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Association of shiftwork and immune cells among police officers from the Buffalo Cardio-Metabolic Occupational Police Stress study

Michael D. Wirth[a,b,c], **Michael E. Andrew**[d], **Cecil M. Burchfiel**[d], **James B. Burch**[a,b,e], **Desta Fekedulegn**[d], **Tara A. Hartley**[d], **Luenda E. Charles**[d], **John M. Violanti**[f]

[a]Cancer Prevention and Control Program, University of South Carolina, Columbia, SC, USA

[b]Department of Epidemiology and Biostatistics, University of South Carolina, Columbia, SC, USA

[c]Connecting Health Innovations, Columbia, SC, USA

[d]Biostatistics and Epidemiology Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Morgantown, WV, USA

[e]WJB Dorn VA Medical Center, Columbia, SC, USA

[f]Department of Epidemiology and Environmental Health, School of Public Health and Health Professions, University of Buffalo, The State University of New York, Buffalo, New York, USA

Abstract

Shift workers suffer from a constellation of symptoms associated with disruption of circadian rhythms including sleep abnormalities, and abnormal hormone secretion (e.g. melatonin, cortisol). Recent, but limited, evidence suggests that shift workers have elevated levels of circulating white blood cells (WBCs) compared to their day working counterparts. Interestingly, recent reviews highlight the strong linkage between the immune system and circadian rhythms which includes, but is not limited to, circulating cell populations and functions. The elevated levels of these WBCs may be associated with the increased chronic disease risk observed among this group. The purpose of this analysis was to examine the cross-sectional association between long- and short-term (3, 5, 7, and 14 days) shiftwork (SW) and counts of WBCs among officers in the Buffalo Cardio-Metabolic Occupational Police Stress (BCOPS) cohort. Data collection for this analysis took place among 464 police officers working in Buffalo, New York, USA between 2004 and 2009. Precise SW histories were obtained using electronic payroll records. Officers were assigned a shift type based on the shift (i.e. day, evening, night) that they spent a majority (i.e ≥50%) of their time from 1994 to the data collection date for long-term SW. The same process was applied to SW over 3, 5, 7, and 14 days prior to data collection. A fasted blood sample collected in the morning of a non-work day was used for characterization of WBCs (total), neutrophils, monocytes, lymphocytes,

CONTACT Michael D. Wirth, MSPH, PhD, wirthm@mailbox.sc.edu Cancer Prevention and Control Program, University of South Carolina, 915 Greene Street, Room 233, Columbia, SC 29208, USA.

Declaration of interest

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eosinophils, and basophils. Potential confounding factors included demographic characteristics (e.g. age, sex, race), occupational characteristics (e.g. rank), health behaviors (e.g. smoking, alcohol consumption, diet), anthropometrics, and other biomarkers (e.g. lipids, hemoglobin A1C, leptin). Generalized linear models were used to estimate least square means of the immune cells according to SW categorization for long- and short-term SW histories. Compared to the day shift group, those working long-term night shifts had greater absolute numbers of total WBCs, neutrophils, lymphocytes, and monocytes (all $p < 0.05$). Those working mainly on the night shift over 7-days had elevated counts of WBCs, lymphocytes, and monocytes ($p < 0.05$) compared to those mainly working day shifts. Results based on 3-, 5-, and 14-day SW were similar to the 7-day results. This study corroborates other studies with similar findings. However, this analysis provided insights into the effect of both long- and short-term SW on the number of circulating WBCs. SW may lead to disruption of circadian-influenced components of the immune system, which in term, may result in various chronic diseases. These findings, plus previous findings, may provide evidence that SW may lead to immune system dysregulation. Future research is needed to understand whether increases in immune cells among shift workers may be associated with the increased disease risk among this group.

Keywords

police officers; shiftwork; white blood cells

Introduction

Over the last couple of decades, circadian rhythm research, as it relates to human health, has gained attention from academic and research communities. There are several review publications describing the effects of disruptions of various circadian rhythms on health (Bollinger & Schibler, 2014; Fuhr et al., 2015; Hastings et al., 2014). In short, various behavioral and physiological mechanisms display roughly 24-hour cycles. These rhythms are primarily driven by the body's central pacemaker, the suprachiasmatic nuclei (SCN), located in the ventral hypothalamus (Bollinger & Schibler, 2014; Fu & Lee, 2003). To stay synchronized with the outside environment, environmental cues (i.e. zeitgebers) are used to adjust circadian clocks (Bollinger & Schibler, 2014). The strongest environmental cue is light, which is perceived by the retina, and is transmitted through the retinohypothalamic tract to the SCN (Bollinger & Schibler, 2014; Lowrey & Takahashi, 2011). The molecular pacemaker is driven by a set of core clock genes (Fu & Lee, 2003). Positive and negative feedback loops alternate between the morning and evening. The molecular basis for these clocks has been extensively described elsewhere (Antle & Silver, 2005; Cermakian & Boivin, 2009; Hastings et al., 2014). In addition to the central clock, peripheral clocks have been found in most tissue types and can be synchronized through various means (e.g. metabolites, cytokines, neuronal signals) by the SCN (Bollinger & Schibler, 2014; Nader et al., 2010). The peripheral clocks also can respond to other zeitgebers including feeding time, exercise, and body temperature (Archer & Oster, 2015; Cuesta et al., 2016). Recent research has brought attention to the circadian biology of the human immune system (Labrecque & Cermakian, 2015; Logan & Sarkar, 2012; Pritchett & Reddy, 2015).

