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Different exposure metrics of rotating night shift work and hyperhomocysteinaemia among Chinese steelworkers: a cross-sectional study

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ABSTRACT

Objective To examine the associations of rotating night shift work with hyperhomocysteinaemia (HHcy) odds by different exposure metrics.

Design Cross-sectional study.

Setting Occupational physical examination centre for steel production workers, Tangshan, China.

Participants A total of 6846 steelworkers, aged 22–60 years, from the baseline survey of a Chinese occupational cohort.

Primary and secondary outcome measures Different exposure metrics of night shift work, including current shift status, duration of night shifts (years), cumulative number of night shifts (nights), cumulative length of night shifts (hours), average frequency of night shifts (nights/month), average length of night shifts (hours/night) and percentage of hours on night shifts, were used to examine the effects of past and current night shift work on HHcy odds. The total homocysteine concentration in the plasma above 15 µmol/L was defined as HHcy.

Results Compared with those who never worked night shifts, current night shift workers had elevated odds of HHcy (OR 1.23, 95% CI 1.06 to 1.44). Considering a person's lifetime work schedule and compared with individuals who never worked night shifts, duration of night shifts >28 years (OR 1.35, 95% CI 1.12 to 1.61), average frequency of night shifts >7 nights/month (OR 1.25, 95% CI 1.07 to 1.47) and percentage of hours on night shifts >30% (OR 1.23, 95% CI 1.05 to 1.43) were associated with higher HHcy odds. The duration of night shifts >20 years and the average frequency of night shifts >7 nights/month could significantly increase the odds of HHcy regardless of whether the average length of night shifts was greater than 8 hours/night. After stratification by sex, no significant association was found in female workers between different exposure metrics of night shift work and HHcy.

Conclusions Long duration and high frequency of night shift work are associated with higher HHcy odds among male steelworkers.

Strengths and limitations of this study

- The present study highlights the effects of different exposure metrics of night shift work on hyperhomocysteinaemia (HHcy), since complete avoidance of rotating night shift work is difficult due to socioeconomic realities.
- This study included detailed information on shift work by interview and verified by company records, which makes this exposure assessment reliable.
- Few studies have evaluated the impact of night shift work on HHcy among females; this study is an exception.
- This study cannot evaluate the long-term trend of homocysteine based on a one-time measurement.
- Given the cross-sectional nature of this study, we cannot infer the temporality of night shift work and HHcy.

INTRODUCTION

It has long been recognised that shift workers are at risk of suffering adverse health outcomes due to misalignment between the circadian clock and behavioural cycles. The disruption of the circadian rhythm has been implicated in the pathogenesis of many diseases. The discovery of the molecular mechanism of the circadian clock, which has been recognised with the awarding of the Nobel Prize in Physiology and Medicine in 2017, has brought the study of circadian rhythm and its health consequences to a new era.[1] Moreover, the International Agency for Research on Cancer has classified night shift work involving circadian disruption as 'probably carcinogenic to humans' (group 2A) according to the latest evaluation of the carcinogenicity in 2019.[2] Despite the adverse effects of night shift work, our society is increasingly dependent on round-the-clock production and activities to coordinate with the socioeconomic realities. Approximately 20% of workers worldwide are engaged in night shift work.[3] In this circumstance, interpreting the effects of different exposures of night shift work can provide evidence for health-related interventions

among night shift workers, since complete avoidance of rotating night shift work is difficult.

Hyperhomocysteinaemia (HHcy) is a medical condition characterised by an abnormally high levels of total homocysteine (tHcy) in the plasma (tHcy concentrations>15 µmol/L). The pooled prevalence of HHcy was 27.5% in China.[4] HHcy has been claimed to be a significant risk factor for a number of disease states, including cardiovascular disease (CVD),[5] neuropsychiatric illness[6 7] and fractures.[8] A previous meta-analysis showed that shift work was associated with myocardial infarction, ischaemic stroke and coronary events without considering the duration of shift work.[9] In addition, a subsequent study updated the evidence on the association between shift work and CVD mortality and morbidity risk, which even concluded that there was a nonlinear dose–response relationship between duration of shift work and CVD risk.[10]

Rhythmicity in homocysteine levels has been observed in a previous study.[11]The misalignment due to night shift work may be implicated in the pathogenesis of HHcy by disrupting glucose metabolism.[12] Additionally, desynchronisation between circadian clocks and the external world light/dark cycle due to night-time light exposure could contribute to the suppression of melatonin secretion, which in turn leads to HHcy.[13 14] Shift work and HHcy as risk factors for CVD have been supported by a large number of epidemiological studies, but whether shift work-induced disruption of the circadian rhythm also contributes to HHcy remains to be determined.

Previous studies have shown an association between night shift work and HHcy.[15 16] However, it must be noted that there is considerable variability in the detail, quality and definitions of exposure information on night shift work, since implementation of night shift work differs across regions and employment sectors. In addition, considering that reducing homocysteine levels does not improve cardiovascular outcomes and since avoiding night shifts is unrealistic,[17] it is, therefore, necessary to explore the effect of different night shift work exposures on HHcy for the formulation of effective preventive measures. To the best of our knowledge, there is currently no study on the relationship between the duration, frequency, and intensity of night shift work and HHcy. In this study, different exposure metrics including current shift status, duration of night shifts (years), cumulative number of night shifts (nights), cumulative length of night shifts (hours), average frequency of night shifts (nights/month), average length of night shifts (hours/night) and percentage of hours on night shifts, were used to examine the effects of past and current night shift work on HHcy odds.

METHODS

Study design and population

This study reported results from the baseline survey of a Chinese occupational cohort conducted among steelworkers who were prospectively recruited at 11 steel

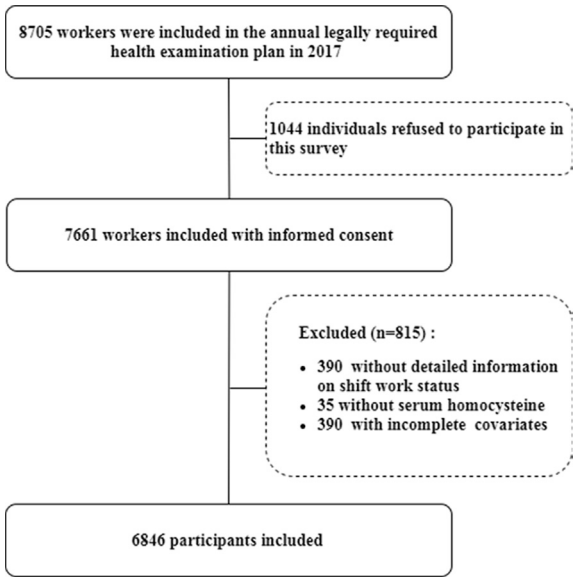

Figure 1 Flow chart of the participant selection.

production departments owned by the HBIS Group's Tangsteel Company in Tangshan City, Hebei Province in north China.[18] All workers at this company underwent a legally required health examination each year. From February to June 2017, 7661 participants were recruited. Dust, heat stress, noise and carbon monoxide are the major occupational hazards to the current workers. Workers without detailed lifetime shift work information, those who provided incomplete covariate data on the questionnaire, and those who did not have homocysteine levels in the plasma were excluded; 6846 participants were included in this study (figure 1). Compared with workers excluded, those who were included were older (44.2±8.0 years vs 34.4±7.7 years, p<0.001), and had a higher proportion of women (8.54% vs 5.90%, p=0.010). All participants gave informed consent before taking part in this study.

Ascertainment of HHcy

Fasting blood was drawn from the participant's forearm vein between 08:00 and 09:30 hours. For night shift workers, blood was drawn in the morning of a day off work. The enzymatic cycling method was used to test the homocysteine concentration in the plasma (HCY kit, Beijing Strong Biotechnologies, Beijing, China). Within-laboratory intra-assay and interassay variable coefficients for serum homocysteine were <5% and <9%, respectively. tHcy concentration in the plasma above 15 µmol/L was defined as HHcy.[19]

Assessment of night shift work

The main work schedule of the present study population has been introduced in detail in our previous research.[18] In brief, shift work in this study refers to rotating night shifts (the mainly four-crew-three-shift system now and historical three-crew-two-shift system). Workers who worked regular working hours at all times were defined as never night workers. In this study, the detailed lifetime

employment history was collected by face-to-face personal interviews and all the reported information was verified with the company's records. Participants who were recruited were asked to report whether they were involved in rotating night shift work (working through 00:00 to 6:00 hours) during their employment (current shift status: 'never', 'ever, 'current'). If yes (ever or current), they would be further asked about the start and end dates of each shift system, the average number of night shifts per month in each shift system, the daily working hours, and usual days off per month. Using that information, different exposure metrics of night shift were derived. Duration of night shifts (years): sum of years spent in all different night shift systems; cumulative number of night shifts (nights): sum of nights spent in all different night shift systems; cumulative length of night shifts (hours): sum of hours spent in all different night shift systems; average length of night shifts (hours/night): cumulative length of night shifts (hours) divided by cumulative number of night shifts (nights); average frequency of night shifts (nights/month): cumulative number of night shifts (nights) divided by cumulative number of months of employment; percentage of hours on night shifts: cumulative length of night shifts (hours) divided by total working hours of employment.[20 21]

Assessment of covariates

Covariates mainly included age, sex, body mass index (BMI), smoking status, drinking status, educational level, physical activity, sedentary behaviour, sleep duration, insomnia, diet (dietary approaches to stop hypertension, DASH), diabetes, dyslipidaemia, hypertension, liver enzyme abnormality and decreased estimated glomerular filtration rate (eGFR) (see online supplemental appendix).

Statistical analysis

Continuous variables are presented as the means and SD, and between-group comparisons were performed using Student's t-test if the data were normally distributed. Otherwise, the median (upper quartile-lower quartile) and Wilcoxon Scores (rank sums) test were used to describe and compare these continuous variables between groups. Categorical variables are presented as numbers and percentages, and the χ^2 test was used to compare differences among groups.

