Serum Vitamin D Metabolites and Bone Mineralization in Young Children With Chronic Low to Moderate Lead Exposure

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ABSTRACT. One hundred five children (49 male, 99 black) with known lead exposure indices from birth and adequate nutrient intake of calcium, phosphorus, and vitamin D were studied at 1 of 3 ages (21, 27, or 33 months) to determine the effects of chronic low to moderate lead exposure on circulating concentrations of vitamin D metabolites and bone mineral content as determined by photon absorptiometry. Univariate multiple regression analyses showed no direct relationship of blood lead levels to vitamin D metabolites or bone mineral content. Structural equation analyses which took into account potential covariates of age, season, race, and sex showed estimated declines in serum concentrations of total calcium (from 9.72 to 9.61 mg/dL), phosphorus (from 5.4 to 4.67 mg/dL), and 25-hydroxyvitamin D (from 27.24 to 25.8 ng/mL) and estimated increases in concentrations of parathyroid hormones (from 73.03 to 83.14 μL Eq/mL), 1,25-dihydroxyvitamin D (from 62.39 to 62.69 pg/mL), and bone mineral content (from 222.66 to 234.91 mg/cm) over the observed range of average lifetime blood lead concentrations (4.76 to 23.61 μg/dL, geometric mean 9.74 μg/dL). However, the only statistically significant effect of average lifetime blood lead concentration was that for phosphorus, and the multivariate test of the combined effects of lead on these six outcomes was not statistically significant ($P = .2$). It is concluded that significant alterations in vitamin D metabolism, calcium and phosphorus homeostasis, and bone mineral content are not present in children whose nutritional status is adequate and who experience low to moderate lead exposure. *Pediatrics* 1991;87:680–687; *lead, 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, bone mineralization, phosphorus.*

ABBREVIATIONS. 25(OH)D, 25-hydroxyvitamin D; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; BMC, bone mineral content; PTH, parathyroid hormone; CT, calcitonin; PbB, average lifetime blood lead concentration.

Lead exposure in children is widespread and may result in multisystem toxicity even at low levels of exposure.[1,2] Disturbances in vitamin D metabolism have been reported in animals[3,4] and children[5–7] with increased lead exposure. Most of the children described in the latter two studies had moderate (>30 μg/dL) to markedly (>60 μg/dL) elevated blood lead concentrations. Some of the subjects had overt clinical lead toxicity coupled with evidence of deficient calcium and vitamin D intake. Although vitamin D metabolites are important for calcium and phosphorus homeostasis and bone mineralization,[8,9] and bone is a major site of lead accumulation,[10] there is no information about whether bone mineralization is affected directly by lead exposure.

As part of an ongoing program in the assessment of the biologic effects of lead exposure,[11] this study was designed to better determine the effect of chronic low to moderate lead exposure on vitamin D metabolism and bone mineralization in a well-defined cohort of young children with known lead exposure history and adequate nutrition from birth. We tested the hypothesis that chronic low to moderate lead exposure would decrease serum concen-

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trations of 25-hydroxyvitamin D [25(OH)D] and 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] and bone mineral content (BMC) as determined by photon absorptiometry, either directly, or indirectly by affecting the circulating concentrations of calcium, phosphorus, magnesium, calcitonin, or parathyroid hormone (PTH).

PATIENTS AND METHODS

The subjects in this study were a subset of a much larger population living in an inner-city area of Cincinnati, at risk of increased lead exposure, and enrolled in the Cincinnati Lead Program Project. One hundred five children (49 males, 99 black) with known lead exposure indices from birth were enrolled into this study. The children were studied at 1 of 3 ages (21, 27, or 33 months ± 2 weeks) during a regularly scheduled lead study clinic visit.