Disruption in rhythms of white blood cells (WBCs) or elevated levels have been associated with several chronic conditions including type 2 diabetes mellitus (T2DM), coronary artery disease, stroke, and leukemia, just to name a few (Costas et al., 2016; Danesh et al., 1998; Ford, 2002; Libby et al., 2016; Vozarova et al., 2002). Recent reviews discuss the wide array of immune components and functions controlled by or, at least, influenced by circadian rhythms. These include, but are not limited to, rhythms of most leukocytes (e.g. monocytes, lymphocytes, neutrophils), cytokine production, recruitment to tissues, phagocytosis, natural killer cell cytotoxicity, and T-cell responses (Labrecque & Cermakian, 2015). Specifically, this paper focused on WBCs (i.e. total, neutrophils, monocytes, lymphocytes, eosinophils, and basophils). In addition to circulating levels of these cell types, some of their other functions exhibit circadian rhythmicity. For example, neutrophils partake in phagocytosis and their release and reuptake from bone marrow show daily patterns; monocytes, which become macrophages, have rhythmic cytokine expression; lymphocytes, which destroy viruses, create antibodies, and secrete cytokines show rhythmicity in proliferation, differentiation, and cytokine production; eosinophils kill parasites and influence allergic inflammatory responses and also show rhythmic cytokine production; lastly, basophils, which modulate allergic inflammatory responses, show rhythms in histamine and cytokine release (Ando et al., 2015; Baumann et al., 2013; Bollinger et al., 2009, 2011; Casanova-Acebes et al., 2013; Perez-Aso et al., 2013; Pritchett & Reddy, 2015; Yu et al., 2013). These cell types also display circadian gene expression (Boivin et al., 2003). It has been proposed that the number of circulating WBCs is under the control of the central clock via neuronal and humoral signaling (Dimitrov et al., 2009; Scheiermann et al., 2012). In general, most of the circulating leukocytes peak between 2000 and 0200 hours (Mazzoccoli et al., 2011; Sennels et al., 2011).

One occupational group that experiences disruption of various circadian rhythms (e.g. melatonin and cortisol rhythms, clock gene expression) is shift workers (Arendt, 2010; Bracci et al., 2014; Wirth et al., 2011). Around 15–20% of workers in the United States and Europe participate in shiftwork (SW) (Straif et al., 2007). Occupations that have high prevalence of SW include healthcare and law enforcement (Straif et al., 2007). This can be somewhat disconcerting considering that SW is associated with a host of abnormal physiological changes and chronic disease risks including cardiovascular disease (CVD), gastrointestinal complaints, inflammation, metabolic syndrome and other metabolic abnormalities, and depression (Lee et al., 2016; Matheson et al., 2014; Proper et al., 2016; Puttonen et al., 2011; Wang et al., 2014). In 2007, the International Agency for Research on Cancer classified SW that involves circadian disruption as a probable human carcinogen (Group 2A) (Straif et al., 2007). Animal models tend to indicate abnormal rhythms of circulating levels of leukocytes when test animals are forced to be active during their typical rest periods (Pritchett & Reddy, 2015). However, this has rarely been studied among human populations. Considering the links between SW and circadian disruption, shift workers may be susceptible to disruptions in regulation of their immune cells. In short, a few studies showed increased leukocytes among shift workers compared to day workers (Kim et al., 2016; Lu et al., 2016; Puttonen et al., 2011).

Police officers suffer disproportionately from various health outcomes including depression, psychological stress, sleep disruption, CVD, T2DM and other metabolic disorders, cancer,

and increased mortality (Franke et al., 1997; Vena et al., 1986; Vila, 2006; Violanti et al., 2006, 1998; Wirth et al., 2013). The Buffalo Cardio-Metabolic Occupational Police Stress (BCOPS) cohort study was designed to examine the biological processes through which stress associated with police work may influence adverse health outcomes (Violanti et al., 2006). Using data from the BCOPS cohort study, this study examined the relationship between short- and long-term SW and immune cells including leukocytes. It was hypothesized that those exposed to more long-term SW (i.e. night or evening shifts) would have elevated absolute levels of these immune cells compared to those working more day shifts. The same hypothesis was proposed for short-term SW over 3, 5, 7, and 14 days. Although the mechanisms linking SW to leukocyte counts have yet to be fully elucidated, both long-term and short-term SW were explored in case they work through slightly different mechanisms, which, in turn, may influence the associations between SW and leukocytes. Lastly, as exploratory analyses, leukocyte percentages also were examined.

Methods

The study population was comprised of police officers working in Buffalo, New York, USA as part of the BCOPS cohort study. All data for this cross-sectional analysis were derived from a single examination occurring between 2004 and 2009 (n = 464). The BCOPS study provides a framework to examine biological processes associated with police work and how this may influence health outcomes (Violanti et al., 2006). The protocol includes characterization of stress biomarkers, psychosocial factors, shiftwork, and markers of adverse health outcomes (e.g. subclinical CVD measures) to examine their associations with chronic diseases and psychological perturbations afflicting police officers (Violanti et al., 2009a, 2006). Data collection included a one-time collection of basic demographics, anthropometric information, blood draw for characterization of numerous markers, questionnaire data (e.g. lifestyle behaviors, work experience, stress, depression, social support), and SW from electronic payroll records from 1994 to the date of the officer's examination (Violanti et al., 2006). The BCOPS study received Institutional Review Board approval from The State University of New York at Buffalo and the National Institute for Occupational Safety and Health. All officers provided written informed consent.

Shiftwork histories

SW histories from 23 May 1994 (or the initiation of employment if occurring since May 23rd, 1994) to the date of study examination and blood draw between 2004 and 2009 were obtained from each officer using electronic payroll records (Violanti et al., 2009b). After 1994, the typical work schedule was four work days, four days off, four work days, and then three days off. All shifts recorded by the electronic payroll records were categorized into day, evening, or night based on the following start times of the shifts: 04:00–11:59 h (morning), 12:00–19:59 h (evening), or 20:00–03:59 h (night). Two forms of SW were characterized for this analysis, long-term and short-term. Long-term SW status (i.e. day, evening, or night) was assigned based on the shift that the officers spent a majority of their time during the time of available electronic payroll records. Previous work indicated that for about 85% of officers, 70% of their time was spent in one specific shift type. Additionally, this process of summarizing SW status showed good consistency over 30, 60, or 90 days,

and 5 years prior to the clinic date (Violanti et al., 2009b; Wirth et al., 2011). Also, the percentage of day shift hours was used as a continuous measure. For short-term SW, a similar process was used as described above for characterization of shifts over 3, 5, 7, and 14 days prior to the date of examination. For both the long-term and short-term shift classifications, in addition to examining day, evening, and night shifts, evening and nights shifts were combined to create a "combined" shift category.

