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Academic stress, hair and saliva cortisol, and their relationship with body mass index and fat percentage in first year medical students

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ABSTRACT

Introduction: Although a large part of the population may be exposed to various pressures that can lead to mental or eating problems and increased perceived stress, the transition from adolescence to adulthood has been shown to be a crucial stage. Medical students are particularly vulnerable during the transition period as they must adapt to new circumstances, which may contribute to increased perceived stress. Cortisol plays an important role between stress, weight gain, and the development of obesity. We designed a study to investigate the association between stress, eating behaviour, cortisol, and body weight in a sample of first-year medical students. *Methods:* We determined 75 first-year medical students' hair and salivary cortisol concentrations by ELISA and related it to self-reported stress, eating behaviour, and anthropometric measurements throughout the academic period. The prevalence of overweight and obesity in females was 25% and 10%, and in males was 35% and 6%, respectively. We report an increase in hair cortisol, higher

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self-reported stress scores, and BMI mainly in females. Finally, we found evidence of positive associations between hair cortisol and BMI in females ($r = 0.348$) and males ($r = 0.423$). *Conclusion:* There is a low association between short-term single-point cortisol measures and long-term cortisol, mainly in males. Hence, short-term cortisol reactivity is moderately associated with long-term cortisol reactivity when both are evaluated simultaneously. These results support the previous evidence of positive associations between cortisol with body fat percentage and BMI, and finally, that eating behaviours are modified by academic stress perception, mainly in females.

KEYWORDS

cortisol, stress, overweight, BMI, students

INTRODUCTION

Overweight and obesity are becoming a major global pandemic, not only among adults, but also among children and adolescents around the world [1, 2]. People who are overweight or obese are more likely to develop physical and mental health problems.

In Latin America, approximately 57% of the adult population is overweight (54% males and 70% female), and 19% is obese (14% males and 24% females) [3]. Similarly, the prevalence of overweight and obesity in Latin American children is alarmingly high (21% in boys and 25% in girls) [4]. In 2014, the prevalence of obesity and overweight in Panama was 21% in males and 30% in females [4]. In 2015, a general prevalence of obesity of 27% (18% in males and 30% in females) was reported for Panamanian population [5].

Weight gain and the development of obesity is a multifactorial condition [1], and its recent increase illustrates its association with changes in lifestyle, eating disorders, biological and psychosocial factors. Although a large part of the population may be exposed to various pressures that might lead to psychiatric or eating problems, the transition from adolescence to young adulthood has been demonstrated to increase the difficulty of choosing healthy dietary choices [6, 7]. In fact, university students have a set of factors that promote dietary intake, such as greater autonomy in the choice of food, reduced budgets, and exposure to different food categories and cultures [8]. This coupled with alcohol consumption and low physical activity can promote weight gain [9]. For example, a prevalence of 29% in overweight and 9% in obesity has been reported in Panamanian medical students [10].

Medical students are particularly vulnerable during the transition period, as they must adapt to new circumstances, academic obligations, and changes in life and identity can contribute to an increase in perceived stress [11].

Stress is defined as the mechanism that links a stressor and its target organ, which results in a physiological and/or behavioural response, characterized by the release of glucocorticoids [12].

Traditionally, studies on stress have relied on the use of various perception scales, with the goal of determining an individual's feelings or thoughts about how much stress overload they perceive in a situation, condition, or space, whether in various dimensions such as personal, sentimental, or economic, and extending to various environments such as work, academic, or family. On the other hand, it has been quite common to measure blood pressure, heart rate, or respiratory rate to explore physiological manifestations of stress. However, the use of physiological markers for stress, particularly cortisol measurements in humans, has recently become popular.

When stress is perceived, a cascade occurs in the hypothalamic-pituitary-adrenal axis (HPA) [13], an increase in the corticotropin-releasing factor (CRF) occurs in the paraventricular nucleus of the hypothalamus, which upon reaching the anterior lobe of the pituitary, stimulating the secretion of adrenocorticotropic hormone (ACTH). Finally, ACTH will act on the adrenal glands cortex inducing the release of glucocorticoids, in particular cortisol.

