Salivary cortisol and alpha-amylase: Is there consistency between psychosocial stress test and burdensome work shifts?

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ABSTRACT

This study examined the consistency of salivary cortisol and alpha-amylase (sAA) total daily secretion between laboratory and eld circumstances. The 95 participants were shift working female health care professionals with high (n = 53) or low (n = 42) psychosocial stress (job strain) measured by the Job Content Questionnaire (JCQ). The Trier Social Stress Test including a 5-min free speech and a men- tal arithmetic task was conducted with four, and eld measurements with three daily saliva samples of cortisol and sAA during circadian rhythm and inter-shift recovery controlled morning shift, night shift, and a day o . The associations of salivary cortisol and sAA area under the curve with respect to ground (AUCg) and area under the curve with respect to increase (AUCi) between laboratory and eld were tested using OLS (Ordinary Least Squares) regression. The sAA AUCg output in the labora- tory was correlated with the output during all eld measurement days and similarly among high and low job strain groups (p < 0.001). SAA AUCi and salivary cortisol AUCg and AUCi were not correlated between laboratory and eld measurement, neither in the whole sample nor among the low or high job strain group. In conclusion, a laboratory measure of sAA AUCg output is promising in predicting stress-related output during burdensome work shifts and leisure time, whereas sAA AUCi or salivary cortisol seem not to have this potential.

Introduction

Establishing simple, reliable, and consistent biomarkers for measuring stress response is a major challenge in psychophysiological research. Using saliva sampling when collecting stress biomarkers is a non-invasive method, and considered to be reliable and relatively low cost.[1,2] As laboratory studies are more cost-effective than field studies, and various intervening factors are easier to control in laboratory than in field, it would be useful to identify laboratory biomarkers for stress which can reliably predict biomarker stress response also in real-life context.

To accurately represent stress physiology, the measurements should encompass the main stress-related pathways, i.e., hypothalamic-pituitary-adrenal axis (HPA- axis) and autonomic (sympathetic) nervous system (SNS).[3] As a primary outcome hormone of the HPA-axis, cortisol is the most frequently measured stress biomarker. In healthy individuals, a diurnal cortisol rhythm shows a substantial peak shortly after awakening and decline over the day resulting in low levels in the evening.[4] An acute stressful situation induces profound cortisol secretion in most of the people.[5] The studies of cortisol profiles during stress show conflicting results[6] but a meta-analysis concluded that chronic stress may lower the cortisol awakening response (CAR) and elevate the evening nadir.[7] Field studies show that shift work including night work is associated with elevated cortisol secretion.[8] Salivary alpha-amylase (sAA) is primarily a digestive enzyme which is produced by the salivary glands. Recently, sAA has been studied as a sympathetic stress biomarker.[9] SAA has systematically been reported to have a rapid stress responsiveness and the typical AA response pro le is consistent with knowledge of the rapid activation and recovery that characterizes the response of SNS to stress.[10] In laboratory settings, sAA has been shown to re ect increased norepinephrine levels in blood induced by corticotrophin releasing

factor. This supports the view that sAA would be useful in the assessment of work stress-related cardiovascular and metabolic changes.[11]

Collecting salivary AA is non-invasive[12] and the enzyme seems not to exhibit significant gender or age differences.[2,13,14] Normal diurnal AA pro le has a dip after awakening and a steady increase during the course of day.[15] Long-term stress is associated with flatter diurnal slopes and decreased daily production of sAA.[2] To date, there is very little research on work-related stress and sAA.[6,16–18] The one study conducted among shift work- ers authors are aware of found no association of sAA profiles with work stress.[18]

Laboratory stress studies may reflect the way in which individuals react to stress outside laboratory.[19] An individual who is highly reactive in the laboratory would be prone to experiencing repeated episodes of heightened arousal in real life, and that will subsequently have an impact on health risks.[20] The associations between laboratory and real-life responses may, however, be dependent on the measured physiological parameters. Real-life stressors also appear to produce responses that are often larger than those seen in the laboratory,[21] and that can be regarded as laboratory measures lack of ecological validity.[22,23]

This article reports one of the first studies which com- bines laboratory measurements of stress reactivity to field measurements of the same biomarkers. This study aimed to explore the consistency of stress biomarkers salivary cortisol and sAA between laboratory and field measurements with a widely used parameter area under the curve (AUC). The study hypothesis was that acute stress reactivity, according to laboratory-measured cortisol and sAA outputs, is associated with the outputs of these biomarkers in work shifts that are experienced burdensome.

