Int Arch Occup Environ Health (2008) 81:391–400
DOI 10.1007/s00420-007-0224-x

ORIGINAL ARTICLE

Relationship between self-reported mental stressors at the workplace and salivary cortisol

Giovanni Maina · Antonio Palmas · Francesca Larese Filon

Received: 6 December 2006 / Accepted: 22 June 2007 / Published online: 16 August 2007

Abstract
Objective To investigate the association between work stress measures and salivary cortisol excretion in working and weekend days.
Methods In a sample of 68 healthy young call-centre operators dimensions of job stress from the demand-control model were related to repeated measures of salivary cortisol on seven samples (at awakening, +30 min, +60 min, + 3h, +6 h, +9 h, and +12 h after awakening) at two working days and a weekend day.
Results The cortisol excretion on work days was higher than during weekend day with gender-specific differences as women only showed higher significant values for area under the curve (AUC_G) and Diurnal cycle (χ^2 (2) = 8.10, $P < 0.05$; χ^2 (2) = 15.75, $P < 0.05$, respectively). There were no associations between job demand, job control and cortisol excretion, while the sociodemographic characteristics of the call-centre operators showed linear relation with the diurnal pattern of cortisol secretory activity.
Conclusions The hypothalamic-pituitary adrenocortical axis activation was higher in working day than in weekend day. This activation measured by salivary cortisol was not related to self-reported mental stressors assessed with job strain model. The availability of more specific psychometric scales would be useful to explore the relationship between salivary cortisol levels and measures of mental stress at workplace.

Keywords Hypothalamic-pituitary-adrenocortical axis · Job strain model · Salivary cortisol · Work stress

Introduction

Over the recent decades work stress has emerged as a major psychosocial influence on physical and mental health in the developed countries resulting in raised social costs and weighting heavily in work organization: in a large investigation analysing the relationship between health risks and medical expenditure on 46,026 American employees, stressed individuals were 46% more costly than those lacking this risk (Goetzel et al. 1998); in the European Union work-related stress is the second most common work-related health problem affecting nearly one out of every three workers (Cox et al. 2000). The increasing interest on the relationship between work stress and physical and mental health has resulted on psycho-social models as well as patho-physiological models. One of the leading theoretical model in the job-stress literature is the “Demand/Control” model developed by Karasek and Theorell (1990) involving two major dimensions of job stress: psychological demand and decision latitude at work. The Karasek’s job strain model proposes that people working in highly demanding jobs also have low control and limited opportunities to use skills will experience high job strain. A high level of job strain has been found to be associated with physiological coronary heart diseases risk factors, such as high blood pressure (Tsutsumi et al. 2001; Niedhammer et al. 1998; Alfredsson et al. 2002), high

G. Maina (✉) · A. Palmas
Dipartimento di Traumatologia, Ortopedia e Medicina del Lavoro, Università di Torino, Via Zuretti 29, 10126 Torino, Italy
e-mail: giovanni.maina@unito.it

F. L. Filon
Dipartimento di Scienze di Medicina Pubblica Unità Clinico Operativa di Medicina del Lavoro, Università di Trieste, Via della Pietà, 19, 34129 Trieste, Italy

serum total cholesterol (Jonsson et al. 1995), and high triglycerides (Netterstrom et al. 1991).

In the investigation of psychosocial influences on health, knowledge on biochemical pathways has been greatly expanded, as well as the availability of physiological measures. The results of the psychobiological research suggest that two neuroendocrine systems are activated as a result of the stress stimuli: the sympathetic-adrenomedullary system (SAM axis), with the secretion of epinephrine and norepinephrine, and the hypothalamic-pituitary-adrenocortical system (HPA axis) with the secretion of cortisol. The measurements of these two systems have formed the basis for psychoneuroendocrine stress research starting with Cannon (Lundberg 1984) and Seyle (Goldstein 1995): the former focusing on the emergency function of the SAM axis, whereas the HPA axis was the central part of theories on stress and the general adaptation syndrome of the latter.

Salivary cortisol levels accurately reflect serum-free cortisol, the physiologically active component (Kirschbaum and Hellhammer 1994). Measurement of cortisol in saliva has many advantages over determination from blood samples: sampling is non-invasive and therefore not painful (avoiding the risk of stress-induced modulation of levels), it does not require trained medical personnel and can be repeated at frequent intervals. In addition the collected samples do not need any special treatment, being stable at room temperature for up to 7 days. These advantages mean that samples can be reliably taken in a normal ambulatory setting or in the subjects' own home during their routine activities. Unlike urinary measurement, detail of dynamic change can be explored using saliva samples as the medium for assay. A moderate to high within-subject stability in the extent of the awakening cortisol response (measured at intervals for up to an hour following awakening) across days and weeks was reported (Pruessner et al. 1997). The response is unrelated to quality of sleep, smoking status, alcohol consumption, time of awakening or post-awakening ambulatory activities (Clow et al. 2004) and phase of the menstrual cycle (Kudielka and Kirschbaum 2003). Increased knowledge has been achieved in the recent years relating both to the well-documented circadian rhytmicity of cortisol output and to the cortisol awakening response (CAR). The CAR, the change in cortisol concentration that typically takes place in the 20–60 min after awakening in the morning, has been recognized over recent years as a distinctive phenomenon in the diurnal profile of cortisol output that is of considerable psychoneuroendocrinological significance (Wust et al. 2000a).

