Kafkas Univ Vet Fak Derg
20 (2): 267-271, 2014
DOI: 10.9775/kvfd.2013.9976

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

Investigation on Serum Hormonal Parameters (Ghrelin, Corticosterone, Insulin, T_3 and T_4) in Chronic Mild Stress Rat Model of Depression [1]

Mohammad NARIMANI-RAD [1] Vahab BABAPOUR [1] Morteza ZENDEHDEL [1]
Mehran MESGARI ABBASI [2] Sara FARHANG [3]

[1] *Present article is summarized from PhD thesis of first author*
[1] Department of Physiology, Faculty of Specialized Veterinary Sciences, Science and Research Branch, Islamic Azad University, Tehran - IRAN
[2] Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz - IRAN
[3] Clinical Psychiatry Research Center, Tabriz University of Medical Sciences, Tabriz - IRAN

Makale Kodu (Article Code): KVFD-2013-9976

Summary

The aim of present study was to investigate the effects of depression induced with chronic mild stress (CMS) protocol on serum hormones (ghrelin, corticosterone, insulin, T_3 and T_4) which is in interaction with stress or depressive disorders. Forty-five 40-45 d old laboratory rats were assigned to two groups; control (n: 10) and CMS (subjected to CMS procedure, n: 30). Rats in the control group were reared in single cages without any environmental stress. Rats in CMS were entered the CMS procedure. This protocol consisted of mild unpredictable stressors (intermittent illumination, stroboscopic light, grouping, food or water deprivation, exposure to an empty water bottle, solid cage, cage tilting, etc.). After CMS protocol sucrose preference (SP) test was used for the identification of depressed animals. Rats with lower than 65% SP were defined as depressed animal. Blood serum was taken from two groups (control and CMS) for the determination of blood hormonal variables by Elisa kits. Analyzed data showed a significant elevation in ghrelin, corticosterone, and insulin levels for CMS group (depressed animals) in comparison with control group, as elevation was very significant for corticosterone. There was not any significant change for T_3, whereas decreased T_4 was observed for CMS groups when compared with the control. It was concluded that chronic mild stress induced depression can cause ghrelin, corticosteroid, and insulin increases, and T_4 decreases. Mentioned effects of CMS induced depression on hormonal indices (with exception to thyroid hormones), can be similar with effects of major depression.

Keywords: *Chronic mild stress, Depression, Glucocorticoids, Insulin*

Depresyonun Kronik Hafif Stres Sıçan Modelinde Serum Hormonal Parametrelerin (Ghrelin, Kortikosteron, İnsülin, T_3 ve T_4) Araştırılması

Özet

Bu çalışmada, depresyon etkilerinin kronik hafif stres (CMS) işlemi ile stres ve depresif bozukluklarla etkileşim halinde olan serum hormonları (ghrelin, kortikosteron, insülin, T_3 ve T_4) üzerine etkilerinin araştırılması amaçlanmıştır. Kırk beş adet 40-45 günlük laboratuvar sıçanları kontrol (n: 10) ve CMS (CMS işlemine tabi, n: 30) olmak üzere iki gruba ayrıldı. Kontrol grubundaki sıçanlar herhangi bir çevresel stres olmadan tek bir kafeste yetiştirildi. CMS grubu sıçanlar CMS işlemine dahil edildi. Bu protokol hafif öngörülemeyen stres oluşturucularının (kesintili aydınlatma, stroboskopik ışık, gruplama, yiyecek veya su yoksunluğu, boş bir su şişesine, katı kafese, kafes eğimine maruziyet, vb) oluşmaktadır. CMS işleminden sonra sükroz tercih (SP) testi depresif hayvanların tanımlanması için kullanıldı. %65 SP'den daha düşük sıçanlar depresif hayvan olarak tanımlandı. Kan serumu ELISA kitleri ile kan hormonsal değişkenlerin tespiti için iki grutan (kontrol ve CMS) alındı. Analiz edilen sonuçlar kontrol grubu ile karşılaştırıldığında çok önemli kortikosteron artışıyla beraber CMS grupta (depresif hayvanlar) ghrelin, kortikosteron ve insülin seviyelerindeki önemli artışı gösterdi. CMS gruplarında kontrol grubu ile karşılaştırıldığında düşmüş T_4'e rağmen T_3 için önemli bir değişiklik gözlemlenmedi. Kronik hafif stres kaynaklı depresyonun ghrelin, kortikosteroid ve insülin artışına ve T_4 azalmasına sebep olabileceği sonucuna varıldı. Hormonsal endeks üzerindeki (tiroit hormonları hariç) CMS kaynaklı depresyonun adı geçen etkileri majör depresyon etkileri ile benzer olabilir.

