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A G15719C Leptin Gene Polymorphism is Associated with Lesser Perception of Stress and Other Physiological Reactions to Stress in Adult Working Pakistani Women

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ABSTRACT

Introduction: The prevalence of obesity and depression is increasing at an alarming rate. The state of obesity and perception of job stress is different in both genders. The circulating levels of stress related hormones cortisol, serotonin and leptin are attenuated in stress.

Objective: To investigate variations in the coding regions of leptin gene in relation to stress perception and other biochemical and physiological parameters of stress perception.

Method: With verbal and written consent, twenty working women were identified from 5 corporate sections of local banks in Karachi. Stress perception was measured by standardized questionnaire. Depending on stress scores the data were classified as no/minimal, mild, moderate and severe stress groups. Changes in appetite were determined by visual analogue scale for hunger and desire to eat during stress. The chemical analytes were measured by ELISA. Leptin gene was sequenced using 3130 Genetic Analyzer of (ABI) Applied Biosystems using BigDye Terminator 3.1 Sequencing Kit protocols at Centralized Science Laboratory University of Karachi. One way ANOVA was applied to analyze data.

Result: The BMI and VAS for hunger and desire to eat increased significantly with the severity of stress ($p<0.05$). Although circulating levels of leptin and cortisol also increased with stress perception ($p<0.05$) but a decrease in cortisol levels was observed in the group with severe stress perception. Plasma serotonin decreased with increase in stress perception. A single nucleotide polymorphism was found in 5' flanking region of exon 2 of leptin gene compared to the reference gene fragment retrieved from gene bank with no minimal perception of stress. The guanosine at position 15719 in the reference fragment was replaced by cytidine. No variation was found in the DNA sequences of exon 2 of women with mild, moderate or severe perception of stress. No variation was found in the segment of exon 3 of leptin gene.

Conclusion: The consistent finding of a G15719C variant on the 5' flanking region of exon 2 of LEP in the group of women who claimed no/minimal stress suggest a role of this gene in stress induced obesity and other physiological reactions to stress.

Keywords: Enter up to 5 Keywords separated with comma

Introduction

Modernization, persistent urge to compete with the high living standards, sedentary lifestyles and availability of low cost high calorie diet has led

the human race to a consistent state of stress and weight gain. Daily life events, the stress at job and even the buzzes from personal electronic gadgets play a role in stress response by an individual [1]. A persistent state of stress may lead to depression and anxiety [2]. Perception of stress is different in both genders. Earlier we observed [3] that women perceive a higher degree of stress perception relative to the men working in same environment [4]. Men tend to have a greater BMI in moderate stress compared to women who exhibit increased appetite and tend to have higher than normal BMI both in moderate and severe stress.

The prevalence of obesity and depression is increasing at an alarming rate in modern societies of both developed and developing countries [5]. Although a strong correlation exists between depression and obesity, extreme weight loss with anorexia nervosa is also observed in significant number of depressed patients [6]. The basis of disparity of eating and stress coping behaviors in depression remains largely unexplained.

The adipocyte hormone leptin administration in ob/ob mice resulted in weight reduction and increased energy expenditure [7]. On the other hand human obesity is also associated with high circulating leptin [8] suggesting its resistance at receptor level. Evidence of both hyper and hypophagia leading to derangements in body weight during depression provides a conceptual understanding of leptin involvement in stress related behavioral changes [9,10, 11].

Hypercortisolism is a feature of both obesity and depression (12). Dallman et al, [13] reported a blunted adrenal glucocoricoid release in obesity. Humans with major depression show decreased sensitivity to glucocorticoids [14] and increased cortisol levels in blood [15]. Plasma cortisol decreases the HPA axis activity by negative feedback mechanism, decreasing corticotropic hormone (CRH) in the hypothalamus. A dysregulated CRH is reported in depression [16] with a blunted ACTH and normal cortisol response to CRH stimulation [17]. The biological effect of leptin in stress remains ambiguous. Leptin administration to starvation and restraint stressed animals, normalized the increases in ACTH and corticosterone levels due to post-stress hyperactivity of HPA axis [18]. Glucocorticoids increase the leptin expression and release while leptin adds to the negative feedback loop regulating HPA axis [19].

Serotonin (5-HT) has also been identified as the food intake and body weight modulator. 5-HT reuptake inhibitors are used as weight reducing agents [20]. Serotonin transporter protein and leptin receptor genes co-express in dorsal raphe nuclei [21]. Reduced expression of 5-HT transporter protein in leptin deficient mice [22] and increased content and metabolism of 5-HT in forebrain by leptin [23, 24] further explain that leptin exerts a positive effect on central 5-HT metabolism. All these data suggest the existence of an interaction between leptin and serotonin in modulation of appetite and stress perception.

Leptin gene is located at chromosome 7q31.3 [Accession number NG_007450.1]. It has 23351 base pairs [25]. The variations of leptin receptor gene have been identified and these result in the development of the leptin resistance leading to a state of hyperleptinemia and obesity [26]. To best of our knowledge the variations in the leptin gene are not correlated with the perception and coping capabilities during the stress.

Aims & Objectives

Previous observations of increased leptin, cortisol and decreased serotonin with increasing stress perception both in men and women and the antianxiety/depressant effects of injected leptin to restrained rats evident from the reversal of stress induced behavioral changes, led us to make an attempt to investigate the possible interactions of the variations in the coding regions of the obesity gene and stress responses leading to behavioral (eating) disorders and chemical (plasma leptin, serotonin, cortisol levels) changes, resulting in clinical depression/anxiety disorders and obesity.

Material and Method

Subjects: Adult working women were identified from the corporate section of five branches of a local bank in Karachi. All the women were not suffering from any chronic, metabolic or nutritional disease. After obtaining the verbal and written consent the

women were asked to fill a questionnaire to evaluate the perception of stress [27]. Stress perception was calculated as described earlier in general analytical methods. Subjective appetite was measured using visual analogue scale (VAS) method [28]. The anthropometric measures were taken to calculate BMI. 3 ml of blood was collected in plain tube to collect serum after centrifugation to estimate serum leptin levels. Another 5 ml of blood was collected in heparinized tube to determine serotonin and cortisol and DNA sequence analysis.