There are different matrices from which cortisol can be detected, such as plasma, nails, feces, urine, saliva, hair or interstitial fluid. Salivary cortisol reflects responsivity to acute stress [14]. On the other hand, hair cortisol is hypothesized to reflect the integrated free cortisol fraction rather than the total serum cortisol concentration [15], and is an indicator of long-term chronic stress [16]. Although cortisol has many physiological functions, it is a critical element in the relation between stress and weight gain [17]. Whereas the reasons are not fully understood, it has been proposed that there is a link between perceived stress and weight gain [18]. Psychological stress can impact the hypothalamic-pituitary-adrenal (HPA) axis, resulting in an increase in cortisol release [2]. This cortisol elevation can promote fat deposition, increased body fat, and weight gain [19].

Given that the transition from adolescence to adulthood is a critical period for the adoption of health-related behaviours to prevent eating disorders, coupled with the possible association between stress and cortisol that promote weight gain and that medical students play an important role in promoting the health of the community; we designed a study to investigate the association between stress, eating behaviour, cortisol, and weight gain related anthropometric measures in a sample of first-year medical students. Our hypothesis is that the academic stress experienced by students is accompanied by an increase in cortisol concentrations and body mass index.

MATERIALS AND METHODS

Study population

Initially, we introduced the research to first-year medical students at the University of Panama. Volunteer participants were included if they were at least 18 years old, did not report suffering from any medical condition, and those who did not report use of any medication. During this phase, 89 students agreed to participate in the study. Then, we applied the self-evaluation scales of generalized anxiety and depression proposed by Cardoze [20], which was intended to identify participants with symptoms of depression and anxiety. Because subjects with anxiety [21] and depression [22] may present altered cortisol levels. We excluded from the analysis 6 participants who exhibited symptoms of moderate anxiety and 5 participants with symptoms of moderate depression. Finally, the atypical cases were detected using the Mahalanobis distance procedure, which allows detecting atypical cases [23]. We identified 3 students with outliers for hair cortisol concentration. The final sample consisted of 75 students, 41 females and 34 males.

Anthropometric measurements

Bioimpedance bases its measurement on the different resistance offered by both water and different body tissues to the flow of an electric current, allowing to determine the body composition of the subjects. In addition, it is correlated with BMI and abdominal skinfolds and low-cost bioimpedance devices are appropriate for assessing body composition in an adult university population.

A bioelectric impedance scale with electrodes for hands and feet (Omron HBF-514C) was used to determine the percentages of body fat (TF%), visceral fat (VF%) and body mass index (BMI) (kg m^{-2}). Subjects were classified by their BMI according to the criteria suggested by the World Health Organization: underweight (<18.5 kg m^{-2}), normal weight (18.5–24.9 kg m^{-2}), overweight (25.0–29.9 kg m^{-2}) and obesity (>30.0 kg m^{-2}) [24]. Anthropometric measurements were determined at the beginning (week #1) and at the end (week #16) of the semester and were performed by a dietitian.

Salivary cortisol concentrations (SCC) and Hair cortisol concentrations (HCC)

Participants collected their saliva samples by themselves 15–20 min after waking up in the morning. We instructed the participants to refrain from brushing, eating, and drinking prior to sample collection. They were asked to gargle with water, then discard it, and finally collect 5 mL of saliva in a disposable tube. They delivered the sample between 7:00–9:00 a.m. and it was stored at −20 ºC until analyses.

Hair strands close to the scalp were collected from the posterior vertex region. Cortisol was determined from the 2 cm hair segment closest to the scalp. Approximately 10 mg of hair [25] was used and trimmed into small pieces, placed in a disposable glass vial, then 1 mL of methanol was added. The sealed vial was incubated overnight (~16 h) at 52 °C while shaking. After incubation, the supernatant was removed and placed in disposable glass culture tubes. A dry nitrogen bath was used to remove the excess solvent, finally, it was resuspended in 200 μL of PBS at pH 8.0 [26].

Three cortisol determinations were made in saliva and hair for each participant, at the beginning (week #1), in the middle (week #8), and at the end of the semester (week #16). Cortisol tests were performed using the procedure described by Sauvé et al. [26], by duplicate, following the SALIMETRICS-ELISA kits.

Self-reported stress and eating behaviour

Participants answered the SEEU-R self-reported academic stress survey [27] and validated for Panamanian students as part of the research with a Cronbach's alpha $\alpha = 0.885$ and 28 items. An alpha value more than 0.70 is considered as satisfactory internal reliability [28]. A higher perceived stress score means higher perceived stress.

