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Shiftwork and Decline in Endothelial Function Among Police Officers

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Abstract

Background—Our objective was to assess the influence of shiftwork on change in endothelial function.

Methods—This longitudinal study was conducted in 188 police officers (78.2% men). Shiftwork status (day, afternoon, night) was assessed objectively using daily Buffalo, NY payroll work history records. Brachial artery flow-mediated dilation (FMD) was assessed using ultrasound. Mean change in FMD% between 2004–2009 and 2010–2015 was compared across shiftwork using analysis of variance/covariance.

Results—Overall, mean FMD% decreased from 5.74 ± 2.83 to 3.88 ± 2.11 over an average of 7 years among all officers; P < 0.0001. Effect modification by gender was significant. Among men (but not women), those who worked day shifts had a smaller mean (\pm SE) decrease in FMD% (-0.89 ± 0.35) compared with those who worked the afternoon (-2.69 ± 0.39 ; P = 0.001) or night shifts (-2.31 ± 0.45 ; P = 0.020) after risk factor adjustment.

Conclusions—Larger declines in endothelial function were observed among men who worked afternoon or night shifts. Further investigation is warranted. Am. J. Ind. Med. Published 2016. This article is a U.S. Government work and is in the public domain in the USA

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Dr. L. Charles conceived of the research question, conducted literature search and review, wrote the manuscript, and revised it for important intellectual content. Ms. S. Zhao conducted data analysis. Drs. Fekedulegn, Violanti, Andrew, and Burchfiel provided interpretation of the data and reviewed the manuscript for important intellectual content. All authors approved of the final version. ETHICS APPROVAL AND INFORMED CONSENT

All participants signed written informed consent. The Institutional Review Boards at the University at Buffalo and the National Institute for Occupational Safety and Health approved the studies.

DISCLOSURE BY AJIM EDITOR OF RECORDS

Steven Markowitz declares that he has no competing or conflicts of interest in the review and publication decision regarding this article.

CONFLICT OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Keywords

endothelial function; flow-mediated dilation; police officers; shiftwork; brachial artery reactivity; law enforcement officers

INTRODUCTION

Shiftwork is frequently required in occupations where services must be provided on a 24-hr basis. Although shiftwork provides many advantages for employers, it is also associated with some disadvantages. Shiftwork has been known to cause or be associated with numerous health problems including obesity, insulin resistance, the metabolic syndrome, and cardiovascular disease (CVD) [Nagaya et al., 2002; Sookoian et al., 2007; Thomas and Power 2010; Landsbergis et al., 2013]. Some of the association between shiftwork and CVD may occur through its association with endothelial function [Shechter et al., 2007]. In the few studies that were identified, night shift work was found to be associated with impaired endothelial function in medical personnel and blue collar workers [Amir et al., 2004; Kim et al., 2011; Suessenbacher et al., 2011; Tarzia et al., 2012].

The endothelium is a monolayer of cells between the arterial wall and circulating blood. It is involved in numerous critical functions, including maintaining vascular homeostasis, regulating vascular tone, controlling coagulation through the production of factors that regulate platelet activity and the fibrinolytic system, and producing cytokines and adhesion molecules that regulate and direct the inflammatory process [Brown and Hu 2001; Gonzalez and Selwyn 2003; Widlansky et al., 2003; Paschos and FitzGerald 2010]. Endothelial dysfunction occurs when there is loss of vasodilator and pro-thrombotic products due to a chronic inflammatory process [Widlansky et al., 2003]. Endothelial dysfunction is a precursor to atherosclerosis and is associated with increased plaque rupture in myocardial infarction and other CVD outcomes [Widlansky et al., 2003; Kapuku et al., 2006; Yeboah et al., 2009]. Flow-mediated dilation (FMD) of the brachial artery is the most commonly used method for measuring endothelial function [Corretti et al., 2002, Kapuku et al., 2006, Yeboah et al., 2009].

Since shiftwork is an integral part of the law enforcement profession, it is important to better understand the health outcomes, including the subclinical CVD measures, that may result from its exposure. Although a few cross-sectional studies have investigated the association between shiftwork and endothelial function, we were not able to identify any studies that used longitudinal designs. Our study is unique in this respect. A longitudinal study design would allow us to determine if shiftwork is a risk factor for endothelial dysfunction. In addition, to the best of our knowledge our study is the only one to investigate this topic in police officers in whom shift work is a significant stressor and who are known to have a high prevalence of CVD compared to the general population.