Dietary intake of calcium, phosphorus, and vitamin D was calculated from a food frequency questionnaire[12] administered under the direct supervision of the research dietitian (S.K.-W.). Calcium, phosphorus, and vitamin D contents of the dietary items were based on published data.[13] All subjects were examined by one investigator (O.G.B.) and anthroprometric measurements were performed by the same research assistants.

Blood samples (7 mL) were taken for the measurement of ionized calcium and serum total calcium, magnesium, phosphorus, 25 (OH)D, 1,25$(OH)_2$D, immunoreactive PTH, and immunoreactive calcitonin (CT). Serum total calcium and magnesium were measured by atomic absorption spectrophotometry; ionized calcium was measured by an ion-selective electrode (radiometer); and phosphorus was measured by the phosphomolybdate method. The respective coefficients of variation for these assays have been discussed elsewhere.[14,15] Normal ranges (mean ± SD or range) in healthy infants and young children in our laboratory are 9.7 ± 0.5 mg/dL for serum total calcium, 2.2 ± 0.2 mg/dL for serum magnesium, 5.0 ± 0.2 mg/dL for ionized calcium, and 3 to 7 mg/dL for serum phosphorus.

Serum 25(OH)D was measured by a competitive protein binding assay (rat serum as binding protein) following preparative chromatography with silicic acid.[16] The intrassay and interassay coefficients of variation are 10% and 13%, respectively. Serum 1,25$(OH)_2$D was measured by a competitive protein binding assay using chick duodenal cytosol as binding protein after extraction; purification steps included high-pressure liquid chromatography.[17] Overall recovery of 1,25$(OH)_2$D is 68 ± 1.2% (mean ± SEM). The assay is highly sensitive for 1,25$(OH)_2D_3$ and 1,25$(OH)_2D_2$ at concentrations above 10 pg/mL. Interassay coefficient of variation is 11%. In our laboratory, the values (mean ± SD) for healthy infants younger than 18 months of age are 45 ± 16 ng/mL for 25(OH)D; and 63 ± 25 pg/mL for 1,25$(OH)_2$D.

Immunoreactive PTH was measured by guinea pig antibody and human PTH standards.[18] Our antiserum measures the whole PTH molecule (1–84) with no cross-reactivity with the 65–84 fragment or the 1–34 fragment. The minimum detectable concentration is 20 μLEq/mL with measurable levels of serum PTH found in 89% of normal adults. The intraassay and interassay coefficients of variation are 8.4% and 16.3%, respectively. The range for normal adults is 33 to 117 μLEq/mL. Serum immunoreactive CT was measured by goat antiserum to human CT.[19] The antibody recognizes the 32 amino acid CT monomer but not the 11–32 fragment and shows no cross-reactivity with glucagon or secretion. "Nonspecific binding" is reduced by using column purified ^{125}I human CT and a double antibody separation method. The lower limit of detection of our assay is 10 pg/mL, with measurable levels of serum CT found in 84% of normal adults. The intraassay and interassay coefficients of variation are 6% and 15%, respectively. The normal adult range is 10 to 107 pg/mL, and similar values have been noted in the limited data available for infants and young children.

All serum samples were stored at −70°C until measurement in batches. Blood lead levels were determined from freshly collected whole blood samples. The technicians were unaware of the lead exposure indices of the children.

Lead in whole blood was measured by anodic stripping voltametry using an ESA model 3010A trace mental analyzer (Environmental Sciences Associates, Burlington, MA).[20] The instrument was calibrated at the beginning, middle, and end of each run with human blood standards whose lead content was determined by isotopic dilution mass spectrometry. Benchtop quality-control samples and pooled human whole blood "controls" with known lead concentrations as determined by isotopic dilution mass spectrometry were used. In addition, our laboratory participates in both the Centers for Disease Control and Pennsylvania State Blood Lead and Protoporphyrin Proficiency Programs. All samples were analyzed in duplicate. The intrassay and interassay coefficients of variation at the lead levels found in this cohort are <8%. The lead exposure index used in this study is the average lifetime blood lead concentration (PbB), calculated from quarterly blood lead level determinations obtained from birth to the age of the child at the time

of study. This index was used because it is a better measure of total cumulative long-term exposure to lead than any of the other characterizations of blood lead level that we considered (eg, concurrent, 3 month prior, or maximum blood lead). It is also correlated with the outcomes used in this study at least as well as any of these other blood lead level characterizations. The average lifetime blood lead correlated between .77 and .85 with these other possible characterizations of lead in blood.