No general association between self-reported mental stress at the workplace and the salivary cortisol response has been observed in studies carried out in this topic. A positive association between magnitude of the CAR and general chronic stress has been observed (Schultz et al. 1998; Pruessner et al. 1999, 2003b), as well as between CAR and work stress (Ockenfels et al. 1995; Kunz-Ebrecht et al. 2004; Schlotz et al. 2004). On the contrary, negative associations have been reported between self-reported mental job stress and cortisol concentration (Steptoe et al. 1998; Yang et al. 2002), as well as no associations between self-reported mental stress and the salivary cortisol response (Evans and Steptoe 2001; Fischer et al. 2000). A plausible explanation for the observed inconsistency may be due to large differences in cortisol sampling procedures, types of mental stressors and the self-report scales used to evaluate them, differences in study design and the absence of control for factors, which are known to or potentially interfere with the cortisol response. Nevertheless, the putative role of the CAR in the regulation of physiological function across the day and its sensitivity to psychosocial variables make it a prime candidate as an intermediary linking mind and health (Clow et al. 2004). Although it is well documented that other steroid hormones are also affected by stress, cortisol is still considered a major indicator of altered physiological states in response to stressful stimulation.

This study was carried out to assess the correlation between self-reported stress assessed by Karasek's questionnaire and salivary cortisol level in working and weekend days in volunteer subjects employed in a call-centre. Recent studies suggest that most jobs in call centres can be characterized as unskilled work, which some authors called an advanced form of Taylorism (Knights and McCabe 1998). Several studies (Isic et al. 1999; Hutchinson et al. 2000) showed that call-centres operators have low levels of job control, and this condition predicted depression among inbound call agents of a national UK bank in cross sectional as well as in longitudinal data (Holman 2002). Furthermore, in a study among US teleservice centre, lack of job control was associated with musculoskeletal disorders (Hoekstra et al. 1995).

Aims of our investigation were to test the following hypothesis: (1) the cortisol excretion on work day is greater than on weekend day; (2) the cortisol output would be more pronounced in the group of working people defined by high job demand and low control; (3) the cortisol awakening response is the most sensitive neuroendocrine measure of psychosocial job stressors compared with measures of the cortisol excretion during the day.

Materials and methods

Subjects

This cross-sectional study has been conducted in a call-centre setting, where subjects have been recruited through e-mail advertisements and on work side meetings asking volunteers to participate in an investigation on daily activities and cortisol. A participation rate of 25.7% was reached, and among 120 adherent subjects, 15 individuals were excluded on the basis of the criteria of selection (hormone replacement therapy, blood glucose levels higher than 120 mg%, medication for psychiatric disorders). 105 subjects (89 women and 16 males) constituted the final sample.

Study protocol

Saliva samples were collected using cotton dental rolls held in the mouth until satured and then stored in Salivette tubes (Sarstedt, Leicester, UK). Participants were instructed to take seven samples on two working days (one judged as pleasant, the other as unpleasant by the subject itself) and a weekend day, with measures at awakening, 30, 60 min thereafter, at the start of work-shift and then every 3 h (Fig. 1). Participants filled in a diary the actual time of day they had taken each sample. Participants were asked to take the first sample while lying in bed and the second after 30 min without brushing their teeth, eating , drinking or smoking. Tubes were returned to the investigators personally, and cortisol concentration was determined by solid phase radioimmunoassay (LOD = 1.5 nmol/l; intra- and interassay variability were 10 and 14%, respectively). Cortisol values higher than 75 nmol/l were excluded (one participant), because these may be due to altered pH-values or blood contamination of the sample (Steptoe et al. 2004). Separate analyses were carried out for cortisol responses to waking and for cortisol levels along the day. Cortisol responses to waking can be erroneously measured if people fail to take the first sample immediately after waking. Because the increase is rapid, a delay in the first sample means that the "waking" sample is taken on this upward curve, and that the overall increase may be correspondingly reduced.

In this study compliance with sampling regime has not objectively been monitored: adherence to protocol was judged by calculating the difference between the time participants stated they had taken each saliva sample and the time demanded by the protocol. Individuals with time differences of more than 10 min as well as individuals with any missing value were excluded (36 subjects): excluded subjects are similar to those studied considering sex, age, educational level, marital status, job contract, hours worked per day and work stress measures. Likewise, no differences were showed between adherent and non-adherent subjects on sociodemographic characteristics. We studied 68 workers, 56 females and 12 males and collected 1,428 samples of salivary cortisol. All subjects gave written, informed consent, and ethical approval for the study was obtained from the local research ethics committee.

Work stress measures

Background information was provided by questionnaire, including measures of gender, age, marital status, educational level, hours worked per day, job contract. Psychosocial job stressors were measured according to the job strain model, using the Italian version of the Job Content questionnaire (JCQ) which contains 11 Likert-scaled items (Cesana et al. 2003). This model predicts that the combination of job demand and job control results in different degrees of perceived strain, stress-related risk, and active–passive behavioural correlates of jobs (Karasek and Theorell 1990). There were five items measuring "job demand" (i.e. "Do you have to work very intensively?"), and six items composing the "job control" scale (i.e. "Do you have a choice in deciding how you do your work?"), each of which was rated on a 4-point scale ranging from 1 ("strongly disagree") to 4 ("strongly agree"). Job demand and job control scales have been obtained by mean of the sum of the items that represent the respective dimension. The Cronbach α for the scales were 0.71 and 0.74 for demand and control, respectively. The scores of each dimension were transformed to range from 25 to 100, where 100 indicated maximum demand and maximum control. The scores were then divided into two categories (high and low) on the basis of median split of each dimension, and four job strain levels (high, active, passive and low) were obtained combining the categories of job demand and job control (see Appendix for details). Table 1 shows the characteristics of the study population.

