ORIGINAL ARTICLE

Salivary cortisol in two professions: daily cortisol profiles in school teachers and firefighters

Olga Susoliakova [1,3], Jindra Smejkalova [1], Marie Bicikova [2], Dana Potuznikova [1], Lenka Hodacova [3], Anna Grimby-Ekman [4], Zdenek Fiala [1]

1 Department of Hygiene and Preventive Medicine, Charles University in Prague Faculty of Medicine in Hradec Kralove, Hradec Kralove, Czech Republic
2 Institute of Endocrinology, Department of Steroids and Proteofactors, Prague, Czech Republic
3 Department of Social Medicine, Charles University in Prague Faculty of Medicine in Hradec Kralove, Hradec Kralove, Czech Republic
4 Department of Occupational and Environmental Medicine, University of Gothenburg, Sahlgrenska Academy, Gothenburg, Sweden

Correspondence to: Olga Susoliakova, MSc.
Department of Hygiene and Preventive Medicine,
Charles University in Prague, Faculty of Medicine
Simkova 870, CZ-500 38 Hradec Kralove, Czech Republic.
TEL: +420 495 816 372; E-MAIL: susoliao@lfhk.cuni.cz

Submitted: 2014-02-17 Accepted: 2014-07-07 Published online: 2014-07-15

Key words: **teachers; salivary cortisol measurements; psychic overload**

Neuroendocrinol Lett 2014; **35**(4):314–321 **PMID:** 25038601 NEL350414A07 © 2014 Neuroendocrinology Letters • **www.nel.edu**

Abstract

BACKGROUND: It's indicated negative-perceived stress could induce worse health status and change of cortisol secretion.
OBJECTIVES: To assess salivary cortisol levels in two occupations with a high psychosocial workload, but different features, teachers and firefighters.
METHODS: The study population consisted of 142 school teachers and 136 firefighters. Four saliva samples were collected from pedagogical participants during their busiest workday. The cortisol measures used were: morning values, evening values, slope of decline, ratio (evening value divided by morning value), and area under the curve (AUC).
RESULTS: The salivary cortisol measurements in both genders were almost equal regarding morning values, slope, and AUC increase. Evening values were lower and the relative reactivity was higher (lower ratio) for female teachers, compared to male teachers. There was a tendency of a lower total daytime output of cortisol (AUC ground) among female teachers. Firefighters had lower levels of cortisol, lower total daytime output, and higher relative reactivity (lower ratio), but lower absolute reactivity, regarding both slope and AUC increase.
CONCLUSION: Overall, male teachers might be the group most affected by stress in this study, even if some of their cortisol values were almost equal to the female teachers' values. Male teachers also seemed to be more affected by stress, according to salivary cortisol, compared to male firefighters, even if there were some inconsistencies.

Abbreviations:

ACTH	- adrenocorticotropic hormone	ED	- emergency department nurses
AUC_g	- area under the curve with the respect to ground	GW	- general wards nurses
AUC_i	- area under the curve with the respect to increase	HPA	- hypothalamic- pituitary adrenal axis
CAR	- cortisol awakening response	Ln	- natural logarithm
CI	- confidence interval	RIA	- radioimmunoassay
CNS	- central nervous system	SES	- socioeconomic status
		WRS	- work- related stress

INTRODUCTION

In the European Union, work-related stress (WRS) is the second most common work-related health problem, after back pain. In the educational sector, WRS is one of the most significant health hazards. School teachers are among those professionals with the highest levels of job stress. Many studies have been conducted to investigate sources of teachers' stress as well as the consequences of stress (OSHA 2002). Since Verdugo & Vere (2003) report that stress is a contributor to illness as well as a cause for leaving the teaching profession. Hasselhorn & Nübling (2004) describes in their study the teaching profession as the occupation with the highest risk for poor mental health, especially in schools for pupils with disabilities and in those engaged in complementary education for apprentices. Teacher stress may be defined as the experience, of teachers, of unpleasant, negative emotions, such as anger, anxiety, tension, frustration, or depression (Kyriacou 2001). European Agency for Safety and Health at Work has mentioned that the problem of teacher stress can be linked to the organizational, physical, or social characteristics of work and the work environment. According to the European Trade Union Committee for Education (2011), there exists five "top stressors" and these are: workload/working intensity, role overload, increased class size per teacher, unacceptable pupil behavior, and bad school management or lack of support from management.

Firefighters, on the other hand, are exposed to work stress with quite different characteristics. Their work stress consists of high peak stressors of short duration. In contrast to teachers' exposure to lasting work stress, the stress exposure in firefighters involves exposure to traumatic situations (Milczarek *et al.* 2011).

