

# Protein Kinase C Activity and the Relations between Blood Lead and Neurobehavioral Function in Lead Workers

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At picomolar concentrations, lead activates protein kinase C (PKC). This activation has been implicated in the neurotoxicity of lead. No prior study has evaluated the association of PKC activity with neurobehavioral function in humans. The purpose of this study was to determine whether PKC activity is associated with neurobehavioral function or modifies the relationship between blood lead levels and neurobehavioral test scores. In this cross-sectional study of 212 current lead workers in the Republic of Korea, we assessed blood lead levels, neurobehavioral test scores, and PKC activity. PKC activity was determined by measuring the levels of phosphorylation of three erythrocyte membrane proteins (spectrin and the 52-kDa and 48-kDa subunits of band 4.9), using an *in vitro* back-phosphorylation assay. When linear regression was used to control for confounding variables, blood lead was a significant predictor of decrements in performance on tests of psychomotor function, manual dexterity, and executive ability. In linear regression models, back-phosphorylation levels were not associated with neurobehavioral test scores, but when dichotomized at the median, back-phosphorylation levels modified the relationship between blood lead and test scores. For spectrin and the 52-kDa and 48-kDa subunits of band 4.9, 5, 2, and 5 of 14 interaction terms, respectively, had associated *p*-values < 0.10, all with positive signs, indicating that blood lead was associated with worse test scores only in subjects with lower back-phosphorylation levels. These data indicate that blood lead levels are associated with decrements in neurobehavioral test scores, mainly in the domains of manual dexterity and psychomotor function, but only in subjects with lower *in vitro* back-phosphorylation levels, which is equivalent to higher *in vivo* PKC activity. We hypothesize that subjects with higher PKC activity in the presence of lead may be more susceptible to the health effects of lead. **Key words:** back-phosphorylation, blood lead, neurobehavioral function, protein kinase C, susceptibility. *Environ Health Perspect* 110:133–138 (2002). [Online 10 January 2002] <http://ehpnet1.niehs.nih.gov/docs/2002/110p133-138hwang/abstract.html>

Protein kinase C (PKC) is a family of phospholipid-dependent serine/threonine protein kinases that are found in almost all eukaryotes and in high concentrations in neural tissues (1–3). Evaluation of the functions of the various PKCs is an active area of research, but it has been established that PKC plays important roles in the nervous system. For example, PKC is involved in the regulation or modulation of neurotransmitter release (4,5), synaptic and neuronal plasticity (6,7), neuronal ion channels (8), cerebral microvascular function (9), and cognition (10–12). Alterations in PKC-dependent phosphorylation have been found in the brains of patients with Alzheimer disease (13,14), suggesting that PKC may also be involved in the pathogenesis of neurodegenerative disease.

Considerable research has focused on the interactions between lead and calcium in the activation of PKC. For example, picomolar concentrations of lead can substitute for micromolar concentrations of calcium in the activation of PKC, as assessed by PKC enzyme activity assays (15). To date, PKC is

the only cellular target known to be affected by lead in the range of free lead concentrations of  $2 \times 10^{-11}$ – $3 \times 10^{-11}$  M (16–18). Translocation of PKC from the cytosol to the membrane has been observed in immature microvessels, rat glioma cells, and bovine endothelial cells after exposure to lead (9). Continuous lead exposure was implicated in interference with training-induced translocation of PKC from the cytosol to the membrane in the rat hippocampus (19). Recently, lead exposure during development was shown to decrease membrane-associated PKC in the rat hippocampus and frontal cortex and to potentiate phorbol-12,13-dibutyrate (PDBu)-activated PKC translocation; PDBu is a phorbol ester that binds to the diacylglycerol binding site on PKC, causing PKC translocation from the cytosol to the membrane (10).

Although a number of experimental studies have evaluated interactions between lead and PKC, none has examined the influence of PKC on neurobehavioral function in adult humans. We previously reported an

association between PKC activity and lead exposure in current lead workers; specifically, we found that higher tibia lead levels and longer job durations were associated with higher *in vivo* PKC activity, as assessed by a back-phosphorylation assay (20). We designed the current study to determine whether *in vivo* PKC activity in erythrocytes is associated with neurobehavioral function. We also evaluated whether PKC activity modifies the relationship between blood lead levels and neurobehavioral test scores, allowing inferences to be made about the mechanisms of lead neurotoxicity in adults.

