

Anticipated academic examinations induce distinct cortisol responses in adolescent pupils

Lucie Martinek¹, Karin Oberascher-Holzinger², Stefan Weishuhn³,
Wolfgang Klimesch¹ & Hubert H. Kerschbaum*²

¹ Department of Physiological Psychology, Institute of Psychology, University of Salzburg, Hellbrunnerstr. 34, 5020 Salzburg, AUSTRIA.

² Department of Molecular Neurobiology and Cellular Physiology, Institute of Zoology, University of Salzburg, Hellbrunnerstr. 34, 5020 Salzburg, AUSTRIA.

³ BORG Salzburg, Akademiestr. 21, 5020 Salzburg, AUSTRIA.

Correspondence to: Prof. Mag. Dr. Hubert H. Kerschbaum
Department of Molecular Neurobiology and Cellular Physiology,
Institute of Zoology, University of Salzburg,
Hellbrunnerstr. 34, 5020 Salzburg, AUSTRIA
TEL: +43 - 662 8044 5667
FAX: +43 - 662 8044 5698
EMAIL: Hubert.Kerschbaum@sbg.ac.at

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Abstract

OBJECTIVES: Although it is widely accepted that academic examinations are accompanied by a cortisol increase, some recent studies reported either no effect or even a decline of the cortisol level. To reevaluate these discrepancies, we investigated whether personality traits predict the cortisol response upon academic examinations.

SETTING AND DESIGN: Nineteen male and female adolescent pupils (17 to 19 years) participated in the study. Two anticipated, mandatory, routine written examinations were used as familiar stressful conditions, whereas an anticipated, mandatory oral examination in front of a board of known and unknown examiners was used as a novel stressful situation.

METHODS: Baseline, pre- and postexamination salivary cortisol were quantified and correlated with psychometric measures, including self-estimated stress level, obtained from a five-point scale, and sensation seeking subscales according to Zuckerman.

RESULTS: Salivary cortisol response, taken as an average of all subjects, showed a transient increase upon examinations. However, comparing individual cortisol responses revealed three distinct cortisol profiles, including a transient increase (Type 1), a transient decline (Type 2), or no response (Type 3). Type 1 predominates in examinations combined with novelty. A moderate negative association was noted between saliva cortisol concentrations on some sensation seeking subscales. Self-reported stress levels did not significantly correlate with salivary cortisol concentration.

CONCLUSION: Our findings show that upon academic examinations the cortisol response varies among subjects. A moderate negative association was unveiled by correlating individual cortisol responses with sensation seeking subscales.

Introduction

Although academic examinations represent stressful challenges to many students, studies on the examination-dependent adrenocortisol response, a sensitive physiological indicator for a stress response, are inconsistent. Some studies report that examinations lead to an increase of cortisol [1, 2, 3, 4, 5, 6, 7, 8], whereas other studies describe either no effect [5, 9, 10] or a decrease of cortisol levels upon examinations [11]. These controversies surrounding the impact of academic examinations on the adrenocortisol response may be due to the variety of examinations conditions, age of subjects, and individual differences on personality traits. Association between personality traits and adrenocortisol response imply two major physiological consequences: (1) individual consistency of the cortisol response upon exposure to stressors and (2) exposure of subjects, differing in their personality traits, to identical stressors show individual differences in the physiological response towards identical stressors. Yet despite promising results in some studies showing an association between personality factors and adrenocortisol response, other studies failed to establish a consistent link [12, 13, 14, 15]. Recently, sensation seeking has been acknowledged as factor interfering with the cortisol response. These findings suggest a moderate association between risk taking and cortisol response [16].

Academic examinations fulfill classical requirements for a psychological stressor, including non-controllable conditions and shortage of time. As written and oral examinations represent psychological stressors during everyday life in school, we monitored the cortisol response upon two written and one oral examination in adolescents, analyzed the consistency of the response within subjects, and evaluated the association between cortisol response and biometric measurements, including sensation seeking according to Zuckerman [17] and self-reported stress level, respectively.

Methods

Nineteen adolescent pupils (8 females, 11 males; 17 to 19 years of age) from a High School ("Gymnasium") in Salzburg, Austria, participated in the present study. The stressors used in the present study consisted of two mandatory written examinations ("Schularbeiten") and one mandatory oral examination ("Matura").

Fifth version of Zuckerman's sensation seeking subscales (SSS-V) was used to estimate individual differences in thrill and adventure seeking, experience seeking, disinhibition, and susceptibility to boredom [17]. The questionnaire was administered on a day without an anticipated examination. In addition, pupils have been asked to rate their stress level by labeling a number between one, representing highly stressed, and five, representing not stressed at all, on days with as well as on days without anticipated examinations.

