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Blood Lead Level to Induce Significant Increase in Urinary δ-Aminolevulinic Acid Level among Lead-Exposed Workers: A Statistical Approach

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Abstract: The present study was initiated to examine the quantitative relationship between blood lead (Pb-B) and urinary δ-aminolevulinic acid (ALA-U) among Pb-exposed workers, and to find a threshold Pb-B level to induce an increase in ALA-U. For this purpose, pairs of venous blood and spot urine samples were collected from 8,274 men and 5,856 women (14,130 workers in total) who were occupationally exposed to inorganic lead. The blood and urine samples were analyzed for Pb-B and ALA-U by atomic absorption spectrometry and colorimetry, respectively, and the correlation between pairs of measures were subjected to statistical analysis. The assumption of the 3rd degree regression for correlation gave a substantially greater correlation coefficient (0.645 for men and 0.619 for women) than 1st or 2nd degree regression, whereas only very small improvement in the coefficient was achieved with 4th to 6th degree ones. Logarithmic conversion of the parameters was not effective in improving the correlation. The assumption of the 3rd degree regression followed by calculation of the local minimum gave 22, 29 and 23 μg/100 ml Pb-B for men, women, and men+women, respectively, as the threshold Pb-B to induce ALA-U increase. Pb-B to elevate ALA-U to the 95% upper normal limit (8 mg/l, common to men and women) was 62, 50 and 58 μg/100 ml for men, women and men+women, respectively. The validity of the 3rd degree regression assumption as a tool to calculate a threshold from experimental or epidemiological data is discussed.

Key words: Biological monitoring, Blood lead, Threshold Pb level, Non-linear regression, Urinary δ-aminolevulinic acid

Introduction

Blood lead (Pb-B) is a traditional marker of occupational as well as environmental exposure to inorganic lead, and has been most popularly used world-wide[1, 2]. The urinary level of δ-aminolevulinic acid (ALA-U), a precursor of porphobilinogen and therefore prophyrin synthesis in heme metabolism, has also been accepted in occupational health as a marker of biochemical effect of Pb[2]. Thus, biological exposure limits for Pb-B are currently set at 30[3], 40[4] or 70 μg/100 ml[5] for workers in general (30 μg/100 ml for <45 years-old women[5]), whereas the limits for ALA-U are set at 5[4] or 15 mg/l[5] for workers in general (6 mg/l for <45 years-old women[5]).

Accordingly, the Pb-B concentration to induce a substantial increase in ALA-U has also been a focus of interest. The lowest Pb-B concentration to induce an increase in ALA-U, or the threshold Pb-B level, has also been discussed as early as mid-1970s[6–9]. The Pb-B values cited by various authors were 16[10], 35[6], 25 to 34[7] or 48 μg/100 ml[9]. Whereas the criterion for setting the threshold Pb-B concentration was different among the authors, it appears that these

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proposed values of 25 to 50 μg/100 ml Pb-B have been accepted since then without major criticism from experiences in occupational health, although reports are also available to suggest that an increase in ALA-U may take place at lower Pb-B[10–14].

The purpose of the present study is to elucidate the relationship of ALA-U with Pb-B, to examine the validity of the proposed Pb-B values[6–14] through a statistical approach, and to identify the threshold Pb-B concentration to induce elevation in ALA-U.

Materials and Methods

Subjects studied

Pairs of venous blood and spot urine samples were obtained from 14,130 workers (8,274 men and 5,856 women) who had been occupationally exposed to inorganic lead (Pb) at least for two years. Men were mostly engaged in lead powder and lead pole plate production in storage battery works, but a few served in secondary lead smelters[15]. Women were either in lead battery assembly lines or in soldering work. Subjects who were clinically ill (e.g., with liver dysfunction) were excluded.