## Materials and Methods

**Study design and subject selection.** From October 1997 to August 1999, we recruited 803 lead workers and 135 nonexposed controls in the Republic of Korea for a 4-year prospective study of the health effects of lead (21). From April to July 1998, we selected from among these lead workers 212 consecutively enrolled subjects (156 males and 56 females) in four lead storage battery plants for a study of erythrocyte membrane protein phosphorylation. This back-phosphorylation study was conducted in only a subset of subjects because of the technical difficulty of the assay and because a power calculation indicated that 200 subjects would be sufficient to assess study aims. The current study is a cross-sectional analysis of data from the first year of the prospective study (October 1997–October 1998). The study subjects were examined at Soonchunhyang University Institute of Industrial Medicine in Chunan. All subjects provided written informed consent, and participation was voluntary. This study was approved by Institutional Review

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**Data collection.** All subjects completed a standardized questionnaire, a medical history questionnaire, an occupational history interview, and a neurobehavioral test battery consisting of examiner-administered tests; underwent assessment of peripheral vibration threshold with the Vibratron II tester (Physitemp Instruments Inc., Clifton, NJ, USA); and provided a 10-mL blood specimen by venipuncture that was stored at  $-70^{\circ}\text{C}$  as whole blood, plasma, and erythrocytes (21).

An occupational physician obtained the medical history, and a trained psychologist and a registered nurse performed the neurobehavioral testing. All study questionnaires and instructions for data collection were translated into Korean and then translated back to English to assess the accuracy of the translation. The standardized questionnaire assessed demographic characteristics, education, tobacco and alcohol use, detailed medical history, subjective symptoms related to lead exposure, and complete job history.

**Neurobehavioral test battery.** We used the World Health Organization Neurobehavioral Core Test Battery (WHO NCTB) (22) and several additional selected tests to assess neurobehavioral function. The digit symbol substitution test is a performance subtest of the Wechsler Adult Intelligence Scale-Revised (WAIS-R) and was used to assess executive abilities, which require learning of associations (23). The digit span test is a verbal subtest of the WAIS-R and the Wechsler Memory Scale-Revised (WMS-R) and was used to assess verbal memory and learning (24). The Benton Visual Retention Test is a test of short-term visual memory using geometrical patterns. The Pursuit Aiming II test was used to measure manual dexterity (25). The simple reaction time test was used to measure how fast subjects reacted to a visual stimulus, an assessment of attention and psychomotor speed (26). Simple reaction time was assessed with the Standard Reaction Time Tester (Software Science, Cincinnati, OH, USA), consisting of 64 trials over 6 min with a static group of random interstimulus intervals between 1 and 10 sec. The Purdue pegboard test (Lafayette Instrument, Model 32020, Lafayette, IN, USA) was substituted for the Santa Ana dexterity test of the WHO NCTB and was used to measure manual dexterity and executive abilities (27). Trail-Making Tests A and B were used to assess executive abilities (28). Raven's colored progressive matrices (Psychological Corporation, San Antonio, TX, USA) were used to measure visual reasoning and problem solving, an assessment of intellectual development (29). The Center for Epidemiological

Studies Depression scale (CES-D), consisting of 20 questions translated into Korean, was used to evaluate depressive mood and affect during the past week (30).

**Laboratory measurements.** Hemoglobin was assayed by the cyanmethemoglobin method (Coulter Model AcTB; Beckman Coulter, Seoul, South Korea), and hematocrit was measured by the capillary centrifugation method (31). Blood lead levels were measured with a Zeeman background-corrected atomic absorption spectrophotometer (Hitachi Z-8100 model; Hitachi Instruments, Tokyo, Japan) using the National Institute of Occupational Safety and Health standard addition method (32) at Soonchunhyang University Institute of Industrial Medicine, a certified reference laboratory for lead in Korea. The institute participates in quality-control programs for industrial laboratories, a regulation of the Korean government (33). Zinc protoporphyrin (ZPP) levels were measured with a portable hematofluorimeter (34).

**Erythrocyte membrane preparation.** Venous blood was collected in a heparinized vacutainer tube. Each sample was centrifuged at 1,000 *g* for 20 min to separate the erythrocytes. Plasma and buffy coats were removed by aspiration, and cells were washed 3 times with equal amounts of saline. Erythrocytes were preserved by rapid freezing in a cryopreservative (35). An equal amount of cryoprotective additive solution (28% glycerol, 3% mannitol, 0.65% NaCl) was added to packed erythrocytes and thoroughly mixed. Erythrocytes were stored at  $-70^{\circ}\text{C}$  in Korea, transferred under dry ice to the Kennedy Krieger Institute in Baltimore, Maryland, USA, and then kept at  $-70^{\circ}\text{C}$  until erythrocyte membranes were prepared.

Appropriate isolation of erythrocyte membranes is critical to the back-phosphorylation assay because the membrane composition depends on the preparation method. Briefly, approximately 3–4 mL glycerolized erythrocytes were thawed on wet ice (36). Osmotic lysis was initiated by adding 20 mM sodium phosphate buffer at pH 7.4 to the erythrocytes to achieve an erythrocyte-to-buffer ratio of 1:15 v/v. After 20 min on wet ice, the hemolysate was centrifuged at 20,000 *g* for 40 min. The supernatant fractions were decanted, and the membrane proteins in the pellet were washed 4 or 5 times with phosphate buffer until the fraction was colorless. The membrane proteins were resuspended in phosphate buffer, and the protein content of the membranes was determined (37). Aliquots of membranes were stored at  $-70^{\circ}\text{C}$  until use.

