

The effect of zinc supplementation on salivary cortisol levels and salivary amylase activity in primary school children with low socioeconomic status

Abstract

In children, both salivary cortisol levels and salivary alpha amylase (sAA) activities are being used to define several psychological/psychiatric problems. Zinc may have critical roles in normal cognitive and emotional functioning. The aim of this study was to evaluate the salivary cortisol levels and sAA activity in low socioeconomic level primary school children receiving placebo or zinc supplementation and to correlate these values with psychometric test scores. The study was designed as a double-blind randomized placebo controlled intervention study with 48 children, who were assigned to 15mg/day placebo (n=26) or zinc sulphate (n=22) for 10 weeks. No correlation was detected between the psychometric test scores and the salivary cortisol levels and sAA activities at the beginning of the study. Changes in psychometric test scores were not statistically significant in placebo and zinc groups at the end of the study. After supplementation, salivary cortisol levels markedly increased (2.54 ± 2.21 vs. 6.44 ± 4.20 nmol/L) in both placebo group and zinc group (2.57 ± 1.90 vs. 4.77 ± 2.17 nmol/L). sAA activities significantly decreased in the placebo (0.29 ± 0.08 vs. 0.24 ± 0.06 mmol p-nitrofenol/min/L) and zinc group (0.28 ± 0.53 vs. 0.24 ± 0.06 mmol p-nitrofenol/min/L). However, the differences between groups in both cortisol levels and sAA activities were not statistically significant. Larger studies should be conducted to show the effect of zinc supplementation on salivary cortisol levels and sAA activities in children as well as on psychometric test scores.

Keywords: salivary cortisol, salivary alpha amylase, zinc, psychometric test scores

Volume 2 Issue 2 - 2016

Pinar Erkekoglu, Yasemin Üçkardeş, Elif N Özmert, Belma Kocer Gumusel, Kadriye Yurdakök

Department of Toxicology, Hacettepe University, Turkey

Correspondence: Pinar Erkekoglu, Hacettepe University, Faculty of Pharmacy, Department of Toxicology, Ankara, Turkey, Tel 00903123052178, Fax 00903123114777, Email belmagumusel@yahoo.com, erkekp@yahoo.com

Received: April 27, 2016 | **Published:** June 06, 2016

Abbreviations: ADHD, attention deficit and hyperactivity disorder; CNS, central nervous system; CPRS, conners' parent rating scale; CTRS, conners' teachers rating scale; HPA, hypothalamo pituitary adrenal axis; HPAS, hacettepe psychological adaptation scale; PNPM, p-nitrophenyl α -maltoide; sAA, α -amylase; SAM, sympathetic adrenal medulary; STAI, state trait anxiety inventory

Introduction

The psychobiology of stress response mainly consists of two components: a. activation of hypothalamo-pituitary-adrenal axis (HPA) and the secretion of glucocorticoids like cortisol; b. activation of locus ceruleus/autonomic sympathetic nervous system and the release of catecholamines, like norepinephrine, into the bloodstream. Stress hormones, like cortisol, regulate body metabolism and behavioral response. Besides, these hormones could affect developing brain. Animal studies showed that elevated basal and stress-induced corticosterone decreased cellular proliferation in the hippocampus.^{1,2} There is also limited evidence on the environmentally-mediated alterations in HPA axis functioning.²

In bio-behavioral research, salivary cortisol levels are being used to evaluate the HPA axis function in children for many years. Saliva offers many advantages as a biomarker fluid. Noninvasive assessment of biomarkers in saliva can create new opportunities to study how biological and social processes interact to influence health and human behavior.^{1,2} Elevations in morning salivary cortisol

levels were documented in children with traumatic life events, like abuse histories and psychosocial problems.^{3,4} Exposure to more extreme developmental contexts, including violence, alters HPA axis functioning.⁵ The duration and type of violence are crucial factors, affecting the alterations in salivary cortisol levels.² Low basal cortisol levels were also associated with increases in aggression, antisocial behavior, and disruptive behavior problems.^{2,5}

There has been a growing interest in salivary α -amylase (sAA) as a non-invasive marker for sympathetic-adrenal-medulary (SAM) activity. sAA is a digestive enzyme that breaks down starch and measuring sAA activity provides a simple means of quantification. Granger et al.² reported that higher basal sAA levels and activity were associated with social behavioral problems, negative affectivity cognitive/academic problems and cardiovascular psychophysiology.² Recently, morning salivary cortisol levels and sAA activities are being used in field studies to define several psychiatric problems such as anxiety, depression and chronic fatigue syndrome.⁶ However, these studies reveal different results.