Salivary cortisol is routinely used as a biomarker of psychosocial stress and related mental or psychic diseases, illustrating hypothalamic–pituitary–adrenal (HPA) axis activity. Saliva collection is non-invasive and salivary biomarkers have the further advantage of being suitable for self-collection (Šimůnková *et al.* 2009). Many early, large-scale studies of cortisol and health focused on "average" cortisol values, particularly urinary measures of cortisol, pooling levels across several hours, and analyzing total exposure to cortisol across 12 or 24 hours (Seeman *et al.* 2002). Free cortisol represents the biologically active hormone fraction and salivary cortisol measures have been considered to be a better indicator of adrenocortical function compared to serum cortisol (Kirschbaum *et al.* 1994).

According to the literature concerning workload and psychosocial stress (Steptoe *et al.* 2000; Masilamani *et al.* 2012), morning cortisol is a suitable marker of adrenocortical activity in teachers. Healthy HPA axis function is thought to require a strong diurnal pattern. Deviations from the typical diurnal cycle of cortisol provide valuable information regarding environmental influences on the HPA axis and the role of the HPA axis in disease processes (Stone *et al.* 2001).

The cortisol awakening response (CAR) is a discrete, but distinctive, part of the cortisol circadian cycle (Clow *et al.* 2004). It is defined as the curve composed by the cortisol levels immediately after awakening and up to about 1 hour after awakening. In healthy subjects, cortisol concentrations increase by between 50% and 160% in the first 30 minutes immediately post-awakening (approximate average increase of 9 nmol, range 4–15 nmol). This response may also play a significant role in the regulatory balance of the immune system (Clow *et al.* 2004). The diurnal cortisol slope is the degree of change (typically, a decline) in cortisol levels from early morning to late evening. A steeper decline in cortisol levels during the day is typically associated with better psychosocial and physical health (Adam & Gunnar, 2001; Adam 2006; Cohen *et al.* 2006).

Another important measure is the total daytime output of cortisol, represented by the area under the curve (AUC) with respect to ground (AUC_g). This is calculated as the area under all the cortisol data points measured across the day. The AUC_g illustrates the average cortisol output, but does not provide any indication of diurnal change (Badrick *et al.* 2007; Nicolson 2004). An AUC reflecting daytime reactivity is the AUC with respect to increase (AUC_i) (Pruessner *et al.* 1997). This is calculated by subtracting the area below the awakening or evening level from the AUC_g. In addition to these measures of salivary cortisol, single-time point measures of morning or evening levels are common in cortisol research (Stewart & Seeman, 2000).

Most studies of WRS have focused on single occupations only (Steptoe *et al.* 2000; Masilamani *et al.* 2012; Perroni *et al.* 2009, Yang *et al.* 2001). It would be beneficial to compare WRS in different professional groups. The aim of the present paper was to assess and compare stress levels according to salivary cortisol in primary school teachers and firefighters.

MATERIALS AND METHODS

Participants

The sample consisted of 142 primary school teachers and 136 firefighters. Participants of both groups were informed of the main purpose of this study. After a personal meeting, they signed the informed consent. All participants received basic instructions on how to fill in the questionnaire and perform saliva collection: for this, they were given a sampling schedule, and instructed not to smoke, use chewing gum or consume alcohol, and not to perform physical exercise during the collection. Further, participants were asked not to brush their teeth before collecting the first sample in the morning. Rinsing of teeth with pure water to remove food residues was allowed during the whole sampling period. Exclusion criteria were: presence of disease, allergy treated by corticoids, psychiatric dis-

orders, endocrine disorders such as diabetes mellitus, and pregnancy in women.

Eight teachers were excluded from the study due to contraindications (e.g., presence of allergy treated by corticosteroids, diabetes mellitus, and depression).

Saliva collection and analysis

Salivette Cortisol Tubes (Sarstedt, Germany) were used for saliva collection. In this study, four samples are used: at 7.00 a.m. (30 minutes after waking); at 11 a.m. (before lunch); at 3 p.m. after work; and at 10 p.m. before sleeping. The teachers were asked to perform their collection during their busiest work day. Firefighters were asked to perform their collection on a day without real emergency situations. Upon completion, saliva samples were centrifuged and stored at –20 °C until analysis. Salivary cortisol (nmol/l) was measured by radioimmunoanalysis with radio ligand I^{125} (a commercial kit Spectria Cortisol radioimmunoassay (RIA) (Orion Diagnostica, Espoo, Finland). The processed samples were measured by a gamma counter (Berthold Company, Bad Wildbad, Germany). The measurements were done by the Department of Steroids and Proteofactors of the Institute of Endocrinology in Prague, Czech Republic, in their certified laboratory. The results for internal controls (low, medium, and high) were also included in the assay.