Anahtar sözcükler: *Kronik hafif stres, Depresyon, Glukokortikoidler, İnsülin*

İletişim (Correspondence)
+98 9144016042
mohammadnarimani@yahoo.com

INTRODUCTION

Depression is one of the common psychological disorders in human society, and it is much more prevalent in industrialized societies. Research undertaken in this regard has largely prescribed anti-depressant medicines or medicinal plants as well as different levels of effective hormones being studied. However, studies with adverse effects of depression on endocrine parameters are very limited. Also, these studies have focused mainly on major depression cases [1-3] not chronic or depression resulting from mild environmental stresses. So, studies on endocrine indices of various models of depression are necessary [4].

Among trusty models of depression, chronic mild stress (CMS) model of depression in rodents has been proposed to model some of the environmental factors that contribute to the induction of depressive disorders in humans [5-7]. In the present protocol (CMS), sequential exposure to a variety of mild stressors causes behavioral deficits in different paradigms that measure sensitivity to rewards. Thus, CMS suppresses the consumption of and preference to palatable sweet solution such as sucrose or saccharin [8], and the rewarding properties of food pellets, sweet solutions and amphetamine, as assessed by the place preference conditioning procedure [9]. Effects of these stresses (CMS) on animal hormonal profile are not reported by any comprehensive study. Albeit sporadic investigations were published in relation to effects on chronic-mild stress on glucocorticoids [10,11] and thyroid hormones [12], however, these experiments with exception to Kioukia *et al*.[12], are not conducted in general case of CMS models. So, the aim of present study was to investigate the effects of CMS on serum hormones which are in interaction with stress or depressive disorders.

MATERIAL and METHODS

Animals

Forty-five 40-45 d old Male Wistar rats were kept into the laboratory animal room for 1 week pre-experimental adaptation period. Animals were weighted and assigned as two groups; control (n: 10) and CMS (subjected to CMS procedure, n: 30). Animals in the control group were reared in single cages without any environmental stresses. Animal in CMS were entered into the CMS procedure *(Table 1)*. With exception to limitations of CMS procedure, the food and water were available ad libitum for all animals. Other environmental conditions included light/dark cycle (12 h: 12 h), light intensity, and ventilation were the same for both groups. Experiments were performed in accordance with the guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23, revised 1985). The protocol was arranged in according to certificate of Tabriz University of medical sciences ethical committee (Reg. no. 5.4.411- April 10, 2011) for present project.

CMS Procedure

CMS has been used to achieve depressive-like symptoms in Wistar rats [7,8]. It was designed to maximize the randomness of the stressors. The protocol was carried out for 4 weeks as described in *Table 1*.

This protocol consisted of mild unpredictable stressors which are: intermittent illumination, stroboscopic light (300 flashes/min), grouping, food or water deprivation, exposure to an empty water bottle immediately following a period of water deprivation, solid cage (300 ml water spilled into bedding) and 45° cage tilting. Grouping indicates housing a rat in pairs with different partners while an individual rat alternately becomes a resident or an intruder. Details of the CMS procedure are presented in *Table 1*.

Sucrose Preference Test

Sucrose preference (SP) test is a measure to evaluate anhedonic effect of CMS [8] and efficiency of protocol to induce depression. In this test, animals were trained to consume a 1% sucrose solution following 18 h of food/water deprivation at week three. Sucrose intake measure with weight losses of sucrose contained bottle at the end

Table 1. *Time and length (h) of stressors used in the CMS procedure*
Tablo 1. *CMS işleminde kullanılan stres oluşturucuların zamanı ve uzunluğu (saat)*

Stressor	Timing						
	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday
Intermitted lighting (light/dark)	00:00-14:00 (1 time/2 h)	-	-	-	-	-	-
Strobe light	-	-	00:00-12:00	-	-	-	-
Cage tilt 45°	-	13:00-23:00		-	-	13:00-23:00	00:00-12:00
Solid cages	-	-	12:00-24:00	-	-	-	13:00-23:00
Feed/Water deprivation	-	00:00-10:00	-	10:00-24:00	-	00:00-12:00	-
Empty water bottle	-	10:00-12:00	-	-	-	-	-
Paired housing	-	-	-	-	10:00- 24:00	-	-

of the test for 24 h during a no-stress period. Rats with lower than 65% SP (net sucrose consumption/ [sucrose consumption+ water consumption] × 100 %) were defined as depressed animal [13-15].

