Published in final edited form as:

Biomarkers. 2017 September ; 22(6): 584–593. doi:10.1080/1354750X.2017.1347961.

Histone 3 modifications and blood pressure in the Beijing Truck Driver Air Pollution Study

Jacob K. Kresovich^{a,b,*}, Zhou Zhang^{a,c,*,#}, Fang Fang^d, Yinan Zheng^{a,e}, Marco Sanchez-Guerra^{f,g}, Brian T. Joyce^{a,b}, Jia Zhong^f, Yana Chervona^h, Sheng Wangⁱ, Dou Chang^j, John P. McCracken^f, Anaite Díaz^k, Matteo Bonzini^l, Michele Carugno^l, Petros Koutrakis^f, Choong-Min Kang^f, Shurui Bian^c, Tao Gao^a, Hyang-Min Byun^m, Joel Schwartz^f, Andrea A. Baccarelli^f, and Lifang Hou^{a,n}

^aDepartment of Preventive Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL, USA

^bDivision of Epidemiology and Biostatistics, School of Public Health, University of Illinois-Chicago, Chicago, IL, USA

^cDriskill Graduate Program in Life Sciences, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

^dDepartment of Epidemiology, College for Public Health and Social Justice, Saint Louis University, Saint Louis, MO, USA

^eInstitute for Public Health and Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

^fDepartment of Environmental Health, Harvard T.H. Chan School of Public Health, Harvard University, Boston, MA, USA

^gDepartment of Developmental Neurobiology, National Institute of Perinatology, Mexico City, Mexico

^hDepartment of Environmental Medicine, New York University School of Medicine, New York, NY, USA

ⁱDepartment of Occupational and Environmental Health, Peking University Health Science Center, Peking University, Beijing, China

^jDepartment of Safety Engineering, China Institute of Industrial Relations, Beijing, China

^kCenter for Health Studies, Universidad del Valle de Guatemala, Guatemala City, Guatemala

^lDepartment of Clinical Sciences and Community Medicine, University of Milan and IRCCS Fondazione Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

CONTACT: Lifang Hou l-hou@northwestern.edu, Department of Preventive Medicine, Feinberg School of Medicine, Northwestern University, 680 North Lake Shore Drive, Suite 1400, Chicago, IL 60611, USA.

*These authors contributed equally to this work.

#Zhou Zhang is responsible for statistical design/analysis. zhou.zhang@northwestern.edu

Supplemental data for this article can be accessed here.

Disclosure statement

The authors declare they have no actual or potential competing financial interests.

^mHuman Nutrition Research Centre, Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom

ⁿRobert H. Lurie Comprehensive Cancer Center, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

Abstract

Context—Histone modifications regulate gene expression; dysregulation has been linked with cardiovascular diseases. Associations between histone modification levels and blood pressure in humans are unclear.

Objective—We examine the relationship between global histone concentrations and various markers of blood pressure.

Materials and methods—Using the Beijing Truck Driver Air Pollution Study, we investigated global peripheral white blood cell histone modifications (H3K9ac, H3K9me3, H3K27me3, and H3K36me3) associations with pre- and post-work measurements of systolic (SBP) and diastolic (DBP) blood pressure, mean arterial pressure (MAP), and pulse pressure (PP) using multivariable mixed-effect models.

Results—H3K9ac was negatively associated with pre-work SBP and MAP; H3K9me3 was negatively associated with pre-work SBP, DBP, and MAP; and H3K27me3 was negatively associated with pre-work SBP. Among office workers, H3K9me3 was negatively associated with pre-work SBP, DBP, and MAP. Among truck drivers, H3K9ac and H3K27me3 were negatively associated with pre-work SBP, and H3K27me3 was positively associated with post-work PP.

Discussion and conclusion—Epigenome-wide H3K9ac, H3K9me3, and H3K27me3 were negatively associated with multiple pre-work blood pressure measures. These associations substantially changed during the day, suggesting an influence of daily activities. Blood-based histone modification biomarkers are potential candidates for studies requiring estimations of morning/pre-work blood pressure.

Keywords

Epigenetics; histone modifications; blood pressure; H3K9ac; H3K9me3; H3K27me3; H3K36me3

Introduction

Hypertension is an important risk factor for both heart disease and stroke, two of the top leading causes of death in the United States (Go *et al.* 2013, Xu *et al.* 2014). According to the American Heart Association's (AHA) 2016 report, 32.6% of adult Americans suffer from hypertension; similarly in China, about one-third of the adult population has hypertension (Gao *et al.* 2013, Lewington *et al.* 2016, Mozaffarian *et al.* 2016). Globally, the prevalence of hypertension is predicted to increase to over 1.5 billion by 2025 (Kearney *et al.* 2004). The public health burden of the disease continues to increase despite our understanding of its risk factors and decades of sustained public health primary prevention interventions. In order to halt this increase, it is essential to develop a more complete

understanding of the potential molecular biomarkers associated with the progression of elevated blood pressure (BP) into clinical hypertension.

Epigenetics (including DNA methylation, histone modifications, and microRNAs) is a mechanism that regulates gene expression without changing the underlying DNA sequence (Friso *et al.* 2015). While DNA methylation and microRNAs have been widely studied in relation to hypertension and blood pressure in humans, histone modifications remain largely unexamined. *In vitro* and *in vivo* studies have reported that post-translational modifications at different histone 3 (H3) lysine (K) residues control the expression of genes associated with the development of hypertension and other cardiovascular diseases (CVDs) (Kaneda *et al.* 2009, Movassagh *et al.* 2011, Kovalchuk *et al.* 2012, Hohl *et al.* 2013, Kim *et al.* 2013, Chaturvedi *et al.* 2014, Peng *et al.* 2014, Vecellio *et al.* 2014, Friso *et al.* 2015).

Researchers have previously shown blood pressure measurements are sensitive to external factors, limiting their capacity to measure underlying (and long-term) cardiovascular health; for example, BP measurements can be influenced by environmental and behavioural factors (Tolonen *et al.* 2015). Previous studies have shown that exposure to air pollution is associated with increased BP (Wilker *et al.* 2009, Wilker *et al.* 2010, Baccarelli *et al.* 2011). Other influencing factors include ambient air temperature (Chen *et al.* 2013, Lanzinger *et al.* 2014, Martinez-Nicolas *et al.* 2015), biological sex (Briant *et al.* 2016, Joyner *et al.* 2016), smoking habits (Virdis *et al.* 2010, Takami and Saito, 2011, Camplain *et al.* 2016), and occupational hazards (Guimont *et al.* 2006, Trudel *et al.* 2016). Epigenetic factors such histone modifications are additionally sensitive to these factors (Liu *et al.* 2015), but may represent a more stable biomarker of underlying cardiovascular health, as estimated by morning blood pressure measurements. Identification of a stable biomarker which represents resting-state BP would be useful in clinical practices due to the reduction in measurement error. Furthermore, a stable marker of blood pressure estimation will allow the earlier detection and targeted screenings of at-risk individuals with the goal of augmenting existing interventions.

Our group previously reported associations between BP and microRNAs (Zhang *et al.* 2017); other researchers have identified associations between BP and DNA methylation (Alexeeff *et al.* 2013, Kato *et al.* 2015). The purpose of this study is to investigate associations between histone modifications and BP measurements in a Chinese population, thus identifying histone marker concentrations as a biomarker of BP measurements. Specifically, we will investigate the histone markers: histone 3 lysine 9 acetylation (H3K9ac), histone 3 lysine 9 tri-methylation (H3K9me3), histone 3 lysine 27 tri-methylation (H3K27me3), and histone 3 lysine 36 tri-methylation (H3K36me3) in relation to various BP measurements among office workers and truck drivers from the Beijing Truck Driver Air Pollution Study (BTDAS).

