The Relationship between Lead in Plasma and Whole Blood in Women

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Studies have suggested that plasma lead levels may better reflect the toxicologically labile fraction of circulatory Pb that is more freely available for exchange with target tissues than do Pb levels in whole blood. Studies have also reported an apparent severalfold variation in the relative partitioning of Pb between whole blood and plasma (or serum) for a given whole-blood Pb level. This may reflect inherent differences in the plasma Pb/whole blood Pb partitioning among individuals and/or methodologic challenges associated with the collection and analyses of samples that generally contain < 1–2 ng total Pb. Here, we conducted a longitudinal assessment of the relationship between Pb in whole blood and plasma in environmentally exposed reproductive-age women (n = 63) living in Mexico City, Mexico. We collected whole blood and plasma samples using trace metal clean techniques and analyzed them for Pb using high-resolution inductively coupled plasma mass spectrometry. A subset of subjects provided repeated blood samples weekly for 4 consecutive weeks (n = 17 subjects) or every 1–2 months over a 9-month period (n = 14 subjects). Plasma Pb concentration was significantly positively associated with whole-blood Pb in a curvilinear fashion over the range of blood Pb values observed here (2.13–39.7 μg/dL). This relationship was best described by the function Plasma Pb = $e^{(-2.392 + 0.0898 \times \text{blood Pb})}$, where $SE_{coefficient}$ = 0.0054, $SE_{constant}$ = 0.063 (n = 63 subjects, n = 141 observations). Results from the short- and long-term repeated collection subjects indicated that the within- and between-subject variance components were not significantly different between the two subsets of subjects. The between-subjects component accounts for 78% of the variance in plasma Pb levels, while the residual variance (22%) may be attributed to other unmeasured factors. Collectively, this study demonstrates that plasma Pb measurements may be applied to general clinical settings, provided that established trace metal clean techniques are adopted. This study also shows that the relative (%) partitioning of whole-blood Pb in plasma naturally varies by a factor of about 2–4-fold among subjects at a given blood Pb level. Because Pb in the plasma is considered to more closely represent the fraction of Pb in the circulation that is readily exchanged with peripheral target tissues (e.g., brain, kidney, skeleton), the routine assessment of plasma Pb may provide a more meaningful measure of toxicologically available Pb. *Key words*: blood, human, ICP-MS, lead, plasma. *Environ Health Perspect* 110:263–268 (2002). [Online 12 February 2002]
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Substantial progress has been made in reducing human lead exposures in the United States and elsewhere over the past two decades. However, many individuals continue to suffer elevated exposures from environmental and occupational sources, and possibly from the mobilization of skeletal body stores accumulated over previous periods of elevated exposures (*1–4*). The potential impact of mobilized skeletal Pb stores on the developing fetus in pregnant women has been of particular concern because laboratory and epidemiologic data have indicated that the developing nervous system is especially sensitive to perturbations with Pb (*5–7*).

Studies that have explored the associations between maternal body Pb burdens and cognitive outcomes in infants postpartum have generally relied on whole-blood Pb levels as the biomarker of maternal body Pb burden and exposure to the developing fetus. However, the use of blood Pb level as a biomarker of exposure has several recognized limitations, including uncertainty over the extent to which whole-blood Pb level reflects the toxicologically available fraction of circulatory Pb. Several investigators have proposed that plasma Pb levels may better reflect the fraction of circulatory Pb that is more freely available for exchange with tissues, including the developing fetus (*3,8–11*). This suggestion is consistent with the toxicokinetic characteristics of readily exchangeable Pb (*9,11,12*), and it has also been substantiated by recent data indicating that plasma Pb/whole-blood Pb ratios are more strongly associated with bone Pb levels than are whole-blood Pb levels (*3,13*). In light of these and other observations on plasma Pb levels, there is a clear need to investigate the role of plasma Pb levels versus whole-blood Pb as a predictor of toxicologic outcomes in humans.

Previous studies have reported an apparent severalfold variation in the relative partitioning of Pb between whole-blood and plasma (or serum) for a given whole-blood Pb level (*3,8,13–19*). Because plasma Pb levels appear to be typically < 0.5 ng/mL (i.e., < 0.5% of whole-blood Pb levels) in environmentally exposed humans, some of these discrepancies may be explained by methodologic difficulties associated with the collection and analyses of samples that generally contain < 1–2 ng total Pb (*10*). These analytical difficulties may introduce variation and bias, which may confound the assessment of inter- and intra-individual variation in plasma Pb levels that may be of toxicologic relevance.

Therefore, we have conducted a longitudinal assessment of the relationship between Pb in whole blood and plasma in environmentally exposed, reproductive age, nonpregnant women living in Mexico City, Mexico. This study was conducted as part of a larger cohort study that is assessing Pb kinetics during pregnancy and lactation and possible associations between maternal body Pb stores, including bone, blood, and plasma Pb levels, with adverse outcomes in infant offspring. These latter assessments are forthcoming and will be presented elsewhere.