The chemical variables were determined by ELISA. The kit for serum leptin [29] and serotonin [30] were obtained from BioSource Europe S.A. The kit for cortisol [31] was obtained from The Equipar Diagnostics.

DNA Sequencing Protocol: The genomic DNA was isolated from white blood cells (WBC) using phenol chloroform method [32]. Samples were subjected to PCR amplification of the target sequence in an Applied Biosystems Thermal Cycler 2720 with 5'-TTTGACGGGATGGTAGCCAG-3', and 5'-AGAGTGGAGCCCTGTGCTTT-3' as forward and reverse primers exon 2 respectively and 5'-TCACCTGGGTGCAGGATACAA-3' and 5'GGGTCTTATGCCTTTGGAAGAG-3' forward and reverse primers for exon 3 respectively. The primers were designed for exon 2 and 3 sequences of the leptin gene, using primer designing website [http://www.yeastgenome.org/cgi-bin/web-primer]. Amplified products were confirmed on gel electrophoresis and then cleaned up enzymatically using ExoSAP. The purified product was quantified by taking absorbance at 260nm using UV spectrophotometer and 100ng of the sample DNA was used for analysis of the sequence of target fragment of gene using 3130 Genetic Analyzer of (ABI) Applied Biosystems using BigDye Terminator 3.1 Sequencing Kit protocols at Centralized Science Laboratory University of Karachi. **Gene Analysis, Discovery and Characterization of "novel SNP".**

The sequences were identified as fragments of human leptin by using blast (33). The partial leptin gene fragments containing exon 2 (425bp) and exon 3 (668bp) of each group (n=5 each) were assembled using DNA star software. The reference sequence of leptin gene was retrieved from the gene bank [34]. The artifactual and low signal peaks responses at the electrophrogram were removed using staden package software. The mapped positions with up to two mismatches were recorded.

Statistical Analysis of Blood Parameters. One way analysis of variance (ANOVA) was applied to evaluate the effect of stress perception score on behavioral (Appetite, VAS for hunger and VAS for desire to eat), physical (BMI) and chemical (Plasma leptin, cortisol and serotonin) parameters. Tuckeys test was applied as a post-hoc test on the data. The differences in the genotype frequencies of LEP for Groups for various stress perception was evaluated using Fisher's exact test if expected values of 20% of cells were smaller than 5.

Results

Adult women volunteers participated in the study with an average age of 34.53 ± 11.74 years ranging from 29 – 59 years. One way ANOVA showed that perception of stress significantly affects the BMI ($F_{3,19}$=4.82, $p<0.05$) (Fig-1). Post-hoc analysis showed significant increase in the BMI of the women who perceived severe stress (32.61±2.54) compared to no/minimal (24.36 ±0.86) stressed women. The BMI of the women with mild (26.71 ± 0.96) or moderate (28.03 ± 1.35) perception of stress were not found significantly different from the other groups. Fig-2 A and B show the behavioral changes related to appetite effected by stress. Data

analyzed with one way ANOVA revealed a significant difference of the VAS values of hunger ($F_{3,19}$=13.70, p<0.01) and desire to eat ($F_{3,19}$=8.812, p<0.01) values. Post-hoc analysis revealed that moderately and severely stressed women had significantly higher values of hunger and desire to eat compared to the women with no/minimal and mild stress perception. Severely stressed women also showed significantly higher VAS for desire to eat compared to mildly stressed women. A significant effect of stress perception was noted on appetite ($F_{3,19}$=7.60, p<0.01). Post-hoc analysis showed the appetite of moderately and severely stressed women was significantly higher compared to no/minimal stress perception group. The chemical variables plasma leptin and cortisol are shown in Fig-3. One way ANOVA showed a significant effect of stress perception of circulating levels of leptin ($F_{3,19}$=30.47, p<0.01) and cortisol ($F_{3,19}$=27.50, p<0.01). Post-hoc analysis showed that plasma leptin levels were significantly greater in moderately and severely stressed women compared to the women with no/minimal (p<0.001) and mild stress perception (p<0.01). Severely stressed women also showed significantly higher concentrations of circulating leptin compared to women with moderate stress (p<0.01). The plasma cortisol levels were significantly higher in women with mild and moderate stress perception (p<0.01) compared to no/minimal stress group. Strikingly the women who reported severe stress perception had significantly lesser concentration of circulating cortisol compared to mild (p<0.05) and moderate (p<0.001) stress perception group. Fig-4 depicts the graphical presentation of plasma serotonin concentrations in the women with various levels of stress perception. One-way ANOVA showed a significant effect of stress perception on plasma serotonin concentrations ($F_{3,19}$=9.624, p<0.01). Post-hoc analysis of the data revealed significantly lower plasma serotonin levels in the women with moderate and severe stress perception compared to no/minimal stress perception groups. The sequence of fragment of leptin gene including protein coding regions exon (Ex) 2 and 3 are given in table 1 and 2 respectively. A single nucleotide polymorphism was found in 5' flanking region of exon 2 of leptin gene compared to the reference gene fragment retrieved from gene bank with no minimal perception of stress within the same job environment. The guanosine at position 15719 in the reference fragment was replaced by cytidine in our samples. No variation was found in the DNA sequences of EXON 2 of women with mild perception of stress.

Six hundred and fifty to six hundred and seventy base pairs of linear DNA fragment of leptin gene including exon 3 were sequenced. No variation was found in the any of the samples belonging to various groups of stress perception. One way ANOVA was applied to analyze the consequences of presence of a SNP G15719C at the 5' flanking region of exon 2 on parameters of obesity and stress. It exerts a significant effect on levels of stress perception ($F_{1,19}$= 27.0, p<0.01), change of appetite during stress perception ($F_{1,19}$= 12.69,p<0.01), VAS for hunger ($F_{1,19}$= 7.95, p<0.05) and VAS for desire to eat ($F_{1,19}$= 8.21, p<0.05). The serum leptin levels were also significantly affected by the presence of G15719C variant ($F_{1,19}$= 5.67, p<0.05), plasma cortisol ($F_{1,19}$= 5.34,p<0.05) and serotonin ($F_{1,19}$= 11.76, p<0.05). The body weight ($F_{1,19}$= 5.164, p<0.05) also significantly affected by the presence of G15719C variant.

