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Associations of acetylcholinesterase activity with depression and anxiety symptoms among adolescents growing up near pesticide spray sites

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Abstract

Background: The cholinergic system has an important role in mood regulation. Cholinesterase inhibitor pesticides (e.g. organophosphates) appear to increase depression and anxiety symptoms in the few existing animal and human studies. Human studies have not described such associations using biomarkers of exposure and studies among children are needed.

Methods: Methods: We studied 529 adolescents (ages 11-17y) in agricultural communities in the Ecuadorian Andes (ESPINA study). Acetylcholinesterase (AChE) activity was measured in a finger-stick sample. Anxiety and depression symptoms were assessed using the CDI-2 and MASC-2 (greater scores reflect greater internalizing symptoms). Models adjusted for age, gender, hemoglobin, income among others.

Results: The median age was 14.38y and 51% were female. The mean (SD) of the following parameters were: AChE 3.7 U/mL (0.55), depression T-score 53.0 (9.4) and anxiety T-score: 57.6 (9.8). Lower AChE activity (reflecting greater cholinesterase inhibitor exposure) was associated with higher depression symptoms (difference per SD *decrease* of AChE [β [95% CI:]]: 1.09 [0.02, 2.16]), was stronger among girls (β=1.61) than boys (β=0.69), and among younger (<14.38y, β= 1.61) vs. older children (β=0.57). The associations were strongest among girls <14.38y (β=3.30 [0.54, 6.05], OR for elevated symptoms per SD decrease in AChE= 2.58 [1.26, 5.27]). No associations were observed with anxiety scores. Analyses of AChE change between 2008 and 2016 concurred with these findings.

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Declarations of Interest: None

Conflicts of Interest: The authors declare no conflict of interest.

Discussion: We observed associations between a biomarker of pesticide exposure and children's depression symptoms. Lower AChE activity may create risk for depression in teenagers, particularly among girls during early adolescence.

Keywords

Depression; anxiety; pesticides; agriculture; adolescents; Ecuador

1. Introduction

According to multiple reports, rates of depression and anxiety among adolescents have rapidly increased in the US and internationally over the past 2 decades (Mojtabai et al., 2016; World Health Organization, 2017a). Suicide is one of the leading causes of death worldwide and has been associated with various psychiatric diseases, including depression. Suicidal behavior has increased 60% globally over the past 45 years (Kim et al., 2011; World Health Organization, 2017b, 2000). Insecticides are well established neurotoxicants; pre- and postnatal exposures to organophosphates and have been linked to neurobehavioral and cognitive delays in children (Bouchard et al., 2011; Eskenazi et al., 2014, 2007; Kofman et al., 2006; Marks et al., 2010; Rauh et al., 2006; Rohlman et al., 2016). However, little is known about the associations with other mental health components in adolescents.

The use of cholinesterase inhibitor insecticides, including organophosphates and carbamates, is ubiquitous worldwide (Alavanja, 2009). Their lethal mechanism of action is the inhibition of acetylcholinesterase (AChE) activity (Kwong, 2002) leading to acetylcholine overstimulation of muscarinic and nicotinic receptors (Aaron CK, 2007; Abou-Donia, 2003). Cholinesterase inhibitors can also induce direct toxicity to neurons and glia, especially during periods of rapid neurodevelopment (Abou-Donia, 2003; Aldridge et al., 2005b; Qiao et al., 2003; Slotkin, 2004).

Studies of agricultural workers and residents living in agricultural areas have described positive associations between exposures to pesticides (mostly organophosphates) with depression (Beard et al., 2014; Beseler et al., 2008; Freire and Koifman, 2013; London et al., 2012, 2005; Malekirad et al., 2013; Meyer et al., 2010; Weisskopf et al., 2013) and depressive symptoms (Beseler and Stallones, 2008; Wesseling et al., 2010) in populations in Latin America, Europe and USA. Most studies assessed depression using self-reports of diagnosis, and exposure by history of pesticide poisoning (Freire and Koifman, 2013). Exposure to high levels of pesticides in rural residents and agricultural workers have also been observed to result in increased number of suicides and suicide attempts. (Freire and Koifman, 2013; London et al., 2012, 2005; Pearce et al., 2007). To our knowledge, no human studies have used biomarkers to assess pesticide exposures in relation to depression and anxiety. Consequently, the absence of precise measures may result in exposure misclassification, threatening the validity of findings. There is a need to estimate environmental determinants of mental health conditions in adolescents because adolescence is a critical period for the onset of internalizing disorders that continue into adulthood, (Beesdo et al., 2009; Paus et al., 2008). Cholinesterase inhibitors used in clinical practice to treat glaucoma and dementia, (i.e. physostigmine or donepezil) have been associated with

increased behaviors of anxiety and depression in mice (Risch et al., 1981) and with borderline personality disorder (Steinberg et al., 1997) or mild cognitive impairment in humans (Reynolds et al., 2011). Conversely, depression symptoms have been noted to improve in depressed patients with Alzheimer's disease who receive cholinesterase inhibitor medication (Rozzini et al., 2007).

Few studies have tested the associations between pesticide exposures and anxiety. Among adult farmers in Iran (Malekirad et al., 2013) and sheep farmers in the United Kingdom with a history of low-level exposure to organophosphate pesticides (Harrison and Mackenzie Ross, 2016), concurrent organophosphate exposure was associated with higher anxiety and depression symptoms compared to those with low exposures. Supporting these associations, higher activities of AChE and paraoxonase-1 (an enzyme that metabolizes some organophosphates) were both inversely associated with anxiety in healthy subjects from a representative study in the U.S. (Sklan et al., 2004).

The objective of this study is to test the associations of AChE activity, as a marker of organophosphate and carbamate pesticide exposures, with anxiety and depression symptoms among adolescents living in agricultural communities in the Andes of Ecuador. We hypothesized that AChE activity will be inversely associated with depression and anxiety symptoms among adolescents.