Bone mineral content and bone width were measured by direct single photon absorptiometry[21] using Norland Cameron bone mineral analyzer model 278 (Norland Corp, Fort Atkinson, WI) modified by the manufacturer for use in pediatric populations. Precision in the measurement of bone "phantoms" during the study period for BMC was <3.5%; for bone width it was <2%.

The left radius was measured at 21, 27, and 33 months. The measurement site for BMC and bone width was standardized to a position equal to the junction of distal ⅓ and proximal ⅔ of the distance from bony prominence at the tip of the olecranon to the tip of the ulna styloid of left forearm, because the length of the ulna can be measured more precisely than that of the radius. The ulna length was measured with a metal tape. The child's forearm was held in a Lucite holding device, surrounded with a segment of water-filled dialysis tubing, and compressed with a Lucite plate. The deionized water-filled bag ensures a constant soft-tissue equivalent material around the bone. A series of four to six determinations of BMC and bone width were done at each site and average value was used.

This study was approved by the Institutional Review Board on Human Investigations. Written informed consent was obtained by a parent of each child admitted to the study.

STATISTICAL METHODS

To analyze the myriad of possible interactions among the dietary intakes, PbB, and the outcome measures of circulating hormones, vitamin D metabolites, and bone indices, the method of structural equations was used. Structural equation analysis is a multivariate method that allows prespecification of a theoretical model for the data and does not require that each of the outcomes of interest be merely correlated, ie, measures that serve as dependent variables in one structural equation may serve as predictors or independent variables in another. The structural model also may be characterized as one in which a set of multivariate outcomes may each have different precursors, thus overcoming the limitation presented by other multivariate procedures. These unique characteristics of the structural equation model provide for variables acting directly, as specified by a single regression-like structural equation, and also indirectly, through two or more structural equation "pathways."

A preliminary set of structural equation analyses were performed using the SAS PROC SYSLIN procedure.[22] Based on a theoretical model (Fig. 1) for the circulating hormones and vitamin D metabolites and bone indices, we controlled in these analyses for the potential confounders of child's age, race, and sex; season of blood sampling; and the dietary intake of calcium, phosphorus, and vitamin D. These variables served as the independent or "exogenous" variables in each of the structural equations for the dependent or "endogenous" variables shown in Fig 1. In addition, an arrow from an endogenous variable indicates that this measure also served as a potential predictor of the dependent variables to which the arrow points. A backward elimination of insignificant structural paths was performed. Confounders and outcome measurements which proved to be unrelated to either lead or any other variables in the theoretical model were not pursued in further analyses. A final structural model was estimated using the LISREL program.[23] Based on this final structural model, in which PbB was allowed to freely affect each of the remaining circulating hormones, vitamin D metabolites, and bone indices, and a model fit with each of PbB's