Discussion

The adipocyte hormone leptin has been reported to have an antianxiety/depression activity [35]. The antianxiety effects of leptin are functionally similar to the antidepressant targeting serotonergic system [36] suggesting the interaction of peripheral leptin expression with central 5-HT

actions. The coexistence of obesity and depression indicates that there is some common factor that regulates the mood swings, behavioral changes and body weight gain. Leptin plays a role in cognitive processes, learning and memory by activation of receptors in limbic areas of brain including hippocampus [37]. Human obesity is associated with the higher circulating leptin [8] resulting in leptin resistance. In the present study we found that severely stressed women are obese consistent with the previous findings [3]. An impaired CNS action of leptin is reported in depression with obesity [38]. The appetite along with the VAS for hunger and desire to eat was also increased in the women with perception of severe stress.

Stress perception is marked with HPA axis activation and high circulating cortisol levels [39]. The increase in corticotrophic hormone (CRH) enhances appetite along with the release of adrenocorticotropic hormone (ACTH). The glucocorticoid induced hyperphagia and increase in body weight has also been reported [40]. Our results confirm that the women who perceive moderate stress exhibit higher levels of cortisol (Fig-3) and an increased VAS for hunger and desire to eat. Cortisol exerts stimulatory effect on leptin synthesis and secretion while leptin in turn inhibits the secretion of cortisol via feedback mechanism acting centrally [41]. Our results showed the reciprocal relation of both circulating cortisol and leptin in the women who perceive severe stress suggesting the effect of higher leptin on cortisol secretion and decrease in their stress coping capabilities.

The exposure to acute stress is associated with high central 5-HT levels. The levels are decreased in repeated stress due to adaptation response [42]. The peripheral 5-HT is synthesized in GIT chromaffin cells, released in blood and stored in platelets. Peripheral 5-HT is implicated in aggregation of platelets. Reduced platelet 5-HT concentrations have been suggested as probable biomarkers in insomnia and alcoholism [43]. The reduction in plasma serotonin levels with the severity of stress perception in our results suggests the possible marker of stress perception. The circulating 5-HT reduces leptin secretion via its receptors on the adipocytes [44] indicating a role of peripheral 5-HT in leptin expression/secretion. A reduction in circulating 5-HT associated with larger leptin levels and severity of stress perception might be the cause of increased appetite exhibited by the women who perceive higher level of stress and increased weight gain.

Present study provides the first evidence of the impact of a single nucleotide polymorphism G15719C on the 5' flanking region of the second exon of leptin gene, on the smaller perception of stress in working women population of Pakistan. Leptin gene deficiency (ob/ob) in mice is associated with morbid obesity [45]. Although many have reported genetic contribution in rodent obesity [46, 47, 48] mutations in leptin gene are rare in obese humans [49]. Mammes et al. [50, 51] demonstrated that the G-2548A variant in the promoter of LEP was associated with decrease in BMI of overweight women. Apart from the link with obesity leptin gene polymorphisms are also linked to the various other disease phenotypes [52]. The rarity of LEP mutations led the researchers to find out the variations in 5' flanking and promoter regions of leptin gene [53, 54]. Several silent mutations have been described [55]. Many variations have been identified highly polymorphic 5' flanking region of LEP of obese women population in USA, but the association was is clear due to low variability of the coding regions [55]. Our results also did not differ in the coding regions (exon 2 and 3) compared to the reference sequence retrieved from the gene bank. The interesting finding in our result was the variation of a single nucleotide G15719C in 5' flanking region of exon 2 of the women who perceive no/minimal stress in the same job environment as the other groups. Although the Leptin molecule is translated from three exons, exon-1 codes for signal sequence required for the entrance of leptin into the endoplasmic reticulum. The exon 2 and 3 contribute to the amino acids in mature leptin molecules. It might be suggested that the G15719C variant is associated for the lesser perception of stress ($p<0.01$). This group also exhibited lesser VAS for hunger and desire to eat ($p<0.01$) as well as had lesser alleviations of the circulating levels of leptin, cortisol and serotonin ($p<0.05$). The BMI within the normal limits along with other findings suggest the well regulated metabolic loops required for coping capabilities

and behavioral changes during stress. Smaller sample size and cross sectional study design were the limitations for the establishment of G15719C variant on LEP as a causal effect of less perception of stress.

Conclusion

Present study therefore hypothesize that the consistent finding of a G15719C variant on the 5' flanking region of exon 2 of LEP in the group of women who perceive no/minimal stress, is involved in stress induced increases in BMI and other physiological reactions to stress.

Conflict of Interest: None declared.

