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Cortisol patterns and brachial artery reactivity in a high stress environment

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ABSTRACT

Chronic stress can result in frequent or persistent challenges of the hypothalamic-pituitary-adrenal (HPA) axis resulting in abnormal cortisol patterns and increased risk for cardiovascular disease (CVD). Police work is an environment replete with stress. The present article describes associations between cortisol, a biomarker of stress, and brachial artery flow mediated dilation (FMD) in police officers. A random sample stratified on gender ($n = 100$, 33% women) was generated from officers in a mid-sized urban department. Four salivary cortisol parameters were derived: after awakening, following a standardized high protein meal challenge, during the entire day, and after a dexamethasone suppression test. Continuous scan B-Mode ultrasound was used to measure percent change in brachial artery FMD following occlusion and release. Elevated cortisol secretion after awakening was significantly associated with impaired FMD in women, reflected by an inverse trend. Adjustment for age, smoking, and alcohol consumption did not appreciably alter this trend. A similar result was not evident among male officers. Responses of other cortisol challenges to the HPA axis were not associated with FMD. In conclusion, increased cortisol secretion after awakening was independently associated with impaired FMD in female police officers only, indicating a possible link between HPA axis stress response and subclinical CVD. However, because associations were not found with other cortisol parameters and were not evident in male officers, replication of these findings with a prospective study design may be warranted.

1. Introduction

Stress may be described as a biosocial process that places undue strain on persons resulting in psychological and biological changes which increase the risk for disease (Cohen et al., 1997). The hypothalamic-pituitary-adrenal (HPA) axis is central to the body's responses to stress and consequently is often a focal point in research concerning stress. The status of the HPA axis can be assessed by measurements of cortisol secretion using various challenge procedures (McEwen, 2000; Rosmond et al., 1998). A normal cortisol pattern may be described as having a high degree of variation, with a morning peak, an evening nadir, and an appropriate response to challenges. HPA dysregulation due to prolonged or extreme stress has been associated with inefficient turning on or shutting off of the cortisol response (McEwen and Seeman, 1999; McEwen, 2004). It has been suggested that abnormal cortisol patterns can result from frequent or persistent challenges of the HPA axis and constitute a major risk for disease including central obesity, insulin resistance, cardiovascular problems, hypertension, and depression (Chrousos, 1998; Haddy and Clover, 2001; McEwen, 1998).

Psychological stress has been associated with an increased risk of cardiovascular events (McEwen, 1998). However, the effect of stress and implications for cardiovascular risk are not well understood. Endothelial dysfunction is an important early stage in atherogenesis, and brachial artery flow mediated dilation (FMD) is endothelium-dependent (Widlansky et al., 2003). The physiology of FMD and methodologic guidelines for its measurement have been discussed (Adams et al., 1996; Corretti et al., 2002; Herrington et al., 2001). Brachial ultrasound measurements of arterial diameter are taken before and during a transient increase in blood flow. This increase in blood flow is caused by the inflation and subsequent release of a blood pressure cuff on the forearm. This procedure provides a noninvasive evaluation of brachial artery flow mediated vasodilation (FMD) (Corretti et al., 2002). Under these experimental conditions the FMD response is a marker for the endothelial response to flow induced shear stress which includes the release of nitric oxide (NO) leading to vasodilation. The magnitude of endothelial dysfunction in coronary arteries has been linked with the degree of coronary atherosclerosis detected by angiography (Zeiher et al., 1991). The presence of endothelial dysfunction has been associated with increased risk of cardiovascular disease (Verma et al., 2003). Although the underlying biologic mechanisms have not yet been established, HPA axis dysfunction and the associated cortisol dysregulation have been linked to endothelial

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dysfunction in two experimental studies in humans. In one study, endothelial dysfunction resulting from an acute mental stress challenge was prevented by blocking the production in cortisol with a pharmacological agent (Broadley et al., 2005). In a second study by Broadley et al. (2006), blocking cortisol production attenuated the endothelial dysfunction seen in patients with treated major depression. The ability of the HPA axis to respond to challenge may be compromised by chronic stress such as that experienced in a high stress occupation like policing.