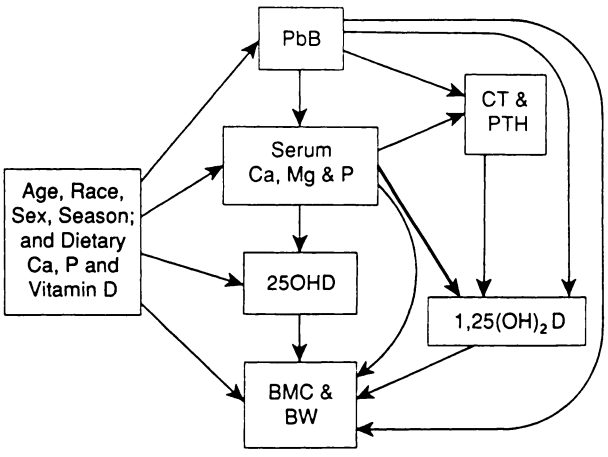

Fig 1. Theoretical structural equation model including all variables proposed for analyses of effects of lead exposure. Arrows indicate direction of potential influence. Abbreviations: Season represents two trigonometric functions of date (see text); PbB represents the average lifetime blood lead concentration. Serum concentrations of Ca, total and ionized calcium; Mg, magnesium; P, phosphorus; 25OHD, 25-hydroxyvitamin D; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; CT, calcitonin; PTH, parathyroid hormone; BMC, bone mineral content; BW, bone width.

effects removed, a multivariate test for the overall effect of lead on these outcomes was calculated as the difference in the two goodness-of-fit χ^2 statistics.

The season of blood sampling was characterized by using two trigonometric functions, the sine and cosine, of the calander date. Statistical tests for seasonal trends were performed by pooling the effects of these two exogenous variables. The natural logarithm of all concentration measures was used to ensure normal distributions of these measured variables. The square root of bone width and BMC was used to obtain approximate normality of the statistical distributions of these variables. Statistical analyses were performed on the University of Cincinnati Computer Centre AMDAHL 5880 mainfram computer. A P value of .05 was used to judge significance of an estimated structural parameter in all statistical tests. Only children with complete PbB, dietary, demographic, serum, and bone indices were used in each of the analyses. Means, standard deviations, and simple Pearson correlations were also calculated as descriptive statistics. Univariate regression analyses were also performed to permit comparison with the results demonstrated for the LISREL model.

RESULTS

The reported daily intake of calcium was in the range of 600 through 1200 mg in 58 subjects, ≤600 mg in 4 subjects, and >1200 mg in 43 subjects: daily intakes of phosphorus over the same ranges were found in 39, 1, and 65 subjects, respectively. Daily intake of vitamin D was in the range of 301 through 600 IU in 54 subjects, ≤300 IU in 47 subjects, and >600 IU in 4 subjects. Major sources of calcium, phosphorus, and vitamin D were the dairy products supplied by the Ohio Women, Infant and Children Program to 74% of the study cohort.

Blood samples were obtained from 105 children for measurement of lead exposure indices and owing to technical difficulties, from 93 children for other analyses; BMC measurements were performed for 88 children. Biochemical and hormonal measurements (Table 1) were normal except for low serum magnesium levels (range 1.5 to 1.7 mg/dL) in 5 children and low serum 25(OH)D levels (range 8 to 11 ng/mL) in 4 children. In the latter group, the vitamin D intake was 91 to 329 IU/d in 3 children and 727 IU/d in 1 child. These children did not show clinical features of vitamin D deficiency and anthropometric measurements were within the normal centiles. The PbB was >20 μg/dL only in 3 children (20.2, 20.5, an 23.6 μg/dL, respectively). However, 23.8% of the total sample of 105 children exceeded the current Centers for Disease Control criteria for elevated blood lead level[24] of 25 μg/dL on at least one quarterly blood lead determination since birth (Table 1).

A preliminary structural equation analysis indicated no significant relationships for the dietary calcium, phosphorus, or vitamin D variables with any of the outcome variables. Furthermore, ionized calcium, magnesium, CT, and bone width were not significantly related, either as outcome variables or as predictors, with any of the variables used in this analysis.

