Exercise heart rate recovery assessment of the cardiac autonomic nervous system in workers occupationally exposed to lead

Mesleki olarak kurşuna maruz kalan işçilerde kardiyak otonom sinir sisteminin kalp hızı toparlanma indeksi ile değerlendirilmesi

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Objective: The aim of the present study was to assess cardiac autonomic function via indices of exercise heart rate recovery (HRR) in workers occupationally exposed to lead.

Methods: A total of 98 lead-exposed workers and 98 healthy controls were enrolled. All underwent exercise testing and transthoracic echocardiography. HRR indices were calculated by subtracting 1st- (HRR1), 2nd- (HRR2), and 3rd-minute (HRR3) heart rates from maximal heart rate (HR). Exercise test parameters– HRR in particular– were compared between groups, and correlation analysis of blood, 24-hour urine lead levels, and test parameters was performed.

Results: Baseline demographic and clinical characteristics were found to be similar between groups. Mean HRR1 (26.2±3.6 vs 29.0±4.1 bpm, p<0.001), HRR2 (42.6±3.9 vs 46.9±3.7 bpm, p<0.001), and HRR3 (56.6±4.5 vs 61.8±4.3 bpm, p<0.001) values were significantly lower in the lead-exposed group than in the healthy controls. HRR1 was found to be significantly correlated with blood (r:-0.415; p<0.001) and 24-hour urine lead levels (r:-0.446; p<0.001). HRR2 and HRR3 were significantly correlated with 24-hour urine lead level (r:-0.396; p<0.001 and r:-0.233; p=0.021, respectively).

Conclusion: Lead-exposed workers had lower HRR indices than normal subjects. Blood and 24-hour urine lead levels were significantly associated with HRR indices. Cardiac autonomic functions may be affected by exposure to lead, and those occupationally exposed should be closely followed for adverse cardiovascular outcome.

Amaç: Bu yazida, mesleki olarak kurşuna maruz kalan işçilerde kalp hızı toparlanma indeksi (KHTİ) ile kardiyak otonom işlevlerin değerlendirilmesi amaçlandı.


Bulgular: Her iki grubun temel demografik ve klinik özelliklerini açığa vuran benzerliği ortaya çıktı. Ortalama birinci dakika KHTİ (26.2±3.6 ve 29.0±4.1 /dk, p<0.001), ikinci dakika KHTİ (42.6±3.9 ve 46.9±3.7 /dk, p<0.001) ve üçüncü dakika KHTİ (56.6±4.5 ve 61.8±4.3 /dk, p<0.001) değerleri kurşuna maruz kalan işçi grupudaki değerlerin altındaydı. Birinci, ikinci ve üçüncü dakika KHTİ ile kan kurşun (r=-0.415, p<0.001) ve 24 saat idrarda kurşun (r=-0.446, p<0.001) düzeyleri ile anlamlı korelasyon gösterdi. İkinci ve üçüncü dakika KHTİ 24 saatlik idrarda kurşun düzeyi ile anlamlı korelasyon gösterdi (r=-0.396, p<0.001 ve r=-0.233, p=0.021).

Lead exposure is among the most common exposures to heavy metals, both environmentally and occupationally, world-wide. Occupational lead exposure is still a main concern, though efforts have been made to reduce the industrial emission of lead. Exposure unfavorably affects many systems, particularly the neuropsychiatric system, and the effects of lead exposure on the cardiovascular system have been the subject of several studies. Lead exposure can lead to a wide range of cardiovascular diseases, including hypertension (HT), ischemic heart disease, diastolic dysfunction, and conduction disturbances. It has additionally been reported that lead exposure may alter activity of the autonomic nervous system (ANS) and autonomic nervous control of the heart.

Cardiac ANS plays an important role in maintaining cardiovascular functions, and is based on a sensitive balance between the parasympathetic and sympathetic systems. Several tools are used to evaluate and measure cardiac ANS functions, such as heart rate recovery (HRR) and heart rate variability (HRV). The relationship between lead exposure and HRV parameters has been described; Park et al. found that higher cumulative lead exposure was associated with significant reduction in HRV parameters. Böckelmann et al. demonstrated that long-term lead exposure caused vagal depression, assessed via HRV. Similar results, demonstrating that occupational lead exposure led to decrease in ANS and HRV parameters, were described by Muzi et al.

