

The Interaction of Serum Serotonin, Cortisol and Leptin in Stress Related Obesity in Working Men at Educational Institutions of Karachi

Zeba Haque* and Darakhshan J. Haleem

Department of Biochemistry, Dow International Medical College, Dow University of Health Sciences, Karachi and Dean Office, Faculty of Science, University of Karachi, Karachi, Pakistan

Abstract: Objective: An interaction of leptin, serotonin and cortisol in the brain and periphery effects body weigh in different stress levels. The study aims to assess the levels of perceived stress in men and estimate its relationship with serum leptin, cortisol and serotonin concentrations and BMI. **Methods:** This cross-sectional study was carried out in Neurochemistry Lab University of Karachi during March –May 2010. Ninety seven adult men with different body mass indices working at undergraduate professional educational institutes of Karachi were selected. Stress levels and the change in appetite were measured subjectively by a standardized questionnaire analyzed by likert scale. Venous sample was drawn at the time of stress evaluation. The levels of chemical modulators were measured using enzyme-linked immunoassay. The subjects were classified on the basis of stress level as having no/little, mild, moderate and severe stress. Blood sampling was done just after the subjects have answered the questionnaire. **Results:** Serum serotonin levels were significantly decreased ($p < 0.001$) while leptin and cortisol levels were increased ($p < 0.001$) with the increasing levels of stress. A significant increase in BMI was observed in moderate stress group ($p < 0.05$), with a decrease in severe stress. Serum leptin was positively correlated with cortisol ($p < 0.05$) while negatively with serotonin ($p < 0.05$) in circulation. **Conclusion:** BMIs of different stress groups were not associated with stress levels. Serum cortisol and leptin increased with the increasing stress levels while serotonin in plasma is decreased that may be considered as the peripheral marker of stress.

Key Words: Obesity, Stress, Leptin, Serotonin, Cortisol, Peripheral markers.

INTRODUCTION

The peptide hormone leptin implicated in regulation of food intake and energy homeostasis in both rodents and humans, is found both centrally and peripherally. The role of peripheral leptin has been more extensively investigated. Leptin receptors are found in multiple tissues [1] signifying its importance in homeostatic regulations. Leptin is secreted by white adipose cells in proportion to the amount of adipose tissue mass. The peripheral effects of leptin primarily include regulation of insulin secretion and energy metabolism in fat cells and skeletal muscles, where it seems to play a role ensuring the maintenance of adequate energy stores and thereby protect against starvation.

Stressful stimuli result in increased cortisol levels in blood via stimulation of hypothalamo-pituitary-adrenal (HPA) axis. Cortisol is a potent stimulator of leptin secretion [2]. As a neuropeptide, leptin inhibits HPA response to stressful stimuli [3] while circulating leptin has been shown to be the predictor of sympathetic cardiovascular activity and parasympathetic withdrawal suggesting that adverse effects of stress on obesity and cardiovascular health are mediated via circulating leptin [4]. The role of central serotonin in response to acute and chronic stress and feeding behavior [5] is well documented. Peripherally serotonin is mainly

aggregated in enterochromaffin cells of GIT and stored in platelets.

The present study concerns circulating levels of leptin, cortisol and serotonin in perceived psychological/physiological stress in healthy adult men and relate it with obesity. The study aims at establishing the interconnection(s) among the three mediators and to test the possibility that serotonin may be used as a peripheral marker of stress response and obesity.

MATERIAL AND METHODS

It was cross sectional study conducted from March to May 2010 in Neuro-Biochemistry Laboratory, University of Karachi. Ninety seven adult men of ages ranging from 30 – 64 years, working in undergraduate professional educational institutes participated in the study. All the apparently healthy men within adult age group (30 – 64 years) working for 8 hours a day were included. The men with diagnosed psychiatric problem or hormonal disorders were excluded from the study. Sampling was done by stratified systematic technique. Ten institutes were identified in the central zone of Karachi and 10 samples from each were selected for the study. Men with smoking/drinking habits, suffering from cardiovascular, metabolic hormonal or psychiatric disorders were not included in the study. Out of hundred three did not continue with the study protocols. All participants submitted written consent, approved by the ethical committee of University of Karachi, for the use of information contained in the questionnaire and for the donation of serum samples.