Methods

Study subjects. We recruited 63 healthy females with no known history of occupational Pb exposure (mean age, 30.6 years ± 7.2 SD) into the study over a 3-year period (January 1996 through January 1999), using two strategies. One called for female volunteers who were willing to provide repeated blood samples over a particular duration. Using this strategy, we were able to recruit 31 volunteers; among them, 17 provided samples every week for 4 consecutive weeks, and 14 subjects provided samples every 1–2 months

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over a 9-month period. We recruited a second sample of 32 subjects from women who were participating in a cohort study to assess Pb kinetics during pregnancy and lactation. The inclusion criteria to participate in the latter study were not being pregnant at recruitment but intent to get pregnant during the following year, having no known history of occupational Pb exposure, and agreeing to participate in a longitudinal study involving pregnancy and lactation. Many of the subjects recruited in the second sample became pregnant after their initial recruitment, although the present study focuses solely on data collected from those subjects prior to their becoming pregnant. Based on previous studies with this population, the main risk factors for Pb exposure in all subjects were residence in Mexico City for more than 10 years (88%) and use of Pb-glazed ceramics (58%).

The research protocol was approved by the Human Subjects Committee of the National Institute of Public Health of Mexico; the University of California, Santa Cruz; and the Harvard University School of Public Health. All participants received informed written consent, including a detailed explanation of the study and procedures used, as well as counseling on ways to minimize their Pb exposure.

Blood and plasma collection. Trained medical staff performed all sample collections at the Center for Environmental Health Research of the American British Cordory Hospital, Mexico City, Mexico. Subjects were instructed beforehand to fast overnight prior to sample collection. Prior to venipuncture, each subject's arm was washed with ultrapure water and disinfected with reagent-grade alcohol. Venous blood was collected using a butterfly catheter (19 gauge) using methods detailed elsewhere (*3,10*). Briefly, an initial whole-blood sample of approximately 3 mL was collected into a low-Pb container (Vacutainer, B-D 367734; Becton-Dickinson, Franklin Lakes, NJ) for total Pb analysis. Subsequently, the catheter tubing was severed at a point distal to the venipuncture, and a second whole-blood sample of 13 mL for plasma separation was collected via gravity-fed phlebotomy (no vacuum) into a polyethylene tube containing 100 USP of sodium heparin (H-3393; Sigma Chemical Company, St. Louis, MO) and centrifuged at 800 × *g* for 10 min at room temperature (*10*). The 5–6 mL plasma fraction was then transferred using a polyethylene pipette into a polyethylene bottle and immediately frozen.

We collected procedural blanks for plasma collection and processing at the Center for Environmental Health Research over the entire 3-year sample collection period (n = 115 blanks), using methods identical to those used for the plasma samples. For this, 6–8 mL Milli-Q ultrapure water was added to the polyethylene centrifuge tube containing heparin and processed as a plasma sample. This procedure did not allow assessment of the butterfly catheters for Pb contamination, but previous work indicated those catheters contributed negligible amounts of contaminant Pb to the sample (*10*). All samples were shipped frozen via overnight carrier to the trace metal facility at the University of California, Santa Cruz.

All blood collections, plasma and whole-blood processing, and sample analyses were conducted under HEPA filtered-air trace metal clean conditions, and all sample collection and processing materials (Teflon, polyethylene, and polypropylene), except butterfly catheters and Vacutainers for whole blood, were acid cleaned using procedures detailed elsewhere (*10,20*). All reagents, including water and acids, were high-purity grade.

Sample analyses. We weighed 5–6 mL of plasma or a 0.5 mL aliquot of whole blood into a Teflon digestion vial and then evaporated and digested it for 8 hr in 3 mL of hot 16 N HNO_3; it was then evaporated to dryness and redissolved in 1 N HNO_3. We measured sample Pb and iron (plasma only) levels independently with a Finnegan Element ICP high-resolution mass spectrometer (ICP-MS; Thermo Finnigan, Bremen, Germany) in multi-isotope counting mode, with both internal and external standardization (*10,21,22*). We used National Institute of Standards and Technology (NIST; Gaithersbrug, MD) standard reference materials (SRM) 955 (blood) and 1577 (bovine liver) to evaluate analytical accuracy. This method yields a measurement precision of ≤ 0.5% RSD (relative standard deviation) for Pb concentrations of > 0.05 ng/mL (*10,21*). The analytical detection limit ranged from 0.001 to 0.01 ng/mL, depending on day of analysis.