Measurement of heart rate after graded exercise is among the techniques most commonly used to reflect autonomic activity. Sympathetic activity increases during exercise and diminishes during recovery, as previously suppressed parasympathetic activity becomes dominant during recovery and reduces heart rate (HR). This decline is blunted by decreased myocardial function and reduced exercise capacity. Abnormal HRR is an independent predictor of increased cardiovascular and all-cause mortality rates. Unlike HRV, HRR in lead-exposed individuals has yet to be evaluated.

The present aim was to evaluate ANS function, using HRR indices, in workers occupationally exposed to lead without clinical presentation of cardiac involvement, compared to control subjects.

METHODS

Study population
Enrolled in the present cross-sectional study were 527 workers occupationally exposed to lead and admitted to the clinic between January 2014 and April 2015. Medical history, physical examination, 12-lead surface electrocardiogram (ECG), laboratory findings, and transthoracic echocardiography were evaluated. Those with coronary artery disease, systolic and/or diastolic dysfunction, valvular heart disease, diabetes mellitus, HT, thyroid dysfunction, hypercholesterolemia, electrolyte imbalance, chronic lung disease, and cigarette smokers were excluded. Also excluded were those with rhythm and/or conduction abnormalities that can be very difficult or impossible to interpret on exercise test (such as bundle branch block), chronotropic incompetence (defined as failure to reach 85% of the age-predicted maximal HR obtained during incremental exercise test), and those using drugs that affect the autonomic system and exercise performance (such as beta-blocking agents, antiarrhythmics, tricyclic antidepressants, and antipsychotics). A total of 429 workers were excluded, primarily due to active smoking.

Of the remaining 98 workers included, 38 (38.7%) were employed in battery production, 34 (34.7%) were employed in metal recycling, and 26 (26.5%) were welders. All worked 8 hours a day, 5 days a week. The majority of workplaces were small or medium-sized, as a result of which environmental exposure/toxic substance testing was rarely performed. Even when performed, accurate values could often not be obtained, due to incorrect methods of measurement and inadequate data.

The control group included 98 healthy subjects matched for age and sex, with no previous history of cardiac disease. All study participants were males aged older than 18 years. Written informed consent was obtained from each subject, and study protocol was approved by the institutional ethics committee.

Collection of biological samples
Blood samples were obtained at the end of the work shift. Blood samples were drawn in 10-mm, red-
capped tubes that did not contain gel (BD Vacutainer; Becton Dickinson Medical, Franklin Lakes, NJ, USA) for the analysis of biochemical parameters, in 10-mm trace element tubes that contained ethylenediaminetetraacetic acid for whole blood lead analysis, and in 5-mm tubes that contained ethylenediaminetetraacetic acid for complete blood cell and erythrocyte sedimentation rate analyses. In preparation for serum analyses, specimens were centrifuged at 1500 x g for 10 minutes after at least 30 minutes of incubation. All samples were analyzed on the same day.

**Analysis methods**

Lead levels were determined in whole blood and 24-hour urine samples using inductively coupled plasma–mass spectrometry (7700 series; Agilent Technologies, Inc., Santa Clara, CA, USA). Blood samples were digested using microwave-induced acid method. Standard solution of lead was prepared by dilution of certified standard solutions (High-Purity Standards, Charleston, SC, USA). Two-level quality control materials were used (Seronorm; Sero AS, Billingstad, Norway). Lead calibration curve ranged from 0–100 μg/dL. Limits of detection and quantification were 0.02 and 0.1 μg/dL, respectively. Relative SD of measurements was 4.2%. Subjects were asked to collect 24-hour urine samples, and were instructed not to collect urine from the first urination of the first day. Urine samples were collected in sterile plastic containers during every subsequent urination, and diluted 1:10 with 5% nitric acid solution.

**Treadmill exercise testing**

Treadmill exercise testing was conducted using modified Bruce protocol. Mason-Likar modification of 12-lead ECG\(^{[13]}\) was continuously recorded at 25 mm/s paper speed. Systolic and diastolic blood pressure (BP) were measured at points of rest and maximum exercise during exercise and recovery phases. Exercise lasted longer than 6 minutes, and maximum HR was at least 85% of maximum age-predicted HR response. After achieving peak workload, all subjects spent at least 3 minutes recovering without a cool-down period. Exercise capacity was measured by metabolic equivalent level at peak exercise. HRR indices were calculated by subtracting the 1st- (HRR1), 2nd- (HRR2), and 3rd-minute (HRR3) heart rates from the maximum HR obtained during stress testing.