Methods

Study sample

Female health care professionals with night shift work (n = 5,615) were identified from the Finnish Public Sec- tor Study (FPSS) cohort. An invitation letter was sent to employees (n = 422) fulfilling the inclusion criteria such as, age 30–58 years, Body Mass Index <35 kg/m2, at least 3 years' work experience in the same ward, and the work- place located in Southern Finland (full list of the inclusion and exclusion criteria in Karhula et al.[24]). No statistically significant differences (p-values >.106) were seen in the background variables between the 95 participants and the 422 invited.[24]

An invitation letter was sent to the workplace of participants fulfilling the inclusion criteria. The most common reasons for exclusion (n = 65) were changing ward or workplace (n = 22) or quitting night shift work (n = 21).

Psychosocial stress at work (job strain) was measured by the Job Content Questionnaire (JCQ)[25] including job demands (JD, three items), i.e., pace and pressure of work tasks, and job control (JC, nine items), regarding, e.g., autonomy over working time and methods. The high (HJS) and low job strain (LJS) groups were formed by grouping the wards with at least five respondents on the JD and JC scales, using median split to identify HJS (high demands and low control) and LJS (low demands and high control) wards. Subsequently, employees were divided into to the HJS or LJS groups based on their individual mean JD and JC scores. To increase contrast between job strain groups, the employees belonging to the quartile with the least strain in the HJS group (n = 86) and the most strain in the LJS group (n = 48) were excluded. The mean values for job demands/job control were 4.56/3.20 in the HJS group and 2.64/4.11 in the LJS group (Mean Difference 1.92/−0.90, p < .001). The reliability coefficients for JCQ for those invited to participate in the study (n = 422) were good

(Cronbach's α = 0.87 for JD and α = 0.80 for JC). The forming of job strain groups has been more precisely described in Karhula et al.[24]

The participants were more often from medical- surgical wards than other wards (e.g., intensive care, emergency, or maternity units) compared to those invited to participate (45% vs. 33%, p < .037).

The study was approved by the Coordinating Ethical Committee of the Hospital District of Helsinki and Uusimaa. Signed informed consent was obtained from each participant. The participants were compensated for travelling expenses and given €50 (approx. $58) as compensation for participation.

Measures

The study included an internet-based questionnaire (Digium QuestBack Company, Espoo, Finland) featuring questions about background, health habits, work conditions, and working hours. The background variables were BMI (body mass index), number of children, level of edu- cation, physical activity (exercising at least on moderate intensity, times per week, at least 30 min/day, dur- ing the past 3 months), sleep length, cigarette smoking, chronotype (Morningness-eveningness questionnaire,[26] stressful life events during the past 12 months,[27] Beck's Depression Inventory (BDI-II),[28] prescription medication use including oral contraceptives and hormone replacement therapy, and fasting blood glucose.

The participants arrived to the Finnish Institute of Occupational Health (FIOH, Helsinki, Finland) at 9 am, where a 12-hr fasting blood sample was collected before breakfast. A 10-min Trier Social Stress Test (TSST) with speech and mental arithmetic[5] was conducted with four saliva samples of cortisol and sAA. The baseline saliva sample was collected at approximately 10:30 am.

The participants were instructed to play a role of a job applicant in an interview for a research nurses' job. The participants had 5-min preparation time before giving a 5-min speech to convince one unknown assessor that she would be the best applicant for the position. In the beginning of the TSST, the T1 saliva sample was collected. Whenever the speech was finished in less than 5 min, the participant was told to continue in a standardized way as described in TSST protocol.[5]

After the speech, the participant was asked to serially subtract the number 13 from 2083 as fast and accurately as possible for 5 min. On every failure, the participants had to restart from 2083. At time 10 min, the task was stopped and the T2 saliva sample was collected. The research nurse debriefed the participant about the goal of the test and that no video analysis would be performed. The T3 saliva sample was collected 15 min after completing the TSST. All the saliva samples were collected using Salivette tubes and cotton rolls, which the participants placed in their mouths for at least 1 min and then replaced into the Salivette tube.

