



# Two-Year Responses of Heart Rate and Heart Rate Variability to First Occupational Lead Exposure

Yu-Ling Yu,\* Lutgarde Thijs,\* Cai-Guo Yu, Wen-Yi Yang, Jesus D. Melgarejo<sup>1</sup>, Dong-Mei Wei<sup>1</sup>, Fang-Fei Wei, Tim S. Nawrot, Peter Verhamme, Harry A. Roels, Jan A. Staessen<sup>1</sup>,† Zhen-Yu Zhang†

**ABSTRACT:** Because of the falling lead exposure, the literature relating autonomous nervous function to blood lead (BL) has limited relevance. In the longitudinal Study for Promotion of Health in Recycling Lead (URL: <https://www.clinicaltrials.gov>; Unique identifier: NCT02243904), we recorded the 2-year responses of heart rate (HR), HR variability (HRV; Cardiax, International Medical Equipment Developing, Budapest, Hungary), and median nerve conduction velocity (Brevio, NeuMed, West Trenton, NJ), a routine test in occupational medicine, to first lead exposure in 195 newly hired workers (91.3% men; mean age, 27.8 years). High- and low-frequency HRV power and orthostatic HRV responses were derived from 5-minute ECGs in the supine and standing positions by Fourier transform and autoregression. BL was determined by inductively coupled plasma mass spectrometry. From baseline to follow-up, BL increased from 4.22 to 14.1  $\mu\text{g}/\text{dL}$  and supine/standing HR from 63.6/75.5 to 67.1/78.8 beats per minute. In analyses stratified by fourths of BL changes, trends in HR and Fourier/autoregressive HRV did not reveal a dose-response curve ( $0.074 \leq P \leq 0.98$ ). In multivariable-adjusted mixed models, HR, Fourier/autoregressive HRV, and nerve conduction velocity changes were unrelated to BL except for a weak inverse association between supine HR and BL changes ( $-0.55\%$ ;  $P=0.029$ ). The expected associations between HRV and HR changes were preserved with no differences at baseline/follow-up. Analyses dichotomized by baseline median BL or cumulative BL index (4.30  $\mu\text{g}/\text{dL}$  or 32.1  $\mu\text{g}/\text{dL} \times \text{year}$ ) suggested an HRV increase versus decrease in the low versus high baseline exposure group. Thus, a  $>3$ -fold BL increment did not affect autonomous neural function as captured by HRV. (**Hypertension**. 2021;77:1775–1786. DOI: 10.1161/HYPERTENSIONAHA.120.16545.) • [Data Supplement](#)

**Key Words:** autonomic nervous system ■ heart rate ■ lead ■ nerve conduction ■ occupational exposure

A review of 102 articles, published in 2000, concluded that effects of lead exposure on the central and peripheral nervous system occurred at blood lead levels of 30 to 40  $\mu\text{g}/\text{dL}$ .<sup>1</sup> However, as documented by successive cycles of the National Health and Nutrition Examination Survey (NHANES), average blood lead levels in US adults decreased from 13.1  $\mu\text{g}/\text{dL}$  in NHANES II (1976–1980)<sup>2,3</sup> to 1.2 to 2.76  $\mu\text{g}/\text{dL}$  in NHANES III (1988–1994),<sup>2,3</sup> and to 1.64  $\mu\text{g}/\text{dL}$  in NHANES IV

(1999–2002).<sup>4,5</sup> Furthermore, a PubMed search conducted without limitation of language or publication date on September 20, 2020, with as search terms “lead exposure” in association with “heart rate variability” or “sympathetic activity” or “parasympathetic activity” or “nerve conduction velocity” revealed that few pertinent articles were published since 2000,<sup>6–15</sup> and that most articles reported on experimental work with focus on potential neurotoxic mechanisms.<sup>10–15</sup> In the general population, low

Correspondence to: Jan A. Staessen, Alliance for the Promotion of Preventive Medicine, Leopoldstraat 59, BE-2800 Mechelen, Belgium. Email [jan.staessen@apremed.org](mailto:jan.staessen@apremed.org)

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\*Y.-L. Yu and L. Thijs contributed equally as co-first authors.

†J.A. Staessen and Z.-Y. Zhang contributed equally as co-last authors.

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## Novelty and Significance

### What Is New?

- Dysregulation of the autonomic nervous function is a forerunner of cardiovascular complications and mortality. In view of the declining lead exposure, the current literature has little relevance with regard to the relation between neural function and blood lead (BL). In 195 workers newly employed in the lead industry and followed up for 2 years, we quantified the associations of heart rate (HR), HR variability (HRV), an established cardiovascular risk factor, and median nerve conduction velocity, a benchmark test in occupational medicine, with the increase in BL.

### What Is Relevant?

- BL increased from 4.28 to 13.8  $\mu\text{g}/\text{dL}$ .
- There was no dose-response association of HR, HRV, and nerve conduction velocity across increasing BL categories.

- Sensitivity analyses dichotomized by baseline BL or CBLI (4.30  $\mu\text{g}/\text{dL}$  or 32.1  $\mu\text{g}/\text{dL}\times\text{year}$ ) suggested an HRV increase versus decrease in the low versus high baseline exposure group.
- In properly adjusted analyses, HRV changes derived by Fourier transform or autoregressive modeling and nerve conduction velocity changes were not associated with the >3-fold BL increase.

### Summary

In a real-world 2-year follow-up study, HRV changes were not related with >3-fold BL increment, making causality unlikely within the BL range studied (<30  $\mu\text{g}/\text{dL}$  in 94% of participants). Our findings confirm the safety of lead exposure thresholds in an occupational setting; they practically exclude HRV dysregulation as mediator of adverse health outcomes at the much lower exposure levels in the general population.

## Nonstandard Abbreviation and Acronyms

<b>CBLI</b>	cumulative blood lead index
<b>HDL</b>	high-density lipoprotein
<b>HRV</b>	heart rate variability
<b>IQR</b>	interquartile range
<b>LDL</b>	low-density lipoprotein
<b>NCV</b>	nerve conduction velocity
<b>NHANES</b>	National Health and Nutrition Examination Survey
<b>PI</b>	percentile interval
<b>SPHERL</b>	Study for the Promotion of Health in Recycling Lead

heart rate variability (HRV) is associated with a higher risk of coronary heart disease,<sup>16,17</sup> atrial fibrillation,<sup>18</sup> sudden cardiac death,<sup>19</sup> and total mortality.<sup>17,20</sup> Given this background and the suspicion that environmental lead exposure causes cardiovascular disease,<sup>21</sup> the objective of the SPHERL (Study for the Promotion of Health in Recycling Lead) was to assess the health effects of starting occupational lead exposure over a 2-year follow-up period. In this article, results for heart rate and HRV are reported along with peripheral nerve conduction velocity (NCV), a more common test of neurological function in lead-exposed workers,<sup>23,24</sup> as a secondary outcome.