We evaluated the eating behaviour through the survey proposed by Castro et al. [29]. The questionnaire was validated by the researchers, with a Cronbach's alpha $\alpha = 0.802$ and 34 items. The scale allowed us the assessment of the eating behaviour. The questionnaire measures healthy eating behaviours (type of food, caloric content, and sugar intake) and eating attitudes (eating for mood and awareness). A higher score on the scale is indicative of better eating behaviour. Both questionnaires were applied at the beginning (week #1) and at the end of the semester (week #16).

Statistical analysis

The level of significance was established $\alpha = 0.05$; all tests were two-tailed. Results in the text are presented as mean ± standard deviation (S.D.) The normality of the data was determined with the asymmetry and kurtosis indices, considering that the values within the threshold ±1.5 indicate slight variations from the normal [30]. For the detection of atypical data, we used the Mahalanobis test (D^2). Three female students with atypical values for HCC were identified. Tests were divided by sex, comparing the self-reported stress, eating behaviour, and anthropometric measures between week #1 and week #16 with *t*-test for paired samples, presenting the effect size as Cohen's d (*d*).

For comparing of HCC and SCC in weeks #1, #8, and #16, a one-factor ANOVA for repeated measures within subjects was used, presenting the effect size with the partial eta squared (η^2). Finally, Pearson correlations were utilized to establish associations between the HCC and SCC means throughout the semester, as well as those of self-reported stress, eating behaviour, BMI, and TF%. Besides, a simple regression was performed with HCC, BMI, and TF%, as well as for self-reported stress and eating behaviour. Analyses were performed using SPSS V.25 (IBM corporation).

Ethics declarations

This research was developed in accordance with the Declaration of Helsinki, and it was approved by the Bioethics Committee of the University of Panama (CE-PT-327-15-02-19-41). We obtained the informed consent of all participants.

RESULTS

Group description

No differences in BMI, weight, TF%, VF%, and eating behaviour were observed between week #1 and week #16. We report significant differences between the perceived stress scores in week #1 and week #16 ($P < 0.001$, $d = 0.37$) of the semester, which was higher in week #16 (Table 1). We found no differences in the group in salivary cortisol concentrations throughout the semester between

Table 1. Descriptive data of anthropometric measurements and cortisol concentrations in the group

	Start of the semester (Week 1)	End of semester (Week 16)		
	Mean ± SD		*P*-Value	*d*
Weight (Kg)	63.78 ± 13.40	63.78 ± 13.27	0.977	0.001
BMI	24.05 ± 4.24	24.10 ± 4.24	0.704	0.011
TF%	29.38 ± 10.88	29.23 ± 10.88	0.708	0.013
VF%	4.96 ± 2.96	5.12 ± 3.52	0.366	0.04
A. stress	31.30 ± 13.82	36.77 ± 15.31	** **< 0.001**	0.37
E. Behaviour	106.25 ± 16.04	105.30 ± 14.50	0.421	0.06
	Week 1	**Week 8**	**Week 16**	η^2
SCC (nmol L^{-1})	17.85 ± 11.40	11.60 ± 8.30	9.51 ± 7.32	0.212
HCC (pg mg^{-1})	64.66 ± 36.84	73.09 ± 28.09	83.70 ± 42.40	0.128

BMI = Body Mass Index, TF% = Total Fat percentage, VF % = Visceral Fat percentage, A. Stress = Academic Stress, E. Behaviour = Eating Behaviour, *P*-values based on Paired Sample *T*-test. **-value is significant at the 0.01 level (2-tailed).

week #1 and week #16. Similarly, we did not report differences in hair cortisol concentrations throughout the semester between week #1 and week #16.

Comparison between sexes

The sample was distributed according to their BMI in underweight (4%), normal weight (59%), overweight (29%) and obesity (8%). Females are distributed in low weight (2%), normal weight (63%), overweight (25%) and obesity (10%). Males are distributed into underweight (6%), normal weight (53%), overweight (35%) and obesity (6%).

Significant differences with a small size effect were observed in the female BMI in week #1 with 23.84 ± 3.64 kg m^{-2} and week #16 with 24.09 ± 3.81 kg m^{-2} ($P = 0.035$, $d = 0.067$). We report significant differences with a moderate size effect between perceived stress scores in week #1 with 32.21 ± 14.56 and week #16 with 39.36 ± 16.15 ($P < 0.001$, $d = 0.530$) of the semester, being higher in week #16 (Table 2). No differences were observed in weight, TF%, VF% and eating behaviour in females. In males, there are no differences between week #1 and week #16 in weight, BMI, TF%, VF%, stress scores and eating behaviour.