Assessment of possible confounders

General information, like gender, age, place of residence, marital status, educational level, and years of work experience, was obtained from the questionnaire. Participants were divided into two age groups: <50 years and ≥50 years. Smoking status was divided into current smokers and non-smokers. Physical activity was categorized into three subgroups, based on the question regarding level of physical activity, namely: higher physical activity (more than 4 days/week), healthy physical activity (more than 1 and less than 4 days/week), and lower physical activity (less than 2 days/week).

Statistical analyses

Analyses were done using IBM SPSS Statistics version 20 (SPSS Inc., Chicago, IL, USA). Means and standard deviation (SD) were used in descriptive statistics. In all analyses, cortisol values were transformed using the natural logarithm (ln) due to skewed data. The results are also presented as back-transformed values. Diurnal cortisol level (slope) was measured as the difference between morning and evening saliva cortisol. The ratio, the relative counterpart of the slope, was defined as the ratio between evening and morning values. Area under the curve with respect to ground and AUC_i were calculated according to Pruessner *et al.* (2003). Population intervals were calculated as mean ± 1.96 SD. These intervals were then back transformed into nmol/l values. Confidence intervals were calculated as mean ± 1.96 SEM. These intervals were then back transformed into nmol/l. To analyze occupational differences, regression analysis was performed, with post hoc tests for group comparisons. The outcome was ln-transformed cortisol values. Explanatory variables were occupational group and gender (three categories: female teachers, male teachers, and male firefighters), age, physical activity, and smoking status. All tests were two-sided and statistical significance was set at $p<0.05$.

RESULTS

Among the teachers, 72% were women and 28% men. The average age of teachers was 41 years, with a 95% CI of (39.8; 43.4). Male teachers were older, mean age 45 years, with a 95% CI of (41.1; 48.6), compared to female teachers, mean age 40 years, with a 95% CI of (39.8; 43.4). The proportions of respondents according to their age showed that 73% were younger than 50 years. The majority of teachers had a university education (95.8%). All firefighters were male. Their average age was 37 years, with a 95% CI of (34.8; 37.6): 93% were <50 years old. In the group of firefighters, 19% had a university degree, whereas 81% responded that they had completed high school (see Table 1).

Salivary cortisol levels

Cortisol levels decrease from the morning through the working day and are at their lowest in the evening, as shown in Figure 1. This general pattern seems be consistent with a normal diurnal cortisol rhythm (Martin *et al.* 2003). Among teachers, the unadjusted pattern of salivary cortisol over the working day seemed similar for women and men (Figure 1). The male firefighters had lower cortisol levels compared to the teachers. This pattern was the clearest for the morning values (Figure 1).

According to adjusted values shown in Table 2, morning and evening cortisol values in both groups were within the range of healthy subjects, but some of the results were lower compared to some other stud-

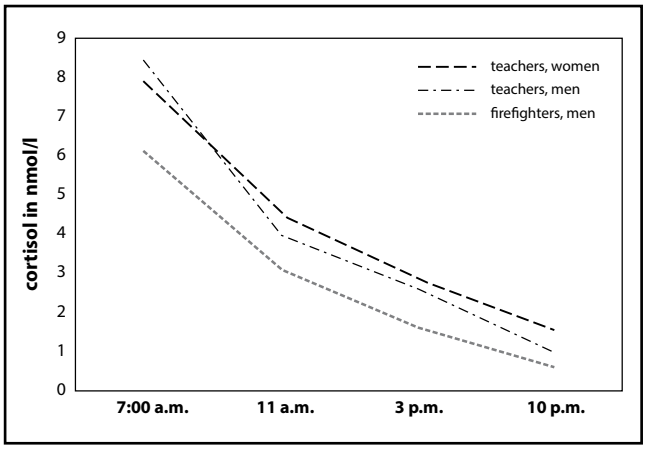

Fig. 1. Diurnal cortisol rhythm in teachers and firefighters.

Copyright © 2014 Neuroendocrinology Letters ISSN 0172–780X • www.nel.edu

Tab. 1. Description of sociodemographic data on participants.