## METHODS

### Study Participants

SPHERL is a longitudinal study (January, 2015–October, 2019) of newly hired lead workers at battery manufacturing and lead

recycling plants in the United States.<sup>22,25</sup> SPHERL complies with the Helsinki declaration for investigation of humans.<sup>27</sup> The Ethics Committee of the University Hospitals Leuven (Belgium) approved the study (No. B322201421631), of which the protocol has been registered and published.<sup>22</sup> There were no exclusion criteria before enrolment. All newly hired workers without previous occupational lead exposure were eligible. The 2-year HRV and NCV responses to first occupational lead exposure were among the predefined study end points.<sup>22</sup> In consultation with the Ethics Committee that approved the study protocol, SPHERL data cannot be made publicly available. Reasons are that the informed consent signed by the workers did not cover data sharing and that an anonymized data set would still contains elements, which could potentially lead to the identification of participants. Only researchers affiliated with the Research Institute Alliance for the Promotion of Preventive Medicine (Mechelen, Belgium) and the Research Unit Hypertension and Cardiovascular Epidemiology (Leuven, Belgium) have access to the study database. However, these researchers are prepared to run additional analyses if a scientifically justified request is addressed to the corresponding author.

### Clinical and Biochemical Measurements

Blood pressure was the average of 5 consecutive readings and was categorized according to the 2017 American College of Cardiology/American Heart Association guideline based on the blood pressure level, irrespective of treatment status.<sup>28</sup> During the clinical examination, nurses counted heart rate over 15 seconds. They also administered the same validated questionnaire at baseline and follow-up to collect information about each worker's medical history, exposure to heavy metals, smoking and drinking habits, and intake of medications. At baseline and follow-up visits, the workers completed the self-administered EuroQol-5 Dimension questionnaire (<https://www.euroqol.org>).

In a medical facility separate from the production sites, the study nurses obtained venous blood samples after participants had fasted for 8 hours. The materials used for blood collection,

including test tubes, needles, and caps, were certified as lead free (Becton, Dickinson and Company, Franklin Lakes, NJ). The nurses thoroughly cleansed the brachial venipuncture site and kept the tubes for the measurement of blood lead closed. For serum measurements, the blood samples were immediately spun and divided into aliquots. All biochemical tests were performed by laboratories adhering to the Clinical Laboratory Improvement Amendments of 1988. Blood lead was determined by inductively coupled plasma mass spectrometry at a single laboratory certified for blood lead analysis in compliance with the provisions of the Occupational and Health Administration Lead Standard, 29CFR 1910.1025 (Occupational Safety and Health Administration). The laboratory participated in the US CDC Blood Lead Proficiency Testing Program. Before analysis, specimens were digested in nitric acid and spiked with an iridium internal standard. The blood lead detection limit was 0.5  $\mu\text{g}/\text{dL}$ . The deviation from known lead standards analyzed along with the samples in each test run was  $<10\%$ . The hematologic measurements included hemoglobin and hematocrit as possible heart rate correlates. Total and HDL (high-density lipoprotein) serum cholesterol, serum triglycerides, blood glucose, and serum insulin were measured as indexes of metabolic status. LDL (low-density lipoprotein) serum cholesterol was estimated from the serum levels of total cholesterol, HDL cholesterol, and triglycerides according to the Friedewald equation.<sup>29</sup> A detailed description of the quality control program implemented for the biochemical measurements, including blood lead, is available in previous publications<sup>26</sup> and in the [Data Supplement](#).

## Heart Rate Variability

HRV was measured from 5-minute ECG recordings in the supine and standing positions, using the Cardiax software, V.4.14.0 (International Medical Equipment Development, Budapest, Hungary). The time interval between the supine and standing ECG recordings was not standardized but lasted from 1 to 3 minutes. The Cardiax software allowed exporting all ECG measurements into an Excel worksheet, which was subsequently imported into SAS version 9.4, using standardized programming statements, thereby excluding any observer-induced bias. The software computes the power spectrum in the frequency domain by fast Fourier transform and by autoregressive modeling and provides the low-frequency (0.04–0.15 Hz) and high-frequency (0.15–0.40 Hz) HRV components in milliseconds and the low-to-high frequency ratio. The Fourier approach consisted of a mathematical transform that decomposes the heart rate signal changing over time into its constituent frequencies. The autoregressive approach derived HRV by regressing heart rate at a given time as response (dependent) variable on its values during a previous period, using the Akaike information criterion as estimator of the in-sample prediction error.<sup>30</sup> Normalized units of low-frequency and high-frequency power were calculated as the low- and high-frequency power divided by the difference (total power–very low-frequency power) $\times 100$ .<sup>31,32</sup>

## Nerve Conduction Velocity

NCV was measured at baseline examination and annual follow-up visits. The study nurses used a handheld device and related software (Brevio Nerve Conduction Monitoring System, NeuMed, West Trenton, NJ) to stimulate the left and right median nerve at a gradually increasing voltage, until the

maximum compound motor action potential of the short thumb abductor muscle was reached. To check the quality of the NCV measurement, 40 workers were randomly selected. Using the Bland and Altman approach,<sup>33</sup> the within-worker bias (right minus left side) was 0.014 ms ( $P=0.83$ ).<sup>25</sup> For analysis, left and right NCV values were averaged.

## Statistical Analysis

For database management and statistical analysis, we used the SAS software, version 9.4, maintenance level 5 (SAS Institute Inc, Cary, NC). Departure from normality was evaluated by the Shapiro-Wilk statistic. We applied a logarithmic transformation (base 10) to normalize the distributions of total power, low-frequency power, high-frequency power, and the low-to-high frequency ratio in the supine and standing positions, the orthostatic HRV responses, and blood lead and serum insulin. We reported the central tendency and spread of continuously distributed variables as mean and SD or for logarithmically transformed variables as geometric mean with the interquartile range (IQR) or fifth to 95th percentile interval (PI), as appropriate. To compare means and proportions, we applied the T statistic or ANOVA for continuously distributed variables, and the Fisher exact test for categorical variables.

In exploratory analyses, we assessed HRV and NCV by fourths of the follow-up-to-baseline blood lead ratio. HRV and NCV responses to the changes in blood lead were expressed for a doubling of the follow-up-to-baseline blood lead ratio. Estimates were derived from mixed models including the first and repeat follow-up visits, using random-effects models to account for clustering of the observations within participants. For each outcome, unadjusted and adjusted models were constructed. The covariables used for initial adjustment were similar to those applied for the cross-sectional analysis of the baseline data<sup>25</sup> but also included the changes from baseline. Thus, models were adjusted for sex, baseline heart rate or HRV, the baseline value and the change during follow-up in age, heart rate (or the orthostatic heart rate response), mean arterial pressure, and serum insulin. To account for changes in measurement conditions, adjusted models also accounted for hemoglobin, room temperature, seasonality, and observer modeled as random effect. The covariables for NCV were sex, baseline nerve conduction latency, baseline value and the changes during follow-up in age, waist-to-hip ratio, mean arterial pressure, the total-to-HDL serum cholesterol ratio, and room temperature during the examination. Within-visit associations were evaluated by simple and multiple linear regression and regression slopes were compared by a Z statistic.

In sensitivity analyses, we stratified the study participants according to the median baseline blood lead level and the median cumulative blood lead index (CBLI).<sup>34</sup> To compute CBLI,<sup>34</sup> age for workers leaving school at less than, at, and above the 12th grade were assumed to be 14, 18, and 23 years; based on NHANES data (1988–1994),<sup>35</sup> the corresponding blood lead levels were set at 2.2, 1.4, and 1.5  $\mu\text{g}/\text{dL}$ , respectively.