Hans Selye (1984) recognized police work as highly stressful. Police work has been described as "civilian combat" (Violanti, 1996). Police officers face the distinct possibility of exposure to psychologically disturbing events in their work including shootings, physical assault, witnessing violence and familial abuse, handling dead bodies, and disaster scenes such as 9/11 or Hurricane Katrina in New Orleans (Paton and Smith, 1996). Although police officers are considered a healthy working population, notably higher mortality rates for cardiovascular disease (CVD) have been found in police cohorts when compared to the general population. CVD has occurred at a higher rate in policemen with fewer years of service, suggesting that work-related factors such as stress may play a part (Violanti et al., 1998). In one of the few large prospective studies of police officers, the Helsinki Police Study identified a number of independent risk factors for coronary heart disease (CHD) (Pyorala et al., 1998). A study of Iowa state police officers found that public safety officers had a higher probability of developing CHD than did the Framingham study population (Franke et al., 1997). Additional studies have found police to have higher rates for heart disease, homicide, and suicide (Forastiere et al., 1994; Dubrow et al., 1988; Quire and Bluont, 1990).

The Buffalo Cardio-Metabolic Occupational Police Stress (BCOPS) baseline study was conducted to establish a population-based sample designed to identify stress and subclinical cardiovascular biomarkers in police work (Violanti et al., 2006). The present article describes findings from the BCOPS study concerning associations between the stress biomarker cortisol and FMD in a sample of police officers.

2. Methods

2.1. Sample

The Buffalo, New York Police Department, an urban force of 934 officers at the time of data collection, was the selected sample site. A random sample stratified on gender ($n=100$) was generated from all police officers in the department using a computer-generated random number table. Female officers were oversampled (42 females, 58 males). No specific inclusion criteria were used for the study, other than the participant would be a sworn police officer and willing to participate in the study. One hundred percent of the originally selected sample participated in the study. None of the selected participants reported taking specific steroid medications. However, seven participants with the following conditions were excluded from brachial ultrasound measures: Raynaud's syndrome ($n=2$), prior myocardial infarction ($n=1$), cardiac pacemaker ($n=1$), hypertension ($n=1$), sprained wrist ($n=1$), and patient discomfort ($n=1$). In addition, participants must not have been taking blood thinners or high doses of aspirin. Among the remaining 93 participants, 12 additional brachial scans were not of sufficient quality to be read due to participant movement, recording problems and inaccessible artery position. In total, 81 participants had acceptable brachial scans for analysis. Additionally, the 12 officers with inadequate brachial scans in this present study were similar to the 81 officers with acceptable brachial scans with respect to demographic and lifestyle characteristics. Six additional subjects were excluded from analyses due to missing salivary cortisol data, leaving 75 participants with complete data on both brachial reactivity and cortisol measures. Demographic and lifestyle characteristics were not significantly different for the 75 participants and the 25 who were excluded or had missing values.

The Center for Preventive Medicine, State University of New York at Buffalo, School of Public Health and Health Professions, Buffalo, NY, served as the data collection site. All phases, testing, and reports of the study were approved by the State University of New York at Buffalo Internal Review Board and the National Institute for Occupational Safety and Health Human Subjects Review Board.

2.2. Measures

2.2.1. Salivary cortisol

Cortisol was measured in saliva samples. Although cortisol can feasibly be measured in blood, urine and saliva, its measurement in saliva has come to be preferred because the cortisol present is unbound thus providing the level of biologically active hormone and the small amounts present in saliva can be easily detected and quantified by immunoassay. 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. 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 was centrifuged to provide a nonviscous 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. All specimens collected had sufficient quantity and quality to conduct assays at each time point.

Four salivary cortisol parameters were measured in the present study. Table 1 provides the cortisol saliva testing schedule. The first measure involved cortisol response to awakening which normally increases rapidly within the first 30 min after awakening, remains elevated for at least 60 min, and then decreases. In chronic stress, this pattern may change, so that levels of cortisol are not elevated upon awakening, and/or are elevated yet fail to return to baseline within a period of several hours. Secondly, a standardized high protein meal challenge is administered at midday in the clinic. The meal challenge consisted of a baseline saliva sample followed by the high protein shake (55 g of protein) and four additional saliva samples at 15-min intervals thereafter (Rosmond and Bjorntorp, 2000). A third measure was diurnal (whole day) cortisol levels, obtained from saliva samples the next day after the clinic visit. The fourth measure was a dexamethasone suppression test (DST), obtained by self-administration of a 0.5-mg dexamethasone tablet taken at bedtime on day 2 after a saliva sample. One additional morning saliva sample was taken the following morning of the third day.

