



Research Article

Uncovering Novel Biomarkers of Inflammation as Potential Screening Targets of Disease Risk in Healthcare Shift Workers: A Pilot Study

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Abstract

Shift work, experienced by nearly 30% of the U.S. workforce, is hazardous to health and has become a pervasive labor practice in the healthcare sector worldwide. It increases the risk of stroke, diabetes, cancer, and cardiovascular disease. Nonetheless, specific screening targets for shift workers still need to be defined. In this study, we have begun uncovering these targets as specific low-grade systemic inflammation markers and functional endotoxin-elicited responses that may foreshadow disease risk in shift workers. One hundred four participants (normothermic and normotensive) were healthy, non-smoking, and drug- and medication-free volunteers recruited from Atlanta area hospitals and medical schools. We assessed the concentration of three proteins in plasma samples from day workers and shift workers (lipopolysaccharide-binding protein, IL-10, and TNF- α), and the relationship between these baseline biomarkers and their response to an *ex-vivo* endotoxin challenge. We show that shift work increases low-grade systemic inflammation and disrupts discrete endotoxin responses. As shift work exposure increases, the correlation between low-grade systemic inflammation markers and their endotoxin responses was disrupted; this effect was more robust for TNF- α than for IL-10. With increased shift work exposure, these events, alone or combined, represent potential systemic and functional signals that may be harnessed to develop screening tools to identify at-risk individuals.

Keywords: Low-grade systemic inflammation; Systemic endotoxemia; LPS; Shift work; *ex-vivo* endotoxin challenge; Inflammatory response.

Introduction

We live in a globalized society where goods and services must be readily available anytime. To accommodate this demand, industries in multiple sectors of the economy have expanded work schedules into the night hours of the day. The healthcare sector is not an exception to this trend and affords a unique opportunity to investigate the adverse effects of this labor practice. Shift work, mainly when it includes extended exposure to light at night, is associated with significant harmful effects on workers'

health by increasing the frequency or severity of stroke, obesity, cardiovascular disease, and cancer, among other pathologies [1-42]. The adverse consequences of shift work exposure increase as this labor practice becomes more pervasive, posing a significant challenge to public health and healthcare providers.

One characteristic shared by many pathological conditions with higher prevalence among shift workers is the development of mechanisms that disrupt the healing or resolution of inflammatory events [43-48]. For example, evidence of aberrant or disrupted inflammatory processes shows that compared to day workers (i.e., workers that have not experienced significant exposure to schedules that extend into the night hours of the day), shift workers have increased leukocyte counts (including at least, monocytes,

lymphocytes, and neutrophils) in the systemic circulation, sleep deficits, elevated blood pressure, and increased levels of distinct biomarkers indicative of chronic low-grade systemic inflammation [19, 49-53]. We recently reported that in plasma from shift workers, the levels of lipopolysaccharide-binding protein (LBP), an indicator of low-grade systemic endotoxemia, are higher than those found in samples from day workers [54]. Increased intestinal permeability and gut leakage have been suggested as potential mechanisms behind increased circulating endotoxin levels [55], but their role in shift workers remains unclear.

Similar to the effects that systemic endotoxemia has on the pathogenesis of other major chronic illnesses [56-59], we hypothesize that in shift workers, chronic exposure to low-grade systemic inflammation disrupts immune cell proliferation, functional responses, activation markers, cell-surface receptors, cytokine release and ultimately the ability of the system to mount and control an inflammatory response when faced with an actual pathogenic challenge.

Despite our knowledge of the physiological and behavioral changes characteristic of shift work exposure, the ability to prevent or predict the development of shift-work-related diseases is still minimal. Since chronic exposure to low-grade systemic inflammation would be expected to increase disease risk, tracking changes in immune reactivity before pathology ensues may provide a pathway to understanding if, when, and how disruption of inflammatory control occurs. A systematic characterization of disrupted or aberrant inflammatory responses and their relationship with low-grade systemic inflammation in otherwise healthy shift workers is lacking. This approach may help identify those immune reactivity markers that may predict the future development of shift-work-related illness.

To test this hypothesis, we investigated immune reactivity by assessing inflammatory responses elicited by an *ex-vivo* lipopolysaccharide (LPS) endotoxin stimulation assay in plasma samples. LPS endotoxin challenge is a vital tool to uncover potential mechanistic pathways disrupted by shift work exposure. LPS has been used extensively to elicit human inflammatory responses [60-64, 84-86], but it has not been used to investigate the disruption of these responses in healthcare shift workers. If the severity of low-grade systemic inflammation caused by shift work disruption can predict the response to a controlled LPS challenge, then these responses could likely be used experimentally to identify what biomarker(s) trigger(s) disruption of inflammation, making shift workers more susceptible to disease.

Materials and Methods

Study Population and Experimental Design

A portion of this study investigating broader baseline effects of shift work exposure, including inflammation, sleep deficits, and

blood pressure changes, has been published before [54]. Using the same samples from the first study, here we present the results of a pilot study in which we repeated baseline inflammatory assessments of a few non-specific markers and added an LPS assay to test for immune reactivity. One hundred four research participants between 21 and 50 years of age signed up for this pilot, cross-sectional study. Research participants were recruited and screened by self-reported intake surveys about general demographics, health status, and previous or current/continuous exposure to shift work schedules. Exclusion criteria included any medical diagnosis of bipolar disorder, anxiety, SAD (seasonal affective disorder), depression, color blindness (including wavelength, a relevant shift-work variable), or any medical diagnosis of conditions in which regulation of inflammation is critical to outcome, including asthma, allergies, hypertension, fibromyalgia, arthritis, colitis, gastritis, etcetera. Thus, eligible research participants were healthy volunteers with a BMI between 18.5 and 29.9 Kg/m², did not smoke, did not report any underlying pathology, nor use any prescription drugs (apart from birth control) or any over-the-counter medication with known interactions with inflammatory responses or sleep patterns (i.e., melatonin). These highly selective criteria intended to rule out most factors and medications that might interfere with inflammation and allowed us to assess inflammation in non-selective markers. Ultimately, this pilot study intended to uncover consistent differences in subclinical or low-grade levels of non-specific markers in healthcare shift workers that can be followed in a future, cohort longitudinal-study.

Research participants working part-time or those working longer than 12 h per shift per 24 h were excluded. Shift work was defined here as any schedule that includes at least six h of daily work scheduled between 7 pm and 7 am (nighttime exposure to light) and with no less than five night- shifts per month (regardless of shift direction) for at least one year before enrollment in the study.

Workers with no exposure to shift work in the three years before the onset of the study, no more than six months of exposure to shift work schedules in a lifetime, and no travel history across more than one time-zone during the three months before the beginning of the study were eligible as controls (day workers) and were included. The institutional review board of the Morehouse School of Medicine approved all study procedures and protocols. Written informed consent was obtained from all volunteers.

