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Cholinesterase and Paraoxonase (PON1) enzyme activities in Mexican-American Mothers and Children from an Agricultural Community

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Abstract

Exposure to organophosphate and carbamate pesticides can lead to neurotoxic effects through inhibition of cholinesterase enzymes. The paraoxonase (PON1) enzyme can detoxify oxon derivatives of some organophosphates. Lower PON1, acetylcholinesterase, and butyrylcholinesterase activities have been reported in newborns relative to adults, suggesting increased susceptibility to organophosphate exposure in young children. We determined PON1, acetylcholinesterase, and butyrylcholinesterase activities in Mexican-American mothers and their 9-year-old children (n=202 pairs) living in an agricultural community in California. We used paired t-tests to compare enzymatic activities among mothers and their children and analysis of variance to determine which factors are associated with enzyme activities. Substrate-specific PON1 activities were slightly lower in children than their mothers; however, these differences were not statistically significant. We observed significantly lower acetylcholinesterase but higher butyrylcholinesterase levels in children compared to their mothers. Mean butyrylcholinesterase levels were strongly associated with child obesity status (BMI Z scores >95%). We observed highly significant correlations among mother-child pairs for each of the enzymatic activities analyzed; however, PON1 activities did not correlate with acetylcholinesterase or butyrylcholinesterase activities. Our findings suggest that by age nine, PON1 activities approach adult levels and host factors including sex and obesity may affect key enzymes involved in pesticide metabolism.

Keywords

organophosphate; paraoxonase; cholinesterase; obesity; children; vulnerable sub-populations

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Introduction

Organophosphates and carbamates are cholinesterase-inhibiting pesticides commonly used in agriculture (CDPR, 2011). Widespread exposure to these compounds has been reported in the U.S. population, including pregnant women, neonates, and children (Adgate, et al., 2001; Bradman, et al., 2003). Several studies suggest that farm workers and their children, as well as rural populations living near agricultural fields, have higher exposures to organophosphate pesticides than other populations (Lu, et al., 2000; McCauley, et al., 2001; O'Rourke, et al., 2000).

Children are particularly vulnerable to pesticides due to their exposure-prone behaviors (Beamer, et al., 2009) as reviewed by Moya et al. (2004). Additionally they have lower metabolic capacities compared to adults (See Ginsberg, et al., 2004 for review). Exposure to organophosphates during critical periods of brain development may contribute to poorer neurobehavioral development in young animals (Campbell, et al., 1997; Eskenazi, et al., 1999). Several recent studies in school-age children have found prenatal exposure to organophosphate pesticides to be associated with poorer intellectual development, deficits in working memory, and attention problems (Bouchard, et al., 2011; Engel, et al., 2011; Marks, et al., 2011; Rauh, et al., 2011). Furthermore, activities of key enzymes involved in the organophosphate toxicity pathway, including paraoxonase (PON1), acetylcholinesterase, and butyrylcholinesterase (Cole, et al., 2003; Eskenazi, et al., 2004), are low at birth and may therefore render young infants more susceptible to exposure than adults. It is critical to determine at what age these activities become comparable to those of adults in order to establish how long this window of increased susceptibility in young children may last.

The toxicity produced by organophosphates and carbamates has been attributed mainly to their potent anti-cholinesterase activity in the nervous system (See Chambers, et al., 2011 for review). Acetylcholinesterase terminates signal transmission in cholinergic neurons by catalyzing the hydrolysis of the neurotransmitter acetylcholine, which allows these neurons to return to their resting state after activation. Inhibition of acetylcholinesterase, due to organophosphate or carbamate exposure, can lead to the accumulation of acetylcholine at the synapse, resulting in overstimulation of cholinergic neurons (Kobayashi, et al. 1986; Kobayashi, et al. 1988).

Butyrylcholinesterase, another class of cholinesterases, is a multifunctional protein. In addition to its cholinergic functions, it is also involved in neurodevelopment, cellular proliferation and morphogenesis (Robitzki, et al., 1997; Willbold and Layer, 1994; Layer, et al., 2005) and has recently been linked to fat utilization and weight status (Martos Estepa, 2000). It is found in most tissues, and can act as a back-up hydrolyzer of acetylcholine in the absence of acetylcholinesterase (Duysen, et al., 2001; Li, et al., 2000). Butyrylcholinesterase also functions as a scavenger of xenobiotic compounds, including organophosphates and carbamates (Raveh, et al., 1993). Importantly, as discussed in the review by Masson and Lockridge (2010), since inactivation of butyrylcholinesterase by xenobiotic compounds has no known adverse effects, scavenging by this enzyme acts to protect acetylcholinesterase, whose inhibition can be lethal. Both acetylcholinesterase and butyrylcholinesterase can be irreversibly inactivated by organophosphate-oxon metabolites. Depression of

acetylcholinesterase and butyrylcholinesterase activity in the blood is a validated biomarker of organophosphate pesticide exposure and is used to monitor occupational exposures (See Timchalk, 2011 for review).