Assessment of plasma contamination. We evaluated plasma sample contamination with Pb by determining the total Pb of procedural collection and processing blanks and separate sample analysis blanks measured over the 3-year duration of the study. Plasma hemoglobin and Fe levels were also measured in order to evaluate the potential contribution of hemolysis to plasma Pb levels. Previous work suggested that plasma Pb and total Fe levels may both become abnormally elevated with hemolysis (*10*), although more recent work in our laboratory has suggested that the wide range of normal plasma Fe levels (e.g., 0.5–2 µg/mL) may limit its use as an indicator of plasma Pb from moderate hemolysis. Therefore, we further evaluated the relative utility of plasma Fe and hemoglobin levels as indicators of Pb contamination from hemolysis. For this, we split a fresh plasma sample (15 mL) from a male volunteer into three subsamples and spiked with increasing amounts of hemolysate (e.g., 0–15 µL) from the separated packed cell volume, and determined the levels of Pb, Fe, and hemoglobin in those plasma samples.

We carried out hemoglobin analyses of plasma samples using an assay based on hemoglobin catalysis of the oxidation of 3,3′,5,5′-tetramethylbenzidine by H_2O_2 (527-A; Sigma). We centrifuged aliquots of plasma (0.5 mL) at 3,000 × *g* for 10 min and analyzed for hemoglobin following the manufacturer's instructions, using a Beckman DU-600 UV-VIS spectrometer (Beckman Instruments, Fullerton, CA). We evaluated the sensitivity, accuracy, and precision of analyses using certified calibration standards (527-11 and H3268, Sigma) and repeated analyses of samples. The hemoglobin assay detection limit was 2.0 mg/dL, with a sample measurement reproducibility of 6.6% RSD at sample concentrations of approximately 10 mg/dL.

Statistical analysis. Based on univariate and bivariate summary statistics and distribution plots for plasma and blood Pb levels, we $\log_e$ transformed plasma Pb levels to improve normality. We explored a number of models to assess the relationship between plasma Pb and blood Pb levels. These included the fit of $\log_e$-transformed and nontransformed plasma Pb versus linear and higher degree polynomials on whole-blood Pb using generalized estimating equations (*23*). This method took into account the intrasubject correlation structure due to the repeated measurements to avoid bias in the estimation of the standard errors. We repeated these analyses for the subsample of subjects whose blood Pb levels were ≤ 10 µg/dL. We chose the best-fit model using the minimum deviance criteria and the parsimonious principle and reanalyzed it with the inclusion of subject age and plasma hemoglobin and Fe levels as additional variables to evaluate their possible impact on plasma Pb levels. We restricted the validity of the best-fit model to the range of measured samples to avoid extrapolation outside the range of observations where the blood Pb–plasma Pb relationship may deviate from that predicted by the model. We performed statistical analyses using STATA (Stata Statistical Software, release 6.0; Stata Corporation College Station, TX).

Intersubject versus intrasubject variation in plasma Pb. We statistically evaluated between- and within-subject variations in blood and plasma Pb levels over both short-term (weeks) and long-term (months) durations for two reasons: first, to assess the extent that a single plasma and blood Pb measurement per subject predicted the "true" values for that subject, and second, to determine

whether intrasubject variation due to measured (e.g., contamination from hemolysis, sample processing, etc.) or unmeasured (e.g., diet, environmental exposures, etc.) factors would confound or preclude comparisons between subjects, or the assessment of temporal changes in plasma Pb levels over pregnancy and lactation, to be conducted in the larger cohort study. We conducted analyses using the variance components estimation of SAS, Version 8 (SAS Institute, Cary, NC).

Results

Table 1 shows summary statistics for the subjects and data. Median plasma Pb and blood Pb levels in this cohort were 0.188 ng/mL and 6.90 µg/dL, respectively, with a median relative plasma Pb of 0.29% of whole-blood Pb levels. We excluded a single sample from one subject from these data and all statistical analyses due to contamination of the plasma sample from hemolysis.

Plasma Pb concentrations were significantly positively associated with whole-blood Pb levels in a curvilinear fashion over the range of blood Pb values observed in our study population (2.13–39.7 µg/dL; Figure 1A). The best-fit model describing this relationship was an exponential function of the form Plasma Pb = $e^{(-2.392 + 0.0898 \times \text{blood Pb})}$, where $SE_{coefficient}$ = 0.0054 and $SE_{constant}$ = 0.063 ($p < 0.01$, n = 63 subjects, n = 141 observations). This model explained 71.8% of the variance in plasma Pb levels. Because the effect of subject age on blood and plasma Pb levels was not significant, we did not retain it in the model. We selected this best-fit model because it was the simplest model that proved equal to or superior to alternate polynomial models, based on model results and quantile–quantile normal plots of the model residuals.

To evaluate whether the blood Pb–plasma Pb relationship reported above was significantly influenced by the presence of the subjects with elevated blood Pb levels, we assessed a series of models for the subsample of subjects whose blood Pb levels were ≤ 10 µg/dL. As with the entire data set, the analysis of the quantile–quantile normal plots of the model residuals indicated that the same general exponential model selected above provided the best fit for this subset of subjects (n = 48 subjects, n = 104 observations): Plasma Pb = $e^{(-2.383 + 0.081 \times \text{blood Pb})}$, where $SE_{coefficient}$ = 0.018 and $SE_{constant}$ = 0.114 (p = 0.012; model explains 22% of the variance in plasma Pb levels; Figure 1B).