Assessment of Low-grade Systemic Inflammation (pre-LPS baseline)

We assessed the plasma concentration of three distinct proteins considered non-specific biomarkers of systemic inflammation: Lipopolysaccharide-binding protein (LBP), Interleukin 10 (IL-10), and Tumor Necrosis Factor Alpha (TNF- α). To control for time of day effects on the parameters that may

exhibit diurnal rhythmicity, the participants were asked to donate two samples of blood collected 12 h apart: one before and one after a daily work shift. Each blood sample was separated into two equal portions, one to assess baseline low-grade systemic inflammation (pre-LPS) and the other to determine inflammatory responses (post-LPS). First, one portion of each sample (500 μ L) was centrifuged at $1500 \times g$ for 10 min at room temperature to determine protein concentration in the systemic circulation. Plasma was then separated and frozen at -80°C until needed for analysis. The concentration of plasma LBP was determined by a solid-phase sandwich ELISA (R&D Systems, Minneapolis, MN, USA), while assessment of the amount of both IL-10 and TNF- α was carried out using a LuminexTM 200TM instrument system, and commercially available Milliplex Kits (Millipore, Burlington, MA, USA) according to the instructions provided by the manufacturers.

Ex-vivo endotoxin assay

The second portion of the blood sample (500 μ L) was set aside before centrifugation for LPS endotoxin assay. At this stage, the whole blood assay provides a more physiological environment to assess endotoxin-elicited responses by preserving the cellular phenotypes responsive to LPS and the cellular interactions that prevail *in-vivo*. Since we did not separate the sample by cell lineage, the LPS responses measured are not specific to only monocytes, lymphocytes, or neutrophils. The conditions and procedures of the assay have been validated previously as a valuable tool for investigating circadian control of *ex-vivo* inflammatory responses in non-shift workers [65]. Here we briefly describe the culture procedure: LPS was diluted in RPMI 1640 culture medium (Life Technologies, Waltham, MA) supplemented with 2 mM L-Glutamine and 10 mM HEPES. In the processing lab, each blood sample was kept at room temperature and then mixed with LPS from E. Coli O111:B4 (Sigma-Aldrich, St. Louis MO) for 2h at 37°C (at a final concentration of 100 $\mu\text{g}/\text{ml}$). During the incubation, the inflammatory response to LPS mounted by the leukocytes in the sample results in the release of cytokines such as IL-10 and TNF- α . After incubation, samples were centrifuged at $1500 \times g$ for 10 min. Then, plasma was collected and frozen for further quantification of protein expression by Luminex, as described above.

Data Analysis

The power analysis and sample size calculation that initially determined the design of this study was based on comparing two groups (day workers vs. shift workers). We first calculated individual daily means. To this end, each participant's two samples (one sample drawn before and one drawn after a daily shift) were analyzed separately, and then an average was calculated. This approach allowed us to control for changes in daily oscillations of each outcome measured and delivered an individual diurnal mean per each assessed biomarker. Then, the daily group mean was

calculated and is reported here \pm standard error (S.E.).

Comparisons among group means were made after a careful assessment of normality distribution. The Kolmogorov-Smirnov test was used to determine normality. Depending on the data distribution, two-tailed *t*-tests for independent samples, Chi-square test, or Wilcoxon rank-sum test assessed if changes in outcome variables (population parameters, low-grade systemic inflammation markers, and LPS-elicited responses) depended on shift work exposure.

Assessment of the significance of endotoxin-elicited responses compared to baseline (pre-LPS) levels was first determined by repeated-measures two-way ANOVA with group and stimulation status (pre-LPS vs. post-LPS) as independent factors. Bonferroni-adjusted multiple comparisons were performed where appropriate, and statistical significance was accepted as $p < 0.05$.

In addition, we investigated whether the LPS response assessed on each biomarker was associated with its initial baseline (pre-LPS) value by correlation analysis (Pearson) and multivariate linear mixed models. The multivariate regression analyses were used to assess whether baseline low-grade systemic inflammation markers and duration of shift work could predict observed LPS responses. In addition to the strict selection criteria that controlled for the influence of underlying conditions and medications on inflammation, we adjusted for all confounding factors, including BMI, gender, age, hours worked per shift, occupation, and shift work exposure.

Results

Study population

Out of the 104 research participants enrolled in the study, a complete data set that included both assessments (systemic inflammation and endotoxin responses) was acquired for 92 of them (39 day workers and 53 shift workers). The endotoxin assay response was not assessed in 12 participants (4 shift workers and 8 day workers) excluded from the analyses. Most healthcare providers (nurses, physicians, medical assistants, etc.), were recruited from Atlanta area hospitals (85%), while the rest (15%) worked in healthcare education as medical students and clinical/basic researchers. All the shift workers enrolled reported some variation of rotating schedule, alternating between working day and night shifts. However, we did not collect more specific information on the length, frequency, or direction of these rotations, as the minimum number of night shifts per month was the defining factor used for classification. Notably, we found that within the population of shift workers, 32.1% reported more than five years (i.e., long-term) of chronic or extended exposure to night shifts.

The study contributes to expanding the inclusion of minority populations into clinical research studies (out of the 92 participants

in the study, 52% self-identified as members of an ethnic/racial minority group). Additional demographics and shift work exposure information are included in Table 1.

	Day workers <i>n</i> = 39	Shift workers <i>n</i> = 53
Asian (%)	0	7.6
Black (%)	61.5	37.7
Hispanic (%)	0	0
Mixed (%)	0	0
White (%)	38.5	54.7
Gender (% females)	84	94
Occupation (% healthcare providers)	87	83
BMI (kg/m ²)	27.3 ± 0.33	26.5 ± 0.22
Age (years)*	35.48 ± 1.18	28.44 ± 0.68
Shift duration (h)*	9.4 ± 0.25	11.84 ± 0.15
Shift-work exposure (years)*	0	4.02 ± 0.69
White blood cells (K/μL)**	5.394 ± 0.21	7.056 ± 0.24
Monocytes (K/μL)**	0.449 ± 0.03	0.543 ± 0.02
Lymphocytes (K/μL)**	1.958 ± 0.08	2.474 ± 0.11
Neutrophils (K/μL)**	2.875 ± 0.16	3.841 ± 0.21
LBP (μg/mL)**	5.401 ± 0.22	6.638 ± 0.27
IL-10 (pg/mL)**	1.751 ± 0.18	3.184 ± 0.36
TNF-α (pg/mL)*	12.54 ± 0.77	15.04 ± 0.75

Table 1: Characteristics of the study population and baseline inflammatory markers Except where indicated, values are expressed as means ± standard error. Significant difference ($p < 0.05^*$, or $p < 0.01^{**}$) between shift workers and day workers assessed by independent t-test, Wilcoxon rank-sum test, or Chi-square test.

Shift work increases systemic inflammation, including low-grade systemic endotoxemia

Our results confirmed that low-grade systemic inflammation is a hallmark of exposure to shift work schedules, as reported in the scientific literature [52-53, 66-69]. Table 1 summarizes the results. We found that compared to day workers, shift workers had mean counts of white blood cells that were higher by 31%, monocyte counts higher by 21%, lymphocytes higher by 26%, and neutrophils, which were higher by 34%. The concentration of LBP, an indicator of systemic endotoxemia [70-72], was higher in shift workers by 23%. Both anti-inflammatory and pro-inflammatory signaling, as indicated by the concentration of IL-10 and TNF-α, was also significantly elevated in shift workers by 81% and 20%, respectively. When comparing occupation, we found that these inflammatory effects of shift work were not different between healthcare provider and healthcare education jobs ($p > 0.05$).

