Lead induced increase of blood pressure in female lead workers

K Nomiyama, H Nomiyama, S-J Liu, Y-X Tao, T Nomiyama, K Omae

Aims: Although lead exposure has, in the absence of mathematical modelling, been believed to elevate blood pressure in females, it is necessary to clarify the relation between lead and blood pressure by eliminating confounding factors in the analysis.

Methods: Blood lead was measured in 193 female workers, including 123 lead exposed workers. Possible confounding factors were controlled by multiple regression analyses.

Results and Conclusion: Blood lead above 40 µg/dl was found to be the most potent factor for elevating systolic/diastolic blood pressure. Aging, urine protein, and plasma triglyceride also contributed to systolic/diastolic/pulse pressure increase, but hypertensive heredity did not. Data suggested that lead induced changes in lipoprotein metabolism may play an important role in the lead induced blood pressure increase in female workers.

Parameters and methods

We recorded details of the age, hypertensive heredity, working plants, and duration of employment of the target population. Blood and urine were also collected. One trained medical doctor determined blood pressure (systolic and diastolic) by sphygmomanometer (mercurial manometer) after 15 minutes or more rest in an adjacent room. Pulse pressure was calculated as the difference between systolic and diastolic blood pressures. Blood lead, haemoglobin, plasma total cholesterol, triglyceride, high density lipoprotein, creatinine, urea nitrogen, calcium, phosphorus, δ-aminolevulinic acid, and alkaline phosphatase, and urine protein, glucose, amino acids, calcium, phosphorus, and δ-aminolevulinic acid were analysed. Renal tubular reabsorption of phosphorus was calculated. Plasma low density lipoprotein was estimated, based on Freeevald's formulae. The ratio of plasma urea nitrogen to creatinine was calculated.

Blood lead was determined as follows: whole blood was diluted 10 times with ultrapurified water. Diluted blood (0.9 ml) was mixed with 0.1 ml concentrated nitric acid (ultrapure grade) and then incubated for deproteinisation in a hot water bath of 70°C for five minutes. In a diluted specimen of the supernatant of the deproteinised blood, lead concentration was measured by flameless atomic absorption spectrophotometry (Hitachi Z-9000) with the Zeeman effect background correction. Daily internal quality control checks were performed.

δ-Aminolevulinic acid (ALA) concentrations in plasma and urine were determined by the method of Oishi and colleagues.

Haemoglobin was determined by the cyanmethaemoglobin method. Total cholesterol, triglyceride, and high density lipoprotein were determined by an automatic biochemical analyser, Abbott VP, using the Cholesterol-FA Test (Wako), Triglyceride G-FA Test (Wako), and HDL FA Test (Wako), respectively. Plasma low density lipoprotein was estimated based on Freewald’s formulae. Plasma creatinine, phosphorus, urea nitrogen, uric acid, glucose, cholesterol, and triglyceride were determined with an Abbott VP using reagent Folin-Wu,” Phospha-B Test (Wako), a-gent BUN (Dainabot),
to detect lead induced lesions of the kidneys. Wako), GPT -FA Test (Wako), and Alkaline Phosphatase biochemical analyser, Abbott VP, employing Phospha-B Test. Urine phosphorus, alanine aminotransferase, and haemoglobin, plasma lipoprotein (LDL) (calculated after Freewald’s formulae), plasma high density lipoprotein (HDL), plasma low density lipoprotein, plasma triglyceride, plasma cholesterol, plasma uric acid, plasma creatinine, plasma urea nitrogen, ratio of plasma urea nitrogen to creatinine, fractional excretion of phosphorus (FEIP), plasma phosphorus, plasma calcium, urine calcium, and plasma alkaline phosphatase. Multiple forward regression analyses were employed for 10 representative parameters selected by factor analysis to evaluate the possible confounders, and multiple regression and the Pearson correlation procedure were used for evaluating the significance of the selected confounders. Multiple logistic regression analyses were employed for evaluating dose-effect and dose-response relations between blood lead and blood pressures after controlling for possible confounders. Some of the data, including blood lead concentrations, were missing because of insufficient sample volume for analyses. However, this is not related to the exposure or health status of workers. All analyses were performed using SAS 6.12 statistical software (SAS Institute, Cary, NC, USA).

## RESULTS

### Possible confounders for blood pressure

From the 22 parameters, 10 representative parameters were chosen by factor analysis, one each from the above 10 factors, as follows: blood lead, age, hypertensive heredity, plasma cholesterol, plasma triglyceride, plasma low density lipoprotein, plasma uric acid, plasma creatinine, plasma urea nitrogen, urine protein, urine amino acids, and fractional excretion of phosphorus. The above parameters were then subjected to multiple forward regression analysis with a level of entry of 0.2. As table 2 shows, these parameters were applied to multiple regression analysis; the potent parameters modulating blood pressure were as follows: (1) for systolic blood pressure: blood lead, age, urine protein, and plasma triglyceride; (2) for diastolic blood pressure: blood lead, age, plasma triglyceride, plasma cholesterol, plasma creatinine, plasma urea nitrogen, ratio of plasma urea nitrogen to creatinine, fractional excretion of phosphorus (FEIP), plasma phosphorus, plasma calcium, urine calcium, and plasma alkaline phosphatase.

