Anxiety Symptoms During Adolescence Predicts Salivary Cortisol in Early Adulthood Among Blacks; Sex differences

Shervin Assari,1,2,* Maryam Moghani Lankarani,1,3 Cleopatra Howard Caldwell,2,4 and Marc Zimmerman4

1Department of Psychiatry, School of Medicine, University of Michigan, Ann Arbor, USA
2Center for Research on Ethnicity Culture and Health, School of Public Health, University of Michigan, Ann Arbor, USA
3Medicine and Health Promotion Institute, Tehran, IR Iran
4Department of Health Behavior and Health Education, School of Public Health, University of Michigan, Ann Arbor, USA

*Corresponding author: Shervin Assari, Department of Psychiatry, School of Medicine, University of Michigan, Ann Arbor, USA. Tel: +1-7342320445, Fax: +1-7344658739, E-mail: assari@umich.edu

Received 2014 February 5; Revised 2015 September 14; Accepted 2015 September 27.

Abstract

Background: Although the link between psychological distress and altered cortisol level has been already shown; very limited information exists about this association among Black youth.

Objectives: We tested sex differences in predictive role of symptoms of anxiety during adolescence on annual decline in morning salivary cortisol levels in early adulthood among Black youth.

Patients and Methods: Data came from wave 1 (year 1994), wave 6 (year 2000), and wave 7 (year 2001) of the Flint adolescent study. In this study 176 Black youth (85 males and 91 females) were followed for 7 years from mean age of 15 at baseline to 22 at the end of follow up. Linear regression was used for data analysis with change in salivary cortisol from 2000 to 2001 as the dependent variable, symptoms of anxiety, at 1994 as independent variable, age, number of employed parents, depressive symptoms and alcohol use at 1994 as controls, and sex as the moderator.

Results: Higher level of anxiety symptoms at 1994 was predictive of a higher decline in morning salivary cortisol from 2000 to 2001 for all youths, while the effects of baseline socio-economics, depressive symptoms, and alcohol use were controlled. Among female participants, anxiety symptoms at 1994 were predictive of a greater decline in morning salivary cortisol level from 2000 to 2001. The association was not found among males.

Conclusions: Our findings suggest sex differences in the predictive role of anxiety symptoms during adolescence on the annual decline in cortisol level during early adulthood. While most research on this topic is among White middle class individuals, our findings shed more light on the longitudinal links between psychological distress and the hypothalamus-pituitary-adrenal (HPA) axis function among Black youth.

Keywords: African Americans, Anxiety, Young Adult, Hydrocortisone/Blood, MH-Hypothalamo-Hypophyseal System, Physiopathology, Male, Female

1. Background

The hypothalamus-pituitary-adrenal (HPA) axis is responsible for maintenance of hemostasis. Following exposure to stress, secretion of corticotropin-releasing hormone (CRH) by hypothalamus stimulates secretion of adrenocorticotrophic hormone (ACTH) by the pituitary gland. Subsequently, release of ACTH results in the release of cortisol from the adrenal glands (1). Cortisol is involved in response to stress (2) and several other important physiological processes such as growth, reproduction (3-5), and energy balance (6).

Altered HPA function seems to be one of the intermediate mechanisms by which psychosocial disadvantage such as long term exposure to high levels of chronic stress increase risk of adverse health outcomes (7-9). In this view, long term exposure to stress over the life course may result in non-reversible modifications to the HPA system with the end product of cortisol, that although is designed to save the individual in response to acute stress, its chronic activation will be damaging for the organism (7, 10-12). This is supported by a large body of research evidence from studies on humans, primates, and rodents that suggest that exposure to stress during critical developmental periods have permanent effects on HPA regulation, with deleterious effects on health (13).

The psychological distress-HPA dysfunction link has been suggested by a considerable amount of research (3, 14-16) yet, most of the available knowledge on this link originates from cross sectional studies or longitu-
3. Patients and Methods

3.1. Design and Setting

Data came from the Flint adolescent study (FAS), a longitudinal study conducted from 1994 to 2012 in Flint, Michigan. The study protocol was approved by the University of Michigan Institutional Review Board and all participants signed consent or assent forms before each interview (24, 32).

3.2. Original Study

The Flint adolescent study (FAS), is a longitudinal study with 12 waves of data collection. The FAS followed 850 Black/African American and White adolescents from their 9th grade to their transition into young adulthood. Data for the current study came from wave 1 (1994), wave 6 (2000), and wave 7 (2001) of the study. Retention rates were 90% from waves 1 to 4 and 75% from waves 4 to 8 (33-35).

3.3. Participants and Sampling

Participants were sampled from four local public high schools. The study enrolled students in the fall semester of ninth grade if they had a grade point average (GPA) of 3.0 or lower in 8th grade and they did not have a diagnosis of developmental disability or emotional impairment. The current analysis only included Black/African Americans who provided saliva samples in waves 6 and 7, and had stayed in the study until wave 9. Youth who consented to saliva sampling were not different from the overall wave 6 sample. The decision of not to include Whites was made because of very few Whites individuals included in waves 6 and 7.

