Abstract

IMPORTANCE Research on fetal epigenetic programming suggests that the intrauterine environment can have long-term effects on offspring disease susceptibility.

OBJECTIVE To examine the association between prenatal maternal occupation and child epigenetic age acceleration (EAA) among a farmworker community.

DESIGN, SETTING, AND PARTICIPANTS This cohort study included participants in the Center for the Health Assessment of Mothers and Children of Salinas, a prospective, Latino, prebirth cohort. Pregnant women were recruited from October 1, 1999, to October 1, 2000, from 6 community clinics in California's Salinas Valley agricultural region. Participants were 18 years or older, English or Spanish speaking, Medicaid eligible, and at 20 weeks' gestation or earlier at enrollment. Mother-child pairs who had blood DNA methylation measured at the ages of 7, 9, and 14 years were included. Data were analyzed from July 2021 to November 2023.

EXPOSURES Prenatal maternal occupation was ascertained through study interviews conducted during prenatal visits and shortly after delivery.

MAIN OUTCOMES AND MEASURES Child EAA at 7, 9, and 14 years of age was estimated using DNA methylation–based epigenetic age biomarkers. Three EAA measures were calculated: the Horvath EAA, skin and blood EAA, and intrinsic EAA. Linear mixed-effects models were used to estimate longitudinal associations of prenatal maternal occupation and child EAA, adjusting for confounders and prenatal organophosphate pesticide exposure.

RESULTS Analyses included 290 mother-child pairs (mean [SD] maternal age at delivery, 26.5 [5.2] years; 152 [52.4%] female infants); 254 mothers (87.6%) were born in Mexico, 33 (11.4%) in the US, and 3 (1.0%) in other countries; and 179 families (61.7%) were below the federal poverty line during pregnancy. Mothers reported engaging in several types of work during pregnancy, including agricultural fieldwork (90 [31.0%]), other agricultural work (40 [13.8%]), nonagricultural work (53 [18.3%]), or no work (107 [36.9%]). Children whose mothers worked in agricultural fields during pregnancy had a mean of 0.66 (95% CI, 0.17-1.15) years of greater Horvath EAA, 0.62 (95% CI, 0.31-0.94) years of greater skin and blood EAA, and 0.45 (95% CI, 0.07-0.83) years of greater intrinsic EAA compared with children whose mothers did not work during pregnancy.

CONCLUSIONS AND RELEVANCE In this cohort study, prenatal maternal agricultural fieldwork was associated with accelerated childhood epigenetic aging independent of organophosphate pesticide exposure.
Abstract (continued)

exposure. Future research on which factors related to agricultural fieldwork accelerate aging in the
next generation can inform targeted prevention programs and policies that protect children’s health.

Introduction

Evidence suggests that exposures in utero can become biologically embedded via epigenetic
mechanisms, affecting fetal development and disease onset later in life.1,2 Genome-wide changes in
dNA methylation (DNAm), a type of epigenetic modification, are strongly correlated with aging.3,4
Gene-specific DNAm from multiple human tissue types has been leveraged to develop biomarkers of
biological aging, known as epigenetic clocks. Epigenetic age acceleration (EAA), which is the
difference between epigenetic age as estimated by these clocks and chronological age, is closely
associated with morbidity and mortality in adults.5-7 However, recent studies demonstrate that
epigenetic aging processes begin as early as conception,8,9 emphasizing the need to consider
 prenatal influences on aging.

Although some environmental and social exposures during pregnancy affect epigenetic age
measured at birth,8-17 data on whether the prenatal environment affects epigenetic aging
throughout childhood are limited. Prenatal smoking, gestational diabetes, and exposure to
phthalates have been associated with altered epigenetic aging in early to middle childhood.9,18-20
However, only a small number of studies have examined prenatal exposures and prospective
measurements of child EAA,21-23 and even fewer have followed up youth into adolescence.21
Moreover, no epigenetic studies have focused on maternal occupation during pregnancy, which is an
important area to consider when assessing pregnancy-related stress and burden. Prenatal maternal
stress alters DNA methylation signatures and downstream gene expression among newborns.24-26 Longitudinal
studies are needed to understand the persistence of these epigenetic modifications in childhood and
beyond,27 especially among low-income populations and mothers from underrepresented
backgrounds, who are disproportionately exposed to occupational stressors during pregnancy that
might affect their children.28

To address these research gaps, we tested the association between prenatal maternal
occupation and epigenetic aging among children in a Latino agricultural community. Pregnant
farmworkers, especially those working in agricultural fields, are particularly vulnerable to
occupational risk factors, including pesticide exposure, heat stress, and physical exertion.29,30
Beyond workplace hazards, farmworker families also often experience food and housing insecurity,
fears related to immigration status, cultural barriers, and inadequate access to medical and social
services.31-34 Given these stressors, we hypothesized that prenatal maternal agricultural work is
associated with accelerated epigenetic aging in childhood.