Zinc is an essential trace element with extensive effects on neurotransmission, receptor function and second messenger systems in telencephalon. Zinc is stored in specific synaptic vesicles by a class of glutamatergic or "gluzinergic" neurons.⁷ Gluzinergic neurons are exclusively found in the cerebral cortex and limbic structures. Thus, zinc may have critical roles in normal cognitive and emotional functioning.² Several researchers have attempted to identify the

mechanisms through which zinc may be involved in mental health disorders such as depression and attention deficit and hyperactivity disorder (ADHD). Different mechanisms have been suggested to explain the relation between zinc and ADHD symptoms, possibly through alterations in the neurotransmitters dopamine and serotonin.^{2,7} Recent studies have suggested that children with ADHD may have blunt HPA function. However, findings on HPA-axis reactivity in ADHD, are rather inconsistent and the effects of systemic zinc supplementation on ADHD symptoms through stress hormones has not been evaluated recently.^{2,8}

While demonstrating the current study, we hypothesized that systemic zinc supplementation would alter salivary stress hormone levels in children as adequate zinc intake is a crucial factor in sustaining normal emotional and behavioral stability.² Thus, this study aimed to evaluate the salivary cortisol levels and sAA activity in low socioeconomic level primary school children receiving placebo or zinc supplementation and to correlate these values with psychometric test scores.

Materials and methods

Chemicals

All chemicals were obtained from Sigma-Aldrich (St.Louis, MO). CORT-CT2 radioimmunoassay kit was obtained from Cisbio Bioassays (Bedford, MA).

Study groups

This study was designed as a double-blind randomized placebo controlled intervention study, conducted as a part of a larger study in Ankara, Turkey in 2005. The research protocol was approved by Hacettepe University Faculty of Medicine Ethics Committee and Turkish Ministry of Health. Written informed consents were obtained from all the parents before the enrolment of the children to this study. Children in each class were randomized to one of the study groups. Forty-eight primary school third grade children (30 girls, and 18 boys), with low socioeconomic level, were randomly assigned to placebo (n=26) or zinc sulphate (15mg/day, n=22) groups for 10 weeks. The placebo and zinc syrups were prepared and labeled by Berko Company (İstanbul, Turkey). Placebo and zinc syrups were given by the teachers at school five days of a week on school days. Until the end of the experiments, the teachers, so as the researchers, who performed the measurement of the cortisol levels and sAA activities, were completely blind about the assignment of children to the study groups.

Socioeconomic, psychometric and other tests

For the evaluation of parental socioeconomic/socio-cultural level, Hollingshead-Redlich Scale was used.⁹ Hacettepe Psychological Adaptation Scale (HPAS), Conners' Parent Rating Scale (CPRS), Conners' Teachers Rating Scale for (CTRS) and State-Trait Anxiety Inventory (STAI) for Children were performed both before and after supplementation.

HPAS was developed by Hacettepe University, Faculty of Medicine, Department of Child and Adolescent Psychiatry to evaluate psychological compliance in children and adolescents. HPAS has 32 articles in which 24 of them are related to the child's psychological adaptation. The answers are given as "No", "Some" and "Yes" which are assessed as 0, 1, and 2, respectively.^{10,11} The Conner's Rating

Scales for Teachers and Parents which was developed by Conners,¹² revised by Goyette et al.¹³ adapted to Turkish population by Sener et al.¹⁴ and Dereboy et al.¹⁵ The Conner's scale consists of 48 questions for assessment of behavior. Evaluation of each question is made by 4-point scale ranging from 'never' (0) to very often (3). The answers are grouped into subscales to determine the behavioral characteristics. Parent's ratings are grouped as attention deficit, hyperactivity, and oppositional behavior and conduct disorders while teacher's ratings are grouped as attention deficit and disruptive behavior disorder symptoms. Cut off points of clinically significant scores for this version are 5 points for attention deficit, 6 points for hyperactivity, 6 points for oppositional behavior and 18 points for conduct disorder in parent's ratings while 18 points for attention deficit and 16 points for disruptive behavior disorders in teacher's ratings. STAI-C was developed by Spielberger¹⁶ and includes 2 subscales (state anxiety and trait anxiety), each consisting of 20 multiple-choice items. Items are scored as 0, 1, or 2, according to the severity of symptoms. State anxiety is anxiety experienced by an individual at a particular time and in a particular situation. State anxiety varies according to external factors. Trait anxiety, on the other hand, is the degree of anxiety an individual experiences in general. The Turkish version was reported to be valid and reliable for use in Turkey.¹⁷