Determination of Pb-B and ALA-U

Pb-B was measured by atomic absorption spectrometry as previously described[15]; the analytical procedure was adjusted so that blood samples with high Pb-B (e.g., 100 μg/100 ml) can be measured without further dilution, and the detection limit was rather high, i.e., 5 μg Pb/100 ml blood. ALA-U was determined after Tomokuni and Ichiba[16], a modification of a colorimetric method[17]. The detection limit was 0.1 mg/l. The values obtained by this colorimetry are essentially the same with the values measured by the HPLC method, when ALA-U is high, e.g., greater than 5 mg/l[18]. Following previous observation[19], ALA-U was expressed without any correction (i.e., as observed).

Statistical analysis

A PC software STAT-VIEW (version 5) was employed for statistical analysis, including calculation for multi-degree regression equations. A log-normal distribution was assumed in some statistical analysis, so that the distribution was expressed in terms of geometric mean (geometric standard deviation) [GM (GSD) in short].

Results

Pb-B levels, and the relation with ALA-U

Distribution of Pb-B values is presented in Table 1 by a 20 μg/100 ml range for men and women, separately and in combination (i.e., men+women). It is clear that Pb-B was below 40 μg/100 ml in majorities of men (93%) and women (98%), whereas 95 men (1.1%) and 21 women (0.4%) had Pb-B in excess of 61 μg/100 ml. Scatter diagrams are shown in Fig. 1 for [A] men, [B] women, and [C] men+women. The relationship of ALA-U with Pb-B appeared to be different between the two sexes. For example, the cases with >50 mg/l ALA-U were observed only among those with >90 μg/100 ml Pb-B in men, but such cases could be detected when Pb-B was as low as 58 μg/100 ml in women.

Equations for the regression of ALA-U with Pb-B

Table 2 summarizes the results of calculations for regression equations of up to the 6th degree with men, women, and men+women. The correlation coefficients tended to increase with a larger degree of the equation from the 1st to 6th. Nevertheless, it was also clear that a substantial increase in the correlation coefficient was obtained only up to the 3rd degree, but quite less so with the 4th to the 6th. In fact, the coefficients to X^4, X^5 or X^6 were generally very small, suggesting that the 3rd degree assumption is sufficient in regression analysis.

Trials were also made to examine if semi-logarithmic conversion could result in high correlation between Pb-B and ALA-U. When the 1st to the 3rd degree regression equation was calculated between Pb-B (as observed) and

Table 1. Pb-B distribution by 20 μg/100 ml in men, women and the combination

Group	Pb-B range (μg/100 ml)						
	Total	≤20	21–40	41–60	61–80	81–100	101–120
Men	8,274	6,327	1393	459	74	17	4
Women	5,856	5,292	459	84	21	0	0
Men+women	14,130	11,619	1852	543	95	17	4

Values are numbers of subjects in each Pb-B range.