LPS-elicited inflammatory responses are disrupted by the history of shift work exposure

For this assessment, we reasoned that the significantly elevated levels of LBP in shift workers should increase the transfer of LPS to monocytes by increasing LBP-sCD14 binding, resulting in disrupted immune reactivity as potentiation of cytokine release upon LPS stimulus. The results of the LPS challenge are presented in Figure 1. Surprisingly, the LPS-elicited responses of both IL-10 and TNF-α were not significantly different between day workers and shift workers. These solid but similar LPS responses in both groups, despite a significantly increased initial baseline in shift workers, prompted us to look deeper into individual baseline to LPS-response relationships. We plotted individual LPS responses (post-LPS) against baseline (pre-LPS) cytokine levels. We discovered a distribution pattern that suggested an effect of differential shift work exposure (i.e., short-term vs. long-term) on the LPS-elicited cytokine responses.

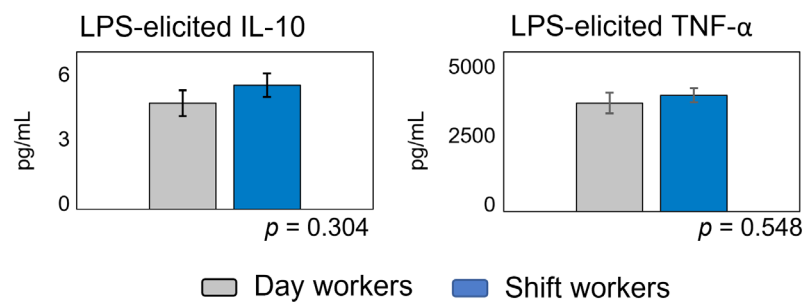


Figure 1: IL-10 and TNF- α , LPS-elicited inflammatory responses in day workers ($n = 39$) and shift workers ($n = 53$). The student's t -test for independent samples compared group means and calculated p values indicated at the bottom right of each panel.

To analyze the role of shift work exposure on the relationship between pre-LPS and post-LPS concentration of IL-10 and TNF- α , we calculated Pearson correlation coefficients (r) followed by multivariate linear regression models and quantified both the strength and the direction of the relationship between these two variables. The results of this exercise are depicted in Figure 2.

In controls (day workers), the baseline (pre-LPS) concentration of both IL-10 and TNF- α predicts the corresponding LPS-elicited response. Pearson correlation coefficient (r) was 0.722, $R^2 = 0.5206$ for IL-10, and 0.679, $R^2 = 0.461$ for TNF- α ,

confirming a positive correlation. A regression equation was calculated, and the corresponding regression lines are depicted on the plots (see Figure 2).

In contrast, we found that this linear relationship is disrupted in shift workers and changes depending on the length of exposure to shift work schedules (see multivariate linear regression analysis below). To differentiate between shift workers with less than five (i.e., short-term) and those with five or more (i.e., long-term) years of exposure, we color-coded the data points and ran the analysis independently for each exposure duration (see Figure 2).

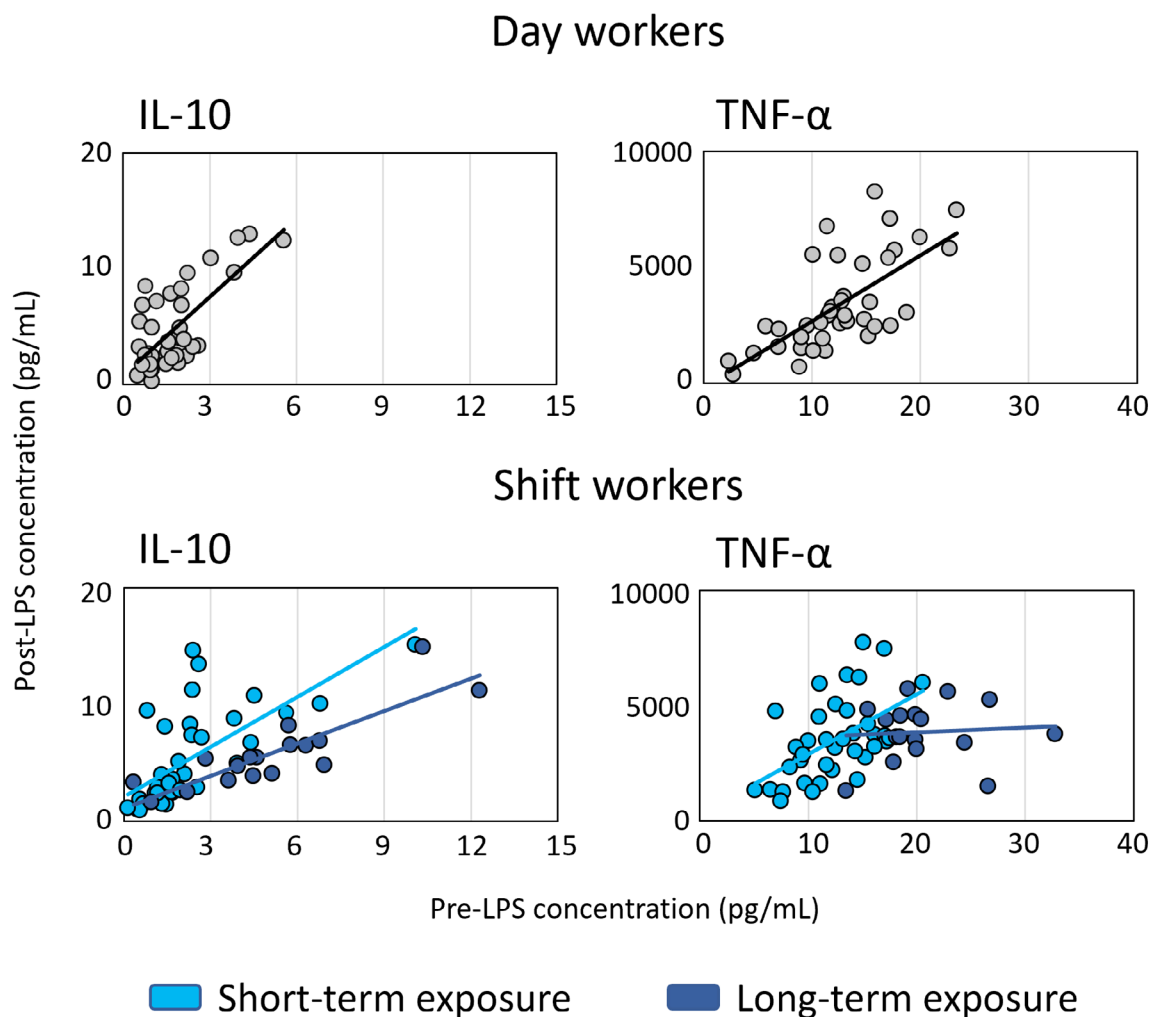


Figure 2: Association between baseline low-grade systemic inflammation (pre-LPS) and inflammatory responses (post-LPS) in day workers (top) and shift workers (bottom) with short-term (≤ 5 years) or long-term (>5 years) exposure to shift work schedules. Regression lines are depicted on each plot. Pearson correlation coefficients (r) and coefficient of determination (R^2) are indicated in the text.