Sample collection

Saliva samples were obtained from students in the morning at school. Salivary samples were immediately transferred to laboratory on ice and samples were frozen stored at 20°C until analysis.

Measurement of salivary cortisol levels

Salivary cortisol levels were determined using a radioimmunoassay kit, according to manufacturer's instructions. Briefly, by using the 2000nmol/L cortisol stock solution, the following standards were prepared with dilution buffer [0.1M Tris-HCl, pH 7.4, 0.2% bovine serum albumin (BSA)]: 1.0 - 4.0 - 20 and 100nmol/L. 150µl of standards, controls and samples to be assayed were dispensed into the correspondingly-labeled coated tubes. ¹²⁵I-cortisol (500µl) was added to each tube and each tube was gently vortexed. The tubes were covered and incubated for 30min at 37°C. Liquid was discarded. The tubes were washed once with 1ml of deionized water. The remaining radioactivity bound to the tubes was measured with a gamma scintillation counter (Perkin Elmer, Waltham, MA) calibrated for ¹²⁵I. All experiments were performed in duplicate.

Salivary amylase activity

The method of Gillard et al.¹⁸ was used for the determination of the sAA activity. This method uses the conversion of p-nitrophenyl α-maltoside (PNPM) into maltoside and p-nitrophenol in the presence of the sAA. Briefly, saliva samples were centrifuged at 200xg and 0.5ml supernatant was used for the experiments. 0.4ml phosphate buffer [50mM sodium dihydrogen phosphate (NaH₂PO₄), 25mM sodium chloride (NaCl), 1.25g/L gelatin, pH 7] and 90µl deionized water was added and mixed gently. The mixture was incubated at 37°C for 10min. Then, the mixture was transferred to a spectrophotometer cuvette and PNPM stock solution (10µl) was added. The linear increase in the absorption was monitored continuously at 400nm and at 37°C for 20min. Quantification was achieved by parallel measurements of a standard curve of known p-nitrophenol concentrations (0, 0.1, 0.2, 0.3, 0.4 and 0.5nmol/L) and results were expressed in nmol p-nitrophenol/min/L. All experiments were performed in duplicate.

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences 11.00 (SPSS 11.00, Chicago, IL). All results were given as mean±SD. The changes in the values of cortisol levels and sAA activities after zinc supplementation were compared by using Sign test ($p < 0.05$).

Results

Children characteristics

The mean age of the study participants was 8.37 ± 0.49 (7.4-10.2) years, 62% being female. Children who had weight/height ratio below 0.90 consisted of 40% of the study population.

After supplementation, there was no statistically significant difference in HPAS neurotic problem scores in either of the groups. HPAS total scores decreased significantly in the zinc group, but not in the placebo group. HPAS behavior problem scores also decreased in both of the study groups.¹⁹ After the treatment, the mean CPRS scores on attention deficit, hyperactivity, oppositional behavior and conduct disorder decreased significantly in both of the study groups ($p < 0.01$), as previously described. The prevalence of children with clinically significant parent ratings on attention deficit ($p = 0.01$) and hyperactivity ($p = 0.004$) decreased in the zinc group while prevalence of oppositional behavior ($p = 0.007$) decreased in the placebo group. There was no change in mean CTRS scores. In STAI, potential scores for both measures ranged from 20 to 80, with higher scores denoting higher anxiety.²⁰

Salivary cortisol levels and salivary amylase activity

Salivary cortisol levels before and after treatment were 2.54 ± 2.21 vs. 6.44 ± 4.20 nmol/L ($p = 0.002$) in placebo group and 2.57 ± 1.90 vs. 4.77 ± 2.17 nmol/L ($p < 0.0001$) in zinc group (Table 1). Changes in salivary cortisol levels within the groups before and after syrup administration was given in Table 2. After the treatment period, the increase observed in zinc group was not statistically significant compared to placebo group.

sAA activities before and after treatment were 0.29 ± 0.08 vs. 0.24 ± 0.06 mmol p-nitrofenol/min/L ($p = 0.004$) in placebo group and 0.28 ± 0.53 vs. 0.24 ± 0.06 mmol p-nitrofenol/min/L in zinc group ($p = 0.016$) (Table 3). After the treatment period, we observed that zinc supplementation did not have any marked effect on sAA activity when compared to placebo group ($p > 0.05$) (Table 4).