The primary objective of our study was to determine if shiftwork at the baseline exam (2004–2009) was associated with change in endothelial function approximately 7 years later (2010–2015). We hypothesized that one or more of the three shifts would be a risk factor(s) for more rapid deterioration in endothelial function versus no difference across shift in this

cohort of officers. Fewer women in this cohort work on the night shift compared with men. Also, levels of male and female sex hormones may impact the vasculature [Green et al., 2016]. Therefore, a secondary objective was to determine if gender significantly modified the relationship between shiftwork and change in endothelial function.

MATERIALS AND METHODS

Study Design and Participants

We used a longitudinal study design. Participants were police officers employed by the Buffalo, New York, Police Department who participated in two examinations: a baseline exam (2004–2009) and a follow-up exam (2010–2015). From June 2004 through October 2009, 464 active-duty and retired police officers (from an estimated 710 officers in 2004) were recruited and examined in the baseline Buffalo Cardio-Metabolic Occupational Police Stress (BCOPS) study. The BCOPS study was undertaken to investigate associations between work-related stressful exposures and subclinical measures of CVD [Violanti et al., 2006]. Female officers who were pregnant at the time of examination were excluded (n = 2). The 464 officers reviewed and signed informed consent forms before the examinations.

In the follow-up study (2010–2015), 281 officers participated after signing consent forms. Of the 281 officers, 235 had complete data on brachial artery FMD. We merged the data from the baseline and follow-up examinations, retaining only those officers who were activeduty, had complete information on shiftwork at baseline, and FMD at both the baseline and follow-up exams. In both studies, officers underwent a 6-hr examination which usually occurred on one of their training days. The length of the examination limited the number of officers that could be processed in 1 day. Our final sample size was 188 police officers, 41 women and 147 men. Data for both exams were collected at the Center for Health Research, School of Public Health and Health Professions, University at Buffalo, State University of New York [Violanti et al., 2006]. The Institutional Review Boards at the University at Buffalo and the National Institute for Occupational Safety and Health approved the studies.

Assessment of Shiftwork

Electronic work history data from the City of Buffalo, NY payroll records were available for each day from May 1994 to the date of each officer's exam (2004–2009). The database contained information regarding the activities for each officer and included the start and end time of work, the type of activity (i.e., regular work, overtime work, court appearances), the type of leave (i.e., weekend, vacation, work-related injury, other types of sick leave), and the number of hours worked on each activity. The Buffalo NY police department instituted fixed or permanent shifts for their officers in 1994, however officers occasionally worked other shifts for other officers on leave. All officers worked weekdays and weekends and were scheduled 4 days on and 3 days off. The time officers started their shift for the regular time work was used to classify each record into one of the following three shifts: day shift, if the start time of the record was between 0400 and 1159; afternoon shift, if the start time was between 1200 and 1959; and night shift, if the start time was between 2000 and 0359. The majority (>90%) of officers who were classified as day shift workers began work at 0700 or 0800. An officer's dominant shift was defined as the shift on which he/she worked the

highest percentage of hours. For example, the dominant shift would be night shift for an officer who worked 10% on the day shift, 5% on the afternoon shift, and 85% on the night shift. We used data on the dominant shift worked during the previous year.

Assessment of Brachial Artery Flow-Mediated Dilation (FMD)

Additional details regarding measurement of brachial artery FMD can be found elsewhere [Violanti et al., 2009]. Briefly, sonographers screened the officers to determine if they had engaged in smoking, consumed beverages containing alcohol or caffeine, or used medications associated with lipid-lowering and/or blood pressure within 6 hr of testing. Ultrasound tests were performed by a registered nurse (RN) technician trained and certified in carotid intima media thickness and brachial artery reactivity ultrasound on the morning of the day of clinic testing. Officers 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. 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 mm Hg above systolic BP (not to exceed 230 mm Hg) for 4 min. The cuff pressure was then released until completely deflated. Brachial FMD was scanned for 3 min following deflation. The same procedure was used in both the baseline and follow-up exams.

Covariates

Demographic characteristics, lifestyle behaviors, medical history and medication use were obtained from all officers through self- and interviewer-administered questionnaires. Second job status was obtained by asking the question "Have you ever worked a second job while you were a police officer?" Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. Trained and certified technicians from the Osteoporosis Research Center at the University at Buffalo measured percent body fat using dual-energy x-ray absorptiometry of body composition (DXA Hologic QDR 4500A machine; Hologic, Inc., Waltham, MA). Blood pressure was determined using the average of the second and third of three separate measurements of resting systolic and diastolic blood pressure obtained with a standard sphygmomanometer. Hypertension was defined as a systolic blood pressure of 140 mm Hg or a diastolic blood pressure of 80 Hg mm, or use of antihypertensive medications.

