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Subclinical Markers of Cardiovascular Disease Among Police Officers: A Longitudinal Assessment of the Cortisol Awakening Response and Flow Mediated Artery Dilation

Running title: Cardiovascular Disease Biomarkers among Police

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DECLARATION OF INTEREST

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Abstract

Objective: To examine the association of the cortisol awakening response (CAR) with change in brachial artery flow-mediated dilation (FMD%) in police officers over a seven-year period.

Methods: Baseline CAR was obtained from four saliva samples taken fifteen minutes apart immediately after awakening. Analysis of covariance was used to compare the change in FMD% ($FMD\%_{Follow\text{-}up}$-$FMD\%_{Baseline}$) across tertiles of area under the cortisol curve with respect to increase (AUCI). Regression analysis was use to assess trend.

Results: Officers (n=172; 81% men) had a mean±SD age of 41±7.6 years. Men in the lowest AUCI tertile (i.e., atypical waking cortisol pattern) had a significantly larger seven-year mean decline in FMD% (mean±SE: -2.56±0.64) compared to men in the highest tertile (-0.89±0.69) (p=0.0087).

Conclusion: An awakening cortisol AUCI predicted worsening of FMD% approximately seven years later among male officers.

Keywords: police stress, cardiovascular disease, awakening cortisol, brachial reactivity, flow-mediated dilation

1. Introduction

A prospective study of the cardiovascular health of police officers in the United States has received limited attention. Exposure to police work has been shown to be associated with a younger average age at death and a higher prevalence of cardiovascular disease (CVD) compared to that of the general population.(1, 2) After controlling for traditional risk factors, CVD nearly doubles by the 6th decade and exceeds a two-fold increased risk by the 7th decade.(3-5) Police officers have previously been shown to have more subclinical CVD including increased levels of atherosclerosis and poorer endothelial function compared with a similarly aged civilian population sample from the same geographical region.(6, 7)

Some of the increased CVD prevalence is thought to be related to high levels of stress inherent in police work.(8-10) The occupation of law enforcement is replete with stressful factors such as traumatic events, physical harm, long work hours, shiftwork, lack of support from supervisor and colleagues, police suicide and other tragic events.(10) A biological system responding to stressful environmental challenges is the hypothalamic pituitary adrenal (HPA) axis. According to the allostasis model, repeated stress which leads to continual stimulation of the HPA axis may lead to its dysfunction, resulting in elevated or reduced levels of the response to subsequent stressors.(11-17) A biomarker of how well the HPA axis responds to stress is the amount or pattern of cortisol released in response to various challenges.(18) Cortisol is an adrenal steroid referred to as a "stress hormone" that can be conveniently measured in saliva.(19) During prolonged periods of repeated exposure to stressors or exposure to very intense stressors, the ability of the HPA axis to regulate cortisol secretion may become compromised, which can

result in inability of the body to regulate the stress response.(20, 21) This breakdown can be manifested as a change in the pattern of cortisol secretion.(22)

Prior cross-sectional studies that examined the effect of occupational stressors on cortisol levels and patterns among police officers include: (a) examination of the effect of various critical incident police scenarios on cortisol secretion (23) that revealed a higher cortisol elevation in officers during a higher stress scenario; (b) positive association between perceived stress and increased secretion of cortisol (24); (c) inverse linear association between stress index of the most stressful events and slope of the awakening cortisol regression line suggesting that as the stress index increased, the pattern of the awakening cortisol tended to flatten (25); (d) lower total are under the curve values among officers working short-term night or afternoon shifts and in officers who switch their shift more frequently (26); and (e) noticeable differences in levels of measurable cortisol across varying levels of traumatic stress symptoms.

In addition to HPA axis dysregulation in either magnitude or pattern, impaired Brachial Flow Mediated Dilation (FMD) has been explored as a biomarker of subclinical CVD.(27-30) FMD assesses endothelial function and can be measured via B-mode ultrasound. The endothelium functions to regulate inflammatory processes within the artery walls.(31) Healthy endothelial cells prevent abnormal fatty deposits from forming within the walls of arteries and when diminished by HPA axis dysregulation the risk for cardiovascular disease and subclinical atherosclerosis may increase.(32-34) Few studies have observed longitudinal associations between cortisol dysregulation and FMD in police officers. Considering the chronic stress and trauma experienced by police officers and the increased risk of CVD in this profession, the

present study examined whether cortisol patterns at baseline, biomarkers of HPA-axis function, predicted a seven-year change in FMD among police officers.

