RESEARCH ARTICLE

Male High School Students Express Higher Blood Cortisol Levels During Examination Periods than Female High School Students.

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Abstract

The academic formation of an individual with good study opportunities in Mexico begins approximately at 3 years of age and remains constant until graduation from high school. At this stage, there are learning periods, that include classes and homework, and evaluation periods, that covers exams and class presentations. Evaluation periods concentrate two or three daily exams during one week. The present project was designed to determine through cortisol concentrations if there are differences in the expression of stress between males and females in high school in a private school in the city of Puebla, Mexico. We recruited 14 female and 13 male adolescent volunteers between the ages of 17 and 18. We obtained two blood samples, the first one was taken during the class period and the second one was taken on the week of final evaluations of the semester. The blood was processed according to the instructions of an ELISA Bio-cortisol (MEXLAB) kit in order to determine serum cortisol concentrations, and according to a Bio-glucose (MEXLAB) kit to determine serum glucose concentrations. The results show that males have higher blood cortisol levels during the class and examination periods compared to females. We also show that cortisol levels in males are significantly higher during the examination period than during the class period; whereas females did not show differences in cortisol levels between periods. In the case of glucose levels, we observed differences related to sex but not differences related to the evaluated periods. These data suggest that school activities at the high school level generate more stress in males than in females.

Introduction

The onset of adolescence is accompanied by cognitive and emotional changes, as well as by high academic and family demands, including homework and home responsibilities, and thus adolescents frequently identify school and family as sources of stress and worry.[1] When stress emerges in the context of an educational process, it is usually referred to as academic stress and it is considered a great strain that students experience during learning periods. This academic stress can be a natural survival response in the academic environment, which involves different potential stressors, such as exams, class presentations, class participation, tutoring sessions, academic overload, classroom overcrowding, lack of time to complete the work, mandatory work, homework, group work, and competitiveness among classmates.[2] In relation to an educational context, the classroom is an environment where children spend a great amount of time while growing up. From kindergarten until high school graduation, individuals undergo experiences that can be predictors of well-being and success in school.[3]

Cortisol is the end product of the activity of the hypothalamic-pituitary-adrenal (HPA) axis and has been widely used as a stress biomarker,[4] and it is thus considered the stress hormone. This hormone expresses an increase in its levels in the 20 – 45 minutes during which an individual gets up in the morning, followed by a gradual decrease throughout the day. Low or high cortisol concentrations can compromise the function of the HPA axis; however, when an individual experiences a real or perceived threat, cortisol is released, which reflects reactivity in the HPA axis[3,5] that is important for the movement of physiological resources that are necessary to deal with challenges.[6]

A previous study showed a significant increase in saliva cortisol concentrations during an examination week, which was related to the perception that periods of academic examination are commonly perceived by students as stressful experiences[7] regardless of their frequency. The objective of the present study was to evaluate blood cortisol concentrations in third-year high school students and determine if there is sexual dimorphism, as has been described in university students, where males exhibit higher cortisol levels than females as a result of written and oral exams.[8]

Materials and Methods

This project was developed as part of the high school projects that aim to get adolescents involved in scientific activities. The high school students responsible for the project actively participated in its development under close supervision and previous training in the use of laboratory equipment. We invited 64 third-year high school students from the 2019-2020 school cycle of the Instituto Mexicano Madero Plantel Centro to participate in the project through informed and signed consent from their parents. Once these documents were obtained, the participants were instructed to arrive with 8 hours of fasting in order to obtain a first blood sample through venipuncture at 7 hours on a

day of a week of regular academic activities, which include class sessions, homework, presentations, laboratory classes, tutoring, and sport activities, among others. We obtained 3 ml of blood from each participant, which was centrifuged at 4000 rpm for ten minutes to separate the serum from the cellular fraction. Serum samples were kept at 4 ºC and transported to the laboratory where they were frozen at -20 ºC until processing. The second sample was also taken with 8 hours of fasting at 7 hours but on the third day of the week of final exams of the semester. The time elapsed between the obtention of the first and the second sample was of two weeks.

Both samples were unfrozen and processed together. We followed the instructions of a Bio-Cortisol and a Bio-Glucose (MexLab) kit in order to measure cortisol and glucose concentrations, respectively, using the ELISA technique. We used 25 µl of serum in triplicate to measure cortisol concentrations and 10 µl of serum, also in triplicate, to measure glucose concentrations.

Glucose and cortisol concentration data were analyzed with a two-way ANOVA, and we considered as independent variables sex and activity period: 1) classes and 2) exams. Differences were considered significant when $P < 0.05$.