Correlations between psychometric test scores and salivary biomarkers

Table 1 Salivary cortisol levels before and after syrup administration

Group	Salivary cortisol levels before administration of syrup (nmol/l)	Salivary cortisol levels after administration of syrup (nmol/l)
Placebo Group (n=26)	2.54 ± 2.21	$6.44 \pm 4.20^*$
Zinc Group (n=22)	2.57 ± 1.90	$4.77 \pm 2.17^*$

*Represents that salivary cortisol levels were significantly different in each group after the administration of the syrup ($p < 0.05$).

Table 2 Changes in salivary cortisol levels within the groups before and after syrup administration

Group	Cortisol levels	n	p
Placebo Group	Increased	18	0.001
	Decreased	1	
	Unchanged	7	
Zinc Group	Total	26	0.0001
	Increased	21	
	Decreased	1	
	Total	22	

P values were determined by Sign test and shows the significance of changes between the basal and second cortisol levels.

Table 3 Salivary α -amylase activities before and after syrup administration

Groups	sAA activity before administration of syrup (nmol p-nitrofenol/min/l)	sAA activity after administration of syrup (nmol p-nitrofenol/min/l)
Placebo Group (n=26)	0.29 ± 0.08	$0.24 \pm 0.06^*$
Zinc Group (n=22)	0.28 ± 0.53	$0.24 \pm 0.06^*$

*Represents that sAA activities were significantly different in each group after the administration of the syrup. p values were determined by Sign test.

Table 4 Changes in salivary α -amylase activities of the groups before and after syrup administration.

	sAA activity	n	p
Placebo	Increased	20	0.003
	Decreased	6	
	Total	26	
Zinc	Increased	18	0.025
	Decreased	4	
	Total	22	

P values were determined by Sign test and shows the significance of changes between the basal and second sAA activities

No correlations were detected between the psychometric test scores applied and the salivary cortisol levels as well as the sAA activities at the beginning of the study ($p > 0.05$, for all). Besides, there were not any correlations between the psychometric test scores in both of the study groups at the end of the study.

Discussion

Scientists are mainly focusing on testing innovative theoretical models of individual differences in behavior as a function of multi-level bio-social processes in the recent years. Development of new biomarkers is a constantly evolving field of research endeavor in psychoneuro endocrinology.²¹ Salivary biomarkers have received special attention since they are readily accessible and easily obtained.²²

The theoretical framework proposes that cortisol levels and sAA activities, the HPA and SAM components of the stress system, are good tools for the evaluation of social behaviors. There are several

studies in literature showing these parameters can be important tools in measuring stress, social behavior and school achievement in children and adolescents. Susman et al.²³ considered cortisol levels and sAA activities as vulnerabilities that also interact with the putative stressful transition of timing of puberty to predispose adolescents toward antisocial behavior.²³ Berry et al.²⁴ showed that these parameters were good indicators of academic achievement in pre-kindergarten.²⁴

Non-invasively collected salivary cortisol and its links to emotions and behaviors have been extensively validated in laboratory settings.²⁵ Starting from the prenatal period, regulatory patterns of cortisol are related to psychological functioning. For example, in three-year-old children low basal, maternal prenatal cortisol levels were related to difficult infant temperament.²⁶ In elderly, higher basal cortisol levels were associated with cognitive declines.²⁷ Concerning antisocial behavior, lower basal salivary cortisol levels are observed in individuals who exhibit disruptive behavior problems,²⁸ including oppositional defiant behavior,²⁹ externalizing behavior problems,³⁰ and abuse.³¹ Furthermore, low basal cortisol is both a concurrent correlate and risk factor for future alcohol use.³² However, there are debated results from hyperactivity studies: Hyperactivity was characterized in the HPA axis response to stressors in some studies,³² whereas hypo-reactivity was characteristic of antisocial boys in other studies.³³ Age, dysfunction in the serotonin system, developmental differences between children and adults, composition of the sample and outcome measures may be the underlying factors of the disparities.²⁹