Blood Sampling and Analysis

Healthy animals (control) and depressed animals recognized by SP test were subjected to blood sampling for identification of effects of CMD on serum hormonal variables. Blood samples taken from orbital sinus were centrifuged at 1.200 x g for 7 min at 18°C, and serum was prepared for determination of blood hormonal variables (ghrelin, corticosterone, insulin, T_3 and T_4) with an auto-analyzer (Alcyon 300; Abbott Park, IL, US) and Elisa commercial kits.

Present experiment was arranged with two treatments and four replicates for each. Data were analyzed with SAS (SAS Inst. Inc., Cary, NC, US) and the differences between treatments were assessed by unpaired t-test, and $P<0.05$ was considered to be significant.

RESULTS

The hormonal measures of serum are presented in *Table 2*. There is a significant elevation in ghrelin, corticosterone, and insulin levels for CMS group (depressed animals) in comparison with control group, as elevation was very significant ($P<0.01$) for corticosterone *(Table 2)*. There was not any significant change for T_3, whereas decreased T_4 was observed for CMS groups when compared with control ($P<0.05$).

DISCUSSION

Nowadays, after 13 years from discovery of ghrelin in rat [16], it has been identified that ghrelin has considerable role in mental health, chronic or acute stress [17]. Chuang and Zigman [17] have stated that serum ghrelin can decrease following chronic stress and negative energy balance. In present study *(Table 2)*, ghrelin was in greater level in depressed group (CMS) when compared with control ($P<0.05$). According to Chuang and Zigman [17] report, the regulatory potential of ghrelin may be the reason for ghrelin elevation in onset of stress for moderating metabolic damages of stress or stress induced depression. Also, ghrelin can moderate depressive symptoms.

In previous studies, ghrelin elevation was observed in acute stress [18] water privation condition [19] or tail cutting-stress [20]. Ochi *et al.*[21] have shown that stress can elevate levels of different forms of ghrelin include pre-proghrelin, ghrelin mRNA, acyl-ghrelin and des-acyl-ghrelin. The present finding about elevation of acyl-ghrelin in stress-induced depression condition is in agreement with Ochi *et al.*[21]. In their study, the number of ghrelin- Ip cells (ghrelin-producer cells) has increased following 5 days solid-cage stress (part of CMS protocol). In Lutter *et al.*[18], animals under social stress have greater acyl-ghrelin concentration witch ghrelin level rise to peak point on day-10 of stress protocol. Whereas ghrelin level returned to normal after protocols finished. In this regard, Rouach *et al.*[22] reported that individuals with psychological stress have greater levels of plasma ghrelin. Another evidence for regulatory role of ghrelin in stressful condition is that ghrelin elevation occures along with epinephrine raises induced with stress [23]. Monteleone *et al.*[24] reported considerable increase in salivary ghrelin following social stress protocol. Investigations on major depression cases show constant level for ghrelin [2]. Findings of present study for ghrelin levels of depressed (CMS) animals *(Table 2)* are in agreement with Chuang and Zigman [17], Asakawa *et al.*[19], Kristenssson *et al.*[20] and Monteleone *et al.*[24] reports witch indicate that ghrelin can increase in chronic psychological or environmental stress, and various protocols for depression induce.

It is suggested that exposure to various stressors in CMS protocol is a main cause of ghrelin elevation. Ghrelin has regulatory role for elimination of depressive effects on animal mental or metabolic health. In other word, ghrelin elevation may be an efficient defense mechanism to avoiding depression related damages in CMS animals or in chronic depression cases. But in major depression this mechanism (increase in ghrelin level) may not be efficient [2].