2.2.2. Brachial FMD

The use of ultrasound to measure endothelial function in the systemic arteries has become an important method for the detection of early arterial abnormalities (Kumiko et al., 2004). Brachial artery

Table 1
Timing of salivary cortisol sampling, BCOPS Pilot Study.

Cortisol sample number	Day	Approximate time	Cortisol sample characteristics
1	1	11:10 am	Baseline (prior to protein lunchtime challenge)
2	1	11:20–12:30	15 min after lunchtime challenge
3	1	11:20–12:30	30 min after lunchtime challenge
4	1	11:20–12:30	45 min after lunchtime challenge
5	1	11:20–12:30	60 min after lunchtime challenge
6	2	Awakening	First awakening sample
7	2	Awakening	15 min after awakening
8	2	Awakening	30 min after awakening
9	2	Awakening	45 min after awakening
10	2	Lunchtime	Immediately before eating midday meal
11	2	Dinnertime	Immediately before eating evening meal
12	2	Bedtime	Before bedtime and before taking dexamethasone tablet
13	3	Awakening	Post dexamethasone awakening sample

FMD was measured in the present study to assess endothelial dysfunction with B-mode ultrasound. The use of B-mode ultrasound imaging enabled visualization of small changes (up to 0.1 mm) in the arterial diameter, and the ability to continuously monitor these changes from baseline through inflation and deflation phases of the scan. Therefore, continuous instead of intermittent scan was employed to provide a more accurate measure of FMD. It is possible to analyze the entire brachial reactivity curve when continuous scanning is used throughout the brachial study. This enabled a better understanding of the process of the reactive mechanism and allowed analysis of the significant portions of the curve with the entire time sequence available for analysis. The continuous scan began at baseline and continued throughout the inflation and deflation phases of the scan, with the probe maintained at a fixed position on the skin for the entire duration (approximately 8 min) (Joseph et al., 2005). Images were recorded on high-resolution video tapes.

Participants rested in a supine position with the head resting on a pillow in a quiet darkened temperature-controlled room for 15 min prior to the test. Ultrasound tests on all participants were performed on the morning of the day that they were scheduled for clinic testing. Sonographers screened participants to determine if they had engaged in smoking, had consumed beverages containing alcohol or caffeine, or had used medications associated with lipid lowering and/or blood pressure within 6 h of testing. A blood pressure (BP) cuff was placed on the left upper arm to record baseline systolic and diastolic BP before the test and remained there to repeat the measurement at the end of the test. A BP cuff on the forearm was inflated to 40 mmHg above systolic BP (not to exceed 230 mmHg) for 4 min. The cuff pressure was then released until completely deflated. Brachial FMD was scanned for 3 min following deflation.

Since data were available for both heart rate (beats/min) and the cardiac cycle at maximum diameter, it was possible to calculate time to peak diameter in seconds, which was equivalent to [{(1/heart rate)*60} (cardiac cycle at maximum diameter)]. Based on this formulation, it was estimated that peak dilation occurred at 26.27 ± 18.72 s after cuff deflation in men and 19.59 ± 19.91 s after cuff deflation in women. Similarly, a total of 25.33 ± 17.26 (men) and 19.03 ± 18.52 (women) cardiac cycles elapsed from the time of cuff deflation until the maximum diameter of the artery was reached.

Quality control of brachial scanning involved a blinded double reading at every 10th scan, one reading by each of two certified readers. Four representative scans were selected for initial quality control assessment of brachial reading at Wake Forest University Ultrasound Reading Center, Wake Forest, North Carolina. Each reader selected and stored 40 frames for each of the scans. These images provided the basis for comparing mean diameter measurements from the same image by different readers (inter-reader reliability). The mean diameter was blind-measured for these frames by each reader and then compared. The above process was then repeated using four different representative scans selected by each reader. The intraobserver coefficient of variation for replicate measurements of FMD, expressed as absolute change from baseline, was less than 1.5%.