Methods

Study Population

This cohort study used data from the Center for the Health Assessment of Mothers and Children of
Salinas (CHAMACOS), a longitudinal, prebirth cohort composed of primarily Mexican American
children born in California’s agricultural Salinas Valley. Eligible pregnant women (≥18 years of age,
English or Spanish speaking, ≤20 weeks’ gestation at enrollment, Medicaid eligible, and planning to
deliver at the county hospital) were recruited between October 1, 1999, and October 1, 2000, from
6 community clinics, as described elsewhere.35,36 Of 601 initial enrollees, 526 (87.5%) were followed
up to the delivery of live, singleton newborns. The study continued to follow up mother-child pairs
after delivery. A phlebotomist collected child blood samples via venipuncture at study visits.
conducted when the children were 7, 9, and 14 years old. We restricted these analyses to 290 mother-child pairs who reported prenatal maternal occupation, had available child chronological age data (estimated to the day of the study visit), and provided blood samples during at least 1 study visit: 7 (n = 182), 9 (n = 239), and 14 (n = 185) years of age. Details on the number of repeated measures per participant and overlapping participants between time points are presented in eTables 1 and 2 in Supplement 1. The University of California, Berkeley Committee for the Protection of Human Subjects approved all study activities. Written informed consent was obtained from mothers. Child verbal assent was obtained from children aged 7 and 9 years; written assent was obtained from children aged 14 years. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline for cohort studies.

**DNAm and Epigenetic Aging Measures**

DNA methylation was measured from blood samples of children aged 9 years (Illumina Infinium HumanMethylation450 BeadChip; Illumina Inc) and from blood samples of children aged 7 and 14 years with the EPIC BeadChip (Illumina Inc), according to the manufacturer’s protocol. DNA methylation profiling and quality control are described in the eMethods in Supplement 1.

Epigenetic age measures from 6 clocks (Horvath pan-tissue, skin and blood, Hannum, PhenoAge, DNAmTL, and GrimAge) were estimated from the DNAm data at each time point using 3 publicly available methods: (1) the methylCIPHER R package, (2) the online Clock Foundation calculator, and (3) a principal component–based estimation. The performance of each clock and its estimation method was evaluated by Pearson correlation coefficients (r) and median absolute error (MAE) between epigenetic age and chronological age (eTable 3 in Supplement 1). The estimation method producing the highest Pearson r followed by the lowest MAE was systematically chosen for each clock for statistical analyses (eFigure 1 in Supplement 1). In primary analyses, epigenetic age from the Horvath pan-tissue clock (referred to here as the Horvath clock) and skin and blood clock were selected due to goodness of fit with chronological age in our sample (r > 0.8, MAE ≤ 2 years), original training data including pediatric populations, and applicability throughout the human lifespan. Secondary analyses were conducted using the other epigenetic aging biomarkers (Hannum, PhenoAge, DNAmTL, and GrimAge).

Epigenetic age acceleration was calculated for each clock as the residuals from a linear regression of epigenetic age on chronological age. We used Horvath EAA, skin and blood EAA, and intrinsic EAA (IEAA) as the primary outcomes. Intrinsic EAA is based on the Horvath clock but independent of changes in blood cell type composition and indicative of cell-intrinsic aging. Details on how IEAA was calculated are available in the eMethods in Supplement 1.

**Maternal Occupation During Pregnancy**

Trained bilingual staff members interviewed pregnant mothers at a median (IQR) of 13 (10-17) weeks’ and 26 (25-27) weeks’ gestation and 1 to 7 days after delivery. During each interview, mothers were asked if they were currently working and, if so, whether they had engaged in specific tasks (yes or no) at each of their jobs, if multiple. Each job was classified as agricultural fieldwork, other general agricultural work, or nonagricultural work. Agricultural fieldwork included harvesting, thinning, or weeding crops. Other agricultural work included applying and handling fertilizers, handling pesticides, operating equipment or tractors, serving as foreperson, or working in a packing shed, nursery, or greenhouse.