In major depression, hypothalamic- pituitary-adrenal (HPA) axis is hyperactive and releases greater amount of

Table 2. *The hormonal variables of serum in rats subjected to CMS procedure*
Tablo 2. *CMS işlemi uygulanan sıçanlarda serum hormonsal değişkenleri*

Group	Variable				
	Ghrelin pg/ml	Corticosterone ng/l	Insulin mU/l	T_3 ng/ml	T_4 µg/ml
Control	250.38 [b]	160.62 [b]	6.25 [b]	0.40	2.00 [a]
CMS (depressed)	304.75 [a]	190.50 [a]	8.50 [a]	0.55	1.65 [b]
P value	0.0223	0.0042	0.0312	0.2782	0.0259
SEM*	12.582	4.717	0.568	0.088	0.084

** Standard error of the mean; - Different letters (a or b) shows significant difference between means*

glucocorticoide [25]. Abdul Aziz *et al.*[11] reported that pregnant CMS models have greater amniotic corticosterone, and the level can rise significantly at d 13 of gestation. Control group (pregnant non-CMS) had normal level of corticosterone at d 13 of gestation, and they have an increase in corticosterone rate at d 18. Also, in a study [26], infants from mothers with exposure to stress during pregnancy had greater level of plasma cortisol. It was reported that variety in kind and number of chronic stresses can activate hippocampal receptors and releases great amount of corticosterone [27]. In present study which was conducted with similar protocol with Raudkivi *et al.*[27], the various chronic stresses can affect corticosterone level *(Table 2)*. It seems that continuous exposing to variable chronic stresses without dietary energy intake (feed deprivation) for long time of CMS protocol cause corticosterone raises in CMS group.

Depressed individuals are susceptible to insulin-related disorders such as hyperinsulinemia or type 2 diabetes [28]. An epidemiological study showed that insulin- resistance indices are common in depressed people or individuals with mental potent for depression [29].

Less consumption of glucose sources (such as sucrose) in depressed models may be a potential factor for tribulation in insulin release, insulin sensitivity, and depression treatment process. In this regard, Ramasubbu [30] reported correlation between insulin-resistance disorder and depression and this correlation was independent from age, weight, nutritional status, plasma GH or glucagon level and cortisol circadian rhythm. In Pan *et al.*[29] study, it was observed that hypercortisolemia is a booster factor for insulin-resistance in depressed models. In according to Castillo-Quan *et al.*[31] suggestion, depression has insulin-resistance disorder similar with type-2 diabetes. So, it can be cause of hypercortisolemia. In present study, there is a significant increase in insulin and corticosterone level for CMS group *(Table 2)*. Whereof corticosterone and insulin is a major role in metabolic equilibrium of body, any change in these hormones can cause serious metabolic disorder [31]. It can be suggested that the stressors in CMS protocol can cause less dietary glucose intake. Also presents protocol can cause hyperinsulinemia witch is a symptom of depression.

Commonly, T_3 level is not affected by depression, whereas in some studies on depression cases it is reduced due to incidence of depression [32,33]. It is indicated that decreases in T_3 in major depression cases may be because of secondary effect of depression such as starvation, swoon and anti-depressant drugs [34]. But T_4 level can be change due to depression or environmental stresses [35]. In an idea [36], loss of serotonin was announced as main cause of change in thyroid activity. Findings of present study about thyroid hormones (T_3 and T_4) *(Table 2)* are in according to Kirkegaard [32] who reported decreases in T_4 level following depression induction. In present study, T_3 level remained unchanged following CMS protocol, which was unlike to Baumgartner *et al.*[33] reports in major depression (decreased T_3). In Olivares *et al.*[35] study, animals had decreased T_4 level and transient hypothyroidism following social stress protocol. Findings of present study *(Table 2)* conducted with CMS protocol was in agreement with Olivares *et al.*[35]. It seems that T_4 decreases in CMS animals *(Table 2)* were in related to serotonin losses, minor energy intake and subsequent declines in basal metabolism.

It was concluded that chronic mild stress protocol and induced depression can cause ghrelin, corticosteroid, and insulin increases, and T_4 decreases. Mentioned effects of CMS induced depression on hormonal indices (with exception to thyroid hormones), can be similar to the effects of major depression. It seems that T_4 decreases in CMS animals are in relation to serotonin losses, minor energy intake (because of continuous stresses) and subsequent declines in basal metabolism. Further studies on CMS effect on other hormones are necessary to completing hormonal profile of CMS protocol.

