Acute and chronic effects of exams week on cortisol production in undergraduate students

Running head: Cortisol in undergraduates

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ABSTRACT

Undergraduate students experience many levels of stress throughout their university trajectory. The pressure to achieve good results highlight the exams week in the Universities as a stimulus that triggers psychosocial stress and increased release of cortisol. Considering the increasingly experience an undue amount of stress, we aimed in this study to investigate the short- and long-term effects of exams week on HPA axis in undergraduates by the cortisol production. Twenty-eight undergraduates aged 18-24 years from biological sciences during the final exams' week at the end of the school year collected hair and saliva for cortisol measures. Hair cortisol was significantly higher in exams month. They exhibited cortisol rhythmicity and preserved CAR (cortisol awakening response) even under psychosocial stress. The exams week is a trigger for psychosocial stress, however, did not generate significant short-term changes in cortisol rhythmicity. Thus, it is evident that young people adapt to the stressful stimulus, avoiding a possible trigger for mental illness.

Keywords: Cortisol; hair; rhythmicity, saliva; psychosocial stress; undergraduate students.

INTRODUCTION

Stress can be defined as a mismatch between demands and the perceived ability to cope with the stressor (Gallagher et al., 2014; Moore, Truscott, St Clair, & Klingborg, 2007). It is founded on an individual's perceptions and cognitive appraisal of his or her situation and cannot be predicted objectively (Moore et al., 2007). In general, the stress response is adaptive and has a crucial role in the adaptation to external demands (García-León, Pérez-Mármol, Gonzalez-Pérez, García-Ríos, & Peralta-Ramírez, 2019). The key mediator of this response is considered to be the neuroendocrine hypothalamus-pituitary-adrenal (HPA) axis and its main downstream effector, the glucocorticoid cortisol (Stalder et al., 2017).

Systemic cortisol levels are highly variable, which is due to the circadian rhythm, acute stress and pulsatile secretion (Wester & van Rossum, 2015). Measurements of cortisol in saliva, blood or urine only assess acute short sampling periods, usually 12 or 24 h, limiting the observation of long-term chronic effects, in addition to depending on the patient's correct adherence to the collection protocols (Stalder et al., 2017; Wester & van Rossum, 2015). Hair analysis has been used for decades in forensic science to measure exposure to environmental toxins and drugs and nowadays has gained attention as a viable biomarker for chronic stress and long-term activity of the HPA axis (D'Anna-Hernandez, Ross, Natvig, & Laudenslager, 2011; Sauvé, Koren, Walsh, Tokmakejian, & Van Uum, 2007; Wester & van Rossum, 2015). In addition, the hair cortisol evaluated hair segments grew in the past, making this technique particularly interesting to assess endocrine activity of past events (Stalder et al., 2017).

Undergraduate students experience many levels of stress throughout their university trajectory. There is empirical evidence that the stressful experience at the university was linked to reduced academic performance, low grade averages, and low rates of graduation and higher dropout, in addition to increased psychological distress (Dwyer & Cummings, 2001; Elias, Ping, & Abdullah, 2011; Portoghese et al., 2019). The pressure to achieve good results highlight the exams week in the Universities as a stimulus that triggers psychosocial stress and increased release of cortisol (Karatsoreos & McEwen, 2014; Liston, McEwen, & Casey, 2009). Considering that undergraduate students increasingly experience an undue amount of stress, we aimed in this

study to investigate the short- and long-term effects of exams week on HPA axis in undergraduates by the cortisol production.

METHODS

Subjects

Volunteers aged between 18 and 24 years were invited during class and poster for general inclusion criteria. They signed the free and informed consent, declaring understanding on the procedures that would be performed during the protocol. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and ethical approval was granted from Research Ethics Committee of the School of Medical Sciences/University of Campinas (CAAE: 41225614.8.0000.5404). This study was performed with collaboration of biological sciences undergraduate students from University of Campinas (Unicamp). This undergraduate course was chosen because the full-time and evening courses have the same curriculum and practically all college subjects are taught in the same institute, avoiding the stress of displacement within the university. All samples were collected during the final exam/test week at the end of the school year.