Fig. 1 Sampling schedule

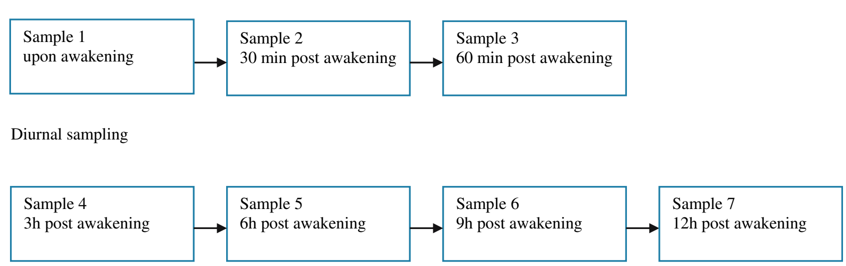

Table 1 Characteristics of 68 call-centre operators

Characteristics	Mean ± SD no (%)
Gender (%)	
Males	12 (17.6)
Females	56 (82.4)
Age (years)	
Males	29.34 +/- 4.5
Females	30.03 +/- 4.9
Educational level (%)	
Not graduate	61 (89.7)
Graduate	7 (10.3)
Marital status (%)	
Not married	43 (63.2)
Married	25 (36.8)
Job contract (%)	
Confirmed unlimited contract	55 (80.9)
Temporary contract	13 (19.1)
Hours worked per day (%)	
Full-time	34 (50.0)
Part-time	34 (50.0)
Job demand[a,*]	
High	243 (234–307)
Low	55 (5–63)
Job control[a,**]	
High	280 (273–289)
Low	205 (187–210)
Job strain[a,***]	
High	205 (203–251)
Active	143 (91–161)
Passive	280 (241–319)
Low	193 (107–201)

*Wilcoxon test $P < 0.001$; **Wilcoxon test $P < 0.001$; ***Kruskal–Wallis test $P < 0.001$

[a] Medians and CI 95% of score values

Statistical analysis

The CAR has been quantified by calculation of: the area under the curve (AUC) relative to zero (or ground: AUC_G), the area under the curve with respect to increase (AUC_I) (Pruessner et al. 2003a) and the mean increase (MnInc) (Wust et al. 2000b). The cortisol excretion during the day has been calculated by mean of the area under the curve with respect the ground (AUC_G) using the samples from 4 to 7($AUC_{G\text{-work}}$), and the area under the curve with respect to the ground using the samples from 1 to 7 (Diurnal cycle) (Pruessner et al. 2003a). Given the skewed distribution of the salivary cortisol measures, logarithmic transformations were performed: the transformation did not generate distribution suitable for parametric analysis, and then all statistic analysis were carried out using non-parametric tests. The cortisol values have been analysed in various ways: firstly, a two-way (time by day) within-subject non-parametric test (Friedman test) was computed to examine differences in each of the seven cortisol samples as well as in CAR, $AUC_{G\text{-work}}$, Diurnal cycle values obtained according to the above-reported calculations. The difference between the two first cortisol samples has been analysed by mean of the Wilcoxon test. The same test has been applied to explore differences between the high and low scores of the demand and control dimensions; the differences among job strain scores have been tested by mean of the Kruskal–Wallis test. Differences on sociodemographic characteristics and work stress measures between included and excluded subjects as well as between adherent and non-adherents were explored by chi-square test. The Spearman rank correlations were used to explore associations between job demand, job control, job strain and cortisol levels. Variables found to be associated with cortisol level were entered into a general linear model (GLM). The categorical predictors were job demand (two levels), job control (two levels), and job strain (four levels). Age, gender, educational level (two levels determined by years of school completed), marital status (two levels: married, not married), working hours per day (two levels: full-time, and part-time), job contract (two levels: confirmed unlimited, temporary contract) were introduced as covariate in the model. The dependent variables were CAR, $AUC_{G\text{-work}}$, and diurnal cycle values. In the first analysis all the factor terms were entered simultaneously into the model, including the covariates, to explore the main effects for all the variables; in the second step interactions between covariates and factors as well as any covariate by covariate interactions were analysed to test the homogeneity of regression slopes assumption. The selection of covariate factors and interactions to be included in the final model was based on a stepwise procedure deletion model: the limit of the significance of covariates to be included in the final model was set at 0.30, that is, variables or interactions not reaching a P-value below 0.30 were eliminated from the model in the next step. This higher limit prevented much of the bias that otherwise may have arisen from this selection method. Significance was set at $P < 0.05$, two-sides for all the analysis. All data analysis was carried out using SPSS 14.0.

Results

Cortisol values showed the expected diurnal rhythm with high morning and low evening values. The cortisol excretion in weekend was lower than in working days in both sex, but only in females there was a significant effect of day in samples no. 5 (χ^2 (2) = 1433, $P < 0.001$; the