Statistical Analysis

Descriptive statistics were obtained for all variables using the chi-square test of independence and Student's t-test. Associations between selected variables and shiftwork were obtained using analysis of variance (ANOVA) and the chi-square test. We used multivariate linear regression and analysis of covariance (ANCOVA) to assess age-adjusted associations between the same selected variables and change in FMD%. Change in FMD% was calculated by subtracting FMD% at the follow-up exam (2010–2015) from that at the baseline exam (2004–2009). Associations between shiftwork and change in FMD% were assessed using ANOVA and ANCOVA. Effect modification was assessed for gender and gender-stratified results were obtained. Comparisons for change in FMD% between each of the shifts were conducted in the fully-adjusted models. Confounders were selected based on

their significant associations with both the independent and dependent variables and/or if these variables were shown to be confounders in previous studies. Confounders included age, percent body fat, education level, and 2^{nd} job status. Statistical significance was indicated if the *P*-value was <0.05. All analyses were conducted in SAS v. 9.3 (SAS Institute, Cary, NC).

RESULTS

In our study, police officers (n = 188) ranged in age from 27 to 66 years (mean \pm standard deviation = 41.3 \pm 7.2 years) (Table I). The majority of officers were male (78.2%), white or Hispanic (81.6%), and held the rank of patrol officer (72.3%). There was a mean decline in brachial FMD% of -1.86 ± 3.2 among all officers between the baseline exam (2004–2009) and the follow-up exam (2010–2015). The decline in FMD% was similar between female (-1.89 ± 4.66) and male (-1.85 ± 2.70) officers. Fifty percent of the officers worked on the day shift, 26.1% on the afternoon shift, and 23.9% on the night shift. Gender was significantly associated with shiftwork, with a smaller percentage of women (12.2%) compared with men (27.2%) working the night shift.

Associations between selected variables and shiftwork are presented in Table II. Age was significantly associated with shiftwork. Officers who worked the night shift were on average younger (37.7 ± 7.0 years) than those who worked other shifts. Having a second job was also associated with shiftwork where a larger percentage of officers working on the night and afternoon shifts reported having a second job. Percent body fat was inversely associated with change in FMD%, age-adjusted b coefficient = -0.084, P = 0.035 (Table III). The association between educational level and change in FMD% was borderline significant; officers who reported the lowest level of education tended to have a greater decline in FMD% compared to those with more education.

In Table IV, the mean change in FMD% is shown across shiftwork at baseline, stratified by gender. Shiftwork status was significantly associated with change in FMD% but only among male officers (P = 0.003). After adjustment for age, percent body fat, education, and 2nd job status, male officers working the day shift had a smaller mean (\pm SE) decrease in FMD% (-0.89 ± 0.35) compared with those who worked the afternoon (-2.69 ± 0.39 ; P = 0.001) or the night (-2.31 ± 0.45 ; P = 0.020) shifts. Taking into consideration that a few of the officers may have changed their dominant shifts between the baseline and follow-up examinations, we conducted additional analyses to determine if these results would differ among officers who held the same shifts in both examinations. Our results were similar. Shiftwork status was significantly associated with change in FMD% only among male officers, and again those working the afternoon and night shifts had significantly larger declines in FMD% compared with those working the day shift (data not shown).

DISCUSSION

We sought to determine whether shiftwork resulted in greater decline of endothelial function over an average of 7 years among a cohort of police officers. Our results showed that male (but not female) officers who worked the afternoon and night shifts had larger average

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decreases in FMD% during the follow-up period compared with those who worked the day shift.