### Statistical analysis

Twenty two parameters related to blood pressure were subjected to factor analysis for classifying into 10 factors. The parameters were age and duration of employment, hypertensive heredity (number of persons suffering from hypertensive disease), blood lead, plasma triglyceride, plasma cholesterol, plasma high density lipoprotein (HDL), plasma low density lipoprotein (LDL) (calculated after Freewald’s formulae), haemoglobin, plasma δ-aminolaevulinic acid (ALA), urine ALA, urine protein, urine glucose, urine amino acids, plasma creatinine, plasma urea nitrogen, ratio of plasma urea nitrogen to creatinine, fractional excretion of phosphorus (FEIP), plasma phosphorus, plasma calcium, urine calcium, and plasma alkaline phosphatase. Multiple forward regression analyses were employed for 10 representative parameters selected by factor analysis to evaluate the possible confounders, and multiple regression and the Pearson correlation procedure were used for evaluating the significance of the selected confounders. Multiple logistic regression analyses were employed for evaluating dose-effect and dose-response relations between blood lead and blood pressures after controlling for possible confounders. Some of the data, including blood lead concentrations, were missing because of insufficient sample volume for analyses. However, this is not related to the exposure or health status of workers. All analyses were performed using SAS 6.12 statistical software (SAS Institute, Cary, NC, USA).

### Table 2

Factors elevating blood pressures in female workers, 16–58 years of age (upward stepwise regression analysis)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Parameter estimate</th>
<th>$R^2$</th>
<th>Partial correlation</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood lead</td>
<td>0.1260</td>
<td>0.0938</td>
<td>0.2651</td>
<td>0.0003</td>
</tr>
<tr>
<td>Age</td>
<td>0.4763</td>
<td>0.0908</td>
<td>0.2359</td>
<td>0.0014</td>
</tr>
<tr>
<td>Urine protein</td>
<td>0.0417</td>
<td>0.0768</td>
<td>0.2125</td>
<td>0.0041</td>
</tr>
<tr>
<td>Plasma triglyceride</td>
<td>0.0501</td>
<td>0.0549</td>
<td>0.2068</td>
<td>0.0052</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood lead</td>
<td>0.1045</td>
<td>0.1174</td>
<td>0.3481</td>
<td>0.0001</td>
</tr>
<tr>
<td>Plasma triglyceride</td>
<td>0.0200</td>
<td>0.0359</td>
<td>0.1746</td>
<td>0.0224</td>
</tr>
<tr>
<td>Age</td>
<td>0.2215</td>
<td>0.0665</td>
<td>0.1852</td>
<td>0.0153</td>
</tr>
<tr>
<td>Urine protein</td>
<td>0.0452</td>
<td>0.0201</td>
<td>0.1627</td>
<td>0.0335</td>
</tr>
<tr>
<td>Plasma low density lipoprotein</td>
<td>0.0198</td>
<td>0.0025</td>
<td>0.1205</td>
<td>0.1165</td>
</tr>
<tr>
<td>Hypertension heredity</td>
<td>0.0246</td>
<td>0.0481</td>
<td>0.1965</td>
<td>0.0080</td>
</tr>
<tr>
<td>Pulse pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine protein</td>
<td>0.0217</td>
<td>0.0419</td>
<td>0.1413</td>
<td>0.0578</td>
</tr>
<tr>
<td>Plasma triglyceride</td>
<td>0.0476</td>
<td>0.0722</td>
<td>0.1934</td>
<td>0.0091</td>
</tr>
<tr>
<td>Blood lead</td>
<td>0.0414</td>
<td>0.0375</td>
<td>0.1406</td>
<td>0.0590</td>
</tr>
<tr>
<td>FEIP</td>
<td>0.1097</td>
<td>0.0187</td>
<td>0.1069</td>
<td>0.1522</td>
</tr>
</tbody>
</table>

Factors shown whose p values were less than 0.2.
### Table 3: Blood lead and blood pressure relations in female workers