3.4. Procedure

Data were collected during structured face-to-face interviews conducted either at school or at alternative community locations. A self-administered questionnaire assessed more sensitive information and was distributed at the conclusion of each interview to facilitate confidentiality. This study followed students, irrespective of drop out from schools. On average, each interview lasted 50 - 60 minutes.

3.5. Measures

Salivary cortisol level: Saliva samples were collected from a subset of samples (N = 201). This number was a proportion of all participants who were present in wave 6 (N = 573), provided consent to the procedure, and were eligible for the saliva collection. Eligibility for saliva collection included not being pregnant, and not having eaten, drank or used tobacco in the hour prior to the collection. Participants provided saliva samples at the beginning of the interview, which happened before 11.00 A.M. in all cases. Samples were placed on ice prior to transportation and assay. Cortisol was specifically assessed by high sensitivity salivary cortisol enzyme immunoassay by Salimetrics, Incorporated. The saliva samples were thawed and centrifuged at 1,500 rpm for 15 minutes before assay. The assay follows standard enzyme immunoassay procedures as previously described (24, 33). The intra- and inter-assay coefficient of variations ranged from 3.88 to 7.12 % and 6.69 to 6.88 %, respectively. The lower limit of sensitivity of this assay is 0.007 μg/dL (25).

Symptoms of anxiety (independent variable): Symptoms of anxiety were measured by the brief symptom inventory (26). Six items assess the frequency of feeling uncomfortable due to symptoms of anxiety during the past week. Response options are on a Likert scale that ranged from 1 (not at all uncomfortable) to 5 (extremely uncomfortable). Items were averaged to form a scale. This scale has been shown to have high internal consistency and test-retest reliability (27, 28). The Cronbach’s alpha is 78 for the Black youth at wave 1 in the present study (Appendices).

Covariates: Age, family socioeconomic status (parental
Symptoms of depression: Depressive symptoms were measured by six items from the brief symptom inventory (26). These items assess the frequency of feeling uncomfortable during the past seven days due to symptoms of depression such as feeling hopeless about the future, and having no interest in things. Response options on the Likert scale ranged from 1 (not at all uncomfortable) to 5 (extremely uncomfortable). These six items were averaged to form the final scale. Researchers have found that this scale has high internal consistency and test-retest reliability and is valid to use with adolescents (27, 28). Cronbach’s alpha was 79 for the current sample at wave 1 (Appendices).

Alcohol use: Participants were asked to report if they had ever used alcohol in their lifetime. If alcohol was reported, frequency of alcohol use in the past 30 days (0 = never, 1 = once a month or less, 2 = 2 - 3 times a month, 3 = about once a week, 4 = 2 - 6 times a week, 5 = about once a day, 6 = more than once a day) was then assessed. Participants who had never used alcohol were coded as never in the past 30 days. Because of relatively low rates of marijuana use (skewed), the variable was recoded into four categories for analysis (0 = never, 1 = 3 or fewer times a month, 2 = 1 - 6 times a week, 3 = daily use) (Appendices).

3.6. Data Analysis

We used SPSS 20.0 (PASW, Chicago, Il) for data analysis. Means and standard deviations (SD) were reported for baseline age, anxiety symptoms, depressive symptoms, alcohol use, and cortisol levels at waves 6 and 7. Pearson’s correlation test was used to investigate unadjusted bivariate associations between the study variables. We used paired t-test to compare mean cortisol levels at 2000 and 2001. For multivariable analysis, first we ran a logistic regression to the pooled sample. Then we ran logistic regression models specific to each sex. Level of anxiety symptoms among males and age, employed parent, depressive symptoms, and alcohol use at baseline among males or females (Table 3).

As Table 2 shows, in the pooled sample, age was correlated with depressive symptoms and alcohol use, but not anxiety symptoms. Cortisol at 2001 was lower among females; however, cortisol and sex were not associated at 2000. Cortisol at 2000 was also not correlated with age, symptoms of anxiety, symptoms of depression, or alcohol use at 1994. Symptoms of anxiety, depression and alcohol use were all positively correlated at baseline (Table 2).

As Table 3 shows, age was positively correlated with anxiety and depressive symptoms among males but not females. Age was positively correlated with alcohol use among males but not females. Number of parents working was negatively associated with depressive symptoms among males, and was positively associated with anxiety and depressive symptoms among females. Cortisol was not correlated with age, anxiety, depression, or alcohol use at baseline among males or females (Table 3).