If mothers reported working in the fields during pregnancy and not participating in other agricultural tasks, their occupation was classified as agricultural fieldwork. Maternal occupation was categorized as other agricultural work if agricultural tasks other than fieldwork were reported during any study interview. Nonagricultural work was assigned if mothers reported working, but never in agricultural settings. Mothers were categorized as not having worked during their pregnancy if they reported not having a job during all 3 interviews. During interviews, working mothers also self-reported the physical difficulty of their jobs (not at all, not very, somewhat, or very strenuous) and the average hours per day spent standing on their feet and stooping or bending at work.
Covariates

Mother-Child Sociodemographic Characteristics
Covariates were selected a priori using a directed acyclic graph and included maternal age at delivery, prepregnancy body mass index, maternal educational level (6th grade or less, 7th-12th grade, high school graduate or more), marital status (married, living as married, separated, divorced, or single), parity (nulliparous or multiparous), prenatal smoking and alcohol consumption (no or yes), poverty status during pregnancy (poverty line or below, between the poverty line and 200%, or higher than 200% of the poverty line as determined by US Census Bureau thresholds), and child sex.

Prenatal Organophosphate Pesticide Exposure
From 1999 to 2000, the prenatal period for children in the CHAMACOS study, nearly a half-million pounds of organophosphate pesticides were applied in Salinas Valley. In this study, we assessed prenatal organophosphate pesticide exposure using 2 methods. Dialkylphosphate metabolites, a proxy of exposure to organophosphate pesticides, were measured from maternal urine samples collected during the 2 prenatal study interviews, as described elsewhere. Metabolite levels below the limit of detection were randomly imputed based on a log-normal probability distribution. Urinary dialkylphosphate concentrations were averaged across both samples. Using California Pesticide Use Reporting data from 1999 to 2001, we also estimated kilograms of organophosphate pesticides applied within 1 km of each mother’s residence from estimated conception date to delivery, as described elsewhere.

Statistical Analysis
We described participant characteristics with means (SDs) for continuous measurements and numbers (percentages) for categorical variables. Linear mixed-effects regression models were used to examine associations between prenatal maternal occupation and longitudinal measures of child Horvath EAA, skin and blood EAA, and IEAA. Models included random slopes and intercepts to account for within- and between-child variability in the outcome. Models were adjusted for child chronological age as recommended, mother-child sociodemographic characteristics, and prenatal organophosphate pesticide exposure. We adjusted for both log_{10}-transformed urinary dialkylphosphates and log_{2}-transformed California Pesticide Use Reporting estimates because a previous study in our cohort showed that these were not highly correlated and provided complementary measures of organophosphate pesticide exposure. The threshold for statistical significance was defined using 95% CIs.

As a sensitivity analysis, statistical interaction terms between child age and prenatal maternal occupation were added to the previously described models as recommended. A likelihood ratio test was used to assess whether model fit was improved by including interaction terms. Additional sensitivity analyses adjusted for (1) maternal years in the US at child’s birth, (2) prenatal paternal occupation, (3) the number of farmworkers living in the household during pregnancy, and (4) replaced maternal occupation with physical exertion at mother’s work during pregnancy as the main exposure. Data were analyzed from July 2021 to November 2023. Analyses were performed using R, version 4.3.1 (R Foundation for Statistical Computing).

Results

Participant Characteristics
Among 290 mother-child pairs (mean [SD] maternal age at delivery, 26.5 [5.2] years; 152 female [52.4%] and 138 male [47.6%] infants) included in the analysis, 254 mothers (87.6%) were born in Mexico, 33 (11.4%) in the US, and 3 (1.0%) in other countries (specific countries not reported because of small sample size and possible identification of participants), and 282 (97.2%) self-identified as Mexican or Mexican American (race not reported for the other 2.8% to protect patient anonymity). A total of 179 mothers (61.7%) were living at or below the federal poverty line during pregnancy, with
279 (96.2%) living below 200% of the poverty line. Ninety mothers (31.0%) reported agricultural field work; 40 (13.8%), other agricultural work; 53 (18.3%), nonagricultural work; and 107 (36.9%), no work during pregnancy. The Table describes sociodemographic characteristics for our analytic sample overall and by each time point. eTable 4 in Supplement 1 describes characteristics for our sample by prenatal occupation category. eTable 5 in Supplement 1 shows a comparison of characteristics between included and excluded mother-child pairs. Mothers included in our analyses were slightly older and had lived in the US for a longer duration compared with those excluded.