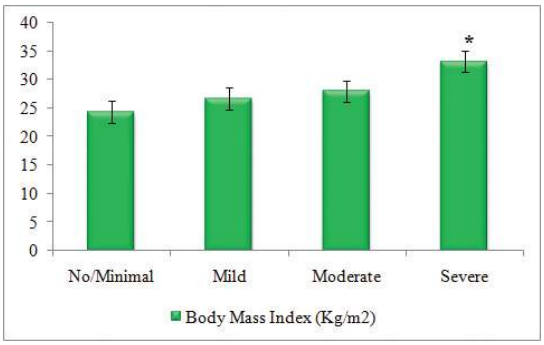
References

1. Hébert S, Lupien SJ. The sound of stress: blunted cortisol reactivity to psychosocial stress in tinnitus sufferers. *Neurosci Lett.* 2007, Jan 10;411(2):138-42.
2. Lovibond PF, Lovibond SH.The structure of negative emotional states: comparison of the Depression Anxiety Stress Scales (DASS) with the Beck Depression and Anxiety Inventories. *Behav Res Ther.* 1995 Mar; 33(3):335-43.
3. Haque Z, Haleem DJ. Role of peripheral serotonin in stress induced obesity. Medical Channel, 2011; 17(4): 5 - 10
4. Haque Z, Haleem DJ. The interaction of serum serotonin, cortisol and leptin in stress related obesity in working men at educational institutions of Karachi. KUJS 2010; 38: 23 – 27.
5. WHO, World health organization report on onesuty facts March 2011; www.who.int/mediacentre/factsheets/fs311/en/index.html
6. Bossola M, Ciciarelli C, Di Stasio E, Panocchia N, Conte GL, Rosa F, Tortorelli A, Luciani G, Tazza L. Relationship between Appetite and Symptoms of Depression and Anxiety in Patients on Chronic Hemodialysis. *J Ren Nutr*. 2011 Jun 16. [Epub ahead of print]
7. Friedman JM. Leptin at 14 y of age: an ongoing story. *Am J Clin Nutr.* 2009 Mar;89(3):973S-979S.
8. Haque Z, Rahman MA. Serum leptin levels in female patients with NIDDM. *J Coll Physicians Surg Pak.* 2003 Mar; 13(3):130-4.
9. Teychenne M, Ball K, Salmon J. Physical activity and likelihood of depression in adults: a review. *Prev Med.* 2008 May;46(5):397-411.
10. Rondina Rde C, Gorayeb R, Botelho C. Psychological characteristics associated with tobacco smoking behavior. *J Bras Pneumol.* 2007 Oct; 33(5):592-601.
11. Kamphuis MH, Geerlings MI, Tijhuis MA, Giampaoli S, Nissinen A, Grobbee DE, Kromhout D. Physical inactivity, depression, and risk of cardiovascular mortality. *Med Sci Sports Exerc*. 2007 Oct; 39(10):1693-9.
12. Bornstein SR, Schuppenies A, Wong ML, Licinio J. Approaching the shared biology of obesity and depression: the stress axis as the locus of gene-environment interactions. Mol Psychiatry. 2006; 11(10):892-902.
13. Dallman MF, la Fleur SE, Pecoraro NC, Gomez F, Houshyar H, Akana SF. Minireview: glucocorticoids – food intake, abdominal obesity, and wealthy nations in 2004. Endocrinology 2004; 145: 2633–2638.
14. Pariante CM. Glucocorticoid receptor functions in vitro in patients with major depression. Stress 2004; 7: 209–219.
15. Dallman MF. Fast glucocorticoid actions on brain: back to the future. Front Neuroendocrinol. 2005; 26(3-4):103-8.
16. Arborelius L, Owens MJ, Plotsky PM, Nemeroff CB. The role of corticotropin-releasing factor in depression and anxiety disorders. *J Endocrinol* 1999; 160: 1–12.
17. Heim C, Newport DJ, Bonsall R, Miller AH, Nemeroff CB. Altered pituitary-adrenal axis responses to provocative challenge tests in adult survivors of childhood abuse. *Am J Psychiatry* 2001; 158: 575–581.
18. Ahima RS, Prabakaran D, Mantzoros C, et al. Role of leptin in the neuroendocrine response to fasting. *Nature* 1996; 382: 250 –252.
19. Sukumaran S, Jusko WJ, DuBois DC, Almon RR. Mechanistic modeling of the effects of glucocorticoids and circadian rhythms on

adipokine expression. J Pharmacol Exp Ther. 2011 Jun; 337(3):734-46.
20. Halford JC, Harrold JA, Boyland EJ, Lawton CL, Blundell JE.Serotonergic drugs: effects on appetite expression and use for the treatment of obesity. *Drugs*. 2007;67(1):27-55
21. Finn PD, Cunningham MJ, Rickard DG, Clifton DK, Steiner RA. Serotonergic neurons are targets for leptin in the monkey. *J Clin Endocrinol Metab.* 2001 Jan; 86(1):422-6.
22. Collin M, Håkansson-Ovesjö ML, Misane I, Ogren SO, Meister B. Decreased 5-HT transporter mRNA in neurons of the dorsal raphe nucleus and behavioral depression in the obese leptin-deficient ob/ob mouse. *Brain Res Mol Brain Res.* 2000 Sep 30;81(1-2):51-61.
23. Calapai G, Corica F, Corsonello A, Sautebin L, Di Rosa M, Campo GM, Buemi M, Mauro VN, Caputi AP. Leptin increases serotonin turnover by inhibition of brain nitric oxide synthesis. *J Clin Invest*. 1999; 104(7):975-82.
24. Hastings JA, Wiesner G, Lambert G, Morris MJ, Head G, Esler M. Influence of leptin on neurotransmitter overflow from the rat brain in vitro. *Regul Pept.* 2002 Feb 15; 103(2-3):67-74.
25. http://www.ncbi.nlm.nih.gov/nuccore/NG_007450.1
26. Jeon JP, Shim SM, Nam HY, Ryu GM, Hong EJ, Kim HL, Han BG. Copy number variation at leptin receptor gene locus associated with metabolic traits and the risk of type 2 diabetes mellitus. *BMC Genomics*. 2010 Jul 12; 11:426.
27. Memon M.S., Kazi F.A. and Arain AA. A comparative study of job oriented occupational stress of peak workload on physicians and clerks. *Proc: ISBBP. Biochem, Biophy* 1997, 2: 261 – 266.
28. Silverstone, J.T. & Stunkard, A. J. The anorectic effect of dexamphetamine sulphate. Br. *J. Pharmacol Chemother*. 1968. 33; 513 – 522
29. Friedman JM, Halaas JL. Leptin and regulation of body weight in mammals. *Nature.* 1998 Oct 22; 396 (6704): 763 – 770.
30. Kema IP, de Vries EG, Schellings AM, Postmus PE, Muskiet FA. Improved diagnosis of carcinoid tumors by measurement of platelet serotonin. *Clin Chem* 1992. Apr; 38(4): 534 – 40.
31. Arakawa, H., Maeda M., Tsuji A. Chemiluminescence enzyme immunoassay of cortisol using peroxidase as label. *Anal Biochem*. 1979; 97(2): 248-54.
32. Sambrook J, Fritsch EF, Maniatis T (1989) commonly used techniques in molecular cloning. Molecular cloning: a laboratory manual, 2nd edn, vol. 1, Cold Spring Harbor Laboratory Press, NY
33. NCBI/Blast home; http://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE_TYPE=BlastHome
34. http://www.ncbi.nlm.nih.gov/nuccore/NG_007450.1 , http://www.ensembl.org/Homo_sapiens/Gene/Summary?g=ENSG00000174697;r=7:127881337-127897681;t=ENST00000308868
35. Lu XY, Kim C, Frazer A, and Zhang W Leptin: A potential novel antidepressant. *Proc Natl Acad Sci U S A.* 2006 January 31; 103(5): 1593–1598.
36. Lucki I. The forced swimming test as a model for core and component behavioral effects of antidepressant drugs. *Behav Pharmacol.* 1997 Nov;8(6-7):523-32.
37. Harvey J. Leptin regulation of neuronal excitability and cognitive function. *Curr Opin Pharmacol.* 2007 Dec;7(6):643-7
38. Yamada N, Katsuura G, Ochi Y, Ebihara K, Kusakabe T, Hosoda K, Nakao K. Impaired CNS leptin action is implicated in depression associated with obesity. *Endocrinology*. 2011
39. Cheng KC, Chiu YC, Lee YN, Liao SK, Lee SH. Relationships between stress perception and stress biomarkers in family caregivers. *Hu Li Za Zhi*. 2011; 58(3):43-52.
40. Nakayama S, Nishiyama M, Iwasaki Y, Shinahara M, Okada Y, Tsuda M, Okazaki M, Tsugita M, Taguchi T, Makino S, Stenzel-Poore MP, Hashimoto K, Terada Y. Corticotropin-releasing hormone (CRH) transgenic mice display hyperphagia with increased Agouti-related protein mRNA in the hypothalamic arcuate nucleus. *Endocr J.* 2011; 58(4):279-86.
41. Spinedi E and Gaillard RC. A regulatory loop between the hypothalamo-pituitary-adrenal

