Latency of the Achilles tendon reflex for detection of reduced functions of the peripheral nervous system in workers exposed to lead

Yasutaka Ogawa, Mamoru Hirata, Akira Okayama, Yoko Endo Ichikawa, Shigeru Goto

Abstract
The latency of the soleus muscle potential, evoked by a tap of the Achilles tendon, was used in the mass assessment of healthy male workers exposed to lead. Three groups were studied: a control, a low exposure, and a high exposure group. Mean blood lead concentrations were 9.3, 19.2, and 53.1 μg/100 ml respectively. Latencies were adjusted for age and height and then compared among the three groups. The mean corrected latency of the high exposure group showed a 4% increase compared with the other two groups (p < 0.01). The latencies of the other two groups showed no between group differences. These results were consistent with previous neurophysiological studies done by many researchers on workers exposed to lead. They suggested that nerve conduction velocities decreased from 3 to 13% among workers whose mean blood lead concentrations were more than 30 μg/100 ml. The method was simple, time conserving, non-invasive, and non-aversive, and provided a quantitative measure of the nerve conduction velocities of peripheral nerves. Thus it may be useful for early detection of occupationally related impairment of peripheral nerves.

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assessing the peripheral nerve dysfunction of workers exposed to lead to evaluate its usefulness.

Materials and methods

Subjects

One hundred and thirty three healthy male workers were chosen from five factories. Workers from two factories did not use lead as product materials and were classified as the non-exposed control group (C). Workers from another factory used lead powder (PbO₂) with protective tools and had regular education about how to decrease the exposure. Consequently, workers from this factory were classified as a low lead exposure group (L). Workers from the other two factories, which were small scale industries, were repeatedly exposed to lead fumes or lead stearate powder. Both workplaces provided less than ideal working conditions for workers exposed to lead because of limited space and insufficient installations for suppressing the leakage of noxious materials. Therefore the workers from these two factories were classified as a high lead exposure group (H). According to the record of these factories, none of the workers were exposed to other chemicals or metals known to have peripheral neurotoxicity in the workplace.

On examination of health records, workers with diabetes mellitus or blood glucose concentrations above 140 mg/100 ml, along with alcohol drinkers whose consumption amounted to more than 80 g/day were excluded. Physical health examinations showed no workers with peripheral nerve disorder, nor any with radicular lesions.

Methods

Our study was performed in accordance with the Japanese industrial safety and health law—that is, each factory under study was required to provide periodic health examinations for workers exposed to lead in the workplace. Examination room temperatures were controlled so as not to be under 20°C during the measurements. The tendon reflex of the right soleus muscle (the Achilles tendon reflex) was examined (fig 1). The subjects were in the standing position with the right knee flexed at a 90 degree angle and resting on a stool with the subject's ankle hanging freely over the end of it. The tendon percussions were made by a hand held hammer (Medelec patellar hammer) that produced a short circuit mechanically when the hammer was tapped; the signal worked as a trigger pulse. Reflex potentials of soleus muscle were recorded from surface electrodes (stainless steel: size 0·8 cm²) placed on the skin with electrolyte gel and surgical tape. An active recording electrode was placed on the mid-dorsal line 3 cm above the lower margin of the gastrocnemius muscle of the calf (modified from Hugon⁶). A reference recording electrode was placed 2 cm above the fibular head of the Achilles tendon for monopolar montage.⁹ A ground electrode was placed at the ankle. The evoked muscle potentials were amplified, bandpass filtered (2 Hz and 10 kHz), and displayed with a portable EMG recorder (Medelec MS7). They were then photographed with a Polaroid camera.

Measurements were taken after the subjects had adapted to the room temperature for 10 minutes. Each subject was instructed to relax and a minimum of five stimuli were applied with sufficient strength to evoke maximal responses. The intervals between tendon taps were at least five seconds in duration. Amplitudes were measured from the peak negative to the peak positive deflection and the wave of maximum amplitude was selected for data analysis. Latencies of the waves (Lts) were measured from the stimulus artifact to the first deflection of the potential from the baseline. The skin temperature at the belly of the calf was monitored by an infrared detector (Optex HR-1PS, Japan). The height of each worker was measured.

Venous blood samples were regularly taken from the workers for blood chemistry during the periodic health examinations. They were collected into heparinised lead free glass tubes and the concentrations of whole blood lead (Pb-B) were determined using electrothermal atomic absorption spectrophotometry.¹⁰

Analytical methods

Firstly the Lts of three groups were compared by analysis of covariance (ANCOVA) using height, skin temperature, and age as covariates. Then stepwise multiple regression analyses were performed with height, skin temperature, and age as independent variables (in, α = 0·15; out, α = 0·15). Each Lt was corrected to Ltₜ, which was a value of Lt at the mean

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**Figure 1** Diagram of experimental arrangement.
value of each independent variable. It was assumed that the resulting regression line would explain the variation depending on the variables. Lat was further corrected to Latc, which was calculated on the assumption that the latency was in proportion to the height of the subject.

