

Associations of Long-term Shift Work with Waking Salivary Cortisol Concentration and Patterns among Police Officers

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Abstract: The objective of this study was to evaluate whether long-term shift work is associated with both the total hormonal secretion after awakening and the pattern of the cortisol levels during the first hour following awakening, among 65 randomly selected police officers who represent a high stress occupation. Dominant shift (Day, Afternoon, or Midnight) was ascertained using daily payroll records of each participant's work activities during the 6–8 yrs prior to saliva sampling. Four salivary samples were collected at 15 min intervals upon first awakening. After accounting for potential confounders, salivary cortisol concentrations averaged across all four time points and total area under the curve differed significantly across shift with midnight shift workers showing suppressed awakening cortisol response relative to the afternoon and day shift. The percent of hours worked on midnight shift was inversely correlated with total awakening cortisol output. In contrast, the pattern of cortisol secretion during the first hour following waking appeared not to be affected as no significant interaction effect was found between time since awakening and shift work. The results show that long-term midnight shift work is associated with decreased absolute mean level and total volume of cortisol released over the waking period.

Key words: Shift work, Waking, Cortisol, Police, Saliva, HPA-axis

Introduction

Research indicates that shift work may lead to disruption of circadian rhythms^{1–3)} and adverse health outcomes including metabolic disorders and cardiovascular disease

(CVD)^{9–11)}. In fact, long-term shift work, particularly evening or rotating shift work, has been linked to chronic stress with possible adverse consequences on the neuro-endocrine-immune systems¹²⁾. Policing by its nature is a stressful occupation where officers are exposed to a high level of traumatic and non-traumatic stressors¹³⁾ and shift work, a major stressor in police work as it is in many occupations, is an integral part of law enforcement. Consequently police officers are shown to have higher rates

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of CVD and CVD risk factors compared to the general population^{14, 15}). They represent an important group in which to examine the relationship between long-term shift work and cortisol levels yet studies in this high risk and understudied occupational group are limited.

Following acute stressors, the activation of the hypothalamic-pituitary adrenal (HPA) axis and the subsequent increase in cortisol secretion is a known physiologic stress response¹⁶⁻¹⁸). However, few studies have investigated the influence of long-term (or extended) shift work on the HPA axis and the awakening cortisol. Also, the findings from previous studies on the association between shiftwork and awakening cortisol have been inconsistent¹⁹⁻²⁸); both, high and low waking cortisol levels have been reported among shift workers.

In a prospective study of nurses, morning serum cortisol levels did not differ between shift working and day time nurses after adjusting for baseline values and job seniority²⁶), while another study on a similar study population²¹) showed plasma morning cortisol concentrations were lower after night work than in the day of rest. In an experimental study of healthy young men²⁴), cortisol concentrations upon awakening were significantly higher in morning than in evening-oriented subjects. A study²³) reported that implementation of night work in former day workers initially lead to blunted cortisol profiles that normalized after a short period of adjustment. In a study of textile factory workers where total cortisol levels were assessed using hair samples, shift workers had significantly higher cortisol levels compared to day workers²⁷).

Although a number of factors including occupation, type of job performed, specific work schedule, level of job-related stress, and design for cortisol sampling may play a part in explaining differences in results across studies, the studies may also share some common limitations. Previous investigations on the association between shift work and waking cortisol have relied heavily upon self-report data concerning shift work. More importantly, information on the duration or length of time (in years) that study participants have been working on a given shift prior to collection of blood, saliva or hair for determining cortisol levels was not available in these studies. Data from a random sample of active duty police officers enrolled in the Buffalo Cardio-Metabolic Occupational Police Stress (BCOPS) pilot study²⁹) were used. The objective of the current study, therefore, was to evaluate how long-term shift work, ascertained retrospectively from payroll records of participant's activity, influences the amount of and pattern of secretion of salivary cortisol at awakening.

We present comparisons of the absolute mean waking cortisol, the integrated volume of cortisol released over the waking period, and the pattern of cortisol during the first hour after waking among long-term day, afternoon, and night shift officers.