Before the beginning of the study, pupils were instructed about harvesting saliva samples using salivate tubes containing a polyester wool swab (Sarstedt, Wiener-Neudorf, Austria). Pupils removed a salivette from a sterile tube, chewed the swab for up to three minutes, and put the soaked swab back into the tube. Within 15 to 30 minutes after harvesting, swabs soaked with saliva were centrifuged at 4500 rpm for fifteen minutes and the saliva samples were stored at -20°C until quantification for cortisol. Saliva samples were taken approximately five to ten minutes before and five to ten minutes after the examination. On days without announced examinations, saliva samples were harvested at similar time points.

Cortisol was quantified using the cortisol saliva LIA form IBL (Hamburg, Germany). The microtiter wells were pre-coated with an anti-cortisol antibody. Then 20 μl of standard, probe, or control were pipetted into the wells. Following application of 100 μl of an enzyme conjugate (cortisol conjugated with horse radish peroxidase), the microtiter wells were sealed and incubated for three hours at room temperature. Then, the wells were washed four times by an Elisa Washer (Anthos, Salzburg, Austria). After removing the wash buffer, 50 μl of a substrate solution mixture (luminal enhancer and peroxide solution mixed 1 : 1) were added into the wells. The relative luminescence units were measured within ten to forty minutes. Each individual samples were run in duplicate in the same assay.

Significance of self-reported stress level between baseline and anticipated stress as well as gender differences between self-reported stress levels were estimated using the U-test according to Mann and Whitney. The strength of relationship between saliva cortisol level and self-reported stress level as well as boredom susceptibility, thrill and adventure seeking, disinhibition, and experience seeking, respectively, was estimated using a non-parametric correlation according to Spearman.

Results

Figure 1 A shows the averaged saliva cortisol concentration at six discrete time points on a day without an announced examination. Mean saliva cortisol concentration peaked about 60 minutes after awaking and then declined. Figure 1 B compares saliva cortisol concentration between two written ("Schularbeit 1" and "Schularbeit 2") and one oral examination ("Matura"). The comparability of cortisol levels between baseline and examination was ensured by matching the time of harvesting salivary samples. The averaged saliva cortisol concentration demonstrated a significantly higher cortisol concentration before an anticipated examination compared to the averaged saliva cortisol concentration on days without an anticipated examination. Cortisol level in saliva, which has been harvested before the oral examination ("Matura"), was significantly higher than the cortisol concentration of saliva harvested before written examinations ("Schularbeit").

To determine whether the averaged cortisol response shown in Fig. 1 B resulted from a homogenous response pattern, which varies only in the intensity of the cortisol response, or disguised a heterogeneous response profile, which varies in intensity and direction of the cortisol response relative to baseline, we analyzed the cortisol response upon anticipated examinations on an individual basis. Upon anticipation of an examination, the cortisol response revealed three distinct profiles (Fig. 2). Type 1 response represents the expected physiological response to a stressor, revealing an increase in saliva cortisol level before examination and a decline at the end of the examination. In contrast, the reverse physiological response has been found in Type 2 response. It is characterized by a saliva cortisol concentration depth before the examination and an increased saliva cortisol concentration after the examination. Unlike the preceding types of responses, Type 3 response shows a decline of saliva cortisol concentration before and after examination. However, type 3 response may simply reflect the endogenous decline of the cortisol concentration during the morning (see Fig. 1 A). Thus, these pupils did not show a change in the cortisol concentration in response to an anticipated examination. Table 1 summarizes the quantitative distribution of the cortisol profile among pupils. Type 1 response dominated written examinations and is the only type of response in anticipation of an oral examination (Table 1). As shown in Table 1, some pupils had consistent whereas others had a variable cortisol profile.

Given that cortisol is a major stress hormone in humans, we next investigated whether individual cortisol concentration correlates with the momentary self-reported stress level. Pupils were asked to rate their stress level when they collected baseline and preexamination salivary samples, respectively. Analysis of these data yielded two basic results. First, with little surprise, self-reported stress level is enhanced before an anticipated examination. Second, intensity of individual behavioral stress level is gender specific. Female adolescents reported a higher stress level in absence of an announced examination ($P < 0.021$, Mann Whitney U-Test) as well as in anticipation of an examination ($P < 0.014$, Mann Whitney U-Test). Although in anticipation of an examination rating of self-reported stress level and averaged cortisol concentration increased, we could not identify a significant correlation between saliva cortisol concentration and self-reported stress level (Table 2).

Figure 2. Representative cortisol profiles of three pupils. Type 1 shows a transient cortisol increase before examination, Type 2 a transient cortisol decrease before examination, and Type 3 a decrease before and after examination.