2. Materials and methods

2.1 Study population and design

Participants were officers enrolled in the Buffalo Cardio-Metabolic Occupational Police Stress (BCOPS) study, a study with a prospective component aimed at investigating the associations of occupational stressors with psychological and physiological health of police officers. Details of the BCOPS study including recruitment, data collection and variables assessed are described elsewhere.(35, 36) The current analysis utilized a longitudinal design. At the baseline examination, a total of 710 police officers who worked with the Buffalo, New York Police Department were invited to participate; 464 (65.4%) officers agreed to participate and were examined during the period of 2004 to 2009. No specific inclusion criteria were indicated for the study, only that participants be sworn police officers and willing to participate. Of the 464 initially examined, 276 were evaluated again after seven-years between 2011 and 2015. A written informed consent was collected from each participant. The study was approved by the Internal Review Board of the University at Buffalo, State University if New York, Buffalo, NY .

2.2 Measures

Data including demographic, lifestyle, physical, occupational, and psychosocial characteristics were collected from each BCOPS study participant at baseline and follow-up examinations using standardized instruments and protocols. The salivary cortisol data collected

at the baseline examination was used to derive parameters that describe the Cortisol Awakening Response (CAR) and served as the exposure variable of interest for the current analyses. The change in brachial artery flow mediated dilation (FMD) from the baseline to the follow-up examination was assessed using ultrasound and served as the outcome variable of interest. Demographic and life style characteristics collected at the baseline served as potential confounders for adjustment of the main association of interest.

2.3 Assessment of salivary cortisol

The assessment of salivary cortisol in this study is standardized and used in other studies related to this project (25, 59). To assess the salivary CAR, subjects were instructed to collect saliva samples immediately upon awakening, and 15, 30, and 45 minutes, thereafter. 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 my micro-injuries of the oral cavity. The saliva samples were collected during a single day and occurred the day after the clinic examination. 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 two minutes allowing for saturation of the roll. The roll was then returned to the tube and samples were returned to the clinic and subsequently sent to the laboratory. Upon delivery the tubes were centrifuged to provide a non-viscous saliva sample for assay of cortisol. Samples were maintained at -20^{o}C until sent to the Technical University of Dresden for analysis of cortisol by a commercially available chemiluminescence immunoassay (IBL, Hamburg,

Germany). The four cortisol values from the saliva samples and the corresponding times of collection were used to derive the area under the curve with respect to increase against the baseline (AUCI) which served as exposure variable in the current analyses.

2.4 Assessment of brachial artery flow-mediated dilation (FMD)

Brachial flow-mediated dilation (FMD), a measure of endothelial function, was assessed using ultrasound. 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 hours 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. Further details on the use of B-mode ultrasound for assessment of FMD in BCOPS study can be found in Violanti et al.(36) The same standardized protocol was used to assess FMD at both the baseline and follow-up examinations. At each examination, percent change in brachial artery diameter (FMD %) was calculated. The calculation of FMD as a percentage change involves the peak diameter in response to cuff deflation and baseline diameter (Harris et al., 2010**)**. It is calculated utilizing the following equation:

$$FMD(\%) = \frac{\left(Peak\ Diameter - Baseline\ Diameter\right)}{Baseline\ Diameter} \times 100$$

The outcome variable of interest for the longitudinal analyses in this paper was calculated by subtracting FMD% at baseline from FMD% at the follow-up examination.

2.5 Assessment of covariates

Concurrent with CAR and FMD measures, questionnaires were administered to collect demographic and lifestyle characteristics including age, gender, race/ethnicity, years of police service, rank, years of education, marital status, smoking, alcohol consumption, and physical activity. Height and weight were measured with shoes removed and recorded to the nearest half centimeter and rounded up to the nearest quarter of a pound, respectively. Height and weight were converted to meters and kilograms, respectively. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Hours of physical activity were assessed using the Seven-Day Physical Activity Recall questionnaire developed in the Stanford Five-City Project.(37) Alcohol consumption was measured from data collected using Food Frequency Questionnaire (FFQ) where, among other things, the officers also reported how often they drank the following amounts of alcoholic beverages: beer (12 Oz), red wine (6 Oz), white or rose wine (6 Oz), and liquor and mixed drinks (1.5 Oz). The number of drinks per week was derived as the sum of consumption of these amounts from the four types of alcoholic beverages.