Methods

Study participants and design

For this study, participants from the Buffalo Cardio-Metabolic Occupational Police Stress (BCOPS) Pilot Study²⁹) were evaluated. The Buffalo, New York Police department, a midsized urban police force, was the selected sample site. A random sample stratified on gender (n = 100, 42 females, 58 males; n=65 participants with non-missing data were used for statistical analyses) was selected from all police officers in the department using a computer-generated random number table. No specific inclusion criteria were used for the study, other than that the participant would be a sworn police officer. One hundred percent of the random sample voluntarily agreed to participate in the study. The subjects were examined between 2001 and 2003 and data collection was performed at The Center for Preventive Medicine, School of Public Health and Health Professions, State University of New York at Buffalo. The State University of New York at Buffalo Internal Review Board and the National Institute for Occupational Safety and Health (NIOSH) Human Subjects Review Board (HSRB) approved the study and informed consent was obtained from all participants. The study is a retrospective cohort in design (or a historical cohort design) as past payroll records (from 1994 to date of examination) were used to assess the exposure of interest (dominant shift worked) prior to the outcome variable (awakening cortisol response). The historical exposure data was transformed into a single integrated measure and was analyzed cross-sectionally with the outcome data that was collected on the date of examination. Further details about the study have been described previously²⁹).

Measures, Shift work

Work history data, from May of 1994 to date of each officer's exam (2001-2003), were available in an electronic format for 96 of the 100 participants from payroll records of Buffalo police officers. The database from payroll records contained a day-by-day account of activities for each officer over the entire time period. Information included the start time of work, the type of activity (regular work, court work, overtime work), the type of leave (weekend,

sickness, work-related injury, vacation), and the number of hours worked on each activity. The distribution of work start times for the regularly scheduled activity was examined and 30% of the records started at 7am, 24% at 8am, 28% at 4pm, and 13% at 9pm. The time a participant started his/her regular work was used to classify the shift for that day into one of the following three shifts: day shift if the start time was between 4 am and 11 am; afternoon shift if the start time was between 12 pm and 7 pm; and midnight shift if the start time was between 8 pm and 3 am. For the time period spanning from 1994 to date of exam, total hours worked as well as hours worked at the day, afternoon and midnight shift were computed for each participant. Taking into account the length of time a participant was working (from first date when electronic work history records were available to date of exam), the computed hours were standardized to a weekly basis (hours worked per week) and percent of total hours worked on each shift was calculated. A dominant shift for each subject was determined by comparing the percent of total hours worked at day, afternoon, and midnight shifts and was defined as the shift with the largest percentage of the total hours. For example, from 1994 to date of exam, an officer who worked 20% on the day shift, 60% on the afternoon shift, and 20% on midnight shift is classified as an afternoon shift worker (the dominant shift). Shift work variables of interest for analyses include (a) the dominant shift, a categorical variable with three levels (day, afternoon, and midnight), and (b) the percent of hours worked on midnight shift, a numeric variable that ranges from 0 to 100.

Salivary Cortisol

For the assessment of the awakening cortisol, subjects were instructed to collect saliva samples immediately after awakening, and 15, 30, and 45 min thereafter. Officers were provided with Salivettes (Sarstedt, USA), a commercially available collection device consisting of a dental roll and a centrifuge tube, for the collection of saliva samples. Participants were asked to refrain from taking stimulant medication, smoking, eating and drinking, and brushing their teeth before completing salivary sampling to avoid contamination of saliva with food or blood caused by micro-injuries of the oral cavity. At the designated collection time, the officers removed the dental roll from the centrifuge tube and placed it in their mouth for approximately 2 min allowing for saturation of the roll. Once in the laboratory, the tube is centrifuged to provide a non-viscous saliva sample for assay; centrifuged samples

are maintained at -20°C until assayed for cortisol by a commercially available chemiluminescence immunoassay (IBL, Hamburg, Germany) at the Technical University of Dresden. The saliva samples were analyzed within a year from the date of collection (between 2001 and 2003). In samples frozen at -20° , cortisol concentrations remain stable for 1 yr³⁰) and are relatively insensitive to repeated thawing and refreezing. Using the four cortisol samples various summary measures were derived. Previous assessment of the current study population³¹) showed that derived awakening cortisol parameters can be classified into two components: those that measure total hormonal excretion (primarily, average of the waking samples (AVE), the peak morning sample (PK) and total area under the curve (AUC_G)) and those that measure the profile (pattern) of cortisol over time (primarily, reactivity (RT), slope of the regression line fitted to all four waking samples (SP), and area under the curve with respect to increase (AUC_I). Reactivity (RT) refers to the change in salivary cortisol during the observation period and was defined as the difference between the last awakening cortisol value and the first. These parameters were derived and used as response variables of interest in this study.