REFERENCES

1. Linkowski P, Mendlewicz J, Kerkhofs M, Leclercq R, Golstein J, Brasseur M, Copinschi G, Van Cauter E: 24-hour profiles of adrenocorticotropin, cortisol, and growth hormone in major depressive illness: Effect of antidepressant treatment. *J Clin Endocrinol Metab*, 65 (1): 141-152, 1987.

2. Kluge M, Schüssler P, Schmid D, Uhr M, Kleyer S, Yassouridis A, Steiger A: Ghrelin plasma levels are not altered in major depression. *Neuropsychobiology*, 59, 199-204, 2009.

3. Joffe RT, Marriott M: Thyroid hormone levels and recurrence of major depression. *Am J Psychiat,* 157 (10): 1689-1691, 2000.

4. Steiger A, Dresler M, Schüssler P, Kluge M: Ghrelin in mental health, sleep, memory. *Mol Cel Endocrinol*, 20, 88-96, 2011.

5. Willner P: Validity, reliability and utility of the chronic mild stress model of depression: A 10-year review and evaluation. *Psychopharmacology (Berlin),* 134, 319-329, 1997.

6. Willner P: Chronic mild stress (CMS) revisited: Consistency and behavioural-neurobiological concordance in the effects of CMS. *Neuropsychobiology*, 52, 90-110, 2005.

7. Jayatissa MN, Bisgaard C, Tingström A, Papp M, Wiborg O: Hippocampal cytogenesis correlates to escitalopram-mediated recovery in a chronic mild stress rat model of depression. *Neuropsychopharmacology*, 31 (11): 2395-2404, 2006.

8. Willner P, Towell A, Sampson D, Sophokleous S, Muscat R: Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. *Psychopharmacology (Berlin)*, 93, 358-364, 1987.

9. Papp M, Willner P, Muscat R: An animal model of anhedonia: Attenuation of sucrose consumption and place preference conditioning by chronic unpredictable mild stress. *Psychopharmacology (Berlin)*, 104, 255-259, 1991.

10. Paternain L, García-Diaz DF, Milagro FI, González-Muniesa P, Martinez JA, Campión J: Regulation by chronic-mild stress of glucocorticoids, monocyte chemoattractant protein-1 and adiposity in rats fed on a high-fat diet. *Physiol Behav*, 103, 173-180, 2011.

11. Abdul Aziz NH, Kendall DA, Pardon MC: Prenatal exposure to chronic mild stress increases corticosterone levels in the amniotic fluid and induces cognitive deficits in female offspring, improved by treatment with the antidepressant drug amitriptyline. *Behav Brain Res*, 16, 29-39, 2012.

12. Kioukia N, Bekris S, Antoniou K, Papadopoulou-Daifoti Z, Christofidis I: Effects of chronic mild stress (CMS) on thyroid hormone function in two rat strains. *Psychoneuroendocrinology*, 25 (3): 247-257, 2000.

13. Strekalova T, Spanagel R, Bartsch D, Henn FA, Gass P: Stress-induced anhedonia in mice is associated with deficits in forced swimming and exploration. *Neuropsychopharmacology*, 29 (11): 2007-2017, 2004.

14. Taliaz D, Loya A, Gersner R, Haramati S, Chen A, Zangen A: Resilience to chronic stress is mediated by hippocampal brain-derived neurotrophic factor. *J Neurosci*, 31 (12): 4475-4483, 2011.

15. Shao, WH, Song-Hua F, Yang L, Guo-En Y, Jian-Jun C, Jian Z, Hong-Bo X, Hai-Peng L, Bo W, Peng Z, Liang F, Peng X: Metabolomic identification of molecular changes associated with stress resilience in the chronic mild stress rat model of depression. *Metabolomics,* 9, 433-443, 2013.

16. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H and Kangawa K: Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*, 402, 656-660, 1999.

17. Chuang JC, Zigman JM: Ghrelin's roles in stress, mood, and anxiety regulation, *Int J Pept*, Article ID 460549, 2010.

18. Lutter M, Sakata I, Osborne-Lawrence S, Rovinsky SA, Anderson JG, Jung S, Birnbaum S, Yanagisawa M, Elmquist JK, Nestler EJ, Zigman JM: The orexigenic hormone ghrelin defends against depressive symptoms of chronic stress. *Nat Neurosci*, 11, 752-753, 2008.

19. Asakawa A, Inui A, Kaga T, Yuzuriha H, Nagata T, Fujimiya M, Katsuura G, Makino S, Fujino MA, Kasuga M: A role of ghrelin in neuroendocrine and behavioral responses to stress in mice, *Neuroendocrinology*, 74, 143-147, 2001.