Results

Initially, 36 students participated in the study; however, 9 students did not complete their participation and thus the results of the study correspond to a sample of 14 females and 13 males. Figure 1 shows the results of the glucose levels of the students. The data show a significant difference related to sex ($F_{(1,50)} = 4.821$, $P = 0.0328$). However, the multiple comparison analysis does not show differences between groups. There was no effect of academic activity period ($F_{(1,50)} = 0.6281$, $P = 0.4318$) or the interaction between variables ($F_{(1,50)} = 0.7899$, $P = 0.3784$) on the levels of glucose. Figure 2 shows the results of the cortisol levels of the students (Sex: $F_{(1,50)} = 163.9$, $P < 0.0001$; Activity periods: ($F_{(1,50)} = 59.76$, $P < 0.0001$; Interaction: $F_{(1,50)} = 36.11$, $P < 0.0001$). There were several differences when comparing the data. The first difference is an increase in cortisol levels in male students during the class period compared to female students during the same period (white bars). The second difference is an increase in cortisol levels in male students during the examination period compared to female students during the same period (black bars). Finally, the third difference is observed in male students exhibiting significantly higher cortisol levels during the examination period than during the class period (white bar and black bar of males). Differences were significant with $P < 0.0001$.

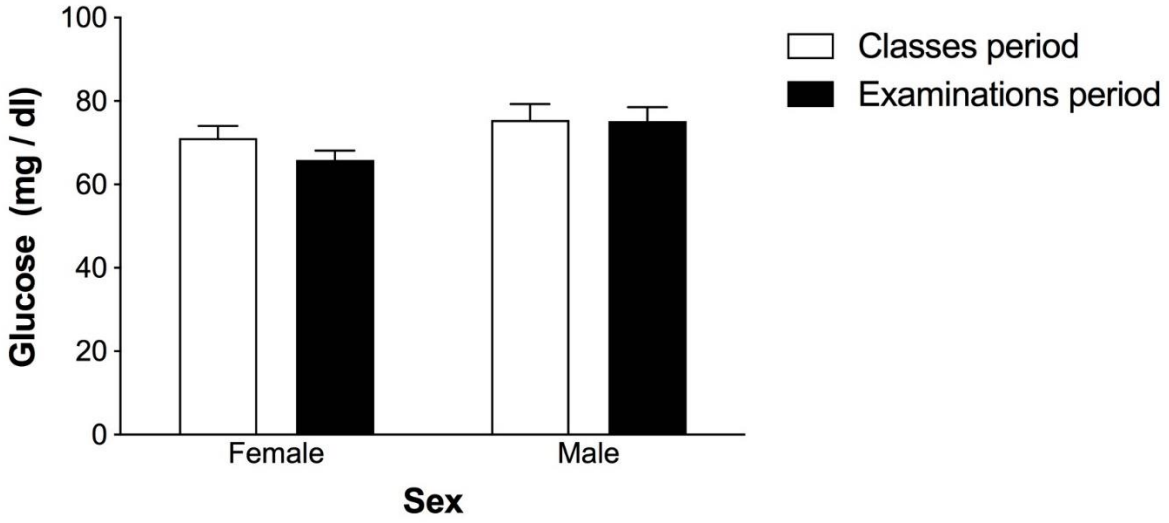

Figure 1. Blood glucose levels (mg/dl) of high school students during a class period and an examination period.