**Transthoracic echocardiography**

Standard echocardiographic imaging was performed in left lateral decubitus position with an Esaote cardiac ultrasound scanner (Esaote Group, Genoa, Italy). Images were obtained using 2.5–3.5 MHz transducer in parasternal and apical views. Left ventricular end-diastolic and end-systolic diameters were determined with M-mode echocardiography under 2-dimensional guidance in parasternal long-axis view, according to recommendations of the American Society of Echocardiography.\(^{[14]}\) Left ventricular ejection fraction was calculated in apical 4-chamber view, according to modified Simpson’s rule.\(^{[14]}\) Left atrial dimension was calculated in parasternal long-axis view, and right ventricular end-diastolic diameter was calculated in apical 4-chamber view. Pulmonary systolic arterial pressure was estimated by continuous wave Doppler as peak regurgitation velocity plus assumed right atrial pressure in relation to the size and respiratory excursion of the inferior cava vein, visualized in subcostal view. Measurements of mitral inflow included peak early filling (E-wave) and late diastolic filling (A-wave) velocities, E:A ratio, deceleration time of early filling velocity, and isovolumic relaxation time (determined by placing the cursor of continuous wave Doppler in the left ventricular outflow tract to simultaneously display the end of aortic ejection and onset of mitral inflow).

**Statistical analysis**

Statistical analysis was performed using SPSS software (version 20.0; SPSS Inc., Chicago, IL, USA). Variables with normal distribution were analyzed using Kolmogorov-Smirnov test. Variables with normal distribution were presented as mean±SD, while those without normal distribution were presented as median with minimum and maximum range. Categorical variables were presented as number and percentage. Comparison of continuous variables was performed with t-test for independent variables with normal distribution, and with Mann-Whitney U test for those without normal distribution. Pearson correlation analysis was used to test normalcy of distribution, and Spearman correlation analysis was used for variables without normal distribution; p<0.05 was considered statistically significant. In power analyses, it was determined that at least 38 members of each group were required in order to obtain 95% confidence interval and 5% error.
RESULTS

General characteristics of study population

Baseline characteristics are shown in Table 1. All subjects were male. Mean age of lead-exposed and control groups were similar (38.6±7.3 vs 37.0±8.0 years, respectively, p=0.154). No statistically significant difference was found between the groups regarding age, height, weight, body mass index, or systolic and diastolic BP. Left ventricular ejection fractions were similar and normal among the lead-exposed and control groups (mean 64.5±3.5% vs 65.0±3.0%, respectively, p=0.265). Likewise similar were left ventricular end-diastolic diameter, left ventricular end-systolic diameter, left atrial diameter, right ventricular diameter, pulmonary systolic arterial pressure, mitral E-wave, A-wave, deceleration time, and isovolumic relaxation time. In the lead-exposed group, median duration of exposure to lead was 13 months, with a range of 1–35 months.

Main laboratory and serologic data are shown in Table 2. Mean white blood cell, platelet, and neutrophil counts, and levels of hemoglobin, creatinine, blood urea nitrogen, low-density lipoprotein, high-density lipoprotein, triglyceride, triiodothyronine, thyroxine, C-reactive protein, thyroid stimulating hormone, alanine aminotransferase, and aspartate aminotransferase (AST) were similar between the groups, as were median erythrocyte sedimentation rates. Median blood and 24-hour urine lead levels were found to be significantly higher in the group exposed to lead, compared to the control group, measuring 34.1 µg/dL (range 11.4–90) and 21.1 µg/L (2–77), respectively (p<0.001).

Exercise test parameters and heart rate recovery indices

All subjects had normal resting 12-lead ECG and completed the exercise stress test without complication. No rhythm abnormalities or ischemic changes were observed during the tests. All subjects achieved at least 85% of the age-predicted maximum HR. Duration of the treadmill exercise test was similar in the lead-exposed and control groups (9.9±2.9 vs