Multiple logistic regression models were used to examine the association of different exposure metrics of night shift work in relation to HHcy prevalence. Potential confounders, including age, sex, BMI, smoking status, dyslipidaemia, hypertension and decreased eGFR, were included in the multiple analysis. For continuous exposure variables, restricted cubic spline (RCS) models were also used to visually examine the association between night shift work and tHcy (as continuous and categorical variables, respectively) with adjustment for potential confounders. For the independent effects and interactions of duration, frequency and intensity of night shifts on HHcy, the medians of duration of night shifts, average frequency of night shifts and average length of night shifts were chosen as cut-off points to secure a reasonable number of observations in each exposure category and cross-classification group.

We tested for the presence of multiplicative interaction by including a cross-product term between the duration of night shifts and the average frequency of night shifts in our fully adjusted multivariable model. To assess additive interaction between duration of night shifts and average frequency of night shifts on odds of HHcy, indicators including relative excess risk due to interaction and attributable proportion due to interaction were used.[22] $p<0.05$ was regarded as significant for two-sided tests. Several sensitivity analyses were performed to test the robustness of our results, including further adjustments for major occupational hazards, and the elimination of the last 1% quantile of the duration of night shifts. All statistical analyses were performed using SAS V.9.3 (SAS Institute).

Patient and public involvement

No patients were involved in the development of the research question and outcome measures, design, recruitment to and conduct of the study. The study results will be disseminated to participants by our customised app.

RESULTS

General characteristics of the participants

Table 1 shows the general characteristics of study participants according to HHcy status. The present study of 6846 included participants consisted of 91.5% males, with a mean age of 44.2 years. Among all workers included, the proportions of ever and current night shift workers were 21.8% and 63.2%, respectively. Overall, workers with HHcy were more likely to be current night shift workers, men, current smokers and those with hypertension or hyperlipidaemia. As shown in online supplemental table S1, the prevalence rates of HHcy in males and females were 32.4% and 10.8%, respectively. Online supplemental table S2 shows the basic characteristics of the participants according to the duration of night shifts. Current smoking, current drinking and sedentary behaviour were more likely to be reported among long-term shift workers. In terms of current health status, long-term shift workers also showed higher prevalence rates of diabetes, dyslipidaemia, hypertension and a lower eGFR. In addition, the prevalence of HHcy also showed age difference (online supplemental table S3).

Different exposure metrics of night shift work and HHcy

The prevalence rates of HHcy among ever night shift workers and current night shift workers were 28.9% and 31.9%, respectively, which were higher than the rate among those who never worked night shifts (27.3%). After classification by different exposure metrics of night shift work, higher HHcy prevalence rates were observed

Table 1 Basic characteristics according to HHcy status

Characteristics	Total N=6846	Non-HHcy n=4753	HHcy n=2093	P value
Current shift status, n (%)				0.004
Never	1027 (15.0)	747 (15.7)	280 (13.4)	
Ever	1493 (21.8)	1062 (22.3)	431 (20.6)	
Current	4326 (63.2)	2944 (61.9)	1382 (66.0)	
Duration of employment, year	23.9 (9.0)	23.8 (8.9)	24.2 (9.2)	0.202
Age, (year), mean±SD	44.2±8.0	44.1±8.0	44.5±8.1	0.052
Age (year), n (%)				0.001
22–29	399 (5.8)	269 (5.7)	130 (6.2)	
30–39	1745 (25.5)	1242 (26.1)	503 (24.0)	
40–49	2979 (43.5)	2111 (44.4)	868 (41.5)	
50–60	1723 (25.2)	1131 (23.8)	592 (28.3)	
Sex (male), n (%)	6261 (91.5)	4231 (89.0)	2030 (97.0)	<0.001
BMI (kg/m^2), mean±SD	25.2±3.4	25.1±3.4	25.4±3.4	0.007
BMI (kg/m^2), n (%)				0.167
<25	3466 (50.6)	2441 (51.4)	1025 (49.0)	
25–30	2830 (41.3)	1941 (40.8)	889 (42.5)	
≥30	550 (8.0)	371 (7.8)	179 (8.6)	
Smoking status, n (%)				<0.001
Never	2809 (41.0)	2101 (44.2)	708 (33.8)	
Ever	547 (8.0)	399 (8.4)	148 (7.1)	
Current	3490 (51.0)	2253 (47.4)	1237 (59.1)	
Drinking status, n (%)				0.082
Never	3926 (57.4)	2764 (58.2)	1162 (55.5)	
Ever	392 (5.7)	275 (5.8)	117 (5.6)	
Current	2528 (36.9)	1714 (36.1)	814 (38.9)	
Education level, n (%)				0.242
Primary or illiterate	86 (1.3)	60 (1.3)	26 (1.2)	
Middle or high school	5304 (77.5)	3656 (76.9)	1648 (78.7)	
University or college	1456 (21.3)	1037 (21.8)	419 (20.0)	
Physical activity (MET-h/week), median (IQR)	121.8 (84.0–150.7)	121.8 (84.0–150.0)	121.8 (84.0–153.3)	0.382
DASH score	21.7 (2.2)	21.7 (2.2)	21.6 (2.1)	0.065
Sedentary behaviour (hour), median (IQR)	2.6 (1.3–4.3)	2.6 (1.3–4.3)	2.6 (1.3–4.1)	0.470
Sleep duration, hour	6.8 (1.2)	6.8 (1.2)	6.8 (1.2)	0.606
Insomnia, n (%)	2675 (39.1)	1856 (39.1)	819 (39.1)	0.949
Diabetes, n (%)	719 (10.5)	499 (10.5)	220 (10.5)	0.988
Dyslipidaemia, n (%)	2781 (40.6)	1865 (39.2)	916 (43.8)	<0.001
Hypertension, n (%)	1755 (25.6)	1133 (23.8)	622 (29.7)	<0.001
Liver enzyme abnormality, n (%)	1384 (20.2)	931 (19.6)	453 (21.6)	0.051
Plasma creatinine (μ mol/L)	77.2±15.8	76.2±15.9	79.4±15.5	<0.001
eGFR (mL/min/1.73 m2)	101.7±11.4	102.4±11.0	100.1±12.2	<0.001
Range of eGFR (mL/min/1.73 m2), n (%)				<0.001
G1 (≥90)	5842 (85.3)	4128 (86.9)	1714 (81.9)	
G2 (60–89)	982 (14.3)	615 (12.9)	367 (17.5)	
G3a–G5 (<60)	22 (0.3)	10 (0.2)	12 (0.57)	

Values are expressed as the mean±SD or median (IQR) or number (%); p values were from Pearson's χ^2 test for categorical variables and Student's t-test or Wilcoxon scores (rank sums) for continuous variables.
BMI, body mass index; DASH, dietary approaches to stop hypertension; eGFR, estimated glomerular filtration rate; HHcy, hyperhomocysteinaemia; MET, metabolic equivalent units.

in the groups with higher exposure categories of night shift work (online supplemental figure S1). As shown in table 2, compared with those who never worked night shifts, significantly elevated odds of HHcy were observed in the highest exposure categories (model 1). After adjustment for age and sex, these estimates were attenuated but remained significant (model 2). Model 3 additionally adjusted for BMI, smoking status, drinking status, dyslipidaemia, hypertension and decreased eGFR, and the results were overall comparable to Model 2. In the multivariable adjustment model (Model 3), current shift workers had higher HHcy odds than those who never worked night shifts (OR 1.23, 95% CI 1.06 to 1.44). Participants in the highest exposure categories of duration of night shifts, cumulative number of night shifts, cumulative length of night shifts, average frequency of night shifts, average length of night shifts and percentage of hours on night shifts had increased odds of HHcy, with ORs (95% CI) of 1.35 (1.12 to 1.61), 1.34 (1.11 to 1.61), 1.33 (1.11 to 1.61), 1.25 (1.07 to 1.47), 1.27 (1.01 to 1.60) and 1.23 (1.05 to 1.43), respectively, when compared with those who never worked night shifts (table 2, model 3). The odds of HHcy associated with other categories among different metrics of night shift work and their linear trends can also be observed in online supplemental figure S2. In the RCS models, positive correlations between homocysteine (as a continuous variable: plasma tHcy concentration (μmol/L), or a binary variable: HHcy (yes/no)) and duration of night shifts (continuous, years), and cumulative number of night shifts (continuous, nights), cumulative length of night shifts (continuous, hours) were observed (figure 2).

We then evaluated the independent effects of current shift status, duration of night shifts (years), average frequency of night shifts (nights/month) and average length of night shifts (hours/night) in relation to HHcy among night shift workers (ever or current). After mutually adjusting for current shift status duration of night shifts (years), average frequency of night shifts (nights/month), average length of night shifts (hours/night) and other confounders, the highest levels of exposure to night shift work independently increased the odds of HHcy, but this increase was insignificant (online supplemental table S4). Cross-classification results showed that exposure to duration of night shifts >20 years and average frequency of night shifts >7 nights/month can significantly increase the odds of HHcy (table 3). The duration of night shifts>20 years and the average frequency of night shifts >7 nights/month were jointly associated with higher odds of HHcy (OR 1.20, 95% CI 1.03 to 1.39). However, no significant multiplicative or additive interactions between the duration of night shifts and the average frequency of night shifts on the odds of HHcy were observed (online supplemental table S5).

The results of stratified analysis by sex showed that the association between different exposure metrics of night shift work and HHcy among male workers was comparable to the total study population. However, no significant associations between different exposure categories of night shift work and HHcy were observed among female workers (online supplemental table S6).

Sensitivity analyses

Considering that dust, heat stress, noise and carbon monoxide are the major occupational hazards to the current steelworkers, we additionally adjusted for these exposures on the basis of model 3 in table 2, and the results were similar to model 3 in table 2 (online supplemental table S7). Moreover, to avoid the influence of the maximum value on the fitting result of RCSs, we removed the last 1% quantile of the duration of night shifts (years), the cumulative number of night shifts (nights) and the cumulative length of night shifts (hours), and the relationships remained robust (online supplemental figure S3).

DISCUSSION

This study examined the cross-sectional association of night shift work with HHcy odds among 6846 steelworkers, and found that elevated odds of HHcy were associated with the highest exposure categories of night shift work when applying different exposure metrics for night shift work. The present study also provided additional evidence concerning dose–response relationships between the duration of night shifts and HHcy among steelworkers, which have never been reported in previous studies. Moreover, long duration of night shifts (>20 years) and high frequency of night shifts (>7 nights/month) were jointly associated with higher odds of HHcy.