## RESULTS

Of 746 workers invited, 601 (80.6%) consented. However, in the interval between signature of the informed consent and the baseline examination (median, 19 days;

fifth–95th PI, 9–59 days), 95 laborers left the work place, did not meet the exposure eligibility criteria, or withdrew (Figure 1). From January 25, 2015, until September 19, 2017, 506 workers underwent the baseline examination, of whom 289 (57.1%) had one and 236 (46.6%) 2 follow-up visits (Figure 1). Of 289 participants with at least one follow-up visit, 94 disqualified from analysis, because blood lead (N=4) or HRV (n=41) had not been measured at baseline or follow-up, because they had frequent extrasystoles (N=2) or became diabetic during follow-up (N=13), or because they were on antihypertensive (N=14) or neuro-active drugs (N=20), including benzodiazepines, neuroleptics, antiepileptics, sympathomimetics, amphetamines, or recreational drugs. Of the remaining 195 workers with HRV data, 192 (98.5%) also underwent an NCV assessment at baseline and follow-up.

### Baseline Characteristics of Workers

Of the 195 participants in the HRV cohort (Table 1), 178 (91.3%) were men, 93 (47.7%) were White, 89 (45.6%) were Hispanic, and 13 (6.7%) had another self-reported ethnicity. At baseline, age averaged 27.8 years, body mass index 28.5 kg/m<sup>2</sup>, and systolic and diastolic blood pressure 119.7 and 79.1 mmHg, respectively. At baseline, serum total, HDL, and LDL serum cholesterol averaged 169.9 mg/dL (SD, 36.7 mg/dL), 45.8 mg/dL (SD, 12.6 mg/dL), and 94.7 mg/dL (SD, 28.5 mg/dL), serum triglycerides 148 mg/dL (IQR, 86–169 mg/dL), blood glucose 93.1 mg/dL (SD, 11.1 mg/dL), and serum insulin 6.73  $\mu$ IU/mL (IQR, 3.60–13.0  $\mu$ IU/mL). The prevalence of smoking and regular consumption of alcohol-containing beverages amounted to 51 (26.2%) and 88 (45.1%), respectively (Table 1).

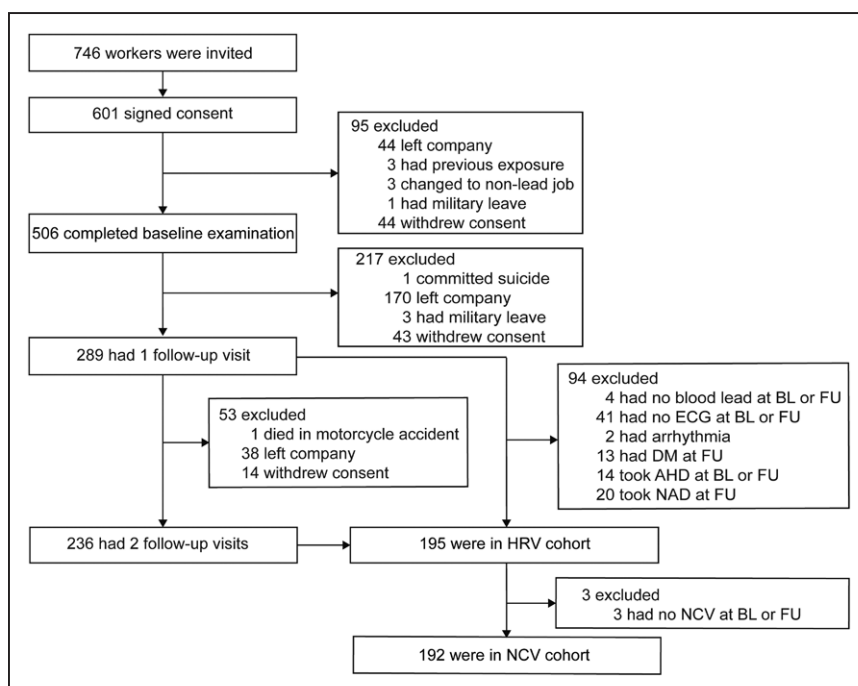
### Blood Lead Concentration

Median follow-up was 2.0 years (PI, 1.0–2.2 years). The geometric mean blood lead concentration was 4.22  $\mu$ g/dL (IQR, 2.50–8.30  $\mu$ g/dL; PI, 1.00–15.2  $\mu$ g/dL), at baseline, 14.1  $\mu$ g/dL (IQR, 9.95–22.2  $\mu$ g/dL; PI, 4.20–30.2  $\mu$ g/dL) at the first follow-up visit, and 14.1  $\mu$ g/dL (IQR, 11.0–23.1  $\mu$ g/dL; PI, 3.10–30.4  $\mu$ g/dL) at the last follow-up visit. The last follow-up-to-baseline blood lead concentration ratio averaged 3.34 (95% CI, 2.98–3.76;  $P$ <0.001; Figure S1 in the [Data Supplement](#)). Smokers compared with nonsmokers had a higher ( $P$ =0.049) baseline blood lead concentration: 5.13  $\mu$ g/dL (IQR, 2.90–9.50  $\mu$ g/dL) versus 3.94  $\mu$ g/dL (IQR, 2.30–7.70  $\mu$ g/dL).

### Heart Rate

#### Baseline-to-Follow-Up Changes

Heart rate counted by the study nurses during the clinical examination averaged 73.6 (IQR, 64.0–81.0) beats per minute at baseline (Table 1) and over the 2-year follow-up increased by 5.1 (CI, 3.4–6.8;  $P$ <0.001) beats per minute, averaging 77.5 (IQR, 70.0–86.0) beats per minute at the first and 78.3 (IQR, 71.0–86.0) beats per minute at the last follow-up. Compared with baseline (Table 2), the ECG-derived heart rate increments were 2.9 (CI, 1.7–4.1;  $P$ <0.001) and 3.2 (CI, 1.4–4.9;  $P$ <0.001) beats per minute with a nonsignificant change compared with baseline in the orthostatic heart rate response, amounting to 0.3 (CI, –1.0 to 1.6;  $P$ =0.65) beats per minute. Expressed on a percent scale—to allow comparison with the HRV changes—the changes from baseline in the supine and standing ECG-derived heart rate and



**Figure 1. Flow chart.**

AHD indicates antihypertensive drugs; BL, baseline; DM, diabetes; FU, follow-up; HRV, heart rate variability; NAD, neuro-active drugs, including benzodiazepines, neuroleptics, antiepileptics, sympathomimetics, amphetamines, and recreational drugs; and NCV, nerve conduction velocity.



