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# The Effect of Stressful Conditions on Biochemical and Hematological Parameters Among University Students

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**Abstract**

University students are more susceptible to stress and other mental health problems, which can have a negative impact on their health and academic performance. The main objective of the present study was to explore the influence of exam stress on various biochemical and hematological parameters among university students. The study involved 100 undergraduate students (n=100) aged between 18 and 35. Blood samples were collected from each student three times. The first sample was taken one week before the exam, the second on the day of the exam, and the third 10 days after the exam. Various tests were carried out to evaluate the impact of academic stress on different parameters, such as Glutamate oxaloacetate transaminase (GOT), Glutamate pyruvate transaminase (GPT), glucose, iron, magnesium, sodium, potassium, thyroid-stimulating hormone (TSH), cortisol, insulin, complete blood count, and blood pressure. Blood samples obtained during exams indicated a substantial decrease in the levels of liver enzymes (GOT, GPT), serum magnesium, insulin, and white blood cell count as compared to pre-post examination findings ($P<0.001$). A significant increase was recorded in electrolyte levels (sodium and potassium; $P<0.001$), blood pressure (systolic and diastolic; $P<0.001$), glucose level ($P<0.001$), Hormones level (TSH; $P<0.001$, cortisol; $P<0.001$), and platelet counts ($P<0.002$). The study has revealed that exam stress can significantly impact certain blood test results, leading to physical changes that could affect a student's academic performance. This study is significant because it explores the impact of stress on tests that have not been previously examined

**Keywords**: Stress, hematological parameters, biochemical parameters, Examination period, university students.

## 1. Introduction

Stress is a chain of events that commences with a stressor stimulus and culminates in a hypothalamus-triggered reaction in the brain, which prompts physiological responses throughout the body's systems (Colon-Emeri *et al.*, 2023). It is a process of perceiving and coping with external events, not just a stimulus or a response (Beer *et al.*, 2021). The impact of a stressor on an individual can also be quite variable, ranging from minor to life-threatening (Stewart *et al.*, 2019). Academic stress is a common occurrence in the daily lives of students and is a significant issue in our society (Wu *et al.*, 2020). A student may be worried for a variety of reasons or stressors, including academic overloads, insufficient study time, a heavy workload each semester, a lack of enthusiasm, and high family expectations all contribute to moderate stress among students (Wuthrich *et al.*, 2020; Karaman *et al.*, 2019).

Stress has been reported to cause a variety of symptoms and illnesses, depending on the reasons and degree of stress (Effati-Daryani *et al.*, 2020). According to physiological studies, any type of stress has a major influence on the hematogenic and endocrine systems (Burtkhanovich and Khasanov, 2023; Alhmoud *et al.*, 2021**)**. During the examination period, psychological stress can affect students in various ways and disrupt their body's balance, leading to changes in blood pressure levels. This can result in an increase in both systolic and diastolic blood pressure (Stock *et al.*, 2020). Previous studies have examined whether stress due to the examination is enough to alter certain hematological parameters. (Alhmoud *et al.*, 2021, Dawson *et al.*, 2020). The increase in platelet count may cause health problems, from bleeding problems to the formation of various clots, and the reduction in white blood cells (WBCs) can impact the effectiveness of the immune system. A recent study has shown that the stress of exams can impact specific blood parameters, including neutrophils, platelet counts, packed cell volume (PCV), and MCV (Seiler *et al.*, 2020). These parameters were found to be elevated, while lymphocyte counts were found to decrease. However, there were no significant changes observed in hemoglobin, red blood cells, or other indicators (Alhmoud *et al.*, 2021).

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Academic examination stress is reported to decrease immune functioning; students showed increased salivary cortisol concentrations and reported greater acute perceived stress during the examination period compared to the non-examination period (Seiler *et al.*, 2020). Previous investigation has provided new insight into the complex relationship between examination stress, cortisol, and immune functioning (Concerto *et al.*, 2017). Individual exam performance is influenced by a variety of changeable and non-modifiable factors, including pre-examination anxiety (Alsulami *et al.*, 2018).