During the 3-week field study, the participants' sleep patterns and sleep-wake rhythm were measured using a sleep diary and actigraphy (Actiwatch AW7, Cambridge Neurotechnology Ltd., Cambs, UK), which was analyzed by Actiwatch Activity and Sleep Analysis 7 software. Field measures of salivary cortisol and sAA were con- ducted on 3 non-consecutive days (24 hr), including one morning shift (mostly 07:00–15:00), one night shift (mostly 21:00–07:00), one day o . To optimize similarity in participants' circadian rhythm (circadian disruption due to working shifts) and recovery, the pre-selected measurement days were: (1) at least the third consecutive morning shift, where the effect of several consecutive early awakenings was demonstrated; (2) the first night shift after a morning or evening shift, where the

change of sleep-wake cycle was most evident; and (3) the second consecutive day off , where the participants' circadian rhythm would be close to normal.

The participants collected saliva samples immediately after awakening (AW) and 30 min after awakening (AW30), and before going to sleep (around 8 am after the night shift). The participants wrote down the exact time of the sample collection to the test tube and to the field measurement diary, and were instructed to refrain from, e.g., heavy exercise, eating, drinking, and brushing teeth before the sample collection. The mean saliva sample collection times were during the morning shift measurements on average at 05:27 (AW), 05:57 (AW30), and 22:36 in the evening, during the night shift measurements at 08:08 (AW), 08:41 (AW30), and 08:06 the next morning before going to sleep, and during the day of measurements at 07:56 (AW), 08:21 (AW30), and 21:34 in the evening. The AW and AW30 samples were collected on average 6 and 35 min after sleep end (determined from actigraph data) in the morning shift, 8 and 38 min after sleep end in the night shift, and 6 and 35 min after sleep end on the day off .[29]

Each measurement days' saliva samples were stored in the participants' home refrigerators and mailed to FIOH by regular post, as these samples tolerate the prevailing temperatures during shipment.[2,30] The samples were analyzed in FIOH laboratories using a LIA kit (LIA, IBL, Hamburg, Germany) and a measurement range of 0.43–110 nmol/l for salivary cortisol, and sAA Kinetic enzyme assay kit (item no. 1–1902, Salimetrics, USA) and measurement range of 2.0–900 U/mL for sAA.[6,10] The cortisol was pipetted first from the sample, and while cortisol was incubated, the sAA was analyzed. The field samples were also collected using Salivette and analyzed in the same way as the TSST saliva samples. 99% of the saliva samples were collected and analyzed successfully.

Participants

There were 42 participants in the HJS group and 53 in the LJS group (mean age 47.2 years). More nurses volunteered to the LJS group (81%) than to the HJS group (60%, p = .020) and fewer nursing assistants, respectively (p < .01). There was no difference in any of the background variables between the groups with one exception. The LJS group participants were physically active more often than the HJS group participants (p < .02). The descriptive statistics are shown in Table 1, and have been previously published by Karhula et al.[24,29]

Statistical analysis

A logarithmic transformation of the stress biomarker original values was used due to skewed distributions. The area under the curve with respect to ground (AUCg, nmol/l × min) and the area under the curve with respect to increase (AUCi, nmol/l × min)[31] were calculated for logarithmic transformed stress biomarkers assuming linearity between the consecutive measurement points (Figure 1). The use of AUC simpli es a multivariate data into univariate space,[32] and reduces the number of statistical comparisons between the group.[23] OLS (Ordinary Least Squares) regression was used to test the consistency of biomarker AUCg results.[33] Education and physical activity were included to the regression model, as there was a statistically significant difference in those ground characteristics between the job strain groups. Physical activity has earlier been shown to have a stress-bu ering capacity[34] and work tasks and demands differ between nurses and nursing assistants. Statistical analyses were conducted using the IBM SPSS Statistics 20 software package (Chicago, IL).