Demographic and Lifestyle Characteristics

Questionnaires were administered to collect demographic and lifestyle characteristics including years of education, sleep duration, physical activity, marital status, years of police service, and police rank. Hours of sleep and physical activity were assessed using the Seven-Day Physical Activity Recall questionnaire developed in the Stanford Five-City Project³²). Smoking and alcohol intake information were gathered at a baseline study conducted two-years earlier (n=115) and 87 of the 100 officers in the present study participated at the baseline. Officers were categorized as current, former and never smokers, and alcohol intake was based on the number of drinks reported per week using categories of 0, <1, 1–6, and ≥ 6 drinks per week. Body mass index was derived using physical measurements of weight in kg divided by height in meters squared.

A number of psychosocial factors including depression, traumatic events, major stressful life events, and PTSD symptoms were assessed. Stressful life events encountered during the previous year were assessed using a 41-item life events questionnaire. Participants were asked to respond (yes or no) to each question and a total life event score was computed by summing the responses for the 41 events. Traumatic Police incidents (TPI) score was as-

essed by asking participants to respond (yes/no) to 9 traumatic events (being involved in a shooting, witnessing the shooting of another police officer, seeing abused children, seeing victims of serious traffic accident, seeing someone die, seeing dead bodies, seeing severely assaulted victims, seeing victims of homicide, seeing other disturbing events) experienced during the past year. A total score was computed by summing the responses to the 9 questions. Depressive symptoms were measured using the Center for Epidemiologic Studies-Depression (CES-D) scale. Participants were asked to complete a 20-item questionnaire designed to measure symptoms of depression during the past 7 days. The Impact of Event Scale (IES) was used to measure psychological symptoms of PTSD experienced by participants during the past 7 days and was assessed using a 15-item questionnaire on a 4 point Likert scale.

Statistical analyses

Only officers with complete data on all four waking cortisol samples and time of collection of the samples were used for statistical analyses ($n=65$). Descriptive statistics were used to describe the demographic and life style characteristics of the study sample by shift work. The primary relationship of interest between shift work and awakening cortisol was examined by comparing mean values of the cortisol parameters across the three shift work categories using analysis of variance (ANOVA). Analysis of covariance (ANCOVA) was used to examine the effect of adjustment for various covariates (age, gender, education, smoking, alcohol consumption, physical activity, body mass index, and awakening time) on the relationship between shift work and awakening cortisol. Due to the skewed nature of the cortisol concentration, the derived parameters (except for RT and AUC_1) were log transformed prior to analyses and back-transformed means and their 95% confidence intervals were reported. The distributions of AUC_1 and RT were fairly normal and hence no data transformation was applied for these two variables.

Differences in cortisol response profile over time across the three shift work groups was tested using two approaches: (a) by comparing the mean values of derived cortisol parameters that are known to reflect pattern over time (RT, SP and AUC_1) across the three groups and (b) by modeling cortisol response over time using a repeated measures analysis and testing the interaction between time since awakening and shift work. The MIXED procedure in SAS was used to model the repeated measures with the spatial power covariance structure as a model for correlations among measurements made on the same subject. In

addition, correlation analyses between the derived measures of awakening cortisol and the continuous measure of shift work (percent of hours worked on midnight shift) were performed with adjustment for possible confounding covariates.

Residuals from the fitted models (ANOVA, ANCOVA, multiple regression models) were tested for normality and homogeneity of variance using formal and graphical procedures. Due to the small number of female participants in the midnight shift ($n=1$), stratified analyses by gender were not conducted. Instead adjustment for the effect of gender was made in all analyses. All statistical analyses were performed using the SAS software version 9.2 (SAS Institute, Inc., Cary, NC) and significance level was set at 5% except for interaction terms (15%).

Results

Demographic and lifestyle characteristics

The demographic and lifestyle characteristics of the participants by the dominant shift are shown in Table 1. The study population consists of 65% males and the majority was white (71%) and had a rank of patrol officer or sergeant (57%). The mean age was 43.2 yrs (range: 29–63). Participants on the day shift were significantly older, had longer years of service, had a higher percentage of women (47%), and African Americans (35%) compared to the afternoon and midnight shift groups. The average time of awakening did not differ across shift (day: 7:29AM, afternoon: 7:55AM, and midnight: 8:12AM, p -value=0.4841). Participants on the afternoon and midnight shift had higher traumatic police incident scores than those on the day shift.