Although the primary function of the paraoxonase 1 (PON1) enzyme is likely related to its antioxidant properties, PON1 also plays an important role in the organophosphate toxicity pathway as recently reviewed in the literature (Camps, et al., 2009; Costa and Furlong, 2011). PON1 detoxifies several but not all oxon derivatives of organophosphate pesticides, thereby preventing inhibition of acetylcholinesterase and butyrylcholinesterase. Certain *PON1* genotypes have been suggested to be associated with increased susceptibility to adverse birth outcomes and neurodevelopment in children exposed to organophosphate pesticides (Engel, et al., 2011; Harley, et al., 2011). Cholinesterases and PON1 have been associated with organophosphate-induced health outcomes and some studies suggest a relationship between activities of these enzymes, both of which are involved in the organophosphate detoxification pathway (Araoud, et al., 2010; Benmoyal-Segal, et al., 2005; Bryk, et al., 2005; Hofmann, et al., 2009). For instance, one study reported inverse associations between PON1 and acetylcholinesterase activities in healthy adult subjects (Bryk, et al., 2005) while another found greater butyrylcholinesterase inhibition among organophosphate-exposed agricultural pesticide handlers with lower PON1 levels (Hofmann, et al., 2009); these findings suggest a potential toxicological interaction in which exposed individuals with low PON1 are more likely to experience cholinesterase inhibition.

In this study, we examine PON1 and cholinesterase enzyme activities in mothers and their 9-year-old children participating in the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS), a longitudinal birth cohort study (Bradman, et al., 2011; Eskenazi, et al., 1999). We evaluate whether these enzyme activities have reached adult levels by age 9, determine whether host factors such as sex and obesity may also affect enzymatic activities, and examine the relationship between PON1 and cholinesterase enzymatic activities. We previously reported in this cohort a broad variability in PON1 activity among women and their newborn infants (65-fold range) (Holland, et al., 2006), with wide inter-individual variation of acetylcholinesterase (14.5 -fold range) and butyrylcholinesterase (6.5- fold range) (Eskenazi, et al., 2004), key enzymes in the organophosphate toxicity pathway. Furthermore, we have also demonstrated that even at age 7, some children continue to have lower PON1 levels and activities than adults, suggesting greater susceptibility to organophosphate pesticides even in school aged children (Huen, et al., 2010). Establishing whether these enzymatic activities reach adult levels by age 9 will help to determine if children remain at an increased risk as they grow older.

Materials and methods

Study subjects and data collection

The Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) is a longitudinal birth cohort study examining the associations of pesticides and other environmental exposures on neurodevelopment, growth, and respiratory disease in children from primarily Mexican-American families (Bradman, et al., 2011 ; Eskenazi, et al., 2003). The Salinas Valley in Monterey County, CA, is intensively farmed with approximately

200,000 kg of organophosphates applied annually (CDPR, 2011). Six hundred and one pregnant women were enrolled in 1999–2000 and 325 children and their mothers were followed through 9 years of age. In this analysis, we included only women and children of Hispanic origin (96.8% of all participants) to avoid potential confounding by ethnicity; the majority of these families (87%) were Mexican-American. We also limited the analysis to women and their children (n=202 mother-child pairs) for whom PON1 enzymatic activities were analyzed at the time of the 9-year-old visit. Among those mother-child pairs, 195 also had cholinesterase activities determined.

Mothers were interviewed during pregnancy and when children were 7 years old about their sociodemographic characteristics, health status, diet, habits, and exposure-related risk factors, including work and housing history. Mothers' and children's weight and height without shoes were determined at the age 9 visit. Height was measured using a stadiometer, and weight was determined using a Tanita TBF-300A Body Composition Analyzer. Body Mass Index (BMI) or weight divided by squared height (kg/m^2), was compared to CDC reference data (National Center for Health Statistics, 2005) to generate BMI Z-scores standardized by sex and age. Study protocols were approved by the University of California, Berkeley Committee for Protection of Human Subjects. Written informed consent was obtained from all mothers, and verbal assent was obtained from the children at 9 years of age.