No differences were observed between males and females in the perception of stress at the beginning ($P = 0.218$, $d = 0.145$) or at the end of the semester ($P = 0.744$, $d = 0.380$). However, when comparing the perceived stress in females between the beginning and end of the semester, a significantly higher score is observed towards the end of the semester ($P < 0.001$, $d = 0.530$). Additionally, the self-reported stress scale used has two questions that assess the quality of sleep (Have you stayed awake through exams or work? And have you suffered from sleep disorders during exam periods?). When comparing their means at the beginning ($\overline{x} = 2.95$) and at the end ($\overline{x} = 3.87$) of the semester, they showed significant differences ($P < 0.001$), indicating a higher frequency of wakefulness and sleep disorders towards the end of the semester.

Finally, we found no differences ($P = 0.345$, $d = 0.203$) in salivary cortisol concentrations throughout the semester between female ($\overline{x} = 13.55$ nmol L^{-1}) and male ($\overline{x} = 12.30$ nmol L^{-1}). Similarly, we did not report differences ($P = 0.856$, $d = 0.035$) in hair cortisol concentrations throughout the semester between female ($\overline{x} = 74.22$ pg mg^{-1}) and male ($\overline{x} = 73.33$ pg mg^{-1}).

SCC and HCC throughout the semester

The mean SCC for females were 18.74 ± 11.42 nmol L^{-1}, 11.64 ± 7.56 nmol L^{-1}, and 10.26 ± 7.99 nmol L^{-1}, for weeks #1, #8, and #16, respectively. Our repeated measures ANOVA in females with a Greenhouse-Geisser correction determined that SCC means was significantly different in the semester (F (1.87, 75.86) = 11.23, $P < 0.001$, $\eta^2 = 0.219$). Post hoc Bonferroni correction tests indicate that throughout the semester a significant decrease ($P = 0.004$) in SCC in females was observed between week #1 (18.74 ± 1.78 nmol L^{-1}) and week #8 (11.64 ± 7.56 nmol L^{-1}). In addition, a significantly reduction ($P < 0.001$) in SCC is also observed in week #1 and week #16 (10.26 ± 7.99 nmol L^{-1}). On the other hand, in males we determined that SCC mean differed during the semester (F (1.51, 49.93) = 8.70, $P < 0.001$, $\eta^2 = 0.209$). Post hoc Bonferroni correction tests indicate that there are no differences ($P = 0.112$) in males SCC between week #1 (16.77 ± 11.46 nmol L^{-1}) and week #8 (11.58 ± 9.23 nmol L^{-1}). Instead, we observed a significant reduction ($P = 0.001$) in SCC between week #1 and week #16 (8.59 ± 6.40 nmol L^{-1}). Overall, we report in females a reduction of 83% SCC towards the end of the semester. Similarly in males we observed a reduction of 95% SCC towards the end of the semester.

Table 2. Descriptive data of anthropometric measurements and cortisol concentrations in males and females

	Female (n = 41)				Male (n = 34)			
	Start of the semester (Week 1)	End of semester (Week 16)			Start of the semester (Week 1)	End of semester (Week 16)		
	Mean ± SD		*P*-Value	*d*	Mean ± SD		*P*-Value	*d*
Weight (Kg)	58.21 ± 9.14	58.65 ± 9.54	0.128	0.047	70.50 ± 14.71	69.98 ± 14.57	0.169	0.035
BMI	23.84 ± 3.64	24.09 ± 3.81	**0.035**	0.067	24.31 ± 4.92	24.12 ± 4.77	0.437	0.039
TF%	35.93 ± 6.88	36.32 ± 6.95	0.128	0.056	21.49 ± 9.53	20.70 ± 8.33	0.335	0.088
VF%	3.95 ± 1.22	4.07 ± 1.17	0.096	0.1	6.17 ± 3.88	6.38 ± 4.81	0.593	0.048
A. stress	32.21 ± 14.56	39.36 ± 16.15	** < **0.001**	0.53	30.20 ± 13.02	33.64 ± 13.83	0.105	0.256
E. Behaviour	106.65 ± 17.27	105.60 ± 16.57	0.601	0.062	105.76 ± 14.65	104.58 ± 11.72	0.547	0.088
	Week 1	**Week 8**	**Week 16**	**η^2**	**Week 1**	**Week 8**	**Week 16**	**η^2**
SCC (nmol L^{-1})	18.74 ± 11.42	11.64 ± 7.56	10.26 ± 7.99	0.219	16.77 ± 11.46	11.58 ± 9.23	8.59 ± 6.40	0.209
HCC (pg mg^{-1})	61.72 ± 33.12	75.57 ± 32.34	85.37 ± 46.30	0.111	68.20 ± 41.12	70.10 ± 22.02	81.69 ± 36.78	0.067