2.3. Statistical analysis

Percent change in brachial artery diameter and salivary cortisol parameters were examined for normality and presence of outliers. Apparent outliers in salivary cortisol data can exert undue influence on results (Neylan et al., 2005). In this study a small number of cortisol measurements fell clearly outside the expected range of the assay. Several methods were used to deal with extreme outliers including the following: assays were repeated to confirm outlier status, and high performance liquid chromatography (HPLC) methods were used to examine samples from participants with repeated cortisol outlier values (e.g. greater than 200 nmol/l) to confirm that high values were not due to authentic cortisol (such examination resulted in the elimination of data from three participants). Consistent with the study by Neylan et al. (2005), any remaining cortisol values greater than 2.3 standard deviations above the mean were set to missing.

Areas under the curve (AUC) for diurnal, lunch and awakening cortisol responses were calculated using the trapezoidal rule for numerical integration (Pruessner et al., 2003). The diurnal AUC was calculated using the actual times that the samples were taken in order to account for time differences between participants. Therefore, the times the cortisol samples were taken during the course of the day may vary across participants. A correction for these differences was implemented by converting the cortisol measurements obtained throughout the day into an area measure for each participant. Therefore, the actual measurements were not used for analyses. The computation of the area measure (AUC_G AUC_G = total area under the curve above the *x*-axis representing seven repeated samples from first awakening to bedtime) standardizes the collection times by converting them into length of time since awakening. AUC_i was determined for the awakening and lunch response and represents the increase in cortisol above the baseline level over the time of sampling. While calculation of AUCs tended to produce data that were approximately normal, the individual cortisol measurements for pre- and post-dexamethasone challenge were skewed. Calculation of the log transform resulted in an approximate normal distribution for these data. The pre- versus post-dexamethasone challenge was indicated by the difference between log base 2 of the post dexamethasone first waking sample and first waking sample without dexamethasone. Participants were categorized into tertiles based on their AUC levels and DST response. Cortisol tertiles were created separately for men ($n = 42$) and women ($n = 33$).

Unadjusted, age-adjusted and risk factor-adjusted (age, education, smoking and alcohol consumption) mean values of percent change in FMD were assessed across tertiles of the cortisol parameters using analyses of covariance. Tests for linear trend in the unadjusted or adjusted means across values of cortisol parameters were calculated for women and men separately using linear regression models.

Fig. 1 represents the mean brachial diameter response curve based on a nonparametric regression model. In order to parameterize the brachial curve, a local regression smoothing model was fit to the brachial artery data for each participant and the maximum diameter from the fitted model was determined. This was done using Proc LOESS in SAS (SAS, 1999). Percent change in brachial artery diameter was calculated as maximum from the fitted model minus the mean of baseline measurements divided by the mean of baseline measurements.

3. Results

3.1. Characteristics of the sample

Table 2 provides selected characteristics of the police sample by gender. Although average age was similar for women and men (43.7

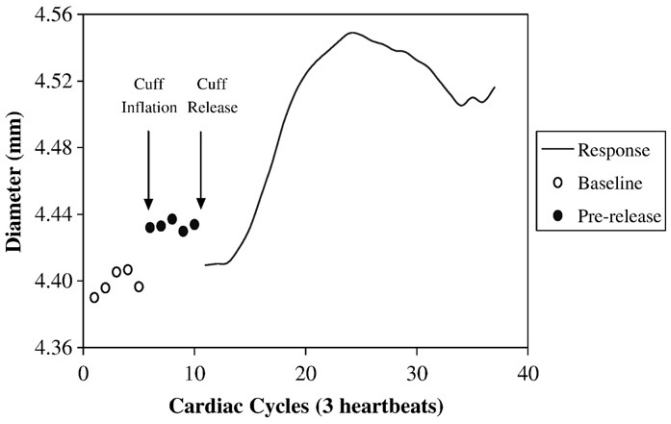

Fig. 1. Average brachial diameter response from nonparametric regression model.