Blood collection and processing

Blood specimens were collected from mothers and children when the children were approximately 9 years old. Heparinized whole blood was collected in BD vacutainers (Becton, Dickinson and Company, Franklin Lakes, NJ), shipped overnight with cooling elements from the collection site in Salinas, CA to our laboratory, where the blood samples were then centrifuged, and divided into plasma, buffy coats, and red blood cells. Aliquots of whole blood were stabilized using a 1:10 dilution in 0.1 M NaPO_4 buffer (pH 8.0) containing 1% Triton X-100 for subsequent cholinesterase activity determination. All samples were stored at -80°C until ready for analysis.

Determination of PON1 enzymatic activities

PON1 enzyme activity was measured in heparinized plasma samples. We measured PON1 enzyme activities against three different substrates [(paraoxon (PO), phenyl acetate (ARY), and chlorpyrifos-oxon (CPO)] in plasma samples using spectrophotometric methods as described previously (Huen, et al., 2009). Initial linear rates of hydrolysis (0-2 min) in mOD/min were converted to U/ml for arylesterase (ARYase) and units per liter (U/l) for chlorpyrifos-oxonase (CPOase) and paraoxonase (POase) activities using the following molar extinction coefficients: $1.310 \text{ mM}^{-1} \text{ cm}^{-1}$, $5.56 \text{ mM}^{-1} \text{ cm}^{-1}$, $18 \text{ mM}^{-1} \text{ cm}^{-1}$, respectively. We use these three measurements as markers of PON1 molecular phenotype. The ARYase assay serves as an indirect measure of PON1 enzyme quantity which we refer to in this paper as PON1 levels. ARYase rates do not vary between *PON1*_{192Q} and *PON1*_{192R} alloforms as they do for paraoxon hydrolysis (POase) (Furlong, et al., 2006; Huen, et al., 2010). PON1 quantity measured using ELISA- and Western-blot-based methods are highly correlated with ARYase activity ($r > 0.85$) (Connelly, et al., 2008;

Kujiraoka, et al., 2000). In contrast, the POase and CPOase are substrate-specific activities, measuring rates of hydrolysis towards oxon derivatives of the pesticides parathion and chlorpyrifos, respectively. These measures are influenced both by quantity and catalytic efficiency of the enzyme (Furlong, et al., 2006). All assays were performed in triplicate. Internal controls (aliquots of the same sample run on all assay plates) were used for every assay and the inter-assay variability (average CV) for these samples ranged between 7% and 9%.

Determination of Cholinesterase enzymatic activities

Acetylcholinesterase and butyrylcholinesterase activities were analyzed using a modification (Wilson, et al., 2002) of the microplate assay based on the original Ellman procedure (Ellman, et al., 1961). Blood samples stabilized with 0.1 M NaPO₄ buffer (pH 8.0) containing 1% Triton X-100 were thawed, mixed thoroughly, and diluted 1:25 with 0.1 M NaPO₄ buffer (pH 8.0) without Triton X-100 for a final 1:250 dilution of sample. One hundred micro-liters of 1:250 diluted samples were distributed to the wells of a microtiter plate. The reaction was initiated with 100 µL of a 2X assay mix of either acetylcholinesterase or butyrylcholinesterase substrate resulting in 1 mM final substrate concentration of acetylcholine (Sigma, Missouri, USA) or butyrylthiocholine (Sigma, Missouri, USA). 5,5'-Dithio-bis(2)-nitrobenzoic acid (DTNB) (Sigma, Missouri, USA) at a final concentration of 0.32 mmol/L was used as the chromogenic indicator of thiocholine formation. Assays were followed continuously at ambient temperature (24-26°C) at 412 nm for 12 min in a SpectraMax PLUS Microplate Reader (Molecular Devices Corp, Sunnyvale, CA). The initial linear rates of hydrolysis were obtained in units of optical density per minute. The path length for each well of the plate was measured immediately after the reaction, and rates were converted to rates of change in A₄₁₂ per minute, and then to units of enzyme activity per milliliter (U/ml) using a molar extinction coefficient of 13,600 mM⁻¹ cm⁻¹. For quality assurance, aliquots of the same cholinesterase sample stabilized with 0.1 M NaPO₄ buffer (pH 8.0) containing 1% Triton X-100 were used for standardization. All assays were performed in triplicate. Internal controls (aliquots of the same sample) were run on every plate and the inter-assay coefficient of variability (average CV) was 7% and 8% for acetylcholinesterase and butyrylcholinesterase enzymatic activities, respectively.

Statistical analysis

Paired t-tests (two-tailed) were used to determine differences in POase, CPOase, ARYase, acetylcholinesterase, and butyrylcholinesterase activities between women and their children. Correlations of activities within mother-child pairs were determined by calculating Spearman's rho.

