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Acetylcholinesterase Activity and Neurodevelopment in Boys and Girls

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KEY WORDS

acetylcholinesterase, AChE, ADD, ADHD, agriculture, agricultural communities, attention, boys, children, Ecuador, floriculture, flower, girls, growth, inhibition, inhibitory control, memory, neurobehavioral development, neurodevelopment, plantation, pesticide

ABBREVIATIONS

AARS—auditory attention and response set
AChE—acetylcholinesterase
ADHD—attention-deficit/hyperactivity disorder
CI—confidence interval
IN-inhibition—inhibition-inhibition
IN-naming—inhibition-naming
IN-switching—inhibition-switching
RR—relative risk
SD—standard deviation

Dr Suarez-Lopez conceptualized and designed the study, conducted analyses, and drafted the initial manuscript; Drs Himes and Jacobs had active participation in data analysis and reviewed and revised the manuscript; Drs Alexander and Gunnar reviewed and revised the manuscript; and all authors approved the final manuscript as submitted.

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WHAT'S KNOWN ON THIS SUBJECT: Prenatal and postnatal organophosphate (cholinesterase inhibitor) pesticide exposure has been associated with delays in attention, memory, intelligence, and inhibitory control. Two recent studies reported decreased attention and working memory with greater exposure to organophosphates in boys but not in girls.

WHAT THIS STUDY ADDS: This is the first study to report associations between decreased acetylcholinesterase activity, a stable marker of cholinesterase inhibitor pesticide exposure, and lower overall neurodevelopment, attention, inhibitory control, and memory. These associations were present in boys but not in girls.

abstract

BACKGROUND: Organophosphate exposures can affect children's neurodevelopment, possibly due to neurotoxicity induced by acetylcholinesterase (AChE) inhibition, and may affect boys more than girls. We tested the hypothesis that lower AChE activity is associated with lower neurobehavioral development among children living in Ecuadorian floricultural communities.

METHODS: In 2008, we examined 307 children (age: 4–9 years; 52% male) and quantified AChE activity and neurodevelopment in 5 domains: attention/executive functioning, language, memory/learning, visuospatial processing, and sensorimotor (NEPSY-II test). Associations were adjusted for demographic and socioeconomic characteristics and height-for-age, flower worker cohabitation, and hemoglobin concentration.

RESULTS: Mean ± standard deviation AChE activity was 3.14 ± 0.49 U/mL (similar for both genders). The range of scores among neurodevelopment subtests was 5.9 to 10.7 U (standard deviation: 2.6–4.9 U). Girls had a greater mean attention/executive functioning domain score than boys. In boys only, there were increased odds ratios of low (<9th percentile) neurodevelopment among those in the lowest tertile versus the highest tertile of AChE activity (odds ratios: total neurodevelopment: 5.14 [95% confidence interval (CI): 0.84 to 31.48]; attention/executive functioning domain: 4.55 [95% CI: 1.19 to 17.38], memory/learning domain: 6.03 [95% CI: 1.17 to 31.05]) after adjustment for socioeconomic and demographic factors, height-for-age, and hemoglobin. Within these domains, attention, inhibition and long-term memory subtests were most affected.

CONCLUSIONS: Low AChE activity was associated with deficits in neurodevelopment, particularly in attention, inhibition, and memory in boys but not in girls. These critical cognitive skills affect learning and academic performance. Added precautions regarding secondary occupational pesticide exposure would be prudent. *Pediatrics* 2013;132:e1649–e1658

Cholinesterase inhibitors (ie, organophosphates and carbamates) are among the most commonly used insecticides worldwide. They are known to suppress the activity of acetylcholinesterase (AChE),[1] an enzyme that metabolizes acetylcholine, and its inhibition can be neurotoxic.[2] There is growing evidence linking prenatal organophosphate exposure with mental and motor developmental delays, pervasive developmental disorder, and decreased attention, working memory, and intelligence[3–6] in children. Postnatal organophosphate exposures have been associated with decreased learning, attention and inhibitory control, and increased risk of attention-deficit/hyperactivity disorder (ADHD) behaviors.[5,7,8] Furthermore, 2 recent studies reported poorer attention and working memory with greater exposure to the organophosphate chlorpyrifos in boys but not in girls.[5,9]

There is limited information regarding the association between AChE activity and neurodevelopment in children; most studies have focused on metabolite or direct quantification of pesticides in blood or urine. Because organophosphates have short half-lives in blood (<1 day)[10–12] and exposure varies by day and season, quantification of their metabolites in urine has greater daily variability within children than between children,[13,14] which increases the likelihood of exposure misclassification. In addition, urinary measurements may overestimate exposures to the parent compound because metabolite elevations also reflect exposure to less toxic ambient metabolites.[15]

Quantification of AChE activity circumvents these problems. First, AChE levels can be interpreted without accounting for factors that affect organophosphate metabolism (eg, paraoxonase) because AChE inhibition is a physiologic response to exposure in relation to the individual's sensitivity and ability to metabolize cholinesterase inhibitors. Next, erythrocytic AChE is similar to neuronal AChE activity,[2] and brain AChE has been reported to be diminished after chlorpyrifos exposure in rats.[16–20] In addition, erythrocytic AChE has a long recovery time (82 days),[21] low intraindividual variability,[22] and is considered to be a stable assessment of past exposures to cholinesterase inhibitors,[23] although it is less sensitive and specific than most pesticide quantification methods.[23,24] Finally, we previously found that a single measure of AChE activity can be an adequate indicator of pesticide exposure in epidemiologic studies of children.[25]

We hypothesized that lower AChE activity would be associated with lower neurodevelopment (particularly attention, inhibitory control, and memory) with stronger associations among boys. We tested these hypotheses among children living in floricultural communities in the Ecuadorian Andes in The Secondary Exposure to Pesticides Among Infants, Children and Adolescents (ESPINA: Estudio de la Exposición Secundaria a Plaguicidas en Infantes, Niños y Adolescentes) study.