After removing these 7 measures, a theoretical model for the remaining system of variables was postulated based on the remaining 12 variables. Effects of the exogenous variables of child's race, sex, and age, and two trigonometric functions (sine and cosine) of the calendar day in which blood was drawn on the endogenous variables of PbB, serum total calcium, phosphorus, PTH, 25(OH)D, $1,25(OH)_2D$, and BMC were tested in a structural equation model. Average lifetime blood lead concentration was treated as a possible precursor of each of the other 6 endogenous variables, with the remainder of the chain being total calcium and phosphorus; PTH; 25(OH)D and $1,25(OH)_2D$; and BMC. A backward elimination of any insignificant pathways for predictors other than PbB in this theoretical causal chain was performed, using the covariances based on the 73 children with complete data on all 12 variables (Table 2). The estimated total effects from the final structural model of PbB on each of the other endogenous variables are shown in Table 3.

The final structural model demonstrating all remaining significant structural coefficients is shown in Fig 2. Both PbB and BMC increased significantly with age, and BMC values were lower in girls. Blacks had lower levels of serum 25(OH)D and higher levels of serum total calcium. A seasonal relationship was noted for both serum total calcium and BMC. Serum total calcium tended to be highest in late fall and lowest in late spring; conversely, BMC tended to be highest in early spring and lowest in early fall.

The only significant relationships among the endogenous variables occurred for the effect of PbB on phosphorus ($\beta = -0.091$, $t = -2.48$); calcium on 25(OH)D ($\beta = 4.616$, $t = 3.85$) and $1,25(OH)_2D$ ($\beta = 1.59$, $t = 2.04$); PTH on $1,25(OH)_2D$ ($\beta = 0.38$, $t = 3.19$); and 25(OH)D on BMC ($\beta = 0.63$, $t = 2.20$). The goodness of fit of this model was $\chi^2 = 48.16$, $df = 37$, $P = .10$. The structural model was rerun with each of the six previously mentioned (Table 3) lead effects removed, resulting in a goodness-of-fit $\chi^2 = 56.75$, $df = 43$, $P = .08$. The difference in the two

TABLE 1. Lead Exposure, Serum Vitamin D Metabolites, Calcitropic Hormone Concentrations, and Photon Absorptiometry Measurements at Distal One Third of Left Radius for All Studied Children*

Indices	n	GM	GSD	Range
Average lifetime blood lead, μg/dL	105	9.74	1.44	4.76–23.61
Maximum lifetime blood lead, μg/dL	105	18.53	1.53	6–63
Concurrent blood lead, μg/dL	105	15.01	1.52	6–44
Phosphorus, mg/dL	93	5.07	1.13	3.4–6.7
Calcium, mg/dL	93	9.70	1.05	8.6–10.6
Ionized calcium, mg/dL	85	5.21	1.04	4.52–5.64
Magnesium, mg/dL	93	2.02	1.08	1.5–2.3
Parathyroid hormone, μLEq/mL	92	76.28	1.37	24–128
Calcitonin, pg/mL	89	21.15	1.86	10–93
25-Hydroxyvitamin D, ng/mL	92	27.46	1.56	8–87
1,25-Dihydroxyvitamin D, pg/mL	86	62.20	1.40	23–148
Indices	**n**	**M**	**SD**	**Range**
Bone mineral content, mg/cm	88	229.07	34.91	129.7–327
Bone width, mm	88	7.83	1.06	5.3–11.1

* GM, geometric mean; GSD, geometric standard deviation; M, arithmetic mean; SD, arithmetic standard deviation.

TABLE 2. Simple Correlations Among Exogenous and Endogenous Variables Used in the Structural Equation Analysis*

