The relationship between serum cortisol, adrenaline, blood glucose and lipid profile of undergraduate students under examination stress.

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Abstract.

Background: Stress is an extremely adaptive phenomenon in human beings and cortisol is a known stress hormone. Examination has been described as a naturalistic stressor capable of affecting human health.

Objectives: To estimate the relationship between serum cortisol, adrenaline, fasting blood glucose (FBG) and lipid profile during examination stress.

Methods: Two hundred and eight (208) apparently-healthy undergraduate students (aged, 24 ± 6 years) were involved in the study. Exactly 5 mls of venous blood was collected from each subject 1-3 hours before a major examination. A second assessment was done on the same students 3-4 weeks before any examination (control samples). Cortisol and adrenaline were assayed using ELISA techniques, FBG was assayed using enzymatic method while lipid parameters were assayed using standard enzymatic- spectrophotometric methods.

Results: There was statistically significant increase in serum cortisol, adrenaline, Total cholesterol, HDL-cholesterol and LDL-cholesterol levels in students under examination stress compared to the non examination period ($p=0.001$, 0.013, 0.0001, 0.0001 and 0.0001, respectively). FBG showed no significant increase. There was also significant positive correlation ($r=0.297$, $p=0.032$) between serum cortisol and TC/HDL ratio (cardiac risk factor) before examination stress but not during the stress period.

Conclusions: Significant positive correlation was observed between cortisol and TC/HDL ratio before examination stress.

Key words: academic examination, stressors, cortisol, lipid profile.

DOI: http://dx.doi.org/10.4314/ahs.v15i1.18

Introduction

Physical and psychological stresses can induce a wide range of immunological alterations in the cell mediated and humoral immunity[1,2]. Although the basic neurochemistry of the stress response is now well understood, much remains to be discovered about how the components of this system interact with one another, in the brain, and throughout the body[3]. Both negative and positive stressors can lead to stress. The intensity and duration of stress changes, depending on the circumstances and emotional condition of the person suffering from it and examples of stressors ranges from sensory input such as pain, to life experiences such as poverty. Besides releasing typical stress metabolites, characteristic enzymes and hormones, primary factors of psychological stress situations, possible reactions and recognizable symptomatic organic changes also show multi-factorial appearances[1]. Exposure to psychological stressors can modulate the primary antibody response and increased permanent stress levels can lead to pathological organ changes, psychological alterations as well as psychosomatic diseases[4].

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In humans, a range of stressful events have been associated with lowering the immune system functioning, including examinations, battle task, vigilance, sleep deprivation and divorce[5]. Academic examinations have often been used in stress research because they are predictable, standardized, and discrete examples of real-life stressors. They are associated with changes in the mental and physical health such as increasing anxiety, increasing negative mood and changes in the immune functioning[5].

While a few studies have found significant positive correlations between psychological and hormonal measures of stress, others have found no significant correlations between these measures, or even negative correlations between these measures[6,7]. Since serum cortisol has similar primary structure (cyclopentanoperhydrophenanthrene ring system) as steroids and lipids, and lipids are usually metabolized to release energy, there may be a possibility of examination stress affecting the lipid profile in the body. Any alteration in the plasma lipid which leads to increase in cardiac risk factor may ultimately predispose the student to risk.

Academic examinations have been reported to also have a significant impact on the student's well-being[8]. We tested the hypothesis that examination stress may lead to increase in stress hormones cortisol and adrenaline and an alteration in plasma lipid profile and fasting blood glucose and that there may be a positive relationship between cortisol and the other parameters. The present study is therefore aimed at evaluating the relationship between cortisol, adrenaline, lipid profile and fasting blood glucose (FBG) during academic examination stress in Nigerian undergraduate students. The findings from this study will highlight the possible need for continuous assessment of students which will help reduce risk associated. To the clinicians, the findings will enhance the understanding of the students' plights and possible medical conditions in the management of students.

Material and method

Subjects

The subjects consist of 208 (132 males and 76 females) apparently healthy undergraduate students aged between 18 and 30 years. They were recruited from Medical laboratory science and Medical students from University of Nigeria, Enugu Campus and Enugu state University of Science and Technology respectively, after an informed consent. Approval was given by the ethics committee of the institution and questionnaire containing clear study protocol was administered to the students and only those who completed and returned the questionnaire were enrolled for the study. Subjects who enrolled but could not complete the protocol were dropped from the study.

Students were included in the study if they were:
a) Undergraduate medical or medical laboratory students aged between 18 and 30 years, who are not on contraceptives or steroid therapy.
b) Students living inside the normal students' hostel.
c) Apparently healthy unmarried students not under any visible stress like surgery, illness, non-payment of school fees, abnormal menstruation, trauma etc.
d) Students likely to sit for a major professional examination for the first time within the next one year.
In this study, Major examination for medical/ medical laboratory students is defined as the respective professional examinations, which if not passed by the student, may lead to loss of a session, withdrawal or expulsion from course of study.