METHODS

Study Description

The ESPINA study enrolled residents of Pedro Moncayo County, Pichincha, Ecuador. This county has substantial floricultural activity, with a production area of ~1800 hectares (5.3% of the county's surface area),[26] and employs ~21% of adults.[25] Organophosphates are among the most frequently used insecticides in the Ecuadorian floricultural industry[27] and account for a substantial proportion of intoxications in Ecuador.[28]

In 2008, we examined 313 children between 4 and 9 years of age who comprised new volunteers (27%) and a subset of children who participated in the 2004 Survey of Access and Demand of Health Services in Pedro Moncayo County conducted by Fundacion Cimas del Ecuador in colaboration with community members (73%). The 2004 survey was representative of Pedro Moncayo County and obtained information regarding 71% of the county's population. Detailed participant recruitment information has been described elsewhere.[25] The current analysis includes 307 (98%) children who had information of all neurobehavioral outcomes of interest.

Informed consent of surveyed parents was obtained, in addition to parental permission for participation of each of their selected children and child assent of participants aged ≥7 years. This study was approved by the institutional review boards of Fundacion Cimas del Ecuador and the University of Minnesota.

Measures

Children's parents and other adult residents were interviewed at home to obtain socioeconomic status, demographic characteristics, and pesticide exposure information of household members. Children were examined in 7 schools of Pedro Moncayo County during the summer months when school was out of session, to ensure a quiet, familiar, and child-friendly environment that was easily accessible.

Examiners were unaware of participants' exposure status. Children's height was measured to the nearest 1 mm, using a height board and following recommended procedures.[29] Erythrocytic AChE and hemoglobin concentrations were measured by using the EQM Test-mate ChE Cholinesterase Test System 400 (EQM AChE Erythrocyte Cholinesterase Assay Kit 470; EQM Research, Inc, Cincinnati, OH) from a single finger-stick blood sample, following standard procedures.[30]

Neurobehavioral development was assessed by using the NEPSY-II test[31] by trained examiners. We conducted a general assessment battery of examinations that included 11 age-appropriate subtests in 5 domains: attention and executive functioning (subtests: auditory attention and response set [AARS], inhibition, statue), language (comprehension of instructions, speeded naming), memory and learning (memory-for-faces immediate and delayed, narrative memory), sensorimotor (visuomotor precision), and visuospatial processing (design copying, geometric puzzles). Descriptions of each subtest can be found in Supplemental Table 4 and elsewhere.[32,33] Most children completed the general assessment battery within 50 to 80 minutes. The NEPSY-II assesses different subtests or different items within a subtest at different ages, which explains the varying sample sizes for each subtest. Table 1 displays the age range of children in our study who were assessed by using each subtest.

Three subtests required translation into Spanish (AARS, comprehension of instructions, and narrative memory). Translation of the NEPSY test has been found to be relatively unaffected by language and culture.[7,34,35]

Participants were examined alone except in cases in which the child experienced separation anxiety from their parents. In such cases (5 participants), 1 relative was allowed to be in the examination room and was instructed to remain silent and to sit between 2 and 4 m away and outside of the child's line of sight.

The distance of homes to the nearest flower plantation was calculated by using ArcGIS 9.3 (Esri, Redlands, CA) from geographical coordinates obtained from portable global positioning system receivers. Detailed data collection information has been described elsewhere.[25]

Statistical Analysis

To include all children in multivariable analyses, we created a "missing" race category to account for 14 children with missing information. In addition, we imputed information of maternal education, income, and residential distance to the nearest flower plantation. Maternal education was imputed for 15 children with missing information based on the household head's education in 2004 and for 3 children not examined in 2004. We entered values from a random normal distribution variable based on maternal education mean ± standard deviation (SD) values. Income was imputed for 17 children according to 3 variables significantly associated with income (maternal education, type of housing in 2004 [ie, house, apartment, room, shack], and building materials in 2004 [ie, brick, adobe, wood]) and for 4 children not examined in 2004 from

TABLE 1 Adjusted Linear Differences of Neurobehavioral Development Scores per U/mL (~2 SD) Decrease of AChE Activity Among Children