The dominant shift and distribution of percent of hours on each shift

Of the current sample used for analyses ($n=65$), the percentage of participants with dominant day, afternoon, and midnight shift were 52.3% ($n=34$), 35.4% ($n=23$), and 12.3% ($n=8$) respectively (Table 1). These estimates are consistent with the distribution of dominant shift for the larger sample ($n=96$); 53.1%, 34.4%, and 12.5% for day, afternoon, and midnight shifts respectively (not restricted to those having complete cortisol measurements). Within each dominant shift, the distribution of percent of hours worked on that specific shift was examined: 31 of the 34 with dominant day shift spent over 70% of their work hours on the day shift; 16 of the 23 with dominant afternoon shift spent over 70% of their work hours on the

Table 1. Demographic and lifestyle characteristics of study participants by shift work

		Day shift (n=34)	Afternoon shift (n=23)	Midnight shift (n=8)	P-value ¹
Age, years		46.8 ± 7.5	38.7 ± 6.1	40.5 ± 7.1	0.001
BMI, kg/m ²		27.3 ± 4.5	28.7 ± 3.6	28.6 ± 4.8	0.439
Physical activity, hours/wk*		7.5 ± 9.6	7.4 ± 13.5	3.5 ± 2.3	0.609
Length of work history (yrs)		8.0 ± 0.8	7.5 ± 1.3	6.6 ± 1.9	0.008
Hours of sleep/day*		5.9 ± 2.0	6.6 ± 0.93	6.3 ± 1.6	0.399
CES_D score ⁺		7.9 ± 8.1	7.0 ± 5.1	6.1 ± 2.8	0.747
IES score ⁺		22.0 ± 14.7	18.8 ± 16.3	13.4 ± 12.0	0.321
Life events stress score		3.1 ± 3.1	2.3 ± 1.8	2.8 ± 1.7	0.535
TPI score ⁺		3.0 ± 2.4	5.0 ± 2.0	4.9 ± 2.0	0.004
Waking time		7:29AM ± 1.7 hrs.	7:55AM ± 1.7 hrs.	8:12AM ± 2.0 hrs.	0.484
Gender	Male	18 (52.9)	17 (73.9)	7 (87.5)	0.094
	Female	16 (47.1)	6 (26.1)	1 (12.5)	
Race	White	19 (55.9)	21 (91.3)	6 (75.0)	0.043
	Black	12 (35.3)	2 (8.70)	2 (25.0)	
	Hispanic	3 (8.8)	0 (00.0)	0 (00.0)	
Education	≤ High school	5 (14.7)	3 (13.0)	2 (25.0)	0.437
	College < 4 years	9 (26.5)	6 (26.1)	4 (50.0)	
	College + 4 years	20 (58.8)	14 (60.9)	2 (25.0)	
Marital status	Single	6 (17.7)	3 (13.0)	3 (37.5)	0.451
	Married	21 (61.8)	17 (73.9)	5 (62.5)	
	Divorced	7 (20.6)	3 (13.0)	0 (00.0)	
Rank	Police officer/Sergeant	14 (41.2)	15 (65.2)	8 (100.0)	0.044
	Lieutenant/Captain	11 (32.4)	4 (17.4)	0 (00.0)	
	Detective/Other	9 (26.5)	4 (17.4)	0 (00.0)	
Smoking*	Current	4 (13.8)	3 (15.0)	2 (25.0)	0.945
	Former	11 (37.9)	8 (40.0)	3 (37.5)	
	Never	14 (48.3)	9 (45.0)	3 (37.5)	
Alcohol drinks/week*	0	6 (20.7)	1 (5.0)	2 (25.0)	0.589
	< 1	6 (20.7)	5 (25.0)	1 (12.5)	
	1–5	14 (48.3)	10 (50.0)	5 (62.5)	
	≥ 6	3 (10.3)	4 (20.0)	0 (00.0)	

Results are means ± SD for the continuous variables, and n (%) for categorical variables. ¹P-values are from χ^2 tests of independence or Fisher's exact test for categorical variables and from ANOVA testing differences in means across dominant shift for continuous variables. *Number of participants with complete data on smoking and alcohol consumption were: 29, 20, and 8 for day, afternoon, and midnight shift respectively; BMI: 31, 16 and 8; Hours of sleep and physical activity: 31, 17 and 8. ⁺The Center for Epidemiologic Studies Depression Scale (CES-D); The Impact of Event Score (IES); Traumatic Police Incidents (TPI) score.

afternoon shift; 7 of the 8 subjects with dominant midnight shift spent over 70% of their time on midnight shift. The data suggests that dominant shift as a categorical variable captures the shift during which a participant spent the majority of his/her work hours.