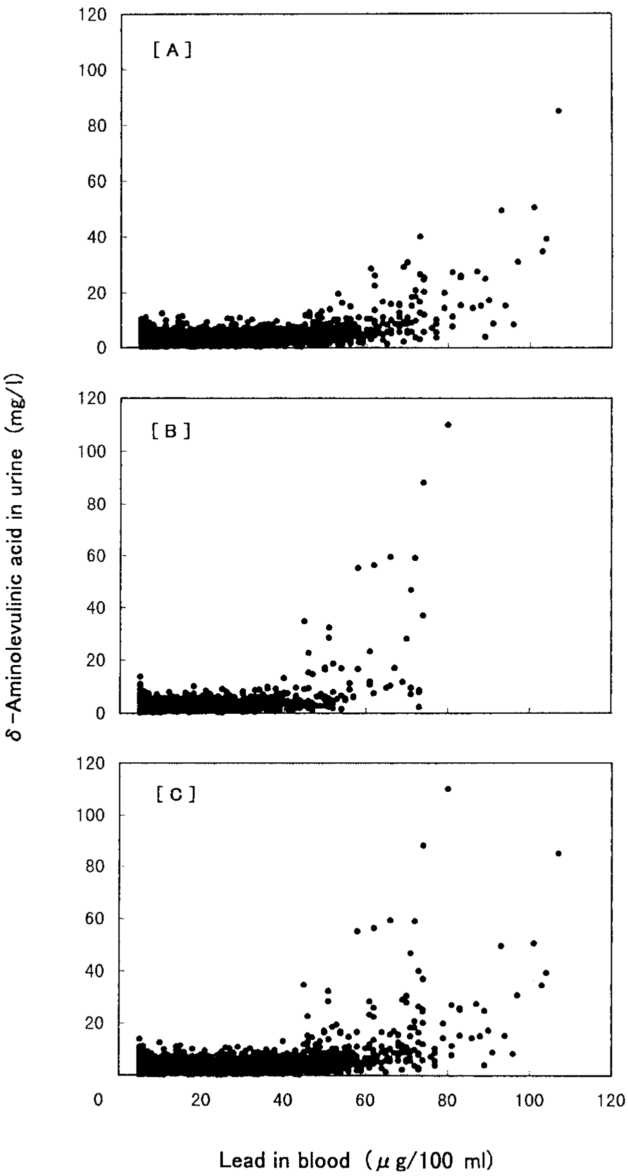

Fig. 1. Scatter diagrams between Pb-B and ALA-U in [A] 8,274 men, [B] 5,856 women, and [C] 14,130 men+women.

Each dot shows one case. For parameters of the 1st to 6th degree regression curves, see Table 2.

$\log_{10}$ [ALA-U], it was found that the calculation after logarithmic conversion gave lower correlation coefficient for men (0.236), women (0.131) and men+women (0.203) than the calculation without the conversion (0.645, 0.619, and 0.552, in the order). Thus, no logarithmic conversion was applied in subsequent analysis. Further comparison of the coefficients, r (without logarithmic conversion) among men, women and men+women showed that the r was >0.6 when men and women were treated separately, but <0.6 when the two sexes were combined, in agreement with the observation that ALA-U increases at lower Pb-B in women than in men as described above (Fig. 1).

Threshold Pb-B to induce elevation in ALA-U

The threshold Pb-B level to induce elevation in ALA-U was calculated with an assumption of the 3rd degree regression. In the 3rd degree equation of $Y=a+bX+cX^2+dX^3$ (in which d>0), X for the local minimum can be calculated as the value when the differential equation is equal to zero, i.e., $dy/dx=b+2cX+3dX^2=0$, or in other words, $X=[(c/3d)^2-(b/3d)]^{1/2}-(c/3d)$. The calculation gave 21.7 μg/100 ml for men, and 28.6 μg/100 ml for women, and 22.7 μg for men+women.

Pb-B at which ALA-U exceeds the 95% upper normal limit

The results of the calculation for the local minimum suggest that the ALA-U e.g. in men should be free from the effects of Pb-B when Pb-B was below the local minimum of 21.7 μg/100 ml. The cases (for men, women, and men+women, separately) with Pb-B below the local minimum were identified and the ALA-U of such cases were subjected to the calculation for GM (GSD). The 95% upper normal limits for ALA-U ('normal' being in the sense that the values were free from the effect of Pb-B) were then calculated as GM × 2GSD (the center column of 'the 95% limit' in Table 3). It was possible to determine graphically the Pb-B values at which the 3rd degree regression curves cross the corresponding 95% upper limits; the values thus obtained are shown in the rightmost column of 'the cross' in Table 3. The Pb-B was about 62 μg/100 ml for men, 50 μg/100 ml for women, and 58 μg/100 ml for men+women.