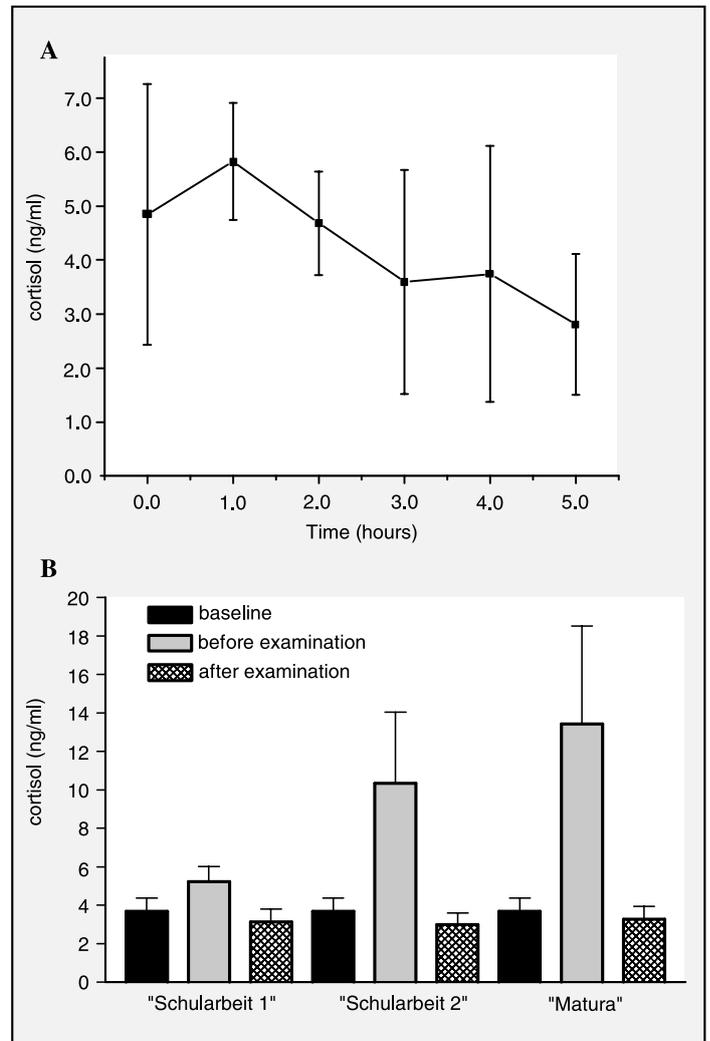


Figure 1. Anticipated examinations modulate saliva cortisol concentration. (A) Time – dependent variation in saliva cortisol concentration (mean \pm standard deviation; $n = 8$) during the morning. Time indicates hours after awaking. First saliva samples were taken immediately after awaking (about 0630h). (B) Saliva cortisol concentration (mean \pm standard deviation) on days without examinations as well as on days with anticipated examinations ("Schularbeit 1", "Schularbeit 2", "Matura"). Notice that the standard deviation for saliva cortisol concentration is about four times the magnitude in "Schularbeit 2" and "Matura" compared to "Schularbeit 1". The increase of the standard deviation is due to a greater heterogeneity of the saliva cortisol concentration among the pupils. In particular one ("Schularbeit 2") or two pupils ("Matura") have had a saliva cortisol concentration of larger than 30 ng / ml. One of these pupils has had an elevated cortisol concentration at "Schularbeit 2" and "Matura".

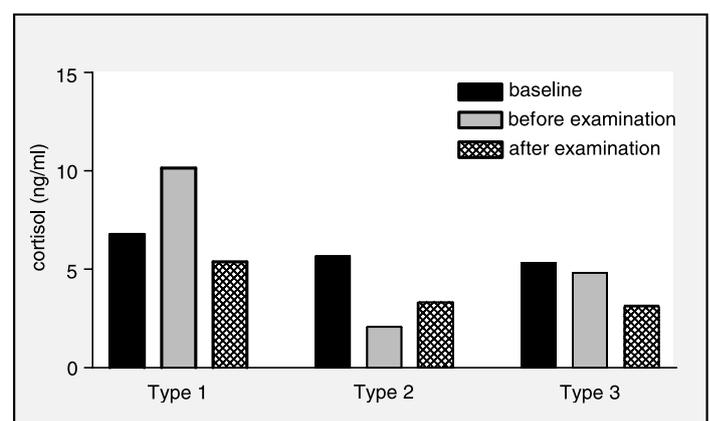


Table 1 Distribution and consistency of the cortisol profile in anticipation of an examination

	Schularbeit 1		Schularbeit 2		Matura		"1 + 2"*		"1 + 2 + M"*	
	%	n	%	n	%	n	%	n	%	n
Type 1	47	8 / 17	64	7 / 11	100	8 / 8	36	4 / 11	50	4 / 8
Type 2	29	5 / 17	18	2 / 11	0	0 / 8	18	2 / 11	0	0 / 8
Type 3	24	4 / 17	18	2 / 11	0	0 / 8	0	0 / 11	0	0 / 8