We used ANOVA to test for differences in maternal and child POase, CPOase, ARYase, acetylcholinesterase, and butyrylcholinesterase activities by demographic and exposure variables, such as maternal country of birth, child sex, maternal and child BMI, maternal work in agriculture, and presence of agricultural workers in the household. The Cuzick's non-parametric test for trend was used to assess trends in cholinesterase levels by weight status in children and mothers.

To examine the relationship between cholinesterase and PON1 activities in all subjects, we calculated Spearman correlation coefficients (ρ). All analyses were performed in STATA 11.0 (College Station, TX). P-values less than 0.05 were considered significant and p-values less than 0.10 were reported as marginally significant.

Results

Demographics

Table 1 describes the sociodemographic characteristics of the study participants and reports the association of each characteristic with cholinesterase enzyme activities (acetylcholinesterase and butyrylcholinesterase). The mean maternal age at the time of the 9-year blood collection was 35 ± 5 years of age. Most mothers (88%) had been born in Mexico and more than half had resided in the United States for fewer than 5 years when their children were born (data not shown); 81% had not graduated from high school. According to data collected at the 7-year visit, all women were living within 200% of the poverty level or below. Approximately 44% of women had worked in the fields or in other agricultural jobs (e.g. in a packing shed, nursery, or greenhouse) during the previous year and 64% of the women reported living with agricultural workers in their homes at the time of the 7-year visit. Approximately 89% of mothers were overweight (BMI 25-30) or obese (BMI ≥ 30) at the time of 9-year visit blood collection. The mean (SD) age of the children was 9.2 (0.25) years and ranged from 8.9 to 10.3 years; 48% were boys and 52% were girls. More than half of the children were overweight (15%) or obese (37%) at 9 years of age. The mean (SD) BMI in boys was 20.6 (4.4) (range=14.1 to 38.3) and in girls was 20.5 (5.1) (range=13.9 to 35.8).

PON1, acetylcholinesterase, and butyrylcholinesterase activities in mothers and children

Average POase, CPOase, and ARYase levels were 1.5-7.9% higher in women (932 ± 528 U/l, 7585 ± 2027 U/l, and 133 ± 29 U/ml, respectively) than in children (864 ± 465 U/l, 7355 ± 1813 U/l, and 131 ± 31 U/ml, respectively; Table 2). However, among mother-child pairs, this difference was not statistically significant for ARYase activity. POase and CPOase were slightly higher in mothers (two tailed $p = 0.06$ and 0.09 , respectively). Interestingly, the range of POase activities among children was close to 40-fold, while this range was only about 18-fold in mothers. Ranges of variability of CPOase and ARYase activities were comparable between mothers and children.

Acetylcholinesterase activities were 5.9% higher in mothers (5.4 ± 1.1 U/ml) than in their 9-year-old children (5.1 ± 0.9 U/ml); this difference was statistically significant ($p < 0.0005$; paired t-test) (Table 2). The distributions of acetylcholinesterase activities in mothers and children are shown graphically in Figure 1. The box plot demonstrates a wider range of variability among mothers compared to children. In contrast to acetylcholinesterase, the mean butyrylcholinesterase levels were 9.5% higher in 9-year-olds (2.3 ± 0.5 U/ml) than in their mothers (2.1 ± 0.5 U/ml) (two-tailed p -value for paired t-test < 0.0005) (Table 2).

For each of the five enzymatic activities (POase, CPOase, ARYase, acetylcholinesterase, butyrylcholinesterase), we observed highly significant but moderate correlations within

mother-child pairs, with Spearman rhos ranging from 0.34 (ARYase) to 0.50 (POase) for all enzymatic activities (see Supplemental Table 1; $p < 0.0005$ for all five activities).

Enzyme activities and demographic and host factors

Among children, we observed slightly higher CPOase levels in boys compared to girls ($p = 0.05$), but no significant associations of sex with ARYase or POase activities (Table 3). ARYase but not POase or CPOase was marginally associated with obesity ($p = 0.07$), with mean levels for obese children higher than in normal or overweight children (136.1 vs 128.9 and 121.1 U/L, respectively). Factors related to exposure such as maternal work in agriculture and living with agriculture workers in the household were not significantly associated with PON1 enzymatic activities. Furthermore, other demographic and host factors such as maternal education, maternal country of birth and poverty level were not associated with children's PON1 activities. We did not observe any significant associations of maternal PON1 activities with demographic and host factors.