	Exogenous Variables					Endogenous Variables						
	Age	Sex	Race	sin(date)	cos(date)	ln(PbB)	ln(Ca)	ln(P)	ln(PTH)	ln[25(OH)D]	ln[1,25(OH)$_2$D]	(BMC)$^{1/4}$
Exogenous												
Age	5.18											
Sex	−0.12	0.50										
Race	−0.03	0.03	0.28									
sin(date)	−0.01	0.02	0.27	0.71								
cos(date)	0.19	−0.01	−0.13	−0.05	0.71							
Endogenous												
ln(PbB)	0.25†	−0.22	−0.08	−0.29†	−0.08	0.37						
ln(Ca)	−0.16	0.06	0.43†	0.68†	−0.21	−0.23†	0.05					
ln(P)	−0.03	0.15	0.07	0.03	−0.03	−0.29†	0.12	0.11				
ln(PTH)	0.09	−0.04	0.07	−0.09	−0.17	0.10	−0.16	−0.04	0.31			
ln[25(OH)D]	−0.18	0.04	−0.05	0.36†	0.01	−0.09	0.37†	−0.16	−0.21	0.46		
ln[1,25(OH)$_2$D]	−0.08	−0.01	0.12	0.10	−0.28†	−0.04	0.18	−0.08	0.32†	−0.17	0.32	
(BMC)$^{1/4}$	0.24†	−0.29†	−0.02	−0.12	0.25†	0.20	−0.01	−0.02	−0.22	0.13	−0.19	1.19

* Standard deviations of each variable are shown on the main diagonal of the correlation matrix. Abbreviations: PbB, average lifetime blood lead concentration; Ca, calcium; P, phosphorus; PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D; 1,25(OH)$_2$D, 1,25-dihydroxyvitamin D; BMC, bone mineral content.

† Significant correlations ($P < .05$) among endogenous and exogenous or other endogenous variables.

TABLE 3. Total Estimated Effect of ln(PbB) on Each of the Six Outcome Variables*

Endogenous Variable	Reduced Structural Equation†		Range of Effect‡	R^2	
	B_0	B_1		With PbB	Without PbB
ln(Ca)	2.28 − 0.007 (lnPbB)		9.72 to 9.61	.540	.535
ln(P)	1.83 − 0.091 (lnPbB)		5.40 to 4.67	.084	.00
ln(PTH)	4.16 + 0.081 (lnPbB)		73.03 to 83.14	.009	.00
ln[25(OH)D]	3.36 − 0.034 (lnPbB)		27.24 to 25.80	.184	.184
ln[1,25(OH)$_2$D]	4.13 + 0.003 (lnPbB)		62.39 to 62.69	.176	.175
(BMC)$^{1/4}$	14.53 + 0.253 (lnPbB)		222.66 to 234.91	.249	.241

* Abbreviations are explained in footnote to Table 2.

† B_0 and B_1 are, respectively, the intercept and predicted change in the endogenous variable with ln(PbB), averaged over any other predictors in each structural equation.

‡ Ranges of effects are shown in the original units for each endogenous variable calculated over the observed range in ln(PbB) of 1.561 to 3.162.

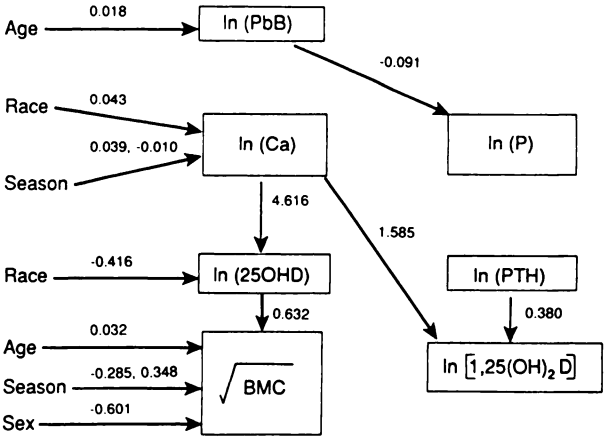

Fig 2. Final structural model paths (β values) for the relationships among lead exposure, serum total calcium, phosphorus, vitamin D metabolites, and bone mineral content based on the hypothesized model shown in Fig 1. Arrows indicate positive effect unless − symbol (ie, negative effect) precedes the numerical β value. Abbreviations are explained in legend to Fig 1. Only the significant β values are shown. Insignificant relationships for PbB are as follows: PbB → Ca ($\beta = -0.007$, $t = -0.68$); PbB → PTH ($\beta = 0.081$, $t = 0.80$); PbB → 25OHD ($\beta = -0.001$, $t = 0.01$); PbB → $1,25(OH)_2D$ ($\beta = -0.016$, $t = 0.16$); and PbB → BMC ($\beta = 0.25$, $t = 0.72$).