Shift work and awakening cortisol response

Salivary cortisol concentrations (nmol/liter) averaged across all four time points (AVE) differed significantly among the day, afternoon and midnight shift workers ($P = 0.038$, Table 2, Model I), with midnight shift workers (6.5,

95% CI: 4.0–10.6) showing diminished awakening cortisol secretion compared to the afternoon (13.3, 10.0–17.8) or the day (12.7, 10.0–16.1) shift. Total area under the curve (AUC_G, nmol/liter × minutes) also differed significantly across the three shift work categories ($P = 0.041$) with those in the midnight shift (314.6, 191.5–517.1) showing lower mean values than those in the afternoon (647.5, 483.1–867.9) or the day shift (606.7, 476.8–772.0). Peak morning cortisol (PK) concentration (nmol/liter), although not significantly different among the shift groups ($P = 0.070$), was smaller for midnight shift workers (9.4,

Table 2. Mean values (and 95% CI) of derived awakening cortisol parameters by shift work

	Day shift (D)	Afternoon shift (A)	Midnight shift (M)	P-value ¹	P-value ² for multiple comparisons and contrasts		
					M vs. A	M vs. D	M vs. D&A
Model I	(n=34)	(n=23)	(n=8)				
^a AVE	12.7 (10.0–16.1)	13.3 (10.0–17.8)	6.5 (4.0–10.6)	0.038	0.015	0.018	0.011
^b AUC _G	606.7 (476.8–772.0)	647.5 (483.1–867.9)	314.6 (191.5–517.1)	0.041	0.015	0.021	0.012
^c AUC _I	77.1 (–20.1–174.2)	85.2 (–32.9–203.3)	46.6 (–153.7–246.9)	0.946	0.741	0.785	0.751
^d PK	17.5 (13.7–22.3)	18.3 (13.6–24.6)	9.4 (5.7–15.6)	0.070	0.028	0.032	0.021
^e SP	0.30 (0.2–0.4)	0.24 (0.17–0.34)	0.21 (0.12–0.37)	0.734	0.671	0.273	0.385
^f RT	–0.32 (–4.4–3.8)	1.41 (–3.5–6.4)	–0.16 (–8.57–8.2)	0.860	0.749	0.974	0.904
Model II	(n=26)	(n=14)	(n=8)				
^a AVE	10.7 (7.8–14.7)	15.7 (10.3–23.8)	6.2 (3.5–11.0)	0.030	0.009	0.121	0.021
^b AUC _G	515.3 (375.8–706.7)	757.3 (498.1–1151.4)	306.3 (172.9–542.5)	0.035	0.011	0.142	0.026
^c AUC _I	47.9 (–48.8–144.7)	153.7 (25.4–282.0)	101.0 (–74.2–276.1)	0.437	0.611	0.620	0.961
^d PK	14.8 (10.6–20.7)	21.1 (13.5–32.9)	9.1 (5.0–16.8)	0.076	0.025	0.198	0.049
^e SP	0.28 (0.19–0.40)	0.27 (0.16–0.43)	0.22 (0.11–0.42)	0.820	0.612	0.542	0.538
^f RT	–1.2 (–5.5–3.2)	2.7 (–3.1–8.5)	2.5 (–5.4–10.4)	0.572	0.973	0.444	0.713

All cortisol parameters except AUC_I and RT were log-transformed prior to statistical analyses. The means and 95% CIs obtained in the log-scale were then back transformed. Model I: unadjusted means (95% CI) of cortisol parameters across shift work. Model II: adjusted means (95% CI) of cortisol parameters across shift work. Adjustment was made for age, gender, education, smoking, alcohol consumption, physical activity, body mass index and awakening time. ¹Overall p-value comparing differences across the three shift categories; ²p-value for pairwise differences and contrasts of interest. ^a AVE: Average of morning samples, nmol/L; ^b AUC_G: Total area under the curve (expressed as nmol/liter × minutes); ^c AUC_I: Area under the curve with respect to increase (expressed as nmol/liter × minutes); ^d PK: Peak morning sample, nmol/L; ^e SP: Slope of fitted regression line, nmol/L; ^f RT: Reactivity, nmol/L.

5.7–15.6) than the afternoon (18.3, 13.6–24.6) or day (17.5, 13.7–22.3) shift workers. Adjustment for potential confounders (age, gender, education, smoking, alcohol consumption, physical activity, body mass index and awakening time) did not alter the overall significant association between the measures of total hormonal secretion and shift work (Table 2, Model II: adjusted p-values were 0.030, 0.035, and 0.076 for AVE, AUC_G and PK respectively). However, adjustment for the confounders slightly attenuated the significant pair-wise comparison between the midnight and day shifts (Table 2, Model II). Interactions between shift work and potential effect modifiers (CES-D score, PTSD score, life events score, and traumatic police incidents) were tested for each derived awakening cortisol parameter and none of the interactions were statistically significant at 15% level. Further adjustment for the psychosocial stress measures did not attenuate the association (adjusted p-values were 0.022, 0.023, and 0.048 for AVE, AUC_G and PK respectively).