Consistent with our findings, several previous studies have found that shift work is related to higher levels of plasma homocysteine.[15 16 23] In addition, what we already know is that HHcy is an independent risk factor for CVD, and largely confirms epidemiological data showing that night shift workers are at increased risk of CVD,[24] which indirectly supports the association between night shift work and HHcy. It is noteworthy that the previous studies on the relationship between night shift work and HHcy often have a coarse assessment of night shift work (usually divided into two categories: day workers and shift workers). However, simply exploring the relationship between night shift work (yes/no) and HHcy is not enough to provide guideline recommendations regarding the risk related to shift schedules, since complete avoidance of rotating night shift work is difficult when socioeconomic realities are taken into consideration. Our findings highlight the effect of different exposure metrics of night shift work on HHcy and extend the positive association between night shift work and odds of HHcy. In addition to the duration of night shifts, we also found that the frequency and length of night shifts are related to odds of HHcy. A high shift rotation frequency increases the likelihood of 'quick returns' between shifts,[25] which can serve as a 'big problem in life' among some shift workers.[26] In previous studies, high shift rotation frequency has been associated

Table 2 Associations of different exposure metrics of night shift work with HHcy odds

Exposure metrics	HHcy		OR (95% CI)		
	No, (n (%))	Yes, (n (%))	Model 1	Model 2	Model 3
Current shift status, n (%)					
Never	747 (15.7)	280 (13.4)	1.00	1.00	1.00
Ever	1062 (22.3)	431 (20.6)	1.08 (0.91 to 1.29)	1.07 (0.89–1.28)	1.08 (0.90–1.29)
Current	2944 (61.9)	1382 (66.0)	1.25 (1.08 to 1.46)	1.23 (1.05–1.44)	1.23 (1.06–1.44)
Duration of night shifts, (years)					
Never	747 (15.7)	280 (13.4)	1.00	1.00	1.00
Q1 (1–12)	747 (15.7)	280 (13.4)	1.16 (0.97 to 1.39)	1.13 (0.95–1.36)	1.15 (0.96–1.38)
Q2 (13–20)	1022 (21.5)	445 (21.3)	1.04 (0.87 to 1.25)	1.04 (0.86–1.25)	1.05 (0.87–1.27)
Q3 (21–27)	1072 (22.6)	419 (20.0)	1.17 (0.97 to 1.40)	1.17 (0.97–1.41)	1.19 (0.98–1.43)
Q4 (28–43)	910 (19.2)	398 (19.0)	1.47 (1.24 to 1.74)	1.38 (1.15–1.65)	1.35 (1.12–1.61)
P trend			<0.001	0.001	0.003
Cumulative number of night shifts (nights)					
Never	747 (15.7)	280 (13.4)	1.00	1.00	1.00
Q1 (43–1131)	1012 (21.3)	443 (21.2)	1.17 (0.98 to 1.39)	1.14 (0.95–1.36)	1.15 (0.96–1.38)
Q2 (1132–1848)	1050 (22.1)	405 (19.4)	1.03 (0.86 to 1.23)	1.03 (0.85–1.24)	1.04 (0.86–1.26)
Q3 (1854–2584)	1006 (21.2)	450 (21.5)	1.19 (1.00 to 1.42)	1.20 (1.00–1.44)	1.21 (1.01–1.45)
Q4 (2585–5239)	938 (19.7)	515 (24.6)	1.47 (1.23 to 1.74)	1.37 (1.14–1.65)	1.34 (1.11–1.61)
P trend			<0.001	0.001	0.004
Cumulative length of night shifts (hours)					
Never	747 (15.7)	280 (13.4)	1.00	1.00	1.00
Q1 (344–9488)	1006 (21.2)	446 (21.3)	1.18 (0.99 to 1.41)	1.15 (0.96–1.39)	1.16 (0.97–1.40)
Q2 (9490–15259)	1054 (22.2)	404 (19.3)	1.02 (0.86 to 1.22)	1.02 (0.84–1.23)	1.04 (0.86–1.25)
Q3 (15 265–21293)	1007 (21.2)	449 (21.5)	1.19 (1.00 to 1.42)	1.18 (0.99–1.42)	1.20 (1.00–1.45)
Q4 (21 295–53541)	939 (19.8)	514 (24.6)	1.46 (1.23 to 1.74)	1.37 (1.14–1.65)	1.33 (1.11–1.61)
P trend			<0.001	0.003	0.006
Average frequency of night shifts					
Never	747 (15.7)	280 (13.4)	1.00	1.00	1.00
<3 nights/month	1110 (23.4)	466 (22.3)	1.12 (0.94 to 1.33)	1.11 (0.93–1.32)	1.11 (0.93–1.33)
3–7 nights/month	1746 (36.7)	265 (12.7)	1.11 (0.91 to 1.36)	1.09 (0.89–1.34)	1.10 (0.91–1.35)
>7 nights/month	2260 (47.6)	1082 (51.7)	1.28 (1.09 to 1.49)	1.25 (1.07–1.47)	1.25 (1.07–1.47)
P trend			0.001	0.003	0.004
Average length of night shifts					
Never	747 (15.7)	280 (13.4)	1.00	1.00	1.00
≤8 hours/night	3082 (64.8)	1360 (65.0)	1.18 (1.01 to 1.37)	1.16 (1.00–1.36)	1.17 (1.01–1.37)
8–9 hours/night	551 (11.6)	259 (12.4)	1.25 (1.03 to 1.53)	1.21 (0.99–1.48)	1.23 (1.00–1.52)
>9 hours/night	373 (7.9)	194 (9.3)	1.39 (1.11 to 1.73)	1.30 (1.04–1.63)	1.27 (1.01–1.60)
P trend			0.003	0.022	0.031
Percentage of hours on night shifts, n (%)					
Never	747 (15.7)	280 (13.4)	1.00	1.00	1.00
<20%	573 (12.1)	246 (11.8)	1.15 (0.94 to 1.40)	1.14 (0.93–1.40)	1.15 (0.94–1.42)
20%–30%	747 (15.7)	309 (14.8)	1.10 (0.91 to 1.34)	1.08 (0.89–1.31)	1.09 (0.90–1.32)
>30%	2686 (56.5)	1258 (60.1)	1.25 (1.07 to 1.46)	1.23 (1.05–1.44)	1.23 (1.05–1.43)
P trend			0.004	0.009	0.010

Model 1: unadjusted; model 2: adjusted for age and sex; model 3: adjusted for age, sex, BMI, smoking status, dyslipidaemia, hypertension and decreased eGFR. The cut-off points of average frequency of night shifts, average length of night shifts and percentage of hours on night shifts were chosen to secure a reasonable number of observations in each category.
BMI, body mass index; eGFR, estimated glomerular filtration rate; HHcy, hyperhomocysteinaemia.;

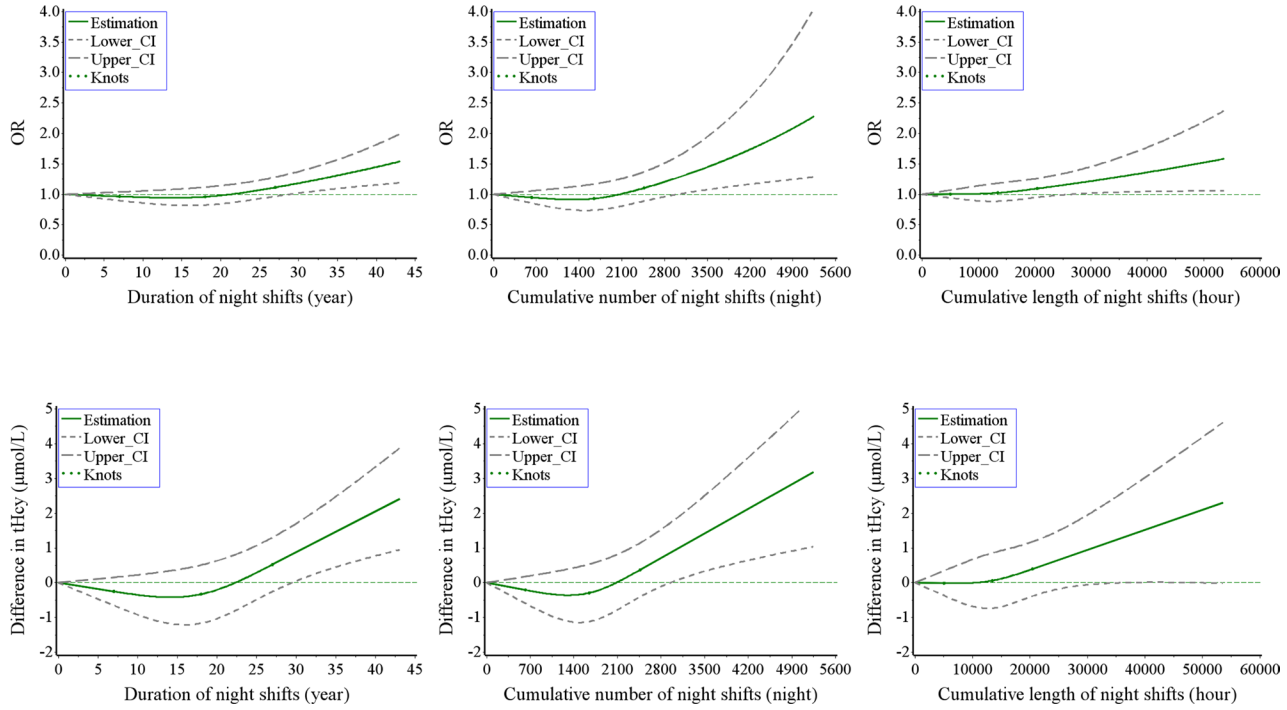

Figure 2 Associations of duration, cumulative number and cumulative length of night shifts with tHcy (as a continuous or a binary variable) from restricted cubic spline models. 'Difference in tHcy' indicates difference of tHcy (μmol/L) levels in the plasma where the reference values for duration, cumulative number and cumulative length of night shifts are all 0 (never worked night shifts). Adjusted for age, sex, BMI, smoking status, dyslipidaemia, hypertension and decreased eGFR. BMI, body mass index; eGFR, estimated glomerular filtration rate; HHcy, hyperhomocysteinaemia; tHcy, total homocysteine.