Our results are consistent with those of previous studies, even though the type and assessment of shiftwork in these studies were different from that of the present study. Amir et al. (2004) conducted an experimental study in 30 healthy Israeli physicians to observe the effect of night shift work on endothelial function [Amir et al., 2004]. FMD was performed first, on a regular workday with no previous or subsequent night shift (baseline), and second, after a continuous workday of 24 hr, including the night shift. FMD significantly decreased after the continuous 24-hr workday compared with the baseline measurements and this decrease was independently related to a longer history of shiftwork. The change in FMD after night shift was also greater in physicians with better FMD at baseline, in those who were younger in age, and also slightly among women. Another study investigated endothelial function in 22 healthy, female nurses [Kim et al., 2011]. They were examined on a regular workday and again after three sequential night shifts. Levels of FMD decreased significantly after the sequential night shifts compared with baseline measurements. The decrease in FMD after night shift work was independently related to a longer history of shiftwork. Nitric oxide (NOx) also decreased significantly after three sequential night shifts compared with baseline measurements. The authors suggested that three sequential night shifts may deteriorate endothelial function through decreased production of nitric oxide. Our findings are also in agreement with those of a cross-sectional study [Suessenbacher et al., 2011] of male shift workers (n = 48) and non-shift workers (n = 47). Peripheral endothelial function was assessed using the EndoPAT technique to determine peripheral arterial tone index. The results showed that the shift workers had a reduced PAT index (i.e., worse endothelial dysfunction) compared with non-shift workers.

A study by Etsuda et al. (1999) found that endothelial function, as measured by FMD, showed diurnal variation [Etsuda et al., 1999]. FMD was significantly attenuated in the morning or at noon (at 8:00 am or 12:00 noon) compared with that in the evening (at 5:00 pm) when examined in healthy young men. Other researchers also reported that FMD was markedly decreased in the early morning and recovered by late morning in 30 participants [Otto et al., 2004]. Circadian variation, which is widely observed in all living organisms and even in cultured cells [Paschos and FitzGerald 2010; Chen and Yang 2015] plays a major role in the diurnal variation of endothelial function. Circadian rhythms are driven by a group of genes called CLOCK (Circadian Locomotor Output Cycles Kaput) genes which are tightly related to cardiovascular functions. Functional circadian CLOCK genes are expressed in the heart, cultured endothelial cells, and vascular smooth muscle cells. The circadian CLOCK functions as the central clock in the suprachiasmatic nucleus (SCN) in the hypothalamus, and its peripheral tissues serve as the peripheral clock. Circadian disruption results in desynchronization between central and peripheral clocks and dysregulation of CLOCK genes. Due to the fact that circadian clocks control a large number of tissue specific CLOCK controlled genes (CCGs), disruption of this mechanism, which is more likely to occur in afternoon or night shift workers, has a domino effect resulting in a wide range of adverse biochemical and physiological outcomes, potentially contributing to CVD or vascular problems [Paschos and FitzGerald 2010; Chen and Yang 2015].

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Although it is not completely clear why this same association was not observed among the female officers, there are a few possible reasons. Estrogen levels have been shown to be directly associated with better endothelial function [Teede 2007; Green et al., 2016]. One study showed that FMD declined in men at an earlier age than in women and that menopause was correlated with a steep FMD decline [Celermajer et al., 1994]. It is possible that had our study included a larger percentage of post-menopausal women, we may have observed similar significant associations.

The study is limited by a relatively small sample size among women. In order to detect observed differences across the three shift groups with 80% power, we would need 50 women in each of the three shifts. Therefore, the small sample of women precluded any meaningful interpretation of the results observed among women. Also, even though we adjusted for four confounding variables, we cannot fully exclude residual confounding such as confounding by chronotype, that is, an individual's propensity to sleep at certain times during the 24-hr period. Depending on chronotype, certain officers may be able to adjust to night shift work more easily than others. It is possible that officers who elected to remain on the afternoon and night shifts may have had greater tolerance for shiftwork. If this is the case, our results may be biased toward the null value. In other words, less tolerance for shiftwork might have resulted in an even greater decline in endothelial function than what was observed; our results may be under-estimated. We were unable to adjust for chronotype status because this information was not collected. Our study also has several strengths. A major strength is the use of objective shiftwork information which was obtained from the City of Buffalo daily payroll records, thus precluding recall bias. In our review of the literature, we did not identify another study that used objective data to investigate associations of shiftwork with endothelial function. Another strength is the use of a longitudinal study design which allows us to report that shiftwork is a risk factor for decline in endothelial function. To the best of our knowledge, this was the only longitudinal study to investigate the influence of shiftwork on change in endothelial function among police officers. The results of this study may be generalized to other police officers who work in similar departments.