The parameters that quantify awakening cortisol dynamics (pattern) over time, i.e., reactivity (RT), slope of the regression line fitted to the waking samples (SP), and the area under the curve with respect to increase (AUC_I) did not differ significantly among the day, afternoon and

midnight shift workers (Table 2, Model II). This result is consistent with the pattern displayed in Fig. 1 and also with the lack of interaction between time since awakening and shift work (p=0.392) from the repeated measures data analysis. We also compared the cortisol awakening response (defined as the difference between the awakening and peak cortisol samples) among the three groups. After accounting for potential confounders, there were no significant differences across shift (Day: 1.63 nmol/l, 95% CI: 0.77–3.45; Afternoon: 1.04 nmol/l, 95% CI: 0.39–2.82; Midnight: 0.75 nmol/l, 95% CI: 0.19–2.91; p=0.618).

The simple and partial (adjusted) correlation coefficients between the percent of hours per week worked on the midnight shift and the six derived measures of awakening cortisol response are presented in Table 3. The average cortisol over all four time points (AVE: r=–0.42, p=0.010), the peak cortisol (PK: r=–0.37, p=0.024) and the total area under the curve (AUC_G: r=–0.41, p=0.012) showed negative and significant correlation with percent of hours worked on the midnight shift, whereas the parameters measuring morning cortisol pattern over time (RT, SP, and AUC_I) were not significantly correlated.

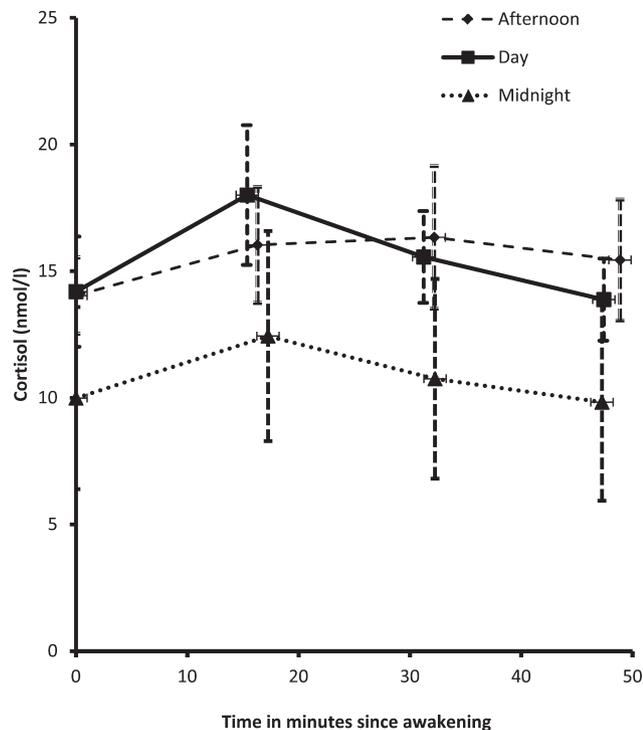


Fig. 1. Profile of awakening salivary cortisol among day, afternoon, and midnight shift workers (mean \pm SE).

Discussion

Previous research linking shift work and HPA axis function as measured by awakening cortisol response was contradictory with some studies reporting increased activation^{21, 23, 24}) and others reporting the opposite^{26, 27}). The central aim of this study was to examine differences in waking salivary cortisol secretion (hormonal output) and profile (pattern over time) across three work shift schedules (day, afternoon, and midnight) among police officers who represent a high stress occupation. The participants were officers who worked on their respective shift for 6–8 yrs (i.e., long term shift workers). Within this context, we found, after controlling for age, gender, education, smoking status, alcohol consumption, physical activity, BMI, and awakening time that officers working in the midnight shift had lower levels of cortisol after awakening compared to those in the afternoon or day shifts. On the other hand, the general pattern (profile) of the repeated measures of salivary cortisol during the first hour after awakening were similar across all three shifts; characterized by a peak cortisol 20–30 min after waking and a gradual decline thereafter. The results from the current study suggest that working dominantly on the midnight shift (for extended