<table>
<thead>
<tr>
<th>Dose-effect relation</th>
<th>n</th>
<th>Mean (SD)</th>
<th>Adjusted difference (95% CI)</th>
<th>p value</th>
<th>Mean (SD)</th>
<th>Adjusted difference (95% CI)</th>
<th>p value</th>
<th>Mean (SD)</th>
<th>Adjusted difference (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood lead* (µg/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (&lt;11.4)</td>
<td>70</td>
<td>110.3 (9.8)</td>
<td>2.1 (-4.3 to 8.5)</td>
<td>0.4582</td>
<td>72.7 (6.0)</td>
<td>2.6 (-1.7 to 6.8)</td>
<td>0.2745</td>
<td>37.7 (7.0)</td>
<td>1.9 (-3.2 to 6.5)</td>
<td>0.6689</td>
</tr>
<tr>
<td>&lt;20 to 40</td>
<td>17</td>
<td>115.2 (9.1)</td>
<td>2.1 (-4.3 to 8.5)</td>
<td>0.4582</td>
<td>76.1 (5.6)</td>
<td>4.1 (1.3 to 6.8)</td>
<td>0.0079</td>
<td>39.4 (7.7)</td>
<td>1.7 (-1.0 to 4.4)</td>
<td>0.2244</td>
</tr>
<tr>
<td>&gt;40 to 60</td>
<td>59</td>
<td>116.9 (16.4)</td>
<td>7.5 (3.0 to 12.0)</td>
<td>0.0011</td>
<td>79.1 (9.5)</td>
<td>6.3 (3.4 to 9.1)</td>
<td>0.0001</td>
<td>40.7 (9.5)</td>
<td>3.4 (0.5 to 6.2)</td>
<td>0.0218</td>
</tr>
<tr>
<td>&gt;60</td>
<td>47</td>
<td>119.8 (16.4)</td>
<td>3.0 (-1.4 to 7.3)</td>
<td>0.1463</td>
<td>82.2 (12.0)</td>
<td>4.6 (1.7 to 7.5)</td>
<td>0.0001</td>
<td>41.4 (12.0)</td>
<td>4.6 (1.7 to 7.5)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

*Blood lead values on control workers were less than 11.4 µg/l, and those on exposed workers were more than 22.5 µg/l.
†Adjusted by the selected possible confounders for SBP, DBP, and PP in Table 2.

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### DISCUSSION

**Lead exposure and blood pressure increase**

Lead exposure has been reported by many scientists to increase blood pressure. However, only some epidemiological studies examined the dose-effect or dose-response relations between blood lead levels and blood pressure. In this study, we found that blood lead concentrations were significantly higher in workers with blood lead concentrations of 37 µg/dl (95% CI: 28–46) compared to the control group. This is consistent with previous studies that have shown a linear relationship between blood lead concentration and blood pressure. The results also support previous findings that blood lead concentrations greater than 22.5 µg/dl were significantly higher than those in the control group (blood lead <11.4 µg/dl).

**Partial correlation between blood lead and blood pressure**

Partial correlation analyses were performed to examine the relationship between blood lead and blood pressure, adjusted for confounders such as age, plasma triglyceride, and blood pressure. The partial correlation coefficients were found to be significantly correlated to blood pressure, indicating a positive relationship between blood lead and blood pressure.

**Relations between other confounders and blood pressure**

Partial correlation analyses were also performed to examine the relationships between other confounders and blood pressure, such as plasma triglycerides, age, and blood pressure. Significant partial correlations were observed between plasma triglycerides and systolic/diastolic and pulse pressure. The partial correlation coefficients were found to be significantly correlated to blood pressure, indicating a positive relationship between these confounders and blood pressure.

**Contribution of blood lead to blood pressure**

Blood lead was selected by multiple forward regression analysis as a significant predictor of blood pressure. The prevalence of an increased blood pressure above 125 mm Hg was significantly higher in the group with blood lead concentrations above 37 µg/dl compared to the control group. This is consistent with previous studies that have found similar findings.

**Prevalence and odds ratio**

The prevalence of an increased blood pressure above 125 mm Hg was significantly higher in the control group (blood lead <11.4 µg/dl) compared to the exposed group (blood lead >60 µg/dl). The odds ratio (95% CI) was also statistically significant, indicating a higher risk of increased blood pressure in the exposed group.

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It seems quite natural that there should be a disparity among the results because most authors did not analyze their data by adjusting for potential confounders. This suggests that the relationship between blood lead and blood pressure may be confounded by other factors. Further studies are needed to clarify the role of blood lead in the development of hypertension.
data by the use of mathematical modelling in order to eliminate confounding factors that elevate blood pressure. In addition, although several authors did apply mathematical modelling, the blood lead might be relatively low. On the other hand, the occupational exposures to lead in some Asian and African countries are still higher than those in western countries. We therefore carried out an epidemiological survey on male lead workers, and found that lead exposure was the most potent factor in the diastolic blood pressure increase, and that diastolic blood pressure in the male workers with blood lead concentrations above 40 µg/dl was increased, after mathematical modelling. Other potent factors in raising blood pressure were hypertensive heredity and age. We carried out the present survey on lead induced increase of blood pressure in female workers, as it has been suggested by Harlan that lead exposure did not raise blood pressure levels in females residents, and Wu and colleagues recently confirmed that lead exposure did not appear to raise blood pressure in female workers. Contrary to the works of Harlan and Wu and colleagues, however, our data yielded the following facts: (1) the potent factors increasing the systolic and diastolic blood pressures calculated by a combination of factor analysis and multiple forward regression analysis were blood lead, age, plasma triglyceride, and urine protein, and did not include hypertensive heredity in common (table 2); and (2) the systolic and diastolic blood pressures were increased in female workers with blood lead concentrations of 40–60 µg/dl or higher, as shown by dose-effect and dose-response relation data (table 3).