Difference in ALA-U at the local minimum and at the local maximum

As discussed above, it is possible to calculate Pb-B for the local minimum taking advantage of the equation of $X=+[(c/3d)^2-(b/3d)]^{1/2}-(c/3d)$. Similarly, Pb-B for the local maximum can be calculated as $X=-[(c/3d)^2-(b/3d)]^{1/2}-(c/3d)$. By definition, b, c and d are as in the equation of $Y=a+bX+cX^2+dX^3$, and the values of b, c and d are given in Table 2. From this equation for Y, it is possible to calculate ALA-U at the local maximum and minimum as Y when X (i.e., Pb-B) is at the local maximum and minimum, respectively. The calculation with the equations above gave the local maximum and minimum of [Pb-B=14.3 (μg/100 ml), ALA-U=3.27 (mg/l)] and [21.7, 3.26] for men, [10.3, 3.36] and [28.8, 2.57] for women, and [0.4, 3.21] and [22.7, 2.93] for men+women, respectively. Thus, the difference in ALA-U at the local maximum and the local minimum

Table 2. Coefficients to X of various powers in the regression equations, and correlation coefficients

Group	Degree [a]	Coefficient (a to g) to							Corr. coeff.
		X^0	X^1	X^2	X^3	X^4	X^5	X^6	(r)
Men	1st	2.56	0.06099						0.362
	2nd	3.97	−0.14733	0.00379					0.604
	3rd	2.91	0.06520	−0.00378	0.00007				0.645
	4th	3.50	−0.07980	0.00429	−0.00009	9E-07			0.649
	5th	2.49	0.20763	−0.01764	0.00056	7E-06	3E-08		0.654
	6th	5.26	−0.68820	0.07003	−0.00308	0.00006	−6E-07	2E-09	0.667
Women	1st	2.43	0.10119						0.304
	2nd	4.73	−0.34057	0.00943					0.572
	3rd	2.36	0.22179	−0.01465	0.00025				0.619
	4th	4.83	−0.46550	0.03150	−0.00082	8E-06			0.633
	5th	0.58	0.89311	−0.09316	0.00377	−0.00006	4E-07		0.645
	6th	10.40	−2.65090	3.01652	−0.01698	0.00044	−6E-06	3E-08	0.668
Men+women	1st	2.57	0.06788						0.302
	2nd	3.99	−0.16518	0.00442					0.534
	3rd	3.21	0.00127	−0.00173	0.00005				0.552
	4th	3.11	0.02718	−0.00320	0.00008	−2E-07			0.552
	5th	2.66	0.15598	−0.01318	0.00038	−4E-06	2E-08		0.553
	6th	5.90	−0.90799	0.09232	−0.00406	0.00008	−8E-07	3E-09	0.567

Coefficients (a-g) are in the regression equations, $Y=a+bX+cX^2+dX^3+eX^4+fX^5+gX^6$, where X is Pb-B ($\mu$g/100 ml) and Y is ALA-U (mg/l); for the numbers of subjects, see Table 1. [a] Degree of the regression equation.

Table 3. The 95% upper normal limit for ALA-U, and Pb-B at the local minimum and at the point of the 95% upper limit for ALA-U

Group	ALA-U (mg/l) [a]			Pb-B (μg/100 ml) at	
	No.	GM	The 95% limit [b]	The local minimum	The cross [c]
Men	6,413	2.8	7.7	22	62
Women	5,533	2.8	8.1	29	50
Men + women	11,868	2.8	7.9	23	58

[a] Calculated with those whose Pb-B was below the corresponding local minimum. As the local minimum varies depending on the group, the addition of men only and women only does not meet the number of men+women. [b] The 95% upper normal limit, calculated as GM × 2GSD. [c] At the cross with the 95% upper normal limit for ALA-U.

was 0.01, 0.79 and 0.28 mg/l for men, women and men+women, respectively.