**Determination of back-phosphorylation levels.** An *in vitro* back-phosphorylation assay was used to determine *in vivo* PKC

activity. The principle of this assay is that proteins that were phosphorylated by PKC *in vivo* will not undergo phosphorylation in the *in vitro* back-phosphorylation assay after addition of exogenous PKC. Therefore, the levels of *in vitro* back-phosphorylation are inversely related to *in vivo* PKC activity.

Erythrocyte membranes were thawed at  $4^{\circ}\text{C}$  and sonicated for 30 sec on wet ice (Sonifier Cell Distributor, model W185D; Branson Ultrasonic Devices, Inc., Danbury, CT, USA). Membrane proteins were diluted with 20 mM phosphate buffer to 0.16  $\mu\text{g}/\mu\text{L}$  concentration. Phosphatidyl serine (PS; BPS-578; Avanti Polar Lipids Inc., Alabaster, AL, USA) was dried under nitrogen gas at  $4^{\circ}\text{C}$ , resuspended in 50 mM Tris/HCl (pH 7.4), and sonicated for 30 sec at  $4^{\circ}\text{C}$ . PDBu (1  $\mu\text{g}/\mu\text{L}$ ) was added to PS to achieve concentrations of 0.0125  $\mu\text{g}/\mu\text{L}$  and 0.25  $\mu\text{g}/\mu\text{L}$ , respectively. A solution of cofactors was made by taking equal volumes of PS/PDBu, membrane protein, and 12 mM  $\text{CaCl}_2$  and 30 mM dithiothreitol, both in 50 mM Tris/HCl (pH 7.4).

$[\gamma\text{-}^{32}\text{P}]$ -Adenosine triphosphate (ATP) at 0.08  $\mu\text{Ci}/\mu\text{L}$  was freshly made in 45 mM  $\text{MgCl}/150 \mu\text{M}$  ATP for each assay. PKC was prepared at 0.1 unit/25  $\mu\text{L}$ . To initiate the reaction, 25  $\mu\text{L}$  of cofactor solution, 25  $\mu\text{L}$  of  $[\gamma\text{-}^{32}\text{P}]$ -ATP, and 25  $\mu\text{L}$  of PKC were mixed. The final quantities of reactants were 1  $\mu\text{g}$  of membrane protein, 0.1 unit of PKC, and 2.6  $\mu\text{Ci}$  of  $[\gamma\text{-}^{32}\text{P}]$ -ATP.

Reaction mixtures were incubated at  $30^{\circ}\text{C}$  for 30 min in a heat block (VWR Scientific Products, West Chester, PA, USA) and terminated by adding an equal amount of ice-cold acetone. The supernatant fraction was removed by centrifugation at 20,000 *g* for 20 min at  $4^{\circ}\text{C}$ , and the pellets were isolated and resuspended in 30  $\mu\text{L}$  loading buffer. Samples were boiled for 3 min and subjected to SDS-PAGE. Gels were stained with 0.005% Coomassie Brilliant Blue solution (in 40% methanol, 7% acetic acid) overnight at room temperature, then destained and dried.

$[\gamma\text{-}^{32}\text{P}]$  incorporation into membrane proteins was measured by phosphoimaging with a bioimaging analyzer (Fuji BAS 2500, Mac BAS version 2.5; Fujifilm Medical Systems, Stamford, CT, USA) from dried gels after the gel was exposed for 30 min to an imaging plate (Fuji, BAS-III). The incorporated radioactivity in the back-phosphorylation assay is expressed as units of photostimulated luminescence (PSL).

Preliminary experiments revealed that membrane proteins of molecular masses of 240, 230, 80, 52, and 48 kDa were phosphorylated by the exogenously added PKC. Without addition of PKC, phosphorylation was not observed. Based on molecular mass, the 240- and the 230-kDa proteins were

identified as  $\alpha$ - and  $\beta$ -spectrin, respectively, and the 52- and the 48-kDa proteins as the two subunits of band 4.9 (38). Although other proteins underwent phosphorylation by PKC, spectrin and band 4.9 were observed most consistently and were thus measured to determine *in vivo* PKC activity.

Although the amount of protein subjected to SDS-PAGE was approximately equivalent for each sample (1  $\mu$ g), we noticed some variability between samples after the gels were stained. For this reason, we measured the staining of spectrin by computerized densitometry to determine the amount of spectrin on each gel. The measured optical density was used to adjust for the variability in loaded protein in the statistical analysis. Spectrin is composed of two subunits,  $\alpha$  and  $\beta$ , but we measured the optical density of spectrin as one protein because the two subunits were difficult to distinguish.