Table 3. Pearson correlations (*r*) between percent of hours worked on midnight shift and derived awakening cortisol parameters

Derived cortisol Parameters	Simple correlations		Partial correlations	
	<i>r</i>	P-value ¹	<i>r</i>	P-value ²
^a AVE	–0.32	0.010	–0.42	0.010
^b AUC _G	–0.32	0.009	–0.41	0.012
^c AUC _I	–0.04	0.749	–0.09	0.584
^d PK	–0.3	0.014	–0.37	0.024
^e SP	–0.13	0.319	–0.18	0.306
^f RT	0	0.991	0.04	0.829

All cortisol parameters except AUC_I and RT were log-transformed prior to statistical analyses. ¹ unadjusted p-value (n=65). ² p-value adjusted for age, gender, education, smoking, alcohol consumption, physical activity, body mass index and awakening time (n=48). ^a AVE: Average of morning samples, nmol/L; ^b AUC_G: Total area under the curve (expressed as nmol/liter \times minutes); ^c AUC_I: Area under the curve with respect to increase (expressed as nmol/liter \times minutes); ^d PK: Peak morning sample, nmol/L; ^e SP: Slope of fitted regression line, nmol/L; ^f RT: Reactivity, nmol/L.

period of time) may influence aspects of HPA axis regulation leading to a suppressed release of cortisol following awakening.

Repeated or chronic challenges of the HPA axis by environmental stressors may cause alterations in HPA axis activity leading to small diurnal variations and flattening of the diurnal cortisol curve as a consequence of significant reductions in awakening cortisol accompanied by a rise in evening cortisol³³). A meta-analysis³⁴) presented a similar argument that following a stress the HPA axis exhibits initial activation in the form of elevated cortisol release. However, following prolonged exposure to the stressors, the body's biological systems could mount a counter-regulatory response (dysregulation) such that the cortisol output rebounds to less than normal. The results from the current study are consistent with this hypothesis and in agreement with other studies that investigated cortisol rhythms in shift workers and found that the cortisol awakening response is decreased and evening cortisol levels are increased during shift work^{19, 25, 35}). In a study of police officers from the current sample, the association between short-term shift work (3, 5, 7 and 14 days prior to saliva collection) and waking cortisol was examined and waking salivary cortisol levels were lower among night or afternoon shift officers compared to those on day shift²⁸). In an experimental study of 8 morning-oriented vs. 8 evening-oriented healthy young men, cortisol concentrations upon awakening were higher in the morning oriented than in the evening oriented subjects²⁴). In a study of workers at an electronic manufacturing facility, cortisol

profiles in permanent night workers appear to be blunted during night work and days off and implementation of night work in former day workers seems to lead to initially blunted cortisol profiles that normalize after a short adjustment period²³). Among nurses, a study showed significant reduction in morning salivary cortisol (at 6AM) after 7 days of consecutive night shifts compared to those working days²⁰).

Results from other studies differed from ours^{26, 27, 36, 37}). In a longitudinal study, morning cortisol was measured in 68 shift-working and 28 daytime nurses at baseline and at 12 months. Cortisol levels at 12 months did not differ between the two groups after adjustment for baseline values and job seniority²⁶). A study of total cortisol levels from hair samples²⁷) compared 33 shift workers and 89 day workers where shift workers were shown to have higher hair cortisol levels than day workers but the hair cortisol in this study measured total amount of cortisol released over the last 3 months while our measure concerns how much is released in response to the awakening signal. In a study of chronic stress associated with unemployment, unemployed subjects had higher morning and lower evening salivary cortisol levels compared to employed subjects³⁵). In a sample of 100 university students, those who perceived a higher level of chronic stress due to workload had higher early morning cortisol increase after awakening than those who did not³⁶).

The mechanism linking shift work and awakening cortisol response can be complex and some studies^{34, 38}) elude that some of the variability in results across studies is attributable to differences in stressors (e.g. the specific work schedule and flexibility, job type, perceived stress, years worked on the specific shift, etc.) and personal characteristics (e.g. individual differences in tolerance to shift work). A number of previous studies that examined associations of perceived stress, job strain, and fatigue/burnout with awakening cortisol response indirectly support the current results^{34, 39, 40, 41–43}). A meta-analysis of studies on the relationship between psychosocial factors and waking cortisol found that waking cortisol is positively associated with job and general life stress but was negatively associated with fatigue, burnout and exhaustion⁴²). In a study of school teachers where saliva samples were collected at 15, 30, and 60 min following awakening, the study found that those scoring high on burnout had lower overall cortisol secretion⁴⁴). Studies of middle-aged women⁴²) and hospital nurses⁴⁰) found perceived stress level was negatively correlated with waking salivary cortisol concentration where those in the high stress group secreted lower levels of

waking cortisol compared to those in the low stress group. A longitudinal study of retail industry workers³⁹) found that salivary cortisol was inversely associated with job strain; higher concentrations were observed among subjects with low job strain compared to those experiencing high job strain. In the current study the negative correlation between percent of hours worked in midnight shift and the total awakening cortisol secretion suggests that those who spend the majority of their weekly work hours on the midnight shift tend to have lower morning cortisol output.