*Address correspondence to this author at the Department of Biochemistry, Dow International Medical College, Dow University of Health Sciences, Karachi, Pakistan; Tel: +92-302-2611892 Fax: 92-21-99261077; E-mail: z.haque@duhs.edu.pk

Stress levels were monitored subjectively by a standardized questionnaire [6]. The questionnaire contained 24 statements/questions that measured the environmental, psychological and physiological aspects of stress the method of scoring was based on likert scale. The total scores of 52 - 58, (55 +/- 3) were taken as no/little stress or the control subjects, 59 - 65 (62±3) were considered as mild, The response 3 and 4 were collectively taken as moderate stress that summed up to 66 - 79, (69- 3 & 76 + 3) and the total of 80 - 86 (83+/- 3) were taken as severe stress groups. Body mass index (BMI) of all the subjects was calculated by dividing weight in kilograms with height taken in meter squares taken in light clothing without slippers. 5 ml of venous blood was drawn from antecubital vein, after the person has answered the questionnaire for perceived stress. Blood was allowed to clot at 4°C and serum was separated and stored at -70°C for estimations of serotonin, leptin and cortisol by ELISA. The chemical variables were determined by ELISA. The kit for serum leptin [7] and serotonin [8] were obtained from BioSource Europe S.A. The kit for cortisol [9] was obtained from The Equipar Diagnostics. All the study protocols were followed from 9:00 - 11:00 hours to analyse the association of hormonal levels with the perceived stress levels in a post prandial state. Statistical Analysis was done applying ANOVA with Tuckey's test as a post hoc test to compare individual variables. SPSS version 15 was used to analyze data. The study was approved by the departmental ethical committee of the University of Karachi.

RESULT

The mean ± SD of age of the participants was 38.38 ± 7.20 years. The proportion of participants with severe, moderate, mild and no stress were 24.74% for both severe and moderate, 26.80% and 23.71 respectively. Out of ninety seven men, only three were found to be obese, 18 were overweight and 76 had normal weight. ANOVA showed significant differences among BMI of various stress groups

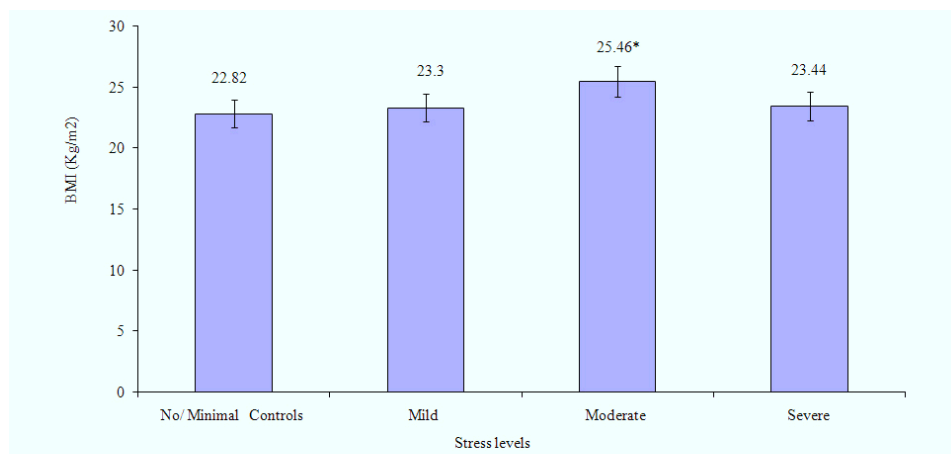


Fig. (1). Changes in BMI in Various Stress Levels. Compared with no/minimal stressed group considered as controls a BMI was significantly higher in the group of men who perceived moderate stress. Values are expressed in mean ± S.E.M. $p < 0.05$ was considered as significant difference.