**Assay variability and adjustment.** We observed variability in back-phosphorylation levels in the same preparation of erythrocyte membranes. To control for this variability, a single standard sample was assayed on each of the 7 days that experimental samples were assayed. During the period, the intraday coefficient of variation ranged from 10.9% to 27.6%, with a mean of 17.1%. However, the interday coefficient of variation of the standard sample was 33.9%. Thus, all samples were adjusted by the ratio of the standard sample on that date to the mean of all standard samples. This method of adjustment controlled, at least in part, for interday

variability. All study samples were assayed in duplicate on separate gels, and the mean of the two results was calculated. The correlation between duplicates was high (Pearson's  $r = 0.87$ ,  $p < 0.001$ ), and the mean percent difference between duplicates was 25.2%.

**Statistical analysis.** The two major objectives of the analysis were to examine whether *in vitro* back-phosphorylation levels, a surrogate for *in vivo* PKC activity, were predictors of neurobehavioral test scores or effect modifiers of the relationship between blood lead levels and neurobehavioral test scores. Prior analysis indicated that among the lead dose measures [i.e., blood lead, dimercaptosuccinic acid (DMSA)-chelatable lead, tibia lead by X-ray fluorescence], blood lead levels were the strongest and most consistent predictors of neurobehavioral test scores in this population (21). Thus, analysis of effect modification by back-phosphorylation levels focused on blood lead levels.

We constructed separate linear regression models for each neurobehavioral test, controlling for age, sex, job duration, and education. To address the first objective, we used linear regression to separately model each neurobehavioral test in relation to back-phosphorylation levels for spectrin, and the 52-kDa and 48-kDa subunits of band 4.9 as explanatory variables. To address the second objective, we added cross-product terms between blood lead and back-phosphorylation levels to each model, adjusting for age, sex, job duration, and education. For these models, we divided back-phosphorylation levels at the median

into higher and lower groups. Four neurobehavioral tests (standard deviation of reaction time, trails A, trails B, and CES-D) were log-transformed to better approximate normality.

For consistency of presentation, we selected the final regression models of neurobehavioral test scores with the same set of confounding variables. Variables that were considered potential confounders (i.e., tobacco and alcohol use, body mass index, hemoglobin, hematocrit) were added in a forward stepwise procedure and were retained in the final models if they were found to be predictors of neurobehavioral test scores or if they significantly changed the  $\beta$  coefficient of the back-phosphorylation or blood lead terms in the models (a determination based on change of both the magnitude and statistical significance of the coefficient). To evaluate nonlinear associations, we included linear and quadratic terms for selected continuous variables in the models. Neurobehavioral test scores were standardized so that a negative regression coefficient indicated worse performance with increasing blood lead levels.

We evaluated all regression models for violation of the assumptions of linear regression. We also evaluated regression diagnostics such as residual plots, added variable plots, and variance inflation factors to determine whether influential points, multicollinearity, nonlinearity, departures from normality, or nonhomogeneous variance accounted for the study results.

## Results

The study participants had a wide range of blood lead, tibia lead, and ZPP levels, ranging from 5.4 to 69.3  $\mu$ g/dL, 0.8 to 290.8  $\mu$ g Pb/g bone mineral, and 26 to 386  $\mu$ g/dL, respectively (Table 1). Back-phosphorylation levels of spectrin, the 52-kDa subunit of band 4.9, and the 48-kDa subunit of band 4.9 ranged from 29.5 to 1737.0 PSL units, 31.2 to 502.1 PSL units, and 35.8 to 576.5 PSL units, respectively (Table 1).

Summary statistics for neurobehavioral test scores can be found in Table 2. Males had significantly better performance than females on all neurobehavioral tests except the Purdue pegboard, and older age was consistently associated with lower neurobehavioral test scores. Education level and tobacco and alcohol use were not significant predictors of neurobehavioral test scores. Back-phosphorylation levels were not associated with neurobehavioral test scores in crude (Table 3) or adjusted analysis (controlling for age, sex, job duration, and education; data not shown). Blood lead levels were significant negative predictors of neurobehavioral test scores in the domains of psychomotor function, manual dexterity, and executive ability (Table 4).