2.6 Statistical analysis

Of the 276 officers examined at both the baseline and follow-up examinations, the current analyses included officers who had complete data for AUCI at baseline and FMD% at both exams (n = 172; 139 men and 33 women). The AUCI measure assessed at the baseline examinations served as the exposure variables of interest while the change in FMD% (follow-up minus baseline) served as the outcome variable of interest for analyses. Both the exposure and outcome variables are continuous in nature and hence two statistical approaches were used to

examine the association of CAR at baseline and seven-year change in FMD%. Linear regression was used to examine and test for linear trend in seven-year change in FMD% across increasing values of AUCI. Analysis of covariance (ANCOVA) was used to compare seven-year mean change in FMD% across the tertiles of AUCI. The primary association of interest was also examined stratifying by gender. In all analyses, adjustment for potential confounding factors including age, gender, race/ethnicity, education, marital status, smoking, alcohol consumption, police rank, and physical activity were made. Prior to statistical adjustment, all covariates were tested for potential effect modification by including an interaction term between the exposure and the covariate. The statistical analyses were performed using the SAS software version 9.3 (SAS Institute, Cary, NC, USA) and significance level was set at p=0.05.

3. Results

3.1 Demographic and lifestyle characteristics

The demographic and lifestyle characteristics of the participants (n = 172) at the baseline examination are presented in Table 1 stratified by gender. The study population consists of 81% males and the majority was white (80%), married (75%), overweight or obese (81%, BMI ≥ 25), never smokers (64%), and had a rank of patrol officer (70%). At the time of the baseline examination, the cohort was relatively young with a mean age was 41.3 years (range: 22-63, SD = 7.6). The demographic and lifestyle characteristics did not significantly vary between male and female officers except for marital status, where the proportion of married male officers (81%) was larger than married females (49%), body mass index, where males had significantly larger BMI than females (30.2 vs. 26.0, p <0.0001), and number of alcoholic drinks per week with

males consuming significantly more than females (6.2 vs. 3.2, p=0.0114). The mean area under the curve with respect to the baseline cortisol (AUCI) was 139 (95% CI: 94 -184) and did not vary significantly by gender (men: 138 [95% CI: 87 – 188]; women: 144 [95% CI: 41 – 247], p=0.9109).

3.2 AUCI at baseline and seven-year mean change in FMD%

There was a significant decline in brachial reactivity over the seven-year period (FMD% at baseline = 5.8 ± 2.9; FMD% at follow-up = 3.7 ± 3.4; seven-year change = -2.4 ± 3.4; p-value <0.0001, Table 1). The seven-year decline in brachial reactivity was independent of increase in age of the participants during the follow-up (data not shown). The seven-year reduction in FMD% was also significant in both men (FMD% at baseline = 5.6 ± 2.6, FMD% at follow-up = 3.6 ± 1.9, seven-year change = -2.0 ± 2.9; p-value<0.0001) and women (FMD% at baseline = 6.5 ± 3.7, FMD% at follow-up = 4.2 ± 3.0, seven-year change = -2.3 ± 5.0; p-value=0.0123). The data in Table 2 shows association of the demographic and lifestyle characteristics with the exposure variable (AUCI) and the outcome variable (seven-year change in FMD %). None of the covariates in Table 2, except for age, were associated with the exposure or outcome.

The association between waking AUCI at baseline and the seven-year mean change in brachial reactivity (FMD %) is shown in Table 3. Overall (all subjects), the unadjusted association between AUCI and the seven-year change in FMD% was significant (trend p-value = 0.0442) but the association was no longer significant following adjustment for demographic and life style factors (Models 2 and 3). Among women, both the unadjusted and multivariable-