Immune markers

Participants were asked to fast for 12 hours before examination and blood collection in the morning (i.e. prior to 0830). Lavender-top vacuum vials with EDTA anti-coagulant were used. Counts of WBCs were made using the Coulter STKS (Arnott & Copson, 1994; Charles et al., 2007). Specifically, the following immune markers were obtained: WBCs (total), neutrophils, monocytes, lymphocytes, eosinophils, and basophils. The units for all WBCs were $\times 10^9$/L. For the specific WBCs, absolute and percentages were examined. However, eosinophils and basophils had values that were zero or close to zero. Therefore, absolutes for these were not examined.

Covariates

Potential covariates included, but were not limited to, age, sex, race/ethnicity, education, police rank, health behaviors (tobacco use, alcohol consumption, dietary factors, physical activity), anthropometrics (e.g. body mass index [BMI = kg/m^2]), blood pressure, lipid and metabolic biomarkers (e.g. cholesterol, glucose, leptin), and several psychosocial measures including the Center for Epidemiologic Studies Depression Scale (CES-D) (Radloff, 1977), Perceived Stress Scale (PSS) (Cohen et al., 1983), Impact of Events Scale (IES) (Horowitz et al., 1979), and Spielberger Police Stress Survey (SPSS) (Martelli et al., 1989). It should be noted that none of the psychosocial measures were statistical confounders and were, therefore, not included in any models.

Statistics

All analyses were performed using SAS version 9.4 (Cary, North Carolina, USA). Descriptive statistics were compiled using frequencies and chi-square tests for categorical variables. For continuous variables, means and standard deviations were presented with tests of significance based on *t*-tests or Wilcoxon ranks sums test depending on normality of the variables. Prior to the main analyses, a confounder selection process was applied. Any potential covariate with a p value of <0.20 in a series of bivariable analyses (i.e. WBC = SW + covariate) was added to a full model. From here, a backward confounder reduction process was used to remove covariates one at a time. Those that changed the beta coefficient of SW by at least 10% were retained; additionally, any statistically significant covariate remained in the models. After the final models were derived, the assumptions of linear regression were examined using the model's residuals. There were no significant deviations from the assumptions of linear regression.

General linear model (GLM) procedures were conducted for the main analyses. The outcomes are listed in the Immune Markers section. GLM allowed for calculation for least

square (LS) means and 95% confidence intervals (95% CI) of the various immune markers according to the SW categories. The primary comparison of interest was between day and night shifts. Other comparisons were displayed in Table 1; however, considering these were not the comparisons of primary interest, no adjustments were made for multiple testing. These analyses were performed for long-term SW and short-term SW over 3, 5, 7, and 14 days prior to data collection. However, results between the short-term SW days were similar. Hence, only the results for the 7-day SW are presented in Table 3. The full data of short-term SW can be found in Supplemental Table S1. For long-term SW, the percent of day shift hours was examined as a continuous factor using GLM. Three additional *post hoc* analyses were conducted. To determine the influence of recent illness and injuries, analyses were conducted after removing any officer reporting sick leave time off in the previous 20 days (n = 13). Considering that 33 of the officers were retired at time of data collection and were not working recently prior to blood draw, analyses were conducted after removing these individuals. Lastly, considering data was collected sometime between 2004 and 2009, the year and season of data collection were considered as two potential covariates for adjustment.

Results

Of the 464 police officers, 34 were missing information on SW information as they were new recruits and 5 others were missing information on WBCs. For the 3-, 5-, 7-, and 14-day analyses, 85, 80, 78, and 76, respectively, were missing information for various reasons including being new recruits, were retired at the time of this data collection, or did not work over these periods (e.g. injury or personal leave), and were excluded from analyses. Most participants were males (75%), European-American (79%), had some level of college education (87%), held the rank of police officer (65%), and were overweight (mean BMI = 29.3 ± 4.8 kg/m^2) with an average age of 42.1 ± 8.6 years (Table 1). Compared to those primarily working day shifts, those primarily working evening or night shifts were more likely to be male (60% vs. 86%, $p < 0.01$), European-American (70% vs. 84%, $p < 0.01$), hold the rank of police officer (54% vs. 74%, $p < 0.01$), and currently use tobacco (23% vs. 31%, $p < 0.01$). Additionally, those working more evening or night shifts were more likely to be younger, consume less fruit and vegetable servings per day, consume more calories, and have higher triglyceride levels (all $p < 0.05$, Table 1).

Table 2 displays the LS means of the WBCs according to SW category. Compared to those in the day shift category, those working the night shift had greater absolute total WBCs (6.16 vs. 5.54, $p < 0.01$), neutrophils (3.59 vs. 3.11, $p < 0.01$), lymphocytes (1.90 vs. 1.76, $p = 0.05$), and monocytes (0.49 vs. 0.44, $p = 0.01$). They also had lower basophil percentages compared to day shift workers (0.47% vs. 0.58%, <0.01). When the night shift group and evening shift group were combined, this group still had statistically significantly greater absolute WBCs and neutrophils, as well as a lower basophil percentage. A similar pattern was observed when comparing only the evening shift group to the day shift group (Table 2). For long-term SW history, the percentage of day shift hours, as a continuous measure, also was analyzed. As the percentage of day shift hours increased, WBCs ($\beta = -0.018$, $p = 0.02$) and neutrophils ($\beta = -0.013$, $p = 0.04$) decreased (data not tabulated).

As noted in the Methods, the results among short-term SW over 3, 5, 7, and 14 days were very similar. Hence, Table 3 displays results for 7-day SW only. For the absolute cell counts, the 7-day night shift group had higher values of total WBCs (5.99 vs. 5.58 × 10^9/L, p = 0.05), lymphocytes (2.05 vs. 1.72 × 10^9/L, $p < 0.01$), and monocytes (0.51 vs. 0.44 × 10^9/L, $p < 0.01$). Additionally, percentages of neutrophils were lower, but the lymphocyte percentage was higher compared to the day shift group ($p \leq 0.02$). When examining the relationship between the combined evening and night groups vs. the day shift group, the statistically significant relationships with absolute WBCs, lymphocytes, and monocytes, as well as neutrophil and lymphocyte percentage remained. Additionally, the combined group had a higher percentage of monocytes compared to the day shift group (8.83% vs. 8.16%, p = 0.01).