Table 1. Demographic characteristics, blood pressure and echocardiographic parameters of the lead-exposed and control groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Lead-exposed group (n=98)</th>
<th>Control group (n=98)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>38.6±7.3</td>
<td>37.0±8.0</td>
<td>0.154</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>171.8±6.4</td>
<td>172.9±6.1</td>
<td>0.220</td>
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<tr>
<td>Weight (kg)</td>
<td>76.2±9.8</td>
<td>75.3±11.9</td>
<td>0.550</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.8±3.2</td>
<td>25.1±3.6</td>
<td>0.163</td>
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<tr>
<td>Systolic blood pressure at baseline (mmHg)</td>
<td>115.9±16.5</td>
<td>118.9±12.7</td>
<td>0.161</td>
</tr>
<tr>
<td>Diastolic blood pressure at baseline (mmHg)</td>
<td>72.3±18.9</td>
<td>72.8±12.4</td>
<td>0.834</td>
</tr>
<tr>
<td>Left ventricular ejection fraction (%)</td>
<td>64.5±3.5</td>
<td>65.0±3.0</td>
<td>0.265</td>
</tr>
<tr>
<td>End-diastolic diameter (mm)</td>
<td>46.0±2.8</td>
<td>45.8±3.2</td>
<td>0.624</td>
</tr>
<tr>
<td>End-systolic diameter (mm)</td>
<td>28.1±2.8</td>
<td>27.7±3.5</td>
<td>0.399</td>
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<tr>
<td>Left atrial diameter (mm)</td>
<td>33.5±3.5</td>
<td>32.9±2.9</td>
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<tr>
<td>Right ventricle diameter (mm)</td>
<td>25.3±2.2</td>
<td>25.0±2.3</td>
<td>0.278</td>
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<tr>
<td>Systolic pulmonary arterial blood pressure (mmHg)</td>
<td>22.8±4.7</td>
<td>23.1±4.9</td>
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<tr>
<td>E wave (cm/s)</td>
<td>79.8±15.3</td>
<td>76.4±14.7</td>
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<tr>
<td>A wave (cm/s)</td>
<td>67.7±12.4</td>
<td>66.8±12.1</td>
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<tr>
<td>Mitral E/A</td>
<td>1.19±0.46</td>
<td>1.14±0.35</td>
<td>0.392</td>
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<tr>
<td>Deceleration time (ms)</td>
<td>176.6±42.7</td>
<td>178.1±46.0</td>
<td>0.813</td>
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<tr>
<td>Isovolumic relaxation time (ms)</td>
<td>95.1±13.7</td>
<td>97.4±15.1</td>
<td>0.265</td>
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</tbody>
</table>

Numerical variables were expressed as mean±standard deviation.
10.5±3.2 min, respectively), as were baseline HR (76.5±8.5 vs 74.4±9.2 bpm, respectively), maximal HR (159.7±11.2 vs 162.8±13.8 bpm, respectively), maximum systolic BP (160.6±17.8 vs 165.1±18.4 mmHg, respectively), maximum diastolic BP (76.7±13.7 vs 74.5±12.6 mmHg, respectively) and peak exercise capacity (12.8±3.4 vs 13.1±3.7 metabolic equivalent level, respectively) (Table 3). Mean HRR1 values were significantly lower in the group exposed to lead than in the control group (26.2±3.6 vs

<table>
<thead>
<tr>
<th>Variables</th>
<th>Lead-exposed group (n=98)</th>
<th>Control group (n=98)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of exercise test (min)</td>
<td>9.9±2.9</td>
<td>10.5±3.2</td>
<td>0.170</td>
</tr>
<tr>
<td>Baseline heart rate (bpm)</td>
<td>76.5±8.5</td>
<td>74.4±9.2</td>
<td>0.105</td>
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<tr>
<td>Maximal heart rate (bpm)</td>
<td>159.7±11.2</td>
<td>162.8±13.8</td>
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</tr>
<tr>
<td>Maximal systolic blood pressure (mmHg)</td>
<td>160.6±17.8</td>
<td>165.1±18.4</td>
<td>0.083</td>
</tr>
<tr>
<td>Maximal diastolic blood pressure (mmHg)</td>
<td>76.7±13.7</td>
<td>74.5±12.6</td>
<td>0.243</td>
</tr>
<tr>
<td>Peak exercise capacity (METs)</td>
<td>12.8±3.4</td>
<td>13.1±3.7</td>
<td>0.555</td>
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<tr>
<td>Heart rate recovery1 (bpm)</td>
<td>26.2±3.6</td>
<td>29.0±4.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heart rate recovery2 (bpm)</td>
<td>42.6±3.9</td>
<td>46.9±3.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heart rate recovery3 (bpm)</td>
<td>56.6±4.5</td>
<td>61.8±4.3</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

bpm: Beats per minute; METs: Metabolic equivalent level; Min: Minute; SD: Standard deviation.

Numerical variables were expressed as mean ± standard deviation or median (minimum–maximum).
29.0±4.1 bpm, p<0.001, respectively), as were HRR2 levels (42.6±3.9 vs 46.9±3.7 bpm, p<0.001, respectively) and HRR3 levels (56.6±4.5 vs 61.8±4.3 bpm, p<0.001, respectively) (Figure 1).