**Table 1.** Characteristics of 212 Korean lead workers, 1998.

Characteristic	Male (n = 156)	Female (n = 56)	p-Value <sup>a</sup>
Age (years)	36.3 $\pm$ 0.8	47.0 $\pm$ 0.9	< 0.01
Exposure duration (years)	8.8 $\pm$ 0.6	6.2 $\pm$ 0.5	< 0.01
Education (years), no. (%)			0.61
$\leq$ 6	36 (23.1)	14 (25.0)	
7–9	40 (25.6)	12 (21.4)	
10–12	69 (44.2)	27 (48.2)	
$\geq$ 13	11 (7.1)	3 (5.4)	
Tobacco use, no. (%)			< 0.01
Never/former	50 (32.1)	56 (100)	
Current	106 (67.9)	0 (0)	
Alcohol use (drinks per week), no. (%)			< 0.01
0 to < 1	41 (26.3)	42 (75.0)	
$\geq$ 1	115 (73.7)	14 (25.0)	
Weight (kg)	62.2 $\pm$ 0.7	57.3 $\pm$ 1.2	< 0.01
Height (cm)	167.2 $\pm$ 0.5	153.6 $\pm$ 0.6	< 0.01
Body mass index (kg/m <sup>2</sup> )	22.2 $\pm$ 0.2	24.3 $\pm$ 0.5	< 0.01
Blood lead ( $\mu$ g/dL)	32.0 $\pm$ 13.0	19.8 $\pm$ 9.2	< 0.01
Tibia lead ( $\mu$ g Pb/g bone mineral)	37.8 $\pm$ 39.6	25.5 $\pm$ 14.7	0.03
ZPP ( $\mu$ g/dL)	68.7 $\pm$ 47.8	72.1 $\pm$ 29.7	0.61
Spectrin back-phosphorylation <sup>b</sup>	537.6 $\pm$ 25.3	549.4 $\pm$ 36.1	0.80
Band 4.9 (52 kDa) back-phosphorylation <sup>b</sup>	192.3 $\pm$ 5.9	216.2 $\pm$ 11.7	0.05
Band 4.9 (48 kDa) back-phosphorylation <sup>b</sup>	238.8 $\pm$ 6.6	253.6 $\pm$ 11.4	0.26
Spectrin quantity on gels, optical density <sup>c</sup>	22.8 $\pm$ 0.8	22.8 $\pm$ 1.2	0.96

Values shown are mean  $\pm$  SD except where indicated.

<sup>a</sup>p-Values were obtained by Student's *t*-test for continuous variables and by  $\chi^2$  test for categorical variables. <sup>b</sup>The back-phosphorylation levels were measured in units of PSL using a phosphorimager. <sup>c</sup>Spectrin quantity was measured in optical density units using computerized densitometry after staining the gels with Coomassie Brilliant Blue.

We then divided back-phosphorylation levels at the median into high and low groups for spectrin and the 52-kDa and 48-kDa subunits of band 4.9. We then evaluated the effect of back-phosphorylation levels on the relationship between blood lead levels and neurobehavioral test scores by adding cross-product terms to the linear regression models. The signs of the  $\beta$  coefficients of the interaction terms between blood lead and back-phosphorylation levels of spectrin, the 52-kDa subunit of band 4.9, and the 48-kDa subunit of band 4.9 were positive for 10 of 14 neurobehavioral tests for each (Table 5). A positive  $\beta$  coefficient for the interaction term means that subjects with lower back-phosphorylation levels had larger declines in neurobehavioral test scores with increasing blood lead levels than did subjects with higher back-phosphorylation levels.

For spectrin, 4 of 14 interaction terms had associated  $p$ -values  $< 0.05$ , and one other

had an associated  $p$ -value  $< 0.10$ . For the 52-kDa subunit of band 4.9, 1 of 14 had an associated  $p$ -value  $< 0.05$  and one other had an associated  $p$ -value  $< 0.10$ . For the 48-kDa subunit of band 4.9, 3 of 14 interaction terms had associated  $p$ -values  $< 0.05$ , and 2 others had associated  $p$ -values  $< 0.10$ . The signs of the  $\beta$  coefficients for all borderline or statistically significant interaction terms were positive. The results indicated that higher blood lead levels were only associated with worse neurobehavioral test scores among subjects with lower back-phosphorylation levels, or correspondingly, higher *in vivo* PKC activity.

Significant effect modification by back-phosphorylation levels was mainly observed in the cognitive domains of manual dexterity and psychomotor function. In these domains, blood lead was a statistically significant predictor of worse neurobehavioral test scores only among subjects with low back-phosphorylation levels (Figure 1).

## Discussion

This study was designed to assess associations of *in vivo* PKC activity with neurobehavioral test scores in current lead workers. PKC activity, as assessed with the back-phosphorylation assay, was not associated with neurobehavioral test scores. However, PKC activity modified the relationship between blood lead levels and test scores, the first evidence in humans, albeit indirect, that PKC may play a role in the neurobehavioral effects of lead. A large body of experimental evidence already suggests that PKC is an important target for lead and is involved in the mechanism of the neurotoxicity of lead.

The 212 subjects reported herein were a consecutive sample from 803 lead workers enrolled in a 4-year prospective study. In cross-sectional analysis of the 803 subjects, blood lead was a stronger and more consistent predictor of neurobehavioral test scores than was tibia lead or DMSA-chelatable lead (21). In the subset of 212 subjects, blood lead was also a consistent predictor of lower neurobehavioral test scores (Table 4). However, after accounting for *in vivo* PKC activity in the effect modification models, blood lead only predicted lower neurobehavioral test scores among subjects with lower *in vitro* back-phosphorylation levels, and thus higher *in vivo* PKC activity. The associated  $p$ -values for these relationships were  $< 0.10$  for 5, 2, and 5 of 14 comparisons for back-phosphorylation levels of spectrin and the 52-kDa and 48-kDa subunits of band 4.9, respectively, clustered in the domains of manual dexterity and psychomotor function. Although this evidence is not overwhelming because of the large number of associations that were evaluated, we feel these observations are unlikely to be due to chance. No such associations were observed with tibia lead, ZPP, and exposure duration (data not shown).