Performance of Epigenetic Clocks
Epigenetic aging measures from the Horvath and skin and blood epigenetic clocks were good estimates of child chronological age across 7, 9, and 14 years as measured by Pearson correlation coefficients (Horvath \( r = 0.84 \) [MAE = 1.5 years]; skin and blood \( r = 0.92 \) [MAE = 2.0 years]) (Figure 1). Other clocks performed relatively well in accuracy but had weaker correlations with chronological age and higher MAEs (eFigure 1 in Supplement 1); therefore, Horvath, skin and blood, and IEAA were used in our primary analyses. Correlations between chronological age and epigenetic age estimates by each time point are presented in eFigure 2 in Supplement 1.

Association Between Prenatal Maternal Occupation and Child EAA
Unadjusted mean child Horvath, skin and blood, and IEAA measures were consistently elevated (>0 years) at 7, 9, and 14 years of age among children whose mothers engaged in agricultural fieldwork during pregnancy (Figure 2). In longitudinal adjusted models, children whose mothers were agricultural fieldworkers during pregnancy had a mean of 0.66 (95% CI, 0.17-1.15) years greater Horvath EAA, 0.62 (95% CI, 0.31-0.94) years greater skin and blood EAA, and 0.45 (95% CI, 0.07-0.83) years greater IEAA compared with children whose mothers did not work during pregnancy (Figure 3). Associations between other agricultural work and nonagricultural work during pregnancy with child EAA were consistently close to the null (Figure 3).

In secondary adjusted analyses, prenatal maternal agricultural fieldwork was also associated with greater mean child EAA from the Hannum (1.43 years; 95% CI, 0.34-2.52 years) and PhenoAge (0.74 years; 95% CI, 0.18-1.31 years) clocks and with a negative mean age-adjusted estimate of child DNAmTL (−0.05; 95% CI, −0.09 to −0.01), indicating shorter telomere length—a hallmark of increased biological aging (eFigure 3 in Supplement 1).

Sensitivity Analyses
Estimates from models with statistical interaction terms provide evidence that the association of prenatal maternal agricultural fieldwork with child Horvath EAA may be greater with increasing child chronological age (eTable 6 in Supplement 1). Compared with children with mothers who did not work during pregnancy, children with mothers who engaged in agricultural fieldwork during pregnancy had a mean Horvath EAA that was 0.38 (95% CI, −0.16 to 0.92) years greater at 7 years of age, 0.70 (95% CI, 0.21-1.18) years greater at 9 years of age, and 1.49 (95% CI, 0.64-2.34) years greater at 14 years of age.

Prenatal maternal agricultural fieldwork remained associated with increased mean child EAA in models that additionally adjusted for (1) mothers’ years in the US as a proxy for social support and immigration-related stressors, (2) prenatal paternal occupation, or (3) the number of farmworkers living in the household during pregnancy. Mothers’ self-reported physical difficulty at work and mean hours per day standing at work were not associated with child EAA. A 1-hour increase in mothers’ mean hours per day stooping or bending at work was associated with increased mean child skin and blood EAA (0.10 years; 95% CI, 0.01-0.18 years) and IEAA (0.11 years; 95% CI, 0.01-0.20 years).
Table. Sociodemographic Characteristics of 290 Mother-Child Pairs Included in the Study at Child Ages of 7, 9, and 14 Years