In correlation analysis, HRR1 was found to be significantly correlated with both blood and 24-hour urine lead levels (r: -0.415; p<0.001 and r: -0.446; p<0.001, respectively) (Figure 2). While HRR2 and HRR3 were not found to be correlated with blood lead levels (r: -0.134; p=0.189 and r: -0.098; p=0.337, respectively), these indices were significantly correlated with 24-hour urine lead levels (r: -0.396; p<0.001 and r: -0.233; p=0.021, respectively) (Figure 3). Additional correlation analysis was performed between work duration and blood/urine lead levels, but no significant correlation was found (r: 0.102, p=0.320 for blood lead level; r: 0.029, p=0.774 for 24-hour urine lead level).

Results of the present cross-sectional study suggest that HRR indices are decreased in workers occupationally exposed to lead, compared to normal subjects. In addition, it is suggested that blood and 24-hour urine lead levels are significantly and negatively correlated with HRR indices. ANS activity is essential in the maintenance of cardiovascular system functions. Although the negative effects of lead exposure on cardiac ANS activity have been evaluated using HRV in several studies, the present is the first to evaluate effects of lead exposure on HRR, another method of assessing cardiac ANS.

HRR reflects decreased HR in the recovery phase, and attenuated HRR reflects reduced parasympathetic nervous system activity.[10,15] Although duration of exercise test, baseline and maximum HR, maximal systolic and diastolic BP, and peak exercise capacity

![Figure 1](image-url1)

**Figure 1.** Differences between the lead-exposed and control groups in terms of (A) HRR1, (B) HRR2, and (C) HRR3.

![Figure 2](image-url2)

**Figure 2.** Graphics of correlation between HRR1 and (A) blood lead level, and (B) 24-hour urine lead level. Solid lines represent the correlation slope, dotted lines represent 95% confidence intervals.
Exercise heart rate recovery assessment of the cardiac autonomic nervous system

It may be asked by what type of mechanism lead negatively effects ANS. First, nervous anatomy may play a role. It has been asserted that neurotoxic effects of lead preferentially occur in the long nerves, such as the vagus nerve, as the cardiac vagus branch is more affected than the sympathetic branch.[8] Second, it is thought that lead leads to tissue damage mediated by inflammation and oxidative stress.[20] Oxidative stress is known to produce proinflammatory mediators and reactive oxygen species, inhibit nitric oxide, and alter calcium homeostasis. The reactive oxygen species formed initiates an inflammatory process by causing damage to cell membranes through lipid peroxidation.[21] Lead exposure down-regulates nitric oxide production, which causes an increase in sympathetic activity and a reduction in vagal activity.[22,23] Decreased calcium homeostasis caused by the resemblance of lead to this ion inevitably disrupts the cardiac messenger system.[5] As a result, lead-related oxidative stress may be linked to sympathetic excitation and vagal withdrawal.[6]

Limitations

Nearly 95% of the total body lead burden is located in bone.[24] In the present study, only blood and 24-hour urine lead levels were analyzed. Lead in the bone may better predict long-term lead toxicity than concurrent blood lead level, which reflects only relatively recent exposure. Furthermore, due to the cross-sectional design of the present study, no follow-up data was available, and no cardiovascular outcomes were defined.
These limitations render the speculation of long-term cardiovascular complications difficult.

In order to delineate possible confounding factors, strict exclusion criteria were applied at the beginning of the study, and only the effects of blood and 24-hour urine lead levels were evaluated. As criteria were narrowed, the groups became extremely homogeneous, restricting any comparisons to real-world conditions, and reducing any possible generalization of findings. Exposure to other conditions was not considered, and workplace testing was not analyzed. In addition, potential contributing factors of each occupational sector were not considered. Another limitation was the uniform gender of the study participants; all were male.

In conclusion, significantly reduced indicators of cardiac ANS– HRR1, HRR2, and HRR3– were found in workers occupationally exposed to lead, compared to the control group. Blood and 24-hour urine lead levels were significantly associated with HRR indices. When the prognostic significance of HRR is considered, individuals exposed to lead should be followed closely for adverse cardiovascular outcomes. Further studies are needed in order to understand the clinical and therapeutic implications of cardiac involvement, and the pathogenesis and consequences of autonomic dysfunction in cases of lead exposure.

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REFERENCES

14. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, et al. Recommendations for chamber quantification: a report from the American Society of Echocardiography’s Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 2005;18:1440–63. Crossref
Exercise heart rate recovery assessment of the cardiac autonomic nervous system

Keywords: Cardiac autonomic dysfunction; exercise test; heart rate recovery; lead.

Anahtar sözcükler: Egzersiz testi; kalp hızı toparlanma indeksi; kardiyak otonomik disfonksiyonu; kurşun.