**Table 1. Baseline Characteristics of 195 Workers**

Characteristic	Number (%)	Characteristic	Mean (SD or IQR)
Women	17 (8.7)	Age, y	27.8 (8.12)
Men	178 (91.3)	Body mass index, kg/m <sup>2</sup>	28.5 (5.8)
White ethnicity	93 (47.7)	Waist-to-hip ratio	0.96 (0.08)
Hispanic ethnicity	89 (45.6)	Systolic pressure, mm Hg	119.7 (9.8)
Other ethnicities	13 (6.7)	Diastolic pressure, mm Hg	79.1 (8.5)
Current smokers	51 (26.2)	Mean arterial pressure, mm Hg	92.6 (8.3)
Drinking alcohol	88 (45.1)	Heart rate, beats per minute	73.6 (11.8)
Hypertension $\geq$ stage 1	92 (47.2)	Hemoglobin, g/dL	15.0 (1.2)
Hypertension $\geq$ stage 2	25 (12.8)	Hematocrit, %	45.4 (3.3)
Treated hypertension	...	Total-to-HDL cholesterol ratio	3.93 (1.33)
Intake of neuro-active drugs	...	Insulin, $\mu$ IU/mL	6.73 (3.60–13.0)

Hypertension was graded according to the 2017 ACC/AHA guideline based on the blood pressure level, irrespective of treatment status. Mean arterial pressure was diastolic pressure plus one-third of the difference between systolic and diastolic pressure. An ellipsis indicates not applicable. Participants on antihypertensive and neuro-active drugs were excluded from analysis (see Figure 1). Neuro-active medications included benzodiazepines, neuroleptics, antiepileptics, sympathomimetics, amphetamines, and recreational drugs. ACC indicates American College of Cardiology; AHA, American Heart Association; HDL, high-density lipoprotein; and IQR, interquartile range.

orthostatic heart rate response were 4.7% ( $P<0.001$ ), 4.2% ( $P<0.001$ ), and  $-0.42\%$  ( $P=0.62$ ), respectively. Heart rate was associated with the observer examining the worker (Figure S2), room temperature (Figure S3), and season (Figure S4), confounders for which the multivariable association analysis was adjusted for. Workers did not report any change in their self-assessed quality of life or self-graded physical health from baseline to last follow-up (Table S1).

### Categorical Analysis

In analyses by increasing fourths (quartiles) of the distribution of the follow-up-to-baseline blood lead ratio (Table 3), trends in heart rate and orthostatic heart rate responses did not reach significance ( $0.088\leq P\leq 0.68$ ). Across escalating categories of lead exposure (Table 3), significant increases were noted in the supine heart rate ( $+9.3\%$ ;  $P<0.001$ ) in the lowest category, in the standing heart rate in the lowest ( $+10.3\%$ ;  $P=0.001$ ) and top category ( $+4.6\%$ ;  $P=0.043$ ), whereas the orthostatic heart rate response ( $-3.8\%$ ;  $P=0.021$ ) decreased in the low-middle category.

### Association Analysis

In unadjusted analyses, for a doubling of follow-up-to-baseline blood lead ratio (Table 4), the supine heart rate decreased by 0.76% ( $P=0.014$ ) with a similar trend

in the standing heart rate ( $-0.70\%$ ;  $P=0.067$ ), but no change in the orthostatic heart rate response ( $+0.06\%$ ;  $P=0.82$ ). In multivariable-adjusted analyses (Table 4), only the inverse association between the baseline-to-follow-up change in supine heart rate and the follow-up-to-baseline change in the blood lead concentration ratio remained significant ( $-0.55\%$  for a doubling of blood lead ratio;  $P=0.029$ ).

### Heart Rate Variability

In this article, HRV derived by fast Fourier transform is presented as the main study outcome, while the largely confirmatory autoregressive HRV results are given in the Data Supplement.

### Baseline-to-Follow-Up Changes

Over follow-up (Table 2), with no difference between the first and second follow-up visit, the Fourier-derived supine total power decreased by 16.5% and supine high-frequency power decreased by 20.6% ( $P<0.001$ ), resulting in an increase in the low-to-high-frequency ratio by 27.6% (Table 2;  $P<0.001$ ). In line with the supine measurements, standing Fourier-derived high-frequency power decreased by 12.5% ( $P=0.002$ ) with an opposite change in the low-to-high-frequency ratio ( $+13.6\%$ ;  $P=0.014$ ). The orthostatic heart rate responses did not change during follow-up. The autoregression-derived analysis of HRV (Table S2), consistent with the Fourier analysis showed decreases in the supine ( $-19.3\%$ ;  $P<0.001$ ) and standing ( $-11.3\%$ ;  $P=0.002$ ) high-frequency power with corresponding increases in the low-to-high frequency ratios:  $+30.1\%$  and  $+9.4\%$  ( $P\leq 0.049$ ), respectively. Compared with the Fourier analysis, the orthostatic autoregressive HRV responses over follow-up were directionally opposite for low-frequency power ( $-7.63\%$ ;  $P=0.019$ ) but similar and significant for high-frequency HRV ( $+9.87\%$ ;  $P=0.051$ ) and for the low-to-high frequency HRV ratio ( $-15.9\%$ ;  $P=0.001$ ).

### Categorical Analysis

In analyses by increasing fourths (quartiles) of the distribution of the follow-up-to-baseline blood lead ratio (Table 3), trends in the Fourier HRV indexes did not reach significance ( $0.074\leq P\leq 0.97$ ). In the lowest, the low-middle and the high-middle categories of the follow-up-to-baseline lead concentration ratio (Table 3), there was a decrease in the supine Fourier high-frequency HRV power ( $-24.3\%$ ,  $-21.7\%$ , and  $-22.7\%$ , respectively;  $P\leq 0.029$ ) with corresponding increases in the supine low-to-high frequency power ratio ( $+44.3\%$ ,  $+28.5\%$ , and  $+29.1\%$ ;  $P\leq 0.014$ ).

In line with the Fourier approach, none of the trends in the autoregressive HRV indexes across increasing lead exposure categories (Table S3) was significant ( $0.076\leq P\leq 0.98$ ). In the supine position, in all 4 categories of lead exposure, the supine high-frequency HRV

**Table 2. Baseline and Follow-Up Heart Rate and Heart Rate Variability Derived by Fourier Transform**

Characteristic	Baseline	Follow-up		Δ in percent (95% CI)	P value
		Year 1	Year 2		
Supine position					
Heart rate, beats per minute	63.6 (57.0 to 71.0)	66.4 (59.0 to 74.0)	67.1 (61.0 to 74.0)	4.7 (2.8 to 6.6)	<0.001
Total power, ms <sup>2</sup>	1611 (864 to 3157)	1302 (633 to 2425)	1398 (733 to 2514)	-16.5 (-27.4 to -4.4)	0.009
Low-frequency power, nu	45.6 (38.5 to 60.1)	46.5 (38.8 to 60.2)	44.4 (35.1 to 61.5)	1.2 (-4.7 to 7.6)	0.69
High-frequency power, nu	19.3 (13.2 to 29.5)	16.1 (10.9 to 26.3)	14.2 (9.8 to 24.7)	-20.6 (-27.3 to -13.3)	<0.001
Low-to-high frequency ratio	2.4 (1.5 to 3.6)	2.9 (1.7 to 4.7)	3.1 (1.9 to 5.1)	27.6 (16.5 to 39.7)	<0.001
Standing position					
Heart rate, beats per minute	75.5 (67.0 to 85.0)	78.4 (70.0 to 88.0)	78.8 (71.0 to 86.0)	4.2 (1.8 to 6.6)	<0.001
Total power, ms <sup>2</sup>	1401 (833 to 2585)	1348 (781 to 2379)	1268 (672 to 2292)	-5.7 (16.8 to 6.9)	0.36
Low-frequency power, nu	56.3 (47.1 to 72.6)	55.6 (46.0 to 70.2)	56.5 (47.3 to 72.1)	-0.6 (-5.9 to 4.9)	0.82
High-frequency power, nu	9.2 (6.1 to 14.6)	8.1 (5.6 to 13.0)	8.0 (5.8 to 11.9)	-12.5 (-19.7 to -4.7)	0.002
Low-to-high frequency ratio	6.1 (3.8 to 10.0)	6.9 (4.2 to 10.9)	7.0 (4.8 to 10.7)	13.6 (2.7 to 25.6)	0.014
Orthostatic responses					
Heart rate, beats per minute	1.19 (1.10 to 1.26)*	1.18 (1.08 to 1.27)*	1.17 (1.09 to 1.25)*	-0.42 (-2.10 to 1.28)	0.62
Total power, ms <sup>2</sup>	0.87 (0.51 to 1.51)*	1.04 (0.55 to 2.09)*	0.91 (0.48 to 1.50)*	13.0 (-1.50 to 29.6)	0.081
Low-frequency power	1.23 (0.98 to 1.56)*	1.20 (0.87 to 1.60)*	1.27 (0.93 to 1.78)*	-1.84 (-9.23 to 6.15)	0.64
High-frequency power	0.48 (0.30 to 0.78)*	0.50 (0.30 to 0.87)*	0.56 (0.33 to 0.90)*	10.2 (-1.55 to 23.5)	0.091
Low-to-high frequency ratio	2.58 (1.60 to 4.06)*	2.38 (1.35 to 4.05)*	2.04 (1.27 to 3.12)*	-11.0 (-21.0 to 0.35)	0.057