Results of the regressions are presented in Table 4. As this table shows, sex and anxiety were the only variables that were correlated with change in cortisol over time in the pooled sample. Age, employed parent, depressive symptoms, and alcohol use were not correlated with change in cortisol over time in the pool sample. Among males, alcohol use, and among females, symptoms of anxiety were associated with change in cortisol over time. Age, symptoms of anxiety, employed parent, and depressive symptoms among males and age, employed parent, depressive symptoms, and alcohol use among females were not associated with change of cortisol over time (Table 4).

| Variables                  | All           | Males         | Females        |
|----------------------------|---------------|---------------|----------------|
| Age, y                     | 13.87 - 16.88 | 13.87 - 16.85 | 13.89 - 16.88  |
| Number of working parents  | 0.00 - 2.00   | 1.42 ± 0.69   | 1.46 ± 0.66    | 0.00 - 2.00   | 1.38 ± 0.71 |
| Anxiety symptoms           | 1.00 - 4.67   | 1.6 ± 0.64    | 1.00 - 4.00    | 1.47 ± 0.52   | 1.00 - 4.67  | 1.71 ± 0.71 |
| Depressive Symptoms        | 1.00 - 4.83   | 1.66 ± 0.71   | 1.00 - 3.67    | 1.48 ± 0.53   | 1.00 - 4.83  | 1.82 ± 0.81 |
| Alcohol Use                | 1.00 - 21.00  | 4.9 ± 4.65    | 1.00 - 21.00   | 4.6 ± 4.62    | 1.00 - 21.00 | 5.18 ± 4.66 |
| Morning cortisol level (pg/mL), at year 2000 | 0.001 - 1.15 | 0.24 ± 0.39   | 0.01 - 1.15    | 0.26 ± 0.18   | 0.03 - 1.03  | 0.23 ± 0.19 |
| Morning cortisol level (pg/mL), at year 2001 | 0.01 - 1.31  | 0.22 ± 0.23   | 0.02 - 1.31    | 0.27 ± 0.26   | 0.01 - 1.14  | 0.17 ± 0.18 |

Statistics obtained in the year 1994.
Table 2. Correlation Between Socio-Economics, Mental Health, and Cortisol Among Blacks (Pooled Sample)

|       | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|
| 1-Age | 1   | -0.120<sup>a</sup> | -0.140<sup>a</sup> | -0.005 | 0.015 | 0.038 | 0.086<sup>b</sup> | 0.106<sup>a</sup> |
| 2-Sex (female) | 1   | -0.06 | -0.061 | -0.222<sup>a</sup> | 0.193<sup>a</sup> | 0.240<sup>a</sup> | 0.062 |    |
| 3-Number of working Parents | 1   | 0.055 | -0.004 | -0.02 | -0.036 | 0.017 |    |    |
| 4-Cortisol pre, pg/mL | 1   | 0.112 | -0.049 | -0.068 |    |    |    |    |
| 5-Cortisol post, pg/mL | 1   | -0.153<sup>a</sup> | -0.145<sup>a</sup> | 0.01 |    |    |    |    |
| 6-Anxiety symptoms 1 | 1   | 0.731<sup>a</sup> | 0.213<sup>a</sup> |    |    |    |    |    |
| 7-Depressive symptoms 1 | 1   | 0.254<sup>a</sup> |    |    |    |    |    |    |
| 8-Alcohol use 1 | 1   |    |    |    |    |    |    |    |

<sup>a</sup>p < 0.01.  
<sup>b</sup>p < 0.05.

Table 3. Correlation Between Socio-Economics, Mental Health, and Cortisol Among Blacks Based on Sex<sup>d</sup>

|       | 1   | 2   | 3   | 4   | 5   | 6   | 7   |
|-------|-----|-----|-----|-----|-----|-----|-----|
| 1-Age | 1   | -0.132<sup>b</sup> | 0.009 | -0.013 | -0.045 | 0.073 | 0.129<sup>b</sup> |
| 2-Number of working parents | 1   | -0.162<sup>c</sup> | 0.074 | -0.013 | -0.109 | -0.176<sup>c</sup> | -0.044 |
| 3-Cortisol pre (pg/mL), at year 2000 | -0.029 | 0.034 | 0.057 | -0.068 | 0.002 | -0.025 |    |
| 4-Cortisol post (pg/mL), at year 2001 | -0.019 | 0.021 | 0.156 | 1   | -0.106 | -0.085 | 0.107 |
| 5-Anxiety symptoms 1 | 0.146<sup>c</sup> | 0.060 | -0.024 | -0.140 | 1   | 0.626<sup>c</sup> | 0.150<sup>c</sup> |
| 6-Depressive symptoms 1 | 0.157<sup>c</sup> | 0.070 | -0.093 | -0.127 | 0.763<sup>c</sup> | 1   | 0.398<sup>c</sup> |
| 7-Alcohol use at 1994 | 0.100 | 0.078 | -0.017 | -0.082 | 0.250<sup>c</sup> | 0.285<sup>c</sup> | 1   |

<sup>a</sup>males upper diagonal, females lower diagonal.  
<sup>b</sup>p < 0.05.  
<sup>c</sup>p < 0.01.