goodness-of-fit statistics, $\chi^2 = 8.59$, is not significant on 6 degrees of freedom, $P = .20$. This multivariate statistic indicates that no overall effect of lead on the system of circulating hormones, vitamin D metabolites, and BMC was demonstrated in these data. None of the excluded variables from any of the structural equations was significantly related with the residuals from either model, indicating that these models could not be significalty improved by including any of the previously eliminated "pathways." For comparative purposes, univariate multiple regression analyses for the five endogenous variables that were significantly predicted by at least one other variable are shown in Table 4.

DISCUSSION

Children with high blood lead levels (>60 μg/dL), in comparison with those with low blood lead levels (<30 μg/dL), are reported to have significantly lower (5% to 10%) mean serum concentrations of total and ionized calcium, 25% to 77% lower 25(OH)D, and 50% lower $1,25(OH)_2D$.[5-7] Animals with lead intoxication also are reported to have decreased serum concentrations of calcium, 25(OH)D, and $1,25(OH)_2D$.[3,25-27] The present study of young children with low to moderate lead exposure shows a significant decrease only for the serum phosphorus concentrations. In previous reports of lead exposure in children, serum phosphorus concentrations either did not change[5] or were not reported.[6,7] However, our data are consistent with the lowering of serum phosphorus concentrations reported in adults with occupational lead exposure[28] and are supported by animal studies which have shown that tissue lead burden and lead toxicity are most pronounced in the presence of dietary calcium and phosphorus deficiency.[3,4,25-27] In this study, the clinical significance of a modest decrease in mean serum phosphorus concentration of about 0.7 mg/dL within the normal ranges is uncertain. In addition, the multivariate test for lead's effect on all measured variables (including serum phosphorus concentration) is not statistically significant.

The changes in serum concentrations of 25(OH)D and $1,25(OH)_2D$ with elevated PbB were found to be statistically insignificant. The magnitude of lead's effect in the present study is less than that reported by Rosen et al[6] and is approximately ¼ that for calcium, ⅛ that for PTH, ⅕ that for 25(OH)D, and essentially 0 for $1,25(OH)_2D$. Based on earlier data[6,7] our sample size would have been adequate to detect a statistically significant effect of PbB on serum concentrations of $1,25(OH)_2D$ with a power of >90%. However, post hoc power calculations showed that to detect a direct effect of PbB (ie, independent of lead's effect on serum calcium concentration) on serum $1,25(OH)_2D$ concentration with a power of 80% would require a sample size of 17 000. Thus, for practical purposes, there is no direct effect of PbB on serum $1,25(OH)_2D$ concentration in our subjects. The seasonal effect on serum calcium concentration and the racial effect on serum 25(OH)D concentration are consistent with other reports.[15,29] The racial effect on serum calcium concentration has not been previously reported.

Photon absorptiometry is an accepted technique to measure bone mineralization in adults[21,30] and in children.[31-33] Thus, BMC as determined by photon absorptiometry may be considered as a biologic marker for disturbed calcium, phosphorus, and vitamin D metabolism. To our knowledge, there is no previous report of BMC measurement in children with chronic lead exposure. There was no significant direct or indirect (total) effect of lead level on BMC found in this study.

