Hypertension

NERVOUS SYSTEM

# 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, Dong-Mei Wei, Fang-Fei Wei, Tim S. Nawrot, Peter Verhamme, Harry A. Roels, Jan A. Staessen,† 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 μg/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 μg/dL or 32.1 μg/dL×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 μg/dL.[1] 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 μg/dL in NHANES II (1976–1980)[2,3] to 1.2 to 2.76 μg/dL in NHANES III (1988–1994),[2,3] and to 1.64 μg/dL in NHANES IV (1999–2002).[4,5] 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,[6-15] and that most articles reported on experimental work with focus on potential neurotoxic mechanisms.[10-15] 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@appremed.org

This article was sent to Marc L. De Buyzere, Guest Editor, for review by expert referees, editorial decision, and final disposition.

*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.

The Data Supplement is available with this article at https://www.ahajournals.org/doi/suppl/10.1161/HYPERTENSIONAHA.120.16545.

For Sources of Funding and Disclosures, see page 1785.



*Hypertension* is available at www.ahajournals.org/journal/hyp

## 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 μg/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 μg/dL or 32.1 μg/dL×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 μg/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

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

heart rate variability (HRV) is associated with a higher risk of coronary heart disease,[16,17] atrial fibrillation,[18] sudden cardiac death,[19] and total mortality.[17,20] Given this background and the suspicion that environmental lead exposure causes cardiovascular disease,[21] 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,[23,24] 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.[22,25] SPHERL complies with the Helsinki declaration for investigation of humans.[27] The Ethics Committee of the University Hospitals Leuven (Belgium) approved the study (No. B322201421631), of which the protocol has been registered and published.[22] 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.[22] 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.[28] 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 µg/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.[29] A detailed description of the quality control program implemented for the biochemical measurements, including blood lead, is available in previous publications[26] 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.[30] 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)×100.[31,32]

## 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,[33] the within-worker bias (right minus left side) was 0.014 ms ($P$=0.83).[25] 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[25] 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).[34] To compute CBLI,[34] 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),[35] the corresponding blood lead levels were set at 2.2, 1.4, and 1.5 µg/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$^2$, and systolic and diastolic blood pressure 119.7 and 79.1 mm Hg, 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 μIU/mL (IQR, 3.60–13.0 μIU/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 μg/dL (IQR, 2.50–8.30 μg/dL; PI, 1.00–15.2 μg/dL), at baseline, 14.1 μg/dL (IQR, 9.95–22.2 μg/dL; PI, 4.20–30.2 μg/dL) at the first follow-up visit, and 14.1 μg/dL (IQR, 11.0–23.1 μg/dL; PI, 3.10–30.4 μg/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 μg/dL (IQR, 2.90–9.50 μg/dL) versus 3.94 μg/dL (IQR, 2.30–7.70 μg/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

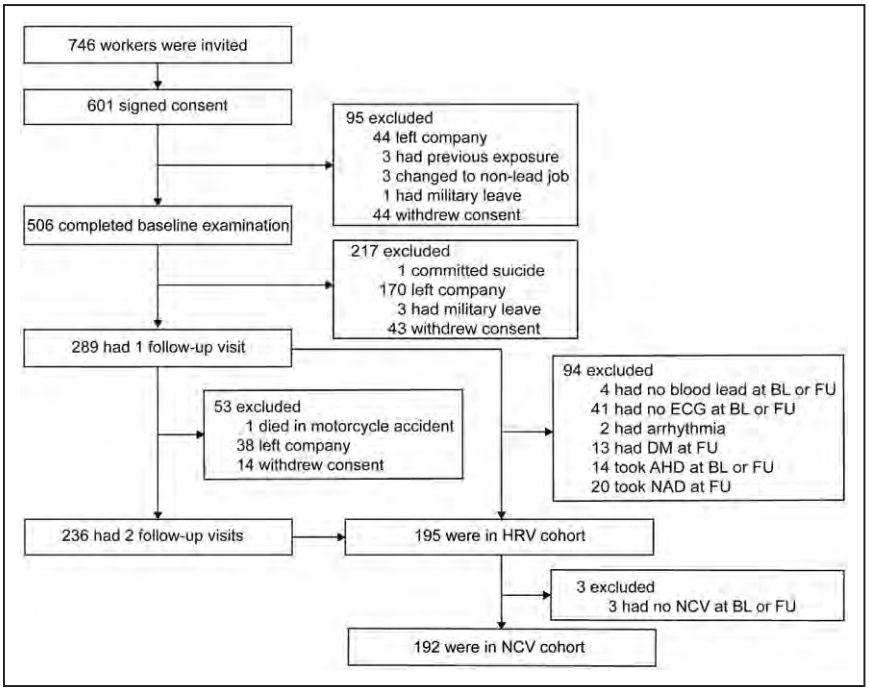



**Figure 1. Flow chart.**
AHD indicates antihypertensive drugs; BL, baseline; DM, diabetes; FU, follow-up; HRV, heart rate variability; NAD, neuro-active drugs, including benzodiazepines, neuroleptics, antiepileptics, sympathomimetics, amphetamines, and recreational drugs; and NCV, nerve conduction velocity.

**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/m² | 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≥stage 1 | 92 (47.2) | Hemoglobin, g/dL | 15.0 (1.2) |
| Hypertension≥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, µIU/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$^2$ | 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$^2$ | 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$^2$ | 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 μg/dL; Table S5) and the median CBLI (32.1 μg/dL×year; Table S6). The strata×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 ($0.11 \le P \le 0.93$; 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,[36,37] which is under control of multiple host and environmental factors. Factors contributing to heart rate and HRV include genetic make-up,[38,39] the respiratory cycle, the oxygen tension in breathed air,[40] body position,[41] habitual physical activity,[42] psychosocial context,[43] circadian rhythmicity,[44] as reflected by the nocturnal decrease in heart rate,[45,46] or biorhythm alteration associated with rotating shift work shift.[43,47] 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**

| | Unadjusted | | Adjusted | |
|---|---|---|---|---|
| Variable | 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.[48]

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[36] 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,

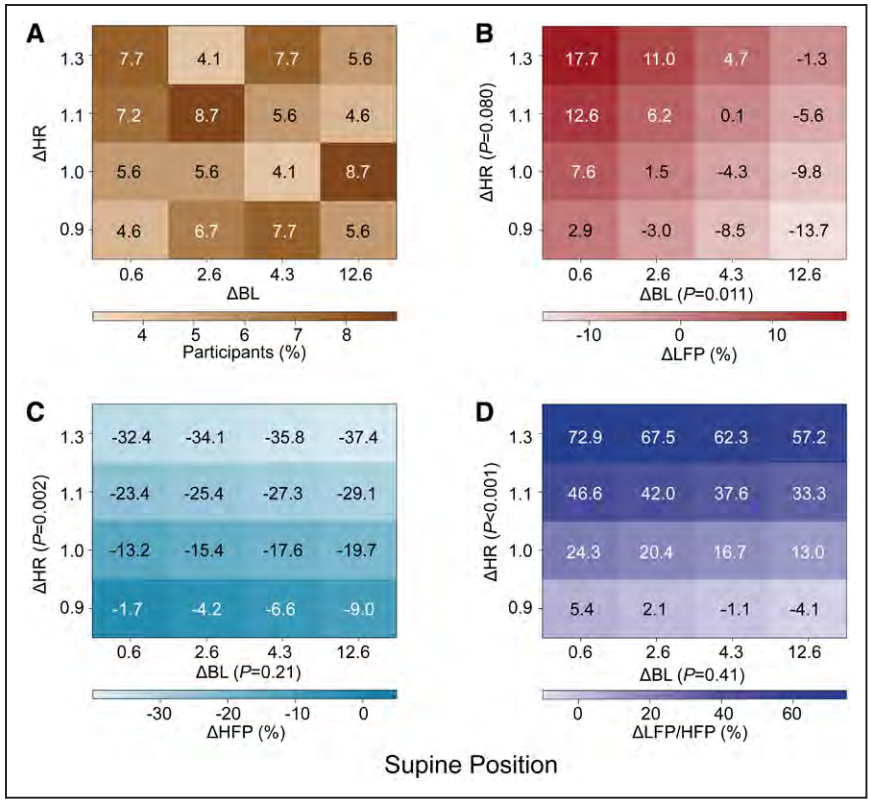



**Figure 2. Heat maps relating the percent changes in the heart rate variability indexes with the follow-up-to-baseline heart rate ratio (ΔHR) and the follow-up-to-baseline blood lead concentration ratio (ΔBL) in the supine position.**

**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). ΔLFP indicates the percent change in low-frequency power (**B**); ΔHFP, percent change in high-frequency power (**C**); and ΔLFP/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.[49] 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.[50] 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.[30,37] Alternatively, experts consider the low-frequency HRV component as a measure reflecting the balance between the sympathetic and parasympathetic autonomous nervous outflow.[30,32,37,51] 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.[36] 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.[36]

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,[30,37] 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.[52,53] 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.[52] Both bone[53,54] and blood[53–55] lead increase with advancing age. Bone lead correlates with blood lead[53,54] and explains around 20% of the variance in blood lead, depending on seasonality[53] and hormonal and other endogenous and environmental stimuli influencing the balance between bone formation and resorption.[54] 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.[26]

## Added Value to the Literature

Araki et al[1] 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 μg/dL and 30 to 40 μg/dL, respectively.[1] 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 μg/dL and 64.0 μg/dL, respectively.[56] Such levels far exceeded current occupational (https://www.osha.gov/laws-regs/preambles#lead) and environmental[2–5] 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.[57] 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.[58] A closer inspection of both meta-analyses[1,56] 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[42,43,47,59] 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 ≈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,[37] 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× the wavelength of the lower frequency bound of the investigated HRV component.[37] Thus, recordings of ≈1 minute are needed to assess the high-frequency HRV power, while ≈2 minutes allow addressing the low-frequency component. To standardize the investigation of short-term HRV, 5-minute recordings are recommended in stationary conditions.[37] Our HRV results were backed up by median nerve NCV, a standard test in occupational medicine.[23,24] 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,[22] 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 enrolment 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 mm Hg) 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,[22] 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.[60] 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[61] or even in a simplified version,[62] 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

Received October 27, 2020; accepted March 4, 2021.