Clinical significance

- For the first time, whole blood histone modification concentrations have been identified in association with blood pressure measurements.

- Our findings show daily activities and behaviours influence the sensitive nature of histone modification concentrations as a biomarker of blood pressure.
- We showed diminishing utility of histone biomarkers of blood pressure throughout the day, particularly among individuals with occupational exposures.
- Blood-based histone modification biomarkers are potential candidates for future studies requiring estimations of morning/pre-work blood pressure measurements.
- Morning, pre-work blood pressure measurements better reflect underlying cardiovascular health and long-term health risks.

Methods

Study population and design

The BT DAS recruited 60 healthy office workers and 60 truck drivers between 15 June and 27 July 2008. All study participants worked and lived in the Beijing metropolitan area and had held their current jobs for more than two years prior to enrolment. Truck drivers and indoor office workers were matched by age (5-year intervals), sex, smoking status, and education level. In-person interviews using a detailed questionnaire were conducted to collect information on demographics, lifestyle, and other exposures. Information on time-varying factors, including tea and alcohol intake as well as smoking status, was obtained for past exposure and for exposure on examination days. All participants were examined on two independent workdays separated by a 1–2 week period; blood samples were collected at the end of each workday. Individual written informed consent was obtained from all participants prior to enrolment in the study. Institutional Review Board or equivalent approval at the participating institution (i.e. Harvard School of Public Health, Northwestern University, and Peking University Health Science Center) was obtained prior to study participant recruitment. All experimental protocols were approved by the participating institutions and all methods were carried out in accordance with relevant guidelines and regulations.

Blood pressure measurements

BP of each individual was measured by a trained research assistant before and after work on each examination day after five minutes of rest according to the standardized protocol issued by the AHA (Pickering *et al.* 2005). BP was measured by a mercury sphygmomanometer on the right arm using the appropriate cuff size. Three readings were taken, each separated by at least one minute. BP measurements were calculated as the mean of the second and third readings. Mean arterial pressure (MAP) was estimated by taking 1/3 of the difference between systolic blood pressure (SBP) and diastolic blood pressure (DBP) and adding it to the DBP value. Pulse pressure (PP) was calculated as the difference between SBP and DBP (Baccarelli *et al.* 2011).

Blood sample collection, processing, and histone modification analysis

Whole blood samples were collected in an EDTA tube at the end of each examination day. Histones were extracted from buffy coat through acid extraction according to previously designed protocols (Shechter *et al.* 2007) with slight modifications. Briefly, buffy coat was

processed with red blood cell lysis solution for 10 min at room temperature. Pellets of white blood cells (WBCs) were collected by centrifugation at 2500×g for 15 minutes and lysed in 500 µL of Triton extraction buffer (TEB) (1× PBS; 0.5% Triton [v/v] and 2mM phenyl-methylsulfonyl fluoride) supplemented with protease inhibitor mixture (Roche Applied Sciences, Indianapolis, IN) on ice for 10 minutes. The pellet was collected by centrifugation at 6500×g for 10 min at 4 °C and resuspended in 0.2 N HCl and kept overnight at 4 °C. The supernatant was collected by centrifugation at 16,000×g for 10 min at 4 °C and added an equal volume of 50% of trichloroacetic acid solution (TCA) to precipitate the histone proteins. The histones were washed three times on cold acetone and centrifuged at 16,000×g for 20 minutes. The histones were air-dried for 20 minutes at room temperature and suspended in 100 µL of ddH₂O.

Total histone protein in each sample was quantified by the bicinchoninic acid protein assay (Smith *et al.* 1985). Histone modifications were quantified by sandwich enzyme-linked immunosorbent assay (ELISA), as previously described by Arita *et al.* (2012). Briefly, polystyrene 96-well microplates (Thermo Fisher Scientific, Pittsburgh, PA) were coated with 100 µL of histone H3 antibody (Abcam ab16061, Cambridge, MA) at a concentration of 1:20,000 in PBS and incubated overnight at 4 °C. Plates were washed with PBST (1× PBS, 0.05% Tween-20) and blocked for 1.5 hours at room temperature with 3% milk in PBST. After washing plates with PBST, 100 µL of standard recombinant protein for the standard curve (total H3 (31207), H3K9ac (31253), H3K9me3 (31213), H3K27me3 (31216), or H3K36me3 (31219); Active Motif, Carlsbad, CA) and samples were added per triplicate to plates and incubated at room temperature for 1.5 hours with agitation on an orbital shaker at 450 rpm (Titramax 101, Schwabach, Germany). After incubation, wells were washed three times with PBST, and 100 µL diluted primary antibody [total H3, 1:40,000 (Sigma H0164, St. Louis, MO); H3K9ac, 1:500 (Active Motif 39137); H3K9me3, 1:500 (Abcam ab8898); H3K27me3, 1:1000 (Active Motif 39155); and H3K36me3, 1:1000 (Abcam ab9050)] in 1% PBST milk and incubated at room temperature for 1 hour with agitation at 450 rpm. After three washes with TBST, 100 µL diluted secondary antibody (Santa Cruz Biotechnology sc-2004, Santa Cruz, CA) in TBST was added to each well and incubated at room temperature for 1 hour without agitation. Wells were washed four times with TBST and 100 µL of 3,3',5,5'-tetramethylbenzidine solution (Thermo Fisher) was added to each well and incubated at room temperature for 30 minutes in the dark. The reaction was stopped by adding 100 µL 2M H₂SO₄ to each well. All assays were performed in triplicate. Optical density was read at 450nm using an Infinite M200 PRO reader (TECAN, Männedorf, Switzerland). Relative percent histone modifications were derived from standard curves specific to each histone modification, and levels were normalized to total H3 levels. The within- and between-assay coefficients of variation of each assay were 5.28% and 13.37% for total H3, 3.49% and 11.88% for H3K9ac, 3.11% and 12.41% for H3K9me3, 6.37% and 8.13% for H3K27me3, and 5.60% and 10.57% for H3K36me3. Histone quantification was conducted on 12, 14, 14, and 10 plates for H3K27me3, H3K36me3, H3K9me3, and H3K9ac, respectively. We conducted ANOVA tests for each histone modification across plates; the corresponding *p*-values were 0.32, 0.27, 0.08, and 0.20, respectively, indicating the histone modifications were not significantly different across plates. We additionally observed missing values of histone modification measurements for some individuals, due to

the limited sample amounts. In total, we collected 217, 187, 231, and 228 non-missing measurements for H3K9ac, H3K9me3, H3K27me3, and H3K36me3, respectively.