with employee performance and health, including poor sleep quality, disrupted social relationships[27] and type 2 diabetes.[28] In terms of shift length (between 8 and 12 hours shifts), the effect of long shifts on employee performance and satisfaction has been widely explored in occupational sectors, with inconsistent results.[29] Based on the results of previous large cross-sectional studies conducted among nurses (sample size ranging from 3710 to 22 275 nurses) in the USA that concluded that long shifts were negatively associated with employees' job performance and psychological well-being,[30–32] our study expanded the evidence of adverse effects of long shifts on physical health. The added finding in this study is that when the duration, frequency, and length of night shifts are considered simultaneously, significantly higher odds of HHcy are limited to participants who have been exposed to duration of night shifts >20 years and have a frequency of night shifts >7 nights/month. This implies that for long duration night shift workers, modifying the rotation frequency of night shifts (ie, <7 nights/

Table 3 Association between night shift work and HHcy odds by cross-classification analysis

Duration of night shifts	Average frequency of night shifts	Average length of night shifts	HHcy		
			No, (n (%))	Yes, (n (%))	OR (95% CI)
Never	Never	Never	747 (15.7)	280 (13.4)	1.00
≤20 years	≤7 nights/month	≤8 hours/night	911 (19.2)	362 (17.3)	1.08 (0.90 to 1.31)
≤20 years	≤7 nights/month	>8 hours/night	351 (7.4)	154 (7.4)	1.16 (0.91 to 1.47)
≤20 years	>7 nights/month	≤8 hours/night	808 (17.0)	339 (16.2)	1.11 (0.90 to 1.37)
≤20 years	>7 nights/month	>8 hours/night	24 (0.5)	9 (0.4)	0.97 (0.44 to 2.13)
>20 years	≤7 nights/month	≤8 hours/night	294 (6.2)	128 (6.1)	1.11 (0.86 to 1.43)
>20 years	≤7 nights/month	>8 hours/night	190 (4.0)	87 (4.2)	1.15 (0.85 to 1.55)
>20 years	>7 nights/month	≤8 hours/night	1069 (22.5)	531 (25.4)	1.29 (1.08 to 1.55)
>20 years	>7 nights/month	>8 hours/night	359 (7.6)	203 (9.7)	1.43 (1.13 to 1.79)

Adjusted for age, sex, BMI, smoking status, dyslipidaemia, hypertension and decreased eGFR. The cut-off points of the duration of night shifts, average frequency of night shifts and average length of night shifts were the median of the corresponding continuous variables.
BMI, body mass index; eGFR, estimated glomerular filtration rate; HHcy, hyperhomocysteinaemia.;

month) may reduce the odds of HHcy. Moreover, given that both exposure and outcome may be related to sex, we added female-specific findings. However, no association was observed between night shift work and HHcy in females. Considering the relationship between HHcy and CVD, our finding was indirectly supported by a previous study, which reported that shift work was associated with carotid atherosclerosis only in men, but not in women.[33] In fact, due to sex differences in fat-free mass and estradiol concentrations, the level of plasma tHcy in males is consistently higher than that in females in the general population.[34] This could explain the sex discrepancies, at least in part, in the effect of night shift work on HHcy. Considering the small sample size of women in this study, the lack of association between night shift work and HHcy in women should be verified in large-scale cohort studies.

Several possible pathways from night shift work to negative health outcomes have been established, such as circadian misalignment, shift work-related unhealthy lifestyles and stress due to a disturbed social life. Numerous physiological functions exhibit substantial circadian oscillations synchronised with the sleep/wake and light/dark cycles. Rhythmicity in homocysteine levels has been observed in a previous study.[11] The misalignment between the circadian clock and behavioural cycles, including rest/activity and feeding/fasting cycles due to night shift work, may be implicated in the pathogenesis of HHcy by disrupting glucose metabolism.[12 35] Additionally, previous evidence has shown that under conditions where melatonin levels are decreased, there would be a possible increase in tHcy concentrations.[13] Therefore, desynchronisation between circadian clocks and the external world light/dark cycle due to nighttime light exposure could contribute to the suppression of melatonin secretion,[14] which in turn leads to HHcy. Moreover, shift workers usually had poorer health habits than day workers, including smoking and unhealthy diet,[33] which were strongly associated with HHcy.[36 37] Furthermore, chronic stress due to a disturbed social life may also explain the elevated risk of HHcy in shift workers.[38 39]

The major strengths of our study include detailed shift work information, lifestyle information and health status related to both exposure and outcome, and a large sample size. To our knowledge, this is the first study to explore the relationship between different exposure metrics of night shifts and HHcy. Despite the limited sample size, we also conducted an exploratory sex-specific analysis of the relationship between night shift work and odds of HHcy. The present study also has certain limitations. First, given the cross-sectional nature of this study, we cannot infer the temporality of night shift work and HHcy, nor can we evaluate the long-term trend of tHcy based on a one-time measurement. Second, plasma levels of vitamin B_6, folic acid (vitamin B_9) and vitamin B_{12} were not tested, deficiencies of which can directly lead to an increase in homocysteine levels.[40] Nonetheless, this may not be regarded as a major bias, since we considered the distribution of the DASH diet (rich in fruits, vegetables, whole grains and dairy), which has beneficial effects on homocysteine that have been confirmed in previous studies .[41] Third, genetic defects (such as polymorphisms at MTHFR C677T and MTR A2756G) in 5-MTHF reductase can consequently lead to HHcy,[42 43] but due to funding constraints we did not analyse the genotypes of the relevant sites of the participants. However, a previous well-designed case–control study showed that higher levels of plasma tHcy in shift-working bus drivers were not secondary to other biochemical problems, including folic acid, vitamin B_{12} and C677T mutations,[15] meaning that our results still have a certain reference value. Fourth, the absence of chronotype information may have led to a confounding bias. Finally, this study was conducted in the steel production occupational setting, which limits the results of this study to the general population.

CONCLUSIONS

In conclusion, this study showed that when the duration, frequency and length of night shifts are considered simultaneously, significantly higher odds of HHcy are limited to participants with a long duration of night shifts (>20 years) combined with a high frequency of night shifts (>7 nights/month). These findings imply that for long-duration night shift workers, modifying the rotation frequency of night shifts may reduce the odds of HHcy. Further prospective studies are warranted to confirm our findings.

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Contributors SZ raised the study concept and drafted the manuscript. JY designed this work. YW, ZW and HW analysed the data. CX, QL and YZ provided inputs and revisions. JY and WG supervised the fieldwork of this project. All authors agreed to submit this article.

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Supplementary files

Different exposure metrics of rotating night shift work and hyperhomocysteinemia among Chinese steelworkers: a cross-sectional study

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Assessment of covariates

Smoking and drinking status were divided into "never", "ever" and "current". Dietary patterns were assessed based on the DASH diet score.[1] The level of education was divided into three categories: "primary or illiterate," "middle or high school," and "university or college." The calculation of metabolic equivalents was based on the International Physical Activity Questionnaire (IPAQ).[2] The sleep quality assessment was estimated using the Athens Insomnia Scale (AIS).[3] Sedentary behaviour (hours/day) was assessed using a set of open-ended questions on the average working days and rest days time spent over the last four weeks on: television viewing (including DVDs and videos) and any other sitting during leisure time (including reading, studying, using a computer, and playing video games).[4] The durations of sleep and sedentary behaviour were the weighted averages of sleep and sedentary behaviour on working days and rest days, respectively.

Standard study protocols were used to train qualified physicians and nurses prior to this survey. Height and weight were measured three times each. The participants stood upright and barefoot in light clothes. The height and weight data that were ultimately used for analysis were accurate to 0.1

cm and 0.1 kg. Body mass index (BMI) was defined as body weight (kg) divided by the square of the body height (m^2). Blood pressure measurements were performed three times at five-minute intervals using an electronic sphygmomanometer (OMRON, HBP-1100, China), and the participants were required to rest for more than ten minutes. Finally, the mean was obtained for analysis. Elevated blood pressure was defined as current systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or if the patient was receiving antihypertensive therapy. Participants were required to fast overnight before the abdominal ultrasound examination and blood collection. Participants' anterior elbow vein blood was collected and centrifuged at room temperature (3000 r/min, 15 minutes) immediately. All blood samples were tested in the central laboratory of Tangshan Hongci Hospital Laboratory using automatic biochemical analysers (mindrary, BS-800, China) within four hours. Total cholesterol (TC) ≥6.22 mmol/L, low-density lipoprotein (LDL-C) ≥4.11 mmol/L, or high-density lipoprotein (HDL-C) ≤1.04 mmol/L or triglycerides (TG) ≥2.32 mmol/L, or patients undergoing lipid-lowering therapy were considered to demonstrate dyslipidaemia.[5] Alanine aminotransferase (ALT) >40 U/L, aspartate aminotransferase (AST) >40 U/L or glutamyl transpeptidase (γ-GT) >58 U/L was defined as abnormal liver enzymes. Diabetes was defined as fasting blood glucose ≥7.0 mmol/L or if the patient was receiving hypoglycaemic therapy. Assessment of estimated glomerular filtration rate (eGFR) was based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[6] The CKD-EPI formula is as follows: eGFR (mL/min/1.73 m^2) = 141 × min (Scr/κ, 1)$^{\alpha}$ × max (Scr/κ, 1)$^{-1.209}$ × 0.993^{Age} × (1.018 if female) × (1.159 if black). Scr indicates serum creatinine (μmol/L), κ = 0.7 for females and 0.9 for males, α = -0.329 for females and -0.411 for males, min and max indicate the minimum of Scr/κ or 1, the maximum of Scr/κ or 1, respectively. According to the Kidney Disease Improving Global Outcomes 2012 recommendations, the range of GFR (mL/min/1.73 m^2) was classed into five categories: normal or high (GFR ≥90, G1), mildly decreased (GFR: 60–89, G2), mildly to moderately decreased (GFR: 45–59, G3a), moderately to severely decreased (GFR: 30–44, G3b), severely decreased (GFR: 15–29, G4), and kidney failure (GFR <15, G5).[7] Based on these classification criteria, only 22 (0.3%) participants had eGFR<60 mL/min/1.73 m^2 (G3a-G5), so we combined these categories into G2 in the subsequent analysis and defined them as "decreased eGFR" (<90 mL/min/1.73 m^2).