20. Kristenssson E, Sundqvist M, Astin M, Kjerling M, Mattsson H, Dornonville de la Cour C, Håkanson R, Lindström E: Acute psychological stress raises plasma ghrelin in the rat, *Regulat Pept*, 134, 114-117, 2006.

21. Ochi M, Tominaga K, Tanaka F, Tanigawa T, Shiba M, Watanabe T, Fujiwara Y, Oshitani N, Higuchi K, Arakawa T: Effect of chronic stress on gastric emptying and plasma ghrelin levels in rats. *Life Sci*, 82, 862-868, 2008.

22. Rouach V, Bloch M, Rosenberg N: The acute ghrelin response to a psychological stress challenge does not predict the post-stress urge to eat. *Psychoneuroendocrinology*, 32, 693-702, 2007.

23. De la Cour CD, Norlen P, Hakanson R: Secretion of ghrelin from rat stomach ghrelin cells in response to local microinfusion of candidate messenger compounds: A microdialysis study. *Regul Pept*, 143, 118-126, 2007.

24. Monteleone P, Tortorella A, Scognamiglio P, Serino I, Monteleone AM, Maj M: The acute salivary ghrelin response to a psychosocial stress is enhanced in symptomatic patients with bulimia nervosa: A pilot study. *Neuropsychobiology*, 66 (4): 230-236, 2012.

25. Prathiba J, Kumar KB, Karanth KS: Effects of neonatal clomipramine on cholinergic receptor sensitivity and passive avoidance behavior in adult rats. *J Neural Transm Gen Sect,* 100 (2): 93-99, 1995.

26. Dugovic C, Maccari S, Weibel L, Turek FW, Van Reeth O: High corticosterone levels in prenatally stressed rats predict persistent paradoxical sleep alterations. *J Neurosci*, 19 (19): 8656-8664, 1999.

27. Raudkivi K, Mällo T, Harro J: Effect of chronic variable stress on corticosterone levels and hippocampal extracellular 5-HT in rats with persistent differences in positive affectivity. *Acta Neuropsych,* 24, 208-214, 2012.

28. Everson-Rose SA, Meyer PM, Powell LH, Pandey D, Torre´ns JI, Kravitz HM, Bromberger JT, Matthews KA: Depressive symptoms, insulin resistance, and risk of diabetes in women at midlife. *Diabet Care,* 27, 2856-2862, 2004.

29. Pan A, Ye X, Franco OH, Li H, Yu Z, Zou S: Insulin resistance and depressive symptoms in middle-aged and elderly Chinese: Findings from the nutrition and health of aging population in China study. *J Affect Dis*, 109, 75-82, 2008.

30. Ramasubbu R: Insulin resistance: A metabolic link between depressive disorder and atherosclerotic vascular diseases. *Med Hypothes,* 59 (5): 537-551, 2002.

31. Castillo-Quan JI, Herrera-González A, Pérez-Osorio JM: Insulin-cortisol interaction in depression and other neurological diseases: An alternative hypothesis. *Psychoneuroendocrinology,* 32 (7): 854-855, 2007.

32. Kirkegaard C: The thyrotropin response to thyrotropin-releasing hormone in endogenous depression. *Psychoneuroendocrinology*, 6, 189-212, 1981.

33. Baumgartner A, Graf KJ, Kurten I, Meinhold H: The hypothalamic-pituitary-thyroid axis in psychiatric patients and healthy subjects: Parts 1-4. *Psych Res*, 24, 271-332, 1988.

34. Kirkegaard C, Faber J: The role of thyroid hormones in depression. *Euro J Endocrinol*, 138, 1-9, 1998.

35. Olivares EL, Silva-Almeida C, Pestana FM, Sonoda-Côrtes R, Araujo IG, Rodrigues NC, Mecawi AS, Côrtes WS, Marassi MP, Reis LC, Rocha FF: Social stress-induced hypothyroidism is attenuated by antidepressant treatment in rats. *Neuropharmacology*, 62 (1): 446-456, 2012.

36. Prange AJ, Wilson IC, Lynn CW: L-Tryptophan in mania: Contribution to a permissive hypothesis of affective disorders. *Arch Gen Psychiat*, 30, 56-62, 1974.