Wu and colleagues reported a lack of evidence of lead induced increase of blood pressure in female workers, probably as a result of the short exposure (0.5–17 years, mean 3.1 (3.2) years). Indeed, it is true that their observed blood pressure increase in workers. In the present survey on female workers, as it has been suggested by Harlan that lead exposure did not raise blood pressure levels in females residents, and Wu and colleagues recently confirmed that lead exposure did not appear to raise blood pressure in female workers. Contrary to the works of Harlan and Wu and colleagues, however, our data yielded the following facts: (1) the potent factors increasing the systolic and diastolic blood pressures calculated by a combination of factor analysis and multiple forward regression analysis were blood lead, age, plasma triglyceride, and urine protein, and did not include hypertensive heredity in common (table 2); and (2) the systolic and diastolic blood pressures were increased in female workers with blood lead concentrations of 40–60 µg/dl or higher, as shown by dose-effect and dose-response relation data (table 3).

Other factors increasing blood pressure
Age also contributed to systolic, diastolic, and pulse pressures. Systolic and diastolic blood pressures were significantly increased in female workers above 30 years old (data not shown), as was observed in male workers. Lilis and colleagues suggested that lead induced renal dysfunction might lead to a blood pressure increase in workers. In the present survey on female workers, however, while blood lead was significantly associated with urine amino acids or plasma creatinine (p = 0.0079, p = 0.0191), these parameters were not selected as possible confounders by multiple forward regression analyses. Urine protein was significantly associated with systolic/diastolic/ pulse blood pressures (table 2), but partial correlations (PCs) indicated no associations between blood lead and urine protein on blood pressure (systolic blood pressure: PC = -0.0928, p = 0.2142; diastolic blood pressure: PC = -0.0989, p = 0.1982; pulse pressure: PC = -0.0614, p = 0.4118). Furthermore, as was shown in table 2, no parameters indicating renal function other than urine protein were selected by multiple forward regression analyses. It is difficult to conclude therefore that the observed blood pressure increase was related to lead induced renal dysfunction, such as glomerular or tubular dysfunction. However, plasma triglyceride was significantly and closely associated with systolic and diastolic blood pressures and pulse pressure, respectively, even though no significant partial correlation was found between blood lead and plasma triglyceride on systolic/diastolic/pulse blood pressure. Although low density cholesterol was significantly associated with diastolic blood pressure, the partial correlation between low density cholesterol and blood lead was negatively significant (PC = -0.2661, p = 0.0004). All the above confounding results may suggest that lead induced changes of lipoprotein metabolism could induce an increase of blood pressure, but the mechanism is not fully elucidated. Kuźnińska reported that potent atherosclerosis occurred in pigeons given cholesterol 600 mg/day and lead 25 mg/kg simultaneously over a period of 3.5 months, compared to the pigeons of the control, cholesterol, and lead groups. Sroczynski and colleagues gave lead acetate to rats intravenously at a dose of 20 mg Pb/kg every other day for three months, and found increased concentrations of plasma cholesterol and lipoprotein T fraction. Sroczynski and colleagues noted cholesterol digitonin deposits in the inner, median, and outer layers of the aorta in rats administered lead acetate at a dose of 20 mg Pb/kg every other day. Revis and colleagues reported that the plasma high density lipoprotein was increased in pigeons given food containing lead chloride at a dose level of 0.8 mg/kg over a period of six months. Tarugi and colleagues observed greatly increased concentrations of plasma cholesterol ester in rabbits given food containing 0.5% lead acetate over a period of 45 days. Their findings appear to provide support for our proposal concerning the mechanism of lead induced increase of blood pressure, whereby lead induced changes in lipoprotein metabolism may play an important role in the lead induced blood pressure increase in female workers.

Limitations of the study
In view of the nature of the cross sectional study, there are some limitations of this study. The temporal causality has not been established, and past exposure to lead was hard to be estimated. Furthermore, we cannot exclude the possibility of unexpected factors that were not evaluated and can confound the effect of lead exposure.

Conclusion
A possible explanation of the results of the study is that lead induced changes in lipoprotein metabolism may play an important role in the lead induced blood pressure increase in female workers, especially at concentrations higher than 40 µg/dl.

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