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total (N = 290)</th>
<th>Age 7 y (n = 182)</th>
<th>Age 9 y (n = 239)</th>
<th>Age 14 y (n = 185)</th>
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<td><strong>Maternal characteristics</strong></td>
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<tr>
<td>Age at delivery, mean (SD), y</td>
<td>26.5 (5.2)</td>
<td>26.1 (5.0)</td>
<td>26.5 (5.2)</td>
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<td>Prepregnancy BMI, mean (SD)</td>
<td>27.4 (5.4)</td>
<td>27.4 (5.4)</td>
<td>27.5 (5.4)</td>
<td>27.6 (5.7)</td>
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<td><strong>Highest level of education</strong></td>
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<td>6th Grade or less</td>
<td>128 (44.1)</td>
<td>81 (44.5)</td>
<td>107 (44.8)</td>
<td>79 (42.7)</td>
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<tr>
<td>7th-12th Grade</td>
<td>102 (35.2)</td>
<td>64 (35.2)</td>
<td>84 (35.1)</td>
<td>67 (36.2)</td>
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<td>High school or more</td>
<td>60 (20.7)</td>
<td>37 (20.3)</td>
<td>48 (20.1)</td>
<td>39 (21.1)</td>
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<td><strong>Marital status</strong></td>
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<tr>
<td>Married</td>
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<td>79 (43.4)</td>
<td>110 (46.0)</td>
<td>79 (42.7)</td>
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<td>Living as married</td>
<td>105 (36.2)</td>
<td>72 (39.6)</td>
<td>88 (36.8)</td>
<td>76 (41.1)</td>
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<tr>
<td>Separated</td>
<td>11 (3.8)</td>
<td>7 (3.8)</td>
<td>7 (2.9)</td>
<td>7 (3.8)</td>
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<td>Divorced</td>
<td>5 (1.7)</td>
<td>3 (1.6)</td>
<td>5 (2.1)</td>
<td>4 (2.2)</td>
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<tr>
<td>Single</td>
<td>35 (12.1)</td>
<td>21 (11.5)</td>
<td>28 (11.7)</td>
<td>19 (10.3)</td>
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<td>1 (0.4)</td>
<td>0</td>
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<td><strong>Parity</strong></td>
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<tr>
<td>Nulliparous</td>
<td>95 (32.8)</td>
<td>63 (34.6)</td>
<td>74 (31.0)</td>
<td>56 (30.3)</td>
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<tr>
<td>Multiparous</td>
<td>195 (67.2)</td>
<td>119 (65.4)</td>
<td>165 (69.0)</td>
<td>129 (69.7)</td>
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<td><strong>Country of origin</strong></td>
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<tr>
<td>US</td>
<td>33 (11.4)</td>
<td>22 (12.1)</td>
<td>30 (12.6)</td>
<td>25 (13.5)</td>
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<tr>
<td>Mexico</td>
<td>254 (87.6)</td>
<td>158 (86.8)</td>
<td>206 (86.2)</td>
<td>158 (85.4)</td>
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<td>Other</td>
<td>3 (1.0)</td>
<td>2 (1.1)</td>
<td>3 (1.3)</td>
<td>2 (1.1)</td>
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<td>Mexican or Mexican American</td>
<td>282 (97.2)</td>
<td>179 (98.4)</td>
<td>232 (97.1)</td>
<td>182 (98.4)</td>
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<td>Other</td>
<td>8 (2.8)</td>
<td>3 (1.6)</td>
<td>7 (2.9)</td>
<td>3 (1.6)</td>
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<td><strong>Length of time in US at child's birth, y</strong></td>
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<td></td>
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<tr>
<td>≤1</td>
<td>52 (17.9)</td>
<td>31 (17.0)</td>
<td>42 (17.6)</td>
<td>30 (16.2)</td>
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<td>2-5</td>
<td>81 (27.9)</td>
<td>52 (28.6)</td>
<td>67 (28.0)</td>
<td>52 (28.1)</td>
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<td>6-10</td>
<td>86 (29.7)</td>
<td>55 (30.2)</td>
<td>73 (30.5)</td>
<td>54 (29.2)</td>
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<td>≥11</td>
<td>45 (15.5)</td>
<td>27 (14.8)</td>
<td>34 (14.2)</td>
<td>31 (16.6)</td>
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<td>Entire life</td>
<td>26 (9.0)</td>
<td>17 (9.3)</td>
<td>23 (9.6)</td>
<td>18 (9.7)</td>
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<td><strong>Poverty status during pregnancy</strong></td>
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<tr>
<td>At or below poverty line</td>
<td>179 (61.7)</td>
<td>113 (62.1)</td>
<td>150 (62.8)</td>
<td>113 (61.1)</td>
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<td>Between poverty line and 200%</td>
<td>100 (34.5)</td>
<td>64 (35.2)</td>
<td>78 (32.6)</td>
<td>67 (36.2)</td>
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<td>&gt;200% Poverty line</td>
<td>11 (3.8)</td>
<td>5 (2.7)</td>
<td>11 (4.6)</td>
<td>5 (2.7)</td>
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<td><strong>Smoking during pregnancy</strong></td>
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<tr>
<td>No</td>
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<td>174 (95.6)</td>
<td>230 (96.2)</td>
<td>177 (95.7)</td>
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<td>Yes</td>
<td>11 (3.8)</td>
<td>8 (4.4)</td>
<td>9 (3.8)</td>
<td>8 (4.3)</td>
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<tr>
<td><strong>Alcohol consumption during pregnancy</strong></td>
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<tr>
<td>No</td>
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<td>139 (76.4)</td>
<td>182 (76.2)</td>
<td>139 (75.1)</td>
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<td>Yes</td>
<td>67 (23.1)</td>
<td>42 (23.1)</td>
<td>56 (23.4)</td>
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<td>1 (0.5)</td>
<td>1 (0.4)</td>
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<tr>
<td>Agricultural fieldwork</td>
<td>90 (31.0)</td>
<td>59 (32.4)</td>
<td>74 (31.0)</td>
<td>59 (31.9)</td>
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<tr>
<td>Other agricultural work</td>
<td>40 (13.8)</td>
<td>22 (12.1)</td>
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<td>Did not work</td>
<td>107 (36.9)</td>
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<td>91 (38.1)</td>
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<td>Mean (SD) prenatal urinary dialkylphosphate, nmol/g of creatinine</td>
<td>287.9 (348.7)</td>
<td>278.9 (335.1)</td>
<td>280.9 (347.6)</td>
<td>265.4 (309.1)</td>
</tr>
<tr>
<td>Missing, No.</td>
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<td>0</td>
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(continued)
In this study, we tested prospective associations of maternal occupation during pregnancy with epigenetic aging across childhood in the CHAMACOS prebirth cohort. We found that prenatal maternal agricultural fieldwork was associated with accelerated child epigenetic aging independent of sociodemographic characteristics and prenatal organophosphate pesticide exposure. Our findings were consistent across multiple epigenetic clock biomarkers. Each clock was developed using DNAm at different CpG (cytosine-phosphate-guanine) sites, thus capturing different aspects of biological aging. Therefore, our results suggest that prenatal maternal agricultural fieldwork may impact several biological aging processes in children, including those independent of age-related changes in blood cell type composition as reflected by IEAA.