Demographic variables	All (N=142)		Teachers Women (N=102)		Men (N=40)		Firefighters Men (N=136)	
	mean	SD	mean	SD	mean	SD	mean	SD
Average age (yrs)	41.6	10.73	40.3	10.11	45.0	11.69	36.2	8.19
Work experience (yrs)	15.5	10.39	14.4	9.79	18.4	11.40	12.5	8.59
Place of residence	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
City	5	3%	2	2%	3	8%	19	14%
Town	79	56%	59	58%	20	50%	46	34%
Village	58	41%	41	40%	17	42%	71	52%
Educational level								
High school education	6	4%	0	0%	6	15%	110	81%
Lower university degree	0	0%	0	0%	0	0%	11	8%
Higher university degree	136	96%	102	100%	34	85%	15	11%
Marital status								
Married	97	68%	67	66%	30	75%	81	60%
Single	14	10%	9	9%	5	13%	18	13%
Divorced	22	16%	18	17%	4	10%	29	21%
Widowed	2	1%	2	2%	1	2%	2	2%
Partner	7	5%	6	6%	0	0%	6	4%
Smoking status								
Smoker	20	14%	14	14%	6	15%	19	86%
Non-smoker	122	86%	88	86%	34	85%	117	14%
Physical activity								
Higher physical activity	87	61%	69	68%	18	55%	37	27%
Healthy physical activity	54	38%	32	31%	22	45%	98	72%
Lower physical activity	1	1%	1	1%	0	0%	1	1%

SD = standard deviation

ies. For example, in a Swedish study by Aardal and Holm (1995), in healthy females, morning cortisol levels reached approximately 12.0 nmol/l; in healthy males, levels were around 10.7 nmol/l. Evening cortisol was given as 1.8 nmol/l. This discrepancy could be expected considering the difference in method (due to the RIA recovery method), population, and sampling time. Even if mean values were in the healthy range in that study, the individual variation was extensive. For example, morning values of cortisol among teachers ranged from 3.7 nmol/l to 17.9 nmol/l for women and from 3.9 nmol/l to 14.2 nmol/l for men (Aardal & Holm 1995).

Morning cortisol values were similar among female teachers (7.9 nmol/l) and male teachers (8.2 nmol/l) in our study. Male teachers had statistically significantly higher evening cortisol levels (0.5 nmol/l) compared to female teachers (0.2 nmol/l).

Diurnal cortisol levels in absolute values were close to equal in male (6.6 nmol/l) and female teachers (6.4 nmol/l). The relative diurnal change (ratio between evening and morning values) indicates better health the closer to zero it is. The relative diurnal change was 0.02 nmol/l for female teachers, which was statistically significantly lower than the ratio for male teachers, 0.05 nmol/l (Table 2).

The total daytime output of cortisol seemed to be higher for male teachers compared to female teachers, but not statistically significant, while the daytime reactivity, measured by AUC_i, was close to equal comparing male and female teachers.

Considering the age groups, higher evening cortisol was recorded in older men and women compared to younger subjects, but statistical significance between age groups was seen only in male teachers ($p<0.005$) (not shown in the Table 1).

Tab. 2. Salivary cortisol measurements in teachers and firefighters, adjusted for age, physical activity, and smoking status. Statistical analyses were performed on natural logarithm (ln) transformed values, but the results are also presented in nmol/l.

Salivary cortisol (nmol/l)	Teachers (Women: N=102, men: N=40)				Comparing women and men	Firefighters (Men: N=136)		Comparing male teachers and firefighters
	females	95% CI	males	95% CI	*p*-value	males	95% CI	*p*-value
ln morning cortisol	2.1	(1.86; 2.26)	2.1	(1.87; 2.32)	0.629	1.8	(1.60; 2.00)	<0.001
Morning cortisol	7.9	(6.42; 9.59)	8.2	(6.49; 10.22)		6.1	(4.96; 7.43)	
ln evening cortisol	–1.8	(–2.87; –0.71)	–0.7	(–1.96; 0.48)	0.010	–1.8	(–2.90; –0.72)	0.011
Evening cortisol	0.2	(0.05; 0.49)	0.5	(0.14; 1.62)		0.2	(0.05; 0.48)	
ln diurnal slope	1.9	(1.60; 2.12)	1.9	(1.60; 2.19)	0.768	1.7	(1.42; 1.95)	0.042
Diurnal slope	6.4	(4.96; 8.38)	6.6	(4.93; 8.93)		5.4	(4.13; 7.02)	
ln ratio	–3.9	(–4.92; –2.78)	–2.8	(–4.05; –1.62)	0.014	–3.6	(–4.69; –2.53)	0.061
Ratio	0.02	(0.007; 0.06)	0.05	(0.017; 0.20)		0.02	(0.009; 0.07)	
ln AUC_g	3.9	(3.66; 4.11)	4.1	(3.79; 4.31)	0.064	3.6	(3.37; 3.83)	<0.001
AUC_g	48.4	(38.98; 60.94)	57.4	(44.25; 74.44)		37.8	(29.13; 46.06)	
ln AUC_i	3.6	(3.32; 3.81)	3.5	(3.25; 3.80)	0.679	3.4	(3.10; 3.60)	0.067
AUC_i	35.2	(27.60; 45.24)	33.8	(25.80; 44.88)		28.5	(22.19; 36.60)	