The relative partitioning of Pb in plasma (plasma Pb expressed as a percentage of whole-blood Pb) varied from 0.099% to 0.74% over the full range of blood Pb levels observed here. Interestingly, the data suggest a nonlinear U-shaped relationship between whole-blood Pb levels and percentage plasma Pb (Figure 2). The best-fit nonlinear regression to these data shows a significant negative slope relationship between whole-blood Pb and percent plasma Pb for subjects with blood Pb levels between about 2 and 10 µg/dL [$p < 0.001$; b = –0.0198 ± 0.0043 SE; 95% confidence interval (CI), –0.0283 to –0.0113], and a significant positive slope for this relationship in subjects with blood Pb levels > 10 µg/dL ($p < 0.001$; b = 0.011 ± 0.0023 SE; 95% CI, 0.0062–0.0159). However, this latter (positive) slope appears to be statistically unstable, because it is driven heavily by the two subjects with blood Pb levels > 25 µg/dL. If those two subjects are excluded, the slope of the regression for subjects with blood Pb levels > 10 µg/dL is not significantly different from zero (p = 0.216; b = 0.0066 ± 0.0053 SE; 95% CI, –0.0039 to 0.0171). All of the above regression models were adjusted for the repeated measurements structure of the data.

Subject variation in plasma Pb levels. Statistical analyses of data from the short- and long-term repeated collections (Figure 3) shows that the variance components (i.e., within and between subjects) were not significantly different in the short-term compared to the long-term subject data sets. Therefore, we pooled and reanalyzed data for both the short- and long-term repeat collections. Those results showed that the between-subjects component accounts for 78% of the variance in plasma Pb levels, whereas the residual variance of 22% may be attributed to other unmeasured factors. Further, analyses that included the variables plasma hemoglobin and Fe showed that these variables did not contribute significantly to the within- or between-subject variance in plasma Pb levels. By comparison, the between subjects and residual components account for 83% and 17% of the variance in whole-blood Pb levels, respectively.

We evaluated the effect of season on plasma and blood Pb levels using the combined repeated collection data set. For this, we coded the sample collection date as either rainy (June–September) or dry (October–May) season and analyzed in a univariate (plasma Pb) or bivariate (plasma and blood Pb) model. The effect of season on plasma Pb levels was marginally nonsignificant in the univariate model (p = 0.06), with plasma Pb levels tending to be slightly higher during the dry season (0.11 ng/mL; 95% CI,

Table 1. Descriptive summary values for the subjects, including plasma Pb, whole-blood Pb, and plasma hemoglobin and total Fe levels.

Category	Mean[a]	Median (blk corr)[b]	SD	Min	Max
All subjects (n = 63)					
Subject age (years)	30.6	30.0	7.20	17	51
Plasma Pb (ng/mL)	0.317	0.188 (0.183)	0.448	0.060	2.65
Blood Pb (µg/dL)	9.22	6.90 (6.90)	7.29	2.13	39.7
Plasma/whole-blood Pb ratio (%)	0.308	0.290 (0.285)	0.107	0.099	0.736
Plasma hemoglobin (mg/dL)	3.32	3.61	1.32	< DL	9.51
Plasma total Fe	0.937	0.971	0.236	0.400	1.34
Subset of subjects (blood Pb ≤ 10 µg/dL; n = 48)					
Plasma Pb (ng/mL)	0.165	0.151 (0.147)	0.064	0.060	0.290
Blood Pb (µg/dL)	5.94	5.67 (5.67)	2.13	2.13	10.1
Plasma/whole-blood Pb ratio (%)	0.291	0.281 (0.270)	0.087	0.099	0.480
Plasma hemoglobin (mg/dL)	3.20	3.42	1.30	< DL	6.89
Plasma total Fe	0.935	0.959	0.211	0.542	1.31

Abbreviations: DL, detection limit; Max, maximum; Min, minimum.
[a]Data calculated using a single value per subject; when multiple values per subject were available, those values were averaged into a single value for calculation in these summary data. [b]Plasma values corrected for the sample collection and processing Pb blank (mean = 0.024 ng Pb; see text for details).