2. Methods

2.1. Participants

ESPINA is a prospective cohort study of children, established in 2008, that examines the associations of subclinical pesticide exposures on child development. We examined 313 boys and girls aged 4-9 years who lived in the agricultural county of Pedro Moncayo, Pichincha province, Ecuador in 2008. Most participants (73%) were recruited using data from the 2004 Survey of Access and Demand of Health Services in Pedro Moncayo County, which was a representative survey of Pedro Moncayo County and procured data regarding 71% of the county's population. This survey was carried out by Fundación Cimas del Ecuador in collaboration with the Local Rural Governments of Pedro Moncayo and community residents. The need to develop this study was defined by the people in participatory assemblies. The remaining participants (27%) were enrolled through community announcements given by governing councils, leaders, and by word-of-mouth. We aimed to recruit a balanced distribution of participants who lived with a flower plantation worker and participants who did not live with any agricultural workers. Participants were accepted if they fulfilled the following criteria: (1) children living with flower workers: cohabitation with a flower plantation worker for at least one year; (2) children living without agricultural workers: never cohabited with an agricultural worker, never inhabited a house where agricultural pesticides were stored and having no previous direct contact with pesticides. Further details have been published (Suarez-Lopez et al., 2012).

In 2016, we carried out a follow-up examination of ESPINA participants (n=245) and recruited new adolescent participants for a total of 545 participants of ages 11-17 years. As

in 2008, new participants were chosen and asked to participate using the System of Local and Community Information (SILC) which is a large geospatial database that contains information of the 2016 Pedro Moncayo County Community Survey (formerly the Survey of Access and Demand of Health Services in Pedro Moncayo County, see above). The present analyses included 529 participants examined in 2016, of which 517 participants completed the Multidimensional Anxiety Scale for Children 2nd Edition (MASC-2) (MHS Inc, North Tonawanda, NY) and 516 participants completed the Children's Depression Inventory 2nd Edition (CDI-2) (MHS Inc, North Tonawanda, NY). Additionally, we included in separate analyses the information of 223 children (91%) who had AChE levels for both 2008 and 2016 and had all covariates and outcome variables of interest.

We acquired informed consent of participation from parents and obtained parental permission and child assent of each of their selected children to participate. This study was approved by the institutional review boards at the University of California San Diego, University of Minnesota, Universidad San Francisco de Quito and the Ministry of Public Health of Ecuador.

2.2. Setting

Pedro Moncayo County is located on the Ecuadorian Andes and has a mean altitude of 2952 m. The floriculture industry is vital to the economy of Pedro Moncayo county, employing 21% of all adults in the county (Suarez-Lopez et al., 2012) and using 5.3% of the geographic area (1800 hectares) (Gobierno Municipal del Canton Pedro Moncayo, 2011). Flower plantations in Pedro Moncayo are sprayed using hand sprayers by workers. Flower plantations there have reported to use over 20 different insecticides, including organophosphates, carbamates, neonicotinoids and pyrethroids, and over 50 different fungicides (Grandjean et al., 2006; Suarez-Lopez et al., 2017a).

2.3. Examination

In 2008, children were tested in 7 schools of Pedro Moncayo County in July and August when school was out of session, to guarantee a quiet, familiar, and child-friendly environment that was easily reachable. Parents and other adult residents were interviewed in their home to obtain socioeconomic status, demographic characteristics, and pesticide exposure history of household members. In 2016, children were examined in schools twice: first in April and again between July and October during the summer closure or during weekends. Examiners were uninformed of participants' pesticide exposure status. Children's height was measured following recommended procedures (World Health Organization, 2008) to the nearest 1 mm, using a height board, and weight was measured using a digital scale (Tanita model 0108MC; Corporation of America, Arlington Heights, IL, USA).

2.4. Anxiety and Depression Symptom Assessments

Depression symptoms, were assessed using the CDI-2 short assessment, Spanish Version (Kovacs, 2011). This a self-report assessment that contains 12 items. The CDI-2 short has excellent psychometric properties and yields a score that is comparable to the score of the full-length version (Bae, 2012; Kovacs, 2011). The CDI-2 reflects the theoretical model of depressive symptoms in youth, including the degree of ongoing depressive manifestations

(Bae, 2012). Questionnaires were scored using the CDI-2 Scoring Software (MHS Inc). *Anxiety symptoms* were assessed using the MASC-2 Child self-report. The MASC-2 indexes the range and severity of anxiety symptoms, and has good psychometric properties and clinical utilities in identifying youth with anxiety disorders (Fraccaro et al., 2015; Hogrefe, n.d.; van Gastel and Ferdinand, 2008; Wei et al., 2013). The MASC-2 Child self-report is a 50-item assessment that has been successfully used among ethnically diverse and non-English speaking populations (Magiati et al., 2013), and in Hispanic children (Isasi et al., 2014). An extensive examination on the validity, utility and reliability of the MASC-2 for school psychologists and related practitioners, provided evidence to suggest that the test was useful for measuring anxiety symptoms in children and adolescent's (Fraccaro et al., 2015). The MASC-2 English version was translated into Spanish, using terminology appropriate for Pedro Moncayo County, with input from community members. The Spanish translation was reviewed and approved by MHS Inc. Completed questionnaires were scored using the MASC-2 Scoring Software (MHS Inc).

Participants completed both CDI-2 and MASC-2 questionnaires in a quiet room and had the opportunity to ask for clarification of any assessment items to a research staff member present in the room. The scaled T-scores, age and gender standardized scores based on a US sample, for both the CDI-2 and MASC-2 were used in analyses (Hogrefe, n.d.; Kovacs, 2011). T-scores were designed to have a median of 50 and a standard deviation of 10.

2.5. Other measures

In 2008 and 2016, erythrocytic AChE activity and hemoglobin concentration were measured with the EQM Test-mate ChE Cholinesterase Test System 400 (EQM AChE Erythrocyte Cholinesterase Assay Kit 470; EQM Research, Inc, Cincinnati, OH) from a finger-stick blood sample (EQM Research Inc., 2003). Geographical coordinates of homes were obtained from portable global positioning system receivers collected as part of the Local and Community Information System (SILC, Sistema de Información Local y Comunitario), developed by Fundación Cimas del Ecuador. Geospatial information of plantations was created using satellite imagery. The distances of homes to the nearest flower plantation perimeter was calculated with ArcGIS (Esri, Redlands, CA). Additional data collection information has been described previously (Suarez-Lopez et al., 2012).