We found that low-grade systemic inflammation (pre-LPS) is generally higher in shift workers with more years of exposure (dark-blue dots). Of the 53 enrolled shift workers in our study, 17 (32.1%) reported an exposure of more than five years to shift work schedules. After adjusting for age, the pre-LPS concentration of TNF- α and IL-10 is higher in most shift workers with long-term exposure than the group mean. The percentage of participants with long-term exposure to shift work that showed pre-LPS concentration levels higher than the group average was as follows: for TNF- α (94 %; mean baseline = 15.04 pg/mL) for IL-10 (76.5 %; mean baseline = 3.19 pg/mL), both $p < 0.05$. This observation indicates that most shift workers in our study with long-term exposure to

shift work schedules had the highest baseline (pre-LPS) levels of both cytokines assessed. Indeed, multivariate regression analysis showed that exposure duration predicts baseline TNF- α ($\beta = 0.017$, $p = 0.02$, $R^2 = 0.15$) and IL-10 ($\beta = 1.96$, $p = 0.03$, $R^2 = 0.46$). No other outcomes at baseline were predicted by shift work duration.

However, a higher baseline with extended exposure did not lead to higher LPS response; instead, it mainly was shift workers with shorter exposure (light-blue dots) in whom we found the most increased LPS responses (see Figure 2). In shift workers with short-term exposure, the Pearson correlation coefficient (r) was 0.67954, $R^2 = 0.4618$ for IL-10, and 0.2865, $R^2 = 0.461$ for TNF- α . In shift workers with long-term exposure, the Pearson correlation

coefficient (r) was 0.86065, $R^2 = 0.7407$ for IL-10, and 0.07809, $R^2 = 0.0061$ for TNF- α . This data indicates that the relationship between low-grade systemic inflammation and LPS responses may weaken as shift work exposure increases. This disruptive effect was more robust for TNF- α than for IL-10.

Multivariate linear regression analysis that accounted for all confounders confirmed that baseline TNF- α predicted its LPS response in workers with ≤ 5 years of shift work ($\beta = 1.66$, $p = 0.04$, $R^2 = 0.3$) but not in those with a more extended history of exposure ($\beta = 12.4$, $p = 0.3$, $R^2 = 0.07$). IL-10 responses (anti-inflammatory protein with early but slower and more sustained response to LPS [76-78] than TNF- α) showed similar regression trends for both exposure groups (short-term exposure, $\beta = 0.011$, $p = 0.007$, $R^2 = 0.39$; long-term exposure, $\beta = 1.68$, $p = 0.002$, $R^2 = 0.18$).

Leukocyte counts and LBP concentration were also assessed as potential predictors of LPS responses. We conducted a multivariate regression analysis, and after adjusting for age, LBP concentration, shift work exposure duration, and occupation, only monocyte counts in day workers were found to be significant predictors of LPS-elicited outcomes. Thus, we found significant regression equations indicating that in Controls, the LPS responses of IL-10 and TNF- α were predicted by monocyte counts (IL-10: $\beta = 0.01$, $p = 0.008$, $R^2 = 0.3$; TNF- α : $\beta = 5.46$, $p = 0.006$, $R^2 = 0.46$). By contrast, in shift workers, LPS responses of either protein were never predicted by monocyte numbers or any other leukocyte phenotype despite significantly increased cell counts. When run as an independent variable in the regression models, LBP baseline concentrations did not predict any LPS outcomes.

Discussion

Low-grade systemic inflammation is a hallmark of shift work exposure

In this study, the average exposure to shift work schedules reported by the research participants was less than five years. Even this seemingly short-term exposure to schedules that regularly require working during the nighttime exacerbates multiple biomarkers of low-grade systemic inflammation, including leukocyte counts, cytokine release, and LPS amount (as indicated by LBP levels) in the systemic circulation (Table 1).

Increased systemic inflammation has been associated with exposure to shift work schedules before [52-53, 67-69]. Given the increase in monocytes, lymphocytes, neutrophils, and LBP reported here and in previous studies [49-54], an increase in cytokine release was expected. If inflammation cannot be resolved in shift workers, then it is not surprising that the risk of illness will increase.

Extended exposure to shift work uncovers disrupted inflammatory responses

Using *ex-vivo* LPS stimulation, in this study, we sought to uncover whether even subclinical but significant changes in the severity of low-grade systemic inflammation can predict the emergence of disrupted immune reactivity. Previous studies have reported an increased risk of pathology with extended exposure to shift work schedules, including high blood pressure and metabolic syndrome [73-75].

The results of our assessments show that individual shift workers had the highest concentration of LPS-elicited cytokines compared to the concentration recorded in controls. However, the overall result of our stimulation assay suggested that, as a group, shift workers had a lessened or weakened potentiation of cytokine release than expected. Since over 32% of enrolled shift workers reported long-term exposure to shift work schedules, we reasoned that exposure duration should be considered in a careful and more granular approach to our initial observations.

Thus, we analyzed inflammatory responses to the LPS-endotoxin challenge (Figures 1 and 2) and uncovered that these responses changed with chronic or extended exposure to shift work schedules. Specifically, the moderate correlation between pre-LPS TNF- α release and its post-LPS concentration observed in both day workers and shift workers with short-term exposure was no longer present after long-term exposure to shift work schedules. In contrast, IL-10 cytokine release (pre- vs. post-LPS) maintained similar correlations regardless of exposure duration to shift work. This disrupted correlation reflects changes in immune reactivity that can be assessed early in shift work exposure. We can only speculate that the relationship between pro- and anti-inflammatory signaling is a target of shift work exposure.

In addition, the mismatch between endotoxin responses of different cytokines (i.e., TNF- α and IL-10) also suggests that shift work exposure may disrupt specific cytokine systems. This idea is not new, as aberrant expression of discrete cytokines may lead to pathology. For example, in stressed individuals, isolated peripheral blood cells show high production of IL-6 both at baseline and in response to immune challenges [91-92]. We must acknowledge that other inflammatory markers that determine immune reactivity may also play a role and were not included in this study but will be assessed in a future, population-based longitudinal study.

IL-10 and TNF- α are paramount in the pathogenesis of systemic endotoxemia. TNF- α increases inflammation and tissue damage, typically counterbalanced through robust anti-inflammatory action by IL-10. However, under pathological conditions, downregulation of inflammatory cytokines like TNF- α

and upregulation of anti-inflammatory mediators such as IL-10 is known to disrupt the regulation of inflammatory events [93]. A time course of the LPS challenge with earlier and later data points could capture the interaction among activation and deactivation dynamics of these and additional cytokine systems and how these are affected by extended shift work.

In addition, a longitudinal study that follows shift workers as they experience chronic exposure could shed light on whether the length of exposure is the only factor (unlikely) determining if and when inflammatory responses, particularly pro- and anti-inflammatory dynamics, begin to show aberrant correlations.