Theoretical frameworks, such as the DOHaD (Developmental Origins of Health and Disease),57,58 posit that maternal stressors during pregnancy can cumulatively influence offspring's health in later life. Mothers in our study population, an immigrant farmworker community, face a unique combination of adverse social and chemical exposures during pregnancy. Agricultural fieldworkers, in particular, are at the bottom of a labor hierarchy on farms that is largely defined by race, class, and citizenship.59 Moreover, female agricultural fieldworkers are routinely exposed to...
sexual harassment in the fields. Those who are pregnant are particularly vulnerable to harsh working conditions, such as irregular access to restrooms and drinking water, pesticide exposure, and prolonged, physically demanding work in high temperatures, all of which have been independently linked to adverse pregnancy and birth outcomes. Exposures to medium- and long-term heat and organochlorine pesticides have been associated with greater EAA in adults. Maternal psychosocial stress, including prenatal anxiety and perceived discrimination, have intergenerational consequences on child epigenetic aging. Given these findings, we hypothesize that the accumulation of multiple stressors associated with prenatal maternal agricultural fieldwork, not any one stressor alone, accelerated child epigenetic aging in our cohort.

To our knowledge, this is the first study to assess the association of prenatal maternal occupation with child epigenetic aging. Numerous studies have shown that chemical, psychosocial, and ergonomic hazards in prenatal maternal work environments have downstream deleterious effects on birth outcomes and child health. Our findings suggest that epigenetic pathways may be involved in these observed associations. Our study also considers a population that is typically underrepresented in research, especially in genomics. Diversifying cutting-edge research on biomarkers of aging will enable us to better understand how the social environment influences deviations in these biomarkers and develop health interventions for vulnerable populations. Moreover, our work provides support for workplace accommodations to ensure the safety of pregnant farmworkers, as well as expanded options for their paid leave during pregnancy.