After removing officers reporting sick leave days within the past 30 days, there was no change in results. Likewise, after removing individuals who were retired at time of blood collection, there was no change in results. After adjustment for year of data collection and season of blood draw, the statistically significant difference in lymphocytes between day and night workers (p = 0.05) became slightly attenuated (p = 0.07). However, it should be noted that the magnitude of effect (i.e. difference in lymphocytes between day and night workers) only changed by 0.01 after the additional adjustment for year and season. Similarly, the difference in total WBCs between day and night workers over 7 days (see Table 3) became nonsignificant (p = 0.07). However, the magnitude of effect (i.e. difference in WBCs between day and night workers) only changed by 0.02 after the additional adjustment (data not tabulated).

Discussion

According to the current study, night work was associated with elevated levels of absolute leukocytes (i.e. total WBCs, neutrophils, lymphocytes, and monocytes) when characterized over a period of years (i.e. long-term SW). The only WBC percentage that differed between night and day shifts for long-term SW was basophils. For short-term SW, similar associations were observed. However, no difference in absolute neutrophils by shift type was found, but night work was associated with a lower percentage of neutrophils and higher percentage of lymphocytes. The reported range of total leukocytes, although it varies from data source to data source, typically falls between 4.0 and 11.0 × 10^9 cells with neutrophils accounting for about 60%, lymphocytes 30%, eosinophils 2%, basophil 0.5%, and monocytes about 5% (Hollowell et al., 2005).

Only a few studies have examined WBC levels according to shift type. Using a population (n = 1,654) of steel workers from Taiwan, Lu and colleagues observed 7.5%, 7.4%, 11.9%, and 7.0% higher counts of total WBCs, neutrophils, monocytes, and lymphocytes, respectively, among rotating shift workers, including afternoon and night shifts (Lu et al., 2016). Those same comparisons in the current study indicated 11.2%, 15.4%, 8.0%, and 10.2% higher values, respectively. Although not identical, both studies show increases in these WBCs. In a study of Finnish airline workers, Puttonen and colleagues found higher total leukocyte counts of between 4.6% and 11.7% among shift workers, which was defined as rotating shift workers with night shifts included, compared to day workers depending on SW system and

gender. These were statistically significant (Puttonen et al., 2011). Two other studies, one among male manual workers in the Republic of Korea, and another among male factory workers in Buenos Aires, found statistically significantly higher levels of total leukocyte counts among rotating shift workers compared to day workers (Kim et al., 2016; Sookoian et al., 2007). However, caution is warranted when comparing these studies, considering that different occupations (i.e. steel workers, airline employees, factory workers, and police officers) and five different countries (i.e. China, Finland, Republic of Korea, Argentina, and the United States) are represented by these studies. Nonetheless, the pattern of an increase in night workers is consistent among the five studies. It should also be noted that the other four studies above collected blood draws in the morning hours (typically between 0600 and 1030), consistent with the current study.

Abnormal WBC levels or rhythms have been associated with several chronic diseases, such as T2DM, that continue to plague many developed and developing countries (Danesh et al., 1998; Ford, 2002; Libby et al., 2016; Vozarova et al., 2002). For example, among women, those with a total leukocyte count above 9.1×10^9/L, compared to less than 5.7×10^9/L, had a 68% (95% CI = 1.21–2.34) greater hazard ratio for incident diabetes after adjustment for a host of factors that included physical activity, smoking, hypertension medicine, blood pressure, and age (Ford, 2002). Abnormal rhythms or amounts of circulating leukocytes may lead to or enhance current disease processes that could ultimately lead to a major chronic disease diagnosis or event. For example, excess leukocytes may lead to an abnormal accumulation in early atherosclerotic plaques. Over time, this accumulation may aid the thickening of the arterial walls. This increase in leukocyte accumulation in plaques can increase inflammation in the affected area (Conti & Shaik-Dasthagirisaeb, 2015; Libby et al., 2016). In addition, it should be noted that several leukocytes produce pro-inflammatory cytokines, which may raise levels of chronic, systemic inflammation (Pritchett & Reddy, 2015). Chronic inflammation is an underlying substrate through which mechanisms for chronic diseases progress. These include, but are not limited to CVD, cancer, T2DM, stroke, and metabolic syndrome, as well as mortality (Ahmadi-Abhari et al., 2013; Keibel et al., 2009; Libby, 2007). Shift workers suffer disproportionately from all of the above listed conditions.

The results for 3-, 5-, 7-, and 14-day SW status were similar. However, there were several noticeable differences between the short and long-term SW. For example, based on long-term SW, those classified as evening shift workers had about a 15% increase in neutrophils compared to long-term day shift workers. However, no such observation was noted for short-term SW classification. In fact, the values for evening and day shift workers over 7-days were nearly identical. Interestingly, total WBCs were still more elevated among evening shift workers over 7-days compared to day workers. Because neutrophils tend to be the most abundant leukocytes, differences in other leukocytes should be driving the difference in total WBCs between evening and day shift workers over 7 days. This also may explain why there are differences in associations between SW and the immune cell percentages between short-term and long-term SW. Neutrophils may respond more to the effects of chronic SW, whereas they may not be as responsive to more acute or short-term effects of SW. The cross-sectional nature of this analysis precludes definitive characterization of this relationship, and these findings should thus be considered exploratory in nature. Another uncertainty is that

those in the short-term evening shift category may not necessarily be the same individuals classified as evening shift workers for long-term SW. However, it should be noted that there was a 76% agreement between long-term SW and 7-day SW classification for day, evening, and night shifts. When evening and night shifts were combined this agreement became 82%. In other words, 82% of police officers were categorized into the same shift type for the long-term and 7-day SW. Additionally, the long-term SW measure is more of an average of shift type over a greater proportion of the career of the officer. Short-term SW is representative of the primary shifts worked immediately prior to data collection. Therefore, this could have led to differential findings between the short- and long-term analyses.