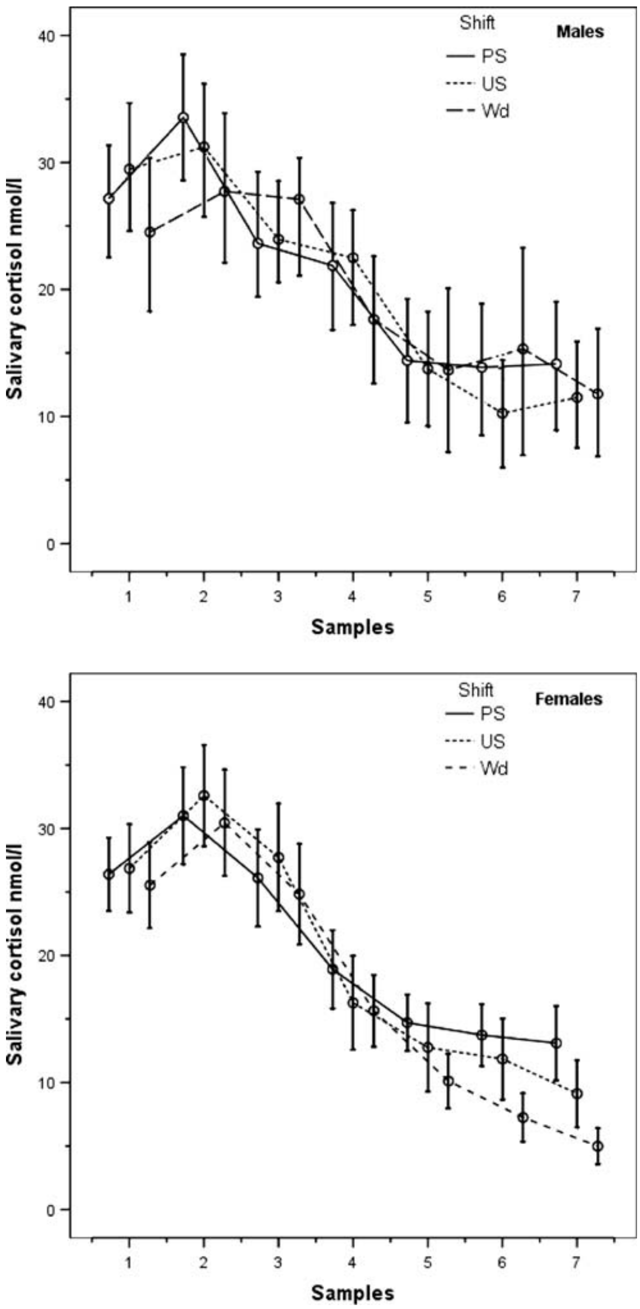

Fig. 2 General profile of cortisol levels. *PS* Pleasant shift, *US* Unpleasant shift, *Wd* Weekend

median scores on pleasant, unpleasant and weekend day were 13.24, 11.06 and 9.55 nmol/l, respectively), no. 6 (χ^2 (2) = 22.30, $P < 0.0001$; the median scores on pleasant, unpleasant and weekend day were 11.06, 8.87, 4.37 nmol/l, respectively), and no. 7 (χ^2 (2) = 29.45, $P < 0.0001$; the median scores on pleasant, unpleasant, and weekend day were 10.51, 6.41, 1.36 nmol/l, respectively) (Fig. 2). In both sex the CAR values, computed as AUC_G, AUC_I and MnInc, were higher in work days than in weekend days, but this difference did not achieve the statistical significance. On the other side, the cortisol values showed the typical increase from the awakening to 30 min post-awakening samples in males (6.0 and 23.5% in unpleasant and in pleasant shift, respectively) and in females (17.5 and 21.35% in pleasant and unpleasant shift, respectively): the difference was statistically significant (males: $Z = 2.17$; $P < 0.05$; females: $Z = 4.88$; $P < 0.001$) (Fig. 3). The cortisol excretion during the day showed higher values in working days, but only in women there was a significant effect of day: in $AUC_{G\text{-work}}$ (χ^2 (2) = 8.10, $P < 0.05$; the median scores on pleasant, unpleasant, and weekend day were 6416.77, 4760.70, 4371.72 nmol/l, respectively) (Fig. 4), and in diurnal cycle (χ^2 (2) = 15.75; the median scores on pleasant, unpleasant, and weekend day were 11684.07, 13988.17, 8627.05 nmol/l, respectively) (Fig. 5). Although the two categories (high and low) of the job demand and job control dimensions as well as the four levels of job strain exhibited significant differences in score values (Table 1), the work stress measures and cortisol values showed no widespread associations (Table 2). Indeed, job control was inversely related to the sample no. 3 and the CAR (area under the curve with respect to ground) in males only, and job demand was statistically positively associated with samples nos. 2, 3, 5 and the CAR indices in females, but with the sample no. 5 only in males. In both sex no significant association was found between job strain and salivary cortisol values. No significant linear relation between work stress measures and salivary cortisol values could be seen in any of the final general linear

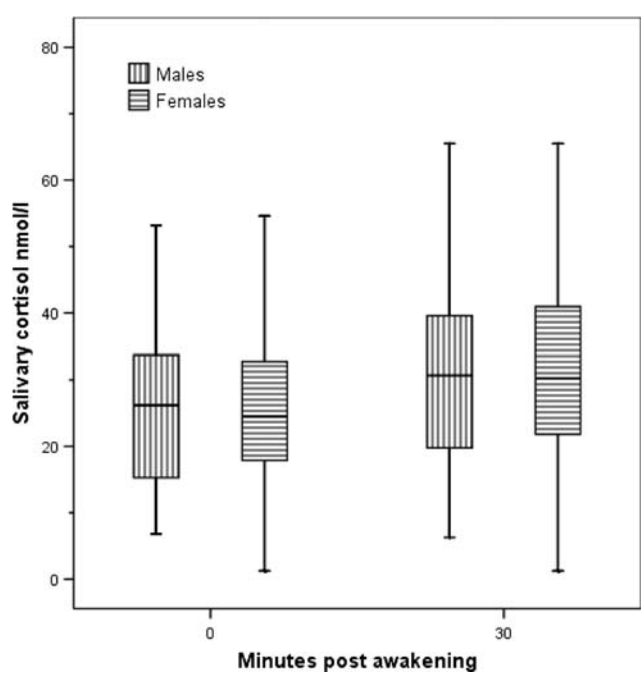

Fig. 3 Values at awakening and 30 min post-awakening (medians and first and third percentile of salivary cortisol concentration)