Calcium-dependent PKCs, the presumed target of lead, are found in erythrocytes and the brain. Erythrocyte membrane proteins are also present in the brain (3,39). Spectrin is found in mouse brain membranes and undergoes phosphorylation (40), and band 4.9 is also found in the nervous system and in rod photoreceptor cells (41). Extensive experimental evidence also documents the interactions between lead and calcium, especially as relevant to PKC and regulation of nervous system functions (15,18,42–44). It would be expected that mechanisms that influence PKC in the brain and erythrocytes are similar, and thus protein phosphorylation in erythrocytes could be a surrogate measure of PKC activity in the brain or, more specifically, of phosphorylation of similar proteins in the central nervous system. However, this discussion must be considered to be speculative because, to our knowledge, no studies

**Table 2.** Summary of neurobehavioral test scores in 212 Korean lead workers, 1998.

Domain, neurobehavioral test	Score	Min–Max	Mean $\pm$ SD
<b>Executive abilities</b>			
Digit symbol substitution	Number correct	9–91	45.9 $\pm$ 16.5
Trail Making A	Seconds	19–278	52.3 $\pm$ 26.6
Trail Making B	Seconds	38–312	104.0 $\pm$ 43.5
Pegboard, assembly	Number of pieces	50–160	106.4 $\pm$ 18.3
<b>Psychomotor</b>			
Simple reaction time (mean)	Milliseconds	0.20–0.78	0.31 $\pm$ 0.07
Simple reaction time (SD)	Root MSD	0.02–0.35	0.08 $\pm$ 0.05
<b>Manual dexterity</b>			
Pegboard, dominant hand	Number of pieces	30–57	44.2 $\pm$ 5.0
Pegboard, nondominant hand	Number of pieces	29–60	43.4 $\pm$ 5.2
Pegboard, both hands	Number of pieces	19–45	34.5 $\pm$ 4.6
Pursuit Aiming II	Number of dots	65–356	180.2 $\pm$ 42.5
Verbal memory/learning, digit span	Number correct	2–22	10.4 $\pm$ 3.7
Visual memory, Benton Visual Retention Scale	Number correct	3–10	7.7 $\pm$ 1.5
Intelligence, colored progressive matrices	Number correct	9–36	25.2 $\pm$ 6.0
Neuropsychiatric, CES-D	Number of rank	0–46	12.1 $\pm$ 7.6

Abbreviations: Max, maximum; Min, minimum; MSD, mean square deviation.

**Table 3.** Pearson's correlation coefficients between back-phosphorylation levels and neurobehavioral test scores in 212 Korean lead workers, 1998.

Domain, neurobehavioral test <sup>a</sup>	Back-phosphorylation		
	Spectrin	52 kDa	48 kDa
<b>Executive abilities</b>			
Digit symbol substitution	–0.01	–0.01	–0.01
Trail Making A	0.01	0.02	–0.01
Trail Making B	–0.06	0.02	0.05
Pegboard, assembly	–0.15*	0.04	0.02
<b>Psychomotor</b>			
Simple reaction time (mean)	–0.06	0.03	0.01
Simple reaction time (SD)	–0.04	0.05	0.04
<b>Manual dexterity</b>			
Pegboard, dominant hand	–0.19**	0.01	0.01
Pegboard, nondominant hand	–0.10	0.08	0.03
Pegboard, both hands	–0.13	0.04	0.03
Pursuit Aiming II	–0.05	–0.01	–0.02
Verbal memory/learning, digit span	0.01	0.02	0.07
Visual memory, Benton Visual Retention Scale	0.04	–0.08	–0.07
Intelligence, colored progressive matrices	–0.03	–0.01	–0.01
Neuropsychiatric, CES-D	0.04	0.08	0.07

<sup>a</sup>Neurobehavioral tests were standardized for the performance direction such that a negative correlation coefficient indicates worse performance with increasing back-phosphorylation levels. \* $p < 0.05$ ; \*\* $p < 0.01$ .

have directly compared the function and activity of PKC in the brain and erythrocytes.