N	Age	Neurodevelopment Score	Score Difference (95% CI) per AChE U/mL Decrease
307	4–9	Total neurodevelopment summary[a]	−0.29 (−0.76 to 0.18)
		Boys, *n* = 158	−0.70 (−1.36 to −0.04)*
		Girls, *n* = 149	0.17 (−0.53 to 0.88)
301	5–9	Attention and executive functioning domain[b]	−0.32 (−1.02 to 0.38)
		Boys, *n* = 155	−0.82 (−1.82 to 0.19)**
		Girls, *n* = 146	0.33 (−0.66 to 1.31)
239	5–9	AARS–auditory attention[a]	−0.14 (−1.29 to 1.01)
126	7–9	AARS–response set[a]	−0.99 (−2.48 to 0.51)
		Boys, *n* = 66	−1.88 (−4.15 to 0.39)**
		Girls, *n* = 60	−0.09 (−2.52 to 2.35)
231	5–9	IN-IN trial[b,c]	−1.18 (−2.22 to −0.13)*
		Boys, *n* = 120	−2.56 (−4.13 to −1.00)*
		Girls, *n* = 111	0.17 (−1.28 to 1.62)
99	7–9	IN-switching trial[a,c]	−0.85 (−2.24 to 0.54)
169	4–6	Statue[a]	0.16 (−1.04 to 1.36)
304	4–9	Memory and learning domain[a]	−0.28 (−0.92 to 0.36)
		Boys, *n* = 156	−0.76 (−1.66 to 0.15)**
		Girls, *n* = 148	0.30 (−0.61 to 1.20)
247	5–9	Memory-for-faces immediate[a]	−0.37 (−1.32 to 0.59)
245	5–9	Memory-for-faces delayed[a,d]	−0.45 (−1.46 to 0.56)
		Boys, *n* = 127	−0.32 (−1.70 to 1.05)
		Girls, *n* = 118	−0.51 (−2.02 to 0.99)
304	4–9	Narrative memory[a]	−0.12 (−0.95 to 0.70)
307	4–9	Visuospatial processing domain[a]	−0.29 (−1.22 to 0.63)
		Boys, *n* = 158	−0.37 (−1.66 to 0.92)
		Girls, *n* = 149	−0.26 (−1.64 to 1.13)
296	4–9	Design copying[a]	−0.74 (−2.24 to 0.75)
306	4–9	Geometric puzzles[a]	−0.05 (−1.01 to 0.92)
306	4–9	Language domain[a]	−0.39 (−1.11 to 0.32)
		Boys, *n* = 157	−0.76 (−1.75 to 0.24)
		Girls, *n* = 149	0.06 (−1.11 to 0.99)
306	4–9	Comprehension of instructions[a]	−0.10 (−0.96 to 0.76)
242	5–9	Speeded naming[a]	−0.81 (−1.84 to 0.22)
		Sensorimotor domain	—
303	4–9	Visuomotor precision[a]	0.34 (−0.70 to 1.38)

Adjustments were made for age, gender, race, height-for-age *z* score, income, maternal education, flower worker cohabitation status, and hemoglobin concentration.

[a] AChE–gender interaction: $P > .10$.

[b] AChE–gender interaction: $P < .01$.

[c] Additionally adjusted for IN-naming score.

[d] Additionally adjusted for memory-for-faces immediate.

* $P < .05$.

** $P = .05$ to $.10$.

values of a random normal distribution variable based on income mean ± SD values. Residential distance to the nearest flower plantation was imputed for 3 children by entering values from a random normal distribution variable.

Participant characteristics according to erythrocytic AChE tertiles and analytical models that included AChE activity were adjusted for hemoglobin concentration to account for varying red blood cell compositions of blood. These variations could alter the values of erythrocytic AChE activity.

Associations between AChE and neurodevelopment were analyzed by using logistic models (dichotomous and polychotomous) and linear regression models in SAS version 9.2 (SAS Institute, Inc, Cary, NC). We assessed effect modification according to gender among statistically significant associations by testing an interaction term and through stratification followed by formal statistical testing of the difference of the stratified coefficients.

Associations between AChE and neurodevelopmental measures were assessed by using a model defined a priori that included hemoglobin and potential confounders (age, gender, race, height-for-age *z* score [to estimate long-term nutritional status], household income, maternal education, and flower worker cohabitation status). We adjusted for flower worker cohabitation status to control for potential secondary occupational exposures to other chemicals that might affect neurodevelopment but that do not inhibit AChE activity.

Subtest-scaled scores were calculated by using the NEPSY-II scoring assistant software (NCS Pearson Inc, San Antonio, TX). Most subtest scores consisted of the available subtests' primary scaled score, which are age-adjusted values based on a national normative sample of US children.[32] For subtests that included time and error components (ie, inhibition, speeded naming, visuomotor precision) and correct and error components (ie, auditory attention and response set), we used the combined scaled scores (scores that combined both components) as primary scaled scores. In subtests that included >1 subtest component and provided >1 primary scaled score (ie, AARS, inhibition, word list interference), each component primary score was analyzed separately (ie, for AARS: AARS–auditory attention and AARS–response set; for inhibition: inhibition-naming [IN-naming] trial, inhibition-inhibition [IN-inhibition] trial, inhibition-switching [IN-switching] trial). We excluded IN-naming trial from the analyses because this part of the test only assesses children's ability to name a set of figures and does not by itself reflect abilities in inhibitory control. We calculated domain scores as the average of 1 primary scaled score per subtest within a domain. In subtests with >1 primary scaled score, we used the average of such scores. We did not calculate a score for sensorimotor domain given that it comprised only 1 subtest (visuomotor precision). We also calculated a total neurodevelopmental summary score, which was the average of the primary scores of all 11 subtests.

Subtest or domain scores <6 (<9th percentile of the NEPSY-II normative sample, which corresponds to a classification of "below and well below expected level")[32] were defined as low. To increase the stability of the statistical models, we set the cutoff of low scores to 6.2 for total neurodevelopment summary given the low proportion of children with scores <6 (6%); for AARS–response set, we set the cutoff to 8 (<25th percentile; NEPSY-II classification: borderline, below, and well below expected level) considering the subtest's small sample size (n = 126) and prevalence of scores <6 (13%).