Besides the effect of shift work on baseline cytokine release, one of the most striking differences between controls and shift workers was the increase in leukocyte counts. This increase did not exacerbate LPS responses in this study. Conditions of chronic inflammation (i.e., aging, obesity, diabetes, etcetera) can result in LPS responses that are lessened [87-88] or heightened [89-90]. One possible explanation for this apparent paradox is based on the differential effect an initial exposure to low-dose LPS can have on the response of monocytes to a future LPS encounter. Indeed, it has been suggested that the degree of LPS exposure can determine a switch between LPS tolerance and priming [55-57, 97].

Our analysis indicated that only monocyte counts were significant predictors of LPS-elicited outcomes but only in day workers and not shift workers. The contribution of other cells in the sample (i.e., lymphocytes, neutrophils) to the LPS response measured should not be overlooked and deserves further investigation. However, the conditions of low-grade systemic endotoxemia (elevated LBP), and elevated white blood cell counts, including monocytes, did not result in a predictable increase in LPS response in shift workers. This suggests that immune reactivity is disrupted by shift work. Exposure of LPS-responsive leukocytes to potential systemic signals before our LPS challenge may alter their activation state; one potential factor to consider is the effect of chronic LBP on monocytes.

Low-grade systemic inflammation is well documented in experimental models of obesity, diabetes, and cardiovascular disease. These pathologies induce the reprogramming of innate immune cell responses [56-58, 94]. Circulating monocytes constantly survey the surrounding environment and express surface molecules that reflect a characteristic activation state [95]. Changes in the monocyte population's proportion and activation state are characteristic of sepsis, rheumatoid arthritis, asthma, coronary heart disease, and other inflammatory conditions [96]. Thus, further research in which the effect of shift work exposure on the reprogramming of LPS-responsive cells (tolerance or priming) is investigated is warranted.

Under the chronic state of activation and low-grade systemic inflammation, we report in this study, a perpetual immune challenge

is unresolved. This state of high alert is not unique, but is prevalent in the healthcare sector. For the most part, the adaptations that a system in these conditions of high alert develops (high blood pressure, reduced sleep, circadian disruption, increased leukocyte counts, and cytokine release) can keep the individual symptom-free and seemingly healthy. However, there is clear evidence on how even short-term (i.e., acute) disruption of behavioral patterns that alter inflammation can have dramatic and perhaps long-lasting effects on regulating signaling pathways that affect leukocyte RNA expression and function, ultimately influencing the development of cardiometabolic diseases [99]. Thus, if even acute disruption can have long-lasting effects, given enough time, the chronic state of activation of the immune system will eventually result in pathological responses. Others have demonstrated the dramatic effects of cumulative shift work exposure on subclinical biomarkers. For example, compared to day workers, shift workers with extended exposure (over 660 night shifts) show a 4% flow velocity increase (arterial stiffness) that contributes to the future development of atherosclerosis [100]. As we show in this report, the biological significance of subclinical inflammatory factors can be uncovered by our *ex-vivo*, whole-blood LPS assay, providing a novel pathway to investigate future and long-term effects of relevant shift work factors pervasive in the healthcare sector, such as extended exposure, psychosocial stress, sleep deprivation, and circadian disruption on the development of disrupted inflammation.

Strengths and Limitations

A major strength of this study is the significant number of participants from racial/ethnic minorities. Most of our study population (52%) self-identified as a racial/ethnic minority. In these populations, chronic activation of stress responses associated with systemic racism, historic socio-economical discrimination, and a higher prevalence and incidence of chronic diseases already increase low-grade systemic inflammation [79-82]. Because shift work is more prevalent among these ethnic/racial groups [83], scientific reports that include large minority populations may better reflect the adverse health effects of this occupational hazard. However, due to the diverse racial/ethnic origin of these cohorts, it is also possible that some differences in the degree of inflammation based on ethnicity could be explained by genetic factors that alter host-immune responsiveness among human populations of different historical and geographic origins [98].

The techniques we used to assess inflammation and LPS responses have been validated previously [65], and our recruiting practices based on long-standing inclusion/exclusion criteria provide strict control of underlying conditions and medications affecting inflammatory markers. These are all strengths of the current study.

In this study, shift work exposure is based on self-reported data; thus, misclassification of exposure may be a limitation to

consider. The overrepresentation of female healthcare workers is also a limitation since premenopausal women might have different inflammatory markers levels than men and post-menopausal women. It is worth noting that compared to the general population, healthcare shift workers are typically better educated and have better than average diet and health habits, and yet, the negative effects of this occupational exposure are pervasive.

The results of our pilot study suggest an effect of long-term exposure to shift work schedules on endotoxin-elicited responses. However, these interpretations must be taken cautiously, as only a third of shift workers fall into this category. A more comprehensive assessment that preserves the racial/ethnic diversity of this study but expands on the representation of short-term and long-term shift workers in the healthcare sector and beyond is necessary.

Conclusions

This study contributes to understanding the association between shift work exposure and increased disease risk in healthcare workers. We have identified potential screening targets (specific low-grade systemic inflammation markers and functional LPS responses) that may foreshadow disease risk in shift workers. We show that shift work increases low-grade systemic inflammation and disrupts discrete endotoxin responses. Multiple factors, including shift work exposure duration, may modulate this effect. As shift work exposure increases, the strength of the correlation between low-grade systemic inflammation markers and their LPS responses is weakened. This effect was more robust for TNF- α than IL-10. As the impact of shift work disruption becomes chronic with increased exposure, these variables alone or in combination with other risk factors are expected to worsen low-grade systemic inflammation, including systemic endotoxemia and disrupted cell reactivity to activation. These effects will ultimately increase susceptibility to illness. Future cohort, longitudinal studies must test these hypotheses by implementing the screening tools developed here for workplace intervention and early (preventive) identification of at-risk individuals.

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Ethical guidelines

The study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the Institutional Review Board of the Morehouse School of Medicine [protocol

code 1141192]. Informed consent was obtained from all subjects involved in the study.