Values are geometric means (interquartile range). Orthostatic responses are expressed as the standing-to-supine ratio. Changes from baseline to follow-up (Δ), given with 95% CI, are presented as percent change. P values denote the significance of the changes from baseline to follow-up. nu indicates normalized units.

\*Significance of the within-participant orthostatic responses:  $P \leq 0.001$ .

power decreased (point estimates ranging from -20.1% to -24.9%;  $P \leq 0.017$ ) with corresponding increases in the supine low-to-high frequency ratio (point estimates ranging from 25.7% to 52.8%;  $P \leq 0.022$ ). In the standing position (Table S3), the autoregressive high-frequency HRV power decreased in the lowest (-20.2%;  $P = 0.033$ ) and highest (-15.5%;  $P = 0.031$ ) increasing exposure categories but not in the middle categories ( $P \geq 0.22$ ).

### Association Analysis

In unadjusted analyses, for a doubling of the follow-up-to-baseline blood lead concentration ratio (Table 4), the supine Fourier low-frequency HRV power decreased by 2.93% ( $P = 0.006$ ). None of the other Fourier-derived HRV indexes was significantly related with the blood lead changes, when unadjusted (Table 4 and Figure S5). The unadjusted and adjusted association analyses of HRV derived by autoregression was confirmatory in that none of the multivariable-adjusted associations with the blood lead increase reached significance; the point estimates ranged from -2.19% to +2.02% ( $0.20 \leq P \leq 0.99$ ; Table S4).

Multivariable-adjusted heat plots were constructed to visualize the complex relations between the changes in the HRV indexes, heart rate, and blood lead in the supine (Figure 2) and standing (Figure S6) positions. In the supine position (Figure 2), the baseline-to-follow-up percent changes in the low-frequency power trended to increase ( $P = 0.080$ ) with the baseline-to-follow-up heart

rate ratio, while the low-to-high frequency ratio increased ( $P < 0.001$ ) and the high-frequency power decreased ( $P = 0.002$ ) with the baseline-to-follow-up heart rate ratio. The percent changes in the low-frequency power decreased with the follow-up-to-baseline blood lead concentration ratio ( $P = 0.011$ ; Figure 2), whereas the corresponding associations were not significant ( $P \geq 0.21$ ; Figure 2) for the percent changes in high-frequency power and the low-to-high frequency ratio. Findings in the standing position were largely confirmatory (Figure S6). However, the percent changes in the baseline-to-follow-up low-frequency power increased with the heart rate ( $P = 0.017$ ) but not with the follow-up-to-baseline blood lead concentration ratio ( $P = 0.63$ ).

### Sensitivity and Validation Analyses

To account for the body burden of lead at enrolment, Fourier-derived HRV was reanalyzed stratified by median baseline blood lead (4.30  $\mu\text{g}/\text{dL}$ ; Table S5) and the median CBLI (32.1  $\mu\text{g}/\text{dL} \times \text{year}$ ; Table S6). The strata  $\times$  follow-up-to-baseline blood lead ratio interaction terms were all nonsignificant except for the orthostatic response of low-frequency power in the analysis stratified by median baseline blood lead (multivariable-adjusted association for a doubling of the follow-up-to-baseline blood lead ratio in the low versus high baseline blood lead group, +13.3% versus -5.95%; interaction  $P = 0.017$ ; Table S5) and for the supine high-frequency power dichotomized by CBLI

**Table 3. Baseline-to-Follow-Up Heart Rate and Fourier Heart Rate Variability Changes by Fourths of Blood Lead Change Distribution**

Characteristic	Follow-up to baseline blood lead ratio				P value
Quartile limits	<1.92	1.92 to 3.29	3.30 to 5.29	>5.29	
Number in category	49	48	49	49	
Supine position					
Heart rate, %	9.3 (5.1 to 13.8)*	3.2 (−0.1 to 6.9)	3.9 (−0.1 to 8.2)	3.7 (−0.6 to 8.2)	0.088
Total power, %	−26.0 (−46.8 to 3.0)	−5.3 (−26.8 to 22.6)	−1.8 (−27.5 to 33.0)	−23.6 (−43.2 to 2.8)	0.84
Low-frequency power, %	9.2 (−5.7 to 26.5)	0.54 (−12.6 to 15.7)	−0.16 (−12.7 to 14.2)	−9.88 (−22.5 to 4.8)	0.074
High-frequency power, %	−24.3 (−38.4 to −6.9)*	−21.7 (−36.8 to −3.1)*	−22.7 (−35.6 to −7.1)*	−25.8 (−39.3 to −9.3)*	0.87
Low-to-high frequency ratio, %	44.3 (16.4 to 78.8)*	28.5 (7.5 to 53.5)*	29.1 (6.0 to 57.2)*	21.5 (−3.0 to 52.2)	0.27
Standing position					
Heart rate, %	10.3 (4.4 to 16.6)*	−0.6 (−5.2 to 4.2)	3.6 (−1.5 to 9.1)	4.6 (0.2 to 9.1)*	0.29
Total power, %	−20.7 (−41.5 to 7.5)	16.1 (−10.9 to 51.3)	−6.9 (−31.2 to 26.0)	−11.5 (−30.7 to 12.9)	0.86
Low-frequency power, %	0.70 (−10.1 to 12.8)	−5.6 (−15.5 to 5.5)	−0.4 (−13.1 to 14.1)	3.58 (−8.6 to 17.4)	0.63
High-frequency power, %	−19.2 (−35.4 to 1.2)	−3.5 (−20.7 to 17.4)	−16.8 (−31.2 to 0.8)	−10.6 (−25.0 to 6.46)	0.73
Low-to-high frequency ratio, %	24.6 (−2.19 to 58.7)	−2.1 (−22.8 to 24.1)	19.6 (−5.4 to 51.3)	15.9 (−5.5 to 42.2)	0.97
Orthostatic responses					
Heart rate, %	0.9 (−3.4 to 5.5)	−3.8 (−6.8 to −0.7)*	−0.01 (−3.6 to 3.7)	0.84 (−2.7 to 4.5)	0.68
Total power, %	7.1 (−21.5 to 46.2)	22.6 (−7.4 to 62.2)	−5.2 (−29.5 to 27.5)	15.8 (−14.3 to 56.5)	0.98
Low-frequency power, %	−7.8 (−24.6 to 12.7)	−6.1 (−21.2 to 12.0)	−0.3 (−17.3 to 20.2)	14.9 (−3.9 to 37.5)	0.089
High-frequency power, %	6.8 (−16.7 to 36.8)	23.2 (−7.38 to 63.9)	7.6 (−17.2 to 40.0)	20.5 (−5.6 to 53.8)	0.70
Low-to-high frequency ratio, %	−13.6 (−33.5 to 12.1)	−23.8 (−41.0 to −1.53)*	−7.4 (−30.4 to 23.3)	−4.61 (−26.4 to 23.7)	0.42