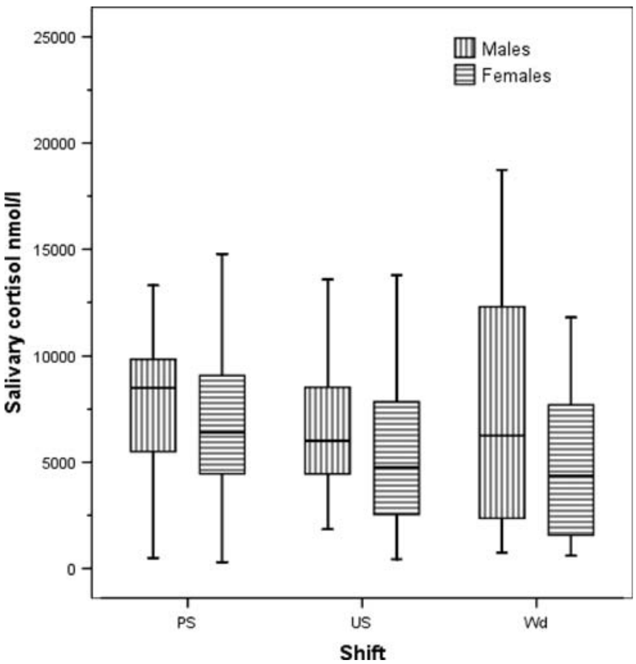

Fig. 4 Salivary cortisol values calculated as area under the curve with respect to ground (AUC_G) during the day using the samples from no. 4 to no. 7 (AUC_D). *PS* Pleasant shift, *US* Unpleasant shift, *Wd* Weekend

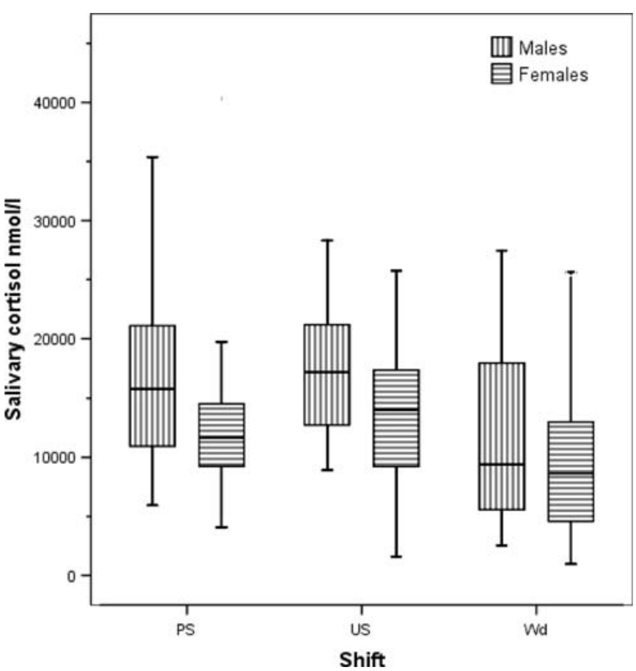

Fig. 5 Cortisol values calculated as area under the curve with respect to ground (AUC_G) during the day using the sample no. 1, and samples from no. 4 to no. 7 (diurnal cycle). *PS* Pleasant shift, *US* Unpleasant shift, *Wd* Weekend

models. Salivary cortisol values in the day, measured as $AUC_{G\text{-work}}$ and diurnal cycle, and sociodemographic characteristics showed a statistically significant linear relation. In all the final build model, the $AUC_{G\text{-work}}$ and the diurnal cycle dependent variables increased as the age increases: in the job control model (Table 3): $F = 16.73$, $P < 0.001$ and $F = 13.26$, $P = 0.001$, respectively; in the job demand model (Table 4): $F = 18.70$, $P < 0.001$ and $F = 13.88$, $P < 0.001$, respectively; and in the job strain model (Table 5): $F = 14.20$, $P = 0.001$ and $F = 8.70$, $P = 0.005$, respectively. Furthermore the covariate gender showed the same linear relation with the diurnal cortisol excretion: the difference between gender group explains a significant size of the variance in both $AUC_{G\text{-work}}$ and diurnal cycle cortisol excretion: in the job control model: $F = 5.33$, $P = 0.025$ and $F = 8.63$, $P = 0.005$, respectively; in the job demand model: $F = 5.08$, $P < 0.028$ and $F = 12.29$, $P < 0.001$, respectively; and in the job strain model: $F = 5.34$, $P = 0.025$ and $F = 10.66$, $P = 0.002$, respectively.

Educational level, marital status, working hours per day and job contract covariates showed a positive linear relation, although without statistical relevance, with the cortisol excretion during the day computed as diurnal cycle in the job control final model (Table 3), indicating that these sources of variance do not play a significant role in the total variance of the cortisol excreted over the day. Examining the job demand final model (Table 4), *F* statistic (7.42) and its associated significance level ($P = 0.009$) of the covariate educational level indicated that it has a significant linear relationship with the diurnal cycle ($F = 7.42$, $P = 0.009$), suggesting that the values of the dependent variable (cortisol excreted over the day) increases as the educational level increases.

Discussion

The first major prediction directing this research was confirmed: in healthy call-centre operators, the salivary cortisol excretion in work day was greater than in weekend day. On the other side, the results for cortisol output over the day present a conflicting picture, as the cortisol values in the post-awakening phase only were higher in the workday, and in females only this difference reached the statistical significance. These findings are only partially consistent with those of the other two studies exploring this topic (Schultz et al. 1998; Kunz-Ebrecht et al. 2004) that reported higher cortisol awakening response in workday in both sex. Several explanations of the reported differences can be proposed. Firstly, both of the two mentioned studies explored the cortisol awakening response only, failing to analyse the cortisol excretion over the day. Secondly, the size of the male group is too small in our study, as it's not possible to expect any significant effect. Thirdly, non-compliance with the sampling schedule is a potential

Table 2 Associations between job strain model and salivary cortisol values using Spearman's Rho correlations