Additional studies are needed to clarify the long-term health implications of altered aging processes in early life. Although the EAA of multiple clocks in adults is associated with morbidity and...
mortality, consequences of altered EAA in pediatric populations have not been characterized but provide immense opportunity for disease prevention. A handful of studies have linked EAA and maturation processes, showing that EAA is associated with higher weight for age, taller height for age, and earlier pubertal onset.69-71 In turn, early pubertal timing is associated with later risk of adult obesity, type 2 diabetes, and cardiovascular disease.72 More longitudinal research is needed to evaluate the persistence of epigenetic aging trajectories and whether accelerated epigenetic aging in childhood and adolescence impacts risk of chronic diseases.

Limitations
Our study has some limitations. Maternal agricultural fieldwork may encompass a variety of exposures, including pesticides, heat, physical exertion, and socioeconomic adversity beyond the workplace, some of which may act as mechanisms on the causal path to epigenetic aging. We found robust associations after controlling for organophosphate pesticide exposure quantified by 2 different exposure assessment methods, as well as immigration-related stressors assessed using maternal years in the US. Although our sensitivity analysis showed positive associations with prenatal occupational bending and stooping and child EAA from some clocks, this measure did not capture physical exertion outside the workplace. Other potential prenatal (eg, mothers’ exposure to heat, harassment at work, and pesticide mixtures) and postnatal (eg, early-life socioeconomic status and pesticide exposure) mechanisms were also not captured in our study. Future research should focus on identifying mediating pathways to inform targeted preventive interventions and policies. In addition, although we adjusted for covariates that are proxies for maternal socioeconomic status and acculturation, there may be residual confounding in our study from unmeasured variables that may have influenced mothers’ choice of occupation (or lack thereof) during pregnancy. Finally, our study sample was largely composed of low-income, immigrant Latino families, which limits generalizability of our results to other populations. Nevertheless, we believe it is crucial to continue expanding social and environmental epigenomics research to more diverse study populations.

Figure 3. Adjusted Associations Between Prenatal Maternal Occupation and Child Epigenetic Age Acceleration (EAA) Compared With Children Whose Mothers Did Not Work During Pregnancy

Regression coefficients in years and 95% CIs (error bars) derived from linear mixed-effects models adjusted for sociodemographic covariates (maternal age at delivery, prepregnancy body mass index, baseline maternal educational level, baseline maternal marital status, parity, poverty status during pregnancy, smoking and alcohol consumption during pregnancy, and child sex) and prenatal organophosphate pesticide exposure (log_{10}-transformed mean prenatal urinary dialkylphosphate concentrations and log_{2}-transformed kilograms of organophosphate pesticides used within 1 km of the maternal residence during pregnancy). IEAA indicates intrinsic EAA.
Conclusions

This longitudinal cohort study found that prenatal maternal agricultural fieldwork was associated with child EAA among a Latino prebirth cohort, independent of prenatal organophosphate pesticide exposure and sociodemographic characteristics. Understanding factors that accelerate early-life biological aging in vulnerable populations, such as farmworker communities, may help to identify targets for adult disease prevention and mitigate health disparities.
REFERENCES


**SUPPLEMENT 1.**

eMethods. Supplemental Methods

eTable 1. Number of Participants Who Have EAA Data Available at 1, 2, or All 3 Timepoints (7, 9, and 14 Years)
eTable 2. Individual and Overlapping Sample Sizes at Three Timepoints (7, 9, and 14 Years)
eTable 3. Systematic Comparison of Three Available Methods for Calculating Epigenetic Clocks in CHAMACOS Children (Ages 7-14 Years, N = 290)
eTable 4. Sociodemographic Characteristics of Mother-Child Pairs Included in the Study by Prenatal Maternal Occupation (N = 290)
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eTable 6. Adjusted Associations Between Prenatal Maternal Occupation and Child Horvath EAA by Child Age Compared to Children Whose Mothers Did Not Work During Pregnancy (Ages 7-14 Years, N = 290)
eFigure 1. Performance of Six Epigenetic Clocks in CHAMACOS Children (Ages 7-14 Years, N = 290)
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eReferences

**SUPPLEMENT 2.**

Data Sharing Statement