2.6 Statistical analysis

We calculated the means and standard deviation (SD) of participant characteristics or the proportions of participants with a certain characteristic (as appropriate) for all participants and stratified by: A) age (upper vs lower median) and tertiles of AChE activity calculated separately for each age group; B) gender and tertiles of AChE activity calculated separately for each gender. We calculated the p-value for trend (p-trend) for participant characteristics across levels of AChE activity using linear regression and modeling AChE activity as a continuous variable.

We used multiple linear regression to estimate associations of AChE activity with depression and anxiety scores. Statistical significance was defined using an alpha of 0.05. We adjusted models for potential confounders defined a-priori including age, gender, z-score for height-

for-age, z-score for BMI-for-age, parental years of education, hemoglobin concentration and race. In this cohort we previously observed that age was positively associated with AChE activity (Suarez-Lopez et al., 2012). Height and BMI were included as markers of chronic and subacute nutritional status which have been found to be associated with mental health status in adolescents (Marmorstein et al., 2014; Rees et al., 2009). Parental level of education is a construct of socioeconomic status and has been found to be independently associated with mental health outcomes (Brooks et al., 2010). As a standard practice, all models were adjusted for hemoglobin concentration because variations in hematocrit can alter the values of erythrocytic AChE activity (EQM Research Inc., 2003). We used the World Health Organization growth standards (World Health Organization Multicentre Growth Reference Study Group, 2006) to calculate the z-scores for height-for-age and BMI-for-age. Considering that only 2 children reported to be of Caucasian race, we combined those children with the Mestizo race.

We used multiple linear regression to test the association between change in AChE activity between 2008 and 2016. Such models were adjusted for age, gender, AChE activity (2008), hemoglobin concentration (2008 and 2016), and 2016 values for z-scores for height-for-age and BMI-for-age, race, parental years of education and family income

We calculated odds ratios (OR) for elevated scores of depression or anxiety associated with each SD decrease in AChE activity, using logistic regression. Elevated scores were defined as T-scores greater than 60 for both the MASC-2 and CDI-2 (Kovacs and MHS staff, 2011; March, 2012). In both linear and logistic regression models we tested for the presence of curvilinear associations using a quadratic AChE term.

We tested for effect modification by age and gender on all associations between AChE activity and scores of depression and anxiety by testing significance of the interaction term. When interaction terms (AChE*covariate) had p-values of <0.1 we conducted stratified analyses. In the presence of 2-level interaction (age and gender), we stratified the associations by 2 categories of both effect modifiers (participants in the upper vs lower median of age, separately for males and females). Finally, we plotted the AChE-mental health associations as adjusted means (least square means) across sextiles of AChE activity by gender (Figure 1), and by age and gender categories where 2-level interactions were present (Figure 2). Sextiles of AChE activity were calculated separately for boys and girls.

To maximize the study's sample, we imputed missing information of maternal education for 21 children and paternal education for 40 children as follows: for 11 children we imputed maternal education using maternal education reported in 2008, and for 30 children using paternal education reported in 2008. For the remaining 10 children with missing maternal education, we conducted a random imputation based on a normal distribution of maternal education. For the same 10 children, we also imputed paternal education using random imputation based on a normal distribution of paternal education. Parental education was calculated as the average number of years of education of both parents. We also imputed household income for 17 children. Income values were imputed using 2008 information for 6 children. In 2008, income was collected as a 7-category variable. We calculated the group's mean and SD of income in 2016 for each of the 7 categories of income in 2008. We

then imputed the 2016 income using a random normal distribution based on the mean (SD) appropriate for the reported 2008 income category. For the remaining 11 children we imputed income based on the average of the mother's and father's education. We calculated the group's mean (SD) income in 2016 for each year of education and imputed the 2016 income using a random normal distribution based on the mean (SD) appropriate for the corresponding parental years of education.

3. Results

3.1. Participant Characteristics

The median age of children at the time of depression and anxiety assessments was 14.38 years (SD= 1.8); 51% of participants were female. The mean (SD) of the following variables were: AChE: 3.71 U/mL (0.55), depression T-score: 53.0 (9.4) and anxiety T-score: 57.7 (9.7). The overall mean (SD) of height-for-age z-score was −1.48 (0.90), and that of BMI-for-age z-score was 0.40 (0.85). AChE activity was lower in children younger than 14.38 years compared to children aged 14.38-17.9 years (3.67 vs 3.74 U/mL, p-difference=0.04, adjusted for hemoglobin). Age in children in this study population was previously found to be positively associated with AChE activity (Suarez-Lopez et al., 2012). Participant characteristics across tertiles of AChE activity stratified by gender and age (upper vs lower median) are presented in Tables 1 and 2. In boys and girls, participants who had lower AChE activity in 2016 also were more likely to have lower AChE values in 2008 (Tables 1 and 2). After adjusting for age, and hemoglobin concentrations in 2008 and 2016, every U/mL increase in AChE activity in 2008 was associated with a 0.78 U/mL increase (95% CI: 0.64, 0.91, model R^2=0.57) in 2016.

The overall prevalence of elevated symptoms of depression and anxiety symptoms were 23% and 44%, respectively. Among participants younger than 14.38 years, the prevalences of elevated symptoms of depression and anxiety were 23% and 43%, respectively, and among older participants, the prevalences were 22% and 45%, respectively. Boys had higher AChE activity than girls (3.77, vs 3.64 U/mL, p-difference= 0.002, adjusted for hemoglobin) and lower prevalences of elevated anxiety and depression symptoms. The prevalences of elevated depression and anxiety symptoms among girls was 25% and 49%, respectively, and among boys was 20% and 38%, respectively.