Zinc is an abundant trace element which has many regulatory roles. It is highly available in brain. It is substantial for normal mammalian brain development and physiology. Direct evidence indicates that zinc can act as a neuromodulator.³⁴ Both deficiency and excess states has been shown to cause alterations in behavior, abnormal central nervous system (CNS) development, and neurological disease. Zinc ions have now been shown to play a role in the neuro-modulation of synaptic transmission as well as in cortical plasticity. Recent evidence has implicated the role of zinc in mental retardation, synaptic plasticity, and cognition.³⁵

Taking all these knowledge and available data in concern, we postulated that zinc could be a modifier of behavior and salivary biomarkers as well as the psychometric scoring in third-grade children. We designed the current study to evaluate the effect of zinc supplementation on psychometric test scores and salivary biomarkers of stress in children. Our hypothesis was that low income may act as a social stressor and zinc could ameliorate the stress factor while placebo would not provide a positive change in both salivary biomarkers and psychometric test scores. In both placebo and zinc groups, we observed significant increases in cortisol levels compared to basal levels. Though we observed marked increases in cortisol levels in both of the study groups at the end of the study, we did not find any difference between the increases among placebo and zinc applications. This might be explained as a conditioned response towards the administration of a drug in both of the study groups. Although the cortisol levels were higher in placebo group when compared to zinc group at the end of the study, there was a high standard deviation in placebo group and this might be another explanation why we did not observe any difference between the two groups at the end of the study. On the other hand, it was demonstrated that salivary cortisol levels show diurnal pattern and many confounding factors may have influence on cortisol secretion. The timing of sampling might be another important factor in the results obtained.²

Salivary amylase is an enzyme and an assumptive biomarker of the adrenergic component of the stress response. sAA is considered an important marker of SAM activity and increases under stressful conditions.² However, sAA can show elevations in response to a stressor independently of serum catecholamines and may reflect a general marker of SAM activity.³⁶ The validity of sAA as an index of SAM activity additionally is supported by the suppression of sAA secretion after experiencing periods of emotionally charged stressors by the adrenergic blocker propranolol.³⁶ Changes in sAA also are observed in response to physiological period and age.³⁷ In line with several researchers, Gordis et al.³⁸ showed that sAA levels increased after stress in adolescents.³⁸ In the present study, we observed marked decreases in sAA activities compared to basal activities in both placebo and zinc groups after the administration of the syrup. There was no difference between the basal sAA activities among the groups. Besides, there was no marked difference in the decrease of in sAA activities between placebo and zinc groups. This might be a reflection of conditioned response to a drug application so as observed in salivary cortisol levels. Besides, we can postulate that drug application in both of the groups has a positive impact on SAM and shows a decrease in the stress conditions of the children although no correlations were determined between sAA activities and applied psychometric test scores.

HPA axis and CNS are connected at multiple neural levels, and thus activities in these two systems do happen to show some degree of symmetry.³⁹ However, there are several unclear factors affecting the degree of symmetry in different individuals with different circumstances. One possible factor may be different habituation rates of these systems given previous exposure to stress. Animal research also supports the evidence from human studies that prolonged stress may lead to asymmetry between these systems.⁴⁰ In addition, in four independent studies intra-individual sAA responses to challenge differed distinctly from salivary cortisol responses, particularly, in associations with social behavior, negative affectivity, cognitive problems, and cardiovascular activity.²³ In the current study, we observed increases in cortisol levels while determining decreases in sAA activities after the treatment period in both groups. However, further studies are needed before accounting this phenomenon. Concerning the current work, the two main limitations were sample size and the timing of salivary collection (not early morning).

In conclusion, large-scale studies with early morning (upon wakening) specimens should be conducted to demonstrate the inverse relationship between cortisol levels and sAA activity in children with or without zinc application. Besides, mechanistic studies should be performed to understand this interrelationship.

Acknowledgements

This study was supported by Hacettepe University Research Fund (Project No: 08 D12 103 001). We thank Düzen Laboratories (Ankara/Turkey) for their help in the measurement of salivary cortisol levels.

Conflict of interest

The author declares no conflict of interest.