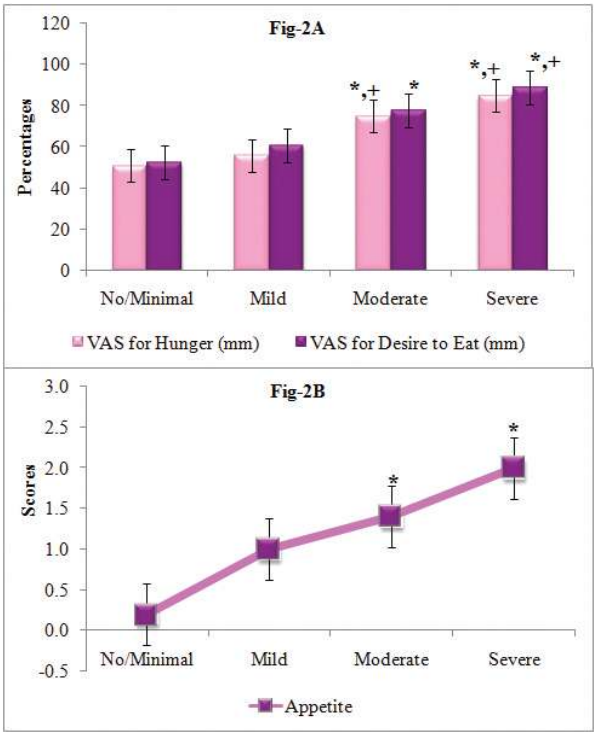
(HPA) axis and circulating Leptin: A Physiological role of ACTH. *Endocrinolgy* 1998; 139(9):4016 – 20.
42. Haleem DJ. Exaggerated feedback control decreases brain serotonin concentration and elicits hyperactivity in a rat model of diet-restriction-induced anorexia nervosa. *Appetite* 2009; 52 (1): 44-50.
43. Nenadic Sviglin K, Nedic G, Nikolac M, Mustapic M, Muck-Seler D, Borovecki F, Pivac N. Insomnia, platelet serotonin and platelet monoamine oxidase in chronic alcoholism. *Neurosci Lett.* 2011 Jun 21. [Epub ahead of print]
44. Stunes AK, Reseland JE, Hauso O, Kidd M, Tømmerås K, Waldum HL, Syversen U, Gustafsson BI. Adipocytes express a functional system for serotonin synthesis, reuptake and receptor activation. *Diabetes Obes Metab.* 2011 Jun;13(6):551-8
45. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature*. 1994 Dec 1; 372(6505):425-32.
46. Damcott CM, Sack P, Shuldiner AR. The genetics of obesity. *Endocrinol Metab Clin North Am* 2003; 32: 761–786.
47. Speakman JR. Obesity: The integrated roles of environment and genetics. *J Nutr* 2004; 134: 2090S–2105S.
48. Haslam DW, James WP. Obesity. *Lancet* 2005; 366: 1197–1209.
49. Clément K, Vaisse C, Lahlou N, Cabrol S, Pelloux V, Cassuto D, Gourmelen M, Dina C, Chambaz J, Lacorte JM, Basdevant A, Bougnères P, Lebouc Y, Froguel P, Guy-Grand B. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature*. 1998; 392(6674):398-401.
50. Mammes O, Betoulle D, Aubert R, et al. Novel polymorphisms in the 5' region of the LEP gene: association with leptin levels and response to low-calorie diet in human obesity. *Diabetes*. 1998; 47:487–9.
51. Mammes O, Betoulle D, Aubert R, et al. Association of the G-2548A polymorphism in the 5_ region of the LEP gene with overweight. *Ann Hum Genet.* 2000; 64:391–4.
52. Ambrosini G, Nath AK, Sierra-Honigmann MR, Flores-Riveros J. Transcriptional activation of the human leptin gene in response to hypoxia. Involvement of hypoxia-inducible factor 1. *J Biol Chem*. 2002 Sep 13;277(37):34601-9.
53. Oksanen L, Kainulainen K, Heiman M, Mustajoki P, Kauppinen-Mäkelin R, Kontula K. Novel polymorphism of the human ob gene promoter in lean and morbidly obese subjects. *Int J Obes Relat Metab Disord.* 1997 Jun;21(6):489-94.
54. Hager J, Clement K, Francke S, Dina C, Raison J, Lahlou N, Rich N, Pelloux V, Basdevant A, Guy-Grand B, North M, Froguel P. A polymorphism in the 5' untranslated region of the human ob gene is associated with low leptin levels. *Int J Obes Relat Metab Disord.* 1998 Mar; 22(3):200-5.
55. Li WD, Reed DR, Lee JH, Xu W, Kilker RL, Sodam BR, Price RA. Sequence variants in the 5' flanking region of the leptin gene are associated with obesity in women. *Ann Hum Genet.* 1999 May; 63(Pt 3):227-34.