3.2. AChE Activity and depression symptoms

As hypothesized, AChE activity was negatively associated with depression scores overall (T-score difference [β] per SD decrease of AChE [95% CI]: 1.09 [0.02, 2.16], Table 3). The associations were stronger among girls (β=1.61) than boys (β=0.69, Table 3, Figure 1). Also, these negative associations were stronger among children younger than 14.38y (β=1.61) compared to older children (β=0.57). In stratified analyses by age and gender, the associations were strongest among girls younger than 14.38y (β=3.30 [0.54, 6.05]). The associations among older girls and boys had the same directionality but were weaker and not statistically significant. We did not observe evidence for curvilinear associations in models using a quadratic AChE term. Figure 2 depicts the associations by gender and age categories.

Similarly, the ORs for elevated depression score per SD decrease of AChE were stronger for girls compared to boys and stronger among younger compared to older children (Table 3). Among girls, the significant ORs (95% CI) were present only among younger girls (2.58 [1.26, 5.27]). Boys had no significant associations with OR for elevated depression scores in either of the age groups.

3.3. AChE activity and anxiety symptoms

AChE activity was not associated with total anxiety symptoms in linear regression analyses (β per SD decrease of AChE [95% CI]: −0.14 [−1.26, 0.98]) nor in any of the anxiety subcomponents including separation anxiety, generalized anxiety disorder, social anxiety and obsessive compulsive (Table 3). There were no differences in the associations across gender and age groups (data not shown). In logistic regression analyses, we observed no increased odds of elevated anxiety scores per SD decrease of AChE (OR=0.97 [0.77, 1.23]).

3.3. Change of AChE activity between 2008 and 2016 and anxiety and depression symptoms

Analyses of change of AChE activity between 2008 and 2016 (n=223) supported the 2016 crosssectional analyses (Table 5). An increase in AChE activity between 2008 and 2016 by 0.55 U/mL (SD of AChE activity in 2016) was associated with a lower score of depression symptoms overall, and this association was stronger in girls than in boys. In subgroup analyses, the associations were strongest among girls younger than 18.38 years, as in the cross-sectional analyses. Change in AChE activity was not associated with anxiety symptom scores. We did not observe evidence for curvilinear associations.

4. Discussion

We observed that adolescents in agricultural communities whose AChE activity indicated greater exposure to cholinesterase inhibitors (i.e., lower AChE activity) had greater depression symptoms than participants with higher AChE levels. These associations were stronger in girls than in boys and in younger compared to older adolescents. Children growing up in the agricultural Pedro Moncayo County, have an increased risk for exposure to agrochemicals, including cholinesterase inhibitor insecticides, compared to urban children. A single measure of AChE activity in this setting is an indicator of exposure to cholinesterase inhibitors as lower values have been observed among a) children living with floricultural workers, in particular with those with greater pesticide “take-home” pathways; b) children living near pesticide spray sites; and c) children examined sooner after the end of a pesticide spray season compared to those examined later (Suarez-Lopez et al., 2018, 2017a, 2012). To our knowledge, this is the first epidemiologic study to describe an association between depressive symptoms and pesticide exposures in adolescents using an exposure biomarker. These findings suggest that cholinesterase inhibition may create risk for depression in adolescents, particularly among women during early adolescence. While the results of this study supported our hypothesis that lower AChE activity was associated with depressive symptoms, we did not observe evidence of an association between AChE activity and anxiety symptoms. These cross-sectional analyses, were supported by longitudinal analyses of a subset of participants, in which change of AChE activity between 2008 and

2016 was also associated with depression (but not anxiety) symptoms, and the associations were strongest among younger girls.

Both carbamate and organophosphate pesticides are cholinesterase-inhibitor agents that decrease the hydrolysis of acetylcholine, thus resulting in the over-stimulation of nicotinic and muscarinic receptors in the peripheral and central nervous systems (Taylor, 2011). In addition to the classic toxic effects caused by AChE inhibition, there is accumulating evidence to suggest that organophosphate pesticides can be directly toxic to neurons and glia (Abou-Donia, 2003; Aldridge et al., 2005b; Qiao et al., 2003; Slotkin, 2004). The cholinergic system has a vast role in the central nervous system and may have modulatory effects on mood and behavior. Clinical studies and animal models have indicated that blockers of both muscarinic and nicotinic cholinergic receptors, including scopolamine, neuromuscular junction inhibitors and M1 muscarinic agonists, can induce antidepressant-like responses (Furey and Drevets, 2006; Mineur et al., 2013). Mice exposed to the AChE inhibitor physostigmine, demonstrated an increase in depression-like and anxiety-like behaviors that was reversed by administration of nicotinic or muscarinic acetylcholine receptor antagonists (Mineur et al., 2013). Likewise, rats exposed to the organophosphate insecticide chlorpyrifos had decreased hippocampal, striatal and prefrontal cortical AChE activity and plasma butyrylcholinesterase activity. Additionally, exposed rats exhibited depressive-like behaviors which were attenuated with further administration of atropine, an anticholinergic (Siqueira et al., 2019). These findings support the adrenergic-cholinergic balance hypothesis of mania and depression (Janowsky et al., 1972; Risch et al., 1981) which originated from reports of depressive symptoms among psychotic patients treated with a cholinesterase inhibitor (Rowntree et al., 1950) and from case reports of individuals poisoned with cholinesterase inhibitor insecticides, who developed depression symptoms and parasympathetic toxicity (Dagytė et al., 2011; Gershon and Shaw, 1961). Currently, it has been acknowledged that the cholinergic system is but one of many factors involved in depression. Alterations in the central cholinergic system may affect neurogenesis and hippocampal function, leading to the cognitive deficits observed in depression (Dagytė et al., 2011).