During exams, the body experiences heightened stress levels that trigger the release of adrenaline and corticosteroids. These hormones can affect insulin production, increase glucagon levels, and prompt the liver to release more glucose (Hinds and Edwin, 2022). Changes in hormonal homeostasis, activation of lipoperoxidation processes with the development of oxidative stress, and the disintegration of antioxidant protection factors are typical for academic stress in students (Al Qteishat *et al.*, 2021). Growth hormone levels increase concurrently, decreasing the insulin responsiveness of body tissues (such as muscle and fat). More glucose is consequently available in the bloodstream. Low insulin results in decreased Na/K ATPase activity, reduced sodium reabsorption, and increased sodium ion exhalation from the distal convoluted tubules (Onyango, 2018)**.** It is possible that an increase in AST and ALT levels after an exam is due to the dietary habits commonly adopted by students during this period (Maradi and Usha, 2019). The purpose of this study was to explore how exam stress affects the health of undergraduate students. Specifically, it aims to identify physiological markers that are influenced by short-term stress and could impact academic performance. Managing stress effectively can help minimize negative effects on overall health.

## 2. Materials and Methods

### *2.1. Study population*

The current study was conducted to examine the changes in the biochemical and hematological characteristics due to exams at Al-Ahliyya Amman University's, Department of Medical Laboratory Sciences, Faculty of Allied Medical Sciences. A total of 100 undergraduate students (n=100) aged between 18 and 35 participated in this study. All students were subjected to a preliminary medical examination. Students with blood hypertension, renal or liver problems, any endocrine disorder, such as diabetes mellitus or thyroid abnormalities, students with any autoimmune disease, and smokers were all disqualified from the study.

### *2.2. Ethical consideration*

The ethical approval for this study was obtained from the Al-Ahliyya Amman University, Faculty of Allied Medical Sciences. Informed written consent was taken from each participant prior to the commencement of the study to explain the benefits and/or any risks related to participation in the study.

### *2.3. Procedure*

A total of 100 students (n=100) underwent intravenous blood collection three times, with 5 ml collected from their median antecubital vein on each occasion. The collection was carried out in the morning, specifically between 8 and 9 am. The first sample was collected a week before the test, the second on the day of the exam, and the third 10 days after the exam. The blood was distributed into two types of blood collecting tubes, vacutainers containing EDTA and heparin-containing tubes, and subjected to centrifugation at 3500 rpm for 5 minutes.

The serum was separated and divided into two Eppendorf tubes. One tube was stored at -20°C as a backup, and the other was used for biochemical analysis. The samples collected in EDTA were used for hematological parameter analysis. Participants were informed of the study's objective and procedure and signed a consent form before blood collection.

The liver enzyme (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)), as well as other parameters such as magnesium and glucose, were analyzed on Human Fully Automatic Biochemistry analyzers (HumaStar 200, Germany). Serum samples were tested in duplicate for cortisol, insulin and TSH levels using commercially available ELISA kits (Ray Biotech) in a single batch. A blood count was performed on an automated hematology analyzer (Sysmex XP-300). Blood pressures were measured in the supine position using the Beurer BM 26 device after a 10-minute rest interval. To ensure the reliability of the data, participants were advised to fast, particularly when measuring the glucose levels in their samples.

### *2.4. Statistical Analysis*

All data were analyzed by the SPSS software (V.28 Inc., Chicago, USA) and the figures were created by Microsoft Excel 2019. Kolmogorov-Smirnov test was used for variables distribution. Normally distributed numerical variables of all data are expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used for comparison of biochemical and hematological parameters before, during, and after examination with Bonferroni post hoc test for multiple comparisons between the readings. Nominal variables were presented as frequency and percentage (%) and a comparison between studied groups was performed using the *Chi*-square test. The significance of the difference was considered at $p<0.05$.

## 3. Results

Table 1 displays the mean, standard deviation (SD), and P-value for the hematological and biochemical parameters. It summarizes the fundamental features of the study sample a week before the exam (pre-exam), during the exam day, and 10 days after the exam.