Baseline-to-follow-up heart rate and heart rate variability changes are expressed as percent change with 95% CI. *P* values are for linear trend across fourths of the distribution of the follow-up-to-baseline blood lead concentration ratio.

\*Significance of the within-participant changes:  $P < 0.05$ .

(low versus high CBLI category, +3.04% versus −5.50%; interaction  $P = 0.022$ ; Table S6). Finally, at baseline and follow-up, in both the supine and standing positions, the slopes of the HRV components on heart rate were similar ( $0.11 \leq P \leq 0.93$ ; Table S7).

To validate the HRV results, peripheral NCV was assessed in 192 workers as the commonly used neurotoxicology benchmark in occupational lead exposure. In crude analyses, the latency time increased from 3.62 ms (IQR, 3.30–3.95 ms) to 3.70 ms (IQR, 3.35–4.01 ms). The difference between baseline and follow-up was 2.13% (CI, 0.41–3.89%;  $P = 0.016$ ). From the lowest to the top fourth of the distribution of the follow-up-to-baseline blood lead concentration ratio, the percent changes in latency time were 2.91% (CI, −0.89 to 6.85%;  $P = 0.14$ ), 0.86% (CI, −2.48 to 4.32%;  $P = 0.064$ ), 2.14% (CI, −0.86 to 5.23%;  $P = 0.057$ ), and 2.57% (CI, −0.93 to 6.19%;  $P = 0.67$ ); the *P* value for linear trend was 0.98. The percent changes in latency time associated with a doubling of blood lead from baseline to follow-up were −0.05% (CI, −0.57 to 0.46%;  $P = 0.84$ ) unadjusted and −0.09% (CI, −0.58 to 0.40%;  $P = 0.72$ ) adjusted.

## DISCUSSION

Heart rate and HRV reflect the complex sinoatrial node responses to alterations in the balance between

the sympathetic and parasympathetic outflow from the central autonomic nervous system,<sup>36,37</sup> which is under control of multiple host and environmental factors. Factors contributing to heart rate and HRV include genetic make-up,<sup>38,39</sup> the respiratory cycle, the oxygen tension in breathed air,<sup>40</sup> body position,<sup>41</sup> habitual physical activity,<sup>42</sup> psychosocial context,<sup>43</sup> circadian rhythmicity,<sup>44</sup> as reflected by the nocturnal decrease in heart rate,<sup>45,46</sup> or biorhythm alteration associated with rotating shift work shift.<sup>43,47</sup> Our study represents an ethically approved real-world experiment among newly hired workers without known previous occupational exposure, starting jobs in lead recycling factories. The health of the labor force employed at these factories was carefully surveyed in compliance with the US Occupational Safety and Health Administration Standard (<https://www.osha.gov/laws-regs/regulations/standardnumber/1910/1910.1025>), which includes regular health check-ups, proper workplace ventilation, and the obligatory use of personal protective equipment.

## Key Findings

The crude observations in our 2-year longitudinal study can be summarized as follows: (1) lead exposure, as captured by the blood lead concentration, increased >3-fold; (2) heart rate as recorded by ECG in the supine and

**Table 4. Associations of Baseline-to-Follow-Up Heart Rate and Fourier Heart Rate Variability Changes With Blood Lead Changes**

Variable	Unadjusted		Adjusted	
	Estimate (95% CI)	P value	Estimate (95% CI)	P value
Supine position				
Heart rate, %	-0.76 (-1.36 to -0.16)	0.014	-0.55 (-1.04 to -0.06)	0.029
Total power, %	3.13 (-1.50 to 7.97)	0.19	3.02 (-0.61 to 6.78)	0.10
Low-frequency power, %	-2.93 (-4.95 to -0.87)	0.006	-0.99 (-2.69 to 0.73)	0.25
High-frequency power, %	-0.13 (-3.10 to 2.92)	0.93	-0.65 (-3.36 to 2.13)	0.64
Low-to-high frequency ratio, %	-2.63 (-5.61 to 0.44)	0.092	-1.64 (-4.57 to 1.38)	0.28
Standing position				
Heart rate, %	-0.70 (-1.44 to 0.05)	0.067	-0.28 (-0.90 to 0.34)	0.37
Total power, %	0.37 (-3.59 to 4.50)	0.86	-0.72 (-4.06 to 2.74)	0.68
Low-frequency power, %	1.02 (-0.88 to 2.96)	0.29	0.81 (-0.66 to 2.30)	0.28
High-frequency power, %	2.30 (-0.74 to 5.44)	0.14	1.19 (-1.35 to 3.80)	0.36
Low-to-high frequency ratio, %	-1.25 (-4.70 to 2.33)	0.49	-0.56 (-3.38 to 2.34)	0.70
Orthostatic change				
Heart rate, %	0.06 (-0.50 to 0.63)	0.82	0.14 (-0.37 to 0.65)	0.60
Total power, %	-2.71 (-7.17 to 1.97)	0.25	-4.04 (-7.49 to -0.47)	0.027
Low-frequency power, %	3.98 (-5.83 to 14.8)	0.43	2.99 (-3.51 to 9.93)	0.36
High-frequency power, %	2.30 (-1.60 to 6.36)	0.25	0.80 (-2.35 to 4.06)	0.62
Low-to-high frequency ratio, %	1.41 (-2.71 to 5.71)	0.50	1.07 (-2.35 to 4.61)	0.54

Estimates are association sizes, given with 95% CI, and relate the baseline-to-follow-up percent changes in heart rate and Fourier heart rate variability to the follow-up-to-baseline blood lead ratio. Association sizes are expressed for a doubling of the blood lead concentration ratio. Mixed models accounted for the within-participant clustering of the baseline and the 1- and 2-year follow-up data. In multivariable-adjusted analyses, the covariables were sex, the baseline heart rate or heart rate variability index, as appropriate, the baseline value and the changes during follow-up in age (equivalent to follow-up duration), mean arterial pressure, serum insulin, hemoglobin, room temperature during the examination, season, and observer (random effect).

standing positions (Table 2) increased by approximately 5 and 3 beats per minute, respectively; (3) using the Fourier HRV approach, low-frequency power did not change over follow-up, while high-frequency power decreased and the low-to-high frequency ratio increased in the supine and standing position with a similar trend in the orthostatic responses (Table 2); (4) the autoregressive approach produced largely confirmatory results (Table S2); and (5) the latency time measured over the median nerve increased by 0.08 ms on average, a clinically irrelevant quantity within the error margin of the technology.<sup>48</sup>