Table 2
Selected characteristics of study population by gender.

Characteristic	Women		Men		Total	
	n	%	*n*	%	*n*	%
Age (years)[a]	33	43.7 (41.6–45.8)	42	44.0 (41.2–46.8)	75	43.9 (42.1–45.6)
Age group (years)						
<40	8	24.2	14	33.3	22	29.3
40–49	20	60.6	15	35.7	35	46.7
≥50	5	15.2	13	30.9	18	24.0
Education						
≤High school	6	18.2	4	9.5	10	13.3
College<4 yrs	9	27.3	14	33.3	23	30.7
College 4+ yrs	18	54.5	24	57.1	42	56.0
Years of service						
1–5	5	15.2	9	21.4	14	18.7
6–10	6	18.2	5	11.9	11	14.7
11–15	8	24.2	7	16.7	15	20.0
16–20	10	30.3	8	19.1	18	24.0
20+	4	12.1	13	31.0	17	22.7
Rank						
Police officer	17	51.5	19	45.2	36	48.0
Sergeant/ lieutenant	6	18.2	9	21.4	15	20.0
Captain	2	6.1	5	11.9	7	9.3
Detective	3	9.1	7	16.7	10	13.3
Other	5	15.1	2	4.8	7	9.3
Smoking status						
Current	5	17.2	5	13.2	10	14.9
Former	13	44.8	13	34.2	26	38.8
Never	11	37.9	20	52.6	31	46.3
Alcohol drinks/week						
0	9	31.0	6	15.8	15	22.4
<1	8	27.6	10	26.3	18	26.9
1–7	10	34.5	14	36.8	24	35.8
>7	2	6.9	8	21.1	10	14.9
Cortisol parameters						
Diurnal AUC_g[a,b,c]	19	5.749 (4.764–6.939)	31	6.207 (5.018–7.675)	50	6.029 (5.212–6.974)
Lunch AUC_i[a,b]	24	0.077 (−0.003–0.158)	35	0.145 (−0.035–0.325)	59	0.118 (0.008–0.227)
Awakening AUC_i[a,b]	22	0.201 (0.104–0.299)	33	0.006 (−0.089–0.100)	55	0.084 (0.013–0.156)
DST[a,c,d]	23	0.134 (0.077–0.221)	32	0.067 (0.045–0.097)	55	0.087 (0.063–0.120)

Abbreviations: AUC_g, area under the curve with respect to ground; AUC_i, area under the curve increase above baseline; DST, dexamethasone suppression test.
[a] Values are means [95% confidence intervals (C.I.)].
[b] Units are (nmol/l) hours expressed in thousands.
[c] Back transformed means.
[d] DST is the ratio of post- to pre-dexamethasone suppression test.

and 44.0 years, respectively), a greater proportion of women were in the 40- to 49-year-old age group (60.6% vs. 35.7%). A greater proportion of male than female officers had more than 20 years of police service (31.0% vs. 12.1%, respectively). The majority of officers were at the rank of police officer (48%). Current and former smoking considered together was more prevalent in women than in men officers (62% vs. 47%, respectively). Male and female officers had similar diurnal AUC_g and lunch AUC_i levels. Compared with male officers, female officers had higher awakening AUC_i (0.201 vs. 0.006 (nmol/l) hours in thousands, respectively) and dexamethasone suppression ratio (0.134 vs. 0.067, respectively).

3.2. Percent change in brachial artery dilation by gender

Table 3 provides mean percent change in brachial artery dilation by gender. While the absolute increase in FMD was slightly greater in men (0.21 mm) than in women (0.17 mm), the overall mean percent change in brachial artery diameter from baseline to maximum was slightly higher for female compared to male officers (4.59% vs. 4.41%).

Table 3
Mean percent change in brachial artery dilation by gender.