Table 4 Summary of Regressions with Cortisol at Year 2001 as Outcome Among Blacks Based on Sex<sup>d</sup>

|                        | All                           | Males                          | Females                          |
|------------------------|-------------------------------|--------------------------------|----------------------------------|
|                        | B (SE) | 95% CI for B | P | B (SE) | 95% CI for B | P | B (SE) | 95% CI for B | P |
| Sex (female)           | -0.206 (0.036)               | -0.162 - 0.02 | 0.012 | -          | -          | -          | -          | -          | -          |
| Age                    | 0.005 (0.026)               | -0.051 - 0.054 | 0.951 | -0.061 (0.043) | -0.107 - 0.063 | 0.602 | 0.036 (0.031) | -0.052 - 0.071 | 0.759 |
| Employed parent at year 1994 | 0.033 (0.026)               | -0.040 - 0.062 | 0.682 | 0.020 (0.043) | -0.078 - 0.093 | 0.863 | 0.005 (0.030) | -0.058 - 0.061 | 0.969 |
| Depressive symptoms at year 1994 | 0.036 (0.035)               | -0.058 - 0.08 | 0.758 | -0.016 (0.062) | -0.130 - 0.017 | 0.915 | 0.157 (0.037) | -0.042 - 0.107 | 0.388 |
| Alcohol use at year 1994 | 0.101 (0.004)               | -0.003 - 0.04 | 0.212 | 0.289 (0.007) | 0.003 - 0.032 | 0.019 | -0.130 (0.005) | -0.035 - 0.004 | 0.269 |
| Anxiety symptoms at year 1994 | -0.243 (0.044)              | -0.179 - 0.004 | 0.04 | -0.238 (0.087) | -0.310 - 0.038 | 0.124 | -0.350 (0.045) | -0.176 - 0.000 | 0.050 |
| Cortisol, pg/mL at year 2000 | 0.085 (0.091)               | -0.081 - 0.277 | 0.282 | 0.076 (0.170) | -0.228 - 0.449 | 0.517 | 0.144 (0.093) | -0.069 - 0.304 | 0.215 |

<sup>d</sup>Abbreviations: B: standardized regression coefficient, CI: confidence interval, SE: standard error.
5. Discussion

High level of anxiety symptoms among female Black adolescents at 2000 was predictive of a higher annual decline in cortisol level 7 years later when they are early adults. Among males, the baseline alcohol use but not level of anxiety symptoms was predictive of later annual decline in cortisol level.

In line with our findings, several (1, 2, 19, 29) if not all (30) studies have shown an association between level of anxiety and cortisol levels in individuals with chronic conditions, healthy adults, and psychiatric disorders. In a study among patients with a chronic medical condition, the correlation coefficient between anxiety symptoms and salivary cortisol was 0.980 (P Value < 0.001) suggesting an extremely strong positive correlation between the two variables (31). In support of our findings, sex differences in the HPA function in response to acute and chronic stress have also been reported previously (36-43).

In line with our study, drinking alcohol has also shown to be linked to the function of HPA axis and cortisol level (44). Animal studies (45, 46) and epidemiological studies (17, 47-50) have documented an effect of alcohol use on HPA axis activity. Epidemiological studies among apparently healthy population have found elevated cortisol levels in heavy drinkers (51, 52) and alcohol-dependent individuals show altered HPA axis function while in withdrawal (44). The slope of cortisol decline over the day may be reduced in heavy drinkers (44). Some research also supports our finding on sex differences in the drinking-cortisol level link (44). For instance, Badrick and colleagues found a positive association between units of alcohol intake per week and cortisol among men but not women. In women the cortisol awakening response was greater in heavy drinkers compared with moderate drinkers (44).

This study makes a unique contribution to the literature by extending the current knowledge on the longitudinal link between anxiety and HPA axis among Black youth who experience poverty, blocked opportunities, high risk environment, and unemployment. Most previous research has enrolled a sample which is mostly White middle class (53).

Our findings suggest that for Blacks who live in disadvantaged urban areas, high anxiety symptoms during adolescence is predictive of later annual decline in cortisol level over early adulthood, especially among females. Findings can be explained by the concept of ‘allostatic over-load’ which suggests that long term exposure to chronic, excessive stress causes in poor regulated stress response that predicts a wide range of poor health outcomes (10-12).

Our findings on the link between anxiety symptoms and cortisol level can be better understood with the more controlled studies such as one by Abelson and colleagues (2) who documented elevated overnight cortisol levels among patients with panic disorder. Authors studied patients with panic disorder for a) activity of HPA axis at rest over a full circadian cycle, b) activation of HPA axis by a panicogenic respiratory stimulant that does not directly stimulate the HPA axis, and c) activation of HPA axis after a panicogenic that does directly stimulate the HPA axis and showed exaggerated paradigm-related ACTH secretion in patients with panic disorder. Authors concluded that the HPA axis dysregulation associated with panic disorder may be due to hypersensitivity to contextual cues (2).

Our findings are also consistent with a study that examined adrenocortical activity among normally developing and at-risk youth with internalizing symptoms (54). The researchers found a link between internalizing problems and gradual or steep declines in basal cortisol production. Internalizing symptoms were associated with immediate and delayed cortisol reactivity to social performance stressors. Authors found no evidence of an association between externalizing problems and cortisol level, however. In that study, the symptoms ranged from subclinical to clinical levels of psychopathology and salivary cortisol levels were measured to assess basal, diurnal variation, and responses to social stressors (54).