### 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.).

### Acknowledgments

We gratefully acknowledge the nursing staff employed at the study sites in the United States and the expert clerical assistance of Vera De Leebeeck and Renilde Wolfs at the Studies Coordinating Centre in Leuven, Belgium.

### Sources of Funding

The International Lead Association (www.ila-lead.org) provided an unrestricted grant to the Research Unit Hypertension and Cardiovascular Epidemiology partially supporting data collection and management and statistical analysis. The Non-Profit Research Association Alliance for the Promotion of Preventive Medicine, Mechelen, Belgium (www.appremed.ord) received a nonbinding grant from OMRON Healthcare Co Ltd, Kyoto, Japan.

### Disclosures

None.

## REFERENCES

1. Araki S, Sato H, Yokoyama K, Murata K. Subclinical neurophysiological effects of lead: a review on peripheral, central, and autonomic nervous system effects in lead workers. *Am J Ind Med.* 2000;37:193–204. doi: 10.1002/(sici)1097-0274(200002)37:2<193::aid-ajim5>3.0.co;2-j
2. Gartside PS. The relationship of blood lead levels and blood pressure in NHANES II: additional calculations. *Environ Health Perspect.* 1988;78:31–34. doi: 10.1289/ehp.887831
3. Pirkle JL, Brody DJ, Gunter EW, Kramer RA, Paschal DC, Flegal KM, Matte TD. The decline in blood lead levels in the United States. The National Health and Nutrition Examination Surveys (NHANES). *JAMA.* 1994;272:284–291.
4. Muntner P, Menke A, DeSalvo KB, Rabito FA, Batuman V. Continued decline in blood lead levels among adults in the United States: the National Health and Nutrition Examination Surveys. *Arch Intern Med.* 2005;165:2155–2161. doi: 10.1001/archinte.165.18.2155
5. Hara A, Thijs L, Asayama K, Gu YM, Jacobs L, Zhang ZY, Liu YP, Nawrot TS, Staessen JA. Blood pressure in relation to environmental lead exposure in the national health and nutrition examination survey 2003 to 2010. *Hypertension.* 2015;65:62–69. doi: 10.1161/HYPERTENSIONAHA.114.04023
6. Böckelmann I, Pfister EA, McGauran N, Robra BP. Assessing the suitability of cross-sectional and longitudinal cardiac rhythm tests with regard to identifying effects of occupational chronic lead exposure. *J Occup Environ Med.* 2002;44:59–65. doi: 10.1097/00043764-200201000-00010
7. Park SK, Schwartz J, Weisskopf M, Sparrow D, Vokonas PS, Wright RO, Coull B, Nie H, Hu H. Low-level lead exposure, metabolic syndrome, and heart rate variability: the VA Normative Aging Study. *Environ Health Perspect.* 2006;114:1718–1724. doi: 10.1289/ehp.8992
8. Böckelmann I, Pfister E, Darius S. Early effects of long-term neurotoxic lead exposure in copper works employees. *J Toxicol.* 2011;2011:832519. doi: 10.1155/2011/832519
9. Zheng G, Tian L, Liang Y, Broberg K, Lei L, Guo W, Nilsson J, Bergdahl IA, Skerfving S, Jin T. δ-Aminolevulinic acid dehydratase genotype predicts toxic effects of lead on workers' peripheral nervous system. *Neurotoxicol.* 2011;32:374–382.
10. Lai CC, Lin HH, Chen CW, Chen SH, Chiu TH. Excitatory action of lead on rat sympathetic preganglionic neurons in vitro and in vivo. *Life Sci.* 2002;71:1035–1045. doi: 10.1016/s0024-3205(02)01789-7
11. Chang HR, Tsao DA, Yu HS, Ho CK. Beta-adrenergic receptor density and adenylate cyclase activity in lead-exposed rat brain after cessation of lead exposure. *Arch Toxicol.* 2005;79:1–6. doi: 10.1007/s00204-004-0605-9
12. Geraldes V, Carvalho M, Goncalves-Rosa N, Tavares C, Laranjo S, Rocha I. Lead toxicity promotes autonomic dysfunction with increased chemoreceptor sensitivity. *Neurotoxicology.* 2016;54:170–177. doi: 10.1016/j.neuro.2016.04.016
13. Simões MR, Preti SC, Azevedo BF, Fiorim J, Freire DD Jr, Covre EP, Vassallo DV, Dos Santos L. Low-level chronic lead exposure impairs neural control of blood pressure and heart rate in rats. *Cardiovasc Toxicol.* 2017;17:190–199. doi: 10.1007/s12012-016-9374-y
14. Toscano CM, Simões MR, Alonso MJ, Salaices M, Vassallo DV, Fioresi M. Subchronic lead exposure produces β1-adrenoceptor downregulation decreasing arterial pressure reactivity in rats. *Life Sci.* 2017;180:93–101. doi: 10.1016/j.lfs.2017.05.009
15. Zhu G, Chen Z, Dai B, Zheng C, Jiang H, Xu Y, Sheng X, Guo J, Dan Y, Liang S, et al. Chronic lead exposure enhances the sympathoexcitatory response associated with P2X4 receptor in rat stellate ganglia. *Environ Toxicol.* 2018;33:631–639. doi: 10.1002/tox.22547
16. Tsuji H, Venditti FJ Jr, Manders ES, Evans JC, Larson MG, Feldman CL, Levy D. Reduced heart rate variability and mortality risk in an elderly cohort. The Framingham Heart Study. *Circulation.* 1994;90:878–883. doi: 10.1161/01.cir.90.2.878
17. Dekker JM, Crow RS, Folsom AR, Hannan PJ, Liao D, Swenne CA, Schouten EG. Low heart rate variability in a 2-minute rhythm strip predicts risk of coronary heart disease and mortality from several causes: the ARIC Study. Atherosclerosis Risk In Communities. *Circulation.* 2000;102:1239–1244. doi: 10.1161/01.cir.102.11.1239
18. Agarwal SK, Norby FL, Whitsel EA, Soliman EZ, Chen LY, Loehr LR, Fuster V, Heiss G, Coresh J, Alonso A. Cardiac autonomic dysfunction and incidence of atrial fibrillation: results from 20 years follow-up. *J Am Coll Cardiol.* 2017;69:291–299. doi: 10.1016/j.jacc.2016.10.059
19. Maheshwari A, Norby FL, Soliman EZ, Adabag S, Whitsel EA, Alonso A, Chen LY. Low heart rate variability in a 2-minute electrocardiogram recording is associated with an increased risk of sudden cardiac death in the general population: the atherosclerosis risk in Communities Study. *PLoS One.* 2016;11:e0161648. doi: 10.1371/journal.pone.0161648
20. Tsuji H, Larson MG, Venditti FJ Jr, Manders ES, Evans JC, Feldman CL, Levy D. Impact of reduced heart rate variability on risk for cardiac events. The Framingham Heart Study. *Circulation.* 1996;94:2850–2855. doi: 10.1161/01.cir.94.11.2850
21. Lanphear BP, Rauch S, Auinger P, Allen RW, Hornung RW. Low-level lead exposure and mortality in US adults: a population-based cohort study. *Lancet Public Health.* 2018;3:e177–e184. doi: 10.1016/S2468-2667(18)30025-2
22. Chia SE, Chia KS, Chia HP, Ong CN, Jeyaratnam J. Three-year follow-up of serial nerve conduction among lead-exposed workers. *Scand J Work Environ Health.* 1996;22:374–380. doi: 10.5271/sjweh.157
23. Chia SE, Chia HP, Ong CN, Jeyaratnam J. Cumulative blood lead level and nerve conduction parameters. *Occup Med.* 1996;46:59–64.
24. Hara A, Gu YM, Petit T, Liu YP, Jacobs L, Zhang ZY, Yang WY, Jin Y, Thijs L, Wei FF, et al. Study for promotion of health in recycling lead - rationale and design. *Blood Press.* 2015;24:147–157. doi: 10.3109/08037051.2014.996409
25. Yu CG, Wei FF, Yang WY, Zhang ZY, Mujaj B, Thijs L, Feng YM, Staessen JA. Heart rate variability and peripheral nerve conduction velocity in relation to blood lead in newly hired lead workers. *Occup Environ Med.* 2019;76:382–388. doi: 10.1136/oemed-2018-105379
26. General Assembly of the World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *J Am Coll Dent.* 2014;81:14–18.
27. Whelton PK, Carey RM, Aronow WS, Casey DE Jr, Collins KJ, Dennison Himmelfarb C, DePalma SM, Gidding S, Jamerson KA, Jones DW, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/

NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *J Am Coll Cardiol*. 2018;71:e127–e248. doi: 10.1016/j.jacc.2017.11.006
28. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA*. 2001;285:2486–2497. doi: 10.1001/jama.285.19.2486
29. Yu YL, Yang WY, Thijs L, Melgarejo JD, Yu CG, Wei DM, Wei FF, Nawrot TS, Zhang ZY, Staessen JA. Two-year responses of cffice and ambulatory blood pressure to first occupational lead exposure. *Hypertension*. 2020;76:1299–1307. doi: 10.1161/HYPERTENSIONAHA.120.15590
30. Malliani A. The pattern of sympathovagal balance explored in the frequency domain. *News Physiol Sci*. 1999;14:111–117. doi: 10.1152/physiologyonline.1999.14.3.111
31. Fagard RH, Pardaens K, Staessen JA, Thijs L. Power spectral analysis of heart rate variability by autoregressive modelling and fast Fourier transform: a comparative study. *Acta Cardiol*. 1998;53:211–218.
32. Fagard RH, Pardaens K, Staessen JA. Influence of demographic, anthropometric and lifestyle characteristics on heart rate and its variability in the population. *J Hypertens*. 1999;17:1589–1599. doi: 10.1097/00004872-199917110-00013
33. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*. 1986;1:307–310.
34. Roels H, Konings J, Green S, Bradley D, Chettle D, Lauwerys R. Time-integrated blood lead concentration is a valid surrogate for estimating the cumulative lead dose assessed by tibial lead measurement. *Environ Res*. 1995;69:75–82. doi: 10.1006/enrs.1995.1027
35. Fadrowski JJ, Navas-Acien A, Tellez-Plaza M, Guallar E, Weaver VM, Furth SL. Blood lead level and kidney function in US adolescents: the Third National Health and Nutrition Examination Survey. *Arch Intern Med*. 2010;170:75–82. doi: 10.1001/archinternmed.2009.417
36. Goldstein DS, Bentho O, Park MY, Sharabi Y. Low-frequency power of heart rate variability is not a measure of cardiac sympathetic tone but may be a measure of modulation of cardiac autonomic outflows by baroreflexes. *Exp Physiol*. 2011;96:1255–1261. doi: 10.1113/expphysiol.2010.056259
37. Malik M, Bigger JT, Camm AJ, Kleiger RE, Malliani A, Moss AJ, Schwartz PJ. Heart rate variability: standards of measurement, physiological interpretation, and clinical use. *Eur Heart J*. 1996;17:354–381.
38. den Hoed M, Eijgelsheim M, Esko T, Brundel BJ, Peal DS, Evans DM, Nolte IM, Segrè AV, Holm H, Handsaker RE, et al; Global BPgen Consortium; CARDIoGRAM Consortium; PR GWAS Consortium; QRS GWAS Consortium; QT-IGC Consortium; CHARGE-AF Consortium. Identification of heart rate-associated loci and their effects on cardiac conduction and rhythm disorders. *Nat Genet*. 2013;45:621–631. doi: 10.1038/ng.2610
39. Nolte IM, Munoz ML, Tragante V, Amare AT, Jansen R, Vaez A, von der Heyde B, Avery CL, Bis JC, Dierckx B, et al. Genetic loci associated with heart rate variability and their effects on cardiac disease risk. *Nat Commun*. 2017;8:15805. doi: 10.1038/ncomms15805
40. Graff B, Szyndler A, Czechowicz K, Kucharska W, Graff G, Boutouyrie P, Laurent S, Narkiewicz K. Relationship between heart rate variability, blood pressure and arterial wall properties during air and oxygen breathing in healthy subjects. *Auton Neurosci*. 2013;178:60–66. doi: 10.1016/j.autneu.2013.04.009
41. Malliani A, Pagani M, Furlan R, Guzzetti S, Lucini D, Montano N, Cerutti S, Mela GS. Individual recognition by heart rate variability of two different autonomic profiles related to posture. *Circulation*. 1997;96:4143–4145. doi: 10.1161/01.cir.96.12.4143
42. Mamen A, Øvstebø R, Sirnes PA, Nielsen P, Skogstad M. High-intensity training reduces CVD risk factors among rotating shift workers: an eight-week intervention in industry. *Int J Environ Res Public Health*. 2020;17:3943.
43. Fazeli MS, Pourrahmat MM, Massah G, Lee K, Lavoie PM, Fazeli M, Esser A, Collet JP. The effect of massage on the cardiac autonomic nervous system and markers of inflammation in night shift workers: a pilot randomized crossover trial. *Int J Ther Massage Bodywork*. 2020;13:6–17.
44. Sheng CS, Cheng YB, Wei FF, Yang WY, Guo QH, Li FK, Huang QF, Thijs L, Staessen JA, Wang JG, et al. Diurnal bood pressure rhythmicity in relation to environmental and genetic cues in untreated referred patients. *Hypertension*. 2017;69:128–135. doi: 10.1161/HYPERTENSIONAHA.116.07958
45. Palatini P, Thijs L, Staessen JA, Fagard RH, Bulpitt CJ, Clement DL, de Leeuw PW, Jaaskivi M, Leonetti G, Nachev C, et al; Systolic Hypertension in Europe (Syst-Eur) Trial Investigators. Predictive value of clinic and ambulatory heart rate for mortality in elderly subjects with systolic hypertension. *Arch Intern Med*. 2002;162:2313–2321. doi: 10.1001/archinte.162.20.2313
46. Hansen TW, Thijs L, Boggia J, Li Y, Kikuya M, Björklund-Bodegård K, Richart T, Ohkubo T, Jeppesen J, Torp-Pedersen C, et al; International Database on Ambulatory Blood Pressure in Relation to Cardiovascular Outcomes Investigators. Prognostic value of ambulatory heart rate revisited in 6928 subjects from 6 populations. *Hypertension*. 2008;52:229–235. doi: 10.1161/HYPERTENSIONAHA.108.113191
47. Dijk DJ, Duffy JF. Novel approaches for assessing circadian rhythmicity in humans: a review. *J Biol Rhythms*. 2020;35:421–438. doi: 10.1177/0748730420940483
48. Kasius KM, Claes F, Verhagen WIM, Meulstee J. Motor nerve conduction tests in carpal tunnel syndrome. *Front Neurol*. 2019;10:149. doi: 10.3389/fneur.2019.00149
49. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. *Circulation*. 1996;93:1043–1065. doi: 10.1161/01.CIR.93.5.1043
50. Akselrod S, Gordon D, Ubel FA, Shannon DC, Berger AC, Cohen RJ. Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. *Science*. 1981;213:220–222. doi: 10.1126/science.6166045
51. Solinsky R, Vivodtzev I, Hamner JM, Taylor JA. The effect of heart rate variability on blood pressure is augmented in spinal cord injury and is unaltered by exercise training [published online march 12, 2020]. *Clin Auton Res*. doi: 10.1007/s10286-020-00677-2 https://link.springer.com/article/10.1007%2Fs10286-020-00677-2
52. Rabinowitz MB. Toxicokinetics of bone lead. *Environ Health Perspect*. 1991;91:33–37. doi: 10.1289/ehp.919133
53. Oliveira S, Aro A, Sparrow D, Hu H. Season modifies the relationship between bone and blood lead levels: the Normative Aging Study. *Arch Environ Health*. 2002;57:466–472. doi: 10.1080/00039890209601439
54. Korrick SA, Schwartz J, Tsaih SW, Hunter DJ, Aro A, Rosner B, Speizer FE, Hu H. Correlates of bone and blood lead levels among middle-aged and elderly women. *Am J Epidemiol*. 2002;156:335–343. doi: 10.1093/aje/kwf042
55. Staessen JA, Lauwerys RR, Buchet JP, Bulpitt CJ, Rondia D, Vanrenterghem Y, Amery A. Impairment of renal function with increasing blood lead concentrations in the general population. The Cadmibel Study Group. *N Engl J Med*. 1992;327:151–156. doi: 10.1056/NEJM199207163270303
56. Krieg EF Jr, Chrislip DW, Brightwell WS. A meta-analysis of studies investigating the effects of lead exposure on nerve conduction. *Arch Toxicol*. 2008;82:531–542. doi: 10.1007/s00204-008-0292-z
57. Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, Amann M, Anderson HR, Andrews KG, Aryee M, et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2012;380:2224–2260. doi: 10.1016/S0140-6736(12)61766-8
58. Staessen JA, Thijs L, Yang WY, Yu CG, Wei FF, Roels HA, Nawrot TS, Zhang ZY. Interpretation of population health metrics: environmental lead exposure as exemplary case. *Hypertension*. 2020;75:603–614. doi: 10.1161/HYPERTENSIONAHA.119.14217
59. Nijjar PS, Puppala VK, Dickinson O, Duval S, Duprez D, Kreitzer MJ, Benditt DG. Modulation of the autonomic nervous system assessed through heart rate variability by a mindfulness based stress reduction program. *Int J Cardiol*. 2014;177:557–559. doi: 10.1016/j.ijcard.2014.08.116
60. Nuyts GD, Elseviers MM, De Broe ME. Healthy worker effect in a cross-sectional study of lead workers. *J Occup Med*. 1993;35:387–391.
61. Ewing DJ, Martyn CN, Young RJ, Clarke BF. Simplified diagnosis of cardiovascular autonomic neuropathy in type 2 diabetes using Ewing's battery. *Diabet Care*. 1985;8:491–498.
62. Pafili K, Trypsianis G, Papazoglou D, Maltezos E, Papanas N. Simplified diagnosis of cardiovascular autonomic neuropathy in type 2 diabetes using Ewing's battery. *Rev Diabet Stud*. 2015;12:213–219. doi: 10.1900/RDS.2015.12.213