More research, potentially laboratory-based studies are needed to elucidate the relationship between SW and immune cell counts. Besides the potential disruption of the circadian system, there are other potential explanations for the observed findings. For example, shift workers tend to request more sick leave and suffer more injuries (Dall'Ora et al., 2016; Dembe et al., 2006; Merkus et al., 2012). Therefore, the increase in WBC counts may be due to increases in injuries and illness, as opposed to the SW itself. Although some information on illnesses and sick leave were available, it is difficult to completely control for these measures as officers may have still be working with injuries or illnesses. Nonetheless, exclusion of officers with prior sick leave did not affect the results of this analysis.

One major strength of this study was the use of electronic payroll records for classification of SW. This allowed for quantification of the actual amount of work hours on different shift types which led to more reliable SW categorization. A wide range of covariates were examined as potential confounders. This is one of the first studies to examine the impact of SW on immune cells. However, there were several noteworthy limitations. The population was comprised of mostly white males. Additionally, results among this unique police officer population may not apply to other shift working populations. Blood was only drawn at one time in the morning. Therefore, it is not possible to fully elucidate whether the shift workers had chronically elevated WBCs or a phase shift in their WBCs, especially given that WBCs typically peak late night or early morning (Mazzoccoli et al., 2011). In addition, the blood draw could have occurred anywhere between 2004 and 2009 and during any season. Although, adjustment for these factors changed the statistical significance for a couple of relationships, the change in effect size was negligible. Finally, a healthy worker effect may have interfered with an ability to detect immune system impacts (Meijers et al., 1989). Officers who struggled the most with evening shifts may have requested transfer to a different shift or left the occupation all together. These individuals may be more likely to suffer from circadian rhythm disruption, possibly including WBC counts.

SW is associated with a range of abnormal behavioral/lifestyle factors, disruption of numerous bodily systems, and increased risk for several chronic diseases (Matheson et al., 2014; Nea et al., 2015; Smith & Eastman, 2012; Wang et al., 2011). One of the primary culprits hypothesized to be the cause of these associations is the disruption of circadian mechanisms observed among this group (Smith & Eastman, 2012). Recent evidence supports the notion that immune cells (e.g. WBCs) exhibit circadian rhythmicity related to their production and functions (Labrecque & Cermakian, 2015; Pritchett & Reddy, 2015). Therefore, it is reasonable to hypothesize that shift workers may have differences in their

immune cell counts compared to their day working counterparts. This research confirms that this hypothesis is in line with previous studies that have examined such a relationship (Lu et al., 2016; Puttonen et al., 2011). However, many avenues of circadian rhythm and SW research have yet to be fully explored. For example, it is not entirely clear whether these increases are due to overall increase in immune cells among shift workers, an abnormal circadian pattern of immune cells among shift workers, or increases in subclinical disease in shift workers. More research is needed to understand and confirm the relationship between WBCs and SW, as well as to determine if abnormal immune cell production is a mechanism that may be associated with the increased disease risk among shift working groups, such as police officers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

Ahmadi-Abhari S, Luben RN, Wareham NJ, Khaw KT. (2013). Seventeen year risk of all-cause and cause-specific mortality associated with C-reactive protein, fibrinogen and leukocyte count in men and women: The EPIC-Norfolk study. Eur J Epidemiol. 28:541–50. [PubMed: 23821244]

Ando N, Nakamura Y, Ishimaru K, et al. (2015). Allergen-specific basophil reactivity exhibits daily variations in seasonal allergic rhinitis. Allergy. 70:319–22. [PubMed: 25443426]

Antle MC, Silver R. (2005). Orchestrating time: Arrangements of the brain circadian clock. Trends Neurosci. 28:145–51. [PubMed: 15749168]

Archer SN, Oster H. (2015). How sleep and wakefulness influence circadian rhythmicity: Effects of insufficient and mistimed sleep on the animal and human transcriptome. J Sleep Res. 24:476–93. [PubMed: 26059855]

Arendt J (2010). Shift work: Coping with the biological clock. Occup Med. 60:10–20.

Arnott AJ, Copson P. (1994). A network-based laboratory data processing system for use with the Coulter STKS haematology analyser. Clin Lab Haematol. 16:65–74. [PubMed: 8039348]

Baumann A, Gonnenwein S, Bischoff SC, et al. (2013). The circadian clock is functional in eosinophils and mast cells. Immunology. 140:465–74. [PubMed: 23876110]

Boivin DB, James FO, Wu A, et al. (2003). Circadian clock genes oscillate in human peripheral blood mononuclear cells. Blood. 102:4143–45. [PubMed: 12893774]

Bollinger T, Bollinger A, Skrum L, et al. (2009). Sleep-dependent activity of T cells and regulatory T cells. Clin Exp Immunol. 155:231–38. [PubMed: 19040608]

Bollinger T, Leutz A, Leliavski A, et al. (2011). Circadian cocks in mouse and human CD4+T cells. Plos One. 6:1–11.

Bollinger T, Schibler U. (2014). Circadian rhythms: From genes to physiology and disease. Swiss Med Wkly. 144:w13984. [PubMed: 25058693]

Bracci M, Manzella N, Copertaro A, et al. (2014). Rotating-shift nurses after a day off: Peripheral clock gene expression, urinary melatonin, and serum 17-beta-estradiol levels. Scand J Work Environ Health. 40:295–304. [PubMed: 24402410]

Casanova-Acebes M, Pitaval C, Weiss LA, et al. (2013). Rhythmic modulation of the hematopoietic niche through neutrophil clearance. Cell. 153:1025–35. [PubMed: 23706740]

Cermakian N, Boivin DB. (2009). The regulation of central and peripheral circadian clocks in humans. Obes Rev Off J Int Assoc Study Obes. 10:25–36.

Charles LE, Fekedulegn D, McCall T, et al. (2007). Obesity, white blood cell counts, and platelet counts among police officers. Obesity. 15:2846–54. [PubMed: 18070777]

Cohen S, Kamarck T, Mermelstein R. (1983). A global measure of perceived stress. J Health Soc Behav. 24:385–96. [PubMed: 6668417]

Conti P, Shaik-Dasthagirisaeb Y. (2015). Atherosclerosis: A chronic inflammatory disease mediated by mast cells. Central-Eur J Immun. 40:380–86.