**Table 1.** Comparison of hematological and biochemical parameters before, during, and after the examination (Paired Samples Test).

| Parameters | N=100 Student | | | P-value |
|---|---|---|---|---|
| | Before | During | After | |
| Liver enzyme | | | | |
| GOT (UI/L) | 24.20±5.33 | 16.77±4.05 | 21.53±5.07 | <0.001 |
| GPT (UI/L) | 27.70±10.93 | 15.60±7.84 | 21.63±7.79 | <0.001 |
| Serum electrolyte | | | | |
| Magnesium (mg/dl) | 2.07±0.36 | 1.22±0.43 | 2.24±0.37 | <0.001 |
| Iron (µg/dl) | 88.82±21.97 | 88.83±28.50 | 92.91±36.66 | 0.828 |
| Sodium (mEq/l) | 132.65±2.44 | 145.25±2.29 | 137.11±2.54 | <0.001 |
| Potassium (mEq/l) | 3.98±0.46 | 4.95±0.38 | 4.15±0.48 | <0.001 |
| Blood pressure | | | | |
| SBP (mm Hg) | 103.23±7.15 | 119.60±7.01 | 101.60±7.91 | <0.001 |
| DBP (mm Hg) | 74.47±5.10 | 79.63±4.30 | 73.93±3.44 | <0.001 |
| Hematological parameters | | | | |
| WBCs (*10^3/ml) | 7.79±0.98 | 6.33±1.18 | 7.55±1.24 | <0.001 |
| Platelets (*10^3/ml) | 294.40±57.57 | 351.20±69.34 | 300.60±67.74 | <0.002 |
| Neutrophil (%) | 58.93±8.08 | 59.14±10.87 | 58.73±9.22 | 0.986 |
| Lymphocytes (%) | 34.27±7.64 | 33.61±10.21 | 34.24±8.72 | 0.948 |
| Hormones | | | | |
| TSH (µIU/ml) | 1.61±0.95 | 2.45±0.96 | 1.12±0.46 | <0.001 |
| Cortisol (µg/dl) | 9.60±3.17 | 15.90±4.76 | 9.70±4.08 | < 0.001 |
| Insulin µU/ml | 7.87±.640 | 5.50±.925 | 6.25±1.38 | <0.001 |
| Blood sugar | | | | |
| Glucose mg/dl | 93.12±2.85 | 105.12±2.90 | 94.12±2.03 | <0.001 |

SBP: Systolic blood pressure. DBP: Diastolic blood pressure. * Significant differences at p-value <0.05.

The findings presented in Figures 1 and 2 indicate a significant difference (P < 0.001) between the GOT and GPT levels prior to the exam (24.20 5.33 U/L and 27.70 10.93 U/L, respectively), during the exam period (16.774.05U/L and 15.607.84 U/L, respectively), and after a 10-day after the exam period (21.53 ± 5.07 U/L and 21.63 ± 7.79 U/L, respectively).

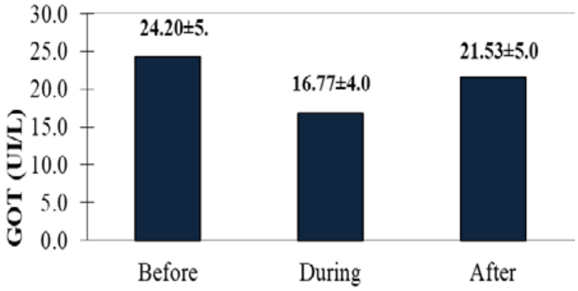


**Figure 1.** The mean levels of serum GOT of the students before, during, and after the examination period. Statistically difference (P<0.001) was observed between the level of GOT during the exam and that before and after on the other hand.

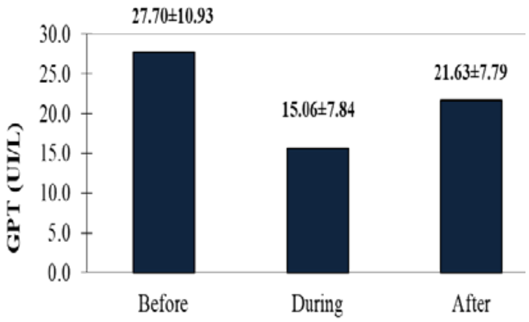


**Figure 2.** The mean levels of serum GPT of the students before, during, and after the examination period. Statistically difference (p<0.001) was observed between the level of GOT during the exam and that before and after on the other hand.

In the course of the present study, magnesium levels were significantly reduced (p,<0.001) during the exam period (1.22±0.43 mg/dl) compared to that before (2.07±0.36 mg/dl ) and after the exam period ( 2.24±0.37 mg/dl) after the exam (Figure 3 and table 1). There was a statically difference between the levels of magnesium before the exam period and after the exam period (Figure 3 and Table 1).