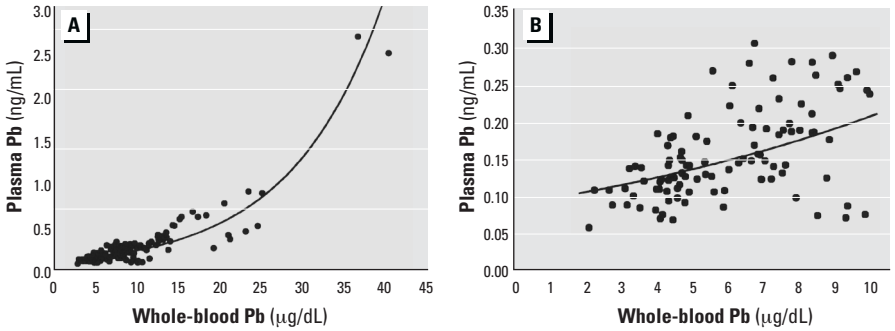

Figure 1. Whole-blood Pb versus plasma Pb concentrations (ng/mL) for (*A*) all subjects (n = 63 subjects; n = 141 observations) and (*B*) subjects with whole-blood Pb levels ≤ 10 µg/dL (n = 48 subjects; n = 104 observations). Curves indicate the best-fit models to the observations: (*A*) Plasma Pb = $e^{(-2.392 + 0.0898 \times \text{blood Pb})}$ and (*B*) Plasma Pb = $e^{(-2.383 + 0.081 \times \text{blood Pb})}$ (see text for details).

–0.005 to 0.23). However, this small effect was attenuated when we included blood Pb levels in the model ($p = 0.18$).

Assessment of plasma Pb contamination. The sample collection/processing Pb blanks measured here averaged 0.024 ng Pb (± 0.002 SE; median, 0.015 ng Pb; range, 0.003–0.091 ng Pb; $n = 115$). Sixty-five of the measured blanks were below the analytical detection limit, yielding calculated mean levels of 0.012 ng Pb ± 0.001 SE (median, 0.013 ng Pb; range, 0.003–0.022 ng Pb; blank values calculated using 0.5 detection limit for the respective analysis days). The remaining 50 blanks that were above the detection limit yielded mean levels of 0.040 ng Pb ± 0.003 SE (median, 0.034 ng Pb; range, 0.009–0.092 ng Pb). Although blank Pb levels were well characterized here, we did not perform quantitative correction of plasma Pb concentrations from external Pb contamination because there is no reliable method to determine the relative contribution of blank contaminant Pb to the plasma fraction of whole blood (*10*). Some fraction of blank Pb undoubtedly partitions with the separated plasma fraction, though the amount is likely small and may vary with the physiochemical nature of the Pb and other biologic and physical factors (*8*).

Nonetheless, we conducted an exercise in which we corrected the plasma sample Pb levels for the measured sample collection/processing blank to conservatively demonstrate the small impact that plasma sample contamination may have had on the values reported here. For this exercise, we subtracted the mean total procedural blank value (0.024 ng Pb) from the amount of plasma sample Pb (nanograms) using the formula

$$\text{Plasma [Pb]}_{\text{corrected}} = \{(\text{Plasma [Pb]}_{\text{uncorrected}} \times \text{plasma sample volume}) - \text{blank Pb}\} \div \text{plasma sample volume},$$

where the units for the [Pb], plasma sample volume, and blank Pb were nanograms per milliliter, milliliter, and nanograms Pb, respectively. The small difference between the corrected versus uncorrected plasma Pb levels (mean difference, –2.74% ± 0.14 SE; median, –2.46%; range, –0.16% to –8.95%) demonstrates that the low blanks measured here would in general have a small effect on the plasma Pb values (Table 1). Further, to ensure that the derived best-fit model describing the blood Pb–plasma Pb relationship (Figure 1) was not biased by uncorrected sample Pb contamination, we performed the best-fit model analyses using the blank corrected plasma Pb data. The model derived from this exercise was statistically indistinguishable from the best-fitted model using the uncorrected plasma Pb data reported above (data not shown).

We attributed the apparent plasma Pb contamination in a single sample from one subject to lysis of erythrocytes during sample collection and processing, based on a measured plasma hemoglobin level of 20 mg/dL. Otherwise, there was no discernable plasma contamination from hemolyzed erythrocytes in any of the remaining samples, as suggested by the normal range in measured plasma hemoglobin and total Fe levels (Table 1). The exercise where we intentionally spiked plasma samples from a male volunteer with hemolysate shows a clear relationship between elevated plasma hemoglobin and plasma Pb levels (Figure 4), thereby substantiating the exclusion of the above plasma sample from this study. Those results also suggest that plasma hemoglobin levels may be more diagnostic than plasma total Fe levels for the assessment of plasma contamination from hemolysis, based on the linear 11-fold increase in plasma hemoglobin levels in those samples, compared to the lower 2.4-fold increase in plasma total Fe levels.

In light of the above results, we explored whether variation in plasma hemoglobin levels could explain some of the variance in plasma Pb levels in the study subjects, under the assumption that mild hemolysis could contribute to increasing plasma Pb levels without discernibly increasing plasma hemoglobin levels. We conducted this analysis by including hemoglobin levels in the longitudinal models for the short-term and long-term repeat collection subjects. The results indicate that variation in hemoglobin levels do not measurably contribute to variation in plasma Pb levels ($p = 0.56$), further substantiating that hemolysis was an unimportant contributor to plasma Pb levels in the present study.