BMI = Body Mass Index, TF% = Total Fat percentage, VF % = Visceral Fat percentage, A. Stress = Academic Stress, E. Behaviour = Eating Behaviour, *P*-values based on Paired Sample *T*-test. * *P*-values are significant at the 0.05 level (2-tailed). ** *P*-value is significant at the 0.01 level (2-tailed).

The HCC mean for females were 61.72 ± 33.12 pg mg^{-1}, 75.57 ± 32.34 pg mg^{-1}, and 85.37 ± 46.30 pg mg^{-1} for week #1, #8, and #16, respectively. The ANOVA of repeated measures in females with Greenhouse-Geisser correction determined that the HCC significantly differed throughout the semester (F (1.81, 72.58) = 5.01, P = 0.011, η^2 = 0.111). Post hoc Bonferroni correction tests indicate no differences (P = 0.101) in HCC in females between week #1 (61.72 ± 33.12 pg mg^{-1}) and week #8 (75.57 ± 32.34 pg mg^{-1}). However, we observed a significant elevation (P = 0.023) in HCC between week #1 and week #16 (85.37 ± 46.30 pg mg^{-1}). On the other hand, in males we determined that HCC mean does not show variation along the weeks of the semester (F (1.66, 54.85) = 2.35, P = 0.113, η^2 = 0.067). Therefore, HCC tend to increase in females, but not in males. Overall, in females we report a 38% HCC increase towards the end of the semester.

Correlation and linear models

In females we obtained the following correlations between HCC and SCC, week #1 (r = 0.113, P = 0.483), week #8 (r = 0.143, P = 0.374) and week #16 (r = 0.204, P = 0.200). In males, week #1 (r = 0.248, P = 0.157), week #8 a significant association (r = 0.363, P = 0.035) and week #16 (r = 0.269, P = 0.124). HCC and SCC correlation with the averages throughout the semester, in females showed a low association (r = 0.104, P = 0.517), but, showed positive significant association in males (r = 0.462, P = 0.006). Therefore, when HCC-SCC are evaluated in both sexes at single point, they do not show an association. On the other hand, the averages throughout the semester show an association between HCC and SCC in males, but not in females.

Females shows an association between HCC, BMI, TF% and VF% (r = 0.348, r = 0.313 and r = 0.324). Males shows an association between HCC, BMI and VF% (r = 0.424 and r = 0.372). Hence, we observed a positive association between HCC and anthropometric measures associated with higher BMI (Fig. 1, Table 3). In addition, there is a negative association between academic stress scores and eating behaviour in females (r = −0.354), but not in males (r = −0.153). Therefore, academic stress causes a change in eating behaviours in females (Table 4).

DISCUSSION

The majority of participant were in the normal range of BMI, our results show that 29% (35% in males and 25% in females) of the participants had BMI above 25 kg m^{-2} and 8% (6% in males and 10% in females) had BMI above 30 kg m^{-2}. These results are higher than the prevalence reported for Panama in 2014 of 11% overweight in young males and 10% overweight in young females, although the same study reported similar percentages of obesity in young men of 5% and 6% in women [4].