Pb-B at which the proportion of the subjects with elevated ALA-U increases

Trials were made to simulate the approaches taken by previous authors[6, 7, 9] to identify the Pb-B concentrations at which the proportion of the subjects with ALA-U above a given concentration (e.g., 10 mg/l) begins to increase. For this purpose, men and women, separately, were classified by 5 μg/100 ml Pb-B ranges (e.g., <15 to 20 μg/100 ml) and the proportion (in %) of the subjects with >10 mg/l ALA-U[7] were calculated for each range. The pairs of the median Pb-B and the proportion (%) was calculated until the percentage was close to 70% (the value of 70% being selected arbitrary; Fig. 2), and the pairs (14 and 11 pairs for men and women, respectively) were subjected to regression analysis with an assumption of the 3rd degree equation for regression. From the parameters of the equations, Pb-B for the local minimum was calculated as described above. The calculation gave 35 and 30 μg/100 ml Pb-B for men and women, respectively. The same analysis was applied also to the cases

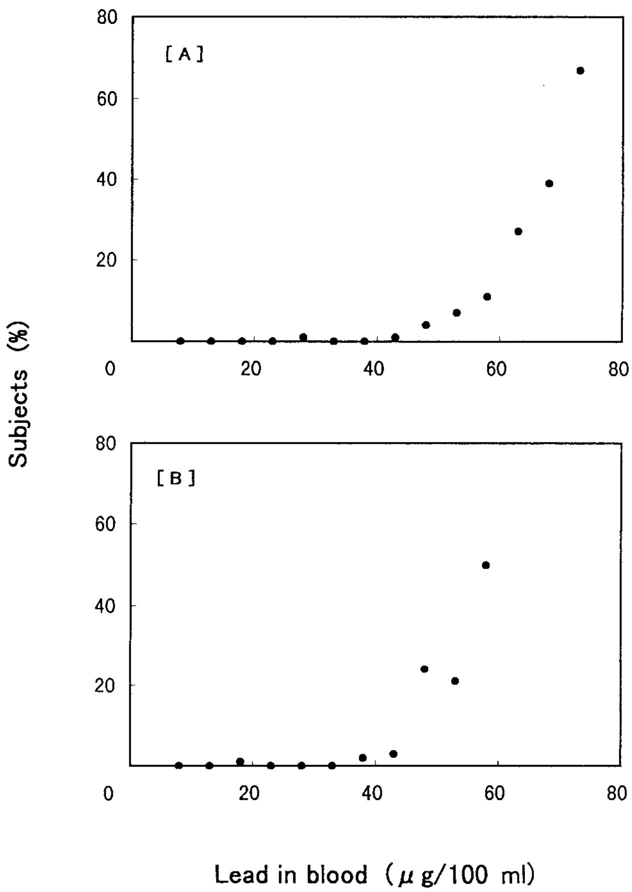

Fig. 2. Relationship between Pb-B (μg/100 ml) and the proportion (percentage) of those with >10 mg/l ALA-U; [A] in men, and [B] in women.

The 3rd degree regression curve calculated is;

$Y = -13.02 + 1.8997X - 0.0745X^2 + 0.0009X^3$ (n=14, *r*=0.994, *P*<0.01) for men, and

$Y = -9.06 + 1.6207X - 0.0806X^2 + 0.0012X^3$ (n=11, *r*=0.970, *P*<0.01) for women.

with >7[9] and >5 mg/l[6, 7] ALA-U. When the results were evaluated together, it was found that the proportions of those with ALA-U greater than 5 to 10 mg/l will increase when Pb-B is 20–40 μg/100 ml in men and 25–30 μg/100 ml in women.

Discussion

More than 10,000 pairs of Pb-B and ALA-U for men and women with occupational exposure to inorganic Pb were subjected to statistical analysis in the present study. It was made clear that ALA-U was elevated when Pb-B was in excess of 22, 29 or 23 μg/100 ml in men, women and men+women, respectively (Table 3). It was also shown that ALA-U was higher than the 95% upper normal limit of 8 mg/l when Pb-B was in excess of 62, 50 or 58 μg/100 ml in the order, and that the Pb-B to induce a substantial increase in ALA-U such as in excess of the 95% upper normal limit was lower in women than in men (Table 3), in agreement with the early observation by Roels *et al.*[11]. The former values of 22 to 29 μg/100 ml as the maximum Pb-B with no elevation in ALA-U may suggest that 30 μg/100 ml Pb-B[3] rather than 70 μg/100 ml[5] is more appropriate as the Pb-B level with no effect on heme metabolism.