Cortisol is secreted according to a well-defined circadian rhythm⁴⁵), and generally morning cortisol level increases rapidly following awakening with the peak level occurring 15 to 30 min after waking. In the current study the non-significant interaction between shift category and time of saliva sampling from repeated measures data analysis and the non-significant association between cortisol parameters that measure overall pattern (RT, SP, and AUC_t) and shift category (% of hours worked on midnight shift) suggest that the pattern of morning cortisol levels during the first 45–60 min after awakening is similar regardless of shift.

The HPA-axis response to shift work is probably highly variable and may be associated with several confounding factors including job stress, individual characteristics, the type of work, duration of work hours, and intervals between shifts²⁶). For example, several studies reported that shift work is overall well-tolerated by nurses and does not impair their health or sleep quality^{46, 47}). Hence future comprehensive studies should consider including the above factors, particularly the type of occupation and level of job-related stress in order to gain a better understanding of the association between shift work and HPA-axis activity in general not just the waking response. Night shift work as a stressor may influence future health risk indirectly through changing health behaviors (e.g. unhealthy eating, less exercise, poor sleep, etc.) and therefore understanding the extent to which these changes in health behaviors influence awakening cortisol secretion¹⁷) and pattern are important in estimating the independent effect of shift work. In future studies it would be worthwhile to compare and contrast the influence of recent night shift work experience and long-term night shift work on awakening cortisol response.

The current study has limitations and strengths. Shortcomings include the retrospective cohort design of the study which precludes causal inference, recruitment limited to police officers only, small sample size for those on the midnight shift, and single day of saliva

sampling⁴⁸). The sub-sample used for statistical analyses (n=65) were compared to those excluded (n=35) with respect to dominant shift (Day: 52% vs. 55%, Afternoon: 35% vs. 32%, Midnight: 12% vs. 13%, p-value=0.9555), demographic, lifestyle, and psychosocial variables. The results showed that the two samples did not differ significantly in all variables except CES-D score (7.4 ± 6.6 vs. 11.2 ± 8.7 , p-value=0.015). The sample used for analysis (n=65) is therefore representative of the whole group (n=100). Generalizability of the findings may be limited to police departments with similar characteristics. To our knowledge, this study is among the first to examine long-term shift work and waking salivary cortisol among police officers, an understudied and high risk population. More importantly, the study provided a unique opportunity to examine the association based on multiple years (8–10 yrs) of objective, day-to-day work records of officers while previous studies relied primarily upon self-reported data concerning shift work. We were also able to account for awakening time and a number of physical (e.g. BMI), lifestyle (smoking, alcohol consumption, physical activity), and psychosocial factors (e.g. depression, traumatic events, life events) that could confound the association between shift work and waking salivary cortisol.

In summary, despite the small sample limitation, our study has shown that independent of confounding factors long-term night work is associated with attenuation of the awakening cortisol response, yet the cortisol pattern through the first hour after waking was not affected by shift work. The findings are consistent with accumulating evidence indicating that working night shift for an extended period of time may lead to chronic stress and reduced HPA axis activity. This study adds to the body of knowledge concerning the association between long-term shift work and awakening salivary cortisol among high stress occupations and may have implications for future health risk of officers working night shifts for an extended period of time. Due to the small sample size of night shift officers these results are preliminary and should, in the future, be replicated with larger samples and other populations. In the context of our finding of a suppressed awakening cortisol in police officers who worked a greater percentage of time on the midnight shift, it is worth noting that a low awakening cortisol level may signify a dysfunction of the HPA axis. Importantly, this type of dysfunction may serve as a pathophysiological link to CVD through a number of mechanisms such as heightened inflammatory responses or by an impact on other established risk factors⁴⁹. Given the susceptibility of this population to CVD, the possible

pathophysiological links between dysfunction of the HPA axis and preclinical or clinical indicators of CVD should be the focus of future investigations in the full BCOPS Study cohort.

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