Our findings on sex differences are also supported by previous research. Kapen et al. (1) explored sex differences in the link between the level of anxiety with basal HPA axis activity in youth with anxiety disorders. Among 8-16 year olds, they found that in girls but not boys, high level of anxiety was associated with a weaker rise in early morning cortisol concentrations. In their study, however, among both males and females, separation anxiety was associated with basal cortisol levels. In that study, separation anxiety and physical anxiety symptoms predicted cortisol concentrations at noon (1).

Current study focused on anxiety symptoms and did not measure anxiety disorders or the sub-type of anxiety disorder. Subtype of anxiety disorder modifies the effect of anxiety on HPA function (1). For instance, change in HPA axis is shown among patients with panic disorder (1). Researchers have also reported results that do not support our findings. Ockenfels et al. (55) did not find an association between chronic stress and overall cortisol excretion or cortisol reactivity. In their study, however, self-reported chronic stress was linked to the diurnal pattern of cortisol excretion. Higher morning and lower evening levels of cortisol were seen among individuals who reported high level of chronic stress (55). The sample for that study, however, was mostly composed of Whites and the study was cross-sectional in design.

Our findings have implications for understanding and elimination of health disparities among Black youth. Blacks including Black youth are exposed to disproportionately higher levels of chronic stressors and report higher levels of psychological distress, even if they less
frequently meet criteria for anxiety and other anxiety disorders (56, 57). Among Blacks, disturbance of the HPA axis may be at least partially responsible for higher levels of hypertension and cardiovascular diseases (58), obesity (59), metabolic disorders (60), diabetes (61) and even cardiovascular risk (62). The results are also important because they link to important concepts of anxiety (63-68) and cortisol (69) in the general population among a minority group. Sex differences found in this study are another emphasis on specificity of risk rather than its universality (70).

Our study has a few limitations. The study did not enroll a national or a representative sample, as it enrolled samples from a single city in Midwest. Measurement of cortisol was also limited in the current study. We used annual decline in average morning concentration of cortisol, however, the HPA function can be better measures using the stress response test and area under the curve of cortisol and ACTH secretion (71). In addition, the sample size was not very large. Finally, the current study measured anxiety symptoms, not anxiety disorder. This is very important as type of anxiety disorder influences the HPA axis function. For instance, some evidence has shown hyper-suppression in PTSD, which is very different from panic disorder or physical and general anxiety disorder (2, 19, 22).

To conclude, our findings suggest that among female Black youth, anxiety symptoms during adolescence predict subsequent annual change in morning cortisol level during early adulthood. The findings help us better understand the longitudinal connection between of symptoms of anxiety during adolescence and rate of the cortisol decline in early adulthood. Our findings suggest that among female but not male Black youth, level of anxiety symptoms during adolescence may have some implications for prediction of trajectory of the HPA axis function several years later. Future research is needed to better understand the complex links between race, sex, chronic stress, psychological symptoms, psychopathology, alteration of HPA function, and health disparities.

Footnotes
Authors’ Contributions: The original idea of this analysis was developed by Shervin Assari, who also analyzed the data and drafted the manuscript. The design and conduction of the original study and supervision of data collection was done by Marc Zimmerman. Marc Zimmerman and Cleopatra Caldwell have also contributed to the content of this manuscript. All authors have read and approved final draft of the manuscript.

Funding/Support: This study was funded by the National Institute on Drug Abuse (NIDA) (grant DA07484). The content of this article does not necessarily reflect the views or policies of the National Institute on Drug Abuse. Shervin Assari is supported by the Heinz C. Prechter Bipolar Research Fund and the Richard Tam Foundation at the University of Michigan Depression Center.

Appendices
Please visit article’s online version for appendices.