To compare the mean values of three groups, analysis of variance (ANOVA) was first applied and then the method of Scheffe was used to compare the differences between groups. Multiple comparison of Pearson’s correlation coefficients were tested by Bonferroni adjusted probabilities.

Results

Three workers from group C and four from group L were unsuccessful in evoking the tendon reflex. The workers whose calf skin temperatures were under 30°C were excluded from the study (one in group L, one in group H). The remaining 124 subjects were used for our analysis. Table 1 shows the age distribution of the analysed workers in the three groups.

Table 2 shows the group means and standard deviations (SDs) of age, height, skin temperature, and Pb-B. The mean age of group L was low compared with that of group C (p < 0.05). The mean skin temperature of group H was low compared with that of C and L (p < 0.01). The mean Pb-Bs of three groups were statistically different between groups (p < 0.01). The concentrations were highest in group H and lowest in group C.

Table 3 shows the correlation coefficients between the variates. Height was positively correlated with latency and negatively correlated with age. There was no correlation between Lat and skin temperature in the range 30.1–35.1°C.

According to ANCOVA, the Lats of three groups were not evenly distributed. By stepwise multiple regression analysis age and height were selected as independent variables and skin temperature was discarded. Hence the corrected model for Lat was:

\[ \text{Lat}_C = \text{Lt} + \{0.057 \times (37.6 - \text{Age}) + 0.188 \times (166.9 - \text{Height})\} \]

where 37.6 and 166.9 are the means of age in years and height in cm of all the subjects. The second corrected model for Lat was:

\[ \text{Lat}_c = (167/\text{Height (cm)}) \times \text{Lt} \]

where 167 is the mean height of all the subjects in cm.

Table 4 shows the means (SDs) of Lat, Lt, and Lt167 for the three groups. Each latency was significantly different between group H and group C, and between group H and group L. Each adjustment of confounding variables decreased the SD of each group. The correlation coefficient between Latc and Lt167 was 0.94 (fig 2).

Discussion

Latency of the muscle action potential, Lat, elicited by the tendon tap, is composed of several components: (1) the speed of stretching a tendon by the tendon tap (the spread time of the tension wave to the muscle spindle) and the muscle spindle activation time; (2) the conduction time of the Ia sensory fibre from muscle spindle to the spinal cord; (3) the synaptic delay at the spinal cord; (4) the conduction time of the a motor fibre from the anterior horn to the neuromuscular junction; (5) the synaptic delay at the neuromuscular junction.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Age distribution for control (C), low exposure (L), and high exposure (H) groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>&lt;25</td>
<td>39.6±0.97</td>
</tr>
<tr>
<td>25-34</td>
<td>33.5±5.6</td>
</tr>
<tr>
<td>35-44</td>
<td>33.0±0.8</td>
</tr>
<tr>
<td>≥45</td>
<td>31.9±1.0</td>
</tr>
</tbody>
</table>

*p < 0.05 between C and L; **p < 0.01 between C and H and between L and H; 0.01; between C, L, and H.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Age, height, skin temperature (S temp), and blood lead concentrations (Pb-B) for control (C), low exposure (L), and high exposure (H) groups</th>
</tr>
</thead>
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<tr>
<td>Age (y)</td>
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<td>≥45</td>
<td>31.9±1.0</td>
</tr>
</tbody>
</table>

| Height (cm) | Mean (SD) | C (n = 68) | L (n = 43) | H (n = 13) |
| <25     | 166.1±6.3 | 168.3±5.6 | 166.6±5.5 |
| 25-34   | 166.3±5.6 | 166.3±5.6 | 166.3±5.6 |
| 35-44   | 33.3±0.8  | 33.0±1.0  | 31.9±0.8  |
| ≥45     | 31.9±1.0  | 31.9±1.0  | 31.9±1.0  |

| Pb-B (μg/100 ml) | Mean (SD) | C (n = 68) | L (n = 43) | H (n = 13) |
| 0-01     | 8.5±2.7  | 19.2±10.7 | 53.3±15.1 |
| 0-05     | 166.1±6.3 | 168.3±5.6 | 166.6±5.5 |
| 0-01     | 33.3±0.8  | 33.0±1.0  | 31.9±0.8  |
| 0-01     | 8.5±2.7  | 19.2±10.7 | 53.3±15.1 |

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Correlation coefficients between age, height, skin temperature (S temp), and the latency of a mechanically elicited electromyogram of soleus muscle (Lt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Height</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>0.465**</td>
<td>-0.140</td>
</tr>
</tbody>
</table>

*p < 0.05; **p < 0.01.