When IN-inhibition and IN-switching were outcomes, we adjusted for IN-naming because the performance in the inhibition subtest depends on the child's ability to name figures. For similar reasons, memory-for-faces delayed was adjusted for memory-for-faces immediate.

RESULTS

Participant Characteristics

Participants had a mean ± SD age of 6.6 ± 1.6 years; 52% were male, 76% mestizo, and 55% lived with a flower worker. Boys and girls had similar hemoglobin-adjusted AChE activity. Participants' characteristics according to gender and AChE tertiles are listed in Table 2. Neurodevelopment scores in our sample were lower but with similar variability than those of the NEPSY-II normative sample, which were designed to have a mean of 10 ± 3 for each subtest. Only 4 of 11 subtests had equivalent or higher mean scores than the normative sample: statue, narrative memory, design copying, and visuomotor precision. Girls had higher mean scores than boys in all but 1 of the subtests within the attention and executive functioning domain, but only attention and executive functioning domain and AARS–auditory attention scores were significantly different. The prevalence data for low neurodevelopment scores are listed in Table 3.

AChE and Neurobehavioral Development

In linear regression analyses, AChE activity had positive (although mostly nonsignificant) associations with all neurodevelopmental domains and subtests except with statue and visuomotor precision (nonsignificant and negative associations) (Table 1). There was significant effect modification according to gender in the

TABLE 2 Children's Characteristics According to Gender and AChE Activity

Characteristic	All	Gender			AChE Tertiles[a]			
		Boys	Girls	$P_{Difference}$	1st	2nd	3rd	P_{Trend}
N	307	158	149		104	101	102	
AChE range	1.44 to 4.69	1.44 to 4.56	1.84 to 4.69		1.44 to 2.93	2.94 to 3.32	3.33 to 4.69	
Demographic and SES characteristics								
Age, y	6.60 ± 1.58	6.63 ± 1.55	6.57 ± 1.62	.72	6.39 ± 1.61	6.38 ± 1.50	7.03 ± 1.66	.001
Gender, male	52	—	—	—	46	50	59	.12
Race, mestizo	76	76	76	.91	75	77	76	.94
Race, indigenous	22	21	23	.67	22	22	23	.99
Monthly income[b]	3.11 ± 0.82	3.09 ± 0.86	3.12 ± 0.77	.74	3.09 ± 0.89	3.14 ± 0.83	3.09 ± 0.92	.92
Maternal education, y	7.28 ± 3.85	7.02 ± 3.62	7.54 ± 4.07	.24	6.81 ± 4.14	7.33 ± 3.86	7.69 ± 4.28	.32
Flower worker cohabitation	55	55	54	.90	67	53	45	.03
Residential distance to nearest flower plantation, m	448 ± 343	438 ± 352	458 ± 334	.60	360 ± 363	484 ± 338	501 ± 376	.01
Anthropometric and blood measurements								
Height-for-age *z* score	−1.24 ± 0.96	−1.33 ± 0.99	−1.15 ± 0.93	.12	−1.34 ± 1.02	−1.27 ± 0.95	−1.11 ± 1.06	.36
AChE, U/mL[d]	3.14 ± 0.49	3.15 ± 0.50	3.13 ± 0.47	.81	2.63 ± 0.27	3.13 ± 0.11	3.67 ± 0.29	—
Hemoglobin, g/L[d]	126.4 ± 11.6	125.5 ± 11.2	127.5 ± 11.9	.13	119.8 ± 9.3	125.0 ± 7.4	134.7 ± 12.0	<.001
Neurobehavioral development[c]								
Total neurodevelopment summary	8.57 ± 1.61	8.43 ± 1.66	8.72 ± 1.55	.11	8.46 ± 1.74	8.64 ± 1.62	8.61 ± 1.80	.26
Attention and executive functioning domain (*n* = 301)	8.53 ± 2.46	8.13 ± 2.52	8.95 ± 2.34	.004	8.38 ± 2.62	8.81 ± 2.44	8.40 ± 2.71	.89
AARS–auditory attention (*n* = 239)	8.19 ± 3.43	7.64 ± 3.25	8.79 ± 3.53	.009	8.10 ± 3.65	8.58 ± 3.45	7.96 ± 3.75	.80
AARS–response set (*n* = 126)	8.87 ± 3.13	8.36 ± 3.44	9.42 ± 2.68	.06	8.13 ± 3.44	9.12 ± 3.21	9.08 ± 3.47	.15
IN-naming trial (*n* = 238)	7.08 ± 3.66	6.77 ± 3.88	7.43 ± 3.39	.16	7.14 ± 3.92	7.01 ± 3.72	7.1 ± 4.03	.88
IN-inhibition trial (*n* = 231)	7.08 ± 3.11	6.85 ± 3.29	7.32 ± 2.89	.25	6.33 ± 3.26	6.79 ± 3.07	7.83 ± 3.32	.07
IN-switching trial (*n* = 99)	7.06 ± 2.59	7.49 ± 2.90	6.64 ± 2.19	.10	6.52 ± 2.87	6.93 ± 2.65	7.39 ± 2.85	.16
Statue (*n* = 169)	10.21 ± 2.86	9.81 ± 3.10	10.61 ± 2.55	.07	10.14 ± 3.02	10.37 ± 2.88	10.07 ± 3.12	.78
Memory and learning domain (*n* = 304)	8.83 ± 2.10	8.74 ± 2.22	8.92 ± 1.98	.46	8.64 ± 2.27	8.97 ± 2.11	8.87 ± 2.34	.60
Memory-for-faces immediate (*n* = 247)	7.47 ± 2.80	7.22 ± 3.04	7.73 ± 2.50	.15	7.21 ± 2.98	7.8 ± 2.83	7.39 ± 3.07	.56
Memory-for-faces delayed (*n* = 245)	8.73 ± 2.98	8.61 ± 3.09	8.85 ± 2.86	.54	8.44 ± 3.17	8.48 ± 3.01	9.15 ± 3.27	.20
Narrative memory (*n* = 304)	9.68 ± 2.73	9.72 ± 2.71	9.64 ± 2.76	.81	9.54 ± 2.95	9.80 ± 2.75	9.70 ± 3.05	.70
Visuospatial processing domain (*n* = 307)	9.56 ± 3.09	9.50 ± 3.19	9.63 ± 3.00	.72	9.31 ± 3.31	9.79 ± 3.08	9.59 ± 3.42	.27
Design copying (*n* = 296)	10.65 ± 4.92	10.39 ± 4.88	10.92 ± 4.97	.36	10.06 ± 5.27	10.49 ± 4.86	11.42 ± 5.45	.06
Geometric puzzles (*n* = 306)	8.57 ± 3.21	8.67 ± 3.43	8.47 ± 2.96	.60	8.51 ± 3.44	9.13 ± 3.21	8.09 ± 3.56	.72
Language domain (*n* = 306)	6.64 ± 2.42	6.62 ± 2.48	6.67 ± 2.37	.84	6.44 ± 2.61	6.77 ± 2.44	6.72 ± 2.70	.14
Comprehension of instructions (*n* = 306)	7.36 ± 2.96	7.24 ± 3.09	7.48 ± 2.82	.49	7.15 ± 3.19	7.58 ± 2.98	7.34 ± 3.30	.24
Speeded naming (*n* = 242)	5.91 ± 3.01	5.99 ± 2.93	5.81 ± 3.11	.64	5.76 ± 3.19	5.64 ± 3.04	6.24 ± 3.30	.13
Sensorimotor domain	—	—	—	—	—	—	—	—
Visuomotor precision (*n* = 303)	9.89 ± 3.33	9.67 ± 3.28	10.13 ± 3.37	.23	10.29 ± 3.58	9.40 ± 3.33	9.97 ± 3.70	.71