Brachial artery diameter	Women		Men		Total	
	n	Mean (S.D.)	*n*	Mean (S.D.)	*n*	Mean (S.D.)
Baseline (pre-inflation) (mm)	33	3.72 (0.49)	42	4.91 (0.54)	75	4.39 (0.78)
Maximum (post-release) (mm)	33	3.89 (0.51)	42	5.12 (0.52)	75	4.58 (0.80)
Absolute difference from baseline to maximum (mm)	33	0.17 (0.12)	42	0.21 (0.15)	75	0.19 (0.14)
Percent change from baseline to maximum[a]	33	4.59 (3.43)	42	4.41 (3.42)	75	4.49 (3.40)

[a] Mean percent change in brachial diameter defined as: [(maximum from smoothing function minus mean of baseline measurements]×100.

3.3. Brachial diameter change by lifestyle variables

Table 4 provides the mean percent change in brachial diameter by lifestyle and demographic characteristics for women and men. In female officers, a non-significant trend of increasing brachial artery percent change was found with increasing consumption of alcoholic beverages ($P=0.108$), while an inverse trend was observed in male officers ($P=0.029$).

Table 4
Mean percent change in brachial artery diameter [a] by demographic and lifestyle characteristics for women and men.

Characteristic	Women			Men		
	N	Mean	S.D.	*N*	Mean	S.D.
Age group (years)						
<40	8	6.05	2.88	14	4.59	2.41
40–49	20	3.96	3.63	15	5.30	4.41
≥50	5	4.76	3.28	13	3.17	2.87
P-linear trend[b]		0.514			0.284	
Education						
≤High school	6	5.74	3.22	4	5.19	4.29
College<4 yrs	9	3.44	4.77	14	3.32	2.49
College 4+ yrs	18	4.78	2.68	24	4.91	3.72
P-linear trend		0.562			0.877	
Years of service						
1–5	5	6.59	3.27	9	4.31	2.17
6–10	6	6.76	2.13	5	5.64	7.13
11–15	8	2.66	2.46	7	4.52	2.20
16–20	10	3.64	4.17	8	4.53	2.62
20+	4	5.08	2.84	13	3.86	3.52
P-linear trend		0.193			0.587	
Rank						
Police officer	17	4.59	3.02	19	3.63	2.42
Sergeant/lieutenant	6	2.69	4.85	9	6.73	5.11
Captain	2	7.05	0.94	5	2.95	1.39
Detective	3	6.20	2.02	7	4.03	3.58
Other	5	4.91	3.92	2	6.25	0.80
P-value[c]		0.488			0.146	
Smoking status						
Current	5	5.06	1.74	5	7.76	6.14
Former	13	5.10	3.89	13	3.96	3.57
Never	11	3.47	3.71	20	4.11	2.28
P-value		0.506			0.088	
Alcohol (drinks/wk)						
0	9	3.29	3.71	6	7.85	5.81
<1	8	3.47	4.03	10	5.03	2.28
1–7	10	5.81	1.93	14	2.79	2.20
>7	2	7.09	6.32	8	4.49	3.24
P-linear trend		0.108			0.029	

[a] Mean percent change in brachial diameter defined as: [(maximum from smoothing function minus mean of baseline measurement) divided by mean of baseline measurements]×100.
[b] For ordinal covariates, a test of linear trend was performed using orthogonal polynomial coefficients.
[c] For nominal covariates, the *P*-calue tests differences in mean percent change across the levels.

Table 5
Gender-specific percent change in brachial artery diameter by cortisol parameter tertile.