Hair cortisol samples

The monthly production of cortisol was evaluated from each of the volunteers in the last 2 months. It was assumed that the average hair growth rate is 1 cm per month according to Dolnick *et al.* (Dolnick, Lindahl, Terrill, & Reynolds, 1969). Twenty-seven undergraduate students from biological sciences course in Unicamp provided hair samples, which were approximately 0.5 cm in diameter with a length between 3 and 5 cm near the scalp. The strands of hair were collected from the posterior vertex of the scalp and were cut off as close to the scalp as possible following Manenschijn *et al.* (Manenschijn, Koper, Lamberts, & van Rossum, 2011). The hair cortisol extraction was performed following Meyer et al. (Meyer, Novak, Hamel, & Rosenberg, 2014). The results are presented in nmol/L and are derived from 40 mg of hair samples sprayed and resuspended in 0.2 ml of assay buffer, and then the conversion factor in pg/mg of hair.

Salivary cortisol samples

Participants were instructed to collect by themselves saliva samples at home, whose Sallivetes® were to be stored under refrigeration. Twenty-eight undergraduate students collect saliva to evaluate the cortisol rhythmicity. The cortisol rhythmicity was evaluated by saliva collections at five times of the day during three consecutive days (Tuesday to Thursday), upon awakening (6 a.m.), 30 minutes after awakening (6:30 a.m.), before lunch (12 a.m.), before dinner (6 p.m.) and before bedtime (11 p.m.).

Cortisol quantification

The samples were centrifuged at 2,800 rpm, 20 min, 4 °C and analyzed using the commercial ELISA kit (Diagnostics Biochem Canada Inc. - Ref CAN-C-290) at the Laboratory of Stress Studies (LABEEST) - UNICAMP, as conducted by Madeira et al. (Madeira et al., 2019). The results were expressed in nmol/L for each sample.

Statistical analysis

Data are presented as means ± SEM. The normality was confirmed by D'Agostino & Pearson and then we performed paired or unpaired Student's t-test for parametric and Wilcoxon or Mann-Whitney for nonparametric data. All statistical analysis was done with Graph Pad Prism version 8.00 for Windows (Graph Pad Software, San Diego, California, USA). The acceptance level of significance was set at $p<0.05$.

RESULTS

Hair cortisol and psychosocial stress

To verify the chronic effect of the test/exams week in cortisol release, we evaluated the hair segments in months with two different expectations: one month without tests/exams (October) and one month of tests/exams with possible emergence of psychosocial stress (November). The results showed that the hair cortisol in November was significantly higher than that observed in October (Figure 1).

Salivary cortisol

These volunteers also presented rhythmicity in cortisol release in the consecutive days, with higher values in the morning and a significantly decrease throughout the day. Even presenting adequate rhythmicity after the test marathon, we can observe an increase in the before bedtime sample at 2nd and 3th day when compared to the before dinner sample, since only 1st day exhibited significant difference from the same point. There was no significant change in the amount and rhythmicity of cortisol produced on different days (Figure 2). The cortisol awakening response (CAR) in these volunteers was preserved, with significantly higher values 30 min upon awakening in all days. CAR values increased throughout the days, but were not significant (Figure 3).

DISCUSSION

The hair cortisol is relevant to provide a retrospective reflection, for periods of several months, of cortisol release. In the last years, a number of evidences have been accumulated on several key features of hair cortisol measurements, including their validity as an indicator of long-term systemic cortisol concentrations, their reliability through repeated assessments, and their robustness over a series of influences. Based on-these findings, researchers started to use this measurement to answer more specific questions about the role of stress in long-term cortisol production and its effect on different health-related conditions. The possibility to measure the long-term production of other steroid hormones (i.e. androgens or estrogens) in hair analysis provides a more intriguing prospect for future research. Taking in accounts its unique characteristics, the hair analysis is a great promise to significantly enhance current understanding about the role of steroid hormones (Braig et al., 2016; Serwinski, Salavecz, Kirschbaum, & Steptoe, 2016; Stalder et al., 2012; Stalder et al., 2017). The results obtained in this study are higher than those described in the literature for a healthy population, according to Parrot and Downey *et al.*. A healthy population presents an average of 13.8 pg/mg and our volunteers presents values of 58.32 and 109.11 for the months of October and November, respectively (Ouellet-Morin et al., 2016; Parrott & Downey, 2017; Short et al., 2016). However, hair cortisol

143 is known to be influenced by a number of factor that contribute to the variability of the hair-
144 cortisol results, as hair bleaching and dye treatment, hair texture and composition, exposure to
145 sun and hair washing (Mezentsef et al., 2017; Russell, Koren, Rieder, & Van Uum, 2012).