Unless otherwise noted, data are presented as % or mean ± SD. SES, socioeconomic status.

[a] Least-squares means adjusted for hemoglobin concentration.

[b] Monthly income categories (US $): 1 = 0 to 50; 2 = 51 to 150; 3 = 151 to 300; 4 = 301 to 500; 5 = 501 to 1000; and 6 = >1000.

[c] Subtest sample size varies according to the age range it was designed to test. Table 1 presents additional details.

[d] Not adjusted for hemoglobin concentration when stratified according to AChE tertiles.

associations between AChE activity and attention and executive functioning domain (P = .01) and AARS–response set (P = .002). Lower AChE activity was associated with lower total neurodevelopmental summary score among boys (–0.70 points per 1-U/mL [~2 SD] AChE decrease [95% confidence interval (CI): –1.36 to –0.04]) but not girls (0.17 point per 1-U/mL decrease [95% CI: –0.53 to 0.88]) (Fig 1, Table 1). Within attention and executive functioning domain, lower AChE activity was associated with lower IN-inhibition trial score (assessment of inhibitory control) in boys (–2.56 points per 1-U/mL decrease [95% CI: –4.13 to –1.00]) but not in girls (0.17 point per 1-U/mL decrease [95% CI: –1.28 to 1.62]). The association between AChE activity and language domain was also stronger in boys, although not statistically significant. Figures 1 and 2 depict the associations between sextiles of AChE activity and select neurodevelopment scores.

In logistic regression analyses, the associations between AChE activity and low neurodevelopment score were positive and stronger in boys than in girls for 4 outcomes (Table 3): (1) the total neurodevelopmental summary; (2) the attention and executive functioning domain and its subtests (IN-inhibition trial and AARS–response set [assessment of complex attention and inhibitory control]); (3) the memory and learning domain score and its subtest: memory-for-faces delayed

TABLE 3 Adjusted Associations Between AChE Activity and Low Neurodevelopment Scores Among Children