The 95% upper normal limit for ALA-U of 8 mg/l (Table 3) observed in the present study is between the two proposed biological exposure limit values of 5[4] and 15 mg/l[5]. Cautions may be necessary with this 95% upper normal limit, however, because of at least two reasons that ALA-U was measured colorimetrically and that urine samples were not from control populations (i.e., those with no occupational exposure to Pb). Regarding the limitation of colorimetry for ALA-U, Tomokuni *et al.*[18] observed that colorimetry tended to give two or more times higher values than fluorometric HPLC especially when ALA-U by the latter was low, e.g. 1 mg/l, although the two values agreed well with each other when ALA-U was >5 mg/l. Very similar observation was also made by Tabuchi *et al.*[10]. Thus, the GM values (2.8 mg/l; Table 3) if measured by fluorometric HPLC might be lower than observed, even though the 95% upper limit may be less affected. With regard to the latter limitation, it should be noted that the ALA-U normal range was calculated from the cases whose Pb-B values were lower than 22 (men) or 29 μg/100 ml (women), after the confirmation that Pb-B below this level did not induce ALA-U elevation.

Trials to estimate the threshold Pb-B levels at which elevation in ALA-U should be induced has been initiated as early as 1970s. Thus, for example, Zielhuis[7] summarized the work of Selander and Cramer[20] on 150 workers (assumedly men) and that of Haeger-Aronsen[21] on 111 men in a figure to show that the proportion of those with >5 or >10 mg/l ALA-U will increase when Pb-B is >25 or >34 μg/100 ml. Similarly, Wada *et al.*[6] observed in their study on 123 subjects (44 occupationally exposed and 79 non-exposed; assumedly men) that the normal upper limit of 5 mg/l ALA-U corresponded approximately 35 μg/100 ml Pb-B. Alessio *et al.*[9] analyzed the relation of ALA-U with Pb-B among 316 men (a combination of non-exposed subjects and Pb-exposed workers[8]) and found that ALA-U increased above the normal upper limit (7 mg/l as AM+2ASD with 4 mg/l as AM) when Pb-B exceeded 48 μg/100 ml.

Roels *et al.*[11] observed that ALA-U was above 4 mg/l in 5% and 10% of Pb-exposed men and women, respectively, when Pb-B was 20 to <30 μg/100 ml. The population in this Pb-B category was however only 27 men and 10 women, unfortunately. In a larger scale study with 221 men [a combination of exposed (about two thirds) and non-exposed subjects (one third)], Ohmori *et al.*[12, 13] reported that the Pb-B range of 20–29 μg/100 ml was the lowest category in which the elevation in ALA-U was observed; one subject out of 31 people in the category had elevated ALA-U (≥6 mg/l) and the proportion increased up to 100% dependent to the Pb-B level of ≤80 μg/100 ml. Tabuchi *et al.*[10] examined Pb-B, ALA-U and other Pb exposure parameters in 199 men occupationally exposed to Pb, and found that an ALA-U increase over the control level was statistically significant among the group with 11–20 μg/100 ml Pb-B (average: 16.2 μg/100 ml) when ALA-U was measured by an HPLC method, whereas it was among the 21–30 μg/100 ml Pb-B group (average: 24.5 μg/100 ml) when a colorimetric method was employed. The results of the study on 62 men by Yoshikawa and Nagamura[14] suggest that Pb-B dose-dependent increase in ALA-U (although probably within a normal range) will start from 15 to 19 μg/100 ml Pb-B on. In contrast, Odachi *et al.*[22] observed that, when 288 Pb-exposed workers were classified by the 10 μg/100 ml Pb-B range and regression lines were calculated for those in each Pb-B range, slope was >0.1 [unit; (mg/l ALA-U)/(μg/100 ml Pb-B)] for those with 30–39 μg/100 ml Pb-B and above, whereas it was <0.06 for those with lower Pb-B; the data may suggest that the increase in ALA-U takes place when Pb-B is in the range of 30–39 μg/100 ml.