Salivary cortisol	Job control		Job demand		Job strain	
	Males	Females	Males	Females	Males	Females
Sample 1	−0.575	0.068	−0.092	0.159	0.048	0.133
Sample 2	−0.637	0.128	0.004	0.285*	0.240	0.053
Sample 3	−0.603*	0.075	0.368	0.405**	0.390	0.107
Sample 4	0.519	−0.120	−0.009	0.111	0.179	0.217
Sample 5	−0.035	−0.142	0.630*	0.295*	0.179	0.101
Sample 6	−0.396	−0.153	0.521	0.079	0.214	0.238
Sample 7	−0.394	−0.002	0.459	0.052	−0.007	0.091
CAR: AUC_G	−0.747**	0.106	0.128	0.312*	0.327	0.029
AUCi	0.060	0.082	0.248	0.330*		0.188
MnInc	−0.037	0.086	0.193	0.376**	0.270	0.200
$AUC_{G\text{-work}}$	0.046	−0.185	−0.541	0.217	0.285	0.199
Diurnal cycle	0.152	−0.028	−0.567	0.230	0.246	0.005

CAR Cortisol awakening response

$AUC_{G\text{-work}}$: salivary cortisol values calculated as area under the curve with respect to ground (AUC_G) during the day using the samples from no. 4 to no. 7

Diurnal cycle: salivary cortisol values calculated as area under the curve with respect to ground (AUC_G) during the day using the sample no. 1, and samples from no. 4 to no. 7

*Spearman's Rho correlation test $P < 0.05$

**Spearman's Rho correlation test $P < 0.01$

Table 3 Relationship between job control, covariates (age, gender, educational level, work hours per day, job contract, marital status) and salivary cortisol output during the day using general linear model (GLM)

"Predictors"	$AUC_{G\text{-work}}$		Diurnal cycle	
	F	*P*	*F*	*P*
Control	0.37	0.55	0.93	0.34
Age	16.73	0.001	13.26	0.001
Gender	5.33	0.025	8.63	0.005
Educational level			2.16	0.147
Hours worked per day			1.98	0.166
Job contract			1.44	0.236
Marital status			1.15	0.289
Final model	6.63	0.001	3.93	0.002

$AUC_{G\text{-work}}$: salivary cortisol values calculated as area under the curve with respect to ground (AUC_G) during the day using the samples from no. 4 to no. 7

Diurnal cycle: salivary cortisol values calculated as area under the curve with respect to ground (AUC_G) during the day using the sample no. 1, and samples from no. 4 to no. 7

confounding factor, as a delay in taking the first sample may mean that cortisol has already begun to rise prior to the first assessment and then the shape of the cortisol awakening response can be altered. The moderate intra-individual stability of the CAR between days observed in this study restricts this possible confusion, as it's unlikely that a subject did a systematic mistake collecting the first sample.

According to the literature data (Pruessner et al. 1997; Wust et al. 2000b; Edwards et al. 2001) the cortisol awakening response showed to be unrelated to age and gender. On the contrary, the cortisol values in the post-awakening period increased as the age increased, and the cortisol values of females were higher than in males. The latter result supports similar findings (Pruessner et al. 1997; Wust et al. 2000b) suggestive of a rather consistent sex difference on the HPA axis activation. Interestingly, the cortisol awakening response values in this research showed values higher than in the other studies: from 26.4 to

Table 4 Relationship between job demand, covariates (age, gender, educational level) and salivary cortisol output during the day using general linear model (GLM)

"Predictors"	$AUC_{G\text{-work}}$		Diurnal cycle	
	F	*P*	*F*	*P*
Demand	0.124	0.73	3.03	0.09
Age	18.70	<0.001	13.88	<0.001
Gender	5.08	0.028	12.29	0.001
Educational level			7.42	0.009
Final model	8.13	<0.001	6.34	<0.001

$AUC_{G\text{-work}}$: salivary cortisol values calculated as area under the curve with respect to ground (AUC_G) during the day using the samples from no. 4 to no. 7

Diurnal cycle: salivary cortisol values calculated as area under the curve with respect to ground (AUC_G) during the day using the sample no. 1, and samples from no. 4 to no. 7

Table 5 Relationship between job strain, covariates (age, gender, educational level, work hours per day, job contract, marital status) and salivary cortisol output during the day using general linear model (GLM)

"Predictors"	$AUC_{G\text{-work}}$		Diurnal cycle	
	F	*P*	*F*	*P*
Job strain	0.19	0.904	0.52	0.673
Age	14.20	0.001	10.66	0.002
Gender	5.34	0.025	8.70	0.005
Work hours/day			2.91	0.094
Final model	3.89	0.004	3.17	0.01

$AUC_{G\text{-work}}$: salivary cortisol values calculated as area under the curve with respect to ground (AUC_G) during the day using the samples from no. 4 to no. 7

Diurnal cycle: salivary cortisol values calculated as area under the curve with respect to ground (AUC_G) during the day using the sample no. 1, and samples from no. 4 to no. 7

33.5 nmol/l, and from 4.7 to 18.5 nmol/l (Clow et al. 2004), respectively. A plausible reason could be related to the different methods used to analyse the samples: in our research the salivary cortisol samples were analysed by mean of the sensitive radioimmunoassays (RIA) method, while a range of various analytical techniques (enzyme-linked immunoassay, time-resolved fluorescence, luminescence, or enzyme-linked read-out systems) have been employed in the studies, that bounding the comparisons among studies. The alternative explanation could be related to a positive association between job stress and cortisol excretion.

The high and low job demand and job control categories as well as the job strain levels significantly differed on score values, but any significant linear relation has been observed between work stress measures and cortisol output in the various general linear models calculated. These findings do not confirm the results pointed out by the studies employing the job strain model as a measure of work stress (Steptoe et al. 1998, 2000).