References
1. Kallen VL, Tulen JH, Utens EM, Trefers PD, De Jong FH, Ferdinand RF. Associations between HPA axis functioning and level of anxiety in children and adolescents with an anxiety disorder. Depress Anxiety. 2008;25(1):31-41. doi: 10.1002/da.20287. [PubMed: 17340603]
2. Abelson JL, Khan S, Liberson I, Young EA. HPA axis activity in patients with panic disorder; review and synthesis of four studies. Depress Anxiety. 2007;24(1):56-76. doi: 10.1002/da.20220. [PubMed: 17645643]
3. Chrousos GP. Stressors, stress, and neuroendocrine integration of the adaptive response. The 1997 Hans Selye Memorial Lecture. Ann NY Acad Sci. 1998;851:311-35. [PubMed: 9668623]
4. de Kloet ER. Hormones, brain and stress. Endor Regul. 2003;37(2):51-68. [PubMed: 12932981]
5. Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endor Rev. 2000;22(1):55-89. doi: 10.1201/edrv.21.1.0389. [PubMed: 10969570]
6. Brillon D, Zheng R, Campbell RG, Matthews DE. Effect of cortisol on energy expenditure and amino acid metabolism in humans. Am J Physiol. 1995;268(3 Pt 1):E501-13. [PubMed: 7900796]
7. McEwen BS, Seeman T. Protective and damaging effects of mediators of stress. Elaborating and testing the concepts of allostatic and allostatic load. Ann N Y Acad Sci. 1999;896:30-47. [PubMed: 10688866]
8. McEwen BS. Brain on stress: how the social environment gets under the skin. Proc Natl Acad Sci U S A. 2012;109 Suppl 2:17810-5. doi: 10.1073/pnas.1212544097. [PubMed: 23045648]
9. Javanbakht A, King AP, Evans GW, Swain JE, Angstadt M, Phan KL, et al. Childhood Poverty Predicts Adult Amygdala and Frontal Activity and Connectivity in Response to Emotional Faces. Front Behav Neurosci. 2015;9:354. doi: 10.3389/fnbeh.2015.00354. [PubMed: 26124712]
10. McEwen BS. Stress, adaptation, and disease. Allostasis and allostatic load. Ann N Y Acad Sci. 1995;780:3-34. [PubMed: 7900796]
11. Seeman TE, Singer BH, Rowe JW, Horwitz RJ, McEwen BS. Price of adaptation—allostatic load and its health consequenc es. MacArthur studies of successful aging. Arch Intern Med. 1997;157(9):2259-68. [PubMed: 9340903]
12. McEwen BS. Allostasis and allostatic load: implications for neuropsychopharmacology. Neuropsychopharmacology. 2000;22(2):108-24. doi: 10.1016/s0893-933x(99)00293-1. [PubMed: 10649824]
13. Flinn MV. Are cortisol profiles a stable trait during child development? Am J Hum Biol. 2009;21(6):769-71. [PubMed: 19902194]
14. Levine S. Influence of psychological variables on the activity of the hypothalamic-pituitary-adrenal axis. Eur J Pharmacol. 2000;405(1-3):139-60. [PubMed: 10331222]
15. Kirschbaum C, Hellhammer DH. Salivary cortisol in psychoneuroendocrine research: recent developments and applications. Psychoneuroendocrinology. 1994;19(4):313-31. [PubMed: 8047637]
16. Pruessner M, Hellhammer DH, Pruessner JC, Lupien SJ. Self-reported depressive symptoms and stress levels in healthy young men: associations with the cortisol response to awakening. Psychosom Med. 2003;65(2):192-9. [PubMed: 12554820]
17. Nemeroff CB, Vale WW. The neurobiology of depression: inroads and allostatic load. The 1997 Hans Selye Memorial Lecture. Ann N Y Acad Sci. 1998;851:311-35. [PubMed: 9668623]
18. Young EA, Lopez JF, Murphy-Weinberg V, Watson SJ, Akil H. Mineralocorticoid receptor function in major depression. Arch Gen Psychiatry. 2003;60(1):24-8. [PubMed: 1250169]
19. Vreeburg SA, Zitman FG, van Pelt J, Derijk RH, Verhagen JC, van Dyck R, et al. Salivary cortisol levels in persons with and without different anxiety disorders. Psychosom Med. 2007;69(4):340-7. doi:10.1097/PST.0b013e3181d1f08c. [PubMed: 17090282]
20. Roy-Byrne PP, Geraci M, Udde TH. Life events and the onset of panic disorder. Am J Psychiatry. 1986;143(1):1244-27. [PubMed: 3772123]

21. de Kloet ER, Joels M, Holsboer F. Stress and the brain: from adaptation to disease. Nat Rev Neurosci. 2005;6(6):463-75. doi: 10.1038/nrn1683. [PubMed: 1599777]

22. Yehuda R, Southwick SM, Clarkson JK, Bremner D, Charney DS, Monson JW. Enhanced suppression of cortisol following dexamethasone administration in posttraumatic stress disorder. Am J Psychiatry. 1999;156(10):1283-9. [PubMed: 9847586]

23. Graeff FG. Anxiety, panic and the hypothalamic-pituitary-adrenal axis. Rev Bras Psiquiatr. 2007;29 Suppl 1:153-6. [PubMed: 17546154]

24. Assari S, Caldwell CH, Zimmerman MA. Sex differences in the association between testosterone and violent behaviors. Trauma Mon. 2014;19(4):181-4. doi: 10.5812/traumamon.19400. [PubMed: 25377519]

25. Schmeelk-Cone KL, Zimmerman MA, Abelson JL. The buffering effects of active coping on the relationship between SES and cortisol among African American young adults. Behav Med. 2003;29(2):85-94. doi: 10.1089/brm.2003.29.2.85. [PubMed: 15478070]

26. Derogatis LR, Melisaratos N. The Brief Symptom Inventory: an introductory report. Psychol Med. 1983;13(1):559-65. [PubMed: 6622662]

27. Derogatis LR, Lipman RS, Covi L. SCL-90: an outpatient psychiatri- c rating scale–preliminary report. Psychopharmacol Bull. 1975(9):25-28. [PubMed: 4663398]

28. Connolly MB, Stimpson P, Shelton RC, Hollow S, Kurtz J, Barber JP, et al. The reliability and validity of a measure of self-understanding of interpersonal patterns. J Counseling Psycho. 1999;46(4):4172.