In our study, *in vivo* PKC activity, as measured by back-phosphorylation levels, was not directly associated with neurobehavioral test scores and did not substantially change the  $\beta$  coefficients for blood lead when added to the regression models. This indicates that PKC activity is unlikely to be in the direct causal pathway between lead dose and neurobehavioral test scores (45). However, when effect modification was evaluated, PKC activity was observed to modify the relationship between blood lead levels and neurobehavioral test scores. These observations suggest that *in vivo* PKC activity may identify a subgroup of

individuals more susceptible to the neurobehavioral effects of lead, as discussed further below. Results from an earlier study indicated that tibia lead and job duration, as measures of cumulative exposure, were associated with back-phosphorylation levels, but blood lead levels were not (20). These contrasting observations, that cumulative exposure and dose measures (i.e., tibia lead, job duration) were associated with PKC activity but that PKC activity only modified the relationship between a short-term dose measure (i.e., blood lead) and neurobehavioral function, demonstrate that much is still unknown about the pools of lead that are assessed by the different lead biomarkers. Reliance on a single

lead biomarker in epidemiologic studies of health outcomes would thus not seem to be an efficient informative strategy.

There are several potential explanations for the observation that blood lead only influenced neurobehavioral test performance in subjects with high *in vivo* PKC activity. First, PKC activity may be a phenotypic measure of probable underlying genetic variation in PKC, a measure of the degree to which factors other than lead, unmeasured in this study, can influence PKC activity. In the presence of lead, there was considerable variability in PKC activity, even after controlling for differences in blood lead levels. Second, *in vitro* back-phosphorylation could

**Table 4.** Multiple regression results of associations of blood lead levels with neurobehavioral test scores in 212 Korean lead workers, 1998.

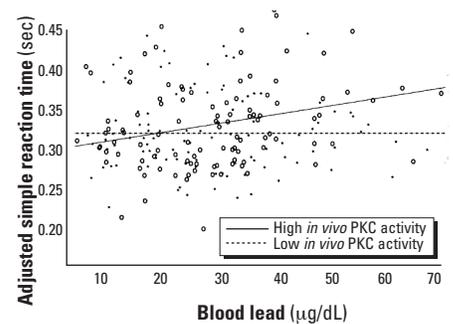
Domain, neurobehavioral test <sup>a</sup>	$\beta$ coefficient <sup>b</sup>	SE
Executive abilities		
Digit symbol substitution	-0.013	0.062
Trail Making A	0.001	0.002
Trail Making B	-0.003*	0.002
Pegboard, assembly	-0.041	0.034
Psychomotor		
Simple reaction time (mean)	-0.0005*	0.0003
Simple reaction time (SD)	-0.006*	0.003
Manual dexterity		
Pegboard, dominant hand	-0.021**	0.010
Pegboard, nondominant hand	-0.021**	0.010
Pegboard, both hands	-0.021**	0.009
Pursuit Aiming II	-0.070	0.102
Verbal memory/learning, digit span	0.008	0.019
Visual memory, Benton Visual Retention Scale	0.001	0.009
Intelligence, colored progressive matrices	-0.028	0.030
Neuropsychiatric, CES-D	0.001	0.006

<sup>a</sup>Neurobehavioral tests were standardized for the performance direction such that a negative  $\beta$  coefficient indicates worse performance with increasing blood lead. <sup>b</sup>The  $\beta$  coefficients for the associations of blood lead levels with neurobehavioral test scores were adjusted for age, sex, job duration, and education. The units of the coefficients are in units of neurobehavioral test per  $\mu\text{g}/\text{dL}$  blood lead. \* $p < 0.10$ . \*\* $0.05$ .

**Table 5.** Multiple regression results of effect modification by back-phosphorylation levels dichotomized at the median on associations of blood lead levels with neurobehavioral test scores in 212 Korean lead workers, 1998.

Neurobehavioral test <sup>a</sup>	Spectrin back-phosphorylation				52 kDa back-phosphorylation				48 kDa back-phosphorylation			
	Blood lead in low <sup>b</sup>		Interaction term <sup>c</sup>		Blood lead in low <sup>b</sup>		Interaction term <sup>c</sup>		Blood lead in low <sup>b</sup>		Interaction term <sup>c</sup>	
	$\beta$ coeff	SE	$\beta$ coeff	SE	$\beta$ coeff	SE	$\beta$ coeff	SE	$\beta$ coeff	SE	$\beta$ coeff	SE
Digit symbol substitution	0.027	0.086	-0.064	0.111	-0.022	0.085	0.019	0.109	0.023	0.087	-0.064	0.111*
Trail making A	0.001	0.003	-0.0001	0.003	0.002	0.003	-0.002	0.003	0.002	0.003	-0.002	0.003
Trail making B	-0.004*	0.003	0.002	0.003	-0.003	0.003	-0.0002	0.003	-0.004*	0.003	0.002	0.003
Pegboard, assembly	-0.103**	0.047	0.111*	0.061	-0.060	0.047	0.031	0.060	-0.104**	0.045	-1.113*	0.062
Simple reaction time, mean	-0.001***	0.0004	0.001**	0.0005	-0.001***	0.0004	0.001**	0.0005	-0.001**	0.0004	0.001*	0.0006
Simple reaction time, SD	-0.012***	0.004	0.012**	0.005	-0.010**	0.004	0.008*	0.005	0.011***	0.004	0.010**	0.005
Pegboard, dominant hand	-0.043***	0.013	0.039**	0.017	-0.031**	0.013	0.017	0.017	-0.044***	0.013	0.041**	0.017
Pegboard, nondominant hand	-0.032**	0.014	0.020	0.018	-0.012	0.013	-0.018	0.017	-0.021	0.014	-0.0006	0.018
Pegboard, both hands	-0.040***	0.012	0.033**	0.015	-0.031***	0.012	0.018	0.015	-0.040***	0.012	0.033**	0.015
Pursuit Aiming II, correct	-0.003	0.140	-0.142	0.183	-0.045	0.143	-0.050	0.180	-0.058	0.144	-0.028	0.182
Digit span	0.020	0.026	-0.023	0.034	-0.010	0.026	0.034	0.033	-0.013	0.026	0.038	0.034
Benton Visual Retention Scale	-0.006	0.012	0.013	0.016	-0.009	0.012	0.018	0.015	-0.012	0.012	0.025	0.015
Colored progressive matrices	-0.033	0.042	0.009	0.055	-0.042	0.042	0.025	0.054	-0.038	0.043	0.019	0.055
CES-D	-0.003	0.008	0.009	0.010	0.0003	0.008	0.002	0.010	-0.002	0.008	0.005	0.010