($df=3, 93$; $F = 4.5$; $p < 0.05$). Posthoc test revealed that indeed BMI was increased in the individuals who perceived stress compared to the group of men with no/minimal perception of stress considered as controls but that was not significant. The BMI of moderately stressed individuals found was significantly higher compared to no/little stress group (Fig. 1).

Fig. (2) depicts serum levels of serotonin, leptin and cortisol in various stress groups. Serum cortisol was found to be progressively and significantly increased with increased severity of stress among the groups ($df=3, 93$; $F=60.7$; $p < 0.01$). Similarly mean serum serotonin was also progressively decreased with the increase in stress scores and a significant difference was observed between the groups ($df=3, 96$; $F=19.45$; $p < 0.001$). Serum leptin concentrations were also different among the four groups of stress ($df=3, 96$; $F = 7.09$; $p < 0.01$), however the difference was found significant only between the two extremes of stress groups from control to the severe stress groups ($p < 0.01$).

Fig. (3) depicts the correlation between serum leptin, cortisol and serotonin. Serum leptin levels showed a significant positive correlation with serum cortisol levels ($p < 0.001$), while serotonin showed a significant negative correlation with both cortisol (correlational coefficient (r) = 0.51, determination coefficient (r^2) = 0.262, $p < 0.01$) and serum leptin ($r = -0.20$, $r^2 = 0.040$, $p < 0.05$).

DISCUSSION

Many authors have reported that subjects who perceived psychological stress had elevated leptin levels [10]. Patients with post traumatic stress disorder are also hyperleptinemic [11] suggesting that leptin concentrations may be used as a marker for perceived daily psychological stress and traumatic disorders. Conversely studies on experimental animals have reported that chronic stress did not increase serum leptin levels [12]. Otsuka et al [10] observed a basal