***HYPERTENSION***

# DATA SUPPLEMENT

*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, Dong-Mei Wei, Fang-Fei Wei, Tim S. Nawrot, Peter Verhamme, Harry A. Roels, Jan A. Staessen, Zhen-Yu Zhang

Correspondence to Jan A. Staessen, Research Institute Alliance for the Promotion of Preventive Medicine, Leopoldstraat 59, BE-2800 Mechelen, Belgium
Email: jan.staessen@appremed.org or jan.staessen@med.kuleuven.be

## Table of Contents

## Biochemical Measurements

The accuracy of the lead tests was verified by use of proficiency samples purchased from the College of American Pathologists (CAP) and the Pennsylvania Department of Blood Lead Programs.1 Proficiency testing was performed in six separate trial runs, including in total 30 test samples annually. All survey materials were handled in the same manner as the study samples and processed with the normal workflow utilizing the same repeat/dilution protocols and calibration and quality control frequency.[1] Compliance with Clinical Laboratory Improvement Amendments (CLIA), CAP and New York State accreditation and regulatory requirements was verified routinely with test level review of the laboratory services by external auditors. Calibrators with certified accuracy (National Institute of Standards and Technology [www.nist.gov]) were included in each batch of study samples and spanned the range of the analytical measurement range. Accuracy was evaluated on Westgard Rules[2] and defined within the total allowable error established with review of the CAP, Centers for Disease Control and Prevention, CLIA 88,[3] and OSHA guidelines. Accuracy, defined as the deviation from known lead standards ran along with the study samples, was within 10%.1 The bias determined according to the Bland and Altman approach4 in 30 spilt blood samples with blood lead concentrations (average in duplicate samples) ranging from 0.70 to 27.9 □g/dL, was 0.08 □g/dL (95% confidence interval, -0.01 to 0.18, *P*=0.078).[5] The repeatability coefficient, defined as twice the SD of the signed differences between duplicate measurements,[4] was 0.52. Expressed as a percentage of the mean blood lead concentration or as a percentage of near maximal variation in blood lead (four times the SD of the logarithmically transformed distribution), the repeatability coefficient was 6.7% and 1.9%, respectively. Lower values indicate better repeatability.

Hemoglobin and hematocrit were measured on EDTA blood samples, using an automated photometric absorbance method.[6] Serum total and high-density lipoprotein (HDL) cholesterol were determined by automated enzymatic methods and serum insulin by ELISA. Over three evaluations, the laboratory obtained a proficiency score of 100% for blood lead and 100% for routine biochemistry.

### References


1. Centers for Disease Control and Prevention. Screening young children for lead poisoning: Guidance for state and local public health officials; Appendix C1: The Lead Laboratory, 2018 (accessed 11 October 2020).
2. Westgard JO, Barry PL, Hunt MR, Groth T. A multi-rule Shewhart chart for quality control in clinical chemistry. *Clin Chem.* 1981;27:493-501.
3. Centers for Disease Control and Prevention. CLIA '88 Focus on Clinic and Office Laboratories, 1988 (accessed 11 October 2020).
4. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet.* 1986;1:307-310.
5. Yu CG, Wei FF, Yang WY, Zhang ZY, Mujaj B, Thijs L, Feng YM, Staessen JA. Heart rate variability and peripheral nerve conduction velocity in relation to blood lead in newly hired lead workers *Occup Environ Med.* 2019;76:382-388.
6. Whitehead RD, Jr., Mei Z, Mapango C, Jefferds MED. Methods and analyzers for hemoglobin measurement in clinical laboratories and field settings. *Ann N Y Acad Sci.* 2019;1450:147-171.

**Table S1**

**. Quality of life at baseline and last follow-up in 195 workers**

| Variable | Baseline | | | | | Last Follow-Up | | | | | *P* Value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | |
| Five dimensions | | | | | | | | | | | |
| Mobility | 0 | 0 | 0 | 2.1 | 97.9 | 0 | 0 | 0 | 0.5 | 99.5 | 0.17 |
| Self-care | 0 | 0 | 0 | 0 | 100.0 | 0 | 0 | 0 | 0 | 100.0 | >0.99 |
| Usual activity | 0 | 0 | 0 | 2.1 | 97.9 | 0 | 0 | 0 | 0.5 | 99.5 | 0.17 |
| Pain/discomfort | 0 | 0 | 3.6 | 15.5 | 80.9 | 0 | 0 | 12.8 | 11.8 | 75.4 | 0.093 |
| Anxiety/depression | 0 | 0 | 1.6 | 7.8 | 90.7 | 0 | 0.5 | 1.5 | 5.7 | 92.3 | 0.58 |
| Health scale | 0.5 | 0 | 0 | 9.7 | 89.8 | 0 | 0 | 1.0 | 14.9 | 84.1 | 0.098 |

Values are the percentages of workers in each scale. *P* values denote the significance of the difference between baseline and the last follow-up. Details on the US version of the EuroQol-5 Dimension questionnaire are available at https://www.euroqol.org.

**Table S2**

**. Baseline and follow-up heart rate variability derived by autoregressive modelling**

| Characteristic | Baseline | Follow-Up | | Δ in Percent (95% CI) | *P* Value |
|---|---|---|---|---|---|
| | | Year 1 | Year 2 | | |
| Supine position | | | | | |
| Total power, ms$^2$ | 885 (510, 1547) | 748 (416, 1384) | 800 (452, 1392) | -12.0 (-21.8 to -1.00) | 0.034 |
| Low-frequency power, nu | 47.3 (39.6, 60.1) | 49.2 (42.2, 61.0) | 48.9 (40.7, 62.7) | 5.0 (-0.1 to 10.4) | 0.052 |
| High-frequency power, nu | 19.6 (13.9, 30.0) | 14.7 (10.6, 22.5) | 14.7 (10.6, 22.5 ) | -19.3 (-25.5 to -12.6) | <0.001 |
| Low-to-high frequency ratio | 2.4 (1.4, 3.9) | 3.3 (2.1, 4.4) | 3.3 (2.1, 4.8) | 30.1 (19.4 to 41.9) | <0.001 |
| Standing position | | | | | |
| Total power, ms$^2$ | 844 (485, 1471) | 867 (538, 1564) | 805 (452, 1392) | 0.05 (-10.1 to 11.4) | 0.99 |
| Low-frequency power, nu | 58.6 (50.3, 72.3) | 57.2 (50.1, 70.4) | 56.7 (48.0, 71.6) | -3.0 (-7.3 to 1.5) | 0.19 |
| High-frequency power, nu | 9.4 (6.6, 13.4) | 8.5 (5.9, 13.0) | 8.4 (5.7, 12.2) | -11.3 (-17.6 to -4.6) | 0.002 |
| Low-to-high frequency ratio | 6.2 (4.1, 9.7) | 6.7 (4.3, 10.5) | 6.8 (4.6, 10.3) | 9.4 (0.1 to 19.7) | 0.049 |
| Orthostatic responses | | | | | |
| Total power, ms$^2$ | 0.95 (0.63, 1.42)‡ | 1.16 (0.70, 2.00)‡ | 1.01 (0.64, 1.70)‡ | 13.7 (1.01 to 28.0) | 0.034 |
| Low-frequency power | 1.24 (1.01, 1.50)‡ | 1.16 (0.94, 1.42)‡ | 1.16 (0.88, 1.52)‡ | -7.63 (-13.5 to -1.34) | 0.019 |
| High-frequency power | 0.48 (0.31, 0.74)‡ | 0.51 (0.33, 0.80)‡ | 0.57 (0.36, 0.90)‡ | 9.87 (-0.03 to 20.8) | 0.051 |
| Low-to-high frequency ratio | 2.57 (1.54, 3.86)‡ | 2.28 (1.45, 3.54)‡ | 2.04 (1.27, 3.12)‡ | -15.9 (-24.3 to -6.63) | 0.001 |

Values are geometric means (interquartile range). nu indicates normalized units. Heart rate changes are given in Table 2. Orthostatic responses are expressed as the standing-to-supine ratio. Changes from baseline to follow-up (□), given with 95% confidence interval (CI), are presented as percent change; *P* values denote the significance of the changes from baseline to follow-up. Significance of the within-participant orthostatic responses: ‡ $P \leq 0.001$.