The clinical importance of the decline in FMD% found in our study is not known with certainty. We were unable to identify studies that investigated differences in FMD% across time-periods or other evidence that showed what level of change in FMD% is clinically meaningful. FMD% decreases with age and therefore we adjusted for age in multivariate analysis. The important finding in our study is that, among male officers, there were significantly larger declines among the officers who worked the night shift (vs. the day shift) and those who worked the afternoon shift (vs. the day shift). Larger decreases in FMD% over time suggest greater endothelial dysfunction. This result is of public health importance in police officers because endothelial dysfunction is a risk factor for cardiovascular disease and in this occupational cohort, the prevalence of cardiovascular disease is reported to be higher than that of the general population.

The results of our study show that among male officers only, those who worked the afternoon or night shifts had a larger decline in FMD% (or worsening of endothelial function) over a 7-year period compared with those who worked the day shift. Since shift

work is essential in law enforcement and many other occupations, managers should consider ways to ameliorate the harmful effects of shiftwork on their employees. Several studies have shown that decline in endothelial function can be reduced or reversed through improvement in diet and physical activity, use of nutritional supplements, estrogen, or medication [Celermajer 1997; Brown and Hu 2001; Lippincott et al., 2008; Harris et al., 2011; van Bussel et al., 2015]. Further investigation of the potential influence of shiftwork status on endothelial function, using longitudinal study designs and larger sample sizes, is warranted.

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TABLE I

Descriptive Statistics of Demographic and Other Variables; BCOPS Study 2004–2009

	<u>All (n=188)</u>	Women(n=41)	<u>Men (n=147)</u>	
Variable	Mean±SD	Mean±SD	Mean±SD	P-value
Age (range: 27–66 yrs)	41.3±7.2	41.5±6.5	41.3±7.4	0.865
Years of service	14.8±7.5	14.2±6.7	15.0±7.7	0.557
BMI (kg/m ²)	29.3±4.6	26.3±4.7	30.2±4.2	<0.0001
Body fat (%)	25.1±6.2	29.9±6.1	23.7±5.4	<0.0001
Brachial (Max-baseline) (mm)	0.17 ± 0.08	0.16±0.10	0.18 ± 0.08	0.280
Brachial FMD (%) (baseline)	5.74±2.83	6.41±3.51	5.56±2.59	0.154
Brachial FMD (%) (follow-up)	3.88±2.11	4.52±3.01	3.71±1.75	0.104
Brachial FMD change (FU-baseline)	$\pm 1.86 \pm 3.21$	$\pm 1.89 \pm 4.66$	-1.85 ± 2.70	0.962
	N (%)	N (%)	N (%)	
Race/ethnicity				0.041
White/Hispanic	151 (81.6)	29 (70.7)	122 (84.7)	
African American	34 (18.4)	12 (29.3)	22 (15.3)	
Education				0.828
12years/GED	18 (9.6)	3 (7.3)	15 (10.3)	
College <4 yrs	104 (55.6)	24 (58.5)	80 (54.8)	
College 4 yrs	65 (34.8)	14 (34.2)	51 (34.9)	
Police rank				0.863
Patrol officer	133 (72.3)	31 (75.6)	102 (71.3)	
Serg/Lieut/Captain	31 (16.9)	6 (14.6)	25 (17.5)	
Det/Exec/Other	20 (10.9)	4 (9.8)	16 (11.2)	
Second job				<0.001
No	121 (64.7)	37 (90.2)	84 (57.5)	
Yes	66 (35.3)	4 (9.8)	62 (42.5)	
Hypertension status				0.127
Hypertensive	39 (20.7)	5 (12.2)	34 (23.1)	
Not hypertensive	149 (79.3)	36 (87.8)	113 (76.9)	
Shift work status (at baseline)				<0.001
Day	94 (50.0)	32 (78.1)	62 (42.2)	
Afternoon	49 (26.1)	4 (9.8)	45 (30.6)	
Night	45 (23.9)	5 (12.2)	40 (27.2)	

BCOPS, Buffalo Cardio-Metabolic Occupational Police Stress.

P values were obtained from the Students' *t*-tests (for continuous variables) and chi-square tests or Fisher's exact tests (for categorical variables). *P* values in bold are statistically significant results.