146 The daily variation of stressors, such as differences in workload levels between week days
147 and weekend days, can mask long-term changes in cortisol rhythms (Adam, Hawkley, Kudielka,
148 & Cacioppo, 2006; Karlamangla, Friedman, Seeman, Stawksi, & Almeida, 2013). The literature
149 already reported associations between cortisol parameters and psychosocial outcomes in studies
150 with 3-day sampling protocols (Hulett, Fessele, Clayton, & Eaton, 2019). Increased evening
151 cortisol over time may be linked to fatigue, as suggested by other studies (Schmidt et al., 2016;
152 Tell, Mathews, & Janusek, 2014; Weinrib et al., 2010).

153 The HPA axis can be assessed by quantifying salivary cortisol 30 min after awakening
154 (Fries, Dettenborn, & Kirschbaum, 2009; Pruessner et al., 1997; Wang et al., 2014). CAR
155 mechanism is independent of cortisol secretion throughout the day, being influenced by factors
156 not yet well clarified. However, that abnormalities in the rhythm of cortisol secretion, either in
157 CAR or in the rhythmicity, are associated with numerous diseases, as well as psychosocial
158 variables and chronic stress (Clow, Thorn, Evans, & Hucklebridge, 2004). In this way, cortisol
159 values 30 min after awakening significantly higher than those upon awakening indicate that the
160 HPA axis is intact. The cortisol produced in CAR also agrees with that described in the literature
161 for a healthy population and reflects a 38% increase in cortisol values upon awakening, being
162 considered healthy up to 100% of this baseline (Dedovic & Ngiam, 2015; Vreeburg et al., 2010).
163 Therefore, we demonstrated that the studied population, even living in a moment of psychosocial
164 stress, exhibited the rhythmicity of daily cortisol production as the CAR preserved and intact.
165 Regarding the evaluation of salivary cortisol, we can affirm that the studied population is healthy,
166 since it presented rhythmicity maintained during the week. However, the amounts of cortisol
167 produced in this undergraduate population are similar to the Canadian elderly population and
168 lower than the healthy third-year students at a US medical school during successive clinical
169 rotations (Souza-Talarico, Plusquellec, Lupien, Fiocco, & Suchecki, 2014; Tseng, Iosif, &
170 Seritan, 2011).

Biological instruments and techniques for the evaluation of chronic stress and the relation with deleterious effects in the organism are fundamental. The possibility of biological quantification of the effects of psychosocial stress in undergraduate students is necessary since the incidence of mental illness in undergraduate students is high (Campos, Oliveira, Mello, & Dantas, 2017). This pilot study presents limitations related to the reduced number of volunteers who participated in the study because of adherence throughout protocol and the data were obtained from a convenience sample offering reduced generalizability of our results.

The exams week is a reality in universities and can become a deleterious factor to the undergraduate health since they maintain high cortisol levels throughout this period. The period is a trigger for psychosocial stress, however, did not generate significant short-term changes in cortisol production rhythmicity. Thus, it is evident that young people adapt to the stressful stimulus, avoiding a possible trigger for mental illness. Beyond that, investigations with this same population and the association or not with the previous experience of allostatic overload, evaluated by cortisol in the hair, highlights information for possible preventive actions by mental health strategy in the University.

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AUTHORS' CONTRIBUTIONS

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Conceptualization, D.M.G.K. and

A.B.A.; Methodology, F.B., F.C. and D.M.G.K.; Investigation, F.B., P.C.S., F.C. and A.L.S.; Formal Analysis, F.B. and D.M.G.K.; Resources: D.M.G.K; Writing – Original Draft, F.B., F.C. P.C.S. and D.M.G.K.; Writing – Review & Editing, F.B. and D.M.G.K.; Visualization, F.B. and D.M.G.K.; Supervision, F.B and D.M.G.K.; Funding Acquisition, D.M.G.K.

