63**: 81-85.

Haleem DJ (2014). Investigations into the involvement of leptin in responses to stress. *Behav. Pharmacol.,* **25**: 384-397.

Haleem DJ, Samad N,Parveen T, Haider S and Haleem MA (2007). Role of serotonin-1A receptors in restraint-induced behavioral deficits and adaptation to repeated restraint stress in rats. *Int. J. Neursci.,* **117**: 243-257.

Harris RB, Zhou J, Redmann SM Jr, Smagin GN, Smith SR, Rodgers E and Zachwieja JJ (1998). A leptin dose-response study in obese (ob/ob) and lean (+/?) mice. *Endocrinol.,* **139**(1): 8-19.

Haque Z and Rehman MA (2003). Serum leptin levels in female patients with NIDDM. *J. Coll. Physicians. Surg. Pak.,* **13**: 130-134.

Haque Z, Yasmeen F and Haleem DJ (2012). A G15719C leptin gene polymorphism is associated with lesser perception of stress and other physiological reactions to stress in adult working Pakistani women. *Int. J. Collabarative Research Internal Medicine Public Health.*, **4**: 296-307.

Hellstrom PM (2013). Satiety signals and obesity. *Current opinion in Gastrienterology*., **19**: 222-227.

Holtkamp K, Herpertz-Dahlmann B, Mika ., Heer ., Heussen N, Fichter M, Herpertz S, Senf W, Blum WF, Schweiger U, Warnke A, Ballauff A, Remschmidt H and Hebebrand J (2003). Elevated physical activity and low leptin levels co-occur in patients with anorexia nervosa. *J. Clin. Endocrinol. Metab.,* **88**: 5169-5174.

Jans MP, van den Heuvel SG, Hildebrandt VH and Bongers PM (2007). Overweight and obesity as predictors of absenteeism in the working population of the Netherlands. *J. Occup. Environ. Med*., **49**: 975-80.

Jing MY, Sun JY and Wang JF (2008).The effect of peripheral administration of zinc on food intake in rats fed Zn-adequate or Zn-deficient diets. *Biol. Trace. Elem. Res.,* **124**: 144-156.

Keller KA, Grider A and Coffield JA (2001). Age-dependent influence of dietary zinc restriction on short-term memory in male rats. *Physiol. Behav.,* **72**: 339-348.

Kim M, Bae S and Lim KM (2013).Impact of High Fat Diet-induced Obesity on the Plasma Levels of Monoamine Neurotransmitters in C57BL/6 Mice. *Biomol. Ther*., **21**: 476-480.

Lang UE, Beglinger C, Schweinfurth N, Walter M and Borgwardt S (2015). Nutritional aspects of depression. *Cell. Physiol. Biochem.* **37**: 1029-43.

Lissner L, Karlsson C, Lindroos AK, Sjostrom L, Carlsson B, Carlsson L and Bengtsson C (1999). Birth weight, adulthood BMI and subsequent weight gain in relation to leptin levels in swedish woman. *Obes. Res.*, **7**: 150-154.

Meye FJ and Adan RA (2014). Feeling about food: the ventral tegmental area in food reward and emotional eating. *Trends in Pharmacol., Sci*., **35**: 31-40.

Maes M, Scharpe S, Meltzer HY, D`Hondt P, Cosyns P and De Broe ME (1994). Hypozincemia in depression. *J. Affect. Disord.,* **31**: 135-140.

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.

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**: 109-118.

Moffett JR and Namboodiri MA (2003). Tryptophan and the immune response. *Immunol. Cell. Biol.*, **81**: 247-265.

Monteiro CA, Moubarac JC, Cannon G, Ng SW and Popkin B (2013). Ultra-processed products are becoming dominant in the global food system. *Obesity. Review*., **14**: 21-28.

Monti V, Carlson JJ, Hunt SC and Adams TD (2006). Relationship of ghrelin and leptin hormones with body mass index and waist circumference in a random sample of adults. *J. Am. Diet. Assoc.*, **106**: 822-828.

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.

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-verities. *J. Clin. Endocrinol. Metab.*, **81**: 3909-3913.