Results

Consistency of stress biomarker levels and AUCg's in laboratory vs. field

The consistency of sAA AUCg's in the di erent shifts and day off between laboratory and field setting was strong (p-values <0.001, and t-values >6.4), both in all participants, and participants in both high and low job strain groups. Contradictory, the AUCi's showed no signi cant consistency between laboratory and field measurements neither in all participants nor in the high and low job strain groups.

The salivary cortisol AUCi's and AUCg's had low consistency between laboratory and field measurements both among all participants and in the high and low job strain groups (Table 2). Additional analysis with age and smoking status as covariates showed same results than presented above both among all participants and in the high and low job strain groups (results not shown).

Discussion

This methodological study compared salivary stress biomarker outputs, cortisol and sAA, between laboratory and eld conditions. The sAA AUCg's correlated systematically and relatively strongly between stress reactivity and burdensome work shifts or a day o , whereas sAA AUCi's and salivary cortisol AUCi's and AUCg's had low consistency between laboratory and field measurements.

To the authors' knowledge, this is the first study combining sAA measurements in laboratory withmeasurements outside laboratory in any kind of field or occupational settings. The lack of previous research results with regard to sAA prevents from comparing the results from this study. Previously, however, sAA has been found to have a good intra-individual stability

when measured repeatedly, e.g., on consecutive days,[12,15,35] a finding which is consistent with the results from this data.

In this study, the salivary cortisol AUC's had a poor consistency between laboratory and field measurements. This result is similar to a study of teachers' cortisol responses[22] in naturalistic and laboratory stress situations. Despite extensive research during the last decades, the e ects of chronic stress on cortisol are still inconsistent.[6] It seems that work stress elicits normal activation responses that most of the people are capable to cope with or recover from before the strain has measurable e ects on cortisol.[36] Some particular stressors, such as high job demands and working at night seem to be related with alterations in cortisol secretion.[8,37,38]

The participants of this study were all relatively healthy shift working females who reported similar working hours, family care-giving responsibilities, stressful life-events, illnesses, prescription medication use, and occurrence of sickness absence during the field study. There was a statistically signi cant difference between the groups in physical activity and education which, when controlled for, had little effect on results.

Saliva samples can be easily collected by participants themselves[1] and, as health care professionals, the participants of this study followed the sample collection protocol particularly carefully. All this is likely to have reduced the bias and confounding of between-group results.

There are very few earlier studies that have measured time from sleep end to collecting the first saliva sample with actigraphy rather than self-reports.[39] In this study, the participants took the rst sample in 6–8 min after sleep end in actigraph data. Smyth and colleagues[40] found that self-determined sleep end is reported on aver- age 4 min later than registered by actigraph, indicating that the participants of this study actually took the first sample very

close to the sleep. This is an important issue, as even moderate sampling delays reduce the cortisol awakening response.[40]

Logarithmic transformation of the original values was used, as the original distributions were mostly skewed. Gardner et al.[10] stated that even after transformation substantial differences in sAA between individuals exist. In parallel, not exactly all distributions were normalized with the logarithmic transformation, the data was analyzed in a similar way instead of using, e.g., nonparametric rank order correlation test. Non-normality does not affect the estimation of the parameters, and the least squared estimates are still the best linear estimates when the other assumptions are met.[31]

When reporting AUC's, it is recommendable to report both AUCg's and AUCi's, as different associations may formulate when using only other of the formula.[31] AUCi has been seldom used in research,[23] even though AUCi is considered to t better for anal- ysis of stress reactivity. This may be due to AUCi is problematic when the repeated measures show a decline over time,[31] which is the case for normal daily cortisol profile.[4]

In this study, the cortisol results with both AUCg and AUCi showed consistently non-significant results, whereas there was inconsistency in the AA AUCg and AUCi results. The possible explanation for this might be that comparing AUCg results between laboratory and field reflects the higher levels of alpha-amylase in general, across different stressful situations. This question warrants further research.