**Table S3**

**. Baseline-to-follow-up autoregressive heart rate variability changes by fourths of the follow-up-to-baseline blood lead concentration ratio**

| Characteristic | Follow-Up-to-Baseline Blood Lead Ratio | | | | *P* Value |
|---|---|---|---|---|---|
| Quartile limits | <1.92 | 1.92-3.29 | 3.30-5.29 | >5.29 | |
| Number in category (%) | 49 | 48 | 49 | 49 | |
| Supine position | | | | | |
| Total power, % | -12.3 (-33.1, 14.9) | -0.1 (-21.3, 26.8) | -1.9 (-25.9, 29.9) | -23.4 (-42.5, 2.2) | 0.50 |
| Low-frequency power, % | 15.3 (2.2, 30.1)* | 3.7 (-7.6, 16.4) | 5.85 (-4.3, 17.0) | -5.6 (-16.8, 7.2) | 0.30 |
| High-frequency power, % | -24.5 (-37.3, -9.2)* | -20.5 (-33.0, -5.7)* | -20.1 (-33.2, -4.5)* | -24.9 (-37.0, -10.4)* | 0.98 |
| Low-to-high frequency ratio, % | 52.8 (25.6, 85.9)* | 30.4 (10.4, 54.2)* | 32.5 (7.8, 62.9)* | 25.7 (4.0, 51.9)* | 0.19 |
| Standing position | | | | | |
| Total power, % | -19.3 (-39.1, 7.1) | 32.1 (4.8, 66.4)* | -5.6 (-26.8, 21.8) | -5.1 (-24.6, 19.4) | 0.79 |
| Low-frequency power, % | 1.0 (-8.2, 11.2) | -8.41 (-16.3, 0.22) | -5.6 (-15.2, 5.2) | 0.7 (-9.1, 11.5) | 0.94 |
| High-frequency power, % | -20.2 (-34.8, -2.4)* | -3.05 (-18.6, 15.4) | -10.1 (-23.9, 6.2) | -15.5 (-27.1, -2.0)* | 0.80 |
| Low-to-high frequency ratio, % | 26.7 (0.4, 59.8) | -5.50 (-22.7, 15.5) | 5.1 (-14.6, 29.4) | 19.1 (-1.0, 43.2) | 0.86 |
| Orthostatic responses | | | | | |
| Total power, % | -7.9 (-30.9, 22.7) | 32.2 (3.4, 69.1)* | -3.8 (-25.3, 24.0) | 23.8 (-8.2, 67.0) | 0.36 |
| Low-frequency power, % | -12.4 (-25.5, 3.1) | -11.7 (-23.3, 1.8) | -10.8 (-22.0, 2.12) | 6.6 (-8.1, 23.7) | 0.076 |
| High-frequency power, % | 5.7 (-14.7, 30.9) | 22.0 (-1.6, 51.2) | 12.5 (-10.1, 40.8) | 12.5 (-5.4, 33.9) | 0.82 |
| Low-to-high frequency ratio, % | -17.1 (-33.3, 3.13) | -27.6 (-41.9, -9.7)* | -20.7 (-39.2, 3.5) | -5.3 (-23.0, 16.6) | 0.35 |

Baseline-to-follow-up heart rate variability changes are expressed as percent change with 95% confidence interval. *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.

**Table S4**

**. Association of the baseline-to-follow-up changes in autoregressive heart rate variability with the follow-up-to-baseline blood lead concentration ratio**

| Variable | Unadjusted | | Adjusted | |
|---|---|---|---|---|
| | Estimate (95% CI) | *P* Value | Estimate (95% CI) | *P* Value |
| Supine position | | | | |
| Total power, % | 0.51 (-3.43 to 4.61) | 0.80 | 2.02 (-1.28 to 5.43) | 0.23 |
| Low-frequency power, % | -2.08 (-3.73 to -0.41) | 0.015 | -0.82 (-2.21 to 0.58) | 0.25 |
| High-frequency power, % | 1.38 (-1.33 to 4.16) | 0.32 | -0.35 (-2.71 to 2.06) | 0.77 |
| Low-to-high frequency ratio, % | -3.04 (-5.85 to -0.15) | 0.039 | -1.03 (-3.60 to 1.61) | 0.44 |
| Standing position | | | | |
| Total power, % | 0.09 (-3.50 to 3.80) | 0.96 | 0.01 (-3.05 to 3.17) | 0.99 |
| Low-frequency power, % | 0.42 (-1.15 to 2.03) | 0.60 | 0.29 (-1.01 to 1.60) | 0.66 |
| High-frequency power, % | 1.94 (-0.76 to 4.70) | 0.16 | 0.02 (-2.39 to 2.49) | 0.99 |
| Low-to-high frequency ratio, % | -1.40 (-4.51 to 1.80) | 0.38 | 0.49 (-2.24 to 3.31) | 0.72 |
| Orthostatic responses | | | | |
| Total power, % | -0.09 (-4.08 to 4.06) | 0.97 | -2.19 (-5.42 to 1.16) | 0.20 |
| Low-frequency power, % | 2.81 (-4.19 to 10.3) | 0.43 | 0.68 (-5.04 to 6.75) | 0.82 |
| High-frequency power, % | 0.93 (-2.34 to 4.30) | 0.58 | -0.66 (-3.21 to 1.97) | 0.62 |
| Low-to-high frequency ratio, % | 1.56 (-2.11 to 5.37) | 0.41 | 1.56 (-1.40 to 4.60) | 0.30 |

Estimates are association sizes, given with 95% confidence interval (CI), relate the baseline-to-follow-up percent changes in autoregressive heart rate variability to the follow-up-to-baseline blood lead concentration 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 variability index, 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, seasonality, and observer (random effect).

**Table S5**

**. Associations of baseline-to-follow-up changes in Fourier heart rate variability with the follow-up-to-baseline blood lead concentration ratio by the median baseline blood lead concentration**

| Variable | Blood Lead <4.30 µg/dL (N=96) | | Blood Lead ≥4.30 µg/dL (N=99) | | *P* Value for Interaction |
|---|---|---|---|---|---|
| | Estimate (95% CI) | *P* Value | Estimate (95% CI) | *P* Value | |
| Supine position | | | | | |
| Low-frequency power, % | -2.85 (-5.67 to 0.05) | 0.054 | -0.49 (-3.99 to 3.12) | 0.78 | 0.48 |
| High-frequency power, % | -0.61 (-5.57 to 4.60) | 0.81 | 0.16 (-4.95 to 5.55) | 0.95 | 0.98 |
| Low-to-high frequency ratio, % | -1.43 (-6.64 to 4.06) | 0.60 | -2.89 (-8.24 to 2.77) | 0.30 | 0.91 |
| Standing position | | | | | |
| Low-frequency power, % | 1.79 (-1.16 to 4.83) | 0.23 | 1.03 (-1.33 to 3.45) | 0.39 | 0.99 |
| High-frequency power, % | 2.60 (-2.23 to 7.67) | 0.29 | 2.37 (-2.58 to 7.56) | 0.35 | 0.84 |
| Low-to-high frequency ratio, % | -1.53 (-6.96 to 4.21) | 0.59 | -1.18 (-6.14 to 4.04) | 0.65 | 0.91 |
| Orthostatic responses | | | | | |
| Low-frequency power, % | 13.3 (-1.07 to 29.9) | 0.066 | -5.95 (-19.0 to 9.21) | 0.38 | 0.017 |
| High-frequency power, % | 4.00 (-2.17 to 10.6) | 0.20 | 1.27 (-4.52 to 7.41) | 0.67 | 0.99 |
| Low-to-high frequency ratio, % | -0.53 (-6.95 to 6.34) | 0.87 | 0.79 (-5.20 to 7.16) | 0.80 | 0.98 |

Estimates are association sizes, given with 95% confidence interval (CI), and relate the baseline-to-follow-up percent changes in Fourier heart rate variability with the follow-up to baseline blood lead concentration 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 1- and 2-year data. Models were additionally adjusted for sex, baseline heart rate variability, 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, seasonality, and observer (random effect). Interaction *P*-values were computed for the baseline blood lead group × follow-up-to-baseline blood lead concentration ratio.