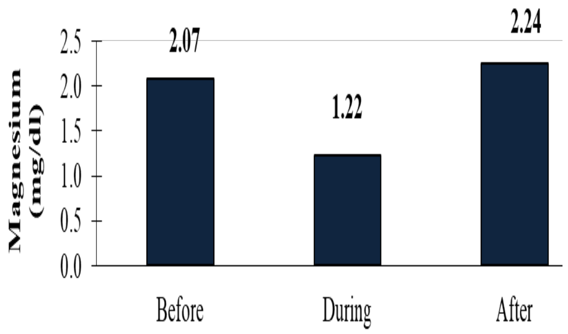


**Figure 3.** The mean levels of serum magnesium of the students before, during and after the examination period.

According to the current data (Table 1), the mean sodium levels before the exam period (132.65±2.44 mEq/l), during the exam period (145.25 ±2.29 mEq/l), and after 10 days of the exam period (137.11 ± 2.54 mEq/l) were significantly different (P< 0.001). The level of sodium significantly increased during the exam period compared to that before and after the exam period (P <0.001). As indicated in (Table 1&2) there was no significant difference in serum potassium levels before and after the exam, despite the serum potassium level on exam day (4.95±0.38) being much higher (P< 0.001) than that levels before the exam (3.98±0.46) and after the exam (4.15±0.48).

During the exam, there was a significant increase in both systolic and diastolic blood pressure levels (P <0.001), with measurements of 119.60±7.01 mmHg and 79.63±4.30 mmHg respectively. Prior to the exam, these levels were 103.23±7.15mmHg and 74.47±5.10mmHg respectively. However, after the exam period, the levels returned to similar values of 101.60±7.91mmHg and 73.93±3.44 mmHg respectively, as illustrated in Figure 4.

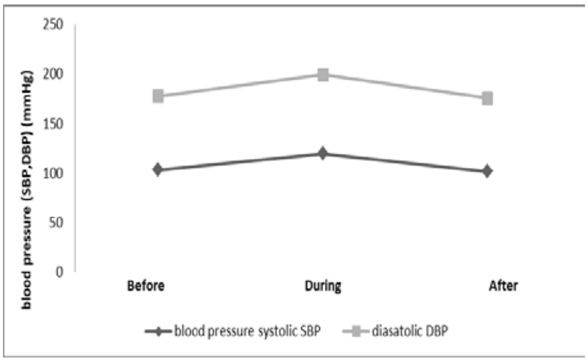


**Figure 4.** The mean levels of systolic and diastolic blood pressure for the students before, during, and after the examination period. A statistical difference (p<0.001) was observed in the systolic and diastolic blood pressure during the exam compared to the levels before and after the exam..

The count of white blood cells (WBC) was higher before (6.33±1.18) and after (7.55±1.24) the exam compared to during the exam (7.79±1.98), with a significant difference (P<0.001).. There was a significant increase in platelet count (P<0.001) during the exam period (351.20±69.34) compared to that before the exam (294.40±57.57) and after the exam (300.60±67.74). The platelet count before and after the exam remained the same with no significant difference (P<0.002),

The present study revealed a significant increase (P<0.001) in glucose level during the exam (105.125±2.9 mg/dl) compared to that before (93.125±2.85 mg/dl and after (94.125±2.031 mg/dl) the exam period. However, there were no significant changes (P=1.00) in glucose levels before and after the exam period (Table 2). Figure 5 reveals a significant rise (P< 0.001) in cortisol levels during exam periods (15.9±4.76 µg/dl) as compared with that before the exam period (9.6±3.17 µg/dl).