Exposure to dust was defined as workers who may be exposed to productive dust (inorganic

dust, organic dust or mixed dust) during production (GBZ/T 229.1–2010).[8] The total dust in the air of the workplace was collected at the breathing zone with a filter membrane, and its concentration was calculated based on the increased weight of the filter membrane and the amount of gas collected. When the dust concentration in the air was ≤50 mg/m^3, a filter membrane with a diameter of 37 mm or 40 mm was used; otherwise, a filter membrane with a diameter of 75 mm was used (GBZ/T 192.1–2007).[9] Exposure to heat stress work was defined as the average wet-bulb globe temperature (WBGT) index of the workplace being equal to or greater than 25°C in the process of production (GBZ 2.2–2007).[10] The WBGT index was measured by a black-wet bulb globe thermometer. If there was no productive heat source in the workplace, three measuring points were selected to take the average value of the WBGT index; if where there was a productive heat source, 3 to 5 measuring points were selected to take the average value of the WBGT index. If the workplace was isolated into different thermal or ventilated environments, 2 measuring points were selected to take the average value of the WBGT index (GBZ/T 189.7–2007).[11] Exposure to industrial toxicants was defined as workers who may be exposed to a variety of harmful chemicals (the toxicant specifically refers to carbon monoxide in this population) during production (GBZ/T 229.2–2010).[12] Carbon monoxide or carbon dioxide in the air of the workplace was pumped into a non-dispersive infrared-ray (NDIR) analyzer and selectively absorbed its infrared rays. The concentration of carbon monoxide was determined according to the absorption value (GBZ/T 160.28–2004).[13] Exposure to noise was defined as workers who were exposed to a noisy environment where the 8-h/d or 40-h/week equivalent A-weighted sound pressure level was ≥80 dB, which may be harmful to health and hearing (GBZ/T 229.4–2012).[14] The workplace production noise was measured by a sound level meter. If the distribution of the sound field in the workplace was uniform (between-field difference of A-sound levels were less than 3 dB(A)), three measuring points were selected to take the average value; otherwise, the workplace was divided into several sound level areas. In each sound field, two measuring points were selected to take the average value (GBZ/T 189.8–2007).[15]

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Figure S1 Associations of different exposure metrics of night shift work with HHcy odds.

Figure S2 Prevalence of HHcy according to different exposure metrics of night shift work.

Figure S3 Associations of duration, cumulative number and cumulative length of night shifts with tHcy (as a continuous or a binary variable) from restricted cubic spline models after deleting the last 1% quantile of the duration of night shifts, cumulative number of night shifts and cumulative length of night shifts.

102 **Table S1** Basic characteristics of participants according to sex

Variables	Total	Female	Male	
	N=6846	n=585	n=6261	*P* value
Current shift status, n (%)				<0.001
Never	1027 (15.0)	118 (20.2)	909 (14.5)	
Ever	1493 (21.8)	140 (23.9)	1353 (21.6)	
Current	4326 (63.2)	327 (55.9)	3999 (63.9)	
Duration of night shifts (years), n (%)				<0.001
Never	1027 (15.0)	118 (20.2)	909 (14.5)	
Q1 (1–12)	1467 (21.4)	122 (20.9)	1345 (21.5)	
Q2 (13–20)	1491 (21.8)	130 (22.2)	1361 (21.7)	
Q3 (21–27)	1308 (19.1)	129 (22.1)	1179 (18.8)	
Q4 (28–43)	1553 (22.7)	86 (14.7)	1467 (23.4)	
Cumulative number of night shifts (nights), n (%)				<0.001
Never	1027 (15.0)	118 (20.2)	909 (14.5)	
Q1 (43–1131)	1455 (21.3)	117 (20.0)	1338 (21.4)	
Q2 (1132–1848)	1455 (21.3)	131 (22.4)	1324 (21.2)	
Q3 (1854–2584)	1456 (21.3)	139 (23.8)	1317 (21.0)	
Q4 (2585–5239)	1453 (21.2)	80 (13.7)	1373 (21.9)	
Cumulative length of night shifts (hours), n (%)				<0.001
Never	1027 (15.0)	118 (20.2)	909 (14.5)	
Q1 (344–9488)	1452 (21.2)	118 (20.2)	1334 (21.3)	
Q2 (9490–15259)	1458 (21.3)	131 (22.4)	1327 (21.2)	
Q3 (15265–21293)	1456 (21.3)	134 (22.9)	1322 (21.1)	
Q4 (21295–53541)	1453 (21.2)	84 (14.4)	1369 (21.9)	
Average frequency of night shifts, n (%)				<0.001
Never	1027 (15.0)	118 (20.2)	909 (14.5)	
<3 nights/month	1576 (23.0)	152 (26.0)	1424 (22.7)	
3–7 nights/month	901 (13.2)	78 (13.3)	823 (13.1)	
>7 nights/month	3342 (48.8)	237 (40.5)	3105 (49.6)	
Average length of night shifts, n (%)				0.002
Never	1027 (15.0)	118 (20.2)	909 (14.5)	
≤8 hours/night	4442 (64.9)	365 (62.4)	4077 (65.1)	
8–9 hours/night	810 (11.8)	57 (9.7)	753 (12.0)	
>9 hours/night	567 (8.3)	45 (7.7)	522 (8.3)	
Percentage of hours on night shifts, n (%)				<0.001
Never	1027 (15.0)	118 (20.2)	909 (14.5)	
<20%	819 (12.0)	86 (14.7)	733 (11.7)	
20%–30%	1056 (15.4)	86 (14.7)	970 (15.5)	
>30%	3944 (57.6)	295 (50.4)	3649 (58.3)	
Age (years), mean ± SD	44.2 ± 8.0	44.0 ± 5.1	44.3 ± 8.3	0.406
Age (years), n (%)				<0.001
22–29	399 (5.8)	3 (0.5)	396 (6.3)	

30–39	1745 (25.5)	131 (22.4)	1614 (25.8)	
40–49	2979 (43.5)	426 (72.8)	2553 (40.8)	
50–60	1723 (25.2)	25 (4.3)	1698 (27.1)	
BMI (kg/m^2), n (%)				<0.001
<25	3466 (50.6)	414 (70.8)	3052 (48.75)	
25–30	2830 (41.3)	143 (24.4)	2687 (42.9)	
≥30	550 (8.0)	28 (4.8)	522 (8.3)	
Smoking status, n (%)				<0.001
Never	2809 (41.0)	513 (87.7)	2296 (36.7)	
Ever	547 (8.0)	20 (3.4)	527 (8.4)	
Current	3490 (51.0)	52 (8.9)	3438 (54.9)	
Drinking status, n (%)				<0.001
Never	3926 (57.4)	526 (89.9)	3400 (54.3)	
Ever	392 (5.7)	23 (3.9)	369 (5.9)	
Current	2528 (36.9)	36 (6.2)	2492 (39.8)	
Education level, n (%)				0.656
Primary or illiterate	86 (1.3)	5 (0.9)	81 (1.3)	
Middle or high school	5304 (77.5)	454 (77.6)	4850 (77.5)	
University or college	1456 (21.3)	126 (21.5)	1330 (21.2)	
Physical activity (MET-h/week), median (IQR)	121.8 (84.0–150.7)	103.8 (80.8–124.5)	121.8 (84.0–153.3)	<0.001
DASH score	21.7 ± 2.2	23.0 ± 2.2	21.5 ± 2.2	<0.001
Sedentary behavior (h), median (IQR)	2.6 (1.3–4.3)	4.0 (2.1–5.4)	2.5 (1.3–4.0)	<0.001
Sleep duration (h), mean ± SD	6.8 ± 1.2	6.8 ± 1.2	6.8 ± 1.2	0.247
Insomnia, n (%)	2675 (39.1)	218 (37.3)	2457 (39.2)	0.348
Diabetes, n (%)	719 (10.5)	31 (5.3)	688 (11.0)	<0.001
Dyslipidemia, n (%)	2781 (40.6)	138 (23.6)	2643 (42.2)	<0.001
Hypertension, n (%)	1755 (25.6)	72 (12.3)	1683 (26.9)	<0.001
Liver enzyme abnormality, n (%)	1384 (20.2)	32 (5.5)	1352 (21.6)	<0.001
eGFR (mL/min/1.73 m^2), mean ± SD	101.7 ± 11.4	102.3 ± 12.5	101.6 ± 11.3	0.207
tHcy (μmol/L), mean ± SD	12.2 (10.1–16.8)	9.2 (7.9–11.2)	12.5 (10.4–17.4)	<0.001
Hyperhomocysteinemia, n (%)	2093 (30.6)	63 (10.8)	2030 (32.4)	<0.001

BMI, body mass index; MET, metabolic equivalent units; IQR, interquartile range; DASH, dietary approaches to stop hypertension. Values are expressed as the mean ± SD or median (IQR) or number (%); P-values were from Pearson's chi-square test for categorical variables and Student's t test or Wilcoxon Scores (Rank Sums) for continuous variables. The cut-off points of the duration of night shifts (range: 1 to 43 years), cumulative number of night shifts (range: 43 to 5239 nights), and cumulative length of night shifts (range: 344 to 53541 hours) were quarters of the corresponding continuous variables. The cut-off points of average frequency of night shifts (range: 0.1 to 10.3 nights/month), average length of night shifts (range: 8.0 to 12.0 hours/night), and percentage of hours on night shifts (range:0.1% to 46.2%) were chosen to secure a reasonable number of observations in each category.