Sample Collection & processing

The study protocol involved collecting fasting blood sample from each participant by 9.00am. The samples were collected from the same students on each of the following periods:
a) One-three hours before any major examination (e.g. first or final professional examination), and
b) Three-four weeks before any major examination (which served as control sample).

Subjects were made to relax before sample collection which was done with minimal stasis and pain. Samples consist of 5mls of venous blood collected aseptically from antecubital vein and dispensed into fluoride oxalate tube for blood sugar estimation and plain venoject® tube for other studies. The blood in the plain tube were transported to the lab in ice pack, centrifuged immediately using refrigerated centrifuge and an aliquot of the separated sera stored frozen (-20^{0}C) until analyzed within 48 hours, for cortisol and adrenaline levels. The rest were stored at 4^{0}C for the estimation of lipid profile within 48 hours. Both samples and reagents were brought to room temperature before analysis.

Commercial control serum (QCA SERISCANN ®, Quimica Clinica Applicada. SA Spain) was included in each assay to evaluate the assay method and technique.

Analytical Method:

Blood glucose was analyzed using the enzymetic method of Trinder[9]
The method of Fossati and Prencipe[10] and McGowan et al[11] which involve the enzymatic hydrolysis of triglyceride to glycerol was employed in triglyceride estimation. The enzymatic colorimetric method by Allain et al[12] was employed for cholesterol estimation. HDL- cholesterol was assayed using the method of Burnstein et al,[13].
LDL-Cholesterol and VLDL-Cholesterol were calculated from the results of total cholesterol, HDL-cholesterol and triglycerides as recommended by Friedwald et al [14] and National Cholesterol Education Programme[15]. Serum cortisol was assayed by ELISA technique[13], whereas adrenaline was estimated using quantitative sandwich immunoassay technique of Burtis et al.,[16]. The results of cortisol and epinephrine were read-off directly from the respective standard curves.

Statistical analysis:

Statistical analysis was performed with graph pad prism computer software and data was analyzed using students' t test at 95 percent confidence limit with $P<0.05$ considered as significant. The results were presented as mean and standard deviation (SD). Relationships between cortisol and other parameters were determined using the Rank correlation analysis.

Results

Table 1 shows the demographic profile of the students.

Table 1: Demographic profile of the students studied.

	Before Exam		During Exam	
Age (years)	**Male**	**Female**	**Male**	**Female**
18-21	32 (24.2%)	20 (26.3%)	32 (24.2%)	20 (26.3%)
22-25	58 (44%)	34 (44.7%)	58 (44%)	34 (44.7%)
26-30	42 (31.8%)	22 (29%)	42 (31.8%)	22 (29%)
Level of study				
300 – 400	68(51.5%)	40 (53%)	68 (51.5%)	40 (53%)
500 – 600	64 (48.5%)	36 (47%)	64 (48.5%)	36 (47%)
Religion				
Christians	132 (100%)	76 (100%)	132 (100%)	76 (100%)
Moslems	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Foot note:
300-400 level: Students in 3rd year and 4th year in the Medical School.
500-600 level: Students in 5th year and 6th year in Medical School

Table 2 shows the levels of serum cortisol, adrenaline, FBG and serum lipid profile before and during academic examination stress in the undergraduate students. The results showed significantly increased serum levels of cortisol, adrenaline, total cholesterol, HDL-cholesterol and LDL-cholesterol ($P = 0.001, 0.013, 0.0001, 0.0001$ and 0.0001, respectively) during examination compared to the non-examination period (Table 2).

Table 2: FBG, cortisol, adrenaline and lipid profile levels of the students before and under examination stress.

	Before [n=208]		During [n=208]			
	Mean	**SD**	**Mean**	**SD**	**T-value**	**P-value**
FBG (mmol/l)	3.562	0.689	3.419	0.665	1.200	0.236
Cortisol (ng/ml)	78.808	27.530	94.039	25.477	3.454	0.001
Adrenaline	31.029	13.765	35.550	14.875	2.575	0.013
T C (mmol/l)	3.364	0.762	4.508	0.716	16.288	0.0001
HDL (mmol/l)	1.046	0.275	1.356	0.370	5.410	0.0001
LDL (mmol/l)	1.896	0.381	2.779	0.644	10.066	0.0001
VLDL (mmol/l)	0.381	0.114	0.371	0.119	0.566	0.574
Triglyceride	0.817	0.221	0.804	0.272	0.379	0.706
TC/HDL Ratio	3.454	1.287	3.473	0.723	0.097	0.923

Legend
FBG → Fasting blood glucose
TC → Total cholesterol
HDL → High density lipoprotein
LDL → Low density lipoprotein
VLDL→ Very low density lipoprotein

Table 3 showed the relationship between serum cortisol and adrenaline, FBG, and lipid profile before and under examination stress. The table showed significant positive correlation (r = 0.297, p = 0.032) between serum cortisol and TC/HDL-cholesterol ratio (cardiac risk factor) before examination stress, but not during examination stress. There was no statistical difference in fasting blood glucose before and during examination stress.

Table 3: The relationship between serum cortisol and fbg, adrenaline and lipid profile values before and under examination stress (n=208).