Costas L, Benavente Y, Olmedo-Requena R, et al. (2016). Night shift work and chronic lymphocytic leukemia in the MCC-Spain case-control study. Int J Cancer. 139:1994–2000. [PubMed: 27416551]

Cuesta M, Boudreau P, Dubeau-Laramee G, et al. (2016). Simulated night shift disrupts circadian rhythms of immune functions in humans. J Immunol. 196:2466–75. [PubMed: 26873990]

Dall'Ora C, Ball J, Recio-Saucedo A, Griffiths P. (2016). Characteristics of shift work and their impact on employee performance and wellbeing: A literature review. Int J Nurs Stud. 57:12–27. [PubMed: 27045561]

Danesh J, Collins R, Appleby P, Peto R. (1998). Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: Meta-analyses of prospective studies. JAMA. 279:1477–82. [PubMed: 9600484]

Dembe AE, Erickson JB, Delbos RG, Banks SM. (2006). Nonstandard shift schedules and the risk of job-related injuries. Scand J Work Env Hea. 32:232–40.

Dimitrov S, Benedict C, Heutling D, et al. (2009). Cortisol and epinephrine control opposing circadian rhythms in T cell subsets. Blood. 113:5134–43. [PubMed: 19293427]

Ford ES. (2002). Leukocyte count, erythrocyte sedimentation rate, and diabetes incidence in a national sample of US adults. Am J Epidemiol. 155:57–64. [PubMed: 11772785]

Franke WD, Cox DF, Schultz DP, Anderson DF. (1997). Coronary heart disease risk factors in employees of Iowa's Department of Public Safety compared to a cohort of the general population. Am J Ind Med. 31:733–37. [PubMed: 9131229]

Fu L, Lee CC. (2003). The circadian clock: Pacemaker and tumour suppressor. Nat Rev Cancer. 3:350–61. [PubMed: 12724733]

Fuhr L, Abreu M, Pett P, Relogio A. (2015). Circadian systems biology: When time matters. Comput Struct Biotec. 13:417–26.

Hastings MH, Brancaccio M, Maywood ES. (2014). Circadian pacemaking in cells and circuits of the suprachiasmatic nucleus. J Neuroendocrinol. 26:2–10. [PubMed: 24329967]

Hollowell JG, Van Assendelft OW, Gunter EW, et al.; Centers for Disease C, Prevention NCfHS. (2005). Hematological and iron-related analytes-reference data for persons aged 1 year and over: United States, 1988–94. Vital Health Statistics Series 11, Data National Health Survey. 3:1–156.

Horowitz M, Wilner N, Alvarez W. (1979). Impact of Event Scale: A measure of subjective stress. Psychosom Med. 41:209–18. [PubMed: 472086]

Keibel A, Singh V, Sharma MC. (2009). Inflammation, microenvironment, and the immune system in cancer progression. Curr Pharm Des. 15:1949–55. [PubMed: 19519435]

Kim SW, Jang EC, Kwon SC, et al. (2016). Night shift work and inflammatory markers in male workers aged 20–39 in a display manufacturing company. Ann Occup Environ Med. 28:48. [PubMed: 27660715]

Labrecque N, Cermakian N. (2015). Circadian clocks in the immune system. J Biol Rhythms. 30:277–90. [PubMed: 25900041]

Lee HY, Kim MS, Kim O, et al. (2016). Association between shift work and severity of depressive symptoms among female nurses: The Korea Nurses' Health Study. J Nurs Manage. 24:192–200.

Libby P (2007). Inflammatory mechanisms: The molecular basis of inflammation and disease. Nutr Rev. 65:S140–146. [PubMed: 18240538]

Libby P, Nahrendorf M, Swirski FK. (2016). Leukocytes link local and systemic inflammation in ischemic cardiovascular disease: An expanded “Cardiovascular Continuum”. J Am Coll Cardiol. 67:1091–103. [PubMed: 26940931]

Logan RW, Sarkar DK. (2012). Circadian nature of immune function. Mol Cell Endocrinol. 349:82–90. [PubMed: 21784128]

Lowrey PL, Takahashi JS. (2011). Genetics of circadian rhythms in mammalian model organisms. Adv Genet. 74:175–230. [PubMed: 21924978]

Lu LF, Wang CP, Tsai IT, et al. (2016). Relationship between shift work and peripheral total and differential leukocyte counts in Chinese steel workers. J Occup Health. 58:81–88. [PubMed: 26549833]

Martelli TA, Waters LK, Martelli J. (1989). The police stress survey: Reliability and relation to job-satisfaction and organizational commitment. Psychol Rep. 64:267–73. [PubMed: 2928440]

Matheson A, O’Brien L, Reid JA. (2014). The impact of shiftwork on health: A literature review. J Clin Nurs. 23:3309–20. [PubMed: 24460821]

Mazzoccoli G, Sothern RB, De Cata A, et al. (2011). A timetable of 24-hour patterns for human lymphocyte subpopulations. J Biol Regul Homeost Agents. 25:387–95. [PubMed: 22023763]

Meijers JMM, Swaen GMH, Volovics A, et al. (1989). Occupational cohort studies: The influence of design characteristics on the healthy worker effect. Int J Epidemiol. 18:970–75. [PubMed: 2695476]

Merkus SL, Van Drongelen A, Holte KA, et al. (2012). The association between shift work and sick leave: A systematic review. Occup Environ Med. 69:701–12. [PubMed: 22767871]

Nader N, Chrousos GP, Kino T. (2010). Interactions of the circadian CLOCK system and the HPA axis. Trends Endocrinol Metab. 21:277–86. [PubMed: 20106676]

Nea FM, Kearney J, Livingstone MB, et al. (2015). Dietary and lifestyle habits and the associated health risks in shift workers. Nutr Res Rev. 28:143–66. [PubMed: 26650243]

Perez-Aso M, Feig JL, Aranzazu M, Cronstein BN. (2013). Adenosine A(2A) receptor and TNF-alpha regulate the circadian machinery of the human monocytic THP-1 cells. Inflammation. 36:152–62. [PubMed: 22923002]

Pritchett D, Reddy AB. (2015). Circadian clocks in the hematologic system. J Biol Rhythms. 30:374–88. [PubMed: 26163380]

Proper KI, Van De Langenberg D, Rodenburg W, et al. (2016). The relationship between shift work and metabolic risk factors: A systematic review of longitudinal studies. Am J Prev Med. 50:e147–157. [PubMed: 26810355]

Puttonen S, Viitasalo K, Harma M. (2011). Effect of shiftwork on systemic markers of inflammation. Chronobiol Int. 28:528–35. [PubMed: 21797781]

Radloff LS. (1977). The CES-D Scale: A self-report depression scale for research in the general population. Appl Psychol Meas. 1:385–401.