When the models took into account the variance accounted for by other factors (gender, age, marital status, educational level, hours worked per day, job contract), the variance that could be attributed to job strain or to the two dimensions (demand–control) of the model was not significantly different from the models in which job strain explains no variance. This lack of association between the measures used in this research to assess the environmental aspects of work stress and the diurnal pattern of cortisol salivary activity is not quite surprising considering that the HPA axis activation is thought to be associated with inability to cope, helplessness, affective distress and perceived uncontrollability (Peters et al. 1998), whereas, the constructs of the job strain model are restricted to the situational component (the assessment of an adverse work environment), and do not take into account the psychological aspect of the stress process (i.e. the cognitive and/or affective characteristics of the working person). Indeed surveys that used psychometric scales assessing both the coping characteristics of working person and the characteristics of the perceived work environment (Steptoe et al. 2000) or that associated with the measures of the psychological aspects of the stress process showed better correlation with biomarkers of stress (i.e. The Perceived Stress Scale; Cohen et al. 1983); Impact of Event Scale (Horowitz et al. 1979); Stress Appraisal (Peacock and Wong 1990); State-Trait Anxiety Inventory (King et al. 1983); Multiple Affect Adjective Checklist (Spielberger 1966).

The results of this study partially confirm our third hypothesis. Indeed, strong associations between job control and the cortisol awakening response indices appeared in females only, whereas males showed a good association between job demand and the total cortisol excretion during the awakening period (computed as area under the curve with respect to ground). No significant associations have been observed between cortisol excretion during the day (calculated both as $AUC_{G\text{-work}}$, and diurnal cycle) and work stress measures. These results agree with the conclusions of the psychoneuroendocrinological literature (Clow et al. 2004) suggesting that the measure of the awakening cortisol response constitutes today the most sensitive tool in the assessment of the physiological response to psychosocial job variables.

Limitations of this study should be recognized. First, the survey design was a typical cross-sectional study performed on a relative limited number of volunteers, so it is not possible to deliver "cause or effects analysis" to describe the complex relationships between the different variables investigated. Secondly, the enrolment in the study of volunteers only could have caused a selection bias: on the other side no significant differences have been observed between adherent and no-adherent subjects about sex, age, sociodemographic characteristics. Third, the investigation was performed with subjects from a single occupation rather than comparing job strain and other work characteristics across occupational groups; therefore the results cannot be generalized to other populations. The reasons for taking this approach is that professions or occupations differing in job strain may also vary in other factors (i.e. social status, physical activity) that might influence neuroendocrinal function independently of work stress. Finally, there was no monitoring of participants' compliance with the study protocol. Participant adherence to the study protocol is particularly important for cortisol awakening response interpretation, because this aspect of cortisol secretory activity is especially sensitive to deviation from instructions, leading to timing error, so even small deviations from the protocol would have resulted in substantial consequence for the values obtained.

In conclusion, the results of this study, although no apparent support for a close association could be found between work stress measures and salivary cortisol excretion, suggest that a lot of efforts must be reserved to elaborate theoretical models suitable for the assessment of job stress. In particular, more specific measures of mental stress, i.e. those involving uncertainty, novelty, lack of control, distress, anxiety, ego-involvement and helplessness, need to be tested in the assessment of physiological response that employs a promising biomarker of mental stress as the salivary cortisol.

Appendix

Formulas for the calculation of AUC variables

For the calculation of AUC_G; (Pruessner et al. 2003a)

$$AUC_G = \sum_{i=1}^{n-1} \frac{m_{(i+1)} + m_i}{2}$$

For the calculation of AUC_I; Pruessner et al. (2003a)

$$AUC_I = \left(\sum_{i=1}^{n-1} \frac{m_{(i+1)} + m_i}{2} \right) - (n-1) \cdot m_1$$

For the calculation of MnInc; Wust et al. (2000b)

$$\mathrm{MnInc} = (\mathrm{B} + \mathrm{C})/2 - \mathrm{A}$$

For the calculation of $AUC_{G\text{-work}}$, see AUC_G formula
For the calculation of Diurnal cycle, see AUC_G formula

Work stress measures

High strain: job control ≤ median job control and job demand > median
Active strain: job control ≤ median job control and job demand ≤ median
Passive strain: job control > median job control and job demand > median
Low strain: job control > median job control and job demand ≤ median

References

Alfredsson L, Hammer A, Nilsoson T (2002) Job strain and major risk factors for coronary heart disease among employed males and females in a Swedish study on work, lipids, and fibrinogen. Scand J Work Environ Health 28:238–248

Cesana GC, Sega R, Ferrario M,Chiodini P, Corrao G, Mancia G (2003) Job strain and blood pressure. A pooled analysis of several general population samples. Psychosom Med 65:1–6

Clow A, Thorn L, Evans P, Hucklebridge F (2004) The awakening cortisol response: methodological issues and significance. Stress 7:29–37

Cohen S, Kamarck T, Mermelstein R (1983) A global measure of perceived stress. J Health Soc Behav 24:385–396

Cox T, Griffiths A, Rial-Gonzales E (2000) Research on work-related stress. Luxemburg: European agency of safety and Health at Work, Office for Official Publications of the European Communities

Edwards S, Clow A, Evans P, Hucklebridge F (2001) Exploration of the awakening cortisol response in relation to diurnal cortisol secretory activity. Life Sci 68:2093–2103

Evans O, Steptoe A (2001) Social support at work, heart rate, and cortisol: a self-monitoring study. J Occup Health Psychol 6:361–370

Fischer JE, Calame A, Dettling AC, Zeier H, Fanconi S (2000) Objectifying psychomental stress in the workplace—an example. Int Arch Occup Environ Health 73:46–52

Goetzel RZ, Anderson DR, Whitmer RM, Ozminshowski RJ, Dunn RL, Wasserman J (1998) The relationship between modifiable health risks and health care expenditures: an analysis of the multi employer HERO health risk and cost database. J Occup Environ Med 40:843–854

Goldstein DS (1995) Stress, cathecolamines, and cardiovascular disease. Oxford University Press, Oxford, pp 1–161

Hoekstra E J, Hurrell J, Swanson NG et al (1995) Ergonomic, job task, and psychosocial risk factors for work-related musculo-skeletal disorders among teleservice center representatives. Int J Hum Comput Interact 8:421–431