Statistical analysis

In the present study, we examined each participant at two time points (before and after work). The skewed distributions of histone modification relative percentage measures were improved by log₂-transformation. To account for this within-person correlation, we employed linear mixed-effects regression models with random intercepts in all analyses. We present the estimated of the marginal means, standard errors (SEs), and *p*-values of BP measures for different characteristics, as well as our comparison of histone modification levels overall and stratified by the two occupational groups (office workers, truck drivers). We additionally examined correlations between the histone modifications within and across examination days using Pearson's *r*. To evaluate the association of histone modifications with BP measures, the following linear mixed-effects model was used:

$$Y_{ij} = \beta_0 + \beta_1 (\log(\text{histone modifications}))_{ij} + \beta_2 X_{2ij} + \dots + \beta_n X_{nij} + \xi_j + e_{ij}$$

where Y_{ij} represents the measured BP metric for the j th participant on the i th examination day; β_0 represents the overall intercept; $\beta_1 \dots \beta_n$ represent the regression coefficients for the time-dependent covariates (i.e. histone modification level and day of the week) or time-independent covariates (i.e. occupation group, gender, age, BMI, and smoking status) included in multivariate models; ξ_j represents the participant-level random effect; and e_{ij} represents the residual error term.

We fitted the linear mixed-effects models adjusting for gender, age (continuous), BMI (continuous), smoking status (current, former, never), number of cigarettes smoked during examination time, hours worked per week, examination day of the week, alcohol intake, temperature, and 8-day ambient PM₁₀. We attempted to further adjust for processing batch by adding it as a covariate to the model even though a principle components analysis did not identify any significant batch effects (Hou *et al.* 2016). Unfortunately, due to the high number of plates, the models performed poorly and we therefore excluded the variable from future analyses. A priori stratification was conducted to evaluate the associations between histone markers and BP by occupational groups. A two-sided *p*-value of 0.05 was considered to determine significance. All analyses were performed in SAS 9.4 (SAS Institute Inc., Cary, NC).

Results

Blood pressure by subject characteristics

A flow diagram depicting the selection of the study population is shown in Supplemental Figure 1. Means of pre-work SBP, DBP, MAP, and PP by covariates are shown in Table 1. Pre-work SBP, DBP, MAP, and PP were significantly higher in males than females (all $p < 0.01$). Pre-work SBP, DBP, and MAP (all $p < 0.01$) significantly varied across categories of BMI, and pre-work PP ($p = 0.06$) marginally varied across categories of BMI, with higher measurements observed for overweight and obese individuals. All pre-work BP

measurements significantly varied across smoking status (all $p<0.01$), with former smokers having the highest measurements of SBP, DBP, and MAP and current and former smokers having higher measurements of PP. All pre-work BP measurements also significantly varied across alcohol use (all $p<0.01$), with regular drinkers having higher measurements compared with nondrinkers. Finally, we observed higher pre-work BP measurements for hypertensive individuals (SBP, DBP, MAP $p<0.01$), with the exception of pulse pressure ($p=0.33$). We did not identify any associations between any of the pre-work BP measurements and occupation, age, or examination day.

Means of post-work SBP, DBP, MAP, and PP by covariates are shown in Table 2. Post-work measurements showed similar patterns with a few exceptions. A marginally significant difference was identified for DBP across occupational groups; truck drivers had higher measurements compared with office workers ($p=0.10$). Post-work SBP, DBP, MAP, and PP were significantly higher in males than females (all $p<0.01$). Post-work SBP, DBP, and MAP, but not PP ($p=0.28$), were associated with BMI, with overweight and obese individuals having higher measurements ($p<0.01$). All post-work BP measurements were associated with smoking status (all $p<0.01$), with former smokers having the highest measurements (save PP, which was highest in current smokers). Post-work SBP, DBP, and MAP were significantly higher among regular drinkers (all $p<0.01$); a marginal association was identified for PP ($p=0.09$). Finally, we observed higher post-work BP measurements for hypertensive individuals (SBP, DBP, MAP; $p<0.01$), with the exception of pulse pressure ($p=0.50$). We identified no associations between the post-work BP measurements and age or examination day.

We examined correlations of the four histone markers within and between examination days (Supplemental Figures 2 and 3). Within examination days, we found moderate-to-strong correlations between the histone markers (r^2 range 0.32 to 0.81). When tested between examination days, we found weak-to-moderate correlations across the markers (r^2 range 0.21 to 0.37).

Comparison of histone modification levels by occupational groups

Figure 1 depicts the log-transformed distributions of the four histone markers by occupational group. Significant differences were found only in H3K27me3, where office workers tended to have a higher mean histone modification level than truck drivers ($p=0.04$). However, after adjustment for gender, age, BMI, smoking habits, alcohol use and examination day, this difference was no longer significant (data not shown).

Association between histone modifications and pre-work blood pressure

Table 3 shows the mean change in pre-work BP measurements per each one-fold increase in histone modification level after adjusting for covariates. In all participants, a one-fold increase in H3K9ac was associated with 2.52mmHg lower mean SBP (95%CI: $-4.22, -0.81$, $p<0.01$) and 1.54mmHg lower mean MAP (95%CI: $-2.95, -0.14$, $p=0.03$). A one-fold increase in H3K9me3 was associated with 2.04mmHg lower mean SBP (95%CI: $-3.32, -0.77$, $p<0.01$), 1.68mmHg lower mean DBP (95%CI: $-2.84, -0.52$, $p=0.01$), and 1.75mmHg lower mean MAP (95%CI: $-2.86, -0.64$, $p<0.01$). Finally, we observed a one-

fold increase in H3K27me3 was associated with 2.28mmHg lower SPB (95%CI: -4.42, -0.13, $p=0.04$).

Upon stratification by occupational group, in office workers a one-fold increase in H3K9me3 was associated with 2.41mmHg lower mean SBP (95%CI: -1.19, -0.64, $p=0.01$), 2.36mmHg lower mean DBP (95%CI: -3.90, -0.83, $p<0.01$), and 2.30mmHg lower mean MAP (95%CI: -3.82, -0.78, $p=0.01$). In truck drivers, a one-fold increase in H3K9ac was associated with 4.02mmHg lower mean SBP (95%CI: -6.81, -1.23, $p=0.01$) and a one-fold increase in H3K27me3 was associated with 3.86mmHg lower mean SBP (95%CI: -7.42, -0.30, $p=0.04$). We did not identify any associations between histone markers and the other BP measurements in truck drivers. We additionally did not find any associations between the histone marker levels and pulse pressure in either group.

Association between histone modifications and post-work blood pressure

Table 4 shows the mean change in post-work BP measurements per each one-fold increase in histone marker levels. In both the overall population and office workers, no significant associations were found between the histone marker levels and BP measurements. However, among truck drivers, we found that a one-fold increase in H3K27me3 was associated with 2.96mmHg higher pulse pressure (95%CI: 0.12, 5.81, $p=0.04$).

Discussion

To the best of our knowledge, the present study is the first epidemiological investigation exploring associations of BP with histone modifications. At both pre- and post-work, higher BP measurements were found among males, overweight and obese participants, former smokers, regular drinkers, and hypertensive participants. We also identified a significant difference in H3K27me3 across occupational groups, although this difference no longer existed after adjustment for participant characteristics. We observed inverse associations between three histone modification markers in peripheral blood (H3K9ac, H3K9me3, and H3K27me3) and pre-work BP measurements. The associations with two of these histone modifications (H3K9ac and H3K27me3) were more pronounced in truck drivers, while the third (H3K9me3) was more pronounced in office workers. We additionally identified a positive association between H3K27me3 and pulse pressure among truck drivers in post-work BP measurements only.