Table S2 Basic characteristics of participants according to duration of night shifts

Variables	Total	Duration of night shifts (years)					*P* value
		Never	Q1 (1–12)	Q2 (13–20)	Q3 (21–28)	Q4 (29–43)	
	N=6846	n=1027	n=1454	n=1469	n=1439	n=1457	
Duration of employment (years), median (IQR)	26.4 (18.5–29.6)	27.4 (17.3–30.4)	15.4 (9.4–27.8)	19.2 (15.9–23.7)	24.8 (23.4–27.4)	30.3 (29.4–34.4)	<0.001
Age (years), mean±SD	44.2±8.0	44.±9.1	39.5±9.1	41.2±6.9	45.5±5.2	50.6±3.8	<0.001
Sex (male), n (%)	6261 (91.5)	909 (88.5)	1336 (91.9)	1340 (91.2)	1296 (90.1)	1380 (94.7)	<0.001
BMI (kg/m^2), mean±SD	25.2±3.4	24.8±3.3	25.3±3.6	25.4±3.6	25.2±3.3	25.2±3.1	<0.001
BMI (kg/m^2), n (%)							<0.001
<25	3466 (50.6)	570 (55.5)	748 (51.4)	702 (47.8)	712 (49.5)	734 (50.4)	
25–30	2830 (41.3)	393 (38.3)	565 (38.9)	617 (42.0)	624 (43.4)	631 (43.3)	
≥30	550 (8.0)	64 (6.2)	141 (9.7)	150 (10.2)	103 (7.2)	92 (6.3)	
Smoking status, n (%)							<0.001
Never	2809 (41.0)	460 (44.8)	623 (42.9)	622 (42.3)	578 (40.2)	526 (36.1)	
Ever	547 (8.0)	53 (5.2)	115 (7.9)	124 (8.4)	146 (10.2)	109 (7.5)	
Current	3490 (51.0)	514 (50.1)	716 (49.2)	723 (49.2)	715 (49.7)	822 (56.4)	
Alcohol consumption, n (%)							<0.001
Never	3926 (57.4)	630 (61.3)	908 (62.5)	870 (59.2)	789 (54.8)	729 (50.0)	
Ever	392 (5.7)	26 (2.5)	87 (6.0)	110 (7.5)	115 (8.0)	54 (3.7)	
Current	2528 (36.9)	371 (36.1)	459 (31.6)	489 (33.3)	535 (37.2)	674 (46.3)	
Education level, n (%)							<0.001
Primary or illiterate	86 (1.3)	9 (0.9)	10 (0.7)	5 (0.3)	26 (1.8)	36 (2.5)	
Middle or high school	5304 (77.5)	710 (69.1)	920 (63.3)	1073 (73.0)	1240 (86.2)	1361 (93.4)	
University or college	1456 (21.3)	308 (30.0)	524 (36.0)	391 (26.6)	173 (12.0)	60 (4.12)	
Physical activity (MET-h/week), median (IQR)	121.8 (84.0–150.7)	116.3 (84.0–152.5)	121.8 (84.0–152.2)	121.8 (84.0–142.0)	121.8 (84.0–150.2)	121.8 (84.0–155.6)	0.06

DASH score, mean±SD	21.7±2.2	21.7±2.1	21.6±2.2	21.5±2.2	21.8±2.2	21.7±2.2	0.592
Sedentary behaviour (hours), median (IQR)	2.6 (1.3–4.3)	2.3 (1.3–3.7)	2.6 (1.3–4.0)	2.5 (1.1–4.5)	2.6 (1.2–4.3)	3.0 (1.5–4.5)	<0.001
Sleep duration (hour), mean±SD	6.8±1.2	7.1±1.3	6.9±1.2	6.9±1.1	6.7±1.1	6.6±1.2	0.001
Insomnia, n (%)	2675 (39.1)	353 (34.4)	560 (38.5)	592 (40.3)	575 (40.0)	595 (40.8)	0.011
Diabetes, n (%)	719 (10.5)	102 (9.9)	119 (8.2)	118 (8.0)	149 (10.4)	231 (15.9)	<0.001
Dyslipidemia, n (%)	2781 (40.6)	388 (37.8)	558 (38.4)	640 (43.6)	586 (40.7)	609 (41.8)	0.013
Hypertension, n (%)	1755 (25.6)	255 (24.8)	294 (20.2)	319 (21.7)	388 (27.0)	499 (34.3)	<0.001
Liver enzyme abnormality, n (%)	1384 (20.2)	171 (16.7)	299 (20.6)	360 (24.5)	276 (19.2)	278 (19.1)	<0.001
eGFR (mL/min/1.73 m^2), mean ± SD	101.7 ± 11.4	102.1 ± 11.3	104.1 ± 12.4	103.8 ± 10.4	100.8 ±10.7	97.8 ± 10.9	<0.001
tHcy (μmol/L), median (IQR)	12.2 (10.1–16.8)	11.7 (9.8–15.8)	12.1 (10.1–16.8)	12.0 (10.0–16.1)	12.1 (10.1–17.0)	12.9 (10.6–17.7)	<0.001
HHcy, n (%)	2093 (30.6)	280 (27.3)	441 (30.3)	418 (28.5)	442 (30.7)	512 (35.1)	<0.001

BMI, body mass index; MET, metabolic equivalent units; IQR, interquartile range; DASH, dietary approaches to stop hypertension; tHcy, total homocysteine; HHcy, hyperhomocysteinemia; eGFR, estimated glomerular filtration rate.

Values are expressed as the mean ± SD or median (IQR) or number (%); P-values were from Pearson's chi-square test for categorical variables and analysis of variance (ANOVA) or Kruskal-Wallis test for continuous variables.

Table S3 Distribution of plasma total homocysteine levels according to age group

Variables	Age group (years)				*P* value
	22–29	30–39	40–49	50–60	
HHcy, n (%)					0.001
No	269 (67.4)	1242 (71.2)	2111 (70.9)	1131 (65.6)	
Yes	130 (32.6)	503 (28.8)	868 (29.1)	592 (34.4)	
tHcy (μmol/L), median (IQR)	12.1 (10.2–17.3)	11.8 (9.9–16.7)	12.0 (9.9–16.4)	12.9 (10.6–17.3)	<0.001

tHcy, total homocysteine; HHcy, hyperhomocysteinemia.

Table S4 Independent effects of different exposure metrics of night shift work on HHcy among night shift workers (ever or current)

Exposure metrics	OR (95% CI)		
	Model 1[a]	Model 2[b]	Model 3[c]
Current shift status			
Ever	1.00	1.00	1.00
Current	1.08 (0.92–1.26)	1.08 (0.92–1.27)	1.07 (0.92–1.27)
Duration of night shifts			
≤20 years	1.00	1.00	1.00
>20 years	1.14 (1.01–1.29)	1.10 (0.94–1.28)	1.07 (0.91–1.25)
Average frequency of night shifts			
≤7 nights/month	1.00	1.00	1.00
>7 nights/month	1.06 (0.92–1.23)	1.07 (0.92–1.24)	1.07 (0.91–1.24)
Average length of night shifts			
≤8 hours/night	1.00	1.00	1.00
>8 hours/night	1.11 (0.97–1.27)	1.08 (0.94–1.24)	1.08 (0.94–1.24)

OR, odds ratio; CI, confidence intervals.

[a] Model 1, adjusted for current shift status, duration of night shifts, average frequency of night shifts, and average length of night shifts.

[b] Model 2, adjusted for current shift status, duration of night shifts, average frequency of night shifts, average length of night shifts, age, and sex.

[c] Model 3, adjusted for current shift status, duration of night shifts, average frequency of night shifts, average length of night shifts, age, sex, BMI, smoking status, dyslipidaemia, hypertension, and decreased eGFR.

Table S5 Interaction between duration and average frequency of night shifts on odds of HHcy

	All	Male	Female
Main effects, OR (95% CI)			
Duration of night shifts (>20 years vs ≤20 years)	1.01 (0.83–1.23)	1.03 (0.84–1.26)	1.77 (0.45–6.91)
Average frequency of night shifts (>7nights/month vs ≤7 nights/month)	1.00 (0.83–1.22)	0.99 (0.81–1.20)	1.71 (0.64–4.59)
Joint effect, OR (95% CI)	1.20 (1.03–1.39)	1.20 (1.03–1.40)	1.12 (0.54–2.36)
Multiplicative interaction, OR (95% CI)	1.18 (0.90–1.55)	1.19 (0.90–1.56)	1.08 (0.21–5.58)
Additive interaction [a]			
Relative excess risk due to interaction, RERI (95% CI)	0.18 (-0.09–0.46)	0.19 (-0.09–0.47)	-1.36 (-4.27–1.56)
Attributable proportion due to interaction, AP (95% CI)	0.15 (-0.08–0.38)	0.16 (-0.07–0.39)	-1.21 (-3.91–1.49)

OR, odds ratio**; CI, c**onfidence intervals; RERI, relative excess risk due to interaction; AP, attributable proportion due to interaction;

[a] If there is no biological interaction, RERI and AP are equal to 0.

Adjusted for age, sex, BMI, smoking status, dyslipidaemia, hypertension, and decreased eGFR.

129 **Table S6** Multivariate-adjusted ORs between HHcy and different exposure metrics of night shift work according to sex

Exposure metrics	Male			Female		
	HHcy		OR (95% CI)	HHcy		OR (95% CI)
	No, [n (%)]	Yes, [n (%)]		No, [n (%)]	Yes, [n (%)]	
Current shift status, n (%)						
Never	641 (15.2)	268 (13.2)	1.00	106 (20.3)	12 (19.1)	1.00
Ever	938 (22.2)	415 (20.4)	1.08 (0.89–1.29)	124 (23.8)	16 (25.4)	1.05 (0.46–2.39)
Current	2652 (62.7)	1347 (66.4)	1.24 (1.06–1.46)	292 (55.9)	35 (55.6)	0.94 (0.46–1.92)
Duration of night shifts (years)						
Never	641 (15.2)	268 (13.2)	1.00	106 (20.3)	12 (19.1)	1.00
Q1 (1–12)	918 (21.7)	427 (21.0)	1.14 (0.94–1.38)	104 (19.9)	18 (28.6)	1.29 (0.57–2.91)
Q2 (13–20)	954 (22.6)	407 (20.1)	1.07 (0.88–1.30)	118 (22.6)	12 (19.1)	0.78 (0.32–1.86)
Q3 (21–27)	792 (18.7)	387 (19.1)	1.20 (0.99–1.46)	118 (22.6)	11 (17.5)	0.84 (0.35–2.04)
Q4 (28–43)	926 (21.9)	541 (26.7)	1.36 (1.13–1.64)	76 (14.6)	10 (15.9))	1.04 (0.41–2.65)
P trend			0.002			0.629
Cumulative number of night shifts (nights)						
Never	641 (15.2)	268 (13.2)	1.00	106 (20.3)	12 (19.1)	1.00
Q1 (43–1131)	911 (21.5)	427 (21.0)	1.15 (0.95–1.39)	101 (19.4)	16 (25.4)	1.22 (0.53–2.80)
Q2 (1132–1848)	932 (22.0)	392 (19.3)	1.05 (0.87–1.28)	118 (22.6)	13 (20.6)	0.79 (0.34–1.88)
Q3 (1854–2584)	879 (20.8)	438 (21.6)	1.23 (1.02–1.48)	127 (24.3)	12 (19.1)	0.86 (0.36–2.05)
Q4 (2585–5239)	868 (20.5)	505 (24.9)	1.35 (1.12–1.63)	70 (13.4)	10 (15.9)	1.14 (0.45–2.91)
P trend			0.003			0.799
Cumulative length of night shifts (hours)						
Never	641 (15.2)	268 (13.2)	1.00	106 (20.3)	12 (19.1)	1.00