**Table S6**

**. Associations of baseline-to-follow-up changes in Fourier heart rate variability with the follow-up-to-baseline blood lead concentration ratio by the median cumulative blood lead index**

| Variable | <32.1 µg/dL × year (N=97) | | ≥32.1 µg/dL × year (N=98) | | P Value for Interaction |
|---|---|---|---|---|---|
| | Estimate (95% CI) | *P* Value | Estimate (95% CI) | *P* Value | |
| Supine position | | | | | |
| Low-frequency power, % | -0.32 (-2.86 to 2.29) | 0.80 | -1.62 (-4.52 to 1.37) | 0.28 | 0.67 |
| High-frequency power, % | 3.04 (-1.06 to 7.31) | 0.15 | -5.50 (-9.40 to -1.40) | 0.010 | 0.022 |
| Low-to-high frequency ratio, % | -3.00 (-7.33 to 1.53) | 0.19 | 1.22 (-3.31 to 5.95) | 0.60 | 0.22 |
| Standing position | | | | | |
| Low-frequency power, % | 1.84 (-0.47 to 4.21) | 0.12 | 0.08 (-2.18 to 2.39) | 0.94 | 0.14 |
| High-frequency power, % | 1.43 (-2.15 to 5.14) | 0.43 | 1.13 (-3.22 to 5.67) | 0.61 | 0.79 |
| Low-to-high frequency ratio, % | 0.37 (-3.85 to 4.78) | 0.86 | -1.18 (-5.84 to 3.72) | 0.63 | 0.90 |
| Orthostatic responses | | | | | |
| Low-frequency power, % | 8.49 (-4.95 to 23.8) | 0.20 | 4.84 (-6.11 to 17. 1) | 0.33 | 0.63 |
| High-frequency power, % | -1.56 (-6.37 to 3.51) | 0.53 | 5.69 (0.65 to 11.0) | 0.027 | 0.15 |
| Low-to-high frequency ratio, % | 3.16 (-2.45 to 9.09) | 0.27 | -3.65 (-8.43 to 1.39) | 0.15 | 0.12 |

Estimates are association sizes, given with 95% confidence interval (CI), and relate the baseline-to-follow-up percent changes in Fourier heart rate variability with the follow-up-to-baseline blood lead concentration 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. Models were additionally adjusted for sex, baseline heart rate variability, 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, seasonality, and observer (random effect). Interaction *P*-values were computed for the baseline cumulative blood lead index group × follow-up-to-baseline blood lead concentration ratio.

**Table S7**

**Associations between Fourier heart rate variability and heart rate at baseline and last follow-up**

| Characteristic | | Unadjusted | | | Adjusted | | |
|---|---|---|---|---|---|---|---|
| | | Estimate (95% CI) | *P* | $P_{slope}$ | Estimate (95% CI) | *P* | $P_{slope}$ |
| Supine position | | | | | | | |
| Low-frequency power, nu | BL | 0.43 (0.14, 0.71) | 0.004 | 0.23 | 0.35 (0.09, 0.61) | 0.009 | 0.11 |
| | FU | 0.19 (-0.08, 0.46) | 0.17 | | 0.06 (-0.19, 0.31) | 0.63 | |
| High-frequency power, nu | BL | -0.57 (-0.85, -0.29) | <0.001 | 0.93 | -0.48 (-0.74, -0.22) | <0.001 | 0.93 |
| | FU | -0.59 (-0.84, -0.34) | <0.001 | | -0.47 (-0.71, -0.22) | <0.001 | |
| Low-to-high frequency ratio | BL | 0.71 (0.44, 0.99) | <0.001 | 0.65 | 0.59 (0.34, 0.85) | <0.001 | 0.42 |
| | FU | 0.62 (0.37, 0.88) | <0.001 | | 0.45 (0.20, 0.70) | <0.001 | |
| Standing position | | | | | | | |
| Low-frequency power, nu | BL | 0.49 (0.19, 0.78) | 0.001 | 0.64 | 0.47 (0.19, 0.75) | 0.001 | 0.54 |
| | FU | 0.39 (0.10, 0.68) | 0.010 | | 0.34 (0.04, 0.64) | 0.025 | |
| High-frequency power, nu | BL | -0.39 (-0.69, -0.10) | 0.010 | 0.83 | -0.36 (-0.64, -0.08) | 0.012 | 0.61 |
| | FU | -0.35 (-0.64, -0.06) | 0.021 | | -0.26 (-0.54, 0.02) | 0.075 | |
| Low-to-high frequency ratio | BL | 0.53 (0.24, 0.83) | <0.001 | 0.90 | 0.51 (0.23, 0.78) | <0.001 | 0.56 |
| | FU | 0.51 (0.22, 0.80) | <0.001 | | 0.39 (0.11, 0.67) | 0.007 | |

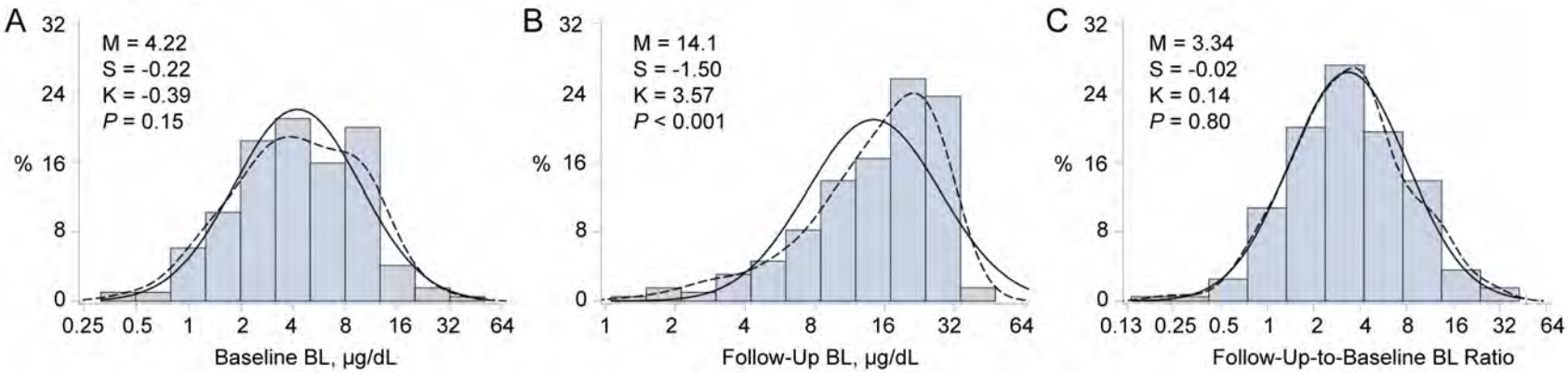
Association sizes, given with 95% confidence interval (CI) and significance, express the change in Fourier heart rate variability per 1-SD increase in heart rate. nu indicates normalized unit. *P*-values in the last column ($P_{slope}$) derived by a z-statistic express the significance of the difference in the association sizes (slopes) between baseline (BL) and last follow-up (FU). Adjusted models accounted for sex, age, mean arterial pressure, serum insulin, hemoglobin, room temperature during the examination, season, and observer.

**Table S8**

**. Baseline characteristics of eligible workers analyzed and not analyzed**

| Characteristic | Number (%) | | Characteristic | Mean of characteristic (SD or IQR) | |
|---|---|---|---|---|---|
| Analyzed | Yes | No | Analyzed | Yes | No |
| Number | 195 | 281 | Number | 195 | 281 |
| Women | 17 (8.7) | 38 (13.5) | Age, years | 27.8 (8.12) | 27.8 (9.50) |
| Men | 178 (91.3) | 243 (86.5) | Body mass index, kg/m$^2$ | 28.5 (5.79) | 29.0 (6.83) |
| White ethnicity | 93 (47.7) | 118 (42.0) | Waist-to-hip ratio | 0.96 (0.08) | 0.96 (0.08) |
| Hispanic ethnicity | 89 (45.6) | 135 (48.0) | Systolic pressure, mm Hg | 119.7 (9.79) | 119.8 (10.6) |
| Other ethnicities | 13 (6.7) | 28 (10.0) | Diastolic pressure, mm Hg | 79.1 (8.53) | 80.7 (8.59)* |
| Current smokers | 51 (26.4) | 78 (27.9) | Mean arterial pressure, mm Hg | 92.6 (8.32) | 93.7 (8.53) |
| Drinking alcohol | 88 (45.6) | 106 (37.9) | Hemoglobin, g/dL | 15.0 (1.15) | 15.0 (1.20) |
| Hypertension ≥ stage 1 | 92 (47.2) | 167 (59.4)† | Hematocrit, % | 45.4 (3.29) | 45.3 (3.58) |
| Hypertension ≥ stage 2 | 25 (12.8) | 36 (12.8) | Total-to-HDL cholesterol ratio | 3.93 (1.33) | 3.78 (1.29) |
| Treated hypertension | … | … | Insulin, μIU/mL | 6.73 (3.60, 13.0) | 6.90 (3.60, 13.0) |
| On neuro-active drugs | … | … | Blood lead, □g/dL | 4.22 (2.50, 8.30) | 4.06 (2.50, 8.30) |

Abbreviations: HDL, high-density lipoprotein. The analyzed (Figure 1) and non-analyzed participants did not include workers on neuro-active or antihypertensive drugs at baseline. 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. Significance of the between-group differences: * $P<0.05$ † $P<0.01$.