Scheiermann C, Kunisaki Y, Lucas D, et al. (2012). Adrenergic nerves govern circadian leukocyte recruitment to tissues. Immunity. 37:290–301. [PubMed: 22863835]

Sennels HP, Jorgensen HL, Hansen AL, et al. (2011). Diurnal variation of hematology parameters in healthy young males: The Bispebjerg study of diurnal variations. Scand J Clin Lab Invest. 71:532–41. [PubMed: 21988588]

Smith MR, Eastman CI. (2012). Shift work: Health, performance and safety problems, traditional countermeasures, and innovative management strategies to reduce circadian misalignment. Nat Sci Sleep. 4:111–32. [PubMed: 23620685]

Sookoian S, Gemma C, Fernandez Gianotti T, et al. (2007). Effects of rotating shift work on biomarkers of metabolic syndrome and inflammation. J Intern Med. 261:285–92. [PubMed: 17305651]

Straif K, Baan R, Grosse Y, et al., Group WHOIAFRoCMW. (2007). Carcinogenicity of shift-work, painting, and firefighting. Lancet Oncol. 8:1065–66. [PubMed: 19271347]

Vena JE, Violanti JM, Marshall J, Fiedler RC. (1986). Mortality of a municipal worker cohort: III. Police officers. Am J Ind Med. 10:383–97. [PubMed: 3788983]

Vila B (2006). Impact of long work hours on police officers and the communities they serve. Am J Ind Med. 49:972–80. [PubMed: 17006951]

Violanti JM, Burchfiel CM, Fekedulegn D, et al. (2009a). Cortisol patterns and brachial artery reactivity in a high stress environment. Psychiatry Res. 169:75–81. [PubMed: 19616310]

Violanti JM, Burchfiel CM, Hartley TA, et al. (2009b). Atypical work hours and metabolic syndrome among police officers. Arch Environ Occup Health. 64:194–201. [PubMed: 19864222]

Violanti JM, Burchfiel CM, Miller DB, et al. (2006). The Buffalo Cardio-Metabolic Occupational Police Stress (BCOPS) pilot study: Methods and participant characteristics. Ann Epidemiol. 16:148–56. [PubMed: 16165369]

Violanti JM, Vena JE, Petralia S. (1998). Mortality of a police cohort: 1950–1990. Am J Ind Med. 33:366–73. [PubMed: 9513643]

Vozarova B, Weyer C, Lindsay RS, et al. (2002). High white blood cell count is associated with a worsening of insulin sensitivity and predicts the development of type 2 diabetes. Diabetes. 51:455–61. [PubMed: 11812755]

Wang F, Zhang L, Zhang Y, et al. (2014). Meta-analysis on night shift work and risk of metabolic syndrome. Obes Rev Off J Int Assoc Study Obes. 15:709–20.

Wang XS, Armstrong ME, Cairns BJ, et al. (2011). Shift work and chronic disease: The epidemiological evidence. Occup Med. 61:78–89.

Wirth M, Burch J, Violanti J, et al. (2011). Shiftwork duration and the awakening cortisol response among police officers. Chronobiol Int. 28:446–57. [PubMed: 21721860]

Wirth M, Vena JE, Smith EK, et al. (2013). The epidemiology of cancer among police officers. Am J Ind Med. 56:439–53. [PubMed: 23255299]

Yu XF, Rollins D, Ruhn KA, et al. (2013). T(H)17 Cell differentiation is regulated by the circadian clock. Science. 342:727–30. [PubMed: 24202171]

Table 1.

Select population characteristics by long-term shift type.

Characteristic	All (*n* = 425)	Day (*n* = 184)	Evening + Night (*n* = 241)	*p* Value Day vs. Evening + Night
Sex				<0.01
Male	319 (75%)	111 (60%)	208 (86%)	
Female	106 (25%)	73 (40%)	33 (14%)	
Race				<0.01
European-American	326 (78%)	127 (70%)	199 (84%)	
Other	93 (22%)	55 (30%)	38 (16%)	
Education				0.38
<College	52 (12%)	25 (14%)	27 (11%)	
some college	149 (35%)	69 (38%)	80 (33%)	
Associates degree	85 (20%)	38 (21%)	47 (20%)	
Bachelors or graduate degree	139 (33%)	52 (28%)	87 (36%)	
Rank				<0.01
Police officer	279 (66%)	100 (54%)	179 (74%)	
Sergeant, Lieutenant, or Captain	64 (15%)	33 (18%)	31 (13%)	
Detective	42 (10%)	22 (12%)	20 (8%)	
Other	40 (9%)	29 (16%)	11 (5%)	
Tobacco use				<0.01
Never	210 (50%)	84 (46%)	126 (53%)	
Former	95 (23%)	56 (31%)	39 (16%)	
Current	115 (27%)	41 (23%)	74 (31%)	
Age (years)	42.7 ± 7.9	46.1 ± 7.6	40.0 ± 7.1	<0.01
Body mass index (kg/m^2)	29.4 ± 4.8	28.9 ± 5.2	29.7 ± 4.5	0.07
Fruit + Vegetable Servings per Day	3.60 ± 2.4	3.85 ± 2.5	3.37 ± 2.2	0.05
Energy Intake per day (kcal)	1848 ± 803	1743 ± 724	1920 ± 851	0.02
Fat Intake per day (g)	69.2 ± 35.5	65.4 ± 30.4	71.8 ± 38.8	0.05
Triglycerides (mg/dL)	132.9 ± 104.5	123.8 ± 108.4	140.1 ± 101.0	<0.01
Glucose (mg/dL)	93.0 ± 11.8	93.4 ± 13.1	92.8 ± 10.8	0.63
HbA1c%	5.58 ± 0.5	5.67 ± 0.5	5.50 ± 0.5	<0.01