Q1 (344–9488)	904 (21.4)	430 (21.2)	1.16 (0.96–1.40)	102 (19.5)	16 (25.4)	1.20 (0.52–2.74)
Q2 (9490–15259)	937 (22.2)	390 (19.2)	1.04 (0.86–1.27)	117 (22.4)	14 (22.2)	0.91 (0.39–2.13)
Q3 (15265–21293)	881 (20.8)	441 (21.7)	1.24 (1.02–1.50)	126 (24.1)	8 (12.7)	0.57 (0.22–1.48)
Q4 (21295–53541)	868 (20.5)	501 (24.7)	1.33 (1.11–1.61)	71 (13.6)	13 (20.6)	1.43 (0.59–3.46)
P trend			0.005			0.942
Average frequency of night shifts						
Never	641 (15.2)	268 (13.2)	1.00	106 (20.3)	12 (19.1)	1.00
<3 nights/month	976 (23.1)	448 (2.1)	1.11 (0.93–1.34)	134 (25.7)	18 (28.6)	1.04 (0.46–2.32)
3–7 nights/month	562 (13.3)	261 (12.9)	1.14 (0.93–1.40)	74 (14.2)	4 (6.4)	0.40 (0.12–1.34)
>7 nights/month	2052 (48.5)	1053 (51.9)	1.26 (1.07–1.48)	208 (39.9)	29 (46.0)	1.11 (0.54–2.30)
P trend			0.003			0.861
Average length of night shifts						
Never	641 (15.2)	268 (13.2)	1.00	106 (20.3)	12 (19.1)	1.00
≤8 hours/night	2754 (65.1)	1323 (65.2)	1.18 (1.01–1.39)	328 (62.8)	37 (58.7)	0.89 (0.44–1.79)
8–9 hours/night	497 (11.8)	256 (12.6)	1.27 (1.03–1.57)	54 (10.3)	3 (4.8)	0.43 (0.11–1.77)
>9 hours/night	339 (8.0)	183 (9.0)	1.22 (0.96–1.54)	34 (6.5)	11 (17.5)	2.48 (0.96–6.43)
P trend			0.058			0.149
Percentage of hours on night shifts						
Never	641 (15.2)	268 (13.2)	1.00	106 (20.3)	12 (19.1)	1.00
<20%	499 (11.8)	234 (11.5)	1.14 (0.92–1.41)	74 (14.2)	12 (19.1)	1.21 (0.50–2.94)
20%–30%	670 (15.8)	300 (14.8)	1.09 (0.89–1.34)	77 (14.8)	9 (14.3)	0.88 (0.33–2.34)
>30%	2421 (57.2)	1228 (60.5)	1.24 (1.06–1.46)	265 (50.8)	30 (47.6)	0.93 (0.45–1.91)
P trend			0.008			0.677

HHcy, hyperhomocysteinemia; OR, odds ratio; CI, confidence intervals.

Adjusted for age, sex, BMI, smoking status, dyslipidaemia, hypertension, and decreased eGFR.

Table S7 Multivariate-adjusted ORs between HHcy and different exposure metrics of night shift work after further adjustment for the main occupational hazards

Exposure metrics	OR (95% CI)		
	All	Male	Female
Current shift status			
Never	1.00	1.00	1.00
Ever	1.08 (0.90–1.30)	1.08 (0.90–1.30)	0.99 (0.43–2.27)
Current	1.25 (1.07–1.47)	1.27 (1.08–1.49)	0.89 (0.43–1.85)
Duration of night shifts (years)			
Never	1.00	1.00	1.00
Q1 (1–12)	1.16 (0.96–1.39)	1.15 (0.95–1.39)	1.20 (0.52–2.73)
Q2 (13–20)	1.06 (0.88–1.29)	1.08 (0.89–1.32)	0.73 (0.30–1.78)
Q3 (21–27)	1.20 (0.99–1.45)	1.22 (1.00–1.49)	0.82 (0.33–2.04)
Q4 (28–43)	1.36 (1.13–1.64)	1.38 (1.14–1.66)	0.97 (0.38–2.49)
P trend	0.003	0.001	0.576
Cumulative number of night shifts (nights)			
Never	1.00	1.00	1.00
Q1 (43–1131)	1.16 (0.97–1.40)	1.16 (0.96–1.40)	1.13 (0.49–2.63)
Q2 (1132–1848)	1.06 (0.87–1.28)	1.07 (0.88–1.30)	0.75 (0.31–1.81)
Q3 (1854–2584)	1.22 (1.01–1.47)	1.24 (1.03–1.51)	0.85 (0.35–2.05)
Q4 (2585–5239)	1.35 (1.12–1.63)	1.36 (1.13–1.65)	1.05 (0.41–2.71)
P trend	0.003	0.002	0.737
Cumulative length of night shifts (hours)			
Never	1.00	1.00	1.00
Q1 (344–9488)	1.17 (0.97–1.41)	1.17 (0.97–1.41)	1.12 (0.48–2.59)
Q2 (9490–15259)	1.05 (0.87–1.27)	1.06 (0.87–1.29)	0.86 (0.36–2.03)
Q3 (15265–21293)	1.21 (1.01–1.47)	1.25 (1.03–1.52)	0.54 (0.20–1.43)
Q4 (21295–53541)	1.35 (1.12–1.63)	1.35 (1.11–1.64)	1.32 (0.54–3.22)
P trend	0.005	0.004	0.854
Average frequency of night shifts (nights/month)			
Never	1.00	1.00	1.00
<3	1.12 (0.94–1.34)	1.12 (0.93–1.35)	0.95 (0.42–2.16)
3–7	1.12 (0.91–1.38)	1.16 (0.94–1.43)	0.37 (0.11–1.28)
>7	1.27 (1.08–1.50)	1.28 (1.08–1.51)	1.07 (0.50–2.45)
P trend	0.002	0.002	0.880
Average length of night shifts (hours/night)			
Never	1.00	1.00	1.00
≤8	1.18 (1.01–1.38)	1.19 (1.02–1.41)	0.82 (0.40–1.68)
8–9	1.25 (1.01–1.54)	1.28 (1.04–1.59)	0.38 (0.90–1.63)
>9	1.28 (1.02–1.61)	1.23 (0.97–1.56)	2.37 (0.91–6.21)
P trend	0.028	0.052	0.169
Percentage of hours on night shifts, n (%)			

Never	1.00	1.00	1.00
<20%	1.16 (0.94–1.43)	1.15 (0.93–1.43)	1.12 (0.45–2.78)
20%–30%	1.10 (0.90–1.34)	1.11 (0.90–1.35)	0.82 (0.31–2.20)
>30%	1.24 (1.06–1.46)	1.26 (1.07–1.49)	0.89 (0.43–1.86)
P trend	0.010	0.006	0.628

OR, odds ratio; CI, confidence intervals.

Adjusted for age, sex (except for gender stratification), BMI, smoking status, dyslipidaemia, hypertension, decreased eGFR, dust exposure (No or Yes), heat stress exposure (No or Yes), noise exposure (No or Yes), and carbon monoxide exposure (No or Yes)

Exposure metrics	Model 1	Model 2	Model 3
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Current shift status			
Never	1.00	1.00	1.00
Ever	1.08 (0.91–1.29)	1.07 (0.89–1.28)	1.08 (0.90–1.29)
Current	1.25 (1.08–1.46)	1.23 (1.05–1.44)	1.23 (1.06–1.44)
Duration of night shifts (years)			
Never	1.00	1.00	1.00
Q1 (1–12)	1.16 (0.97–1.39)	1.13 (0.95–1.36)	1.15 (0.96–1.38)
Q2 (13–20)	1.04 (0.87–1.25)	1.04 (0.86–1.25)	1.05 (0.87–1.27)
Q3 (21–27)	1.17 (0.97–1.40)	1.17 (0.97–1.41)	1.19 (0.98–1.43)
Q4 (28–43)	1.47 (1.24–1.74)	1.38 (1.15–1.65)	1.35 (1.12–1.61)
P trend	<0.001	0.001	0.003
Cumulative number of night shifts (nights)			
Never	1.00	1.00	1.00
Q1 (43–1131)	1.17 (0.98–1.39)	1.14 (0.95–1.36)	1.15 (0.96–1.38)
Q2 (1132–1848)	1.03 (0.86–1.23)	1.03 (0.85–1.24)	1.04 (0.86–1.26)
Q3 (1854–2584)	1.19 (1.00–1.42)	1.20 (1.00–1.44)	1.21 (1.01–1.45)
Q4 (2585–5239)	1.47 (1.23–1.74)	1.37 (1.14–1.65)	1.34 (1.11–1.61)
P trend	<0.001	0.001	0.004
Cumulative length of night shifts (hours)			
Never	1.00	1.00	1.00
Q1 (344–9488)	1.18 (0.99–1.41)	1.15 (0.96–1.39)	1.16 (0.97–1.40)
Q2 (9490–15259)	1.02 (0.86–1.22)	1.02 (0.84–1.23)	1.04 (0.86–1.25)
Q3 (15265–21293)	1.19 (1.00–1.42)	1.18 (0.99–1.42)	1.20 (1.00–1.45)
Q4 (21295–53541)	1.46 (1.23–1.74)	1.37 (1.14–1.65)	1.33 (1.11–1.61)
P trend	<0.001	0.003	0.006
Average frequency of night shifts (nights/month)			
Never	1.00	1.00	1.00
<3	1.12 (0.94–1.33)	1.11 (0.93–1.32)	1.11 (0.93–1.33)
3–7	1.11 (0.91–1.36)	1.09 (0.89–1.34)	1.10 (0.91–1.35)
>7	1.28 (1.09–1.49)	1.25 (1.07–1.47)	1.25 (1.07–1.47)
P trend	0.001	0.003	0.004
Average length of night shifts (hours/night)			
Never	1.00	1.00	1.00
⩽8	1.18 (1.01–1.37)	1.16 (1.00–1.36)	1.17 (1.01–1.37)
8–9	1.25 (1.03–1.53)	1.21 (0.99–1.48)	1.23 (1.00–1.52)
>9	1.39 (1.11–1.73)	1.30 (1.04–1.63)	1.27 (1.01–1.60)
P trend	0.003	0.022	0.031
Percentage of hours on night shifts			
Never	1.00	1.00	1.00
<20%	1.15 (0.94–1.40)	1.14 (0.93–1.40)	1.15 (0.94–1.42)
20%–30%	1.10 (0.91–1.34)	1.08 (0.89–1.31)	1.09 (0.90–1.32)
>30%	1.25 (1.07–1.46)	1.23 (1.05–1.44)	1.23 (1.05–1.43)
P trend	0.004	0.009	0.010