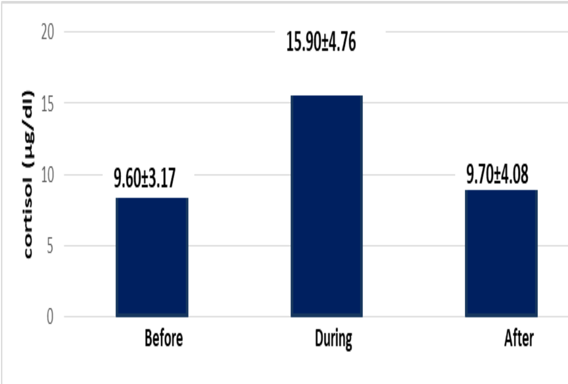


**Figure 5.** The mean level of cortisol before, during ,and after the examination period.

A substantial decrease (P<0.001) in insulin levels during the exam period (5.50±0.925 µU) in comparison to before (7.87±0.64 µU) and after the exam (6.25±1.38 µU) as indicated in Table 1. A significant difference (P <0.001) was observed between insulin levels before and during the exam, and a significant difference (P < 0.015) was also noted between insulin levels before and after the exam, as shown in Table 2.

Table 1 shows the thyroid stimulating hormone (TSH) level before (1.61±0.95 pg/ml), during (2.45±0.96pg/ml), and after the exam period (1.12±0.46 pg./ml). The levels of TSH during the exam were significantly higher (P<0.001) than the levels before and after the exam, there was no significant difference (P=0.075) between the levels before and after the exam (Table 2).

**Table 2.** Bonferroni post-hoc analysis of the difference between (Magnesium, Sodium, potassium , SBP, DBP, glucose, cortisol, and insulin ) of the students before, during and after the examination period.

| Dependent Variable | | Period | p-value |
|---|---|---|---|
| Magnesium (mg/dl)) | Before | During | <0.001 |
| | | After | 0.264 |
| | During | Before | <0.001 |
| | | After | <0.001 |
| | After | Before | 0.264 |
| | | During | <0.001 |
| Sodium (mEq/l) | Before | During | <0.001 |
| | | After | <0.001 |
| | During | Before | <0.001 |
| | | After | <0.001 |
| | After | Before | <0.001 |
| | | During | <0.001 |
| Potassium (mEq/l) | Before | During | <0.001 |
| | | After | 0.355 |
| | During | Before | <0.001 |
| | | After | <0.001 |
| | After | Before | 0.355 |
| | | During | <0.001 |
| SBP(mm Hg) | Before | During | <0.001 |
| | | After | 1.000 |
| | During | Before | <0.001 |
| | | After | <0.001 |
| | After | Before | 1.000 |
| | | During | <0.001 |
| DBP (mm Hg) | Before | During | <0.001 |
| | | After | 1.000 |
| | During | Before | <0.001 |
| | | After | <0.001 |
| | After | Before | 1.000 |
| | | During | <0.001 |
| Glucose (mg/dL ) | Before | During | <0.001 |
| | | After | 1.00 |
| | During | Before | <0.001 |
| | | After | <0.001 |
| | After | Before | 1.00 |
| | | During | <0.001 |
| Cortisol | Before | During | <0.001 |
| | | After | 1.00 |
| | During | Before | <0.001 |
| | | After | <0.001 |
| | After | Before | 1.00 |
| | | During | <0.001 |
| Insulin | Before | During | <0.001 |
| | | After | <0.015 |
| | During | Before | <0.001 |
| | | After | 0.483 |
| | After | Before | 0.015 |
| | | During | 0.483 |
| TSH (pg/ml) | Before | During | <0.001 |
| | | After | 0.075 |
| | During | Before | <0.001 |
| | | After | <0.001 |
| | After | Before | 0.075 |
| | | During | <0.001 |

*Significant differences at p-value <0.05

## 4. Discussion

University students often experience academic-related stress, which can have negative effects on their mental and physical health and lead to academic difficulties (Lassiter *et al.*, 2022). Regularly conducting biochemical and hematological examinations is crucial in monitoring physiological changes that occur during exam periods. This study involved tests that had not been previously examined, including liver enzymes, thyroid hormone, and magnesium levels, alongside other tests that had been antecedently studied. The levels of liver enzymes (GOT, GPT) were significantly decreased during the examination period. Our results are consistent with the study that found lower levels of GOT and GPT before exams compared to that after the exam period (Maradi and Usha, 2019). It's likely that the significant decrease in liver enzymes during stressful situations is caused by an overabundance of cortisol hormone **(**Edoardo *et al.*, 2005).

The findings of the current study observed a significant reduction in serum magnesium levels during the examination period when compared to that before and after the examination period ($P < 0.001$). However, there was no statistically significant difference between magnesium levels before and after the exam ($P= 0.264$). This decrease is likely due to increased urinary magnesium excretion during the examination period, as previously reported (Lopresti *et al.*, 2020). Another study found that a stimulus-induced rise in cortisol levels results in a fall in magnesium levels, creating a shortage that increases the body's susceptibility to stress (Pickering *et al.*, 2020).