Figure 1:



Figur-1: The Effect of Stress Perception on BMI of Women with various Levels of Stress Perception: Values are means ±S.E.M (n= 5 in each group). Significant difference by Tuckey's test *p<0.05 compared with the group with No/Minimal stress perception.

Figure 2:



Figur-2: The Effect of Stress Perception on Eating Behavior of Women with various Levels of Stress Perception: Fig2-A.The variations of VAS for hunger and desire to eat by stress perception, Fig-2 B Variation of Appetite by stress perception. Values are means ±S.E.M (n= 5 in each group). Significant difference by Tuckey's test *p<0.05 compared with the group with No/Minimal stress perception. +p<0.05 compared with women with mild perception of stress.

Figure 3:

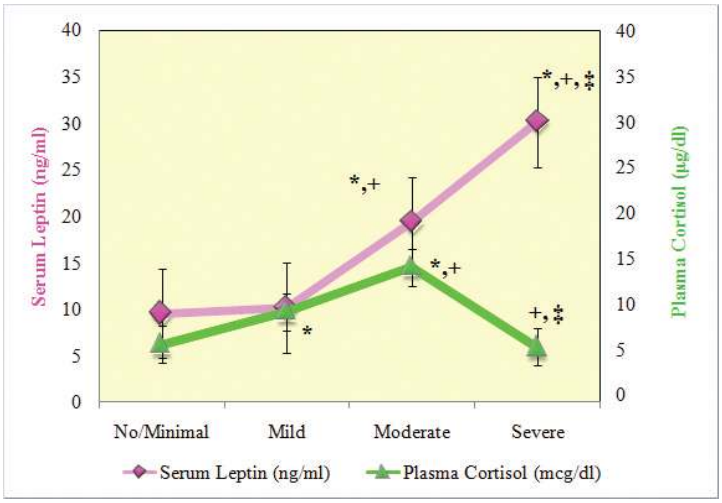
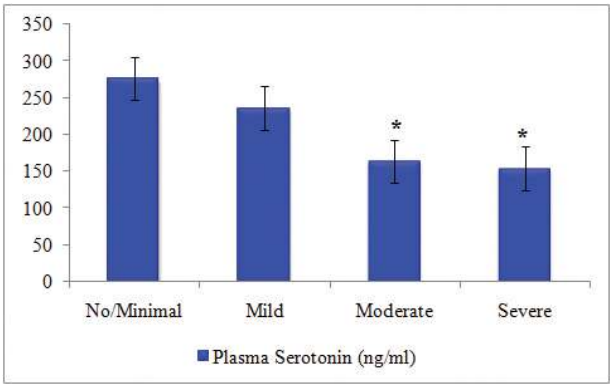


Figure-3: The Effect of Stress Perception on Plasma leptin and Cortisol Concentrations of Women with various Levels of Stress Perception: Values are means ±S.E.M (n= 5 in each group). Significant difference by Tuckey's test *p<0.05 compared with the group with No/Minimal stress perception. +p<0.05 compared with women with mild perception of stress. ‡p<0.01 compared with moderately stressed women.

Figure 4:



Figur-4: The Effect of Stress Perception on Plasma Serotonin Concentrations of Women with various Levels of Stress Perception: Values are means ±S.E.M (n= 5 in each group). Significant difference by Tuckey's test *p<0.05 compared with the group with No/Minimal stress perception.

Table 1:

Reference sequence 15629 – 16054 (425bp)

GTCTGGTAATGTGGTTGGTAATGTGAAGATGGGTGTATTCTGAGATACCGGCTCCT
TGCAGTGTGTGGTTCCTTCTGTTTTCAGGCCCAAGAAGCCCATCCTGGGAAGGAAA
ATGCATTGGGGAACCCTGTGCGGATTCTTGTGGCTTTGGCCCTATCTTTTCTATGTC
M H W G T L C G F L W L W P Y L F Y V
CAAGCTGTGCCCATCCAAAAAGTCCAAGATGACACCAAAACCCTCATCAAGACAA
Q A V P I Q K V Q D D T K T L I K T
TTGTCACCAGGATCAATGACATTTCACACACGGTAAGGAGAGTATGCGGGGACAA
I V T R I N D I S H T
AGTAGAACTGCAGCCAGCCCAGCACTGGCTCCTAGTGGCACTGGACCCAGATAGT
CCAAGAAACATTTATTGAACGCCTCCTGAATGCCAGGCACCTACTGGAAGCTGAG
AAGGATTTGAAAGCACAGGGCTCCACTCTTTCTGGT

No/Minimal Stress (Control) 15629 - 16052 (423bp)

GTCTGGTAATGTGGTTGGTAATGTGAAGATGGGTGTATTCTGAGATACCGGCTCCT
TGCAGTGTGTGGTTCCTTCTGTTTTCAGGCCCAASAAGCCCATCCTGGGAAGGAA
AATGCATTGGGGAACCCTGTGCGGATTCTTGTGGCTTTGGCCCTATCTTTTCTATGT
CCAAGCTGTGCCCATCCAAAAAGTCCAAGATGACACCAAAACCCTCATCAAGACA
ATTGTCACCAGGATCAATGACATTTCACACACGGTAAGGAGAGTATGCGGGGACA
AAGTAGAACTGCAGCCAGCCCAGCACTGGCTCCTAGTGGCACTGGACCCAGATAG
TCCAAGAAACATTTATTGAACGCCTCCTGAATGCCAGGCACCTACTGGAAGCTGA
GAAGGATTTGAAAGCACAGGGCTCCACTCTAAATG