References

1. Malamud D. Saliva as a diagnostic fluid. *Dent Clin North Am.* 2011;55(1):159–178.

2. Granger DA, Kivlighan KT, Sheikh ME, et al. Salivary α -amylase in bio behavioral research. *Ann N Y Acad Sci.* 2007;1098:122–144.
3. Carpenter LL, Shattuck TT, Tyrka AR, et al. Effect of childhood physical abuse on cortisol stress response. *Psychopharmacology (Berl).* 2011;214(1):367–375.
4. Morin IO, Odgers CL, Danese A, et al. Blunted cortisol responses to stress signal social and behavioral problems among maltreated/bullied 12-year-old children. *Biol Psychiatry.* 2011;70(11):1016–1023.
5. McBurnett K, Lahey BB, Frick PJ, et al. Anxiety, inhibition, and conduct disorder in children: II. Relation to salivary cortisol. *J Am Acad Child Adolesc Psychiatry.* 1991;30(2):192–196.
6. Allwood MA, Handwerker K, Kivlighan KT, et al. Direct and moderating links of salivary alpha-amylase and cortisol stress-reactivity to youth behavioral and emotional adjustment. *Biol Psychol.* 2011;88(1):57–64.
7. Digirolamo AM, Ramirez ZM. Role of zinc in maternal and child mental health. *Am J Clin Nutr.* 2009;89(3):940–945.
8. Mead HK, Beauchaine TP, Shannon KE Neurobiological adaptations to violence across development. *Dev Psychopathol.* 2010;22(1):1–22.
9. Hollingshead AB, Redlich FC. Social Class and Mental Illness: A Community Study. *Am J Public Health.* 2007;97(10):1756–1757.
10. Gökler B, Öktem F. Bir gecekondu ilkokulu öğrencilerinde ruhsal uyum taraması (A survey on psychiatric competence in primary school children living in shanties). *Toplum ve Hekim.* 1985;36:24–27.
11. Coşkun A. *Çocuklarda Davranış Derecelendirme Ölçeği ve Hacettepe Ruhsal Uyum Ölçeğinin Malatya İl Merkezindeki İlkokullarda Uygulanması (Child Scoring Scale and Applying Hacettepe Scale to Primary Schools in Malatya City Center)*. Ankara: Hacettepe University Faculty of Medicine Department of Child Psychiatry, Master's Thesis; 1994.
12. Conners CK. A teacher rating scale for use in drug studies with children. *Am J Psychiatry.* 1969;126(6):884–888.
13. Goyette CH, Conners CK, Ulrich RF. Normative data on revised Conners parent and teacher rating scales. *J Abnorm Child Psychol.* 1978;6(2):221–236.
14. Sener S, Dereboy C, Dereboy F, et al. Conners öğretmen derecelendirme ölçeği Türkçe uyarlaması (Applying Conners teacher rating scale, Turkish version). *Cocuk ve Genclik Ruh Sağlığı Dergisi.* 1995;2:131–142.
15. Dereboy C, Sener S, Dereboy F, et al. Conners öğretmen derecelendirme ölçeği Türkçe uyarlaması-2 (Applying Conners teacher rating scale Turkish version-2). *Cocuk ve Genclik Ruh Sağlığı Dergisi.* 1997;4:10–19.
16. Spielberger CD. The measurement of state and trait anxiety: Conceptual Method. *Iss Monogr.* 1976;2:713–715.
17. Özusta Ş. Çocuklar için Durumluk-Süreklilik Kaygı Envanterinin Uyarlama, Geçerlik ve Güvenirlik Çalışması. *Türk Psikoloji Dergisi.* 1995;10:32–44.
18. Gillard BK, Marksman HC, Feig SA. Direct spectrophotometric determination of alpha-amylase activity in saliva, with p-nitrophenyl alpha-maltoside as substrate. *Clin Chem.* 1977;23(12):2279–2282.
19. Uçkardeş Y, Özmert EN, Unal F, et al. Düşük sosyoekonomik düzey ilköğretim çocuklarında çinko desteğinin Hacettepe Ruhsal Uyum Ölçeği skorlarına etkisi. *Çocuk Sağlığı ve Hastalıkları Dergisi.* 2009;52:53–59.
20. Uçkardeş Y, Özmert EN, Unal F, et al. Effects of zinc supplementation on parent and teacher behavior rating scores in low socioeconomic level Turkish primary school children. *Acta Paediatr.* 2009;98(4):731–736.
21. DiGirolamo AM, Ramirez ZM. Role of zinc in maternal and child mental health. *Am J Clin Nutr.* 2009;89(3):940–945.