Low[a] Neurodevelopment (Dependent Variable)				AChE Activity (Independent Variable)		
				Continuous	Tertiles	
N	Age	Neurodevelopment Score	% Low Score	OR (95% CI) per SD Decrease	OR (95% CI) Low Versus High	OR (95% CI) Middle Versus High
307	4–9	Total neurodevelopment summary[a]	7[a]	1.34 (0.72 to 2.48)	1.17 (0.30 to 4.52)	1.11 (0.32 to 3.79)
		Boys, *n* = 158	9[a]	2.49 (1.06 to 5.88)*	5.14 (0.84 to 31.48)**	1.37 (0.23 to 8.25)
		Girls, *n* = 149	6[a]	0.59 (0.21 to 1.69)	0.19 (0.02 to 2.26)	0.96 (0.13 to 6.97)
301	4–9	Attention and executive functioning domain	14	1.70 (1.09 to 2.65)*	1.86 (0.70 to 4.93)	0.88 (0.33 to 2.31)
		Boys, *n* = 155	18	2.33 (1.30 to 4.18)*	4.55 (1.19 to 17.38)*	1.46 (0.40 to 5.28)
		Girls, *n* = 146	10	0.87 (0.40 to 1.90)	0.48 (0.09 to 2.51)	0.46 (0.10 to 2.51)
239	5–9	AARS–auditory attention	23	1.02 (0.68 to 1.55)	0.69 (0.28 to 1.72)	0.52 (0.22 to 1.24)
126	7–9	AARS–response set[b]	35[b]	1.55 (0.91 to 2.65)	2.37 (0.70 to 8.03)	1.90 (0.67 to 5.41)
		Boys, *n* = 66	44[b]	2.04 (0.94 to 4.46)**	5.94 (0.99 to 35.73)**	8.07 (1.47 to 44.42)*
		Girls, *n* = 60	25[b]	1.14 (0.42 to 3.06)	1.49 (0.16 to 13.62)	0.49 (0.09 to 2.68)
231	5–9	IN-inhibition trial[c,d]	33	1.40 (0.95 to 2.06)**	3.04 (1.28 to 7.25)*	2.08 (0.95 to 4.56)**
		Boys, *n* = 120	38	1.78 (1.05 to 3.03)*	6.33 (1.80 to 22.28)*	5.26 (1.74 to 15.86)*
		Girls, *n* = 111	29	1.09 (0.57 to 2.11)	1.71 (0.47 to 6.26)	0.69 (0.20 to 2.45)
99	7–9	IN-switching trial[c,d]	23	0.94 (0.47 to 1.89)	0.82 (0.17 to 3.85)	0.57 (0.15 to 2.12)
169	4–6	Statue	14	1.09 (0.61 to 1.96)	0.52 (0.12 to 2.22)	0.36 (0.09 to 1.49)
304	4–9	Memory and learning domain	10	2.12 (1.25 to 3.59)*	3.49 (1.03 to 11.83)*	1.61 (0.49 to 5.25)
		Boys, *n* = 156	12	3.76 (1.62 to 8.76)*	6.03 (1.17 to 31.05)*	1.69 (0.33 to 8.64)
		Girls, *n* = 148	7	1.10 (0.45 to 2.68)	1.91 (0.25 to 14.51)	1.85 (0.26 to 13.08)
247	5–9	Memory-for-faces immediate	25	1.21 (0.81 to 1.89)	1.63 (0.67 to 3.97)	1.27 (0.57 to 2.85)
245	5–9	Memory-for-faces delayed[e]	35	1.32 (0.92 to 1.90)	2.29 (1.01 to 5.17)*	2.23 (1.05 to 4.74)*
		Boys, *n* = 127	35	1.75 (1.02 to 2.99)*	3.30 (0.98 to 11.10)**	3.50 (1.19 to 10.26)*
		Girls, *n* = 118	34	1.10 (0.65 to 1.87)	1.67 (0.53 to 5.28)	1.18 (0.37 to 3.73)
304	4–9	Narrative memory	7	1.00 (0.52 to 1.90)	0.82 (0.20 to 3.39)	1.01 (0.29 to 3.51)
307	4–9	Visuospatial processing domain	11	0.76 (0.47 to 1.23)	0.37 (0.13 to 1.05)**	0.52 (0.20 to 1.33)
		Boys, *n* = 158	13	0.79 (0.35 to 1.78)	0.36 (0.09 to 1.44)	0.47 (0.14 to 1.57)
		Girls, *n* = 149	10	0.80 (0.42 to 1.51)	0.43 (0.08 to 2.45)	0.68 (0.14 to 3.32)
296	4–9	Design copy	17	1.07 (0.69 to 1.65)	0.75 (0.28 to 2.01)	0.94 (0.39 to 2.31)
306	4–9	Geometric puzzles	17	0.98 (0.62 to 1.55)	0.57 (0.22 to 1.52)	0.48 (0.18 to 1.23)
307	4–9	Language domain	32	1.38 (0.97 to 1.96)**	1.76 (0.81 to 3.82)	1.04 (0.51 to 2.15)
		Boys, *n* = 158	34	1.52 (0.93 to 2.49)**	1.80 (0.60 to 5.36)	1.05 (0.39 to 2.85)
		Girls, *n* = 149	30	1.32 (0.78 to 2.25)	1.70 (0.53 to 5.42)	1.04 (0.34 to 3.14)
306	4–9	Comprehension of instructions	26	1.12 (0.77 to 1.62)	1.04 (0.46 to 2.33)	0.75 (0.35 to 1.64)
242	5–9	Speeded naming	44	1.14 (0.80 to 1.62)	1.27 (0.59 to 2.675)	0.95 (0.47 to 1.91)
		Sensorimotor domain		—	—	—
303	4–9	Visuomotor precision	17	0.87 (0.57 to 1.32)	0.58 (0.23 to 1.50)	0.92 (0.40 to 2.10)

Adjustments were made for age, gender, race, height-for-age *z* score, income, maternal education, flower worker cohabitation status, and hemoglobin concentration.

[a] Low scaled score definition: <6 (<9th percentile of the NEPSY-II normative sample).

[b] Low scaled score definition: <6.2.

[c] Low scaled score definition: <8 (<25th percentile of the NEPSY-II normative sample).