adjusted association between AUCI and the seven-year change in FMD% were not significant (Table 3). The association, however, was significant among men. Among men, the unadjusted (Model 1) mean seven-year change in FMD% declined significantly with increasing tertiles of AUCI (lowest tertile: -2.81 ± 3.3, middle tertile: -2.01 ± 2.6, highest tertile: -1.09 ± 2.6, p-value = 0.0174). The regression approach also showed that there was a significant linear decline in FMD% change with increasing values of AUCI (trend p-value = 0.0316). Following adjustment for demographic and lifestyle variables (Model 3), there was a slight attenuation in the association (trend p-value = 0.0897). The multivariable adjusted (Model 3) seven-year mean change in FMD% (± SE) across increasing tertiles of AUCI were: lowest = -2.56 ± 0.64; middle = -1.86 ± 0.65, highest = -0.89 ± 0.69, p-value = 0.0306). Post-hoc pairwise multiple comparison test showed that officers in the lowest tertile of AUCI had a significantly larger seven-year mean decline in brachial reactivity compared to officers in the highest tertile of AUCI (pairwise comparison p-value = 0.0087). The result suggested that, in men, waking AUCI at baseline significantly predicted seven-year change in FMD%. More specifically, low waking AUCI at baseline predicted worsening of FMD% during the seven-year follow-up. In order to elucidate this association, understanding of AUCI and what it measures is essential. AUCI is a measure of pattern and values of AUCI approaching toward zero and negative values are indicative of 'abnormal' or 'dysregulated' waking pattern. For example, Figure 1 shows the mean awakening cortisol pattern, separately, for male officers in each tertile of AUCI. Officers in the highest tertile of waking AUCI had a typical waking cortisol pattern but officers in the lowest tertile of AUCI appear to have a waking pattern that is 'abnormal' or significantly flatter (especially compared to the pattern for those in the highest tertile of AUCI). In fact the pattern appears to be the opposite of the typical pattern seen in "normal" subjects. The data in Table 3 and Figure 1

suggest that male officers with the worst seven-year mean change in FMD% were those who had atypical or flatter waking cortisol pattern at baseline.

4. Discussion

Results of the present study suggest that the waking AUCI pattern at baseline significantly predicted a seven-year change in FMD% independent of demographic and lifestyle factors. More specifically, an atypical or flatter waking cortisol pattern predicted a significant worsening of FMD% during the seven-year follow-up among male but not in female officers. This finding suggests that atypical cortisol response at baseline leads to impaired brachial FMD% in males over time.

These results were similar to those of Ghiadoni and colleagues (38) who found that episodes of stress were associated with impairment of endothelium relaxation in arteries. They add that the findings reveal an important mechanism linking stress to vascular abnormalities and subclinical CVD.(38) Mangos and colleagues also found cortisol to be an important component of the stress response.(39) These authors found that a short-term high-dose oral cortisol impaired endothelial function. Other investigators found that mental stress impaired artery endothelial function. A receptor antagonist prevented endothelial dysfunction.(40)

The gender difference in FMD% over time was interesting. Women officers generally perform the same work as male officers, thus, the effects of stress exposure on female officers is expected to not vary substantially from that on male officers, but in the present study a baseline

biomarker of stress significantly predicted change in FMD among male officers only. It may be that women officers used more effective types of coping with the stress of police work. Taylor et al (41) concluded that women are more likely to "tend and befriend" than men thus bringing into play a creation of social support networks to help protect against stress. Taylor et al (41) add that oxytocin, together with female reproductive hormones and endogenous opioid peptide mechanisms, may be responsible for this behavior. Secondary effects of female hormones, such as alteration in the lipid profile, may also be involved in endothelial function.(42, 43) Kirschbaum et al (44) concluded that differences between the genders may be related to cognitive differences in how the genders interpret and respond to perceived stressful events.

The type of stress experienced by officers may have affected cortisol patterns and subsequently reduced FMD%. Specific stressful events of the type seen in police work have been associated with blunting of the awakening cortisol pattern.(25) Previous work has demonstrated that highly stressful incidents may initiate a lower or blunted cortisol response.(43, 44) In addition, the biologic changes observed in trauma stress are different than other types of stress.(45) Cortisol levels were lower in studies of persons with traumatic stress, even decades after a traumatic event.