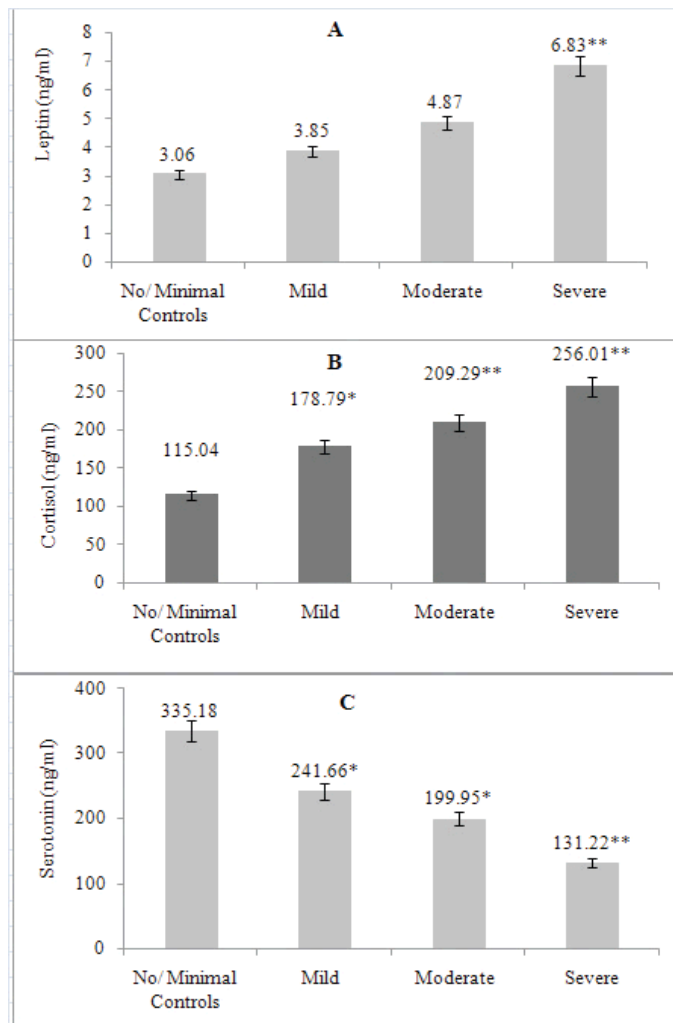


Fig. (2). Comparison of Circulating levels of Leptin (A), Cortisol (B) and Serotonin (C) in various Stress Perception Groups. Values are expressed in means \pm SEM. Significant difference was considered as * $p < 0.05$ and ** $p < 0.01$. Post-hoc analysis showed a significant rise in serum leptin (A) and Cortisol (B) in severe stress perception. The cortisol levels were also increased in mild and moderate stress perception compared to controls (no/minimal stress). Peripheral serotonin also demonstrated a significant decrease with severity of stress.

increase in leptin concentrations (after an overnight fast) in the subjects with a high level of stress perception. In the present study we collected the samples of blood in a postprandial state at the time of answering the questionnaire to analyse the hormonal concentrations at the time of stress awareness. Hypothalamus is a critical target for the satiety effects of leptin. The protein elicits more potent anorectic effect when administered centrally than peripherally [13]. It is transported through blood brain barrier via saturable transport system and achieves most of its metabolic effects by interacting with specific receptors located in the central nervous system. The role of peripheral leptin has been more extensively investigated [13, 14]. Administration of leptin to genetically obese mice reduced their food intake and made them loose weight [15]. Congenital leptin deficiency in humans is associated with severe early onset obesity. Somewhat paradoxically however, plasma leptin levels increased in obese women [16] and decreased in women with anorexia nervosa [17]. Since leptin levels are chronically increased in obese humans, this suggests that obesity may be

associated with malfunctioning of leptin receptors, a condition often described as leptin resistance. It possibly will be considered that people who feel stressed in their lives are exposed to some chronic stressor.

The present study shows that the subjects who reported an awareness of moderate or severe stress in their daily lives had significantly higher leptin levels than little or mild stress groups (Fig. 1). Mild to moderate stress triggers the consumption of palatable food resulting into gradual increase in adipose tissue mass. Serum leptin that corresponds to adipose tissue mass thus gets chronically increased leading to leptin resistance and obesity [18]. Stress hormone cortisol further aggravates the situation by stimulating the secretion of leptin. Complex carbohydrates termed as comfort food, increase the level of tryptophan in brain resulting into calming effect through serotonin [19]. Central serotonin also decrease appetite through 5HT₄ receptors [20] which may be the possible explanation of less BMI observed in severely stressed group. It is however possible that severe stress