Parsad AS, Bao B, Beck FW, Kucuk O and Sarkar FH, (2004). Antioxidant effects of zinc in human. *Free. Radic. Biol. Med.*, **37**: 1182-1190.

Pearson NJ, Johnson LL and Nahin RL (2006).Insomin, trouble sleeping, and complementary and alternative medicine: Analysis of the 2002 national health interview survey data. *Arch. Intern. Med.*, **166**: 1775-1782.

Rapport MM, Green AA and Page IH (1948). Serum vasoconstrictor, serotonin: Isolation and characterization. *J. Biol. Chem.*, **176**: 1243-1251.

Ricconi G, D Orazio N,Menna V, Lambo MS, Guagnano MT and Dill llio C (2003). Role of leptin in anorexia nervosa. *Clin. Ther.*, **154**: 401-404.

Sandstead HH, Penland JG, Alcock NW, Dayal HH, Chen XC, Li JS, Zhao F and Yang JJ (1998). Effects of repletion with zinc and other micronutrients on neuropschological performance and growth of Chinese children. *Am. J. Clin. Nutr.*, **68**: 470-475.

Singh RB,Beegom R, Rastogi SS, Gaoli Z and Shoumin Z (1998). Association of low plasma concentration of antioxidant vitamins, magnesium and zinc with high body percent measured by bioelectrical impedance analysis in indian men. *Magnes. Res.*, **11**: 3-10.

Sleiker LJ, Sloop KW, Surface PL, Kriauciunas A, LaQuier F, Manetta J, Bue-Valleskey J and Stephens TW (1996). Regulation of expression of ob mRNA and protein by glucocorticoids and CAMP. *J. Biol. Chem.*, **271**: 5301-5304.

Stephens TW, Basinski M, Bristow PK, Bue-Valleskey J, Burgett SG, Craft L, Hale J, Hoffmann J, Hsiung HM and Kriauciunas A (1995). The role of neuropeptide Y in the antiobesity of the obese gene product. *Nature*. **377**: 530-532.

Stunes AK, Reseland JE, Hauso O, Kidd M, Tømmerås K, Waldum HL, Syversen U and Gustafsson BI (2011). Adipocytes express a functional system for serotonin synthesis, reuptake and receptor activation. *Diabetes. Obes. Metab.*, **13**: 551-558.

Takeda A and Tamano H (2009). Insight into zinc suganling from dietary zinc deficiency. *Brain. Rev*., **62**: 33-34.

Takeda A, Yamada K, Tamano H, Fuke S, Kawamura M and Oku N (2008). Hippocampal calcium dyshomeostasis and long term potentiating in 2-week zinc deficiency. *Neurochem. Int.*, **52**: 241-246.

Timothy C &Birdsall ND (1998). 5-HTTP: A clinically-effective serotonin precursor. *Alternative. Medicine. Review.*, **3**: 271-280.

Tsuneyama N, Suzuki Y, Sawamura K, Sugai T, Fukui N, Watanabe J, Ono S, Saito M and Someya T (2016). Effect of Serum Leptin on Weight Gain Induced by Olanzapine in Female Patients with Schizophrenia. *PLoS. One.* **11**: e0149518.

Tungtrongchitr R, Pongpaew P, Phonrat B, Tungtrongchitr A, Viroonudomphol D, Vudhivai N and Schelp FP (2003). Serum cooper, zinc ceruloplasmin and superoxide dismutase in Thai overweight and obese. *J. Med. Assoc. Thai.,* **86**: 543-551.

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).Anovel leptin receptor isoform in rat. *F.E.B.S. Letters.,* **392**(2): 87-90.

Watanabe M, Tamano H, Kikuchi T and Takeda A (2010).Susceptibility to stress in young rats after 2-week zinc deprivation. *Neurochem. Int.,* **56**: 410-416.

Weigle DS, Bukowski TR, Foster DC, Holderman S, Karmer JM and Lasser G (1995). Recombinant ob protein reduces feeding and body eight in the ob/ob mouse. *J. Clin. Invest*, **96**: 2065-2070.

Yamada N, Katsuura G, Ochi Y, Ebihara K, Kusakabe T, Hosoda K and Nakao K (2011). Impaired CNS leptin action is implicated in depression associated with obesity. *Endocrinol.,* **152**: 2634-2643.