An impaired FMD% may be one cardiovascular disease risk associated with stress in policing. The metabolic syndrome (46), a collection of five cardiovascular risk components, has also been noted among police officers (6) with estimated prevalence of 25.7% among urban police officers in eastern United States. Baughman et al (6) found that a flatter salivary cortisol response curve following a high protein meal challenge was associated with an increasing

number of metabolic syndrome components. Walvekar et al (24) found a significant positive relationship between blood glucose, HbA1c and serum cortisol among police constables. The most common components of metabolic syndrome in police constables were Hypertriglyceridemia and low HDL Cholesterol, and 22.7 % police constables were hypertensive.

Psychosocial factors in police work may also play a role in affecting cortisol patterns and cardiovascular disease risk. Izawa et al (47) found that an imbalance of effort and reward at work in police officers resulted in lower salivary cortisol levels as well as higher salivary C-reactive protein (CRP) levels. In a prospective resilience study, Galatzer et al (48) found that police officers who continued resilient attitudes over time had typical cortisol patterns while those who reported chronic increasing stress had a blunted cortisol response.

Similar associations of cortisol with disease have been found in populations other than policing.(49-51) Phillips et al (52) in the West of Scotland Study, observed that blunted cardiovascular and cortisol stress reactivity was associated with a range of negative health outcomes, including depression, poor self-reported health, compromised immunity, and addictive behavior.(41, 53) These authors added that in terms of differing types of health outcomes, there appears to be an inverted U-shaped distribution. High cortisol was more associated with obesity and addiction while blunted cortisol was more associated with cardiovascular disease. Carroll et al (54) commented that departure from a "normal" cortisol response may have health consequences in either direction but blunted cortisol responses have been associated more with cardiovascular disease. Seldenrijk et al (55) found that a combination of long-term

psychological distress and cortisol reactivity elevated the odds of developing severe coronary artery calcification (CAC).

A note on the use of AUCI for analysis of the cortisol pattern in the present study is worthwhile. It was possible to use both the area under the curve with respect to the ground (AUCG) and AUCI as predictors. AUCG is the total area under the curve and takes into account both the difference between the single measurements from each other and the distance of these measures from ground. AUCI, however, is calculated with reference to the baseline cortisol measurement and it ignores the distance from the ground for all measurements and emphasizes the changes over time. AUCI is therefore a time dependent parameter and reflects changes in the awakening cortisol pattern; declining magnitude of AUCI as it approaches zero and negative values is indicative of atypical cortisol pattern.(53, 56)

The strengths of this study are the longitudinal design and the study population, a group known to have high levels of occupational stress. The use of quantitative physiologic measures of cortisol and FMD is an advantage in the present study. The availability of the established cohort, which is well-characterized particularly with regard to lifestyle habits, psychosocial factors, biometric characteristics, and subclinical structural/functional parameters, at baseline also provided an advantage.

There are some limitations in the present study. Cortisol dysregulation by itself may not be totally responsible for endothelial function; other factors might include unhealthy lifestyles and other physiological anomalies.(38, 57) Use of a police population may limit generalizability of our findings to other departments that may have different characteristics. Because of

individual differences, it may be not reasonable to assume that there is a single apparent atypical awakening pattern that is a marker for disease. Additionally, other types of cortisol challenges which yield diurnal or suppression patterns may provide important additional information.(58) Procurement of larger samples, including more women officers, would be beneficial. With appropriate recognition of these limitations, some of the findings in this study may be relevant to other occupations characterized as emergency or first responders. Further prospective research is need in this occupation. In conclusion, the present study provides some insight into the long term association of atypical cortisol patterns with FMD, a marker of subclinical cardiovascular disease, among police officers. Additionally, the results point out the need for stress reduction and beneficial wellness strategies in police work.

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Table 1. Demographic and life style characteristics of study participants at baseline and the seven year change in FMD by gender, BCOPS Study (n = 172).