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Latency Lt and corrected latencies Ltc, Lt167, for control (C), low exposure (L), and high exposure (H) groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lt</td>
<td>Lt167</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>27.0±1.0</td>
<td>26.9±1.5</td>
</tr>
<tr>
<td>27.1±1.1</td>
<td>26.9±1.3</td>
</tr>
<tr>
<td>27.2±1.5</td>
<td>26.7±1.0</td>
</tr>
</tbody>
</table>

tp < 0.05 between C and H and between L and H; *p < 0.05 between C and H and p < 0.01 between L and H.
The spread of a tension wave after the tendon tap and the activation of the muscle spindle takes roughly 2 ms + synaptic delay of the neuromuscular junction in ms.\textsuperscript{11} The variation of this component is due to the tapping technique and the length of the muscle. By our measurements intraindividual variations were not more than 3% in coefficient of variation, which is about 0.8 ms. The spinal synaptic delay of the H reflex arc is less than 1 ms\textsuperscript{11} 1; thus the contribution of summed (3) and (5) to Lt may be less than 2 ms. The remaining part of Lt (\approx 23 ms), which is about 85% of Lt, is composed of the nerve conduction time of the afferent Ia fibre and the efferent \alpha fibre.

In animal studies there is evidence that low concentrations of lead depress or block synaptic transmission but do not noticeably affect transmission time.\textsuperscript{14,15} There is also evidence that synaptic delay is dependent on temperature, but it seems that it cannot change more than 0.05 ms/°C around 30°C.\textsuperscript{16} Therefore, there is little possibility for the change in synaptic transmission time to contribute to the increase that appeared in the Lt of group H. This increase is probably due to the slowing of nerve conduction velocities.

According to the assumptions we have made above, if the latency elongation is 4%, as found in this study, the mean nerve conduction velocities of nerves relating to the reflex will decrease by 4-5%. This estimation is consistent with the nerve conduction velocity studies of many researchers. Triebig \textit{et al} showed a 1.5-4.2% decrease among ulnar nerve conduction velocities in workers whose mean Pb-B was 80 µg/100 ml.\textsuperscript{17} Muijser \textit{et al} reported a 2-7% decrease among ulnar and median nerve conduction velocities in workers whose mean Pb-B was 53.2 µg/100 ml,\textsuperscript{18} and He \textit{et al} showed a 3.6-13.7% decrease among ulnar, median, peroneal, and sural nerve conduction velocities in workers whose mean Pb-B (geometric) was 40.0 µg/100 ml.\textsuperscript{19}

Although this study's mean (Pb-B of 20 µg/100 ml) showed no change in Lt compared with 9 µg/100 ml, a mean of 50 µg/100 ml did. This change is also consistent with former studies. Seppäläinen \textit{et al} identified the Pb-B concentration threshold that changes nerve conduction velocity to be 40-50 µg/100 ml.\textsuperscript{20} Schwartz \textit{et al} found the threshold concentration of children to be 30 µg/100 ml.\textsuperscript{21}

The mean skin temperature of group H was significantly lower than the temperatures of the other two groups indicating that this might have influenced Lt.\textsuperscript{22} Despite this fact, this difference does not account for the between group differences in Lt because there was no positive correlation between skin temperature and Lt (in the study’s temperature range). Skin temperature on the calf may not be indicative of deep skin temperatures where the nerve trunk runs. It may in fact be disturbed by the thickness of underlying fat tissue.\textsuperscript{23} Accordingly, further studies are needed in this area.

Nerve conduction time is related to the nerve conduction velocity and the length of the conduction pathway. This may explain the result that there was a high correlation between Lt and height. Guiheneuc and Bathien also showed a linear relation (r = 0.95) between the height of the subject and the interval between M wave and H wave, which did not include (1) and (5).\textsuperscript{24} According to this relation, Lt needs to be corrected for height in epidemiological studies. Our analysis showed that the correction of Lt with height, assuming that Lt is in proportion to height, was simple and effective.

The method we introduced was non-invasive, non-aversive, and easy to perform within a short time. For these reasons, it may be deemed acceptable for the periodic mass monitoring of workers exposed to neurotoxic substances. As previously discussed, this method is not specific for measurement of nerve conduction velocities. Even if some change in latency was found between groups, therefore, we cannot pinpoint the exact portion responsible. This problem requires further examination. If the effects of a material are well known, however, such as for inorganic lead, which mainly affects the fibres of the peripheral nervous system, then our method may be useful for screening the effects on this system. Furthermore, this method included measurements of conductivities of the proximal as well as the distal portion of the peripheral nervous system. For a neurotoxicant such as β,β'-iminodipropionitrile that produces neuropathy in the proximal portion earlier than the distal portion of the peripheral nervous system,\textsuperscript{25} this method may be useful in assessing the dysfunction of the proximal portion.

We thank Miss Midori Kobayashi for her linguistic help.

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**Figure 2** Relation between two corrected latencies Lt, and Lt₀₀:

- Lt₀₀: The corrected latency for the median nerve, which is calculated by subtracting the mean Pb-B concentration from the mean Lt value.
- Lt: The uncorrected latency for the median nerve, which is the time difference between the tendon tap and the muscle response.

The graph shows the relationship between Lt and Lt₀₀, indicating how the corrected latency changes with Pb-B concentration.
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