[d] Additionally adjusted for IN-naming score.

[e] Additionally adjusted for memory-for-faces immediate.

* $P < .05$.

** $P = .05$ to .09.

[assessment of long-term memory]); boys also had a stronger, although nonsignificant, positive association with memory-for-faces immediate (assessing short-term memory [results not shown]); and (4) the language domain (borderline association).

AChE activity in the lowest tertile (versus highest) had ORs for low neurodevelopment among boys that were substantial (between 3.30 and 6.33) (Table 3) for all scores in the first 3 outcomes discussed in the previous paragraph. There was a borderline inverse association between AChE and low visuospatial processing domain (OR: 0.37); however, the association was only observed in analyses of AChE in tertiles among all participants.

Overall, the associations between AChE activity and neurodevelopment were negligibly different when flower worker cohabitation status was not included in the adjustment model.

DISCUSSION

Lower AChE activity was associated with overall lower neurobehavioral development, primarily affecting

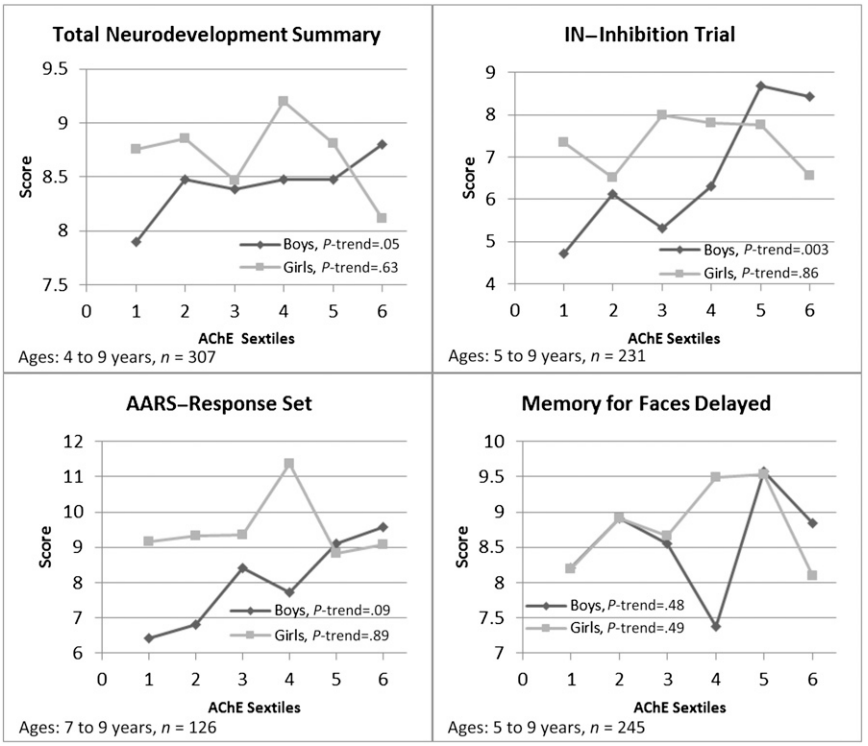

FIGURE 1

Associations between AChE activity and total neurodevelopment summary and select subtests. Adjusted for age, gender, race, height-for-age *z* score, income, maternal education, flower worker cohabitation status, and hemoglobin concentration. IN-inhibition is further adjusted for IN-naming, and memory-for-faces delayed is further adjusted for memory-for-faces immediate. Sample size varies according to the age range that each subtest was designed to assess.

attention, inhibitory control, and memory (and perhaps language) among apparently healthy boys but not girls. Considering that the cholinergic system plays an important role in brain development,[36,37] and subclinically lower AChE activity has been associated with measureable physiologic changes in children,[38] our findings support the hypothesis that AChE inhibition is an important mechanism of neurotoxicity of cholinesterase inhibitor pesticides. Chlorpyrifos has been reported to inhibit brain AChE activity in rats,[16–19] with significant AChE inhibition in the hippocampus,[20] a structure with important roles in memory, spatial navigation, imagination, and perhaps inhibitory control.[39–41] However, AChE inhibition is an indicator of exposure, and other neurotoxic mechanisms may also be present.

Two other studies also reported the gender interaction observed in our study. Higher prenatal organophosphate exposure was associated with greater decreases in attention among boys versus girls in Mexican-American children in agricultural areas in California[5] and in working memory among African-American and Dominican children in New York City.[9] Animal studies have also reported greater detrimental neurochemical and behavioral effects, including working memory, among males after early postnatal exposure to chlorpyrifos.[19,20,42,43] The reason for this gender interaction is not understood and is a topic for future research.

Diminished attention or inhibitory control may affect a child's capacity to plan and concentrate, resulting in poor school performance.[32] Inhibitory control is the ability to abstain from engaging in an inviting or automatic behavior (eg, resisting eating the candy at arm's reach while visiting a candy store) and is considered to be the primary deficit in ADHD.[44,45] Children with ADHD were found to perform significantly worse than controls in the subtests of inhibition and auditory attention and response set, with greater difficulties in the AARS–response set component[32] (a similar pattern to our findings).