Characteristic	All (*n* = 425)	Day (*n* = 184)	Evening + Night (*n* = 241)	*p* Value Day vs. Evening + Night
Leptin (pg/mL)	13195 ± 12082	15109 ± 14060	11671 ± 10000	0.02
Insulin (uU/mL)	8.60 ± 6.3	8.82 ± 7.0	8.41 ± 5.71	0.51
Diastolic blood pressure (mmHg)	77.9 ± 10.0	77.3 ± 10.1	78.4 ± 9.9	0.27

Column percentages may not equal 100% due to rounding. Stratum numbers may not equal column totals due to missing data. All categorical variable *p* values are based on chi-square tests and all continuous *p* values are based on *t*-tests or Wilcoxon rank sums test.

Table 2.

Immune cell absolute values and percentages by long-term shiftwork status among Buffalo, New York police officers.

Dependent Variables	Day (*n* = 184)	Evening (*n* = 141)	Night (*n* = 100)	Evening + Night (*n* = 241)	*p* Values		
					D vs. E	D vs. N	D vs. E + N
White blood cells ($\times 10^9$/L)	5.54 (5.27–5.80)	5.98 (5.65–6.31)	6.16 (5.78–6.55)	6.06 (5.77–6.34)	0.02	<0.01	<0.01
Neutrophil absolute ($\times 10^9$/L)	3.11 (2.93–3.31)	3.44 (3.20–3.69)	3.59 (3.31–3.87)	3.50 (3.28–3.72)	0.03	<0.01	<0.01
Lymphocyte absolute ($\times 10^9$/L)	1.76 (1.68–1.85)	1.82 (1.72–1.93)	1.90 (1.78–2.02)	1.86 (1.76–1.95)	0.32	0.05	0.11
Monocyte absolute ($\times 10^9$/L)	0.44 (0.42–0.46)	0.45 (0.42–0.48)	0.49 (0.45–0.52)	0.46 (0.44–0.49)	0.49	0.01	0.10
Neutrophil (%)	55.6 (54.2–57.0)	57.5 (55.7–59.3)	56.5 (54.4–58.5)	57.1 (55.5–58.7)	0.08	0.47	0.13
Lymphocyte (%)	33.0 (31.7–34.2)	31.6 (29.9–33.2)	31.9 (30.0–33.9)	31.7 (30.2–33.1)	0.22	0.40	0.22
Monocyte (%)	8.15 (7.80–8.50)	7.79 (7.34–8.24)	7.88 (7.37–8.39)	7.82 (7.43–8.22)	0.17	0.35	0.17
Eosinophil (%)	2.71 (2.43–2.98)	2.45 (2.09–2.80)	2.79 (2.39–3.19)	2.58 (2.27–2.90)	0.21	0.72	0.51
Basophil (%)	0.58 (0.54–0.63)	0.50 (0.45–0.56)	0.47 (0.40–0.53)	0.49 (0.44–0.54)	0.01	<0.01	<0.01

Values represent least-square means and 95% confidence intervals via general linear models. **Abbreviations**: D = day, E = evening, N = night. **Adjustments**: All models adjusted for sex, race, age, tobacco use, triglycerides, glycosylated hemoglobin, leptin, and amount of vegetable and fruit servings consumed per day.

Table 3.

Immune cell absolute values and percentages by short-term 7-day shiftwork status among Buffalo, New York police officers.

Dependent Variables	Day (*n* = 200)	Evening (*n* = 104)	Night (*n* = 82)	Evening + Night (*n* = 186)	*p* Values		
					D vs. E	D vs. N	D vs. E + N
White blood cells ($\times 10^9$/L)	5.58 (5.34–5.83)	5.90 (5.56–6.23)	5.99 (5.62–6.36)	5.94 (5.66–6.21)	0.10	0.05	0.03
Neutrophil absolute ($\times 10^9$/L)	3.22 (3.03–3.40)	3.20 (2.94–3.45)	3.22 (2.94–3.50)	3.20 (3.00–3.41)	0.86	0.99	0.92
Lymphocyte absolute ($\times 10^9$/L)	1.72 (1.64–1.80)	1.94 (1.82–2.05)	2.05 (1.92–2.17)	1.98 (1.89–2.08)	<0.01	<0.01	<0.01
Monocyte absolute ($\times 10^9$/L)	0.44 (0.42–0.46)	0.51 (0.48–0.54)	0.51 (0.48–0.55)	0.51 (0.48–0.54)	<0.01	<0.01	<0.01
Neutrophil (%)	56.6 (55.3–57.9)	54.3 (52.5–56.2)	52.8 (50.7–54.8)	53.6 (52.1–55.1)	0.03	<0.01	<0.01
Lymphocyte (%)	32.0 (30.7–33.2)	33.5 (31.8–35.2)	34.5 (32.6–36.4)	33.9 (32.5–35.3)	0.11	0.02	0.02
Monocyte (%)	8.16 (7.80–8.52)	9.01 (8.52–9.50)	8.60 (8.06–9.15)	8.83 (8.43–9.24)	<0.01	0.15	0.01
Eosinophil (%)	2.74 (2.48–3.02)	2.81 (2.44–3.19)	3.14 (2.73–3.55)	2.95 (2.65–3.26)	0.75	0.09	0.25
Basophil (%)	0.55 (0.51–0.60)	0.51 (0.46–0.57)	0.50 (0.44–0.57)	0.51 (0.46–0.56)	0.24	0.15	0.12

Values represent least-square means and 95% confidence intervals via general linear models. **Abbreviations**: D = day, E = evening, N = night. **Adjustments**: All models adjusted for sex, age, tobacco use, triglycerides, glycosylated hemoglobin, leptin, and amount of vegetable and fruit servings consumed per day.