Among the demographic and host factors tested, only child obesity status was associated with children's cholinesterase activity (Table 3). Butyrylcholinesterase activity was higher in obese children (2.5 U/mL) (BMI Z scores $> 95\%$) than in normal weight children (2.1 U/mL) (BMI Z scores $< 85\%$) and overweight children (2.3 U/mL) (BMI Z scores 85-95%). As shown in Figure 2, increased butyrylcholinesterase activities were strongly associated with obesity in children ($p < 0.0005$); however, similar trends were not observed with children's acetylcholinesterase activities. Among mothers, women residing with agricultural workers at the time of the 7-year visit tended to have lower butyrylcholinesterase activity than those who did not ($p = 0.03$). However, we did not observe any significant associations between maternal work in agriculture and cholinesterase levels in either children or mothers.

Correlations between cholinesterase and PON1 enzyme activities

Acetylcholinesterase activity was moderately correlated with butyrylcholinesterase activity within the group of children ($\rho = 0.52$, $p < 0.0005$) (Table 3) and of mothers ($\rho = 0.59$, $p < 0.0005$) (Table 4). CPOase activity was also moderately correlated with POase and ARYase in both children ($\rho = 0.45$ $p < 0.0005$ and $\rho = 0.70$ $p < 0.0005$, respectively) and mothers ($\rho = 0.43$ $p < 0.0005$ and $\rho = 0.75$ $p < 0.0005$, respectively). However, while POase activity was significantly correlated with ARYase activity in mothers ($\rho = 0.27$ $p < 0.0005$) and children ($\rho = 0.18$ $p = 0.01$), the correlation coefficients were relatively small. Furthermore, no PON1 activities were correlated with either acetylcholinesterase or butyrylcholinesterase activities in mothers or children. Overall in our study, the three co-correlated PON1 activities do not appear to be associated with two co-correlated cholinesterase activities.

Discussion

In this study we determined the enzymatic activities of PON1, acetylcholinesterase, and butyrylcholinesterase in a large cohort of Mexican-American mothers and their 9-year old children living in an agricultural community, and investigated the relationship of these enzyme levels with different host factors. In 9-year old children, we observed slightly lower

PON1, significantly lower acetylcholinesterase, and higher butyrylcholinesterase activities relative to their mothers. Furthermore, butyrylcholinesterase activities were strongly associated with obesity in children but not mothers. However, there was no correlation between cholinesterase and PON1 enzyme activities in either mothers or children.

Previously, we found that although children's PON1 activity rose between birth and age 7, it was still significantly lower at age 7 than maternal levels, with the difference between maternal and child levels being most pronounced in children with genotypes associated with low PON1 activities (Huen, et al., 2010). Here we examined PON1 activity in the same mothers and children at 9 years of age. Although PON1 activities tended to be slightly lower in 9-year-old children than in their mothers, for the most part the differences were not statistically significant. This indicates that children's PON1 enzyme activities may be approaching adult levels by age 9.

Several studies have reported lower acetylcholinesterase activity levels in newborns compared to adults (Burman, 1961; de Peyster, et al., 1994; Eskenazi, et al., 2004), but few have looked at the acetylcholinesterase enzyme activities of older children in relation to adult levels. An early study showed that school-age children ranging in age from 7 to 17 years had lower acetylcholinesterase levels than adults; however, likely due to a small sample size (n=39), this trend was not statistically significant (Clark and Beck, 1950). In contrast, in this much larger cohort of CHAMACOS mothers (N=251) and children (N=216), we observed significant differences in acetylcholinesterase between 9-year-old children and adult women.

Unlike acetylcholinesterase, we found higher butyrylcholinesterase levels in 9-year-old children compared to their mothers. This finding is consistent with several earlier reports (Hutchinson and Widdowson, 1952; Simpson and Kalow, 1963). For instance, butyrylcholinesterase levels were previously found to be lower in newborn babies as compared to adults, to increase during the first three weeks of life to levels greater than those of healthy adults, and then to remain elevated through age thirteen (Hutchinson and Widdowson, 1952; McCance, et al., 1949). In addition, Simpson and Kalow observed that in a healthy Canadian population, the mean butyrylcholinesterase levels were significantly lower in parents (n=57) than in their children (n=50) ($p<0.01$), with enzymatic activity levels dropping consistently each year from age 3 to 19 (Simpson and Kalow, 1963). Although it is unknown why children's enzyme activities relative to their mothers are higher for butyrylcholinesterase but lower for acetylcholinesterase, it may be due to differences in their noncholinergic functions during development. Similar patterns of expression of these two enzymes have been observed in murine embryonic stem cells (Sperling, et al., 2008); expression of butyrylcholinesterase, which is involved in cell proliferation, decreased over successive stages of development while expression of acetylcholinesterase, involved in cell differentiation, increased.