Characteristics	All subjects (n = 172)	Men (n = 139)	Women (n = 33)	p-value[¥]
	n (%)	n (%)	n (%)	
Gender				
Men	139 (80.8)	139 (100.0)		
Women	33 (19.2)		33 (100.0)	
Race				0.2531
White	135 (79.9)	111 (81.6)	24 (72.7)	
Black/Hispanic	34 (20.1)	25 (18.4)	9 (27.3)	
Education				0.9075
≤High school/GED	19 (11.1)	16 (11.5)	3 (9.1)	
College <4 yrs	89 (51.7)	72 (51.8)	17 (51.5)	
College 4+ yrs	64 (37.2)	51 (36.7)	13 (39.4)	
Marital status				0.0005
Single	20 (11.6)	12 (8.6)	8 (24.2)	
Married	129 (75.0)	113 (81.3)	16 (48.5)	
Divorced	23 (13.4)	14 (10.1)	9 (27.3)	
Smoking status				0.0852
Current	24 (14.0)	17 (12.2)	7 (21.9)	
Former	38 (22.2)	28 (20.1)	10 (31.3)	
Never	109 (63.7)	94 (67.6)	15 (46.9)	
Rank				0.2094
Patrol officer	120 (69.8)	94 (67.6)	26 (78.8)	
Other[1]	52 (30.2)	45 (32.4)	7 (21.2)	
	mean ± SD	mean ± SD	mean ± SD	
Age (in years)	41.3 (7.6)	41.5 (7.9)	40.8 (6.4)	0.6575
Years of service	14.6 (8.3)	15.2 (8.7)	13.5 (6.6)	0.3069
Body mass index (kg/m^2)	29.4 (4.5)	30.2 (4.2)	26.0 (4.2)	<.0001
Hours of physical activity/week[2]	14.8 (12.3)	14.9 (12.6)	14.1 (11.1)	0.7272
No. of alcohol drinks/week	5.6 (9.2)	6.2 (9.9)	3.2 (4.6)	0.0114
FMD%[3] at baseline(2004-2009)	5.8 ± 2.9	5.6 ± 2.6	6.5 ± 3.7	0.0988
FMD% at follow-up(2011-2015)	3.7 ± 3.4	3.6 ± 1.9	4.2 ± 3.0	0.1509
Seven-year change in FMD%	-2.4 ± 3.4	-2.0 ± 2.9[+]	-2.3 ± 5.0[+]	0.6296
AUCI at baseline	138.9 ± 299	137.6 ± 302	144.1 ± 289	0.9109

Results for continuous variables are means ±SD. [1]Other includes Sergeant, Lieutenant, Captain, and Detective.[2]Physical activity hours include occupational, household and leisure time

activities. [3]FMD = Flow-mediated dilation. [+]The seven-year reduction in FMD was significant in both men (p-value<0.0001) and women (p-value=0.0123). [¥]P-value comparing mean values of continuous variables (t-test) and distributions of categorical variables (chi-square test) between men and women.

Table 2. Association of AUCI at baseline and the seven-year change in brachial reactivity with baseline demographic and life style characteristics of study participants, BCOPS Study (n = 172).

Characteristics	n	AUCI	Seven- year mean change in FMD%
		mean ± SD	
Gender			
Men	139	137.6 ± 302	-1.98 ± 2.91
Women	33	144.1 ± 289	-2.30 ± 4.97
p-value		*0.9109*	*0.6296*
Race			
White	135	142.6 ± 292	-2.07 ± 3.50
Black/Hispanic	34	125.3 ± 327	-1.91 ± 3.13
p-value		*0.7646*	*0.8103*
Education			
≤High school/GED	19	231.4 ± 244	-3.22 ± 3.37
College <4 yrs	89	101.9 ± 296	-1.73 ± 3.70
College 4+ yrs	64	162.8 ± 312	-2.11 ± 2.87
p-value		*0.1665*	*0.2160*
Marital status			
Single	20	161.0 ± 191	-2.53 ± 4.04
Married	129	127.0 ± 288	-2.04 ± 3.13
Divorced	23	186.2 ± 417	-1.60 ± 4.24
p-value		*0.6433*	*0.6670*
Smoking status			
Current	24	91.9 ± 296	-1.90 ± 3.00
Former	38	186.2 ± 300	-2.23 ± 4.24
Never	109	135.6 ± 300	-1.93 ± 3.08
p-value		*0.4621*	*0.8837*
Rank			
Patrol officer	120	118.3 ± 283	-2.14 ± 3.45
Other[2]	52	186.4 ± 329	-1.82 ± 3.28
p-value		*0.1707*	*0.5729*
		Correlation coefficient (p-value)	
Age (in years)	172	0.192 *(0.0115)*	0.110 (*0.1525*)
Years of service	172	0.162 *(0.0341)*	0.079 (*0.3042*)
Body mass index (kg/m^2)	172	-0.037 *(0.6263)*	-0.036 (*0.6350*)
Hours of physical activity/week[3]	172	-0.006 *(0.9371)*	0.061 (*0.4255*)
No. of alcohol drinks/week	169	-0.020 *(0.7956)*	-0.037 (*0.6308*)

[1]P-values are from analysis of variance comparing means of cortisol parameters and seven-year change in brachial reactivity across categories of categorical covariates or from correlation analysis for continuous covariates. [2]Other includes Sergeant, Lieutenant, Captain, and Detective. [3]Physical activity hours include occupational, household, and leisure time activities.