Saliva cortisol concentration has been quantified on days without anticipated examinations as well as on days with anticipated examinations. According to the cortisol response relative to baseline, we found three types of response patterns. Type 1 is characterized by a transient increase and type 2 by a transient decrease of the cortisol concentrations in anticipation of an examination. Type 3 is characterized by a decrease of saliva cortisol concentration before and after an anticipated examination. "1 + 2" and "1 + 2 + M" represent the number of pupils that have had a consistent cortisol response at "Schularbeit 1" and "Schularbeit" 2 ("1 + 2") as well as at "Schularbeit 1", "Schularbeit" 2, and "Matura" ("1 + 2 + M").

Table 2 Correlation coefficients between saliva cortisol concentration and sensation seeking subscales according to Zuckerman as well as self – reported stress level.

	Saliva cortisol concentration								
	"Schularbeit 1"			"Schularbeit 2"			"Matura"		
	baseline	before	after	baseline	before	after	baseline	before	after
SF	-0.055	0.056	0.115	0.254	0.049	0.481	n.d.	n.d.	n.d.
BS	-0.250	-0.187	-0.567*	-0.434	0.220	0.034	-0.545	0.667	-0.409
TAS	0.087	0.106	0.212	-0.883**	0.381	0.358	-0.778*	0.736	0.149
DIS	-0.180	-0.012	-0.029	-0.254	0.830*	0.424	-0.220	0.723	-0.102
ES	-0.009	0.223	-0.170	-0.766*	-0.123	0.264	-0.545	-0.455	-0.060
Total	-0.109	-0.047	-0.236	-0.686*	0.383	0.402	-0.636	0.464	-0.699

*P < 0.05, ** P < 0.01, number of pupils is between 15 and 19 for "Schularbeit 1" or between eight and nine for "Schularbeit 2" and "Matura", respectively. Numbers represent Spearman correlation coefficient. BS: boredom susceptibility; DIS: disinhibition; ES: experience seeking; SF: self – rated stress level; TAS: thrill and adventure seeking; n. d.: not determined

Comparing saliva cortisol concentration with Zuckerman's subscales did not reveal a consistent association among all three examinations (Table 2). However, we found a significant association between cortisol level and thrill and adventure seeking at "Schularbeit 2" and "Matura" as well as a significant association for disinhibition and experience seeking at one examination ("Schularbeit 2") and a tendency towards the same direction of these variables at "Matura" (Table 2). Statistically significant gender specific effects of cortisol level on Zuckerman's subscales have not been identified (not shown).

Discussion

Early as well as recent reports link academic examinations to a cortisol increase [1, 2, 3, 4, 5, 6, 7, 8]. However, this view has been challenged by studies that reported either no [5, 9, 10] or a decreasing effect on the cortisol level upon academic examinations [11]. In the present study, we identified three types of cortisol responses: Type 1 shows a transient increase before examinations, Type 2 is characterized by a transient decrease, and Type 3 shows a decrease before and after examinations.

The magnitude of cortisol response might vary as a function of the intensity of the stressor. A study by Nejtek reports that high emotional stressors increase

whereas low emotional stressors decrease saliva cortisol concentration [18]. This finding commensurate with our observation that the magnitude of increase of saliva cortisol concentration correlates with novelty of the stressor. "Matura", one of the most stressful examinations during the school career, shows only an increase of cortisol, whereas pupils respond at a regular "Schularbeit" either with an increase or a decrease of cortisol.

Personality traits might be associated with neurotransmitters, such as biogenic amines, or hormones, such as cortisol [19]. Whereas some studies report an association with personality traits and magnitude of cortisol response, others failed to establish an association [12, 13, 14, 15, 20, 21, 22, 23, 24, 25]. A strict association between these parameters would predict that a particular personality trait is linked to a particular cortisol response. With regard to sensation seeking, risk taking negatively correlates with the cortisol level upon psychological stressors [16, 26]. These observations support our finding that thrill and adventure seeking, experience seeking, and disinhibition according to Zuckerman [17] negatively correlates with saliva cortisol concentration. Although these associations were not consistent in all three examinations (see Table 2), our investigation suggest that novelty of the stressor might result in more consistent associations,

e. g. thrill and adventure seeking, experience seeking, and disinhibition at “Schularbeit 2” and “Matura” in our experiments. In the present as well as in published studies, no significant correlation has been obtained between self-reported stress level and cortisol concentration, indicating that cortisol does not affect the feeling of being stressed [11, 13, 18, 27].

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