In this analysis, we identified inverse associations between H3K9ac levels and pre-work SBP and MAP measurements. H3K9ac histone markers are associated with transcriptional activation and are regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs) (Barski *et al.* 2007, Kouzarides 2007). Inhibition of HDACs is associated with increases of H3K9ac in active gene regions (Wang *et al.* 2009) and is protective against the development of hypertension in rats (Cardinale *et al.* 2010, Usui *et al.* 2012, Kang *et al.* 2015). Our observation for this histone maker may be due to residual confounding by factors not measured in our study that can affect HDAC activity. For example, physical activity has been shown to inhibit HDACs in rodents and also protect against the development of hypertension (Cornelissen and Fagard 2005, Elsner *et al.* 2011). Additional studies more closely examining the precise mechanism of the identified association are warranted.

Similar to H3K9ac, our study identified inverse associations between H3K9me3 levels and pre-work SBP, DBP, and MAP measurements. H3K9me3 is a repressive transcriptional marker and is regulated by numerous H3K9 methyltransferases such as KMT1, KMT2H, and KMT8A/D as well as demethylases such as KDM3, KDM4, and KDM7 (Barski *et al.* 2007, Kouzarides, 2007, Kim and Kim 2012, Zhang and Liu 2015). In particular, upregulation of H3K9me3-specific demethylase JMJD2A/KDM4A is associated with hypertrophic cardiomyopathy (Zhang *et al.* 2011), a disease resulting in increased ventricular thickness, which is commonly associated with elevated blood pressure (Gersh *et al.* 2011). Therefore, dysregulation of JMJD2A/KDM4A may result in decreased concentrations of H3K9me3 as well as increased risk of hypertrophic cardiomyopathy and elevated blood pressure. While the evidence is limited, these studies together explain our inverse association between H3K9me3 regulation and blood pressure markers.

We additionally identified an inverse association between H3K27me3 levels and pre-work systolic BP measurements. H3K27me3 is a repressive histone marker and is primarily maintained by histone methyltransferases such as EZH2 (Kuzmichev *et al.* 2002). Long-term exposure to PM₁₀ is negatively associated with expression of *EZH2* (Miousse *et al.* 2014), which can lead to depletion of H3K27me3 (Viré *et al.* 2006). Furthermore, exposure to particulate matter is associated with increased BP (Chahine *et al.* 2007, Brook and Rajagopalan 2009, Wilker *et al.* 2009, Wilker *et al.* 2010, Baccarelli *et al.* 2011, Coogan *et al.* 2012, Coogan *et al.* 2016). Thus, H3K27me3 may mediate the development of environmentally induced hypertension via dysregulation of EZH2. Similarly, researchers have identified inverse associations between H3K27me3 concentrations and blood pressure through treatment with resveratrol (Han *et al.* 2015). While these findings are not causal, they suggest that underlying components regulate both histone modification concentrations and blood pressure.

Interestingly, all of our associations related to pre-work BP measurements were modified by occupation, with the associations between pre-work BP and H3K9ac and H3K27me3 being more pronounced among truck drivers, and the associations between pre-work BP and H3K9me3 more pronounced among office workers. These findings may be reflective of long-term occupational exposures to different components of air pollution. In this cohort, while office workers and truck drivers did not differ in short-term or average 14-day levels of PM₁₀ exposure, truck drivers had significantly greater exposure to PM_{2.5}, black carbon, and heavy metals (Sanchez-Guerra *et al.* 2015). As PM_{2.5} has been shown to independently increase H3K27me3 markers and risk of hypertension (Coogan *et al.* 2012, Liu *et al.* 2015, Coogan *et al.* 2016), this may also explain our paradoxical finding of a positive association between H3K27me3 and post-work PP among truck drivers. Additionally, metals such as arsenic and nickel have been shown to affect levels of other histone 3 modifications as well as predispose individuals to the development of increased BP and hypertension (Wang *et al.* 2002, Cantone *et al.* 2011, Abhyankar *et al.* 2012, Farzan *et al.* 2015, Jiang *et al.* 2015). Thus, the observed modification of our findings by occupation may be reflective of long-term differential exposure to air pollution components by participants in these two occupational groups, while the large change in the effect of H3K27me3 on PP in truck drivers from pre- to post-work measurements may be due to acute exposure to additional components of air pollution during the course of the workday.

Though novel, this study is subject to limitations. First, the histone markers were measured in whole blood samples reflecting a mixed cell population, which may limit our ability to understand the exact biological mechanism by specific blood cell type. Nonetheless, whole blood is easy to obtain and process – for this reason most human epigenetic studies use whole blood for epigenetic biomarker measurements, including histone modifications, in the hope of identifying blood-based, cost-effective disease biomarkers (Hou *et al.* 2013, Hou *et al.* 2014, Liu *et al.* 2015, Ma *et al.* 2015, Sanchez-Guerra *et al.* 2015, Hou *et al.* 2016, Zheng *et al.* 2016, Zhang *et al.* 2017). The rationale for our using mixed white blood cells is supported by the need to identify novel biomarkers of BP in easily obtainable blood samples. Such biomarkers have high potential for preventive and/or clinical use, and may provide data to support epigenetic biomarker studies in other large human cohorts that have previously collected ready-to-use unfractionated white blood cells. Furthermore, the blood samples for epigenetic analyses were only measured at the post-work exam. However, due to the relative stability of histone markers over short time periods (Zee *et al.* 2010, Tabassum *et al.* 2015), coupled with our moderate-to-strong correlations between the histone markers within examination days suggest that our post-work histone measurements are likely an acceptable proxy for pre-work histone measurements in blood. Thus, our analysis follows a pseudo-cross-sectional design but our findings will need to be validated using true cross-sectional data collection. Finally, this study was conducted on only 120 participants; as a result, our results need to be interpreted with caution.

Conclusion

Overall, our study suggests that global H3K9ac, H3K9me3, and H3K27me3 levels measured in whole blood are negatively associated with pre-work BP measurements. We additionally identified H3K27me3 to be positively associated with pulse pressure, but only among highly exposed truck workers. Our findings highlight the importance of the acute effects of daily activities and behaviours in influencing the utility of this biomarker. We showed diminishing associations between histone biomarkers and BP throughout the day, and a complete reversal in the association between H3K27me3 in truck drivers (potentially due to acute occupationally based exposures). This reversal of effect additionally seems to be independent of PM₁₀ exposure, although our study is not able to identify the responsible contaminant. Thus, blood-based histone modification biomarkers are potential candidates for future population-based studies with interests in identifying underlying cardiovascular health states. Histone modification concentrations may better reflect long-term cardiovascular health with the same accuracy as morning, pre-work BP measurements. Future studies will be needed to examine the utility (sensitivity and specificity) of these markers although this study suggests a potential clinical significance for population-based studies requiring estimates of morning BP measurements.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding

This work was supported by the National Institute of Environmental Health Sciences [grant numbers R21ES020984, R21ES020010], American Heart Association [grant number 12GRNT12070254], the National Cancer Institute [grant number R25CA057699] and the Robert H. Lurie Comprehensive Cancer Center – Rosenberg Family Cancer Research Fund. MSG was financially supported by the Fundación México en Harvard, A.C. and Consejo Nacional de Ciencia y Tecnología (CONACYT, Mexico).