In females, a higher stress score was observed at the end of the semester, this has been reported in a similar study, where females indicated a higher stress perception at the end of the class period [31]. This supports the idea that, unlike females, males appear to be less sensitive in their perception of subtle bodily symptoms and are less likely to assess bodily or emotional changes as significant [32]. Although the evidence suggests that HCC are usually about 21% higher in male than in female [33]. Our results indicate that the average values of HCC

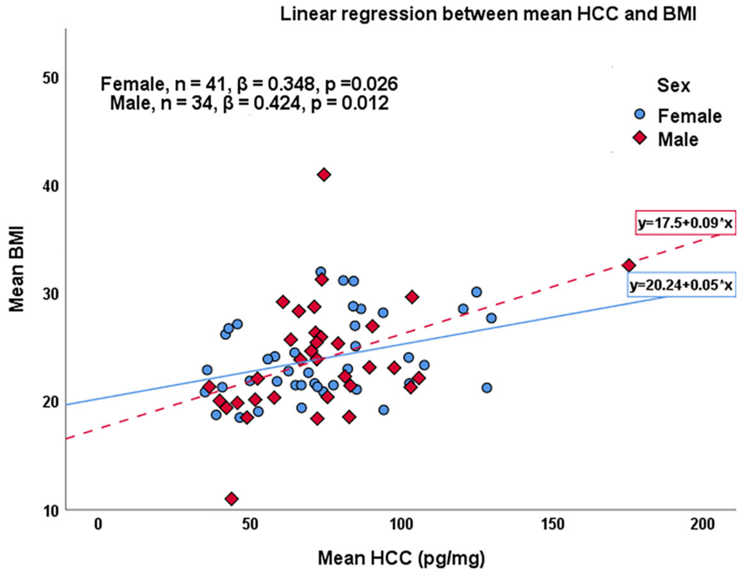

Fig. 1. **Linear regression model between hair cortisol as a predictor of BMI**. The graph shows that hair cortisol is a predictor of BMI in females ($\beta = 0.348$, $P = 0.026$). In addition, hair cortisol is also a predictor of BMI in males ($\beta = 0.424$, $P = 0.012$). Therefore, there is a relationship between cortisol concentrations and body mass index in medical students

Table 3. Correlations between anthropometric measurements, cortisol concentrations and eating behaviour in males and females

	SCC	HCC	BMI	TF (%)	VF (%)	A. stress	E. behaviour
Female							
SCC	—	0.104	−0.005	0.084	0.062	0.028	−0.225
HCC	—	—	**0.348***	**0.313***	**0.324***	0.075	0.017
BMI	—	—	—	**0.958****	**0.968****	0.167	−0.021
TF%	—	—	—	—	**0.957****	0.138	−0.047
VF%	—	—	—	—	—	0.140	−0.002
A. stress	—	—	—	—	—	—	**−0.354***
E. behaviour	—	—	—	—	—	—	—
Male							
SCC	—	**0.462****	0.262	0.307	0.293	−0.055	0.293
HCC	—	—	**0.424***	0.330	**0.372***	0.025	0.147
BMI	—	—	—	**0.882****	**0.909****	0.023	0.093
TF%	—	—	—	—	**0.898****	−0.062	0.132
VF%	—	—	—	—	—	0.067	0.221
A. stress	—	—	—	—	—	—	−0.153
E. behaviour	—	—	—	—	—	—	—

*. Significant correlation at 0.05 level (2-tailed). **. Significant correlation at the 0.01 level (2-tailed).

Table 4. Summary of the linear regression model between cortisol in hair associated with anthropometric measurements and academic stress associated with eating behaviour

	Female					Male				
HCC as a predictor of anthropometric measurements (BMI, TF%, VF %)										
	B	SE B	β	R^2	P	B	SE B	β	R^2	P
BMI	0.05	0.022	0.348	0.121	***0.026**	0.087	0.033	0.424	0.18	***0.012**
TF%	0.084	0.041	0.313	0.098	***0.046**	0.114	0.058	0.33	0.109	0.057
VF%	0.015	0.007	0.324	0.105	***0.039**	0.062	0.027	0.372	0.138	***0.03**
Academic stress as a predictor of eating behaviour										
E. Behaviour	−0.398	0.169	−0.354	0.125	***0.023**	−0.153	0.175	−0.153	0.023	**0.387**

B = unstandardized regression coefficient, SE B = standard error of unstandardized regression coefficient, β = standardized regression coefficient, R^2 = explained variance. *P-values are significant at the 0.05 level (2-tailed).

throughout the semester do not vary between male and females. However, we must consider certain covariates such as the frequency of hair washing, which tends to decrease hair cortisol [34], as well as the use of contraceptives could affect HCC in females [35]. Unfortunately, our data is not enough to clarify whether the frequency of washing or the use of contraceptives affected our determinations.