Mild Stress 15609 - 16051 442bp

GGATTTAGCATTTCCTCACCGTCTGGTAATGTGGTTGGTAATGTGAAGATGGGTGT
ATTCTGAGATACCGGCTCCTTGCAGTGTGTGGTTCCTTCTGTTTTCAGGCCCAAGA
AGCCCATCCTGGGAAGGAAAATGCATTGGGGAACCCTGTGCGGATTCTTGTGGCT
TTGGCCCTATCTTTTCTATGTCCAAGCTGTGCCCATCCAAAAAGTCCAAGATGACA
CCAAAACCCTCATCAAGACAATTGTCACCAGGATCAATGACATTTCACACACGGT
AAGGAGAGTATGCGGGGACAAAGTAGAACTGCAGCCAGCCCAGCACTGGCTCCT
AGTGGCACTGGACCCAGATAGTCCAAGAAACATTTATTGAACGCCTCCTGAATGC
CAGGCACCTACTGGAAGCTGAGAAGGATTTGAAAGCACAGGGCTCCACTCTTAAT

Moderate Stress 15628 - 16052 (424bp)

CGTCTGGTAATGTGGTTGGTAATGTGAAGATGGGTGTATTCTGAGATACCGGCTCC
TTGCAGTGTGTGGTTCCTTCTGTTTTCAGGCCCAAGAAGCCCATCCTGGGAAGGAA
AATGCATTGGGGAACCCTGTGCGGATTCTTGTGGCTTTGGCCCTATCTTTTCTATGT
CCAAGCTGTGCCCATCCAAAAAGTCCAAGATGACACCAAAACCCTCATCAAGACA
ATTGTCACCAGGATCAATGACATTTCACACACGGTAAGGAGAGTATGCGGGGACA
AAGTAGAACTGCAGCCAGCCCAGCACTGGCTCCTAGTGGCACTGGACCCAGATAG
TCCAAGAAACATTTATTGAACGCCTCCTGAATGCCAGGCACCTACTGGAAGCTGA
GAAGGATTTGAAAGCACAGGGCTCCACTCTTAGTG

Severe Stress 15617 – 16053 (436bp)

ATCATTGGATGCGTCTGGTAATGTGGTTGGTAATGTGAAGATGGGTGTATTCTGAG
ATACCGGCTCCTTGCAGTGTGTGGTTCCTTCTGTTTTCAGGCCCAAGAAGCCCATC
CTGGGAAGGAAAATGCATTGGGGAACCCTGTGCGGATTCTTGTGGCTTTGGCCCT
ATCTTTTCTATGTCCAAGCTGTGCCCATCCAAAAAGTCCAAGATGACACCAAAACC
CTCATCAAGACAATTGTCACCAGGATCAATGACATTTCACACACGGTAAGGAGAG
TATGCGGGGACAAAGTAGAACTGCAGCCAGCCCAGCACTGGCTCCTAGTGGCACT
GGACCCAGATAGTCCAAGAAACATTTATTGAACGCCTCCTGAATGCCAGGCACCT
ACTGGAAGCTGAGAAGGATTTGAAAGCACAGGGCTCCACTCTTATCTG

Table-1: Partial Sequences of Leptin gene with Exon 2: Pink highlight in the reference gene fragment shows the protein coding region with the blue letter showing the corresponding amino acid. The yellow highlight shows the protein coding region of the sample sequences. The green highlight shows the variation found in the samples of women with no/minimal perception of stress. Blue highlight shows the conservation of base in consensus with the reference gene.

Table 2:

Reference sequence Exon 3 18007 - 18675 (668bp)

GGGAGGGTGGAAGGAGGCAGCCCAGAGAATGACCCTCCATGCCCACGGGGAAGGCAGAGGGC
TCTGAGAGCGATTCCTCCCACATGCTGAGCACTTGTTCTCCCTCTTCCTCCTGCATAGCAGTCAG
Q S
TCTCCTCCAAACAGAAAGTCACCGGTTTGGACTTCATTCCTGGGCTCCACCCCATCCTGACCTT
V S S K Q K V T G L D F I P G L H P I L T L
ATCCAAGATGGACCAGACACTGGCAGTCTACCAACAGATCCTCACCAGTATGCCTTCCAGAAA
S K M D Q T L A V Y Q Q I L T S M P S R N
CGTGATCCAAATATCCAACGACCTGGAGAACCTCCGGGATCTTCTTCACGTGCTGGCCTTCTCT
V I Q I S N D L E N L R D L L H V L A F S
AAGAGCTGCCACTTGCCCTGGGCCAGTGGCCTGGAGACCTTGGACAGCCTGGGGGGTGTCCTG
K S C H L P W A S G L E T L D S L G G V L
GAAGCTTCAGGCTACTCCACAGAGGTGGTGGCCCTGAGCAGGCTGCAGGGGTCTCTGCAGGAC
E A S G Y S T E V V A L S R L Q G S L Q D
ATGCTGTGGCAGCTGGACCTCAGCCCTGGGTGCTGAGGCCTTGAAGGTCACTCTTCCTGCAAGG
M L W Q L D L S P G C
ACTACGTTAAGGGAAGGAACTCTGGCTTCCAGGTATCTCCAGGATTGAAGAGCATTGCATGGA
CACCCCTTATCCAGGACTCTGTCAATTTCCCTGACTCCTCTAAGCCACTCTTCCAAAGGCATAA
GACCCTAAGCCTCCTTTTGCTTGAAACCAAAGA

No/Minimal Stress 18007 – 18675 (668bp)