A
M = 4.22
S = -0.22
K = -0.39
P = 0.15
%
32
24
16
8
0
0.25 0.5 1 2 4 8 16 32 64
Baseline BL, µg/dL
B
M = 14.1
S = -1.50
K = 3.57
P < 0.001
1 2 4 8 16 32 64
Follow-Up BL, µg/dL
C
M = 3.34
S = -0.02
K = 0.14
P = 0.80
0.13 0.25 0.5 1 2 4 8 16 32 64
Follow-Up-to-Baseline BL Ratio

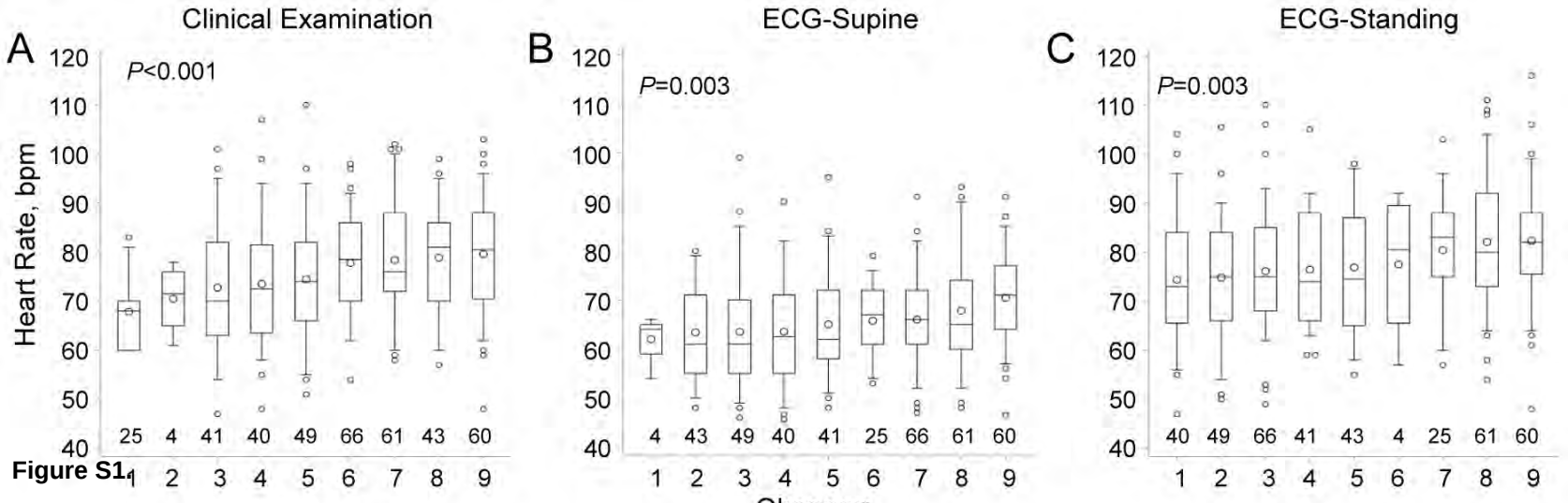



**Figure S1.**

Distributions of baseline and follow-up blood lead concentrations and the last-follow-up-to-baseline blood lead concentration ratio. The solid and dotted lines represent the normal and kernel density distributions. The *P*-values are for departure of the actually observed distribution from normality according to the Shapiro-Wilk statistic. Skewness and kurtosis were computed as the third and fourth moments about the mean divided by the cube of the standard deviation. Abbreviations: M, mean; S, skewness; K, kurtosis; BL, blood lead.

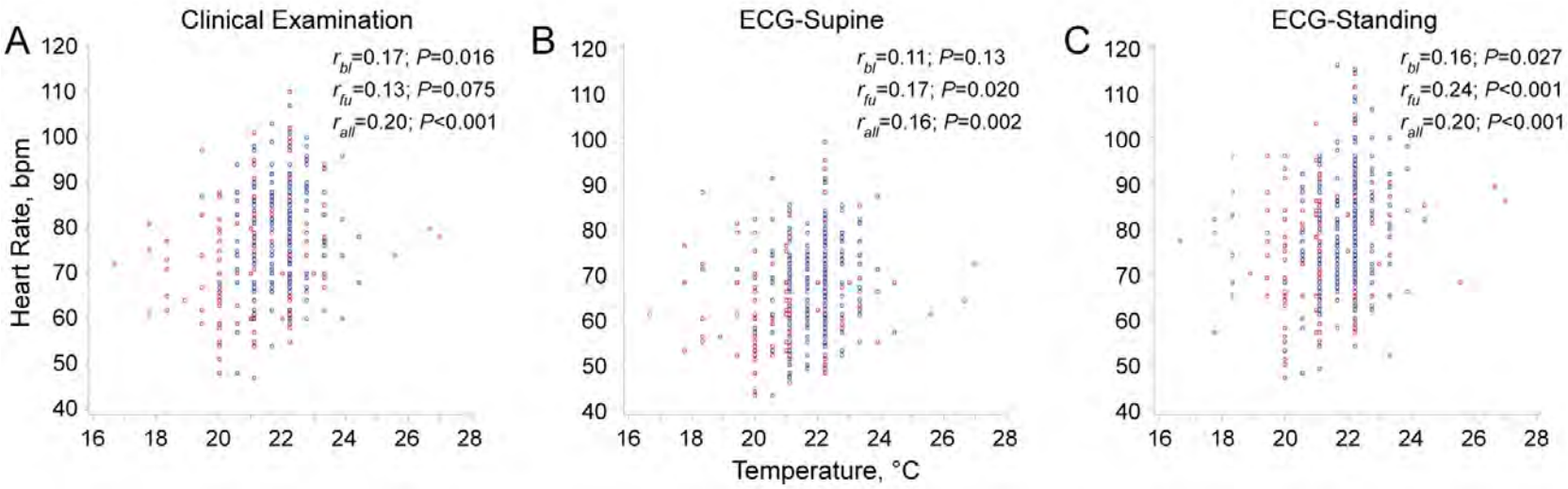



**Figure S2.**

Boxplots showing the distributions of heart rate as assessed by observer during the clinical examination (**A**) and by ECG in the supine (**B**) and standing position (**C**). The central line, the upper and lower lines, and the upper and lower caps represent the median, interquartile range, and the 5th to 95th percentile interval. The arithmetic means and extreme measurements are represented by circles inside the box and outside the whiskers, respectively. The number of data points contributing to each whisker plot is given within the boxes. *P*-values are for the overall between-observer differences derived by ANOVA.

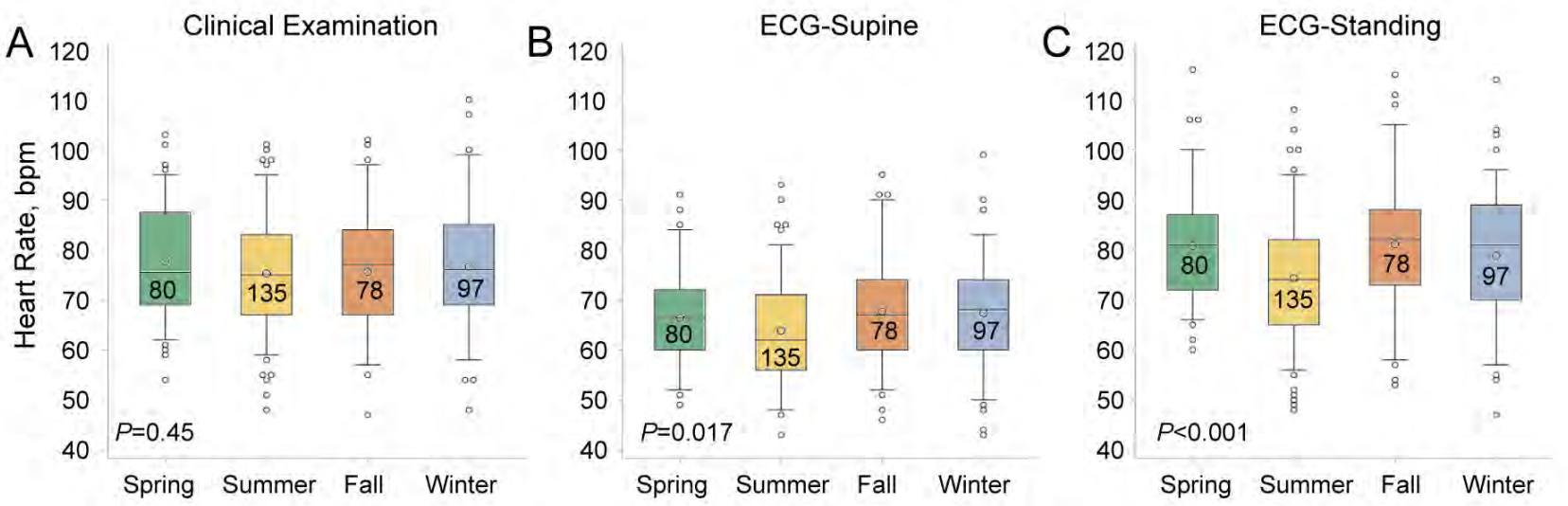



**Figure S3.**

Pearson correlation coefficients between heart rate and room temperature. Heart rate was assessed by the study nurses during the clinical examination (**A**) and derived from the ECG in the supine (**B**) and standing (**C**) position. Red and blue dots indicate heart rate at baseline and follow-up, respectively. Correlation coefficients and significance levels are given for baseline ($r_{bl}$), follow-up ($r_{fu}$) and all data combined ($r_{all}$).