In the present study, BMC values are comparable with those from another report for a small number of young children.[32] The findings that BMC increased with age and that males have higher BMC are consistent with other reports.[31,32] Bone mineral content was found to increase 11.8% between 21 and 33 months, similar to the reported average yearly change in BMC of 10.5% for the older pediatric preadolescent population.[31] However, we were unable to confirm the presence of racial effect on

TABLE 4. Univariate Multiple Regression Analyses for Outcomes Related Significantly With Covariates, Confounders, and/or PbB*

Dependent Variable	Multiple R^2	Regressor	β	SE	t	P
ln(Ca)	.55	Intercept	2.19	0.03		
		sin(date)	0.04	0.006	F = 27.37	.0001
		cos(date)	−0.01	0.005		
		Race	0.04	0.01	2.95	.004
ln(P)	.08	Intercept	1.83	0.08		
		ln(PbB)	−0.09	0.04	−2.55	.007†
ln[25(OH)D]	.19	Intercept	−6.40	2.49		
		Race	−0.42	0.20	−2.11	.04
		ln(Ca)	4.62	1.16	3.99	.0001†
ln[1,25$(OH)_2$D]	.16	Intercept	−1.17	1.88		
		ln(Ca)	1.61	0.76	2.12	.02†
		ln(PTH)	0.38	0.12	3.23	.001†
$(BMC)^{1/4}$	.23	Intercept	12.65	1.40		
		Age	0.05	0.03	1.90	.03†
		sin(date)	−0.33	0.19	F = 3.25	.05
		cos(date)	0.32	0.18		
		Sex	−0.65	0.26	−2.53	.02
		ln[25(OH)D]	0.65	0.30	2.13	.02†

* Abbreviations are explained in footnote to Table 2.
† One-tailed P value.

BMC in young children.[32] This may be a ramification of the predominance of blacks in our study sample or of an indirect effect of race through 25(OH)D, for which blacks were observed to have lower concentrations than whites. The higher BMC values observed in spring in this study have not been previously reported.

Failure to replicate previous findings regarding lead effects[5–7] might be due to the low to moderate lead exposure in this cohort, as only five children had markedly elevated blood lead levels of >60 μg/dL similar to levels of previous studies, and all children's blood lead levels were <45 μg/dL at the time of assessment.

Furthermore, there was generally an adequate dietary intake, particularly that of calcium, phosphorus, and vitamin D. This is in marked contrast to previously studied cohorts,[5,6] which exhibited deficient intakes of calcium and vitamin D. High intake of dairy products in this cohort in part accounts for the high calcium and phosphorus intake compared to the recommended daily allowances.[34] This is consistent with data from several studies[35–37] in which up to one third of children younger than 3 years may have calcium intake greater than 1200 mg/d. Although 41% of the present cohort had dietary intake of vitamin D at less than 75% of the recommended daily allowances,[34] it was apparently adequate to support normal vitamin D status, as indicated by the normal serum 25(OH)D concentrations. The level of dietary calcium intake in this cohort was almost twice as great, and vitamin D intake was generally higher than those reported in children with high lead exposure and lower serum concentrations of calcium and vitamin D metabolites.[6] The generally adequate dietary intake of calcium, phosphorus, and vitamin D in this cohort theoretically may have lowered the potential toxicity of lead exposure, inasmuch as high calcium and phosphorus intake is associated with decreased intestinal absorption and tissue retention of lead in animals[4,25–27] and decreased intestinal absorption of lead in children.[35] Furthermore, there were no changes in the serum concentrations of the physiologically active ionized calcium fraction and calcitropic hormones (PTH and CT).

We conclude that chronic low to moderate lead exposure in young children in the presence of adequate dietary intake of calcium, phosphorus, and vitamin D does not result in clinically and statistically significant alteration in serum vitamin D metabolites and calcitropic hormones responsible for the control of calcium and phosphorus homeostasis; bone mineralization as measured by photon absorptiometry also was unaffected. From comparison of our results with previously published studies,[5–7] we suggest the possibility of a strong lead-nutritional deficiency interaction. The large effect reported by prior reports presumably only occurs with chronic nutritional deficiency and chronically elevated PbB.

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