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Schernhammer ES, Laden F, Speizer FE, Willett WC, Hunter DJ, et al. (2003) Night-shift work and risk of colorectal cancer in the nurses' health study. *J Natl Cancer Inst* 95: 825-828.
2. Schernhammer ES, Kroenke CH, Laden F, Hankinson SE (2006) Night work and risk of breast cancer. *Epidemiology* 17:108-11.
3. Kamdar BB, Tergas AI, Mateen FJ, Bhayani NH, Oh J (2013) Night-shift work and risk of breast cancer: a systematic review and meta-analysis. *Breast Cancer Res Treat* 138: 291-301.
4. Lahti TA, Partonen T, Kyrrönen P, Kauppinen T, Pukkala E (2008) Nighttime work predisposes to non-Hodgkin lymphoma. *Int J Cancer* 123: 2148-51.
5. Conlon M, Lightfoot N, Kreiger N (2007) Rotating shift work and risk of prostate cancer. *Epidemiology* 18: 182-183.
6. Kubo T, Ozasa K, Mikami K, Wakai K, Fujino Y, et al. (2006) Prospective cohort study of the risk of prostate cancer among rotating-shift workers: findings from the Japan collaborative cohort study. *Am J Epidemiol* 164: 549-555.
7. Segawa K, Nakazawa S, Tsukamoto Y, Kurita Y, Goto H, et al. (1987) Peptic ulcer is prevalent among shift workers. *Dig Dis Sci* 32: 449-453.
8. Drake CL, Roehrs T, Richardson G, Walsh JK, Roth T (2004) Shift work sleep disorder: prevalence and consequences beyond that of symptomatic day workers. *Sleep* 27: 1453-1462.
9. Karlsson B, Knutsson A, Lindahl B (2001) Is there an association between shift work and having a metabolic syndrome? Results from a population based study of 27,485 people. *Occup Environ Med* 58: 747-752.
10. Karlsson B, Alfredsson L, Knutsson A, Andersson E, Torén K (2005) Total mortality and cause-specific mortality of Swedish shift- and dayworkers in the pulp and paper industry in 1952-2001. *Scand J Work Environ Health* 31: 30-35.
11. Morikawa Y, Nakagawa H, Miura K, Soyama Y, Ishizaki M, et al. (2005) Shift work and the risk of diabetes mellitus among Japanese male factory workers. *Scand J Work Environ Health* 31: 179-183.
12. Tenkanen L, Sjöblom T, Härmä M (1998) Joint effect of shift work and adverse life-style factors on the risk of coronary heart disease. *Scand J Work Environ Health* 24: 351-357.

13. Haupt CM, Alte D, Dörr M, Robinson DM, Felix SB, et al. (2008) The relation of exposure to shift work with atherosclerosis and myocardial infarction in a general population. *Atherosclerosis* 201: 205-211.
14. Tüchsen F, Hannerz H, Burr H (2006) A 12 year prospective study of circulatory disease among Danish shift workers. *Occup Environ Med* 63: 451-455.
15. Magrini A, Pietroiusti A, Coppeta L, Babbucci A, Barnaba E, et al. (2006) Shift work and autoimmune thyroid disorder. *Int J Immunopathol Pharmacol* 19: 31-36.
16. Hedström AK, Åkerstedt T, Hillert J, Olsson T, Alfredsson L (2011) Shift work at young age is associated with increased risk for multiple sclerosis. *Ann Neurol* 70: 733-741.
17. Loeff B, van Baarle D, van der Beek AJ, Sanders EAM, Bruijning-Verhagen P, et al. (2019) Shift Work and Respiratory Infections in Healthcare Workers. *Am J Epidemiol* 188: 509-517.
18. Mohren DC, Jansen NW, Kant IJ, Galama J, van den Brandt PA, et al. (2002) Prevalence of common infections among employees in different work schedules. *J Occup Environ Med* 44: 1003-1011.
19. Vijayalaxmi M, George A, Nambiar NA (2014) Study of General Health Pattern among Night Shift Work Employees in a Tertiary Care. *JAIR* 3: 176-183.
20. Wei F, Chen W, Lin X (2022) Night-shift work, breast cancer incidence, and all-cause mortality: an updated meta-analysis of prospective cohort studies. *Sleep Breath* 26: 1509-1526.
21. Gehlert S, Clanton M (2020) On Behalf Of The Shift Work And Breast Cancer Strategic Advisory Group. Shift Work and Breast Cancer. *Int J Environ Res Public Health* 17: 9544.
22. Wegrzyn LR, Tamimi RM, Rosner BA, Brown SB, Stevens RG, et al. (2017) Rotating Night-Shift Work and the Risk of Breast Cancer in the Nurses' Health Studies. *Am J Epidemiol* 186: 532-540.
23. Manouchehri E, Taghipour A, Ghavami V, Ebadi A, Homaei F, et al. (2021) Night-shift work duration and breast cancer risk: an updated systematic review and meta-analysis. *BMC women's health* 21: 89.
24. Pahwa M, Labrèche F, Kim J, Harris MA, Song C, et al. (2019) The impact of night shift work on breast cancer: Results from the Burden of Occupational Cancer in Canada Study. *American journal of industrial medicine* 62: 635-642.
25. Fagundo-Rivera J, Gómez-Salgado J, García-Iglesias JJ, Gómez-Salgado C, Camacho-Martín S, Ruiz-Frutos C (2020) Relationship between Night Shifts and Risk of Breast Cancer among Nurses: A Systematic Review. *Medicina (Kaunas)* 56: 680.
26. Arafa A, Eshak ES, Iso H, Muraki I, Tamakoshi A (2021) Night Work, Rotating Shift Work, and the Risk of Cancer in Japanese Men and Women: The JACC Study. *J Epidemiol* 31: 585-592.
27. Nam MW, Lee Y, Mun E, Lee W (2023) The association between shift work and the incidence of reflux esophagitis in Korea: a cohort study. *Sci Rep* 13: 2536.
28. Barber LE, VoPham T, White LF, Roy HK, Palmer JR, et al. (2022) Circadian disruption and colorectal cancer incidence in Black women. *Cancer Epidemiol Biomarkers Prev* EPI-22-0808.
29. Mun E, Kim D, Lee Y, Lee W, Park S (2021) Association between Shift Work and Reflux Esophagitis: The Kangbuk Samsung Health Study. *Int J Environ Res Public Health* 18: 6189.
30. Chang WP, Peng YX (2021) Differences between fixed day shift workers and rotating shift workers in gastrointestinal problems: a systematic review and meta-analysis. *Ind Health* 59: 66-77.
31. Zhang Q, Chair SY, Lo SHS, Chau JP, Schwade M, et al. (2020) Association between shift work and obesity among nurses: A systematic review and meta-analysis. *Int J Nurs Stud* 112: 103757.
32. Sun M, Feng W, Wang F, Li P, Li Z, et al. (2018) Meta-analysis on shift work and risks of specific obesity types. *Obes Rev* 19: 28-40.
33. Liu Q, Shi J, Duan P, Liu B, Li T, et al. (2018) Is shift work associated with a higher risk of overweight or obesity? A systematic review of observational studies with meta-analysis. *Int J Epidemiol* 47: 1956-1971.
34. Ulacia CS, Silva-Costa A, Rotenberg L, Griep RH (2021) Doses of exposure to night shift work and nutritional status among nursing workers. *Rev Bras Med Trab* 19: 419-425.
35. Myers S, Govindarajulu U, Joseph MA, Landsbergis P (2021) Work Characteristics, Body Mass Index, and Risk of Obesity: The National Quality of Work Life Survey. *Ann Work Expo Health* 65: 291-306.
36. Skogstad M, Aass HCD, Sirnes PA, Mamen A, Skare Ø, et al. (2023) Influence of Shift Work on Arterial Stiffness and Systemic Inflammation: A 3-Year Follow-up Study in Industry. *J Occup Environ Med* 65: 284-291.
37. Matre D, Christensen JO, Mork PJ, Ferreira P, Sand T, et al. (2021) Shift work, inflammation and musculoskeletal pain-The HUNT Study. *Occup Med* 71: 422-427.
38. Kwak HS, Park HO, Kim YO, Son JS, Kim CW, et al. (2019) The effect of shift work on high sensitivity C-reactive protein level among female workers. *Ann Occup Environ Med* 31: e5.
39. Skogstad M, Mamen A, Lunde LK, Ulvestad B, Matre D, et al. (2019) Shift Work Including Night Work and Long Working Hours in Industrial Plants Increases the Risk of Atherosclerosis. *Int J Environ Res Public Health* 16: 521.
40. Wang N, Sun Y, Zhang H, Wang B, Chen C, et al. (2021) Long-term night shift work is associated with the risk of atrial fibrillation and coronary heart disease. *Eur Heart J* 42: 4180-4188.
41. Chen Y, Yang L, Liang YY, He Z, Ai QH, et al. (2022) Interaction of night shift work with polymorphism in melatonin receptor 1B gene on incident stroke. *Scand J Work Environ Health* 48: 372-379.
42. Hermansson J, Hallqvist J, Karlsson B, Knutsson A, Gillander Gadin K (2018) Shift work, parental cardiovascular disease and myocardial infarction in males. *Occup Med (Lond)* 68: 120-125.
43. Ryan S, Taylor CT, McNicholas WT (2009) Systemic inflammation: a key factor in the pathogenesis of cardiovascular complications in obstructive sleep apnea syndrome? *Postgrad Med J* 85: 693-698.
44. Bojarski C (2009) Malignant transformation in inflammatory bowel disease: prevention, surveillance and treatment □ new techniques in endoscopy. *Dig Dis* 27: 571-575.
45. Goldberg RB (2009) Cytokine and cytokine-like inflammation markers, endothelial dysfunction, and imbalanced coagulation in development of diabetes and its complications. *J Clin Endocrinol Metab* 94: 3171-3182.
46. Khanna D, Khanna S, Khanna P, Kahar P, Patel BM (2022) Obesity: A Chronic Low-Grade Inflammation and Its Markers. *Cureus* 14: e22711.