In our population of Mexican-Americans with a high prevalence of obesity (Rosas, et al., 2011), we also found a strong positive association between butyrylcholinesterase activities and obesity. These findings are consistent with those of Estepa and colleagues who reported that higher butyrylcholinesterase activities were observed in pre-pubescent (ages 6-9) obese

children (n=46) than in control children (n=49) (Martos Estepa, et al., 2000). Several studies have shown that the enzymatic activity of butyrylcholinesterase is increased in several conditions associated with metabolic syndrome, including obesity, diabetes, and cardiovascular disease (Iwasaki, et al., 2007; Chu, et al., 1978; Inacio Lunkes, et al., 2006; Kutty, et al., 1981; Alcantara, et al., 2005; Annapurna, et al., 1991; Li, et al., 2008). Conversely, decreased butyrylcholinesterase activities have been reported in malnourished humans (Waterlow, 1970). These findings suggest that butyrylcholinesterase activity may be induced in obese individuals.

Several animal studies have begun to elucidate potential roles for butyrylcholinesterase in obesity. For example, butyrylcholinesterase knockout mice have normal weight when fed a standard (5%) fat diet, but have exaggerated obesity compared to their wild type counterparts when put on a high (11%) fat diet (Li, et al., 2008). This suggests that butyrylcholinesterase activity may be increased to improve lipid utilization when fat consumption increases. In addition, it provides a potential mechanism explaining how chronic exposure to organophosphates and carbamates, which are potent inhibitors of butyrylcholinesterase, could contribute to obesity by interfering with lipid utilization. Therefore, the level of obesity in those exposed to pesticides would be expected to be exaggerated with the consumption of high fat diets when butyrylcholinesterase activity normally increases to stimulate fat utilization. In support of these assertions, some animal studies suggest an association between organophosphate exposure and obesity (Slotkin, 2010).

It has been suggested that butyrylcholinesterase activity stimulates fat utilization through its breakdown of the appetite and fat storage-promoting hormone octanoyl ghrelin (De Vriese, et al., 2004). However, deletion of the butyrylcholinesterase gene did not result in increased octanoyl ghrelin levels in mice (Li, et al., 2008). This is probably due to the fact that octanoyl ghrelin degradation does not rely solely on butyrylcholinesterase activity, and that other enzymes compensate for the absence of butyrylcholinesterase. For instance, it has been shown that human platelet-activating factor acetylhydrolase, and rat carboxylesterase and lysophospholipase I also contribute to octanoyl ghrelin degradation (De Vriese, et al., 2004; De Vriese, et al., 2007; Shanado, et al., 2004). To provide more direct evidence of the role of organophosphates in lipid utilization, specifically in disrupting the ghrelin pathway, octanoyl ghrelin levels could be measured in animal models of chronic organophosphate exposure consuming either a standard or high fat diet.

Hofmann and colleagues compared baseline levels of PON1, acetylcholinesterase, and butyrylcholinesterase activity in farm workers prior to a pesticide spray season and after pesticide applications had begun. They reported a decrease in butyrylcholinesterase activity levels after exposure, which was strongly associated with lower levels of PON1 activity in plasma (Hofmann et al, 2009). Though we anticipated similar findings in CHAMACOS participants (i.e. higher butyrylcholinesterase in those with higher PON1 activity), we found no association between butyrylcholinesterase and PON1 enzyme activities. It is possible that organophosphate and carbamate exposure levels in our population of mothers and children may also be lower than in the farmworkers studied by Hoffman and colleagues and therefore, not high enough to influence cholinesterase inhibition.

Our study has a few limitations. We were not able to assess concurrent organophosphate or carbamate exposures in CHAMACOS children and mothers, which could affect cholinesterase levels in subjects. Although maternal organophosphate exposure levels in the prenatal period and postpartum were higher in the CHAMACOS than in the general population (Bradman, et al., 2005), no exposure data is available for mothers and children at the age 9 visit. Another limitation of our study is the lack of baseline (pre-exposure) measurements for cholinesterase activities such as those typically measured for occupational biomonitoring (Timchalk, 2011).