The sample of nursing employees may not represent neither other shift working occupations nor work-aged populations in general, restricting generalizability of the results. The stress biomarkers were measured in 1 day per shift type, similarly to earlier studies,[6,18,19] although it would be recommendable to collect several days' saliva samples for each measurement category.[41] The laboratory and work shift stressors also

differed with regard to time duration, likewise to earlier studies combining laboratory and field conditions,[19,22] which may have contributed to the biomarker output pro les. In the regression analysis, the factors that had a baseline difference among job strain groups (education and physical activity) were included in the regression model. There are other factors influencing to individual differences in salivary biomarkers, e.g., age and cigarette smoking, which were tested in separate analysis with no e ect to the results. However, possibility for confounding by non-measured factors remains.

Only 24% of the invited employees participated in the study. As there was no personal contact with the invited employees, it could not be verified whether they actually were reached or not. However, the participants were representative of those invited, and the field data collected was of very good quality, as 99% of the measurements were collected and analyzed successfully. After the labora- tory stress test, only 4% of the 99 participants of the TSST failed or refused to carry out the field measurements.

Due to practical reasons, only one "staff manager" assessed the TSST, whereas the standard protocol uses three managers.[5] The cortisol responses to the TSST might have been more explicit, if the test had included more than one evaluator. The lack of information concerning the participants' menstrual cycle and menopausal status is also a limitation, since salivary cortisol response patterns may be more evident in the luteal phase,[42] and after the menopause acute sympathoadrenal responsive- ness increases.[43]

In conclusion, a laboratory measure of sAA AUCg may predict stress-related output during burdensome work shifts and leisure time, whereas laboratory sAA AUCi or salivary cortisol output seems not to have this potential. These findings suggest that alpha-amylase output is a laboratory biomarker with a potential of predicting stress response in real-life context.

References

[1] Kudielka, B.M., A. Gierens, D.H. Hellhammer, S. Wust, and W. Schlotz: Salivary cortisol in ambulatory assessment–some dos, some don'ts, and some open questions. Psychosom. Med. 74(4):418–31 (2012).

[2] Rohleder, N., and U.M. Nater: Determinants of salivary alpha-amylase in humans and methodological considerations. Psychoneuroendocrinology 34(4):469–485 (2009).

[3] Lovallo, W.R., and T.L. Thomas: Stress hormones in psychophysiological research: emotional, behavioral and cognitive implications. In Handbook of psychophysiology, J.T. Cacioppo, L.G. Tassinary and G. Bernston (eds.). New York: Cambridge University Press, 2000. pp. 342–367.

[4] Fries, E., L. Dettenborn, and C. Kirschbaum: The cortisol awakening response (CAR): facts and future direc- tions. Int. J. Psychophysiol. 72(1):67–73 (2009).

[5] Kirschbaum, C., K.M. Pirke, and D.H. Hellhammer: The 'Trier Social Stress Test'–a tool for investigating psy- chobiological stress responses in a laboratory setting. Neu- ropsychobiology 28(1–2):76–81 (1993).

[6] Wong, I.S., A.S. Ostry, P.A. Demers, and H.W. Davies: Job strain and shift work influences on biomarkers and subclinical heart disease indicators: a pilot study. J. Occup. Environ. Hyg. 9(8):467–477 (2012).

[7] Miller, G.E., E. Chen, and E.S. Zhou: If it goes up, must it come down? Chronic stress and the hypothalamic- pituitary-adrenocortical axis in humans. Psychol. Bull. 133(1):25–45 (2007).

[8] Thomas, C., C. Hertzman, and C. Power: Night work, long working hours, psychosocial work stress and cortisol secretion in mid-life: evidence from a British birth cohort. Occup. Environ. Med. 66(12):824–831 (2009).

[9] Out, D., D.A. Granger, S.E. Sephton, and S.C. Segerstrom: Disentangling sources of individual differences in diurnal salivary alpha-amylase: reliability, stability and sensitivity to context. Psychoneuroen- docrinology 38(3):367–375 (2013).