Also concurring with our findings, reversible cholinesterase inhibitors used in clinical practice, such as physostigmine, have been found to induce depressive symptoms and exacerbate mood disorders in normal and manic subjects (Davis et al., 1976; Janowsky et al., 1974, 1973; Steinberg et al., 1997). Conversely, mood symptoms improved in depressed patients with Alzheimer’s disease, after treatment with cholinesterase inhibitors including donepezil (Inoue et al., 2016; Rozzini et al., 2007). Cholinesterase inhibitors have been found to improve cognitive performance in adults with Alzheimer’s disease and such improvements may be mediating factors in this association. Given the apparent role of the cholinergic system on mood modulation, anticholinergic drugs have also been investigated as potential treatments for depression but inconsistent antidepressant effects have been found (Goldman and Erickson, 1983; Howland, 2009; Shytle et al., 2002). Additionally, there is evidence of organophosphate pesticides affecting serotonergic mechanisms and serotonin-related emotional behaviors in animal models, which may also explain the observed associations (Aldridge et al., 2005b, 2005a, 2004; Chen et al., 2011).

The findings of our study also concur with previous studies of agricultural populations. Self-reports of occupational pesticide use in adults (mostly organophosphates, and to a lesser extent, carbamates and OCPs) have been found to be associated with greater depressive symptoms, suicidal thoughts and risk of suicide (Beseler et al., 2008, 2006; Beseler and Stallones, 2008; Harrison and Mackenzie Ross, 2016; London et al., 2012, 2005; Weisskopf et al., 2013; Wesseling et al., 2010). For instance, chronic pesticide exposure as a result of pesticide application work was associated with depression diagnosis among agricultural workers enrolled in the Agricultural Health Study in Iowa and North Carolina (Beseler et al., 2008). The findings suggest that both cumulative pesticide exposure and acute high-intensity exposure may lead to depression in pesticide applicators. Suicide rates are notoriously high in farming populations exposed to pesticides and case series and ecological studies support a significant association between organophosphate use and suicide (London et al., 2005). The AChE concentrations in our study population of adolescents (28.6 U/g of hemoglobin) were higher than those of pesticide applicator and non-applicator adolescents of ages 12-22 years living in agricultural areas in Egypt (Crane et al., 2013). The AChE concentrations among the applicators in Egypt before and during a pesticide spray season were 27.1 U/g and 25.4 U/g, respectively, and the concentrations among non-applicators before and during the spray season were 27.3 U/g and 26.5 U/g. Both studies used the same kit and machine model for quantification of AChE activity. In our study, we also observed that those participants who had lower AChE activity in 2008 (after accounting for age and hemoglobin concentration) also had lower AChE activity in 2016 (Table 2). It is plausible that the same participants with lower AChE activity in 2008 and 2016 were also more likely to have greater potential for pesticide exposure (e.g. live with an agricultural worker or live near pesticide spray sites). This is the topic of a separate manuscript.

As in the present study, we previously reported that children with lower AChE activity had poorer neurobehavioral performance among 4-to-9-year-old ESPINA study participants (Suarez-Lopez et al., 2013). Contrary to the findings of the present study (stronger associations in girls compared to boys), the associations observed in that study were stronger among boys in the domains of attention & inhibitory control and memory & learning. This concurs with evidence that pesticide exposures can affect mental health outcomes differently among males and females in human and animal studies. (Dam et al., 2000; Horton et al., 2012; Johnson et al., 2009; Levin et al., 2001; Marks et al., 2010). The reason for this sex interaction is not well understood and is a topic for current research. It has been well established that boys tend to perform lower in profiles of attention, impulsivity (Davies, 2014) and are twice as likely to be diagnosed with attention deficit disorder compared to girls (Visser et al., 2014). This indicates that boys are more susceptible to alterations on this domain and is concordant with the previously described effect modification by sex of the AChE-neurobehavior associations (Suarez-Lopez et al., 2013). Likewise, it is known that adolescent girls have higher rates of depression compared to boys (Perou et al., 2013) and this increased susceptibility to internalizing behaviors may be a contributing factor for the stronger associations observed in the present study between AChE and depression scores in girls. The present study also observed effect modification by age, in which younger children had stronger associations. The strongest associations were present among girls, ages 11.0-14.4 years compared to those 14.4-17.9 years. Childhood is a period of increased

vulnerability to environmental exposures; these findings suggest that the effects of pesticide exposures on mental health are strongest in younger adolescents.

We did not observe associations between AChE and anxiety, which contrasts with the few studies that have observed associations between self-report of exposure to organophosphates and anxiety among agricultural workers (Harrison and Mackenzie Ross, 2016; Malekirad et al., 2013). Also, in mice cholinesterase inhibition in the hippocampus has been found to induce stress, depression-like and anxiety-like symptoms, as observed in experiments that administered general and localized (hippocampal) administration of physostigmine (Mineur et al., 2013).

The cholinergic influence on anxiety has resulted in more mixed findings than studies on depression, suggesting that the cholinergic effect on anxiety may depend other factors such as stress (Mineur et al., 2013). In our study, anxiety symptoms were generally high, with 44% of the total sample scoring in the subclinical to clinical range. Thus, it is possible that other anxietygenerating factors operating in the lives of these adolescents masked any effects of AChE activity.

While the ESPINA study is one of the largest longitudinal studies of children growing up in agricultural communities, the statistical power of our study maybe inadequate to adequately characterized 2-level interactions (gender and age). This may explain why we were unable to detect significant associations with an $\alpha<0.05$ among age and gender categories with weaker associations than those of young girls. Another limitation of our study is the cross-sectional design, as mental health assessments started in adolescence in our cohort. Therefore, the present design hinders the assessment of chronic AChE inhibition on mental health outcomes. At minimum, our findings provide insight on subacute AChE inhibition and its short-term association with internalizing behaviors. There is growing evidence that pesticide exposures may transiently affect neurobehavioral performance in children as observed in animal studies (Levin et al., 2003; Maurissen et al., 2000; Middlemore-Risher et al., 2010) and observational studies of children, including in our study population (Khan et al., 2014; Rohlman et al., 2016; Suarez-Lopez et al., 2017b). Yet, we cannot ignore the possibility 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. Further work is needed to disentangle the effects of chronic vs acute pesticide exposures on these mental health outcomes. Although the longitudinal findings of change of AChE activity between 2008 and 2016 supported the cross-sectional findings, it does not allow us to disentangle whether the effects observed were chronic or subacute, considering that we did not collect depression or anxiety scores in 2008. An alternate interpretation of our findings is that children with greater depressive symptoms may have lower AChE activity due to disrupted brain physiology, independent of environmental exposures. This seems less likely because the influence of brain activity on red blood cell AChE activity is likely small or nil.