29. Zimmerman MA, Meduri V, Paramuksem G, Pachkara KR. Relationship of salivary cortisol and anxiety in recurrent aphthous stomatitis. Indian J Endocrinol Metab. 2015;29(1):156-9. doi: 10.4103/2230-8210.137166. [PubMed: 25593827]

30. Harris A, Endresen Reme S, Tangen T, Hansen AM, Helene Garde A, Eikrehn KI. Diurnal cortisol rhythm: Associated with anxiety and depression, or just an indication of lack of energy? Psychiatry Res. 2005;128(2):269-15. doi: 10.1016/j.psychres.2004.06.006. [PubMed: 16090959]

31. Nanadka LK, Meduri V, Paramuksem G, Pachkara KR. Evaluation of salivary cortisol and anxiety levels in myofascial pain dysfunction syndrome. Korean J Pain. 2015;28(3):320-3. doi: 10.3344/kjp.2015.28.3.320. [PubMed: 26402924]

32. Giannakoulopoulou A, Ramirez-Valles J, Zapert KM, Maton KI. Longitudinal study of stress-buffering effects for urban African-American male adolescent problem behaviors and mental health. J Community Psychol. 2000;28:37-31. doi: 10.1002/(SICI)1097-0044(200001)28:1<37::AID-JCP4>3.0.CO;2-9.

33. Zimmerman MA, Steinman KJ, Rowe KJ. Violence among urban African American adolescents: the protective effects of parental support. In: Arriaga XB, Oskamp S, editors. Addressing community problems: Psychosocial research and interventions. Thousand Oaks, CA: Sage Publications; 1998. pp. 78-103.

34. Kudelka BM, Kirschbaum C, Schirrmacher C. Sex differences in HPA axis responses to stress: a review. Biol Psychol. 2005;69(1):113-2. doi: 10.1016/j.biopsycho.2004.04.009. [PubMed: 15740829]

35. Badrick E, Bobak M, Britton A, Kirschbaum C, Marmot M, Kumari M. The relationship between alcohol consumption and cortisol secretion in an aging cohort. J Clin Endocrinol Metab. 2008;93(9):3770-7. doi: 10.1210/jc.2007-0707. [PubMed: 18073166]

36. Rasmussen DD, Boldt BM, Bryant CA, Mitton DR, Larsen SA, Wilkinson CW. Chronic daily ethanol and withdrawal: I. Long-term changes in the hypothalamo-pituitary-adrenal axis. Alcohol Clin Exp Res. 2000;24(12):2386-49. doi: 10.1002/ace.10443.

37. Ongmann K, Lee S, Weiss B, Rivier C. Mechanisms mediating the influence of alcohol on the hypothalamic-pituitary-adrenal axis responses to immune and nonimmune signals. Alcohol Clin Exp Res. 1998;22(5 Suppl):243S-75. doi: 10.1111/j.1530-0277.1998.tb04078.x. [PubMed: 9727644]

38. Junghanns K, Backhaus J, Tietz U, Lange W, Bernsen J, Wetterling T, et al. Impaired serum cortisol stress response is a predictor of early relapse. Alcohol Alcohol. 2003;38(3):289-93. [PubMed: 12643269]

39. Costa A, Bono G, Martignoni E, Merlo P, Sances G, Nappi G. An assessment of hypothalamic-pituitary-adrenal axis functioning in non-depressed, early abstinent alcoholics. Psychoneuroendocrinology. 1996;21(3):263-75. [PubMed: 8877225]

40. Bernardy NC, King AC, Parsons OA, Lovullo WR. Altered cortisol response in sober alcoholics: an examination of contributing factors. Alcohol. 1996;13(5):493-9. [PubMed: 888947]

41. Groote Veldman R, Meinders AE. On the mechanism of alcohol-induced pseudo-Cushing's syndrome. Endoc Rev. 1996;17(3):262-8. doi: 10.1210/jc.2007-0707. [PubMed: 12711310]. [PubMed: 24487856].

42. Blascovich J, Tomaka J, Lowney JD, Alpern ML, Cacioppo JT. The experience of social rejection versus achievement stress. Psychol Sci. 2001;12(3):175-82. doi: 10.1111/1467-9280.00309. [PubMed: 11485934].

43. Kudelka BM, Schirrmacher C, Sex differences in HPA axis responses to stress: overall cortisol levels, diurnal rhythm, and heart rate variability in apparently healthy men: Evidence for impaired inhibitory control of the HPA axis in heavy drinkers. Int J Psychopharmacol. 2006;59(3):244-50. doi: 10.1016/j.ijpsycho.2005.10.011. [PubMed: 16325235].

44. Gionoulakis C, DaI X, Brown T. Effect of chronic alcohol consumption on the activity of the hypothalamic-pituitary-adrenal axis and pituitary beta-endorphin as a function of alcohol intake, age, and gender. Alcohol Clin Exp Res. 2003;27(3):420-7. doi: 10.1111/j.1530-0277.2003.tb15373.x. [PubMed: 12878586].