22. Nater UM, Rohleder N. Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: current state of research. *Psychoneuroendocrinology.* 2009;34(4):486–496.
23. Susman EJ, Dockray S, Granger DA, et al. Cortisol and alpha amylase reactivity and timing of puberty: vulnerabilities for antisocial behaviour in young adolescents. *Psychoneuroendocrinology.* 2010;35(4):557–569.
24. Berry D, Blair C, Willoughby M, et al. Salivary alpha-amylase and cortisol in infancy and toddlerhood: direct and indirect relations with executive functioning and academic ability in childhood. *Psychoneuroendocrinology.* 2012;37(10):1700–1711.
25. Kirschbaum C, Wüst S, Faig HG, et al. Heritability of cortisol responses to human corticotropin-releasing hormone, ergometry, and psychological stress in humans. *J Clin Endocrinol Metab.* 1992;75(6):1526–1530.
26. Susman EJ, Schmeelk KH, Ponirakis A, et al. Maternal prenatal, postpartum, and concurrent stressors and temperament in 3-year-olds: a person and variable analysis. *Dev Psychopathol.* 2001;13(3):629–652.
27. Seeman TE, McEwen BS, Singer BH, et al. Increase in urinary cortisol excretion and memory declines: MacArthur studies of successful aging. *J Clin Endocrinol Metab.* 1997;82(8):2458–2465.
28. McBurnett K, Lahey BB, Rathouz PJ, et al. Low salivary cortisol and persistent aggression in boys referred for disruptive behavior. *Arch Gen Psychiatry.* 2000;57(1):38–43.
29. Van Goozen SH, Matthys W, Cohen KPT, et al. Salivary cortisol and cardiovascular activity during stress in oppositional-defiant disorder boys and normal controls. *Biol Psychiatry.* 1998;43(7):531–539.
30. Shirtcliff EA, Granger DA, Booth A, et al. Low salivary cortisol levels and externalizing behavior problems in youth. *Dev Psychopathol.* 2005;17(1):167–184.
31. Bergman B, Brismar B. Hormone levels and personality traits in abusive and suicidal male alcoholics. *Alcohol Clin Exp Res.* 1994;18(2):311–316.
32. Susman EJ, Dorn LD, Inoff-Germain G, et al. Cortisol reactivity, distress behavior, and behavioral and psychological problems in young adolescents: a longitudinal perspective. *Journal of Research on Adolescence.* 1997;7(1):81–105.
33. Fairchild G, Van Goozen SH, Stollery SJ, et al. Cortisol diurnal rhythm and stress reactivity in male adolescents with early-onset or adolescence-onset conduct disorder. *Biol Psychiatry.* 2008;64(7):599–606.
34. Prasad AS. Zinc: an overview. *Nutrition.* 1995;11(1S):93–99.
35. Bitanhirwe BK, Cunningham MG. Zinc: the brain's dark horse. *Synapse.* 2009;63(11):1029–1049.
36. Stegeren AV, Rohleder N, Everaerd W, et al. Salivary alpha amylase as marker for adrenergic activity during stress: effect of betablockade. *Psychoneuroendocrinology.* 2006;31(1):137–141.
37. Giesbrecht GF, Granger DA, Campbell T, et al. Salivary alpha-amylase during pregnancy: diurnal course and associations with obstetric history, maternal demographics, and mood. *Dev Psychobiol.* 2013;55(2):156–167.
38. Gordis EB, Granger DA, Susman EJ, et al. Asymmetry between salivary cortisol and alpha-amylase reactivity to stress: relation to aggressive behavior in adolescents. *Psychoneuroendocrinology.* 2006;31(8):976–987.
39. Lovallo WR, Dickensheets SL, Myers DA, et al. Blunted stress cortisol response in abstinent alcoholic and polysubstance-abusing men. *Alcohol Clin Exp Res.* 2000;24(5):651–658.
40. Britton KT, Segal DS, Kuczenski R, et al. Dissociation between in vivo hippocampal norepinephrine response and behavioral/neuroendocrine responses to noise stress in rats. *Brain Res.* 1992;574(1–2):125–130.