References

- Abhyankar LN, et al. Arsenic exposure and hypertension: a systematic review. *Environmental health perspectives*. 2012; 120:494–500. [PubMed: 22138666]
- Alexeeff SE, et al. Association between blood pressure and DNA methylation of retrotransposons and pro-inflammatory genes. *International journal of epidemiology*. 2013; 42:270–280. [PubMed: 23508416]
- Arita A, et al. Global levels of histone modifications in peripheral blood mononuclear cells of subjects with exposure to nickel. *Environmental health perspectives*. 2012; 120:198–203. [PubMed: 22024396]
- Baccarelli A, et al. Effects of particulate air pollution on blood pressure in a highly exposed population in Beijing, China: a repeated-measure study. *Environmental health*. 2011; 10:108. [PubMed: 22188661]
- Barski A, et al. High-resolution profiling of histone methylations in the human genome. *Cell*. 2007; 129:823–837. [PubMed: 17512414]
- Briant LJ, Charkoudian N, Hart EC. Sympathetic regulation of blood pressure in normotension and hypertension: when sex matters. *Experimental physiology*. 2016; 101:219–229. [PubMed: 26682826]
- Brook RD, Rajagopalan S. Particulate matter, air pollution, and blood pressure. *Journal of the American Society of hypertension: JASH*. 2009; 3:332–350. [PubMed: 20409976]
- Camplain R, et al. Smoking behaviors and arterial stiffness measured by pulse wave velocity in older adults: The Atherosclerosis Risk in Communities (ARIC) Study. *American journal of hypertension*. 2016; 29:1268–1275.
- Cantone L, et al. Inhalable metal-rich air particles and histone H3K4 dimethylation and H3K9 acetylation in a cross-sectional study of steel workers. *Environmental health perspectives*. 2011; 119:964–969. [PubMed: 21385672]
- Cardinale JP, et al. HDAC inhibition attenuates inflammatory, hypertrophic, and hypertensive responses in spontaneously hypertensive rats. *Hypertension*. 2010; 56:437–444. [PubMed: 20679181]
- Chahine T, et al. Particulate air pollution, oxidative stress genes, and heart rate variability in an elderly cohort. *Environmental health perspectives*. 2007; 115:1617–1622. [PubMed: 18007994]
- Chaturvedi P, et al. Differential regulation of DNA methylation versus histone acetylation in cardiomyocytes during HHcy in vitro and in vivo: an epigenetic mechanism. *Physiological genomics*. 2014; 46:245–255. [PubMed: 24495916]
- Chen Q, et al. Association between ambient temperature and blood pressure and blood pressure regulators: 1831 hypertensive patients followed up for three years. *PLoS One*. 2013; 8:e84522. [PubMed: 24391962]
- Coogan PF, et al. Air pollution and incidence of hypertension and diabetes mellitus in black women living in Los Angeles. *Circulation*. 2012; 125:767–772. [PubMed: 22219348]
- Coogan PF, et al. PM2.5 and Diabetes and Hypertension Incidence in the Black Women's Health Study. *Epidemiology*. 2016; 27:202–210. [PubMed: 26595125]
- Cornelissen VA, Fagard RH. Effects of endurance training on blood pressure, blood pressure-regulating mechanisms, and cardiovascular risk factors. *Hypertension*. 2005; 46:667–675. [PubMed: 16157788]

- Elsner VR, et al. Effect of different exercise protocols on histone acetyltransferases and histone deacetylases activities in rat hippocampus. *Neuroscience*. 2011; 192:580–587. [PubMed: 21745541]
- Farzan SF, et al. Blood Pressure Changes in Relation to Arsenic Exposure in a U.S. Pregnancy Cohort. *Environmental health perspectives*. 2015; 123:999–1006. [PubMed: 25793356]
- Friso S, et al. Epigenetics and arterial hypertension: the challenge of emerging evidence. *Translational research: the journal of laboratory and clinical medicine*. 2015; 165:154–165. [PubMed: 25035152]
- Gao Y, et al. for the China National Diabetes and Metabolic Disorders Study Group. Prevalence of hypertension in china: a cross-sectional study. *PLoS One*. 2013; 8:e65938. [PubMed: 23776574]
- Gersh BJ, et al. 2011 ACCF/AHA guideline for the diagnosis and treatment of hypertrophic cardiomyopathy: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *The journal of thoracic and cardiovascular surgery*. 2011; 142:e153–e203. [PubMed: 22093723]
- Go AS, et al. Heart disease and stroke statistics–2013 update: a report from the American Heart Association. *Circulation*. 2013; 127:e6–e245. [PubMed: 23239837]
- Guimont C, et al. Effects of job strain on blood pressure: a prospective study of male and female white-collar workers. *American journal of public health*. 2006; 96:1436–1443. [PubMed: 16809603]
- Han S, et al. Resveratrol affects histone 3 lysine 27 methylation of vessels and blood biomarkers in DOCA salt-induced hypertension. *Molecular biology reports*. 2015; 42:35–42. [PubMed: 25234650]
- Hohl M, et al. HDAC4 controls histone methylation in response to elevated cardiac load. *Journal of clinical investigation*. 2013; 123:1359–1370. [PubMed: 23434587]
- Hou L, et al. Particulate Air Pollution Exposure and Expression of Viral and Human MicroRNAs in Blood: The Beijing Truck Driver Air Pollution Study. *Environmental health perspectives*. 2016; 124:344–350. [PubMed: 26068961]
- Hou L, et al. Inhalable particulate matter and mitochondrial DNA copy number in highly exposed individuals in Beijing, China: a repeated-measure study. *Particle and fibre toxicology*. 2013; 10:17. [PubMed: 23628000]
- Hou L, et al. Altered methylation in tandem repeat element and elemental component levels in inhalable air particles. *Environmental and molecular mutagenesis*. 2014; 55:256–265. [PubMed: 24273195]
- Jiang J, et al. Association between Arsenic Exposure from Drinking Water and Longitudinal Change in Blood Pressure among HEALS Cohort Participants. *Environmental health perspectives*. 2015; 123:806–812. [PubMed: 25816368]
- Joyner MJ, Wallin BG, Charkoudian N. Sex differences and blood pressure regulation in humans. *Experimental physiology*. 2016; 101:349–355. [PubMed: 26152788]
- Kaneda R, et al. Genome-wide histone methylation profile for heart failure. *Genes to cells: devoted to molecular: cellular mechanisms*. 2009; 14:69–77. [PubMed: 19077033]
- Kang SH, et al. Histone deacetylase inhibition attenuates cardiac hypertrophy and fibrosis through acetylation of mineralocorticoid receptor in spontaneously hypertensive rats. *Molecular pharmacology*. 2015; 87:782–791. [PubMed: 25667225]
- Kato N, et al. Trans-ancestry genome-wide association study identifies 12 genetic loci influencing blood pressure and implicates a role for DNA methylation. *Nature genetics*. 2015; 47:1282–1293. [PubMed: 26390057]
- Kearney PM, et al. Worldwide prevalence of hypertension: a systematic review. *Journal of hypertension*. 2004; 22:11–19. [PubMed: 15106785]
- Kim GH, Ryan JJ, Archer SL. The role of redox signaling in epigenetics and cardiovascular disease. *Antioxidants: redox signaling*. 2013; 18:1920–1936. [PubMed: 23480168]
- Kim J, Kim H. Recruitment and biological consequences of histone modification of H3K27me3 and H3K9me3. *ILAR journal*. 2012; 53:232–239. [PubMed: 23744963]
- Kouzarides T. Chromatin modifications and their function. *Cell*. 2007; 128:693–705. [PubMed: 17320507]