		Women								Men							
		Unadjusted			Age-adjusted		Risk factor-adjusted[a]			Unadjusted			Age-adjusted		Risk factor-adjusted[a]		
Cortisol parameter	Cortisol tertile	*n*[b]	Mean	S.D.	Mean	S.E.	*n*	Mean	S.E.	*n*[b]	Mean	S.D.	Mean	S.E.	*n*	Mean	S.E.
Diurnal AUC_g	Low	6	5.66	2.03	5.81	1.12	6	6.47	1.35	10	3.28	1.97	3.21	0.77	10	3.20	0.88
	Medium	7	5.32	3.53	5.49	1.04	7	5.51	1.31	11	5.64	3.48	6.01	0.74	11	5.82	0.83
	High	6	4.29	2.23	3.95	1.16	6	3.26	1.17	10	3.33	2.23	2.99	0.78	6	3.24	1.13
P-linear trend[c]			0.324		0.209			0.082			0.871		0.814			0.451	
Lunch AUC_i	Low	8	4.41	5.38	4.52	1.38	8	4.27	1.45	11	3.69	2.31	3.57	1.09	9	3.44	1.17
	Medium	8	4.62	2.30	4.40	1.39	7	6.23	1.93	12	4.24	2.48	4.28	1.03	10	4.39	1.11
	High	8	4.52	3.35	4.64	1.38	6	2.54	2.09	12	4.70	5.09	4.76	1.03	12	5.03	1.00
P-linear trend			0.518		0.344			0.048			0.218		0.185			0.222	
Awakening AUC_i	Low	7	5.32	2.04	5.20	0.98	6	5.85	1.07	11	4.07	2.91	4.11	0.88	10	4.22	1.12
	Medium	8	6.19	3.00	6.26	0.91	8	6.24	1.00	11	4.20	3.53	4.12	0.88	11	3.89	1.00
	High	7	2.30	2.49	2.34	0.98	6	1.69	1.01	11	4.03	2.20	4.06	0.88	8	4.70	1.22
P-linear trend			0.081		0.100			0.020			0.889		0.935			0.699	
$\log_2$ (DST)	Low	7	5.21	3.80	5.48	1.16	6	5.92	1.29	10	3.84	2.22	3.77	0.95	9	4.24	0.96
	Medium	8	5.58	1.72	5.46	1.06	6	4.85	1.18	11	4.28	2.73	4.30	0.90	8	4.28	1.07
	High	8	5.60	3.12	5.48	1.06	7	5.65	1.18	11	4.23	3.65	4.27	0.90	11	4.27	0.95
P-linear trend			0.813		0.999			0.597			0.970		0.937			0.755	

Abbreviations: AUC_g, area under the curve with respect to ground; AUC_i, area under the curve increase above baseline; DST, dexamethasone suppression test.
[a] Risk factor model adjusted for age, education, smoking and alcohol intake.
[b] The number of participants by tertiles is the same for unadjusted and age-adjusted.
[c] Based on linear regression.

3.4. Brachial diameter change and cortisol parameter measures

Table 5 examines associations between percent change in brachial diameter and AUC measures of diurnal, awakening, lunch challenge, and DST cortisol parameters. The AUC cortisol response to awakening revealed a significant inverse trend in female officers such that the mean percent change in brachial artery diameter was lower for those in the highest AUC tertile (greatest cortisol response). Adjustment for age, education, smoking, and alcohol had minimal influence on these trends ($P=0.081$ unadjusted vs. $P=0.020$ adjusted). A nearly significant inverse trend of decreasing mean FMD with increasing diurnal cortisol AUC and the AUC lunch response were also observed in women after full adjustment $P=0.082$ and $P=0.048$, respectively). The responses to the high protein lunch and DST were not associated with percent change in brachial artery diameter in women. Percent change in FMD was not associated with these four cortisol parameters in men. Regarding the DST cortisol parameter, adjustment for the individual variation in the time interval between the pre-dexamethasone collection (at bedtime) and the post-dexamethasone collection (awakening) did not alter the associations observed.

In addition, the associations between the continuous form of the cortisol parameters and the mean percent change in brachial artery diameter were also examined using simple linear regression. The correlation coefficients for women were as follows: diurnal $AUC_G=-0.239$, AUC_I lunch $=-0.139$, AUC_I waking $=-0.380$, and DST $=0.052$. For men, the correlation coefficients were as follows: diurnal $AUC_G=0.030$, AUC_I lunch $=0.213$, AUC_I waking $=0.025$, and DST $=0.007$.

4. Discussion

The objective of this study was to examine associations between cortisol and brachial FMD in a high stress work population. Results indicated that higher secretion of awakening cortisol was significantly associated with impaired endothelial function in women as reflected by an inverse trend with FMD ($P=0.020$). A similar result was not found among male police officers. Adjustment for lifestyle factors of age, smoking, and alcohol consumption did not appreciably alter this trend. Mean awakening cortisol AUC_i levels were higher in women than in men, yet diurnal AUC_g levels were similar. Brachial FMD change from baseline to maximum was measured as a percentage to account for arterial size differences by gender.