Recent research indicates that academic stress can activate the release of adrenaline and corticosteroids in the body, leading to negative effects on insulin levels and the function of the Na/K ATPase pump. This pump system is essential for maintaining the balance of sodium and potassium in the body, and when its function is compromised, more sodium ions are excreted while potassium ions flow out of the cell (Maradi and Usha., 2019). The current study's results support the observation that potassium levels tend to increase during exam periods (Maradi and Usha., 2019).

It has been observed in this study that blood pressure tends to increase during times of exams. However, there was no significant change in systolic and diastolic blood pressure before and after the exam ($P=1.000$). Our findings are in line with the recent report that has demonstrated an increase in blood pressure during exam periods (Singh *et al.*, 2022). Stress triggers the hypothalamus to stimulate the adrenal glands to release epinephrine and cortisol, which in turn elevates heart rate, blood pressure, and metabolic rate (Hinds and Edwin, 2022). According to previous reports, individuals who experience the greatest rise in blood pressure or heart rate in reaction to sudden stress are more likely to develop future health problems (Kivimäki and Andrew , Turner *et al.*, 2020).

There was a reduction in the count of white blood cells during the examination period in comparison to the count before the exams. However, the count of white blood cells returned to its pre-examination phase level after the exams were over. Catecholamines have the ability to lower the activity of specific white blood cells which can lead to a weakened immune system. This effect is most prominent in individuals who experience significant stress reactions such as an increase in heart rate and blood pressure (Sharma *et al.*, 2022). Conversely, individuals with no significant changes in heart rate and blood pressure did not experience any changes in white blood cell activity. This finding is similar to a report that demonstrated a decrease in the total leucocyte count during examination periods (Dhabhar *et al.*, 1996).

There was a significant increase in platelet count ($P<0.001$) during the exam period compared to that before the exam and after the exam. The platelet count before and after the exam remained the same with no significant difference ($P=1.000$), which is consistent with the findings of the previous investigation (Engler *et al.*, 2004). It was found that platelet count increased significantly during the examination period and remained elevated for three days after the end of the exam period (Alhmoud *et al.*, 2021). However, in the current study, platelet count returned to normal within ten days after the examination period ended. This suggests that platelets remained elevated for no more than ten days after the examinations.

During exam periods, the stress hormone cortisol was found to be elevated, which directly affects glucose levels in the bloodstream by triggering glucose synthesis through gluconeogenesis (Maduka *et al.*, 2015). This study confirms the previous report of a significant increase in cortisol levels during exam weeks compared to non-exam weeks (Glaser *et al.*, 1994). A study revealed that glucose levels tend to be higher during exams compared to before and after the exams. This is due to an increase in cortisol levels and a decrease in insulin levels (Chan *et al.*, 2020). Stress hormones such as cortisol can hinder the proper functioning of insulin-producing cells in the pancreas, resulting in decreased insulin production and reduced insulin levels (Rahman *et al.*, 2021). According to a report, patients who experience persistent stress states may develop type II diabetes due to stress-induced hyperglycemia and other mechanisms causing insulin resistance at the tissue level (Sharma *et al.*, 2022). This study examined the impact of exam stress on thyroid gland function by measuring TSH hormone levels. The findings showed a significant increase in TSH levels during the exam period compared to before and after. Our findings are consistent with those of a recent study that found a rise in TSH levels during stress (Hettiarachchi *et al.*, 2014). On the other hand, there is a conflicting study that associates insulin resistance and problems with regulating blood sugar with hypothyroidism and it has indicated that cortisol may decrease the level of TSH in the bloodstream (Gierach *et al.*, 2014).

## 5. Conclusion

This study investigated the impact of exam stress on various biochemical and hematological tests, which affects a significant number of students. The study examined previously untested factors and found that these changes could have an adverse effect on students' academic performance. However, the study did not investigate the potential impact of other hormones, and thus more studies are necessary to gain a complete understanding.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors of this article, affirm that the work presented herein is entirely original. Any responsibility for claims related to the content of this article will rest solely upon us.

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