TABLE II

Associations Between Shiftwork Status at Baseline and Selected Variables; BCOPS Study 2004-2009

	Day (n=94)	Afternoon (n=49)	Night (n=45)	
Variable	Mean±SD	Mean±SD	Mean±SD	P-value
Age (years)	43.9±6.7	39.7±6.3	37.7±7.0	<0.0001
BMI (kg/m ²)	29.5±4.9	29.0±4.2	29.4±4.3	0.864
Body fat (%)	27.0±6.0	23.3±5.2	22.9±6.3	<0.0001
	N (%)	N (%)	N (%)	
Gender				<0.001
Women	32 (34.0)	4 (8.2)	5 (11.1)	
Men	62 (66.0)	45 (91.8)	40 (88.9)	
Race/ethnicity				0.012
White/Hispanic	70 (76.1)	46 (95.8)	35 (77.8)	
African American	22 (23.9)	2 (4.2)	10 (22.2)	
Education				0.039
12 years/GED	12 (12.8)	5 (10.4)	1 (2.2)	
College <4 yrs	58 (61.7)	23 (47.9)	23 (51.1)	
College 4 yrs	24 (25.5)	20 (41.7)	21 (46.7)	
Second job				<0.001
No	73 (77.7)	28 (58.3)	20 (44.4)	
Yes	21 (22.3)	20 (41.7)	25 (55.6)	

BCOPS, Buffalo Cardio-Metabolic Occupational Police Stress.

P values were obtained ANOVA (for continuous variables) and chi-square tests or Fisher's exact tests (for categorical variables).

P values in bold are statistically significant results.

TABLE III

Age-Adjusted Associations Between Selected Variables and Change in FMD% (BCOPS Study 2010–2015 minus 2004–2009)

Variable	Change in FMD%	
	β-coefficient, P-value	
Age (yrs)	0.0625, 0.056	
BMI (Kg/m ²)	-1.5914, 0.693	
Body fat (%)	-0.0840, 0.035	
	Mean±SE	
Gender		
Women	-1.90 ± 0.50	
Men	-1.85±0.26	
<i>P</i> -value	0.930	
Race/ethnicity		
White/Hispanic	-1.88 ± 0.26	
African American	-1.69 ± 0.55	
<i>P</i> -value	0.759	
Education		
12 years/GED	-3.35 ± 0.75	
College <4 yrs	-1.70 ± 0.31	
College 4 yrs	-1.67 ± 0.40	
P-value*	0.051	
Second job		
No	-1.77 ± 0.29	
Yes	-1.99 ± 0.39	
<i>P</i> -value	0.654	

BCOPS, Buffalo Cardio-Metabolic Occupational Police Stress.

P values were obtained from multivariate linear regression (for continuous variables) and ANCOVA (for categorical variables).

P values in bold are statistically significant results.

* *P*-value was obtained from linear contrasts.

TABLE IV

MeanValues of Change in FMD% (BCOPS Study 2010–2015 Minus 2004–2009) Across Shiftwork at Baseline, Stratified by Gender

	Day	Afternoon	Night	P-value
All officers	(n=94)	(n=49)	(n=45)	
Model 1	-1.40 ± 3.54	-2.51 ± 2.84	-2.11±2.74	0.119
Model 2	-1.52 ± 0.34	-2.43 ± 0.46	-1.93 ± 0.49	0.293
Model 3	-1.31±0.36	-2.51 ± 0.48	-2.22 ± 0.54	0.138
Men	(n=62)	(n=45)	(n=40)	
Model 1	-0.97 ± 2.52	-2.76 ± 2.69	$-2.19{\pm}2.60$	0.002
Model 2	-1.11 ± 0.35	-2.71 ± 0.39	-2.02 ± 0.43	0.012
Model 3	-0.89 ± 0.35	-2.69 ± 0.39	-2.31 ± 0.45	0.003
Women	(n=32)	(n=4)	(n=5)	
Model 1	-2.23 ± 4.89	0.30±3.43	-1.44 ± 3.98	0.586
Model 2	-2.31 ± 0.85	0.78 ± 2.52	-1.32 ± 2.13	0.512
Model 3	-2.34 ± 0.92	0.10±2.83	-0.57 ± 2.83	0.650

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P values in bold are statistically significant results.

Results are mean±standard deviation for the unadjusted models and mean±standard error for all adjusted models.

Model 1: Unadjusted.

Model 2: Adjusted for age.

Model 3: Adjusted for age, percent body fat, education, and 2nd job status.

Interaction by gender: P=0.058 (Model 3).

Multiple comparisons (Model 3).

Men:

Day versus afternoon; P=0.001.

Day versus night; P=0.020.

Afternoon versus night; P=0.514.

Women:

Day versus afternoon; P=0.426.

Day versus night; P=0.574.

Afternoon versus night; P=0.871.