Few studies exist that have examined gender differences in the association between hormones, including cortisol, and brachial FMD. Hashimoto et al. (1995) examined the impact of gender and menopausal status on endothelium-dependent vasodilation (Hashimoto et al., 1995). In females, an increase in the percentage FMD was associated with increased levels of serum estradiol concentrations that varied during the menstrual cycle. The question remains as to whether FMD studies with female populations should attempt to monitor the menstrual cycle and use of medications for birth control or hormone replacement therapy as well since this could have some impact on results. Gender differences found in other investigations have prompted interest in the role of natural sex steroids and their impact on atherogenic events. Secondary effects of hormones, such as alteration in the lipid profile, may in turn alter endothelial function (Hashimoto et al., 1995). We did not have sufficient information in the present study to assess such effects in women.

Within the purview of legal and organizational standards, both men and women officers are mandated to participate in all phases of police work, including routine patrol and handling of any particular critical incident which may occur in the line of duty. Thus, the exposure of female officers by prescribed standards should not vary substantially from that of male officers in the day to day performance of police work. There are, however, tangential factors which may lead to increased stress for women officers.

Female officers with police service in the range of 11–20 years appeared to have somewhat lower brachial FMD compared to those with fewer years of service (Table 4). The prevalence of depression in female officers has been found to be approximately two times that of male officers (Darensburg et al., 2006). Social isolation, conflict with colleagues, and negative group climate are relatively strong predictors of depression in policewomen (Dormann and Zapf, 2002). Such stressors may be problematic for women, given that support is perceived as important by women in achieving job satisfaction. The emergence of interpersonal stress as a distinct factor in job related stress for policewomen, is consistent with the increasing emphasis on interpersonal conflicts as stressors in models of job stress. The increased stress associated with managing multiple roles could leave female officers more susceptible to depression (Harris et al., 2001). In this study, 85% of female police officers were under the age of 50 and therefore, some are likely to have children in the home. More female officers reported being single (23.8%) or divorced (23.8%) than male officers

(17.2% and 8.6%, respectively). It is possible that some of these female officers are heads of single-parent households and therefore have sole responsibility for raising children. It is also possible that associations between cortisol response and brachial FMD might be more readily detected in women than in men if women experience greater stress.

Limitations of the study include its sample size and the choice of a unique population. Additionally, we do not have information on exposure to specific, actual stress experiences at work. This study was concerned with field stress exposures of police officers, which are by the nature of this work chronic and well documented in the literature. Exposure to specific occupational stressors at the time of their occurrence is likely difficult to assess. A full history of medication use was not ascertained. We did initially, however, screen out officers who were taking medications which may have affected either cortisol levels or endothelial function. Examples were steroids and blood thinners.

Information gained in the present study may be generalizable to other high stress enviroments such as firefighters, Emergency Medical Technicians, nurses, physicians, air traffic controllers, and the military. In general terms, results found in this study and possible future studies may add to existing knowledge of associations between psychological stress and long-term physiological consequences. Although the cross-sectional design of this study precludes causal inferences, the assessment of cortisol and subclinical CVD at a single point in time provides descriptive health-related characteristics and provides a baseline for future prospective work.

Strengths of this study include the availability of precisely measured subclinical CVD and cortisol biomarkers along with risk factors and psychosocial measures, the use of a standardized protocol, and high response rates and cooperation. Participation of 100% of the randomly selected officers suggests that the sample is highly representative. Few of the previous studies evaluating factors impacting police health included female officers. We know very little about the health of women officers and the present endeavor will help to provide some baseline assessment data for future work.

Recent events such as terrorist acts and natural disasters have increased the potential for stress and trauma in first responders and the general population. The need for examination of more objective health outcomes associated with stress is increasingly apparent (Galea et al., 2002). Future studies and clinical clarifications are needed to enhance present knowledge about how psychological stress may lead to increased morbidity and mortality. The police occupation offers an excellent opportunity to study a population exposed to stress. In number, the police population represents approximately 708,000 sworn officers in the United States alone (Reaves, 2003). The complex nexus of psychological stress and physiological outcomes may eventually be better understood by further investigation in this high stress population.

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