<sup>a</sup>Neurobehavioral tests were standardized for the performance direction such that a negative  $\beta$  coefficient indicates worse performance with increasing blood lead. Four neurobehavioral tests (SD of reaction time, trails A, trails B, and CES-D) were log-transformed to better approximate normality. <sup>b</sup> $\beta$  coefficients for blood lead in subjects with lower back-phosphorylation levels. These values are the adjusted slopes of the relationship between blood lead and neurobehavioral test scores in subjects with lower back-phosphorylation levels. All models were adjusted for age, sex, job duration, and education. Models with spectrin were also adjusted for the amount of spectrin loaded on the gel (spectrin optical density after stained with Coomassie Brilliant Blue). The units of the beta coefficients are in units of test score per microgram per deciliter of blood lead. <sup>c</sup>Effect modification by back-phosphorylation was evaluated by adding interaction terms between blood lead and the back-phosphorylation levels, dichotomized at the median. These  $\beta$  coefficients can be added to those in the lower back-phosphorylation group to derive the adjusted slopes of the relationship between blood lead and neurobehavioral test scores in the higher back-phosphorylation group. The units of the  $\beta$  coefficients are in units of test score per microgram per deciliter of blood lead. \* $p < 0.1$ ; \*\* $p < 0.05$ ; \*\*\* $p < 0.01$ .



**Figure 1.** Associations of blood lead with simple reaction time (mean), adjusted for age, sex, job duration, and education in 212 Korean lead workers, 1998. Solid (adjusted  $\beta = -0.001$ ,  $p < 0.01$ ) and dotted (adjusted  $\beta = -0.0001$ ,  $p = 0.92$ ) lines are for subjects with low and high levels of *in vitro* back-phosphorylation of 52-kDa subunit of band 4.9, dichotomized at the median, respectively. The difference in the two slopes achieved statistical significance ( $p < 0.05$ ).

be a surrogate measure for bioavailable lead, after lead binding by tissue sites; subjects with high PKC activity in the presence of lead may have less efficient binding to proteins that are not directly influenced by lead binding, such as hemoglobin (i.e., lead binds to hemoglobin but this binding does not affect hemoglobin levels) (46). Finally, PKC activity could be a surrogate measure for brain lead levels. Ion channels and transporters that participate in cellular homeostasis, including voltage-dependent calcium channels, are regulated, in part, by PKC (47–49). These channels are involved in the uptake of calcium by neurons and across the blood–brain barrier. These channels are also permeable to lead, and thus lead may stimulate its own uptake, into the brain and into neurons, by activating PKC (47). Subjects with high PKC activity in the presence of lead may have higher brain lead levels for a given blood lead level. In these subjects, blood lead may be a better surrogate for brain lead levels and thus more highly correlated with neurobehavioral test scores.

In this cross-sectional study, blood lead was more closely associated with performance decrements in the cognitive domains of psychomotor function, manual dexterity, and executive ability than in other domains. These observations are consistent with those of earlier cross-sectional studies of occupationally exposed subjects (50–54).

In summary, *in vivo* PKC activity, assessed by back-phosphorylation of erythrocyte membrane proteins, was not a predictor of neurobehavioral deficits in humans with current occupational lead exposure. However, *in vivo* PKC activity did modify the relationship between blood lead levels and neurobehavioral test scores. Subjects with high PKC activity were apparently more susceptible to the neurobehavioral effects of lead. These data are the first evidence in humans that PKC activity may modulate the neurobehavioral effects of lead.

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