45. Yehuda R. Current status of cortisol findings in post-traumatic stress disorder. Psychiatr Clin North Am. 2002;25(3):341-68. [PubMed: 12136504].

46. Klimes-Dougan B, Hastings PD, Granger DA, Usher BA, Zahn- Waxler C. Adrenocortical activity in at-risk and normally developing adolescents: HPA responses to laboratory psychosocial stress in healthy elderly adults, younger adults, and children: impact of age and gender. Psychoneuroendocrinology. 2004;29(1):83-98. [PubMed: 14575731].
and acute stress reactivity. *Psychosom Med.* 1995;57(2):460–7. [PubMed: 8532737]

56. McLaughlin KA, Hilt LM, Nolen-Hoeksema S. Racial/ethnic differences in internalizing and externalizing symptoms in adolescents. *J Abnorm Child Psychol.* 2007;35(5):389–96. doi: 10.1007/s10802-007-9128-x. [PubMed: 17508278]

57. Anderson ER, Mayer LC. Race/ethnicity and internalizing disorders in youth: a review. *Clin Psychol Rev.* 2010;30(3):338–48. doi: 10.1016/j.cpr.2009.12.008. [PubMed: 2071063]

58. Jackson JS, Knight KM, Rafferty JA. Race and unhealthy behaviors: chronic stress, the HPA axis, and physical and mental health disparities over the life course. *Am J Public Health.* 2010;100(5):933–9. doi: 10.2105/AJPH.2008.143446. [PubMed: 19846689]

59. Black PH. The inflammatory consequences of psychologic stress: relationship to insulin resistance, obesity, atherosclerosis and diabetes mellitus, type II. *Med Hypotheses.* 2006;67(4):879–81. doi: 10.1016/j.mehy.2006.04.008. [PubMed: 16781084]

60. Hjemdahl P. Stress and the metabolic syndrome: an interesting but enigmatic association. *Circulation.* 2002;106(2):2634–6. [PubMed: 12438283]

61. Rosmond R. Stress induced disturbances of the HPA axis: a pathway to type 2 diabetes? *Med Sci Monit.* 2003;9(2):RA235–9. [PubMed: 12601046]

62. Walker BR. Glucocorticoids and cardiovascular disease. *Eur J Endocrinol.* 2007;157(5):545–59. doi: 10.1530/EJE-07-0455. [PubMed: 17984234]

63. Fathi M, Alavi SM, Joudi M, Joudi M, Mahdi Khani H, Farashkar F, et al. Preoperative anxiety in candidates for heart surgery. *Iran J Psychiatry Behav Sci.* 2014;8(2):90–6. [PubMed: 25053963]

64. Ahmadi J, Amiri A, Ghanizadeh A, Khademhosseini A, Khademhosseini Z, Gholami Z, et al. Prevalence of addiction to the Internet, computer games, DVD, and video and its relationship to anxiety and depression in a sample of Iranian high school students. *Iran J Psychiatry Behav Sci.* 2014;8(2):75–80. [PubMed: 25053960]

65. Mottaghi M, Atarodi A, Rohani Z. The relationship between coaches’ and athletes’ competitive anxiety and their performance. *Iran J Psychiatry Behav Sci.* 2013;7(2):68–76. [PubMed: 24644452]

66. Farzaneh N, Gobakhli Y, Moghimi-Dehkordi B, Naderi N, Fadai F. Anxiety and depression in a sample of Iranian patients with irritable bowel syndrome. *Iran J Psychiatry Behav Sci.* 2013;7(1):30–6. [PubMed: 24644447]

67. Assari S. Additive effects of anxiety and depression on body mass index among blacks: Role of ethnicity and gender. *Int Cardiovasc Res J.* 2014;8(2):44–51. [PubMed: 24936480]

68. Assari S, Lankarani MM, Lankarani RM. Ethnicity modifies the additive effects of anxiety and drug use disorders on suicidal ideation among black adults in the United States. *Int J Prev Med.* 2013;4(1):1251–7. [PubMed: 24404358]

69. van der Kaay D, van den Akker E. Ultralow-dose dexamethasone to preserve endogenous cortisol stress response in nonclassical congenital adrenal hyperplasia: A new promising treatment. *Int J Endocrinol Metab.* 2014;12(3):e24657. doi: 10.5812/ijem.24657. [PubMed: 25237318]

70. Assari S. Separate and combined effects of anxiety, depression and problem drinking on subjective health among black, Hispanic and non-Hispanic white men. *Int J Prev Med.* 2014;5(3):269–79. [PubMed: 24829760]

71. Modabbernia A, Pousschi H, Malekzadeh R. Neuropsychiatric and psychosocial issues of patients with hepatitis C infection: a selective literature review. *Hepat Mon.* 2013;13(1):e8340. doi: 10.5812/hepatmon.8340. [PubMed: 23550100]