Discussion

Relationship between plasma Pb and whole-blood Pb. We observed a strong curvilinear relationship between plasma Pb and whole-blood Pb levels across the range of blood Pb

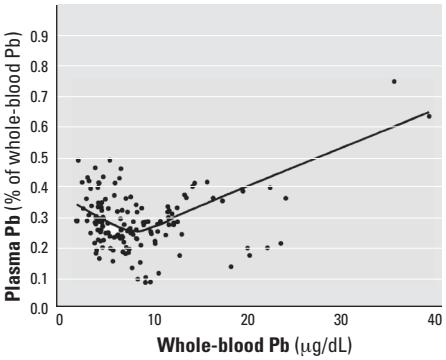

Figure 2. Whole-blood Pb versus plasma Pb expressed as a percentage of whole-blood Pb levels for all subjects ($n = 63$ subjects; $n = 141$ observations). The solid line indicates the best-fit regression with a Lowess smoothing. (See "Results" for regression parameters.)

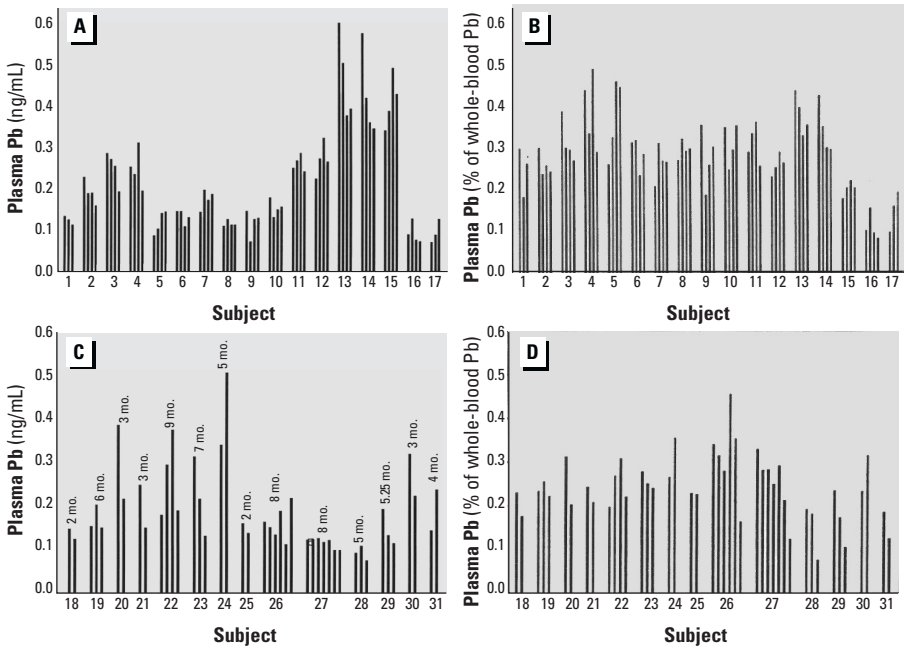

Figure 3. (*A*) Plasma Pb concentrations (ng/mL) and (*B*) corresponding plasma Pb expressed as the percent of whole-blood Pb in subjects sampled repeatedly over short-term durations ($n = 17$ subjects); samples within subjects were collected at weekly intervals over 3–4 consecutive weeks between April 1998 and January 1999. (*C*) Plasma Pb concentrations (ng/mL) and (*D*) corresponding plasma Pb expressed as the percent of whole-blood Pb in subjects sampled repeatedly over long-term durations ($n = 14$); samples within subjects were collected at 1–2 month (mo.) intervals. The numbers above each set of bars indicates the total interval over which all samples were collected for each subject.

levels encountered in these non-occupationally exposed women (2.13–39.7 µg/dL; Figure 1). These data indicate that "typical" plasma Pb levels in this population are approximately 0.29% (median) of whole-blood Pb levels and always < 0.5% of whole-blood Pb when the latter are < 25 µg/dL. However, the relative fraction of whole-blood Pb contained in the plasma varied greatly among subjects, ranging from 0.099% to 0.74% across all subjects and from 0.099% to 0.48% for subjects with blood Pb levels ≤ 25 µg/dL (Table 1, Figure 2). This suggests that the fraction of Pb partitioning in plasma may serve as a superior alternative to whole-blood Pb levels for assessing the toxicodynamics of Pb (*11*).