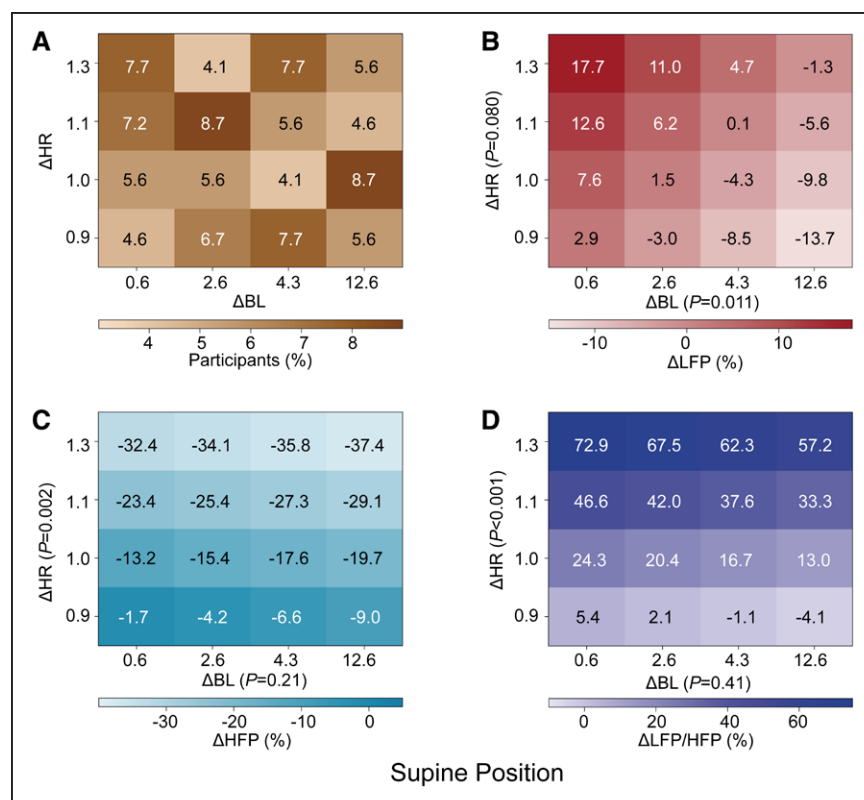
The objective of our current study was to relate the baseline-to-follow-up changes in heart rate, HRV indexes and NCV and the blood lead changes, captured by the follow-up-to-baseline blood lead concentration ratio. The key findings were (1) across escalating categories of the follow-up-to-baseline blood lead ratio, trends in heart rate (Table 3) and in the Fourier (Table 3) and autoregressive (Table S3) HRV indexes were not significant; (2) the analysis of heart rate and the HRV indexes across increasing categories of the blood lead changes did not reveal a consistent dose-response relation with increments in heart rate (Table 3) and the HRV indexes (Table 3 and Table S3) being observed at small as well at large blood lead changes; (3) in multivariable-adjusted analyses, the associations of the baseline-to-follow-up changes

in heart rate (Table 4) and in the Fourier (Table 4 and Figure S5) and autoregressive (Table S4) HRV indexes with the blood lead changes were all nonsignificant with the exception of a borderline significant ( $P=0.029$ ) association for the heart rate change in the supine position (Table 4); and (4) the median nerve NCV and blood lead changes were unrelated. At baseline and follow-up, in both the supine and standing positions, the slopes of the HRV components were similar (Table S7), suggesting that the inputs of the autonomous nervous system into the reflex arches<sup>36</sup> or directly into the heart controlling the firing rate of the sinoatrial node were functionally intact, irrespective of lead exposure. In line with this interpretation and the overall evidence generated by our study, the limited heart rate increases observed clinically and electrocardiographically (Table 2) probably reflect a reset of the heart rate set point related to the alternating work shifts and the associated circadian biorhythm misalignment and to the physically intensive labor, to which workers were exposed starting at job initiation.

### Clinical Interpretation

Total power, the major component in the divisor used for computing normalized HRV units as applied in our current study, increases with sympathetic activation,





**Figure 2.** Heat maps relating the percent changes in the heart rate variability indexes with the follow-up-to-baseline heart rate ratio ( $\Delta HR$ ) and the follow-up-to-baseline blood lead concentration ratio ( $\Delta BL$ ) in the supine position.

**A**, the percent of workers (N=195) in each cell in the cross-classification between the follow-up-to-baseline heart rate ratio (vertical axis) and the follow-up-to-baseline blood lead concentration ratio (horizontal axis).  $\Delta LFP$  indicates the percent change in low-frequency power (**B**);  $\Delta HFP$ , percent change in high-frequency power (**C**); and  $\Delta LFP/HFP$ , percent change in low-to-high frequency ratio (**D**). Mixed models accounted for sex, the baseline value, and the change during follow-up in age, mean arterial pressure, serum insulin, hemoglobin, room temperature during the examination, season, and observer (random effect).

whereas vagal activation produces the opposite effect.<sup>49</sup> Efferent vagal activity is the major contributor to the high-frequency HRV component, as evidenced by clinical and experimental interventions, such as electrical vagal stimulation, muscarinic receptor blockade, and vagotomy.<sup>50</sup> More controversial is the interpretation of the low-frequency component, which is variously interpreted as a marker of sympathetic modulation, in particular if expressed in normalized units.<sup>30,37</sup> Alternatively, experts consider the low-frequency HRV component as a measure reflecting the balance between the sympathetic and parasympathetic autonomous nervous outflow.<sup>30,32,37,51</sup> A more recently proposed interpretation of low-frequency HRV is that it does not primarily reflect sympathetic efferent nervous outflow to the heart but rather baroreflex function.<sup>36</sup> Consequently, according to some experts, manipulations and drugs that change low-frequency power or the low-to-high frequency ratio are not affecting autonomic nervous outflow to the heart in a direct manner but modulate these outflows via baroreflex arches.<sup>36</sup>

Whatever, the interpretation of low-frequency power, the physiological soundness of our current findings is substantiated by the expected heart rate and HRV orthostatic responses (Table 2 and Table S2) and by the associations between the baseline-to-follow-up changes in heart rate and in the supine (Figure 2) and standing (Figure S6) HRV components. The inverse association between the baseline-to-follow-up heart rate and high-frequency HRV component (Figure 2 and Figure S6),

reflecting the balance between sympathetic and vagal tone,<sup>30,37</sup> suggests that independent of lead exposure (Figure 2 and Figure S6) the equilibrium between both arms of the autonomous nervous system moved to a more pronounced vagal control of heart rate, an interpretation also supported by the positive association between the baseline-to-follow-up changes in heart rate and the low-to-high-frequency power ratio (Figure 2 and Figure S6).