5. Conclusion

These are the first data to describe associations between a biomarker of pesticide exposure and internalizing symptoms among children in one of the largest studies of its kind. Lower AChE activity may create risk for depression in teenagers, particularly among girls during early adolescence. Replication of these findings are needed.

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Abbreviations:

AChE	acetylcholinesterase activity

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Highlights

- Low acetylcholinesterase (ACHE) is a marker of increased pesticide exposure
- We examined 529 11-17 year-olds living near flower production sites in Ecuador
- Lower AChE was related to increased depressive symptoms but not to anxiety symptoms
- Associations were stronger in girls vs. boys and in younger vs. older adolescents
- Pesticide exposure may create risk for depression, particularly among young girls

Declaration of Interest Form

We wish to confirm that there are no known conflicts of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome.

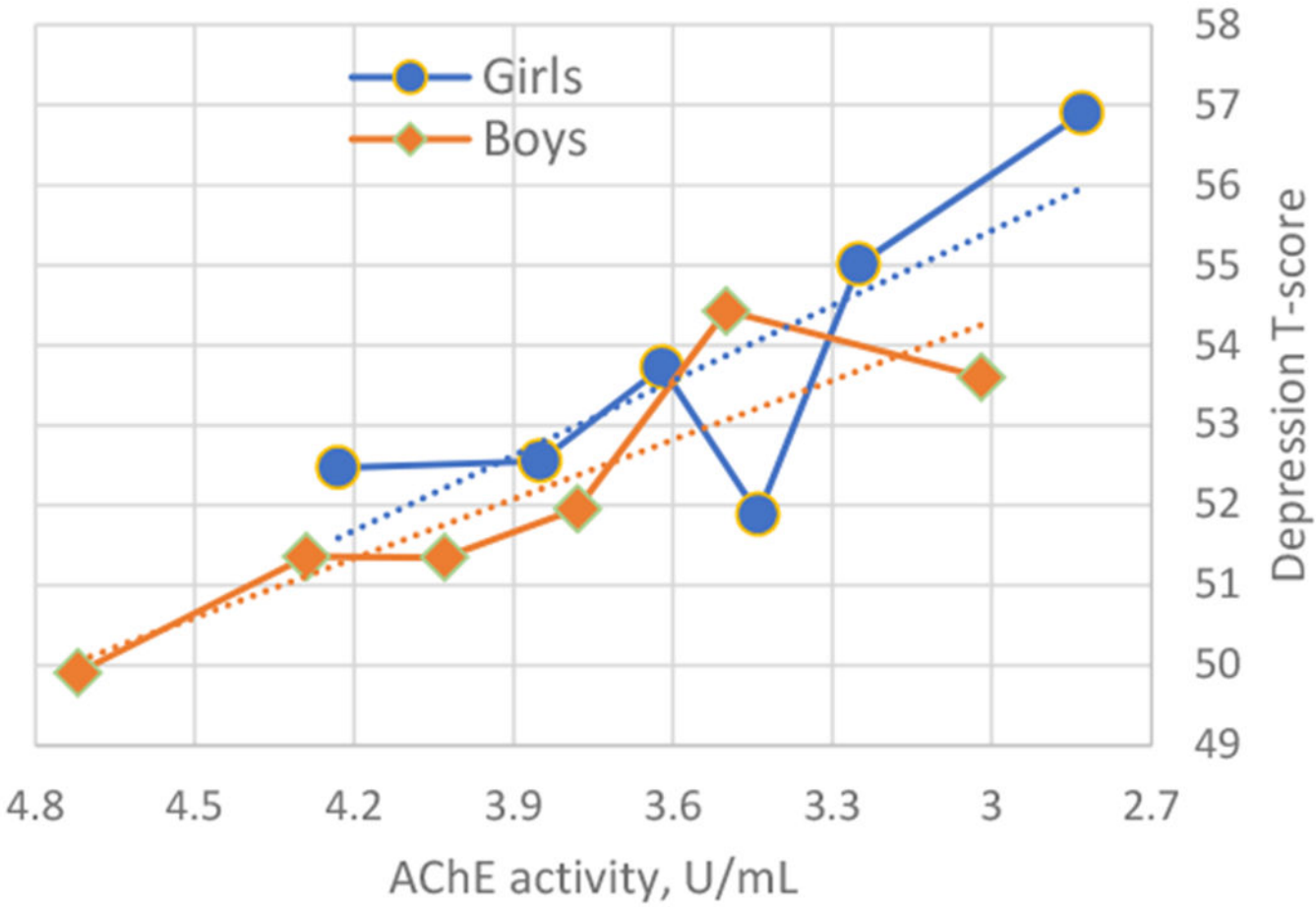

Figure 1.

Adjusted associations of AChE activity (sextiles) and depression T-scores by gender.

Adjustments: age, hemoglobin concentration, z-scores for height-for-age and BMI-for-age, race, parental years of education and family income.

Slopes of the models from linear regression analyses (dashed lines) are listed in Table 3.

Sextiles of AChE activity were calculated separately for boys and girls.