GGGAGGGTGGAAGGAGGCAGCCCAGAGAATGACCCTCCATGCCCACGGGGAAGGCAGAGGGC
TCTGAGAGTGATTCCTCCCACATGCTGAGCACTTGTTCTCCCTCTTCCTCCTGCATAGCAGTCAG
TCTCCTCCAAACAGAAAGTCACCGGTTTGGACTTCATTCCTGGGCTCCACCCCATCCTGACCTT
ATCCAAGATGGACCAGACACTGGCAGTCTACCAACAGATCCTCACCAGTATGCCTTCCAGAAA
CGTGATCCAAATATCCAACGACCTGGAGAACCTCCGGGATCTTCTTCACGTGCTGGCCTTCTCT
AAGAGCTGCCACTTGCCCTGGGCCAGTGGCCTGGAGACCTTGGACAGCCTGGGGGGTGTCCTG
GAAGCTTCAGGCTACTCCACAGAGGTGGTGGCCCTGAGCAGGCTGCAGGGGTCTCTGCAGGAC
ATGCTGTGGCAGCTGGACCTCAGCCCTGGGTGCTGAGGCCTTGAAGGTCACTCTTCCTGCAAGG
ACTACGTTAAGGGAAGGAACTCTGGCTTCCAGGTATCTCCAGGATTGAAGAGCATTGCATGGA
CACCCCTTATCCAGGACTCTGTCAATTTCCCTGACTCCTCTAAGCCACTCTTCCAAAGGCATAA
GACCCTAAGCCTCCTTTTGCTTGAAACCAAAGA

Mild Stress 17995 – 18533 (655 bp)

GGCTACGTGGTGAGGGAGGGTGGAGGAGGCAGCCCAGAGAATGACCCTCCATGCCCACGGGG
AAGGCAGAGGGCTCTGAGAACGATTCCTCCCACATGCTGAGCACTTGTTCTCCCTCTTCCTCCT
GCATAGCAGTCAGTCAGTCTCCTCCAAACAGAAAGTCACCGGTTTGGACTTCATTCCTGGGCTCCACC
CCATCCTGACCTTATCCAAGATGGACCAGACACTGGCAGTCTACCAACAGATCCTCACCAGTAT
GCCTTCCAGAAACGTGATCCAAATATCCAACGACCTGGAGAACCTCCGGGATCTTCTTCACGTG
CTGGCCTTCTCTAAGAGCTGCCACTTGCCCTGGGCCAGTGGCCTGGAGACCTTGGACAGCCTGG
GGGGTGTCCTGGAAGCTTCAGGCTACTCCACAGAGGTGGTGGCCCTGAGCAGGCTGCAGGGGT
CTCTGCAGGACATGCTGTGGCAGCTGGACCTCAGCCCTGGGTGCTGAGGCCTTGAAGGTCACTC
TTCCTGCAAGGACTACGTTAAGGGAAGGAAC

Moderate stress 17994 – 18647 (655bp)

GGCTACGTGGTGAGGGAGGGTGGAAGGAGGCAGCCCAGAGAATGACCCTCCATGCCCACGGG
GAAGGCAGAGGGCTCTGAGAGCGATTCCTCCCACATGCTGAGCACTTGTTCTCCCTCTTCCTCC
TGCATAGCAGTCAGTCTCCTCCAAACAGAAAGTCACCGGTTTGGACTTCATTCCTGGGCTCCAC
CCCATCCTGACCTTATCCAAGATGGACCAGACACTGGCAGTCTACCAACAGATCCTCACCAGTA
TGCCTTCCAGAAACGTGATCCAAATATCCAACGACCTGGAGAACCTCCGGGATCTTCTTCACGT
GCTGGCCTTCTCTAAGAGCTGCCACTTGCCCTGGGCCAGTGGCCTGGAGACCTTGGACAGCCTG
GGGGGTGTCCTGGAAGCTTCAGGCTACTCCACAGAGGTGGTGGCCCTGAGCAGGCTGCAGGGG
TCTCTGCAGGACATGCTGTGGCAGCTGGACCTCAGCCCTGGGTGCTGAGGCCTTGAAGGTCACT
CTTCCTGCAAGGACTACGTTAAGGGAAGGAACTCTGGCTTCCAGGTATCTCCAGGATTGAAGA
GCATTGCATGGACACCCCTTATCCAGGACTCTGTCAATTTCCCTGACTCCTCTAAGCCACTCTTC
CAAAGGCATAAGACCCTA

Severe Stress 17994 – 18652 (660bp)

GGCTACGTGGTGAGGGAGGGTGGAAGGAGGCAGCCCAGAGAATGACCCTCCATGCCCACGGG
GAAGGCAGAGGGCTCTGAGAGCGATTCCTCCCACATGCTGAGCACTTGTTCTCCCTCTTCCTCC
TGCATAGCAGTCAGTCTCCTCCAAACAGAAAGTCACCGGTTTGGACTTCATTCCTGGGCTCCAC
CCCATCCTGACCTTATCCAAGATGGACCAGACACTGGCAGTCTACCAACAGATCCTCACCAGTA
TGCCTTCCAGAAACGTGATCCAAATATCCAACGACCTGGAGAACCTCCGGGATCTTCTTCACGT
GCTGGCCTTCTCTAAGAGCTGCCACTTGCCCTGGGCCAGTGGCCTGGAGACCTTGGACAGCCTG
GGGGGTGTCCTGGAAGCTTCAGGCTACTCCACAGAGGTGGTGGCCCTGAGCAGGCTGCAGGGG
TCTCTGCAGGACATGCTGTGGCAGCTGGACCTCAGCCCTGGGTGCTGAGGCCTTGAAGGTCACT
CTTCCTGCAAGGACTACGTTAAGGGAAGGAACTCTGGCTTCCAGGTATCTCCAGGATTGAAGA
GCATTGCATGGACACCCCTTATCCAGGACTCTGTCAATTTCCCTGACTCCTCTAAGCCACTCTTC
CAAAGGCATAAGACCCTAAGCCT

Table-2: Partial Sequence of Leptin Gene including exon 3: Pink highlight in the reference gene fragment shows the protein coding region with the blue letter showing the corresponding amino acid. The yellow highlight shows the protein coding region of the sample sequences.