Table 3. Seven-year mean change in brachial reactivity (FMD %), from baseline to first follow-up, by tertiles of waking cortisol area under the curve with respect to baseline (AUCI).

					Change in FMD% (follow-up minus baseline)		
					(Model 1)	(Model 2)	(Model 3)
Group	Tertile of AUCI	n	Mean FMD% at baseline (± SD)	Mean FMD% at follow-up (± SD)	Unadjusted mean (SD)	Age, gender, and race-adjusted mean (SE)	Multivariate[1] adjusted mean (SE)
All subjects (n = 172)							
	Low [-1154 – 54]	57	6.12 ± 3.0	3.32 ± 2.1	-2.80 ± 3.6	-2.78 ± 0.53	-2.86 ± 0.63
	Medium [68 – 250]	58	6.00 ± 2.8	4.01 ± 2.4	-1.99 ± 3.3	-2.05 ± 0.54	-2.28 ± 0.65
	High [252 – 1413]	57	5.13 ± 2.7	3.81 ± 1.9	-1.33 ± 3.1	-1.38 ± 0.53	-1.56 ± 0.64
	P-value^{+}				*0.0671*	*0.1039*	*0.1535*
	P-value^{++}				*0.0442*	*0.0729*	*0.1413*
Men (n = 139)							
	Low [-1154 – 54]	46	6.20 ± 2.9	3.38 ± 2.1	-2.81 ± 3.3	-2.56 ± 0.47	-2.56 ± 0.64
	Medium [68 – 250]	48	5.70 ± 2.5	3.69 ± 1.9	-2.01 ± 2.6	-1.85 ± 0.47	-1.86 ± 0.65
	High [252 – 1413]	45	4.81 ± 2.3	3.72 ± 1.7	-1.09 ± 2.6	-0.87 ± 0.50	-0.89 ± 0.69
	P-value^{+}				*0.0174*	*0.0286*	*0.0306*
	P-value^{++}				*0.0316*	*0.0584*	*0.0897*
Women (n = 33)							
	Low [-677 – 32]	11	5.80 ± 3.5	3.07 ± 2.4	-1.91 ± 5.0	-2.93 ± 1.67	-3.67 ± 2.4
	Medium [96 – 233]	10	7.45 ± 4.0	5.54 ± 3.8	-2.22 ± 6.0	-2.16 ± 1.94	-5.47 ± 3.1
	High [268 – 701]	12	6.35 ± 3.7	4.13 ± 2.6	-2.22 ± 4.4	-2.31 ± 1.54	-5.08 ± 2.6
	P-value^{+}				*0.9328*	*0.9390*	*0.8550*
	P-value^{++}				*0.5873*	*0.5290*	*0.8735*

[1]Adjusted for age, gender, race/ethnicity, education, smoking, marital status, rank, alcohol use, and physical activity. $^{+}$P-value: ANOVA/ANCOVA p-value testing for any differences in means across the tertiles.$^{++}$P-value: trend p-value from regression model that utilizes the continuous form of AUCI. **Multiple pairwise comparison test results: All subjects:** model 2: low vs. high p =

0.0337; model 3: low vs. high p = 0.0539; **Men:** model 1: low vs. high p = 0.0045; model 2: low vs. high p = 0.0081; model 3: low vs. high p = 0.0087.

Figure 1. Awakening cortisol profile by tertiles of AUCI for men. Officers who showed the largest seven-year decline in FMD% are those in the lowest tertile of AUCI and have 'atypical' awakening pattern or profile. Slope of the linear regression line fitted to the log-transformed cortisol values were: Lowest tertile = -0.0137, Medium tertile = 0.0078, and highest tertile = 0.0277.