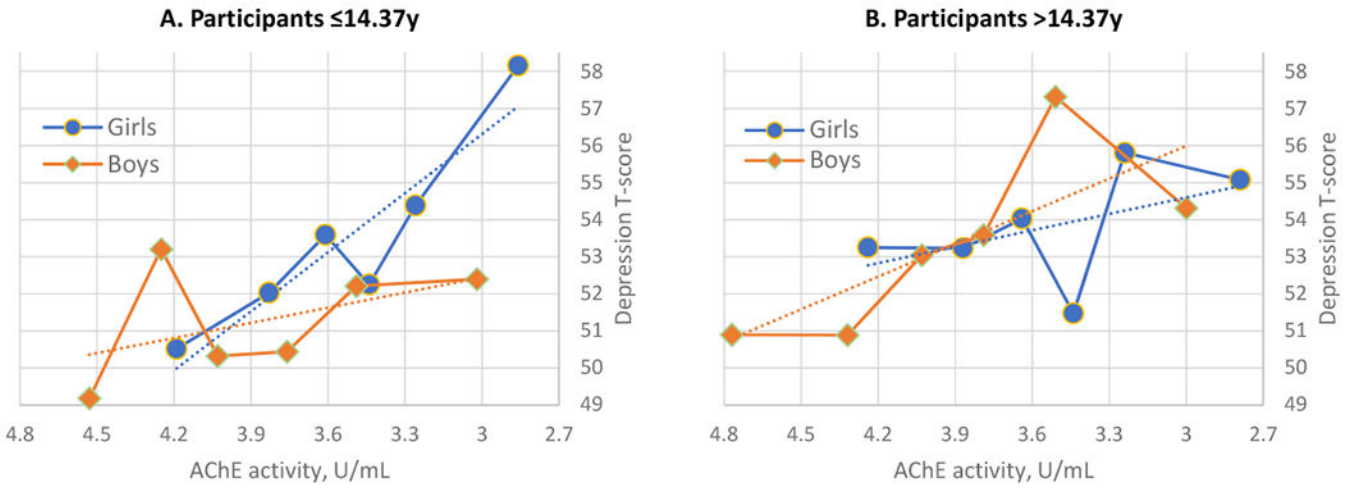

Figure 2.

Adjusted associations of AChE activity (sextiles) and depression T-scores for participants above and below the median for age by gender.

Adjustments: hemoglobin concentration, z-score for height-for-age and BMI-for-age, race, parental years of education and family income.

Slopes of the models from linear regression analyses (dashed lines) are listed in Table 3.

Sextiles of AChE activity were calculated separately for boys and girls.

Table 1.

Participant characteristics by categories of age (upper vs lower median) across tertiles of AChE activity, N=529.

	11.0 – 14.37 years of age (n=264)				14.38 – 17.9 years of age (n=265)			
	AChE Tertiles[a]				AChE Tertiles[a]			
Range (U/mL)	**2.38-3.39**	**3.40-3.76**	**3.77-4.92**	**p-trend**	**1.97-3.53**	**3.54-4.11**	**4.12-5.62**	**p-trend**
N	**89**	**88**	**87**		**88**	**89**	**88**	
Age, years	12.8 (1.0)	12.9 (0.9)	13.3 (0.7)	<0.01	15.8 (1.1)	15.9 (1.0)	16.0 (1.1)	<0.01
Gender (male), %	40	44	68	<0.01	24	47	73	<0.01
Race, % mestizo	70	73	91	0.05	84	74	82	0.05
Parental education, years	7.98 (2.90)	8.11 (3.61)	8.96 (3.95)	0.59	7.52 (3.06)	8.23 (3.65)	7.81 (3.39)	0.57
Household monthly income, USD	548 (217)	609 (583)	557 (307)	0.13	660 (573)	570 (221)	519 (302)	0.13
Height-for-age z-score, SD	−1.54 (1.03)	−1.43 (1.01)	−1.20 (0.84)	0.27	−1.62 (0.82)	−1.56 (0.85)	−1.49 (0.85)	0.27
BMI-for-age z-score, SD	0.36 (0.80)	0.46 (0.83)	0.53 (0.88)	0.62	0.37 (0.96)	0.41 (0.79)	0.24 (0.81)	0.62
AChE activity	3.10 (0.25)	3.59 (0.11)	4.09 (0.24)	<0.01	3.17 (0.34)	3.84 (0.15)	4.48 (0.31)	<0.01
Hemoglobin, mg/dl	12.1 (1.0)	12.7 (0.8)	13.3 (0.8)	<0.01	12.2 (0.87)	13.3 (0.8)	14.2 (1.2)	<0.01
AChE activity (2008)[b]	2.83 (0.39)	2.92 (0.41)	3.20 (0.47)	<0.01	3.00 (0.40)	3.23 (0.41)	3.47 (0.47)	<0.01
Hemoglobin, mg/dl (2008)[b]	12.2 (0.9)	12.2 (0.9)	12.5 (1.2)	0.02	12.8 (1.2)	12.9 (1.0)	13.0 (1.2)	<0.01

Presented values are mean (SD) or percent

[a]Tertile cut-offs specific for the age group

BMI= body mass index, USD=United States Dollars

[b]N=223

Table 2.

Participant characteristics by gender across tertiles of AChE activity, N=529.

	Girls (n=268)				Boys (n=261)			
	AChE Tertiles				AChE Tertiles			
Range (U/mL)	**2.18-3.35**	**3.36-3.71**	**3.73-4.92**	**p-trend**	**1.97-3.64**	**3.65-4.13**	**4.14-5.62**	**p-trend**
N	**87**	**93**	**88**		**87**	**89**	**85**	
Age, years	14.5 (1.8)	14.1 (1.9)	14.9 (1.5)	0.31	13.5 (1.6)	14.4 (1.6)	15.4 (1.7)	<0.01
Race, % mestizo	78	76	82	0.57	71	81	85	0.03
Parental education, years	7.84 (2.80)	7.67 (3.51)	8.33 (3.80)	0.25	8.26 (3.46)	8.36 (3.39)	8.15 (3.72)	0.56
Household monthly income, USD	601 (423)	617 (543)	566 (337)	0.46	583 (451)	539 (235)	561 (307)	0.26
Height-for-age z-score, SD	−1.54 (0.91)	−1.41 (0.81)	−1.44 (0.81)	0.99	−1.63 (1.06)	−1.53 (0.90)	−1.30 (0.89)	0.11
BMI-for-age z-score, SD	0.43 (0.77)	0.59 (0.83)	0.62 (0.68)	0.12	0.24 (0.96)	0.14 (0.79)	0.33 (0.94)	0.97
AChE activity	3.04 (0.28)	3.53 (0.11)	4.04 (0.26)	<0.01	3.25 (0.32)	3.90 (0.14)	4.50 (0.29)	<0.01
Hemoglobin, mg/dl	12.0 (0.8)	12.5 (0.7)	13.2 (0.9)	<0.01	12.4 (1.1)	13.4 (0.8)	14.3 (1.0)	<0.01
AChE activity (2008)[b]	2.94 (0.41)	3.01 (0.31)	3.34 (0.52)	<0.01	2.88 (0.43)	3.10 (0.50)	3.43 (0.41)	<0.01
Hemoglobin, mg/dl (2008)[b]	12.6 (0.9)	12.6 (1.0)	12.9 (1.4)	0.34	12.2 (1.2)	12.5 (1.0)	12.9 (0.9)	<0.01

Presented values are mean (SD) or percent

[a]Tertile cut-offs specific for the gender

BMI= body mass index, USD=United States Dollars

[b]N=223

Table 3.