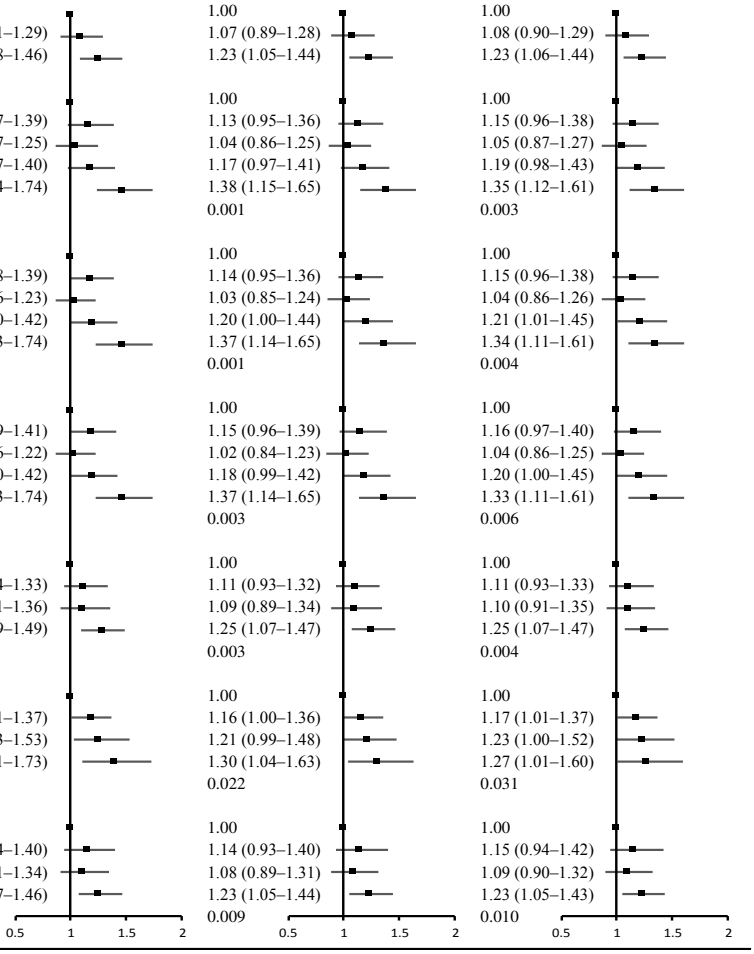

Figure S1 Associations of different exposure metrics of night shift work with HHcy odds. HHcy, hyperhomocysteinemia; OR, odds ratio; CI, confidence intervals. Model 1: unadjusted; Model 2: adjusted for age and sex; Model 3: adjusted for age, sex, BMI, smoking status, dyslipidaemia, hypertension, and decreased eGFR.

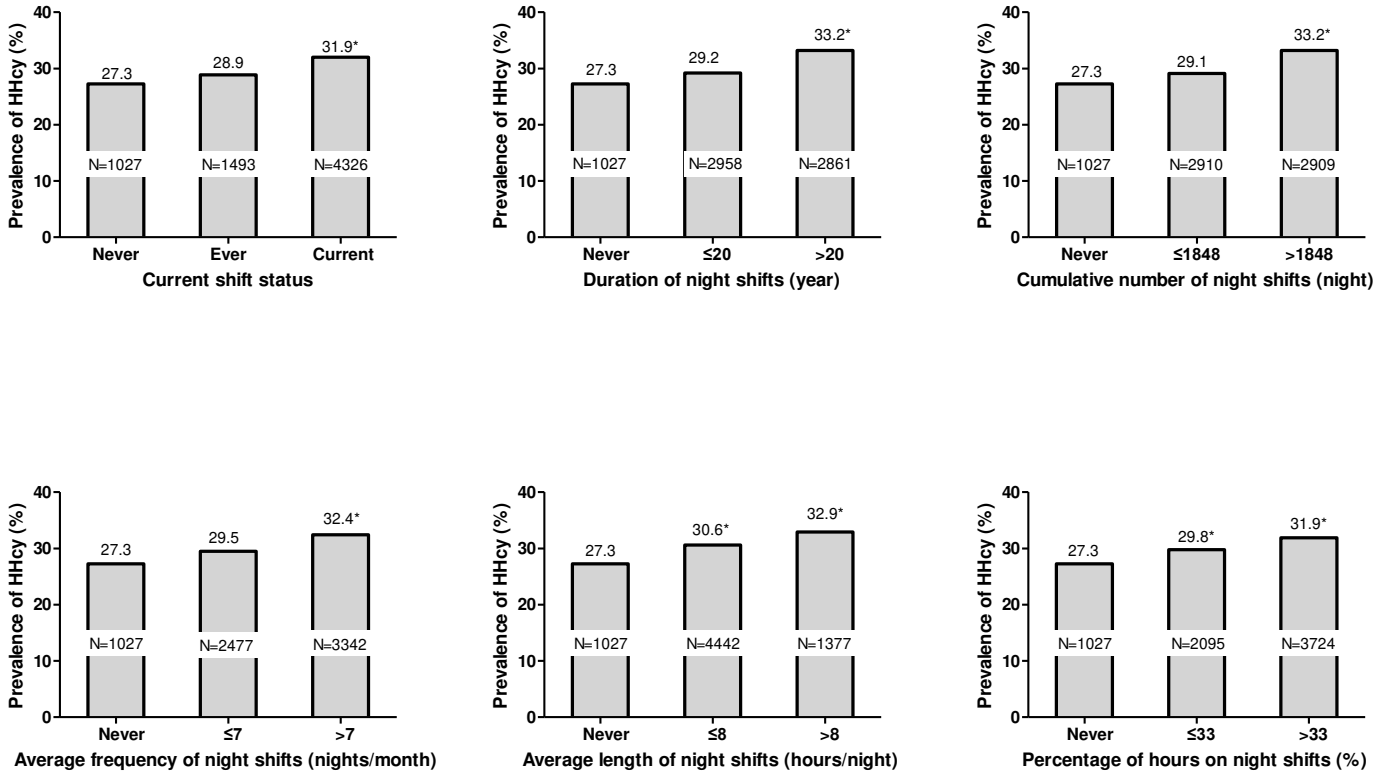

Figure S2 Prevalence of HHcy according to different exposure metrics of night shift work. The number in the middle of each column indicates the number of subjects included in the category. $^{*}P < 0.05$ (vs. the "Never" group).

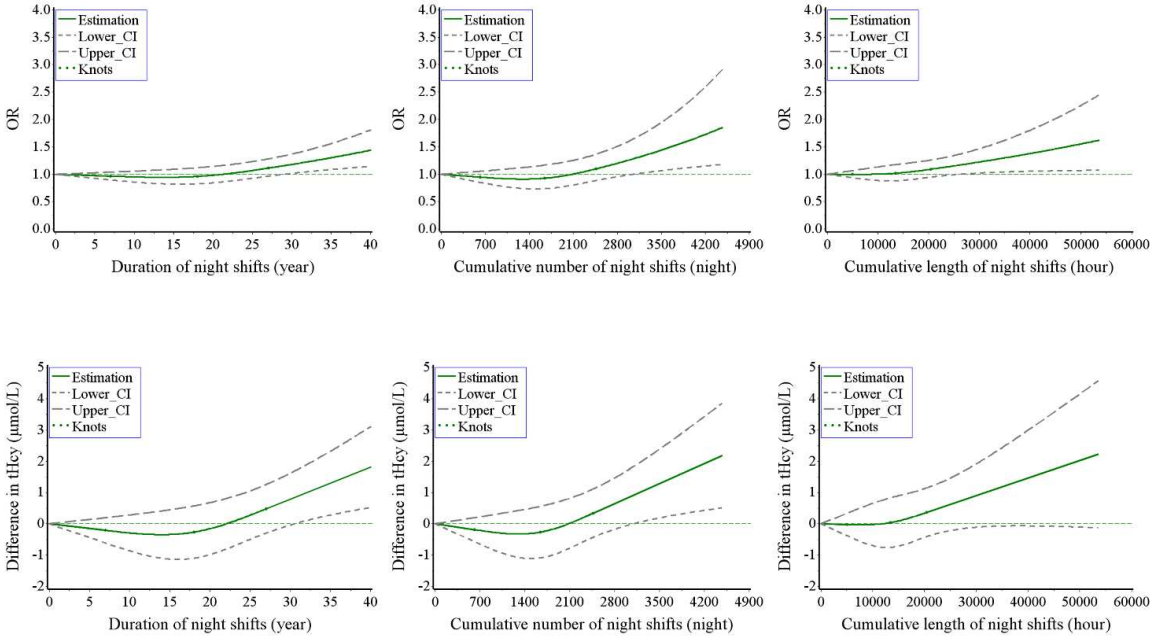

Figure S3 Associations of duration, cumulative number and cumulative length of night shifts with tHcy (as a continuous or a binary variable) from restricted cubic spline models after deleting the last 1% quantile of the duration of night shifts, cumulative number of night shifts and cumulative length of night shifts. "Difference in tHcy" indicates difference of tHcy (μmol/L) levels in the serum where the reference values for duration, cumulative number and cumulative length of night shifts are all 0 (never worked night shifts); Adjusted for age, sex, BMI, smoking status, dyslipidaemia, hypertension, and decreased eGFR. OR, odds ratio; CI, confidence interval; tHcy, total homocysteine; HHcy, hyperhomocysteinemia.

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