AUCg = area under the curve with respect to ground; AUCi = area under the curve with respect to increase; CI = confidence interval.

Occupational differences among male teachers and firefighters were found in all cortisol measurements (Table 2), but were not statistically significant for ratio and AUC_i. Firefighters had lower levels of cortisol, lower total daytime output, and higher relative reactivity (lower ratio), but lower absolute reactivity, regarding both slope and AUC_i.

DISCUSSION

Morning and evening salivary cortisol values were within the normal range in both teachers and firefighters, although the individual variation was quite large, as mentioned in the Results. Overall, the results indicate that in this study, male teachers may be most affected by stress, even if some cortisol measurements were close to equal to the female teachers' values. Male teachers also seemed to be more affected by stress, according to salivary cortisol, than male firefighters, even if there were some inconsistencies. One possible reason why the results do not point to high occupational stress at a group level might be that group stress levels may be too low to be shown in salivary cortisol (Moya-Albiol *et al.* 2010), while the individual variation might show clearly higher values.

Among teachers, the morning saliva cortisol levels were similar between men and women. Male teachers had higher levels compared to female teachers during the working day and also had higher evening cortisol levels, indicating a somewhat flatter slope and a clearly smaller relative slope for male teachers. These results are similar to results shown by Steptoe *et al.* (2000), who recorded higher cortisol values in male teachers. The total daytime output of cortisol also tended to be higher in male teachers compared to female teachers.

In male teachers, less increased levels may reflect the pattern of heightened HPA responsivity to acute challenge. Perhaps, males tend to interpret confrontations with a potentially detrimental situation in a different way compared to females. Kirschbaum *et al.* (1999) propose that males seem to be have elevated adrenocorticotropic hormone (ACTH) responses and a stronger hypothalamic drive in response to stressful stimulation compared to females.

Morning cortisol is a good predictor of unbalanced states and/or pathologies such as burnout (Grossi *et al.* 2005). Effort–reward imbalance has in earlier studies been negatively associated with salivary cortisol levels at awakening and positively associated with cortisol awakening response (Eller & Nielsen 2012). In the present study, the morning values in teachers were about 8 nmol/l. The obtained absolute levels of salivary cortisol in this study were somewhat lower compared to other studies. Masilamani *et al.* (2012) report a concentration of 2.24 nmol/l in Malaysian female teachers compared to 2.64 nmol/l in male teachers. In Moya-Albiol *et al.* (2010), the morning cortisol concentration in teachers scoring high levels of burnout was higher (18.92 nmol/l) in comparison to teachers with lower levels of burnout (3.254 nmol/l). Pruessner *et al.* (1999) observed a similar overall cortisol concentration to the concentration reported, in the mentioned studies, in teachers suffering from burnout (15.3 nmol/l). Note that in the present study, the upper 2.5th percentile of

Copyright © 2014 Neuroendocrinology Letters ISSN 0172–780X • www.nel.edu

the female teachers was estimated to have morning cortisol values of ≥17.87 nmol/l. For male teachers, this upper 2.5th percentile value was 14.17 nmol/l. Our female teachers therefore seemed to have lower average morning cortisol compared to the male teachers, but showed larger variation and, therefore, a larger proportion of individuals with extreme cortisol values. Bellingrath *et al.* (2008) showed dysregulation in school teachers, not only to burnout, but to vital exhaustion and effort–reward imbalance. In our case, a probability of low levels of burnout can be assumed. It would be useful to verify this in further research.

In some instances, low early morning cortisol and a less marked slope to the daytime rhythm have been associated with lower socioeconomic status and evidence of less successful development and decreased wellbeing (Brandstädter *et al.* 1991). Generally, the teaching profession is a profession with potentially lower socioeconomic status.

The evening cortisol level was higher in male teachers, and a difference was seen between younger and older males. This indicates that aging can be a potential confounder regarding increased salivary cortisol, which is supported by Ferrari *et al.* (2001).