In summary, PON1 enzyme activities did not differ meaningfully between Mexican-American mothers and their 9-year old children, suggesting that the window of increased susceptibility to organophosphate pesticides due to lower PON1 levels during childhood likely does not extend past 9 years of age. Conversely, we found significant differences in cholinesterase activities between mothers and children, as well as by obesity status among children. However, our data did not indicate any associations between cholinesterase and PON1 activities in either age group of this agricultural cohort. Our study also supports the importance of butyrylcholinesterase in fat metabolism. It is plausible that chronic organophosphate exposure may be increasing the risk of obesity in our population by competing for the butyrylcholinesterase being produced by the body and preventing it from reaching its substrate.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Box Plots of whole blood (acetylcholinesterase=AChE) and plasma (butyrylcholinesterase=BChE) cholinesterase activities in children and their mothers (n=202 mother-child pairs). P-values are for two-tailed paired t-tests.

Figure 2.

Box Plots of Children's whole blood (acetylcholinesterase=AChE) and plasma (butyrylcholinesterase=BChE) cholinesterase activities by Weight Status. Normal = BMI Z Scores <85% (n=97); Overweight = BMI Z-scores 85-95% (n=30); Obese = BMI Z-scores > 95% (n=73). The p for trend, determined using Cuzick's non parametric test was 0.80 and <0.0005 for AChE and BChE, respectively.

Table 1

Demographic characteristics of cohort of mothers and 9 year old children and their association with cholinesterase enzyme activity (CHAMACOS study population, Salinas Valley, California, 2008-2010).

Characteristics	n*	(%)
Child Sex		
Boy	97	(48)
Girl	105	(52)
Child Obesity Status		
Normal	97	(48.5)
Overweight	30	(15)
Obese	73	(36.5)
Mother's Obesity Status		
Normal	22	(11.1)
Overweight	75	(37.9)
Obese	101	(51)
Mother's Country of Birth		
Mexico	177	(87.6)
US	24	(11.9)
Other	1	(0.5)
Maternal Education		
Less than 6th grade	89	(44.1)
7th through 12th grade	74	(36.6)
Completed High School	39	(19.3)
Poverty [†]		
At or below Poverty Level	139	(69.8)
Within 200% Poverty Level	60	(30.2)
Mother's Work Status [†]		
Not working	43	(21.6)
Agricultural work	87	(43.8)
Other work	69	(34.7)
Agriculture workers in the Household [†]		
No	72	(36.2)
Yes	127	(63.8)

* Total numbers of observations vary due to missing data.

[†] Data collected at time of 7 year visit.

Summary of PON1 , whole blood cholinesterase (AChE), plasma cholinesterase (BChE) enzymatic activities in children at 9 years of age and mothers

Table 2

	Children					Mothers				
	n	Mean	SD	Min.	Max.	n	Mean	SD	Min.	Max.
POase (U/l) ^{c,†}	202	863.8	465.3	56.0	2234.6	202	932.2	528.3	127.0	2284.8
CPOase (U/l) ^{d,†}	202	7354.9	1813.3	2261.2	12791.6	202	7584.5	2027.2	1837.4	12303.1
ARYase (U/ml) ^e	202	130.7	31.4	47.5	206.5	202	132.7	29.3	51.6	221.5
AChE (U/ml) ^a	194	5.1	0.9	2.3	7.5	194	5.5	1.1	2.3	8.0
BChE (U/ml) ^b	195	2.3	0.5	0.8	3.9	195	2.1	0.5	0.7	3.7

^a Paraoxonase (POase): substrate = paraoxon, units = μmol/min/L

^b Chlorpyrifos-oxonase (CPOase): substrate = chlorylpyrifos-oxon, units = μmol/min/L

^c Arylesterase (ARYase): substrate = phenyl acetate, units = μmol/min/mL

^d Acetylcholinesterase (AChE): substrate =acetylcholine, units = μmol/min/mL

^e Butyrylcholinesterase (BChE): substrate = butyrylcholine, units = μmol/min/mL

** P-values are for Wilcoxon signed-rank tests comparing enzyme activities in mothers and their children.

Table 3
Mean PON1 and Cholinesterase activities by demographic characteristics in children and mothers