[10] Granger,D.,K.Kivlighan,M.el-Sheikh,E.Gordis,and L. Stroud: Salivary α-amylase in biobehavioral research. Recent developments and applications. Ann. NY Acad. Sci. 1089:122–144 (2007).

[11] Petrakova, L., K. Boy, L. Mittmann, L. Möller, H. Engler, and M. Schedlowski: Salivary alpha-amylase and noradrenaline responses to corticotropin-releasing hormone administration in humans. Biol. Psychol. 127:34–39 (2017). [Epub ahead of print]

[12] Marchand, A., R.P. Juster, S.J. Lupien, and P. Durand: Psychosocial determinants of diurnal alpha-amylase among healthy Quebec workers. Psychoneuroendocrinol- ogy 66:65–74 (2016).

[13] Strahler, J., A. Mueller, F. Rosenloecher, C. Kirschbaum, and N. Rohleder: Salivary alpha-amylase stress reactivity across di erent age groups. Psychophysi- ology 47(3):587–595 (2010).

[14] van Stegeren, A.H., O.T. Wolf, and M. Kindt: Salivary alpha amylase and cortisol responses to different stress tasks: impact of sex. Int. J. Psychophysiol. 69(1):33–40 (2008).

[15] Nater, U.M., N. Rohleder, W. Schlotz, U. Ehlert, and C. Kirschbaum: Determinants of the diurnal course of salivary alpha-amylase. Psychoneuroendocrinology 32(4):392–401 (2007).

[16] Groer, M., R. Murphy, W. Bunnell, et al.: Salivary measures of stress and immunity in police officers engaged in simulated critical incident scenarios. J. Occup. Environ. Med. 52(6):595–602 (2010).

[17] Limm,H.,H.Gundel,M.Heinmuller,et al.:Stressman- agement interventions in the workplace improve stress reactivity: a randomised controlled trial. Occup. Environ. Med. 68(2):126–133 (2011).

[18] Wingenfeld, K., M. Schulz, A. Damkroeger, C. Philippsen, M. Rose, and M. Driessen: The diurnal course of salivary alpha-amylase in nurses: an investigation of potential confounders and associations with stress. Biol. Psychol. 85(1):179–181 (2010).

[19] Kidd, T., L.A. Carvalho, and A. Steptoe: The relationship between cortisol responses to laboratory stress and cortisol profiles in daily life. Biol. Psychol. 2014; 99c:34– 40 (2014).

[20] Steptoe, A. Psychophysiological contributions to behavioral medicine and psychosomatics. In Handbook of psychophysiology, 3rd ed, J.T. Cacioppo, L.G. Tassinary, and G. Bernston (eds.). New York: Cambridge University Press, 2007. pp. 723–751.

[21] Zanstra, Y.J., and D.W. Johnston: Cardiovascular reactivity in real life settings: measurement, mechanisms and meaning. Biol. Psychol. 86(2):98–105 (2011).

[22] Wolfram, M., S. Bellingrath, N. Fuerhahn, and B.M. Kudielka: Cortisol responses to naturalistic and labora- tory stress in student teachers: comparison with a non-stress control day. Stress Health 29(2):143–149 (2013).

[23] Fekedulegn, D.B., M.E. Andrew, C.M. Burch el, et al.: Area under the curve and other summary indicators of repeated waking cortisol measurements. Psychosom. Med. 69(7):651–659 (2007).

[24] Karhula, K., M. Härmä, M. Sallinen, et al.: Association of job strain with working hours, shift-dependent perceived workload, sleepiness and recovery. Ergonomics 56(11):1640–1651 (2013).

[25] Karasek, R.A., C. Brisson, N. Kawakami, I. Houtman, P. Bongers, and P. Amick: The job content questionnaire (JCQ): an instrument for internationally comparative assessments of psychological job characteristics. J. Occup. Health Psychol. 3:322–355 (1998).

[26] Smith, C.S., C. Reilly, and K. Midki : Evaluation of three circadian rhythm questionnaires with suggestions for an improved measure of morningness. J. Appl. Psychol. 74(5):728–738 (1989).