We found differences in SCC throughout the semester, mainly between the beginning and the end of the semester, being SCC less at the end of the semester. From a methodological perspective, it is important to indicate that there is a rapid habituation of a salivary cortisol response after chronic exposure to a stressful situation reflected in various psychological stress protocols [36, 37]. On the other hand, salivary cortisol is subject to different physiological fluctuations, which makes it difficult to reflect long-term cortisol exposure [38]. In addition, the cortisol awakening response (CAR) is a 38–75% increase in cortisol levels that peak 30–45 min after awakening [39], and there is evidence that it may be influenced by drowsiness, tiredness [40] and shift works. This could be an explanation since in weeks #8 and #16 the students were in exam periods or close to them, so it is common to stay awake [41]. However, for futures studies, we suggest a greater number of cortisol determinations in saliva. In addition, we did not obtain data from CAR, or cortisol throughout the day. Therefore, we do not have information on the magnitude of the complete response. This was because we do not have enough manpower or resources to obtain that data, thus we decided to apply the simplest possible design that could provide us with robust data.

Furthermore, we observed an increase in HCC in females towards the end of the semester, this has been reported by a metadata analysis, which indicates that under stressful events HCC increases between 20 and 43% [33], suggesting a global upregulation of cortisol secretion with present/ongoing stress.

Initially, weekly HCC and SCC values showed a low association, possibly because SCC is an acute and highly fluctuating stress biomarker, while the HCC reflected a long-term activity [42]. On the other hand, the overall HCC and SCC means show an important association in males, but not in females. This has been reported in males [43], as well as in a study that reports associations similar to those we have found between HCC and SCC [44]. This is due to the fact that HCC and SCC are linked in the short term [33].

Our results suggest that, throughout the semester, the HCC tends to increase in females, but not in males, this is consistent with a report where a sample composed mainly of females presented a higher HCC in academic periods compared to holidays [45]. The proximal 1 cm segment of the scalp approximates the cortisol production of the last month, the second most proximal 1 cm segment approximates the production during the previous month, and so on [46]. This allows retrospective examination of cortisol production at times when a stressor is most pronounced [47]. This points to a similarity with our results since the HCC analyses of week #8 mainly reflect the cortisol of the previous month, that is, around weeks #4 or #5, in which students are taking their first midterm exam or are next to do it. In addition, HCC at week #16 reflects the cortisol at weeks #11 or #12, so it is likely that students have a general idea of their subject grades and in some cases have taken or are close to taking another exam. Therefore, academic pressure produces stress, which leads to an increase in HCC. Despite, unbound cortisol in females tend to increase in luteal phase. Nevertheless, the sample of females could be heterogeneous since we do not have information about their menstrual cycles.

Our data indicate an association between eating habits (food choice, caloric and sugar content, and emotional eating) and academic stress in females, similar findings indicate that perceived stress was positively associated with uncontrolled eating and emotional eating [48]. In addition, students with higher stress, compared with those with lower levels of stress, had higher scores for emotional eating, uncontrolled eating, and a higher frequency of fast food [49], as well as higher scores for perceived stress are associated with an increased fat intake. This indicates that the intake of foods with high palatability (fat, sugar, salt) can enhance the processing of rewards and increase the stress-driven eating cycle.

Although the data are small, we believe that sex segregation is feasible since BMI classification, body fat distribution, eating patterns, and perceived stress vary between the sexes. In addition, cortisol concentrations can fluctuate with the female menstrual cycle and cortisol in hair is affected by external factors, demonstrating individual differences (eg. Use of hair cosmetic in females and greater frequency of hair washing in males). Although hair cortisol is an indicator of long-term stress, further longitudinal studies addressing gender differences are needed to better understand its use as a marker of chronic stress [50].

In summary, there is a low association between short-term single-point cortisol measures versus long-term cortisol, mainly in males. Hence, short-term cortisol reactivity is moderately associated with long-term cortisol reactivity when both are evaluated simultaneously. The correlation between cortisol concentrations in hair and saliva indicates that cortisol is a useful marker of the general activity of the HPA axis during the academic period. Hence, hair cortisol is an appropriate marker of continuous physiological stress.

The results support the previous evidence of positive associations between cortisol with body fat percentage, BMI and weight gain, also that eating behaviours are modified by the academic stress perception, mainly in females. Thus, we recommend addressing stress in weight gain prevention efforts and promoting healthier eating behaviours.

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