- Kovalchuk A, et al. Epigenetic bystander-like effects of stroke in somatic organs. *Aging*. 2012; 4:224–234. [PubMed: 22466454]
- Kuzmichev A, et al. Histone methyltransferase activity associated with a human multiprotein complex containing the Enhancer of Zeste protein. *Genes & development*. 2002; 16:2893–2905. [PubMed: 12435631]
- Lanzinger S, et al. Short-term effects of air temperature on blood pressure and pulse pressure in potentially susceptible individuals. *International journal of hygiene and environmental health*. 2014; 217:775–784. [PubMed: 24877761]
- Lewington S, et al. The Burden of Hypertension and Associated Risk for Cardiovascular Mortality in China. *JAMA internal medicine*. 2016; 176:524–532. [PubMed: 26975032]
- Liu C, et al. Characterization of genome-wide H3K27ac profiles reveals a distinct PM2.5-associated histone modification signature. *Environmental health*. 2015; 14:65. [PubMed: 26276146]
- Ma L, et al. Histone Methylation in Nickel-Smelting Industrial Workers. *PLoS One*. 2015; 10:e0140339. [PubMed: 26474320]
- Martinez-Nicolas A, et al. Daytime variation in ambient temperature affects skin temperatures and blood pressure: Ambulatory winter/summer comparison in healthy young women. *Physiology & behavior*. 2015; 149:203–211. [PubMed: 26072176]
- Miousse IR, et al. Epigenetic alterations induced by ambient particulate matter in mouse macrophages. *Environmental and molecular Mutagenesis*. 2014; 55:428–435. [PubMed: 24535919]
- Movassagh M, et al. Distinct epigenomic features in end-stage failing human hearts. *Circulation*. 2011; 124:2411–2422. [PubMed: 22025602]
- Mozaffarian D, et al. Heart Disease and Stroke Statistics-2016 Update: A Report From the American Heart Association. *Circulation*. 2016; 133:e38–e60. [PubMed: 26673558]
- Peng C, et al. Inhibition of Histone H3K9 Acetylation by Anacardic Acid Can Correct the Over-Expression of Gata4 in the Hearts of Fetal Mice Exposed to Alcohol during Pregnancy. *PLoS One*. 2014; 9:1–12.
- Pickering TG, et al. Recommendations for blood pressure measurement in humans and experimental animals: Part 1: blood pressure measurement in humans: a statement for professionals from the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. *Hypertension*. 2005; 45:142–161. [PubMed: 15611362]
- Sanchez-Guerra M, et al. Effects of particulate matter exposure on blood 5-hydroxymethylation: results from the Beijing truck driver air pollution study. *Epigenetics*. 2015; 10:633–642. [PubMed: 25970091]
- Shechter D, et al. Extraction, purification and analysis of histones. *Nature protocols*. 2007; 2:1445–1457. [PubMed: 17545981]
- Smith PK, et al. Measurement of protein using bicinchoninic acid. *Analytical biochemistry*. 1985; 150:76–85. [PubMed: 3843705]
- Tabassum R, et al. Omic personality: implications of stable transcript and methylation profiles for personalized medicine. *Genome medicine*. 2015; 7:88. [PubMed: 26391122]
- Takami T, Saito Y. Effects of smoking cessation on central blood pressure and arterial stiffness. *Vascular health and risk management*. 2011; 7:633–638. [PubMed: 22102787]
- Tolonen H, et al. Challenges in standardization of blood pressure measurement at the population level. *BMC medical research methodology*. 2015; 15:33. [PubMed: 25880766]
- Trudel X, et al. Adverse psychosocial work factors, blood pressure and hypertension incidence: repeated exposure in a 5-year prospective cohort study. *Journal of epidemiology and community health*. 2016; 70:402–408. [PubMed: 26530810]
- Usui T, et al. HDAC4 mediates development of hypertension via vascular inflammation in spontaneous hypertensive rats. *American Journal of physiology. Heart and circulatory physiology*. 2012; 302:H1894–H1904. [PubMed: 22389387]
- Vecellio M, et al. The histone acetylase activator pentadecylidene-malonate 1b rescues proliferation and differentiation in the human cardiac mesenchymal cells of type 2 diabetic patients. *Diabetes*. 2014; 63:2132–2147. [PubMed: 24458358]
- Virdis A, et al. Cigarette smoking and hypertension. *Current pharmaceutical design*. 2010; 16:2518–2525. [PubMed: 20550499]

- Viré E, et al. The Polycomb group protein EZH2 directly controls DNA methylation. *Nature*. 2006; 439:871–874. [PubMed: 16357870]
- Wang SJ, et al. Variation of systolic blood pressure in rats exposed to cadmium and nickel. *Environmental Research*. 2002; 88:116–119. [PubMed: 11908936]
- Wang Z, et al. Genome-wide mapping of HATs and HDACs reveals distinct functions in active and inactive genes. *Cell*. 2009; 138:1019–1031. [PubMed: 19698979]
- Wilker E, et al. Postural changes in blood pressure associated with interactions between candidate genes for chronic respiratory diseases and exposure to particulate matter. *Environmental health perspectives*. 2009; 117:935–940. [PubMed: 19590686]
- Wilker EH, et al. Black carbon exposures, blood pressure, and interactions with single nucleotide polymorphisms in MicroRNA processing genes. *Environmental health perspectives*. 2010; 118:943–948. [PubMed: 20211803]
- Xu J, et al. Mortality in the United States, 2012. *NCHS data brief*. 2014; 168:1–8.
- Zee BM, et al. Global turnover of histone post-translational modifications and variants in human cells. *Epigenetics chromatin*. 2010; 3:22. [PubMed: 21134274]
- Zhang QJ, et al. The histone trimethyllysine demethylase JMJD2A promotes cardiac hypertrophy in response to hypertrophic stimuli in mice. *Journal of clinical investigation*. 2011; 121:2447–2456. [PubMed: 21555854]
- Zhang QJ, Liu ZP. Histone methylations in heart development, congenital and adult heart diseases. *Epigenomics*. 2015; 7:321–330. [PubMed: 25942538]
- Zhang Z, et al. Blood pressure and expression of microRNAs in whole blood. *PLoS One*. 2017; 12:e0173550. [PubMed: 28278198]
- Zheng Y, et al. Traffic-derived particulate matter exposure and histone H3 modification: A repeated measures study. *Environmental research*. 2016; 153:112–119. [PubMed: 27918982]

Figure 1. Histone modification levels in office workers and truck drivers. Relative percentage of histone modification level over total histone 3 (H3) content in blood were log-transformed.

Table 1

Blood pressure by participant characteristics before work.