The relative partitioning of Pb in plasma (percentage plasma Pb) increases with decreasing blood Pb levels of 10 µg/dL or lower, based on the significant negative regression slope for blood Pb levels around 2–10 µg/dL (Figure 2). This interesting relationship, which has not been reported previously, supports that Pb partitioning between plasma and nonplasma components is composed of a relatively high affinity but very low capacity specific binding component in the plasma, and a relatively lower affinity but high capacity binding component in the nonplasma fraction (e.g., erythrocytes). This conceptual competitive binding model is consistent with the relatively shallow slope of the blood Pb–plasma Pb concentration regression at low blood Pb levels (Figure 1). It also suggests that the partitioning of Pb in the nonplasma fraction may serve a somewhat protective role in terms of Pb availability to tissues outside the circulation, by limiting the relative amount of Pb in the plasma fraction as blood Pb levels increase toward 10 µg/dL. We do not believe that this observation is due to disproportionately higher blank Pb contamination at the lower plasma Pb levels because the estimated plasma Pb blank correction (~0.2 ng Pb) needed to eliminate the statistically significant negative slope is nearly eight times the actual mean sample collection/processing Pb blank measured here (0.024 ng Pb).

The best-fit model to the blood Pb versus plasma Pb concentration data does not predict a plasma Pb level of zero at a blood Pb level of zero (Figure 1). This further suggests that the relationship between blood and plasma Pb levels is different at very low blood Pb levels than that observed over the range of blood Pb levels reported here. Both Bergdahl et al. (*24*) and Hernandez-Avila et al. (*3*) similarly suggested that the relationship between blood Pb versus plasma Pb derived from occupationally and environmentally exposed subjects may not extrapolate to lower blood Pb levels. Bergdahl et al. (*24*) also suggested that the presence of a systematic error in their measurement of plasma Pb levels may account for their reported regression intercept of 0.2 ng Pb/mL plasma at a blood Pb of zero. It is unlikely that the presence of a systematic error significantly contributed to our observations because we would need a plasma sample analytical bias on the order of 0.50 ng Pb here to account for the plasma Pb intercept of approximately 0.09 ng/mL at a blood Pb of zero (given an average plasma volume for analysis of 5.7 mL ± 0.09 SE). This value is more than 20 times higher than the mean total procedural Pb blank in this study. Moreover, previous studies in our laboratory intercalibrating the ICP-MS method used here with isotope dilution thermal ionization mass spectrometry (TIMS) have not suggested the presence of a systematic bias for low Pb concentration measurements (*21,22,25*).

Comparisons with previous studies. Several studies have investigated the relationship between plasma (or serum) Pb and whole-blood Pb levels in environmentally and occupationally exposed subjects. For example, we previously reported plasma Pb levels in a small (n = 26) cohort of adult subjects with no history of occupational Pb exposure, using sample collection and analysis methods identical to those reported here. That cohort exhibited slightly higher median plasma Pb (0.27 ng/mL) and blood Pb (7.34 µg/dL) levels and a very similar curvilinear relationship between plasma Pb and blood Pb levels over the range of reported blood Pb values (2.3–42 µg/dL). Generally similar curvilinear relationships between plasma (or serum) Pb and whole-blood Pb levels have also been reported by deSilva (*14*), Manton and Cook (*8*), Schutz et al. (*19*), and Bergdahl et al. (*24*), which collectively substantiate the relative increase in the partitioning of whole-blood Pb into plasma as blood Pb levels increase above 25 µg/dL. However, further direct comparison with those latter studies is difficult in most if not all cases because the researchers used different methods of sample collection and analysis, including varying degrees of sample contamination control, as well as study populations that vary widely in size and Pb exposure history, among other factors. Further, while plasma and serum samples are considered interchangeable regarding their relative Pb content, this has not been systematically validated within a sufficient number of subjects.

Subject variation in plasma Pb levels. This study was unique in that we performed repeated plasma and blood Pb collections from a subset of subjects, in order to better evaluate variation in plasma Pb levels both within and between subjects (Figure 3). A primary benefit of this exercise was its demonstration that the within-subject variance in plasma Pb levels was small relative to the between-subject variance, regardless of whether the repeat collections occurred over weeks or months. This substantiates that the plasma and whole-blood Pb measurements were highly reproducible within a subject and that a single plasma or whole-blood Pb measurement is a relatively good predictor of those values for an individual when compared to the variance in those values across the study population.

The residual variance in plasma Pb levels (22%) that could not be explained by the between-subjects component (78% variance explained) may be attributed to other factors that may affect plasma Pb levels. These may include unmeasured temporal differences in diet and Pb exposure, different bone Pb burdens, slight differences in sample collection and processing, and so forth. However, we cannot attribute it to variation in subject age, season of sample collection, or whole-blood hemolysis during collection because those factors did not contribute significantly to the variance in plasma Pb levels when we included them in the statistical models.