[27] Dohrenwend,B.S.,L.Krasno ,A.R. Askenasy,and B.P. Dohrenwend: Exemplification of a method for scaling life events: the Peri Life Events Scale. J. Health Soc. Behav. 19(2):205–229 (1978).

[28] Beck, A.T., R.A. Steer, and G.K. Brown: Manual for the Beck Depression Inventory-II. San Antonio: Psychological Corporation, 1996.

[29] Karhula, K., M. Härmä, M. Sallinen, et al.: Association of Job strain with cortisol and alpha-amylase among shift-working health care professionals in laboratory and field. Biol. Res. Nurs. 18(1):101–112 (2016).

[30] Clements, A.D., and C.R. Parker: The relationship between salivary cortisol concentrations in frozen versus mailed samples. Psychoneuroendocrinology 23(6):613–616 (1998).

[31] Pruessner, J.C., C. Kirschbaum, G. Meinlschmid, and D.H. Hellhammer: Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology 28(7):916–931 (2003).

[32] Watamura, S.E., B. Donzella, D.A. Kertes, and M.R. Gunnar: Developmental changes in baseline cortisol activity in early childhood: relations with napping and effortful control. Dev. Psychobiol. 45(3):125–133 (2004).

[33] Rawlings, J.O., S.G. Pantula, and D.A. Dickey: Applied Regression Analysis: A Research Tool. 2nd ed. New York: Springer-Verlag, 1998.

[34] Hansen, Å.M., A.K. Blangsted, E.A. Hansen, K. Søgaard, and G. Sjøgaard: Physical activity, job demand-control, perceived stress-energy, and salivary cortisol in white-collar workers. Int. Arch. Occup. Environ. Health 83(2):143–153 (2010).

[35] Out, D., M.J. Bakermans-Kranenburg, D.A. Granger, C.M. Cobbaert, and M.H. van Ijzendoorn: State and trait variance in salivary alpha-amylase: a behavioral genetic study. Biol. Psychol. 88(1):147–154 (2011).

[36] Karlson, B., P. Lindfors, R. Riva, C. Mellner, T. Theo- rell, and U. Lundberg: Psychosocial work stressors and salivary cortisol. In The Role of Saliva Cortisol Measurement in Health and Disease, M. Kristenson, P. Garvin, and U. Lundberg (eds.). Sharjah: Bentham Science Publishers, 2012. pp. 43–66.

[37] Karlson, B., F. Ek, Å.M. Hansen, A.H. Garde, and P. Ørbæk: Cortisol variability and self-reports in the measurement of work-related stress. Stress and Health 27: e1–e24 (2011).

[38] Sjörs, A., T. Ljung, and I.H. Jonsdottir: Diurnal sali- vary cortisol in relation to perceived stress at home and at work in healthy men and women. Biol. Psychol. 99: 193–197 (2014).

[39] Dockray, S., M.R. Bhattacharyya, G.J. Molloy, and A. Steptoe: The cortisol awakening response in rela- tion to objective and subjective measures of waking in the morning. Psychoneuroendocrinology 33(1): 77–82 (2008).

[40] Smyth, N., A. Clow, L. Thorn, F. Hucklebridge, and P. Evans: Delays of 5–15 min between awakening and the start of saliva sampling matter in assessment of the cortisol awakening response. Psychoneuroendocrinology 38(9):1476–1483 (2013).

[41] Hellhammer, J., E. Fries, O.W. Schweisthal, W. Schlotz, A.A. Stone, and D. Hagemann: Several daily measurements are necessary to reliably assess the cortisol rise after awakening: state- and trait components. Psychoneuroendocrinology 32(1):80–86 (2007).

[42] Duchnese, A., and J.C. Pruessner: Association between subjective and cortisol stress response depends on the menstrual cycle phase. Psychoneuroendocrinology 38(12):3155–3159.

[43] Kajantie, E., and D.I. Phillips: The effects of sex and hormonal status on the physiological response to acute psychosocial stress. Psychoneuroendocrinology 31(2): 151–178.