Variables	N (%)	Systolic Blood Pressure			Diastolic Blood Pressure			Mean Arterial Pressure			Pulse Pressure		
		Mean ^a	SE ^a	p-Value ^b	Mean ^a	SE ^a	p-Value ^b	Mean ^a	SE ^a	p-Value ^b	Mean ^a	SE ^a	p-Value ^b
Occupation, n (%)													
Office workers	120 (50%)	111.50	1.45	0.14	77.66	1.14	0.26	88.98	1.20	0.19	33.84	0.78	0.33
Truck drivers	120 (50%)	114.55	1.45		79.48	1.14		91.20	1.20		34.91	0.78	
Sex													
Female	80 (33%)	104.88	1.56	<0.01	73.60	1.30	<0.01	84.09	1.33	<0.01	31.46	0.90	<0.01
Male	160 (67%)	116.76	1.10		80.98	0.92		92.93	0.94		35.80	0.64	
Age (Quartile)													
Q1 [18–27 years]	60 (25%)	112.14	2.06	0.39	77.70	1.60	0.12	89.16	1.69	0.15	34.38	1.11	0.98
Q2 [28–32 years]	62 (26%)	111.58	2.03		76.47	1.58		88.21	1.66		34.73	1.09	
Q3 [33–37 years]	58 (24%)	112.62	2.10		78.65	1.63		90.01	1.71		34.27	1.13	
Q4 [38–46 years]	60 (25%)	116.18	2.06		81.75	1.60		93.42	1.69		34.10	1.11	
BMI													
Under and normal	170 (71%)	109.70	1.08	<0.01	76.06	0.85	<0.01	87.30	0.88	<0.01	33.56	0.64	0.06
Overweight	58 (24%)	119.74	1.85		83.02	1.46		95.27	1.51		36.43	1.10	
Obese	12 (5%)	128.06	4.06		91.71	3.21		103.78	3.33		36.39	2.42	
Smoking habits													
Never smoked	138 (58%)	109.66	1.28	<0.01	76.85	1.04	0.01	87.84	1.07	<0.01	32.83	0.70	<0.01
Former smoker	8 (3%)	124.73	5.32		87.88	4.31		100.15	4.46		36.97	2.90	
Current smoker	94 (39%)	116.74	1.55		80.27	1.26		92.45	1.30		36.40	0.84	
Regular drinker													
Yes	90 (38%)	117.78	1.59	<0.01	81.56	1.28	<0.01	93.65	1.33	<0.01	36.17	0.88	0.01
No	150 (62%)	110.02	1.24		76.74	0.99		87.88	1.03		33.29	0.68	
Examination day of the week													
Monday	35 (15%)	112.10	1.64	0.10	79.04	1.37	0.46	90.12	1.34	0.28	33.02	1.19	0.11
Tuesday	31 (13%)	111.60	1.81		76.63	1.50		88.23	1.48		35.07	1.24	
Wednesday	29 (12%)	114.91	1.91		78.72	1.59		90.74	1.57		35.67	1.29	
Thursday	35 (15%)	110.93	1.83		76.69	1.51		88.18	1.50		33.86	1.21	

Variables	N (%)	Systolic Blood Pressure			Diastolic Blood Pressure			Mean Arterial Pressure			Pulse Pressure		
		Mean ^a	SE ^a	p-Value ^b	Mean ^a	SE ^a	p-Value ^b	Mean ^a	SE ^a	p-Value ^b	Mean ^a	SE ^a	p-Value ^b
Friday	36 (15%)	112.02	1.88		79.29	1.55		90.22	1.54		32.13	1.21	
Saturday	34 (14%)	113.14	2.01		79.00	1.66		90.56	1.65		34.52	1.31	
Sunday	40 (17%)	117.00	1.71		80.28	1.42		92.48	1.40		36.57	1.18	
Hypertension ^c													
Yes	31 (13%)	124.19	1.80	<0.01	90.61	1.41	<0.01	101.15	1.42	<0.01	35.53	1.32	0.33
No	209 (87%)	111.18	0.90		76.78	0.63		88.40	0.69		34.19	0.58	

^a Means and standard errors of blood pressure measured on each of the two examination days were estimated by marginal means and corresponding standard errors from mixed-effects regression models.

^b p-Values were calculated using mixed-effects regression models.

^c Hypertension was defined as systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg.

Table 2

Blood pressure by participant characteristics after work.

Variables	N (%)	Systolic Blood Pressure			Diastolic Blood Pressure			Mean Arterial Pressure			Pulse Pressure		
		Mean ^a	SE ^a	p-Value ^b	Mean ^a	SE ^a	p-Value ^b	Mean ^a	SE ^a	p-Value ^b	Mean ^a	SE ^a	p-Value ^b
Occupation, n (%)													
Office workers	120 (50%)	115.31	1.54	0.66	77.81	1.06	0.10	90.26	1.16	0.23	37.64	0.93	0.22
Truck drivers	120 (50%)	116.26	1.54		80.32	1.06		92.26	1.16		36.01	0.93	
Sex													
Female	80 (33%)	107.78	1.65	<0.01	74.22	1.20	<0.01	85.43	1.27	<0.01	33.52	1.08	<0.01
Male	160 (67%)	119.92	1.17		81.58	0.85		94.19	0.90		38.69	0.76	
Age (Quartile)													
Q1 [18–27 years]	60 (25%)	116.86	2.19	0.83	78.02	1.52	0.40	90.88	1.66	0.71	39.09	1.30	0.10
Q2 [28–32 years]	62 (26%)	114.77	2.15		77.55	1.49		89.96	1.63		37.49	1.28	
Q3 [33–37 years]	58 (24%)	114.77	2.22		80.18	1.54		91.70	1.69		34.58	1.32	
Q4 [38–46 years]	60 (25%)	116.82	2.19		80.52	1.52		92.56	1.66		36.32	1.30	
BMI													
Under and normal	170 (71%)	112.69	1.16	<0.01	76.51	0.78	<0.01	88.54	0.84	<0.01	36.28	0.78	0.28
Overweight	58 (24%)	121.27	1.98		83.76	1.33		96.21	1.45		37.48	1.34	
Obese	12 (5%)	132.85	4.35		92.21	2.92		105.81	3.18		40.80	2.94	
Smoking habits													
Never smoked	138 (58%)	112.61	1.37	<0.01	77.59	0.97	<0.01	89.21	1.05	0.01	35.12	0.84	0.01
Former smoker	8 (3%)	122.83	5.68		88.28	4.04		99.76	4.35		34.77	3.48	
Current smoker	94 (39%)	119.86	1.66		80.45	1.18		93.54	1.27		39.40	1.02	
Regular drinker													
Yes	90 (38%)	120.59	1.69	<0.01	82.43	1.18	<0.01	95.05	1.28	<0.01	38.27	1.07	0.09
No	150 (63%)	112.95	1.31		77.07	0.91		88.99	0.99		35.99	0.83	
Examination day of the week													
Monday	35 (15%)	115.70	1.65	0.73	78.04	1.33	0.54	90.64	1.31	0.59	38.01	1.29	0.31
Tuesday	31 (13%)	115.57	1.80		77.40	1.43		90.04	1.41		38.38	1.41	
Wednesday	29 (12%)	116.86	1.90		79.14	1.49		91.48	1.48		38.25	1.48	
Thursday	35 (15%)	116.87	1.82		80.78	1.42		92.96	1.41		36.23	1.39	

Variables	N (%)	Systolic Blood Pressure			Diastolic Blood Pressure			Mean Arterial Pressure			Pulse Pressure		
		Mean ^a	SE ^a	p-Value ^b	Mean ^a	SE ^a	p-Value ^b	Mean ^a	SE ^a	p-Value ^b	Mean ^a	SE ^a	p-Value ^b
Friday	36 (15%)	113.17	1.88		78.74	1.45		90.21	1.46		33.97	1.41	
Saturday	34 (14%)	116.08	2.02		79.01	1.59		91.40	1.58		36.77	1.50	
Sunday	40 (17%)	116.40	1.73		80.05	1.37		91.97	1.36		36.69	1.31	
Hypertension ^c													
Yes	31 (13%)	122.87	1.89	<0.01	90.43	1.33	<0.01	100.59	1.39	<0.01	35.91	1.51	0.50
No	209 (87%)	114.72	1.01		77.32	0.59		89.85	0.69		36.95	0.69	

^a Means and standard errors of blood pressure measured on each of the two examination days were estimated by marginal means and corresponding standard errors from mixed-effects regression models.