47. Tsalamandris S, Antonopoulos AS, Oikonomou E, Papamikroulis GA, Vogiatzi G, et al. (2019) The Role of Inflammation in Diabetes: Current Concepts and Future Perspectives. *Eur Cardiol* 14: 50-59.
48. Alfaddagh A, Martin SS, Leucker TM, Michos ED, Blaha MJ, et al. (2020) Inflammation and cardiovascular disease: From mechanisms to therapeutics. *Am J Prev Cardiol* 4:100130.
49. Loefer B, Nanlohy NM, Jacobi RHJ, van de Ven C, Mariman R, et al. (2019) Immunological effects of shift work in healthcare workers. *Sci Rep* 9: 18220.
50. Lu LF, Wang CP, Tsai IT, Hung WC, Yu TH, et al. (2016) Relationship between shift work and peripheral total and differential leukocyte counts in Chinese steel workers. *J Occup Health* 58: 81-8.
51. Wirth MD, Andrew ME, Burchfiel CM, Burch JB, Fekedulegn D, et al. (2017) Association of shift-work and immune cells among police officers from the Buffalo Cardio-Metabolic Occupational Police Stress study. *Chronobiol Int* 34: 721-731.
52. Woo SJ, Chae CH, Lim JW (2022) Association between shift work and inflammatory markers in workers at an electronics manufacturing company. *Ann Occup Environ Med* 34: e35.
53. Streng AA, Loefer B, Dollé MET, van der Horst GTJ, Chaves I, et al. (2022) Night shift work characteristics are associated with several elevated metabolic risk factors and immune cell counts in a cross-sectional study. *Sci Rep* 12: 2022.
54. Atwater AQ, Immergluck LC, Davidson AJ, Castanon-Cervantes O (2021) Shift Work Predicts Increases in Lipopolysaccharide-Binding Protein, Interleukin-10, and Leukocyte Counts in a Cross-Sectional Study of Healthy Volunteers Carrying Low-Grade Systemic Inflammation. *Int J Environ Res Public Health* 18: 13158.
55. de Punder K, Pruimboom L (2015) Stress induces endotoxemia and low-grade inflammation by increasing barrier permeability. *Front Immunol* 6: 223.
56. Morris M, Li L (2012) Molecular mechanisms and pathological consequences of endotoxin tolerance and priming. *Arch Immunol Ther Exp (Warsz)* 60: 13-18.
57. Morris MC, Gilliam EA, Li L (2015) Innate immune programming by endotoxin and its pathological consequences. *Front Immunol* 5: 680.
58. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, et al. (2007) Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 56: 1761-1772.
59. Brown GC (2019) The endotoxin hypothesis of neurodegeneration. *J Neuroinflammation* 16:180.
60. Damsgaard CT, Lauritzen L, Calder PC, Kjaer TM, Frøkiaer H (2009) Whole-blood culture is a valid low-cost method to measure monocyte cytokines - a comparison of cytokine production in cultures of human whole-blood, mononuclear cells and monocytes. *J Immunol Methods* 340: 95-101.
61. Chen J, Bruns AH, Donnelly HK, Wunderink RG (2010) Comparative *in vitro* stimulation with lipopolysaccharide to study TNF- α gene expression in fresh whole blood, fresh and frozen peripheral blood mononuclear cells. *J Immunol Methods* 357: 33-37.
62. van der Poll T, Coyle SM, Moldawer LL, Lowry SF (1996) Changes in endotoxin-induced cytokine production by whole blood after *in vivo* exposure of normal humans to endotoxin. *J Infect Dis* 174:1356-1360.
63. Cuesta M, Boudreau P, Dubeau-Laramée G, Cermakian N, Boivin DB (2016) Simulated Night Shift Disrupts Circadian Rhythms of Immune Functions in Humans. *J Immunol* 196: 2466-2475.
64. Brooks D, Barr LC, Wiscombe S, McAuley DF, Simpson AJ, et al. (2020) Human lipopolysaccharide models provide mechanistic and therapeutic insights into systemic and pulmonary inflammation. *Eur Respir J* 56: 1901298.
65. Rahman SA, Castanon-Cervantes O, Scheer FA, Shea SA, Czeisler CA, et al. (2015) Endogenous circadian regulation of pro-inflammatory cytokines and chemokines in the presence of bacterial lipopolysaccharide in humans. *Brain Behav Immun* 47: 4-13.
66. Faraut B, Boudjeltia KZ, Vanhamme L, Kerkhofs M (2012) Immune, inflammatory and cardiovascular consequences of sleep restriction and recovery. *Sleep Med Rev* 16: 137-149.
67. Puttonen S, Viitasalo K, Härmä M (2011) Effect of shiftwork on systemic markers of inflammation. *Chronobiol Int* 28: 528-535.
68. Khosro S, Alireza S, Omid A, Forough S (2011) Night work and inflammatory markers. *Indian J Occup Environ Med* 15: 38-41.
69. Kim SW, Jang EC, Kwon SC, Han W, Kang MS, 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.
70. Lim PS, Chang YK, Wu TK (2019) Serum Lipopolysaccharide-Binding Protein is Associated with Chronic Inflammation and Metabolic Syndrome in Hemodialysis Patients. *Blood Purif* 47: 28-36.
71. Lakatos PL, Kiss LS, Palatka K, Altorjay I, Antal-Szalmás P, et al. (2011) Serum lipopolysaccharide-binding protein and soluble CD14 are markers of disease activity in patients with Crohn's disease. *Inflamm Bowel Dis* 17: 767-777.
72. Gonzalez-Quintela A, Alonso M, Campos J, Vizcaino L, Loidi L, et al. (2013) Determinants of serum concentrations of lipopolysaccharide-binding protein (LBP) in the adult population: the role of obesity. *PLoS One* 8: e54600.
73. Lin YC, Hsiao TJ, Chen PC (2009) Persistent rotating shift-work exposure accelerates development of metabolic syndrome among middle-aged female employees: a five-year follow-up. *Chronobiol Int* 26: 740-755.
74. Cheng WJ, Liu CS, Hu KC, Cheng YF, Karhula K, et al. (2021) Night shift work and the risk of metabolic syndrome: Findings from an 8-year hospital cohort. *PLoS One* 16: e0261349.
75. Sooriyaarachchi P, Jayawardena R, Pavey T, King NA (2022) Shift work and the risk for metabolic syndrome among healthcare workers: A systematic review and meta-analysis. *Obes Rev* 23: e13489.
76. de Waal Malefyt R, Abrams J, Bennett B, Figdor CG, de Vries JE (1991) Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J Exp Med* 174: 1209-1220.
77. Lonnemann G, Endres S, Van der Meer JW, Cannon JG, Koch KM, et al. (1989) Differences in the synthesis and kinetics of release of interleukin 1 α , interleukin 1 β and tumor necrosis factor from human mononuclear cells. *Eur J Immunol* 19: 1531-1536.
78. Wang Y, Yu P, Li Y, Zhao Z, Wu X, et al. (2021) Early-Released Interleukin-10 Significantly Inhibits Lipopolysaccharide-Elicited Neuroinflammation *In Vitro*. *Cells* 10: 2173.