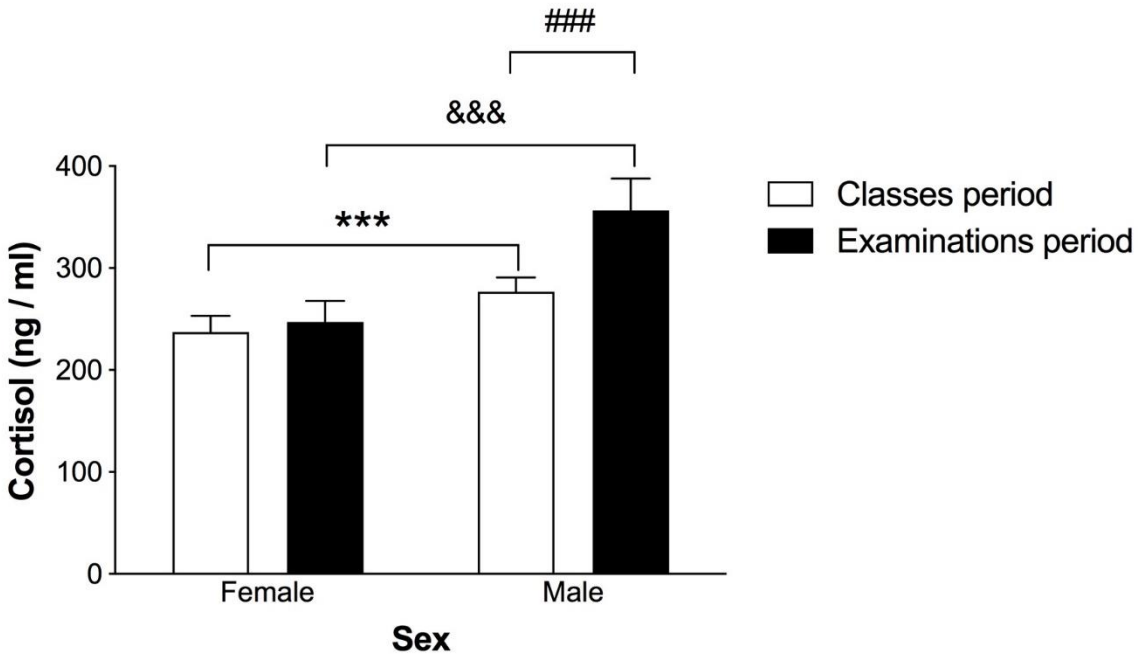

Figure 2. Blood cortisol levels (ng/ml) of high school students during a class period and an examination period. Differences can be observed between males and females and between periods in males: 1) Cortisol concentrations are significantly higher in males than in females in the class period (*** P < 0.0001) ; 2) Cortisol concentrations are significantly higher in males than in females during the examination period ($^{\&\&\&}$ P < 0.0001); 3) There is an increase in cortisol levels in males during the examination period with respect to the class period ($^{\#\#\#}$ P < 0.0001). Graph shows the mean ± SEM; n = 14 females and 13 males.

Discussion

The results of the present study show that there is sexual dimorphism in the expression of cortisol in the blood of high school students, in both the class and examination periods, without alterations in glucose levels. These results are similar to those previously reported in undergraduate medical students between 18 – 30 years old.[9]

In relation to sex, our results contrast with previous studies that mention that females express higher stress and a high emotional reactivity when taking an exam or doing a class presentation, among other academic activities, when compared to males.[1,2] Nevertheless, the results of the present study are similar to those reported by Maestripieri, Baran, Sapienza et al.[6] who found that males exhibit higher cortisol concentrations than females before and after taking a computerized test. The authors suggest that the differences in the hormonal response to psychosocial stress largely depends on the subjectivity with which the stressful stimuli are interpreted as a result of variations in context, the participant's personality, and the interindividual variability that is accentuated when the sample size is small.

The high cortisol levels in males during the class period detected in the present study could be related to male adolescents being occasionally exposed to bullying by their peers during the school day.[3] On the other hand, the high increase in cortisol levels during the examination period could be due to the fear of taking exams.[9] Therefore, we agree with Preuss, Schoofs, Schlotz et al.[10] in the idea that measuring cortisol levels several weeks before examinations and throughout the whole examination period could be a methodologically desirable strategy.

Recent studies in adolescent rodents have shown that exposure to chronic stress causes substantial long-term changes in behavior and the function of the HPA axis in females with respect to males. Thus, adolescent females can benefit more from the environment in order to improve the lasting effects of stress. One example is related to female rats, unlike males, not showing adrenal gland hypertrophy induced by variable chronic stress or hypersecretion of corticosterone as a response to new stressors.[11] According to this, we suggest that the female participants of the present study use control strategies as a response to the stress generated by taking exams that they have developed throughout their primary and secondary education, and thus did not express changes in the release of cortisol during the examination period. Nevertheless, it is possible that there is a protective effect of estrogens, which could be evaluated in future studies.

It has been suggested that the magnitude of the increase in cortisol in response to stress could differ between sexes in relation to the type of stressor.[12] In this sense, it has been demonstrated that oral evaluations have a social stress component generated by an audience that listens to and judges the speaker, whereas written evaluations do not have this component. In addition, an anticipatory effect has been observed in the HPA response of male students, which implies that cortisol concentrations are

significantly high on the day before a written exam.[8,10]

Even though not all stressful events are considered to be experienced in the same way or to have a negative impact on the health of an individual,[13] students subjected to final examinations have been found to show a decrease in cortical plasticity compared to less stressful periods during the semester, which could be related to the increase in circulating cortisol levels.[14] Thus, we suggest, first, that further studies on the subject are necessary and, second, to raise awareness in school administrators in order to avoid, to the extent possible, concentrating evaluation activities within short periods of time.

Finally, and similarly to some previous studies,[3,14] we recognize that the present study has some limitations that should be considered in future investigations: 1) The time elapsed between the moment each student gets up in the morning and the moment when the sample is taken should be similar between participants. We did not control for this variable in the present study, but we consider that females spend more time preparing for activities outside the home, and thus the time between the moment they got up and when the sample was taken was likely to be longer than that of males, which could explain the observed differences. 2) The results are based on a single sample taken in each period of academic activity, and thus analyzing cortisol levels throughout several days during both periods would be more informative, which implies considering taking a different kind of sample, such as saliva. 3) Acquiring the participation of a larger number of students (first and second year of high school), which is difficult because it depends on the authorization of the parents due to the age of students in high school. 4) Considering the fluctuations in sex hormones throughout the estrus cycle as a factor that contributes to the expression of stable cortisol levels under repetitive stressful conditions, such as several examination periods throughout the school cycle or academic formation.

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