^b p-Values were calculated using mixed-effects regression models.

^c Hypertension was defined as systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg.

Table 3

Association between blood pressure measures with one-fold increase of histone modification level before work^a.

Effect	All participants				Office workers				Truck drivers			
	Obs ^b	Estimate	95%CI	p-Value ^c	Obs ^b	Estimate	95%CI	p-Value ^c	Obs ^b	Estimate	95%CI	p-Value ^c
H3K9ac												
Systolic pressure	217	-2.52	-4.22, -0.81	<0.01*	103	-2.30	-4.88, 0.29	0.09	114	-4.02	-6.81, -1.23	0.01*
Diastolic pressure	217	-1.12	-2.57, 0.34	0.14	103	-1.64	-3.75, 0.47	0.13	114	-0.87	-3.39, 1.66	0.50
Mean arterial pressure	217	-1.54	-2.95, -0.14	0.03*	103	-1.84	-3.94, 0.26	0.09	114	-1.91	-4.24, 0.43	0.11
Pulse pressure	217	-1.10	-2.44, 0.24	0.11	103	-0.58	-2.46, 1.31	0.55	114	-1.06	-3.35, 1.23	0.37
H3K9me3												
Systolic pressure	187	-2.04	-3.32, -0.77	<0.01*	93	-2.41	-1.19, -0.64	0.01*	94	-1.66	-3.67, 0.35	0.11
Diastolic pressure	187	-1.68	-2.84, -0.52	0.01*	93	-2.36	-3.90, -0.83	0.01*	94	-0.44	-2.53, 1.65	0.68
Mean arterial pressure	187	-1.75	-2.86, -0.64	<0.01*	93	-2.30	-3.82, -0.78	0.01*	94	-0.90	-2.79, 0.99	0.36
Pulse pressure	187	-0.24	-1.16, 0.68	0.61	93	-0.22	-1.36, 0.93	0.71	94	-0.88	-2.57, 0.80	0.31
H3K27me3												
Systolic pressure	231	-2.28	-4.42, -0.13	0.04*	114	-1.96	-5.07, 1.16	0.22	117	-3.86	-7.42, -0.30	0.04*
Diastolic pressure	231	-1.19	-3.06, 0.68	0.21	114	-1.84	-4.37, 0.70	0.16	117	-0.95	-4.33, 2.44	0.59
Mean arterial pressure	231	-1.51	-3.28, 0.26	0.10	114	-1.85	-4.35, 0.65	0.15	117	-1.78	-4.81, 1.24	0.25
Pulse pressure	231	-0.83	-2.57, 0.92	0.35	114	-0.23	-2.56, 2.11	0.85	117	-0.52	-3.72, 2.69	0.75
H3K36me3												
Systolic pressure	228	-1.46	-3.34, 0.43	0.13	113	-2.01	-4.87, 0.86	0.17	115	-1.06	-4.11, 1.99	0.50
Diastolic pressure	228	-0.07	-1.69, 1.55	0.93	113	-0.06	-2.48, 2.35	0.96	115	0.03	-2.66, 2.72	0.98
Mean arterial pressure	228	-0.49	-2.05, 1.06	0.53	113	-0.55	-2.86, 1.78	0.64	115	-0.30	-2.81, 2.21	0.81
Pulse pressure	228	-0.96	-2.43, 0.50	0.20	113	-1.56	-3.72, 0.61	0.16	115	0.44	-1.97, 2.85	0.72

^a Adjusted for occupational group, sex, age, BMI, work hours per week, day of the week, smoking habits, number of cigarettes smoked during examination time, alcohol drinking status, temperature, and 8-day ambient PM₁₀.

^b Number of non-missing observations used for analysis for histone modification measurements.

^c *p*-Values were calculated using mixed-effects regression models.

Table 4

Association between blood pressure measures with one-fold increase of histone modification level after work^a.

Effect	All participants				Office workers				Truck drivers			
	Obs ^b	Estimate	95%CI	p-Value ^c	Obs ^b	Estimate	95%CI	p-Value ^c	Obs ^b	Estimate	95%CI	p-Value ^c
H3K9ac												
Systolic pressure	217	-0.53	-2.24, 1.17	0.54	103	0.72	-1.67, 3.10	0.56	114	-0.79	-3.81, 2.24	0.61
Diastolic pressure	217	-1.00	-2.31, 0.31	0.14	103	-1.34	-3.25, 0.57	0.17	114	-0.36	-2.30, 1.58	0.72
Mean arterial pressure	217	-0.81	-2.08, 0.46	0.21	103	-0.63	-2.49, 1.23	0.51	114	-0.45	-2.43, 1.53	0.66
Pulse pressure	217	0.55	-0.95, 2.05	0.47	103	1.74	-0.50, 3.97	0.13	114	0.11	-2.22, 2.45	0.92
H3K9me3												
Systolic pressure	187	-0.51	-1.83, 0.81	0.45	93	-0.08	-1.83, 1.66	0.93	94	-0.18	-2.67, 2.30	0.89
Diastolic pressure	187	-0.34	-1.46, 0.79	0.56	93	-0.62	-2.19, 0.95	0.44	94	-0.12	-1.80, 1.57	0.89
Mean arterial pressure	187	-0.30	-1.36, 0.77	0.59	93	-0.33	-1.83, 1.18	0.67	94	0.08	-1.56, 1.74	0.92
Pulse pressure	187	-0.18	-1.37, 1.02	0.77	93	-0.21	-1.87, 1.44	0.80	94	0.47	-1.56, 2.49	0.65
H3K27me3												
Systolic pressure	231	0.09	-2.06, 2.24	0.93	114	0.70	-2.18, 3.58	0.63	117	-0.15	-3.98, 3.67	0.94
Diastolic pressure	231	-0.30	-1.98, 1.39	0.73	114	0.57	-1.76, 2.89	0.63	117	-2.38	-5.08, 0.32	0.09
Mean arterial pressure	231	-0.16	-1.81, 1.49	0.85	114	0.60	-1.65, 2.86	0.60	117	-1.71	-4.46, 1.04	0.23
Pulse pressure	231	0.14	-1.67, 1.96	0.88	114	-0.35	-3.00, 2.30	0.79	117	2.96	0.12, 5.81	0.04*
H3K36me3												
Systolic pressure	228	0.18	-1.70, 2.07	0.85	113	-0.02	-2.76, 2.73	0.99	115	1.22	-1.94, 4.38	0.45
Diastolic pressure	228	0.05	-1.39, 1.49	0.95	113	0.00	-2.19, 2.20	1.00	115	-0.66	-2.81, 1.48	0.55
Mean arterial pressure	228	0.16	-1.25, 1.57	0.82	113	0.15	-1.99, 2.28	0.89	115	0.06	-2.15, 2.26	0.96
Pulse pressure	228	-0.18	-1.80, 1.43	0.83	113	-0.53	-3.04, 1.97	0.68	115	1.49	-0.87, 3.85	0.22

^a Adjusted for occupational group, sex, age, BMI, work hours per week, day of the week, smoking habits, number of cigarettes smoked during examination time, alcohol drinking status, temperature, and 8-day ambient PM₁₀.

^b Number of non-missing observations used for analysis for histone modification measurements.

^c *p*-Values were calculated using mixed-effects regression models.