Lead is a cumulative contaminant, which is for 90% to 95% stored in bone, from where it is recirculated with a half-life of 20 to 25 years.<sup>52,53</sup> Blood lead, for 99% carried by red blood cells, reflects recent exposure over the past 1 to 2 months and the amount of lead released and recirculated from bone.<sup>52</sup> Both bone<sup>53,54</sup> and blood<sup>53-55</sup> lead increase with advancing age. Bone lead correlates with blood lead<sup>53,54</sup> and explains around 20% of the variance in blood lead, depending on seasonality<sup>53</sup> and hormonal and other endogenous and environmental stimuli influencing the balance between bone formation and resorption.<sup>54</sup> Our current study demonstrated that 1 year after starting occupational exposure steady-state blood lead levels were achieved with no further increase 1 year later (Figure S1). Our sensitivity analyses dichotomized by the baseline median blood lead concentration (Table S5) or the median CBLI (Table S6) addressed the lead burden before starting a job in the lead industry, but no consistent pattern emerged. Our current HRV findings are also in keeping with the absence of an association of 24-hour blood pressure or the incidence of hypertension, the primary end point, with the increase in blood lead in the SPHERL cohort.<sup>26</sup>

## Added Value to the Literature

Araki et al<sup>1</sup> reviewed 102 articles to evaluate the effects related to lead on peripheral, central, and autonomic nervous system function. The pooled data suggested that effects on HRV and NCV started at mean blood levels exceeding 40 to 50  $\mu\text{g}/\text{dL}$  and 30 to 40  $\mu\text{g}/\text{dL}$ , respectively.<sup>1</sup> Another meta-analysis of 49 studies, including 2825 exposed and 1629 control individuals, showed that the lowest blood lead levels significantly associated with a NCV reduction in the sensory median nerve and a greater median nerve motor latency time were 33.0  $\mu\text{g}/\text{dL}$  and 64.0  $\mu\text{g}/\text{dL}$ , respectively.<sup>56</sup> Such levels far exceeded current occupational (<https://www.osha.gov/laws-regs/preambles#lead>) and environmental<sup>2–5</sup> lead exposure in the United States, but nevertheless led the Global Burden of Disease Study to infer that occupational and environmental lead exposure was a direct cause of cardiac autonomous nervous dysfunction, mainly causing the aggregate of atrial fibrillation and flutter.<sup>57</sup> The current SPHERL data do not confirm this proposition as no exposed worker developed atrial arrhythmias, once more illustrating that population health metrics need careful interpretation.<sup>58</sup> A closer inspection of both meta-analyses<sup>1,56</sup> highlighted that many of the reviewed studies had implemented a case-control design, in which lead exposure was not measured but inferred from the job description and that investigators failed to consider how physical activity or rotating shift work<sup>42,43,47,59</sup> might have operated alongside the lead exposure.

## Strengths and Limitations

Among the strong points of our study are its longitudinal design with annual follow-up visits and the stringent quality control of the blood lead measurement maintained during the 2-year course of the study (Data Supplement). To avoid confounding of HRV by use of medications, we excluded workers on neuro-active or antihypertensive drugs. We also removed workers with incident diabetes or new-onset treated hypertension from the analysis. However, they only represented  $\approx 5\%$  of the participants potentially eligible for analysis, so that we are confident that removal did not bias our HRV results. Furthermore, the methods to quantify HRV in SPHERL complied with expert recommendations,<sup>37</sup> which favor frequency domain over time-domain approaches. The recording conditions, including observer, room temperature, and season were carefully recorded and accounted for. HRV recordings should last for at least 10 $\times$  the wavelength of the lower frequency bound of the investigated HRV component.<sup>37</sup> Thus, recordings of  $\approx 1$  minute are needed to assess the high-frequency HRV power, while  $\approx 2$  minutes allow addressing the low-frequency component. To standardize the investigation of short-term HRV, 5-minute recordings are recommended in stationary conditions.<sup>37</sup> Our HRV results were backed up by median

nerve NCV, a standard test in occupational medicine.<sup>23,24</sup> Finally, the multivariable-adjusted analyses covered all identifiable sources of heterogeneity between study participants and between baseline and follow-up measurement conditions.

Notwithstanding its strong points, our study also has limitations. First, the attrition rate among the 506 workers who participated in the baseline examination, but defaulted from follow-up amounted to 217 (42.9%), mainly because they left employment (Figure 1). However, according to the SPHERL protocol,<sup>22</sup> the anticipated attrition rate was 50% and as compensation the stated goal was to enroll 500 workers to address the co-primary end points, that is, blood pressure and renal function. We did enroll 506 workers, a stated objective that was met and the attrition rate was slightly lower than predicted (214 workers of 506 entering the study [42.9%]; Figure 1). After excluding workers on neuro-active or antihypertensive drugs at baseline (Table S8), the enrollment characteristics of the 195 workers analyzed and their 281 counterparts not analyzed were similar with the exception of diastolic blood pressure (79.1 versus 80.7 mmHg) and the prevalence of stage 1 hypertension (47.2% versus 59.4%). Second, the median 2-year follow-up did not capture the potential long-term influence of lead exposure on the autonomous nervous system. Therefore, as planned,<sup>22</sup> the cohort will be followed further for an additional 2 years, using a simplified protocol focusing on key traits potentially associated with low-level lead toxicity. Third, the healthy worker effect might partially account for the nonsignificant results in relation to lead exposure in this occupational cohort.<sup>60</sup> Fourth, although the ethnic distribution of the workers was representative for the population residing in the catchment area around the factories, women represented only 8.7% of the study population, which precluded analyses stratified by sex. Finally, measurement of HRV according to the Ewing's methodology in its original format<sup>61</sup> or even in a simplified version,<sup>62</sup> would have added complexity to the SPHERL protocol not compatible with the time workers had to spend at the medical facility of the industrial sites or with the occupational—not research—setting of the study and the work flow of its medical staff.

## Perspectives

At the exposure in our current study, we failed to demonstrate an association of neural function with an over 3-fold increase in blood lead. Ongoing analyses of the same cohort are focusing on a detailed evaluation of the effects of rotating shift work, changing diurnal rhythmicity and taking up physically strenuous labor on cardiovascular regulation. Our review of the literature revealed that confounding between exposure to hazardous materials and changing living and working conditions was rarely addressed. Lead is a hazardous

environmental contaminant, which should be addressed worldwide. The observation that in healthy, mostly young and male workers, there was no relation between neural function, as assessed by HRV and NCV, might inform regulators in setting safe lead exposure thresholds in occupational settings.

## ARTICLE INFORMATION

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### Affiliations

From the Research Unit Hypertension and Cardiovascular Epidemiology, KU Leuven Department of Cardiovascular Sciences, University of Leuven, Belgium (Y.-L.Y., L.T., J.D.M., D.-M.W., Z.-Y.Z.); Department of Cardiology, Guangdong Cardiovascular Institute, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangzhou, China (Y.-L.Y.); Center for Endocrine Metabolism and Immune Diseases, Beijing Luhe Hospital and Key Laboratory of Diabetes Prevention and Research, Capital Medical University, China (C.-G.Y.); Department of Cardiology, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, China (W.-Y.Y.); Department of Cardiology, the First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, Guangdong, China (F.-F.W.); Centre for Environmental Sciences, Hasselt University, Diepenbeek, Belgium (T.S.N., H.A.R.); Centre for Molecular and Vascular Biology, KU Leuven Department of Cardiovascular Sciences, University of Leuven, Belgium (P.V.); Research Institute Alliance for the Promotion of Preventive Medicine (J.A.S.); and Biomedical Science Group, Faculty of Medicine, University of Leuven, Belgium (J.A.S.).

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### Disclosures

None.

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