The relative increase in cortisol concentrations in the evening and at nighttime is usually linked to a flattening of the cortisol circadian profile. The impairment of evening cortisol levels may be a marker of a reduced activity of central cholinergic and serotoninergic pathways. Raff *et al.* (1999) likewise describe higher nocturnal values in elderly males (2.3 nmol/l) compared to females (2.1 nmol/l) but no difference in morning cortisol levels between older subjects and younger controls. In a large study by Cohen *et al.* (2006), associations were reported between lower socioeconomic status and potentially higher evening salivary cortisol.

In the present study, male firefighters had a lower reactivity of daytime cortisol, which is not a healthy sign. However, in our population, the absolute levels in the morning and the total daytime output were lower among male firefighters. Firefighting is an occupation with high variability and unpredictable work demands (Bos *et al.* 2004; Kales *et al.* 2007), but at the same time firefighters are an occupational group highly skewed towards physically fit individuals.

Cortisol values were lower in the firefighters compared to the group of teachers. Perroni *et al.* (2009) evaluated the effect of simulated firefighting on salivary cortisol reactivity. No differences emerged between pre-intervention and post-intervention; in other words, no hormonal changes were attributable to the intense physical stress of the simulated intervention. They concluded that the firefighters' daily work stressors were of mild to moderate intensity, or alternatively that the firefighters may have adapted to stress.

For a comparison with other occupations, Yang *et al.* (2001) assessed WRS, using salivary cortisol, in two groups of nurses. Emergency department (ED) nurses perceived higher levels of stress compared to general ward (GW) nurses. Morning cortisol was significantly lower in ED nurses (9.10 nmol/l, 95% CI [6.62; 12.42]) compared to GW nurses (15.45 nmol/l, 95% CI [11.86; 20.14]). These results are much closer to our results. This implies the possibility of using a single morning cortisol sample to assess self-perceived stress.

Rosmond *et al.* (1998) suggest that the variability in cortisol values in individuals is determined by the difference between morning and evening. Therefore, a large decrease in cortisol values, from morning to evening, is typically associated with a normal diurnal curve.

By contrast, a flattened curve indicates either lower morning values or higher evening values, or both. Adam and Kumari (2009) propose that the steeper decline in the diurnal slope indicates better psychosocial and physical health.

In the group of female teachers included in this study, a steeper decline in the cortisol slope was obtained. In male teachers, it was flattened. These results were most clear for the relative diurnal slope. Among firefighters, the diurnal pattern seemed to be attenuated. According to Kudielka *et al.* (2006), this situation can be explained from a biological perspective. They also argue that adaptation to stress involves a successive reduction in cortisol response (McEwen 2000). When this happens, cortisol levels usually return to normal levels via a process called homeostasis (Kaye & Lightman, 2005).

Pruessner *et al.* (2003) describe overall hormonal output as good reactivity to daily stressors. Greater hormonal output is usually considered to imply worse health status (Hagger-Johnson *et al.* 2004). Based on our results, female teachers showed smaller overall hormonal output and higher reactivity to daily stressors, whereas male teachers had opposite results – greater hormonal output and smaller reactivity to daily stressors. In firefighters, we recorded smaller hormonal output and smaller reactivity compared to male teachers. This could be due to their adaptation to stress, as mentioned.

LIMITATIONS

The samples in this study included no more than 1 working day, which limits the ability to examine real exposure to stress. To capture stress over a longer term, measurements should include several working days and one resting day. We also worked with single measurements, which mean lower predictability of potential work-related stress. Dickerson and Kemeny (2004) reviewed 208 studies of laboratory stressors and determined that those most likely to produce a consistent cortisol response were those that involved uncontrollability or posed a social–evaluative threat (e.g., "others will think badly of you …"). However, even within these parameters, all stress paradigms do not produce the expected cortisol elevation.

ACKNOWLEDGEMENTS

This study was supported by the Faculty of Medicine in Hradec Kralove, Charles University, Prague, Czech Republic (PRVOUK 37/09), and by the Ministry of Education, Youth and Sports of Czech Republic (Specific university research project SVV No. 266901).