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FIGURES

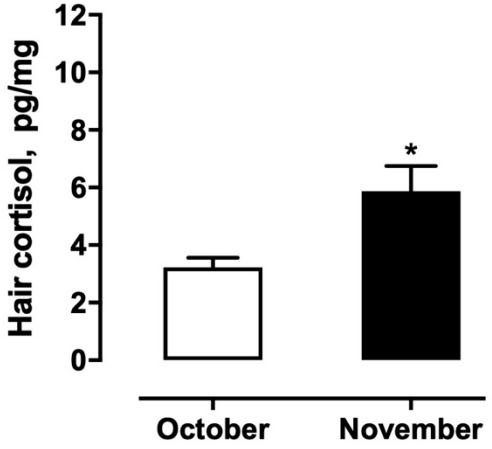


Figure 1 – Hair cortisol from undergraduate students in October (month without exams) and November (month of exams). Data are presented as mean ± SEM. *p<0.05 in the same group.

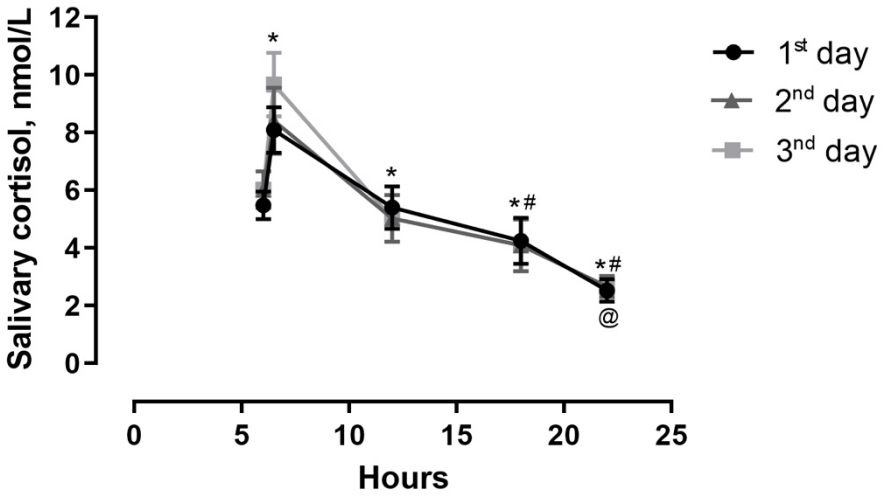
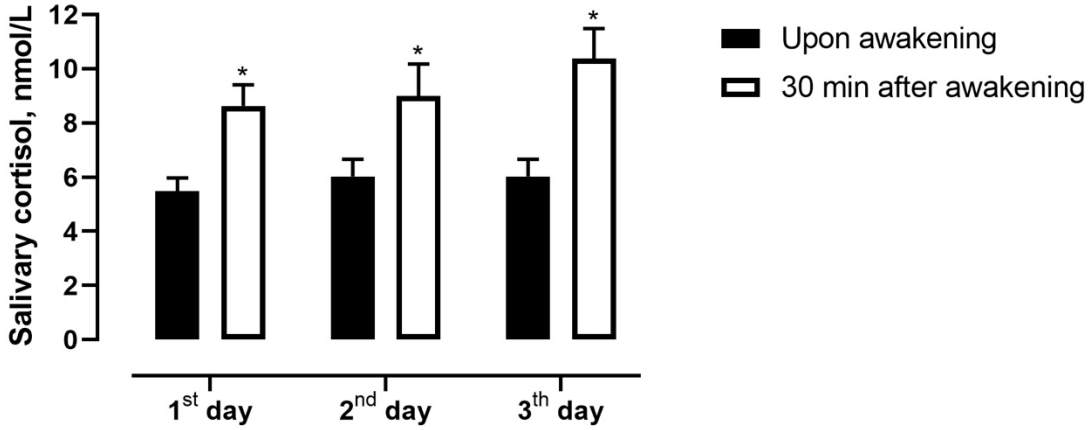


Figure 2 – Cortisol rhythmicity by saliva samples at five different times of the day during three consecutive days: upon awakening (6 a.m.), 30 minutes after awakening (6:30 a.m.), before lunch (12 a.m.), before dinner (6 p.m.) and before bedtime (11 p.m.). Data are presented as mean ± SEM. *p<0.05 vs. 6 a.m. in the same group; #p<0.05 vs. 11 p.m. in the same group; @p<0.05 1st day 6 p.m. vs. 1st day 11 p.m.



345

346 **Figure 3 -** CAR from undergraduate students in different consecutive days. Data are presented as

347 mean ± SEM. *p<0.05 in the same group.