Implications for Pb toxicodynamics and widespread application of plasma Pb measurements. As we noted above, these data demonstrate that the relative (%) partitioning of whole-blood Pb in plasma naturally varies

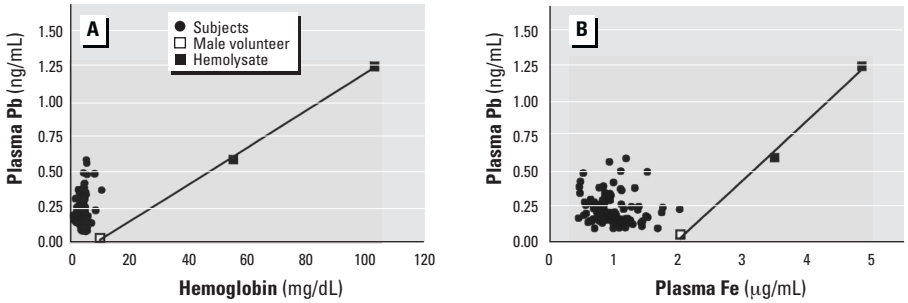

Figure 4. Relationship between (*A*) plasma hemoglobin and plasma Pb and between (*B*) total plasma Fe and plasma Pb levels in subjects and in a male volunteer whose collected plasma was spiked with increasing amounts of hemolysate from packed cells following whole-blood centrifugation. The solid line is the best-fit linear regression for the male volunteer plasma samples only: (*A*) slope = 0.0133, y-intercept = –0.108, r = 0.9995; (*B*) slope = 0.0427, y-intercept = –0.854, r = 0.9977.

between subjects by a factor of about 2- to 4-fold at a given blood Pb level (Figure 2). Because Pb in the plasma is considered to more closely represent the fraction of Pb in the circulation that is readily exchanged with peripheral target tissues (e.g., brain, kidney, skeleton) (*3,11,12,26*), the routine assessment of plasma Pb may provide a more meaningful measure of toxicologically available Pb. This has been suggested previously (*11*) and also has been substantiated by studies showing a stronger statistical association between bone Pb levels and plasma Pb/whole blood Pb ratios, compared to bone Pb and blood Pb levels (*3,13*). In light of this fact, the routine assessment of plasma Pb levels should be considered a marker of bioavailable Pb in clinical and laboratory studies of Pb toxicity and toxicodynamics. Those measurements would complement blood Pb level measurements because the latter provide a relatively longer time-integrated measure of Pb exposure from exogenous and endogenous sources (*3,11,27–29*).

This study also substantiates the potential utility of plasma Pb measurements in our ongoing investigations of bone Pb toxicodynamics in response to increased physiologic stressors on bone metabolism, such as pregnancy, lactation, and nutritional deficiency. As we noted above, initial studies have shown a significant relationship between bone Pb levels and the plasma Pb/whole blood Pb ratio (*3*). However, this observation may also reflect inherent differences in the partitioning of Pb between plasma and whole blood and its effect on bone Pb levels, rather than a specific mobilization of bone Pb into plasma per se. Consistent with this, a recent study by Gulson et al. (*30*) reporting stable Pb isotopic compositions in blood and spot urine samples collected from women over the course of pregnancy suggested that Pb released from the skeleton was not preferentially partitioned into plasma. That study was limited in that it used urine Pb as a surrogate of plasma Pb, and the isotopic composition of the labile fraction of skeletal Pb was not well known in those subjects. Thus, additional research using direct and repeated measurements of plasma Pb over the course of pregnancy would be desirable. Nonetheless, it remains apparent that even if plasma Pb/whole blood Pb ratios did not change over the course of pregnancy, measurements of plasma Pb levels at least one time during pregnancy may provide a measure of Pb exposure to the developing fetus that is more predictive of toxicity than whole-blood Pb levels. To address this, studies now under way will specifically explore the association between maternal plasma Pb levels over pregnancy and neurobehavioral outcomes in infant offspring.

The plasma sample collection procedures we used here may be applied to general clinical settings, provided that well-established trace metal clean techniques are adopted in sample collection and analyses. These procedures were developed in previous methodologic studies (*3,10*) which indicated that the sample collection methods used here were robust and not susceptible to errors associated with sample collection. This study also demonstrates that the ICP-MS method used here possesses the sensitivity for Pb measurements necessary for quantitation of Pb in plasma of environmentally exposed subjects. The wide availability of ICP-MS instrumentation, in contrast to more sophisticated mass spectrometers such as multicollector ICP-MS or TIMS, should favor more routine assessments of plasma Pb levels within clinical settings.

Future studies. Future assessment of the blood Pb versus plasma Pb relationship at blood Pb levels approaching zero may be of fundamental interest because the lowest blood Pb level reported here (2.13 μg/dL) and average current adult blood Pb levels in the United States (~2 μg/dL) (*1*) are still > 100-fold higher than estimated blood Pb levels in preindustrial humans (~0.016 μg/dL) (*31,32*). This may be facilitated using specially reared animal models to assess the relationship between plasma Pb and blood Pb across ranges of blood Pb levels that more closely approximate preindustrial levels. The feasibility of these studies has already been shown (*20*).

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