REFERENCES

1 Aardal E, Holm AC (1995). Cortisol in saliva – reference ranges and relation to cortisol in serum. Eur J Clin Chem Clin Biochem. **33**: 927–932.
2 Adam KE, Kumari M (2009). Assessing salivary cortisol in large-scale, epidemiological research. Psychoneuroendocrinology. **34**: 1423–36.
3 Adam KE (2006). Transactions among adolescent trait and state emotion and diurnal and momentary cortisol activity in naturalistic settings. Psychoneuroendocrinology. **31**: 664–79.
4 Adam KE, Gunnar RM (2001). Relationship functioning and home and work demands predict individual differences in diurnal cortisol patterns in women. Psychoneuroendocrinology. **26**: 189–208.
5 Badrick E, Kirschbaum C, Kumari M (2007). The relationship between smoking status and cortisol secretion. J Clin Endocrinol Metab. **92**: 819–824.
6 Bellingrath S, Weigl T, Kudielka BM (2008). Cortisol dysregulation in school teachers in relation to burnout, vital exhaustion, and effort–reward-imbalance. Biological Psychology. **78**(1): 104.
7 Bos, J, Mol, E, Visser, B, Frings-Dresen, MH (2004). The physical demands upon (Dutch) fire- fighters in relation to the maximum acceptable energetic workload. Ergonomics. **47**(4): 446–460.
8 Brandtstädter J, Baltes-Götz B, Kirschbaum C, Hellhammer D (1991). Developmental and personality correlates of adrenocortical activity as indexed by salivary cortisol: observations in the age range of 35 to 65 years. J Psychosom Res. **35**(2): 173–185.
9 Clow A, Thorn L, Evans P, Hucklebridge F (2004). The awakening cortisol response: methodological issues and significance. Stress: The International Journal on the Biology of Stress. **7**(1): 29–37.
10 Cohen S, Schwartz JE, Epel E, Kirschbaum C, Sidney S, Seeman T (2006). Socioeconomic status, race, and diurnal cortisol decline in the Coronary Artery Risk Development in Young Adults (CARDIA) Study. Psychosom Med. **68**(1): 41–50.
11 Dickerson et al (2004). Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. Psychol Bull. **130**(3): 355–391.
12 Eller NH, Nielsen SF (2012). Effort reward imbalance, and salivary cortisol in the morning. Biol Psychol. **89**(2): 342–348.
13 European Agency for Safety and Health at Work (2002). Facts22 Work related stress. p.1, Available online: http: //osha.europa.eu/en/publications/factsheets/22
14 European Trade Union Committee for Education (2011). Teachers' work-related stress: Assessing, Comparing and Evaluating the Impact of Psychosocial Hazards on Teachers at their Workplace. p. 1–28 Available online: http: //etuce.homestead.com/Publications2011/WRS_Brochure_Final_ENG_doc.pdf
15 Ferrari E, Cravello L, Muzzoni B, Casarotti D, Paltro M, Solerte SB, Fioravanti M, Cuzzoni G, Pontiggia B, Magri F (2001). Age-related changes of the hypothalamic-pituitary-adrenal axis: pathophysiological correlates. Eur J Endocrinol. **144**(4): 319–329.
16 Grossi G, Perski A., Ekstedt M, Johansson T, Lindström M, & Holm K (2005). The morning salivary cortisol response in burnout. J Psychosom Res. **59**: 103–111.
17 Hagger-Johnson GE, Whiteman MC, Wawrzyniak AJ, Holroyd WG (2010). The SF-36 component summary scales and the daytime diurnal cortisol profile. Qual Life Res. **19**(5): 643–51
18 Hasselhorn HM, Nübling M (2004). Arbeitsbedingte psychische Erschöpfung bei Erwerbstätigen in Deutschland. Work- related mental exhaustion among workers in Germany [in German]. Arbeitsmed Sozialmed Umweltmed. **39**: 11.
19 Kales, SN, Soteriades, ES, Christophi, CA, Christiani DC (2007). Emergency duties and deaths from heart disease among firefighters in the United States. New Engl J Med. **356**(12): 1207–1215.
20 Kaye JM, Lightman SL (2005). Psychological stress and endocrine axes. Human Psychoneuroimmunology. p. 25–52.
21 Kirschbaum C, Hellhammer DH (1994). Salivary cortisol in psychoneuroendocrine research: recent developments and applications. Psychoneuroendocrinology. **19**(4): 313–333.
22 Kirschbaum C, Kudielka BM, Gaab J, Schommer NC, Hellhammer DH (1999). Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. Psychosom Med. **61**(2): 154–162.
23 Kudielka BM, Von Känel R, Preckel D, Zgraggen L, Mischler K, Fischer JE (2006). Exhaustion is associated with reduced habituation of free cortisol responses to repeated acute psychosocial stress. Biol Psychol. **72**(2): 147–153.
24 Kyriacou C. (2001). Teacher stress: Directions for future research. Educ Rev. **53**: 27–35.
25 Martin PA, Crump MH (2003). The adrenal gland. In Dooley MP, Pineda MH. McDonald's veterinary endocrinology and reproduction (5th ed.). Ames, Iowa: Iowa State Press.
26 Masilamani R, Darus A, Ting Su A, Ali R, Mahmud Awang Bulbiga A, David K (2012). Salivary Biomarkers of Stress Among Teachers in an Urban Settings. Asia-Pac J Public Health. **24**(2): 278–287.
27 McEwen, BS. Allostasis and allostatic load: Implications for neuropsychopharmacology (2000). Neuropsychopharmacology. **22**(2): 108–124.
28 Milczarek M, Hanke, A, Georgiadou P, Pinotsi D, Kallio H, Lusa S, Malmelin J, Punakallio A, Pääkkonen R, de Meyer S, Nicolescu GI: Emergency Services: A Literature Review on Occupational Safety and Health Risks (2011). European Agency for Safety and Health at Work. p. 1–80
29 Moya-Albiol L, Serrano MÁ, Salvador A (2010). Job satisfaction and cortisol awakening response in teachers scoring high and low on burnout. Span J Psychol. **13**(2): 629–636.
30 Nicolson N (2004). Childhood parental loss and cortisol levels in adult men. Psychoneuroendocrinology. **29**(8): 1012–1018.
31 Perroni F, Tessitore A, Cibelli G, Lupo C, D'Artibale E, Cortis C, Capranica, L (2009). Effects of simulated firefighting on the responses of salivary cortisol, alpha-amylase and psychological variables. Ergonomics. **52**(4): 484–491.
32 Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH (2003). Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology. **28**(7): 916–31.
33 Pruessner, JC, Hellhammer, DH, Kirschbaum C (1999). Burnout, perceived stress, and cortisol responses to awakening. Psychosom Med. **61**(2): 197–204.
34 Pruessner JC, Wolf OT, Hellhammer DH, Buske-Kirschbaun A, Von Aurer K, Jobst S, Kirschbaun C (1997). Free cortisol levels after awakening: a reliable biological marker for the assessment of adrenocortical activity. Life Sci. **61**(26): 2539–49.
35 Raff H, Raff JL, Duthie EH, Wilson CR, Sasse EA, Rudman I, Mattson D (1999). Elevated Salivary Cortisol in the Evening in Healthy Elderly Men and Women: Correlation with Bone Mineral Density. J Gerontol A Biol Sci Med Sci. **54**(9): M479–M483.
36 Rosmond R, Dallman, MF, Björntorp P (1998). Stress-related cortisol secretion in men: relationships with abdominal obesity and endocrine, metabolic and hemodynamic abnormalities. J Clin Endocrinol Metab. **83**(6): 1853–1859.
37 Seeman TE, Singer BH, Ryff CD, Dienberg LG, Levy-Storms L (2002). Social relationships, gender, and allostatic load across two age cohorts. Psychosom Med. **64**(3): 395–406.
38 Steptoe A, Cropley M, Griffith J, Kirschbaum C (2000). Job strain and anger expression predict early morning elevations in salivary cortisol. Psychosom Med. **62**(2): 286–92.