Holman DJ (2002) Employee well being in call centres. Hum Resour Manage 12:35–50

Horowitz MJ, Wilner N, Alvarez W (1979) Impact of even scale: a measure of subjective distress. Psychosom Med 41:209–218

Hutchinson S, Purcell J, Kinnie N (2000) Evolving high commitment and the experience of the RAC call centre. Hum Resour Manage 10:63–78

Isic A, Dormann C, Zapf D (1999) Belastungen und Ressourcen an Call Center- Arbeitsplä tzen [Stressors and resources at call centre jobs]. Zeitschrift für Arbeitswissenschaft 53:202–208

Jonsson D, Rosengren A, Dotevall A (1995) Job control, job demand, and social support at work in relation to cardiovascular risk factors in MONICA J Cardiovasc Risk 6:379–365

Karasek RA, Theorell T (1990) Healthy work, productivity, and the reconstruction of working life. Basic Books, New York, p 348

King M, Burrows G, Stanley G (1983) Measurement of stress and arousal: validation of the stress/arousal adjective checklist. Br J Psychol 74:473–479

Kirschbaum C, Hellhammer DH (1994) Salivary cortisol in Psycho-neuroendocrine research: recent developments and applications. Psychoneuroendocrinology 19:313–334

Knights D, McCabe D (1998) What happens when the phone goes wild? Staff, stress and spaces for escape in a BPR telephone banking call regime. J Manage Stud 35:163–194

Kudielka BM, Kirschbaum C (2003) Awakening cortisol response are influenced by health and awakening time but not by menstrual cycle phase. Psychoneuroengocrinology 28:35–47

Kunz-Ebrecht S, Kirschbaum C, Steptoe A (2004) Work stress, socioeconomic status and neuroendocrine activation over the working day. Soc Sci Med 58:1523–1530

Lundberg U (1984) Human psychobiology in Scandinavia: II. Psychoneuroendocrinology: human stress and coping processes. Scand J Psychol 25:214–226

Netterstrom B, Kristensen TS, Damsgaard MT (1991) Job strain and cardiovascular risk factors: a cross-sectional study of employed Danish men and women. Br J Ind Med 48:684–689

Niedhammer I, Goldberg M, Leclerc A (1998) Psychosocial work environment and cardiovascular risk factors in a occupational cohort in France. J Epidemiol Community Health 52:93–100

Ockenfels MC, Porter L, Smyth J, Kirschbaum C, Hellahammer DH, Stone AA (1995) Effects of chronic stress associated with unemployment on salivary cortisol: overall cortisol levels, diurnal rhythm, and acute stress reactivity. Psychosom Med 57:460–467

Peacock EJ, Wong PTP (1990) The stress appraisal measure (SAM): a multi-dimensional approach to cognitive appraisal. Stress Med 6:227–236

Peters J, Madelon L, Godaert GLR, Ballieux RE, van Vliet M, Willemsen J (1998) Cardiovascular and endocrine responses to experimental stress: effects of mental effort and controllability. Psychoneuroendocrinology 23:1–17

Pruessner JC, Wolf OT, Hellhammer DH, Buske-Kirschbaum A, von Auer K, Jobst S, Kaspers F, Kirschbaum C (1997) Free cortisol levels after awakening: a reliable biological marker for the assessment of adrenocortical activity. Life Sci 6:2539–2549

Pruessner JC, Hellhammer DH, Kirschbaum C (1999) Burnout, perceived stress, and cortisol response to awakening. Psychosom Med 61:197–204

Pruessner JC, Kischbaum C, Meinlschmid G, Hellhammer DH (2003a) Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology 28:916–931

Pruessner M, Hellhammer DH, Pruessner JC, Lupien S (2003b) Self reported depressive symptoms and stress levels in Healthy Young Men: associations with the cortisol response to awakening. Psychol Med 65:92–99

Schlotz S, Hellhammer J, Schulz P, Stone AA (2004) Perceived work overload and chronic worrying predict weekend–weekday differences in the cortisol awakening response. Psychosom Med 66:207–214

Schultz P, Kischbaum C, Prubner J, Hellhammer D (1998) Increased free cortisol secretion after awakening in chronically stressed individuals due to work overload. Stress Med 14:91–97

Spielberger C (1966) Theory and research on anxiety. In: Spielberger C (ed) Anxiety and behaviour. Academic Press, New York, pp 3–20

Steptoe A, Wardle J, Lipsey Z, Mills R, Oliver G Jarvis M, Kirschbaum C (1998) A longitudinal study of work load and variations in psychological well-being, cortisol, smoking, and alcohol consumption. Ann Behav Med 20:84–91

Steptoe A, Cropley M, Griffith J, Kirschbaum C (2000) Job strain and anger expression predict early morning elevations in salivary cortisol. Psychosom Med 62:286–292

Steptoe A, Siegrist J, Kirschbaum C, Marmot M (2004) Effort–reward imbalance, overcommitment and measures of cortisol and blood pressure over the working day. Psychosom Med 66:323–329

Tsutsumi A, Kayaba K, Tsutsumi K, Igarashi M, Jichi Medical School Cohort Study Group (2001) Association between job strain and prevalence of hypertension: a cross-sectional analysis in a Japanese working population with a wide range of occupations: the Jichi Medical school cohort study. Occup Environ Med 58:367–373

Wust S, Federenko I, Hellhammer DH, Kischbaum C (2000a) Genetic factors, perceived chronic stress, and the free cortisol response to awakening. Psychoneuroendocrinology 25:707–720

Wust S, Wolf J, Hellhammer DH, Federenko I, Schommer N, Kischbaum C (2000b) The cortisol awakening response—normal values and confounds. Noise Health 7:77–85

Yang Y, Koh D, Ng V, Lee F, Chan G, Dong F (2002) Salivary cortisol levels and work-related stress among emergency department nurses. J Occup Environ Med 43:1011–1018