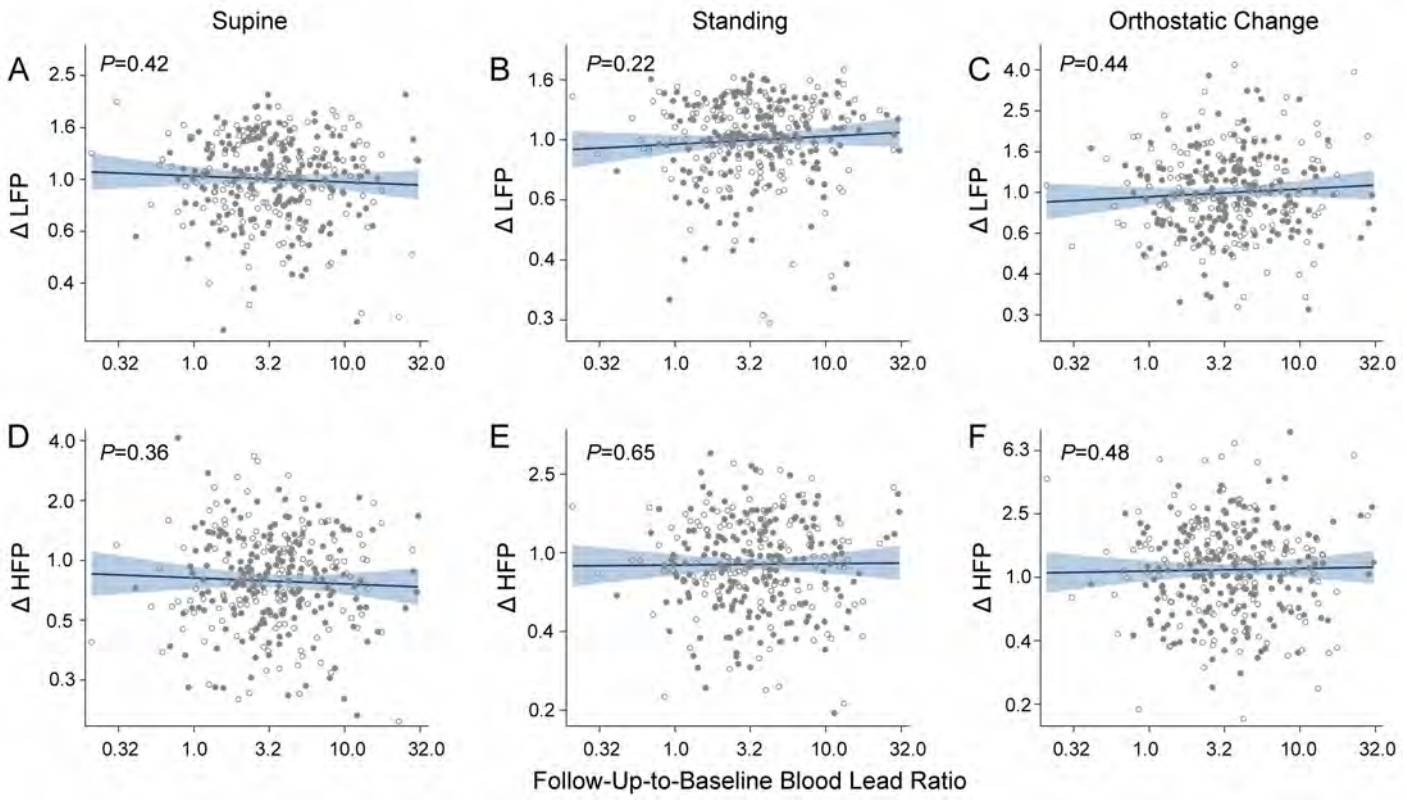



**Figure S4.**

Distributions of heart rate by season. Heart rate was assessed by the study nurses during the clinical examination (**A**) and derived from the ECG in the supine (**B**) and standing (**C**) position. The central line, the upper and lower lines, and the upper and lower caps of the boxplots represent the median, interquartile range, and the 5th to 95th percentile interval. The arithmetic means and extreme measurements are represented by circles inside the box and outside the whiskers, respectively. The number of data points contributing to each plot is given within the boxes. *P*-values are for the overall between-season differences derived by ANOVA.

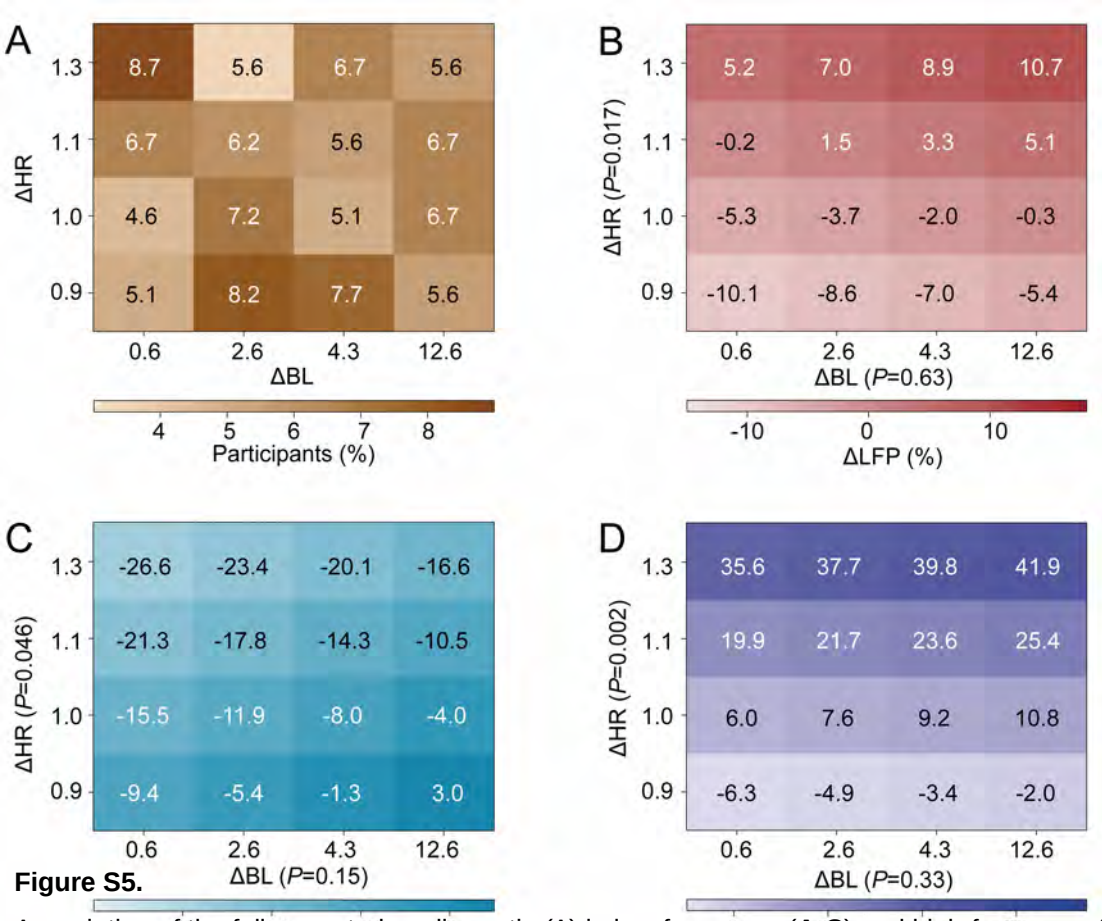



**Figure S5.**

Association of the follow-up-to-baseline ratio (Δ) in low-frequency (**A-C**) and high-frequency (**D-F**) power in the supine (**A, D**) and standing (**B, E**) positions and in the orthostatic responses (**C, F**) with the follow-up-to-baseline blood lead ratio. Open and closed symbols depict the first and second follow-up results, respectively. The regression lines with 95% confidence interval were derived from mixed models accounting for clustering of the observations within participants. Adjusted models accounted for sex, baseline heart rate variability index, the baseline value and the change during follow-up in age, heart rate (or the heart rate orthostatic response, as appropriate), mean arterial pressure, serum insulin, hemoglobin, room temperature, season, and observer (random effect). LFP indicates low-frequency power and HFP high-frequency power.

**Figure S6.**

Heat maps relating the percent changes in the heart rate variability indexes with the baseline-to-follow-up heart ratio (ΔHR) and the follow-up-to-baseline blood lead concentration ratio (ΔBL) in the standing position. Panel **A** show the percent of workers (N=195) in each cell in the cross-classification between the baseline-to-follow-up heart ratio (vertical axis) and the follow-up-to-baseline blood lead concentration ratio (horizontal axis). ΔLFP, ΔHLP and ΔLFP/HFP indicate low-frequency power (**B**), high-frequency power (**C**) and the 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).