 Copyright © 2014 Neuroendocrinology Letters ISSN 0172–780X • **www.nel.edu**

39 Stewart J, Seeman T (2000). Salivary cortisol measurement. Summary of a meeting of the John D. and Catherine T. MacArthur Research Network on Socioeconomic Status and Health. Available online: http: //www.macses.ucsf.edu/research/allostatic/salivarycort.php
40 Stone AA, Schwartz JE, Smyth J, Kirschbaum C, Cohen S, Hellhammer D, Grossman S (2001). Individual differences in the diurnal cycle of salivary free cortisol: a replication of flattened cycles for some individuals Psychoneuroendocrinology. **26**(3): 295–306.
41 Šimůnková K, Stárka L, Dušková M, Hill R (2009). Současné možnosti využití stanovení steroidných hormonů v slinách. (Steroids in saliva: current application possibilities). [in Czech]. DMEV. **12**(3): 155–16.
42 Yang Y, Koh D, Ng V, Lee F CY, Chan G, Dong F, Chia, SE (2001). Salivary cortisol levels and work-related stress among emergency department nurses. Int J Occup Environ Med. **43**(12): 1011–1018.
43 Verdugo R, Vere A (2003). Workplace violence in service sectors with implications for the education sector: Issues, solutions and resources. International Labour Office. p. 1, Available online: http: //www.ilo.org/public/libdoc/ilo/2003/103B09_304_engl.pdf.