79. Zahodne LB, Kraal AZ, Zaheed A, Farris P, Sol K (2019) Longitudinal effects of race, ethnicity, and psychosocial disadvantage on systemic inflammation. *SSM Popul. Health* 7: 100391.
80. Stepanikova I, Bateman LB, Oates GR (2017) Systemic Inflammation in Midlife: Race, Socioeconomic Status, and Perceived Discrimination. *Am J Prev Med* 52: S63-S76.
81. Stepanikova I, Oates GR, Bateman LB (2017) Does one size fit all? The role of body mass index and waist circumference in systemic inflammation in midlife by race and gender. *Ethn. Health* 22: 169-183.
82. Özdemir BC, Dotto GP (2017) Racial Differences in Cancer Susceptibility and Survival: More Than the Color of the Skin? *Trends Cancer* 3: 181-197.
83. World Health Organization IARC (2020) Night shift work. In *IARC Monographs on the Identification of Carcinogenic Hazards to Humans*; IARC: Lyon, France 124: 1-371.
84. Wolf J, Weinberger B, Arnold CR, Maier AB, Westendorp RG, et al. (2012) The effect of chronological age on the inflammatory response of human fibroblasts. *Exp Gerontol* 47: 749-753.
85. Bettiol J, Bartsch P, Louis R, De Groote D, Gevaerts Y, et al. (2000) Cytokine production from peripheral whole blood in atopic and nonatopic asthmatics: relationship with blood and sputum eosinophilia and serum IgE levels. *Allergy* 55: 1134-1141.
86. Tawfik DM, Lankelma JM, Vachot L, Cerrato E, Pachot A, et al. (2020) Comparison of host immune responses to LPS in human using an immune profiling panel, in vivo endotoxemia versus *ex vivo* stimulation. *Sci Rep* 10: 9918.
87. Wutzler S, Maier M, Lehnert M, Henrich D, Walcher F, et al. (2009) Suppression and recovery of LPS-stimulated monocyte activity after trauma is correlated with increasing injury severity: a prospective clinical study. *J Trauma* 66: 1273-1280.
88. Fei F, Lee KM, McCarry BE, Bowdish DM (2016) Age-associated metabolic dysregulation in bone marrow-derived macrophages stimulated with lipopolysaccharide. *Sci Rep* 6: 22637.
89. Huggard D, McGrane F, Lagan N, Roche E, Balfe J, et al. (2018) Altered endotoxin responsiveness in healthy children with Down syndrome. *BMC Immunol* 19: 31.
90. Creely SJ, McTernan PG, Kusminski CM, Fisher FM, Da Silva NF, et al. (2007) Lipopolysaccharide activates an innate immune system response in human adipose tissue in obesity and type 2 diabetes. *Am J Physiol Endocrinol Metab* 292: E740-7.
91. Miller GE, Murphy ML, Cashman R, Ma R, Ma J, et al. (2014) Greater inflammatory activity and blunted glucocorticoid signaling in monocytes of chronically stressed caregivers. *Brain Behav Immun* 41: 191-199.
92. Gola H, Engler H, Sommershof A, Adenauer H, Kolassa S, et al. (2013) Posttraumatic stress disorder is associated with an enhanced spontaneous production of pro-inflammatory cytokines by peripheral blood mononuclear cells. *BMC Psychiatry* 13: 40.
93. Liu D, Cao S, Zhou Y, Xiong Y (2019) Recent advances in endotoxin tolerance. *J Cell Biochem* 120: 56-70.
94. Kapellos TS, Bonaguro L, Gemünd I, Reusch N, Saglam A, Hinkley ER, et al. (2019) Human Monocyte Subsets and Phenotypes in Major Chronic Inflammatory Diseases. *Front Immunol* 10: 2035.
95. Italiani P, Boraschi D (2014) From Monocytes to M1/M2 Macrophages: Phenotypical vs. Functional Differentiation. *Front Immunol* 5: 514.
96. Wong KL, Yeap WH, Tai JJ, Ong SM, Dang TM, et al. (2012) The three human monocyte subsets: implications for health and disease. *Immunol Res* 53: 41-57.
97. Monguió-Tortajada M, Franquesa M, Sarrias MR, Borràs FE (2018) Low doses of LPS exacerbate the inflammatory response and trigger death on TLR3-primed human monocytes. *Cell Death Dis* 9: 499.
98. Quach H, Rotival M, Pothlichet J, Loh YE, Dannemann M, et al. (2016) Genetic Adaptation and Neandertal Admixture Shaped the Immune System of Human Populations. *Cell* 167: 643-656.
99. Aho V, Ollila HM, Rantanen V, Kronholm E, Surakka I, et al. (2013) Partial sleep restriction activates immune response-related gene expression pathways: experimental and epidemiological studies in humans. *PLoS One* 8: e77184.
100. Jankowiak S, Backé E, Liebers F, Schulz A, Hegewald J, et al. (2016) Current and cumulative night shift work and subclinical atherosclerosis: results of the Gutenberg Health Study. *Int Arch Occup Environ Health* 89: 1169-1182.