In order to compare the present observation with values reported in literature, the comparability of the methods employed is a basic issue at least theoretically. In practice, atomic absorption spectrometry or other compatible method was used for Pb-B analysis in all reports cited, and colorimetry was used in common for ALA-U determination (thus the problems associated with ALA-U colorimetry[18] as discussed above should also be shared by all cited reports except for that of Tabuchi *et al.*[10] who used an HPLC method in addition). A major problem is however the fact that different ALA-U levels were selected by various authors to identify the threshold Pb-B level, i.e., from 4[11], 5[6, 7], 6[13], 7[9] to 10 mg/l[7], or 5 mg/g creatinine[23] together with 8 mg/l as employed in the present study (Table 3). Thus, it is not easy to compare the present results with the values reported in literature.

Nevertheless, 22 (for men) to 29 (for women) μg/100 ml Pb-B (Table 3) as the threshold Pb-B to increase ALA-U is in agreement of Roels *et al.*[11] and Ohmori *et al.*[13], although somewhat higher than the levels reported by Yoshikawa and Nagamura[14] and Tabuchi *et al.*[10]. From the viewpoint of Pb-B corresponding to ALA-U at the upper normal limit, the present observation suggests the Pb-B levels of 50 (women) to 62 (men) μg/100 ml (Table 3). The observation (e.g., Fig. 2) that the proportion of those with ALA-U greater than 5 or 10 mg/l will increase when Pb-B exceeds 20–40 μg/100 ml in men and 25–30 μg/100 ml in women is essentially in agreement with Wada *et al.*[6] (35 μg/100 ml Pb-B) and Zielhuis[7] (25–34 μg/100 ml), although it was somewhat lower than the level proposed by Alessio *et al.* (48 μg/100 ml)[9].

The selection of the type of regression equation has been made not theoretically but empirically. Whereas the most simple assumption would be a linear regression between Pb-B and ALA-U[6, 22, 24, 25], a juxta-linear relation was also reported[26]. The linear regression assumption is however apparently inadequate, because it is known through experiences that ALA-U stays unchanged when Pb-B is low, whereas the increase in ALA-U is substantial when Pb-B is above a certain level (e.g., Fig. 1).

Thus, several research groups[9, 22, 27, 28] assumed a linearity between Pb-B (as observed) and Log_{10}[ALA-U] (i.e., a semi-logarithmic approach), and double logarithmic assumption was also made[10]. Whereas the correlation coefficients among 316 pairs of the data analyzed was as high as 0.622 in one report[9], a draw-back is that the semi-logarithmic approach does not allow estimation of the threshold Pb-B at which ALA-U would increase. It should also be noted that the correlation coefficients following this approach were rather small (i.e., <0.25) in the present study, possibly because both Pb-B and ALA-U (and therefore log_{10}[ALA-U]) were low in majorities of men and women (Table 1 and Fig. 1).

As stated above, the present choice of the 3rd degree regression is empirical. Nevertheless, an advantage and also a practical importance of the application of the 3rd degree regression is that the local minimum (the point where the ALA-U turns to upward in association with the increase of Pb-B, in other words, the threshold Pb-B) can be readily calculated as the point where $dy/dx=0$, as practiced in the present study (see the Results section for an example). A theoretical weak point inherent to the assumption of the 3rd degree regression is that ALA-U should decrease as Pb-B increases within the Pb-B range of the local maximum and the local minimum as far as the coefficient to X^3 is >0 (for the equation to calculate the local maximum and the local minimum, see the Results section), the decrease which would not take place biologically. Practical application however showed that the decrease in ALA-U from the local maximum to the local minimum was very small and essentially negligible. Thus, it is expectable that the 3rd degree regression analysis can be applied to experimental or epidemiological data as a tool to find a threshold value in occupational or environmental research.

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