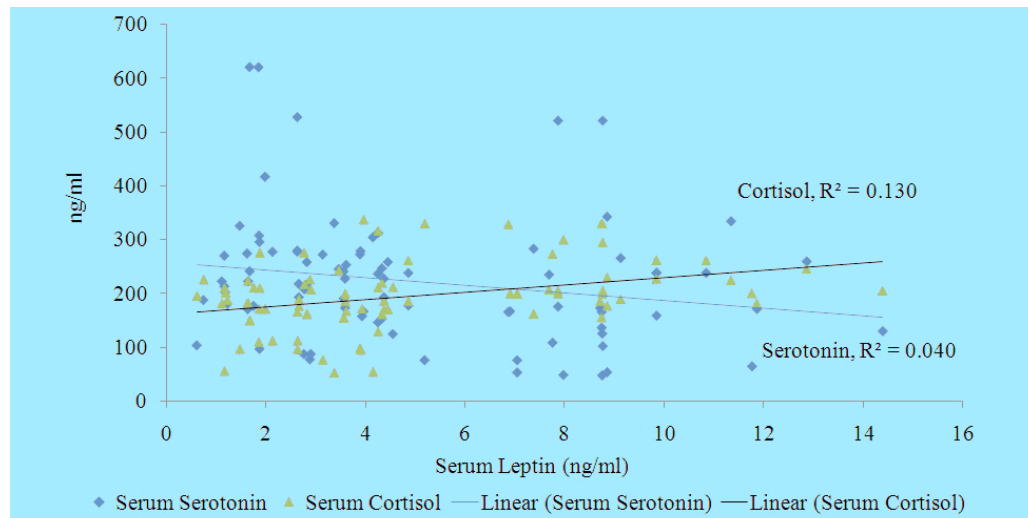


Fig. (3). Correlation of Peripheral Cortisol and Serotonin with Serum leptin in Stress: Stress hormone cortisol significantly increased ($p < 0.05$) with the increase in serum leptin while serotonin in circulation decreased ($p < 0.05$) with the leptin levels.

induced increases of sympathetic activity and stimulated HPA axis overcomes the effect of leptin on adiposity/obesity. Once the stressor has gone the neuro-endocrine response is also terminated because of the negative feedback control of HPA axis activity. Failure to terminate this response is observed in various conditions of over-activity of HPA axis [21]. Disruption of the glucocorticoid negative feedback system is observed in approximately one half of human depression [22]. A similar condition is induced in animals by chronic stress [21]. Dose dependant cortisol induced increases in plasma leptin concentrations have been reported in healthy humans [23]. Leptin response to glucocorticoids occurred at physiological dose and gets abolished by fasting [24]. The disruption is thought to involve a down regulation of receptors in the feedback sites of the brain [25]. Our findings of an increase in circulating levels of cortisol with the severity of stress are more relevant that at the time of sample collection perception of stress was highest in severe stress group and least in the no/little stress group. The result support the notion that leptin might have been increased to attenuate the stress response. The cross sectional nature of the present study implies a possibility that a single raised leptin level may identify an individual to be in greater perceived stressed state rather than someone with a lower leptin level.

A role of brain serotonin in response to stress and regulation of appetite is well documented. Exposure to stress-inducing situations increases the firing of serotonergic neurons in the brain resulting in an increase in the metabolism, synthesis and release in serotonin [26]. This occurs because transport of tryptophan, the precursor of serotonin which is an essential amino acid, to the brain increases [26]. Only 2% of the total body serotonin is present in the brain, while 98% of it is found in the chromaffin cells of GIT or blood platelets. One of the aims of present study was to test the possibility that circulating levels of serotonin may be used as a marker of stress response or obesity. A decrease in serum levels of serotonin with the severity of stress is

relevant that life event stressors precipitate depression [27], 5HT is deficient in depression and drugs that increase serotonin function are antidepressants. It would suggest that decrease in serum serotonin concentrations may be taken as a measure of stress perception, but it may not be taken as a measure of obesity. Most of the tryptophan present in circulation is metabolized by Kynurinine pathway located in the liver. Exposure to a stress inducing situation increases the activity of tryptophan pyrrolase, the rate limiting enzyme for its degradation [28].

Present study provides the evidence that serum leptin and cortisol are increased and the of serotonin decrease with the severity of stress perception. Positive correlation of serum cortisol with leptin observed in the present study (Fig. 3) suggests that stress hormones may result in persistent increased leptin in circulation. A moderate increase of leptin elicits obesity possibly because of leptin resistance [18]. Cortisol increase is dependent on stress levels may overcome the obesity effects of leptin in severely stressed group. A decrease in serum concentration of serotonin with the severity of stress suggests that serum serotonin may be taken as a marker of stress perception that may lead to depression. The sample size of the study did not allow the calculation of definite correlation among the hormonal parameters and BMI. Moreover the gender specific hormones may play a role in the development of stress induced obesity in men. The study concludes that working men with the awareness of moderate level of stress have a higher BMI which may be due to the development of leptin resistance and high cortisol. The levels of serotonin as the measure of obesity merit more studies.

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