T-score differences in anxiety and depression symptoms per SD decrease in AChE activity.

	Score difference (95% CI) per SD[a] decrease in AChE		
	All Children	**11.0-14.37 y**	**14.38-17.9y**
Depression (n=517)			
All	1.09 (0.02, 2.16)[b]	1.61 (−0.15, 3.38)	0.57 (−0.82, 1.96)
Girls	1.61 (0.11, 3.10)[b]	3.30 (0.54, 6.05)[b]	0.76 (−1.04, 2.57)
Boys	0.69 (−0.89, 2.27)	0.36 (−1.99, 2.71)	0.33 (−1.89, 2.54)
Anxiety (n=518)			
All	−0.13 (−1.26, 0.99)	−0.20 (−2.13, 1.74)	−0.38 (−1.77, 1.02)
Separation Anxiety	−0.57 (−1.77, 0.63)	−0.08 (−2.07, 1.92)	−1.19 (−2.72, 0.33)
GAD Index	0.26 (−0.87, 1.39)	1.02 (−0.87, 2.91)	−0.34 (−1.76, 1.07)
Social Anxiety	−0.42 (−1.50, 0.66)	0.21 (−1.65, 2.07)	−0.89 (−2.18, 0.46)
Obsessive Compulsive	−0.05 (−1.18, 1.09)	−1.18 (−3.11, 0.76)	0.23 (−1.20, 1.66)

[a] SD of AChE (overall): 0.55 U/mL

[b] $p<0.05$

GAD= Generalized anxiety disorder

Adjustments: age, gender, hemoglobin concentration, z-score for height-for-age, z-score for BMI-for-age, race parental years of education and family income. Models stratified by median age categories were not adjusted for age.

Table 4.

Odds ratios for elevated scores of depression or anxiety (CDI-2 or MASC-2 T-score ≥60) per SD decrease of AChE activity.

	OR (95% CI) per SD[a] decrease of AChE		
	All Children	**11.0-14.37 y**	**14.38-17.9y**
Depression (N=517)			
All	1.18 (0.88, 1.58)	1.57 (0.97, 2.55)	0.96 (0.66, 1.38)
Girls	1.43 (0.93, 2.19)	2.58 (1.26, 5.27)[b]	0.98 (0.57, 1.67)
Boys	1.03 (0.69, 1.55)	1.02 (0.51, 2.04)	0.89 (0.54, 1.49)
Anxiety (N=518)			
Total Anxiety	0.97 (0.77, 1.24)	0.85 (0.57, 1.27)	1.02 (0.75, 1.37)
Separation Anxiety	0.98 (0.77, 1.25)	0.84 (0.56, 1.26)	1.04 (0.77, 1.40)
GAD Index	1.00 (0.79, 1.28)	0.85 (0.57, 1.27)	1.07 (0.79, 1.46)
Social Anxiety	0.97 (0.77, 1.24)	0.85 (0.57, 1.28)	1.02 (0.75, 1.37)
Obsessive compulsive	0.98 (0.77, 1.25)	0.84 (0.56, 1.26)	1.03 (0.76, 1.39)

[a] SD of AChE (overall): 0.55 U/mL

[b] $p<0.05$

GAD: Generalized anxiety disorder

Adjustments: age, gender, hemoglobin concentration, z-scores for height-for-age and BMI-for-age, race, parental years of education and family income. Models stratified by median age categories were not adjusted for age.

Table 5.

T-score differences in anxiety and depression symptoms per unit change in AChE activity between 2008 and 2016, n=223.

	Score difference (95% CI) per SD[a] change in AChE (2016-2008)		
	All Children	**11.0-14.37 y**	**14.38-17.9y**
Depression			
All	−2.11 (−3.99, −0.24)[b]	−1.92 (−5.20, 1.35)	−2.21 (−4.68, 0.27)
Girls	−3.25 (−6.18, −0.32)[b]	−4.74 (−9.98, 0.49)	−2.65 (−6.57, 1.27)
Boys	−1.13 (−3.68, 1.42)	0.27 (−4.35, 4.86)	−1.53 (−5.01, 1.94)
Anxiety			
All	−0.74 (−2.66, 1.18)	−1.84 (−5.29, 1.60)	−0.15 (−2.61, 2.32)
Separation Anxiety	−0.09 (−2.28, 2.10)	−0.71 (−4.20, 2.78)	−0.20 (−3.20, 2.81)
GAD Index	−0.91 (−2.87, 1.04)	−3.21 (−6.58, 0.16)	−0.28 (−2.79, 2.24)
Social Anxiety	0.09 (−1.85, 2.03)	−0.83 (−4.39, 2.73)	0.17 (−2.18, 2.52)
Obsessive Compulsive	−1.06 (−3.02, 0.90)	−1.23 (−4.47, 2.01)	−0.72 (−3.40, 1.95)

[a] SD of AChE (2016): 0.55 U/mL

[b] $p<0.05$

GAD: Generalized anxiety disorder

Adjustments: age, gender, AChE activity (2008), hemoglobin concentration (2008 and 2016), and 2016 values for z-scores for height-for-age and BMI-for-age, race, parental years of education and family income. Models stratified by median age categories were not adjusted for age.