	BEFORE		DURING	
	r-value	p-value	r-value	p-value
FBG(mmol/l)	0.049	0.731	0.252	0.072
Adrenaline(ng/ml)	0.020	0.888	0.229	0.102
TC(mmol/l)	0.088	0.537	0.036	0.798
HDL(mmol/l)	0.233	0.096	-0.094	0.508
LDL(mmol/l)	0.198	0.160	0.104	0.463
VLDL(mmol/l)	0.123	0.384	-0.046	0.746
TG(mmol/l)	0.139	0.327	-0.086	0.542
TC/HDL	0.297	0.032	0.150	0.289

Legend
FBG → Fasting blood glucose
TC → Total cholesterol
HDL → High density lipoprotein
LDL → Low density lipoprotein
VLDL→ Very low density lipoprotein

Discussion

In the present study, we tested the hypothesis that examination stress may lead to increase in stress hormones cortisol and adrenaline and an alteration in plasma lipid profile and fasting blood glucose and that there may be a positive relationship between cortisol and the other parameters.

The results showed significantly increased serum levels of cortisol, adrenaline, total cholesterol, HDL-cholesterol and LDL-cholesterol (P = 0.001, 0.013, 0.0001, 0.0001 and 0.0001, respectively) during examination compared to the non-examination period (Table 2). This implies an increase in the stress hormones and lipids as a result of the ongoing examination. There was no statistical difference in fasting blood glucose before and during examination stress.

We also tested the hypothesis that there may be a positive correlation between cortisol and the other parameters during examination stress. The result showed significant positive correlation (r = 0.297, p = 0.032) between serum cortisol and TC/HDL- cholesterol ratio (cardiac risk factor) before examination stress, but not during examination stress. This shows that the students were exposed to stress and abnormal lipid profile even before the examination period, probably due to the thought of the impending examination which exposes the students to stress before the actual examination.

The relationship obtained may actually not be connected to the examination since it did not manifest during the examination period. No relationship was however observed between cortisol and the other parameters studied both before and during examination stress. This did not support the postulation of unfavourable relationship between high stress cortisol and lipid profile levels reported by Roy et al[17].

The increase in the stress hormones observed may be as a result of stimulation of the ACTH secretion by the stress stimuli which stimulated the synthesis of adrenaline and cortisol precursors[16]. In response to a stressor, neurons with cell bodies in the paraventricular nuclei of the hypothalamus secrete corticotrophin releasing hormone (CRH) and arginine-vasopressin (AVP) into the hypophyseal portal system[18,19]. The CRH through the HPA then activates the pituitary and adrenal glands. These interactions can lead to immune system changes leading to increase in vulnerability to infection and increase in potential for an outbreak of certain diseases such as psoriasis[5]. Over secretion of stress hormones affect the brain where memories are processed and stored[20], and might cause hormonal and metabolic changes that contribute to heart disease and other health problems[21].

Based on our hypothesis, the present study showed that the stress hormones adrenaline and cortisol secretions, TC, LDL and HDL were raised by examination stress. Since over secretion of stress hormones affects the brain where memories are processed and stored,[20] the findings of this study implies that over stressed students may develop memory problems which will affect the outcome of their examination performance. The examinations studies may not actually be a true test of knowledge of these set of stressed students. The excessive response to stress observed in this study may be as a result of fear associated with the examination. This may also be linked to the warning and knowledge of the fate of the past students who did not excel in the previous similar examinations. Some were expelled, some were asked to withdraw or change courses while others were made to loose one semester or one full session with the attendant additional school fees. It may also be noted that some students were never ready for the respective examinations as a result of not being serious with their studies until examination date is announced. Such students study under duress and severe stress, and are always afraid of examinations.

The result of this study supports the reports of Qureshi et al[22] and Viner[23] who reported an increase in visceral fat due to stress, and that of Glavas and Weinberg[20] who reported an over secretion of cortisol as a result of stressors. The lack of significant positive correlation between elevations in cortisol and elevations in psychological stress during the examination period was in agreement with the study by Weekes et al.,[7] who reported no significant correlations in cortisol and elevations in psychological stress measures. However, the causal relationship between increase in stress hormone and serum lipid levels could not be established by the present study although they have same precursor skeletal structure.

Conclusion

The present study showed significant increase in cortisol, adrenaline, TC, HDL and LDL but no correlation between cortisol and the other parameters during examination stress. There was also significant positive correlation between cortisol and TC/HDL ratio (cardiac risk factor) before examination stress. Although serum cortisol has similar primary structure (cyclopentanoperhydrophenanthrene ring system) as steroids and lipids, the abolition of normal physiological control mechanism observed in examination stress did not relate to the serum concentration of these parameters. This may be as a result of physiological compensatory mechanisms. To the teachers, the findings from this study highlights the need for continuous assessment of students which helps reduce risk associated with single exam that may not actually be a true test of knowledge for a high stress-prone student. To the, clinicians, this calls for understanding of the students' plights and possible medical conditions in the management of students. To the students, the findings will help them manage stress better especially since they will no longer be judged during exams alone, as the outcome may actually be affected by stress.

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