Characteristics	PON1						Cholinesterases					
	Child			Mother			Child			Mother		
	ARY	CPO	PO	ARY	CPO	PO	ACHE	BCHE	ACHE	BCHE	ACHE	BCHE
Child Sex												
Boy	133.8	7618.8	898.5	135.2	7835.7	908.3	5.2	2.3	5.5	2.1		
Girl	127.8	7111.0	831.8	130.4	7352.4	954.3	5.0	2.2	5.4	2.1		
Child Obesity Status												
Normal	128.9	7346.8	903.9	130.6	7473.5	951.6	5.0	2.1	5.4	2.1		
Overweight	121.1	6996.6	756.6	136.2	8028.3	1013.8	5.2	2.3	5.6	2.1		
Obese	136.1	7462.0	848.1	134.2	7550.5	866.8	5.1	2.5	5.5	2.2		
Mother's Obesity Status												
Normal	137.6	7433.9	790.9	132.5	7629.7	941.7	5.0	2.3	5.5	2.1		
Overweight	130.8	7433.6	901.9	134.7	7698.9	1001.2	5.2	2.3	5.6	2.0		
Obese	129.3	7328.3	861.9	132.2	7548.5	896.7	5.0	2.3	5.4	2.1		
Mother's Country of Birth												
Mexico	131.1	7466.3	746.2	139.1	7953.6	896.1	5.1	2.1	5.3	2.1		
US	130.7	7343.2	880.5	131.7	7532.9	940.6	5.1	2.3	5.5	2.1		
Other	114.5	6743.1	735.0	160.5	7853.3	299.4	5.3	2.1	7.9	2.7		
Maternal Education												
Less than 6th grade	133.8	7407.7	867.7	132.2	7618.7	973.2	5.1	2.3	5.5	2.1		
7th through 12th grade	127.5	7224.8	840.1	132.5	7471.9	933.5	5.2	2.3	5.5	2.1		
Completed High School	129.6	7481.0	899.9	134.2	7719.8	836.1	5.0	2.2	5.3	2.2		
Poverty [†]												
At or below Poverty Level	131.9	7386.3	874.0	130.3	7431.4	945.9	5.0	2.3	5.5	2.1		
Within 200% Poverty Level	128.5	7255.5	841.3	137.5	7874.3	911.7	5.2	2.3	5.6	2.2		
Mother's Work Status [‡]												
Not working	125.1	7251.4	852.4	133.0	7861.2	1026.8	5.3	2.4	5.6	2.2		
Agricultural work	126.8	7066.1	848.4	126.0	7224.2	793.7	4.9	2.1	5.5	1.9		

Characteristics	PON1						Cholinesterases					
	Child			Mother			Child			Mother		
	ARY	CPO	PO	ARY	CPO	PO	ACHE	BChE	BChE	ACHE	BChE	BChE
Other work	135.8	7617.0	821.6	137.6	7828.7	864.4	5.0	2.3	5.4	2.1		
Agriculture workers in the Household [†]												
No	132.5	7491.9	843.4	136.2	7666.3	855.8	5.2	2.3	5.6	2.2		
Yes	130.0	7264.6	875.8	130.4	7507.5	980.8	5.1	2.3	5.4	2.1		

**Analysis of variance (ANOVA) used to test for differences in enzyme level by demographic characteristics. P-values < 0.05 shown in bold.

[†]Data collected at time of 7 year visit.

Table 4

Spearman's correlation coefficients between PON1, whole blood (AChE), and plasma (BChE) cholinesterase activities in 9 year old Mexican-American Children (N=202).

	POase	CPOase	ARYase	AChE	BChE
POase ^a	1				
p					
CPOase ^b	0.450	1			
p	<0.0005				
ARYase ^c	0.178	0.6950	1		
p	0.011	<0.0005			
AChE ^d	0.082	-0.004	-0.021	1	
p	0.257	0.956	0.774		
BChE ^e	0.011	0.043	0.085	0.524	1
p	0.882	0.230	0.239	<0.0005	

Bold coefficient values were statistically significant ($p < 0.05$).

Positive or Negative coefficients indicate positive or negative correlations.

^aPOase: Paraoxonase

^bCPOase: Chlorpyrifos-oxonase

^cARYase: Arylesterase

^dAChE: Acetylcholinesterase

^eBChE: Butyrylcholinesterase

Table 5

Spearman's correlation coefficients between PON1, whole blood (AChE), and plasma (BChE) cholinesterase activities in Mexican-American Mothers (N=202).

	POase	CPOase	ARYase	AChE	BChE
POase ^a P	1				
CPOase ^b P	0.429 <0.0005	1			
ARYase ^c P	0.272 <0.0005	0.750 <0.0005	1		
AChE ^d P	0.064 0.379	0.003 0.970	0.118 0.100	1	
BChE ^e P	0.058 0.420	0.037 0.610	0.062 0.388	0.594 <0.0005	1

Bold coefficient values were statistically significant ($p < 0.05$).

Positive or Negative coefficients indicate positive or negative correlations.

^aPOase: Paraoxonase

^bCPOase: Chlorpyrifos-oxonase

^cARYase: Arylesterase

^dAChE: Acetylcholinesterase

^eBChE: Butyrylcholinesterase