Although statistical models for many neurodevelopmental outcomes were conducted, the observed positive associations between AChE and attention, inhibitory control, and memory in boys are not subject to multiple comparison concerns because we hypothesized this (defined associations a priori) based on results of other cohorts.[5,9] These associations are strengthened by their internal consistency; boys had positive associations between AChE and the domains (and subtests within each domain) of attention and executive functioning and

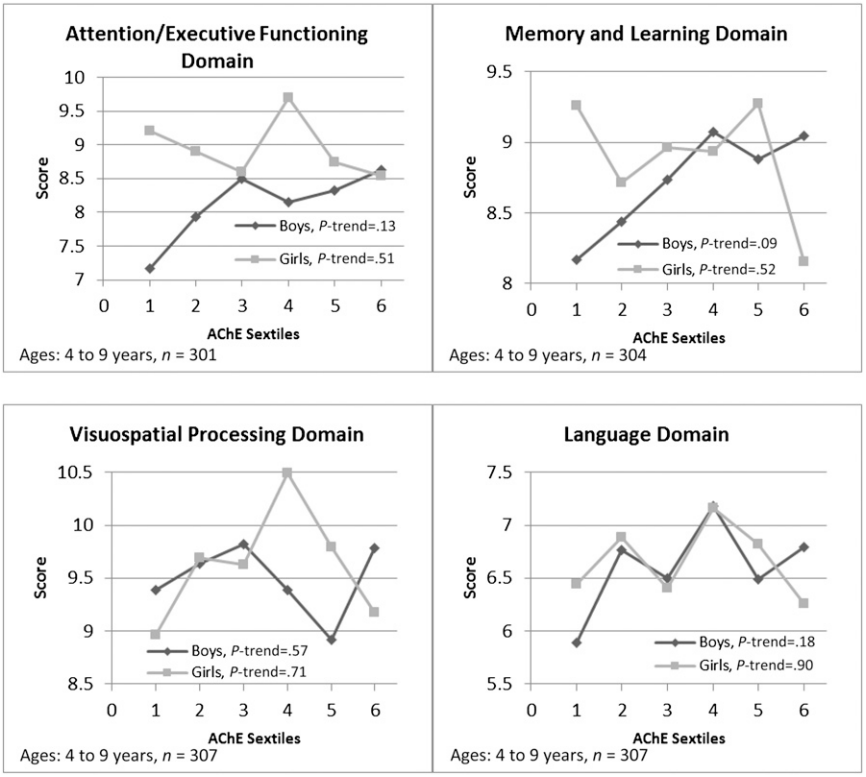

FIGURE 2
Associations between AChE activity and domain scores of neurobehavioral development. Adjusted for age, gender, race, height-for-age *z* score, income, maternal education, flower worker cohabitation status, and hemoglobin concentration.

memory and learning. The observed borderline associations between AChE and language or visuospatial processing domains were not hypothesized in advance and are subject to concerns regarding multiple comparisons.

The amount of pesticides reaching ESPINA study children in 2008 was small enough to not elicit clinical manifestations and was possibly similar to those of US children. Urinary organophosphate metabolite concentrations among children in this area were comparable to those of the National Health and Nutrition Examination Survey,[46,47] and the mean AChE activity in our study population for children living with flower workers (3.08 U/mL) and nonagricultural workers (3.20 U/mL) was consistent with mean levels reported for Hispanic children living in agricultural (3.0 U/mL) and nonagricultural (3.1 U/mL) families in Oregon.[48] The higher AChE values of ESPINA children possibly reflect the older age of our participants (4–9 years vs 3–6 years). In the ESPINA study, age was positively associated with AChE activity.[25]

ESPINA study participants had lower neurodevelopment scores in most subtests than the NEPSY-II normative sample. Explanations for this finding can include differences in cultural and social practices,[49] test-taking abilities, nutrition, and environmental exposures between children of Pedro Moncayo County and the United States.

Limitations of the current study include constrained power to adequately assess associations stratified according to gender and a lack of concurrent and prenatal environmental biomarkers of exposure and historical nutritional information. However, we did control for chronic malnutrition and concurrent anemia by adjusting for height-for-age and hemoglobin concentration. In addition, the cross-sectional nature of our findings hinders the assessment of chronic AChE inhibition on neurodevelopment. At minimum, our findings provide insight on acute AChE inhibition and its short-term association with neurodevelopment. Whether boys' neurodevelopmental performance would improve after full recovery of their AChE activity is unknown. However, we cannot exclude that the observed differences may be due to chronic AChE inhibition considering that AChE measurements are stable and could reflect long-term exposure to pesticides in this population because: (1) AChE has a long recovery time of ~3 months after irreversible inhibition[21]; (2) the sources of secondary pesticide exposures remain relatively constant (eg, distance of homes to agricultural farms, consumption of pesticide-exposed produce, cohabitation with agricultural workers);

and (3) agricultural production and pesticide use are maintained throughout the year because of Ecuador's minimal seasonal temperature changes. It is likely that most children in our study were exposed to pesticides since birth or prenatally, given that most have always lived in agricultural communities and 40% were born into flower workers' families.

An alternate interpretation of our findings is that children with neurodevelopmental delays have lower brain AChE activity due to disrupted brain growth, independent of environmental exposures. This finding seems less likely, however, given that the influence that brain development has on red blood cell AChE activity is probably very small.

CONCLUSIONS

Lower AChE activity, reflecting organophosphate and carbamate pesticide exposure, was associated with lower performance on attention, inhibitory control, and memory in boys. These are critical cognitive skills that affect learning and academic performance. Our findings suggest that boys have greater sensitivity than girls for neurodevelopmental delays from subclinical pesticide exposures.

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