



Immune epigenetic age in pregnancy and 1 year after birth: Associations with weight change

Kharah M. Ross¹ | Judith Carroll² | Steve Horvath³ | Calvin J. Hobel⁴ |
Mary E. Coussons-Read⁵ | Christine Dunkel Schetter⁶

¹Centre for Social Sciences, Athabasca University, Athabasca, AB, Canada

²Department of Psychiatry and Biobehavioral Sciences, Cousins Center for Psychoneuroimmunology, David Geffen School of Medicine, Semel Institute for Neuroscience and Human Behavior, University of California – Los Angeles, Los Angeles, CA, USA

³Department of Biostatistics, University of California – Los Angeles, Los Angeles, CA, USA

⁴Department of Obstetrics and Gynecology, Cedars-Sinai Medical Center, Los Angeles, CA, USA

⁵Psychology Department, University of Colorado – Colorado Springs, Colorado Springs, CO, USA

⁶Department of Psychology, University of California – Los Angeles, Los Angeles, CA, USA

Correspondence

Kharah M. Ross, Centre for Social Sciences, Athabasca University, 1 University Drive, Athabasca, AB T9S 3A3, USA.
Email: kharahr@athabascau.ca

Funding information

National Center for Advancing Translational Sciences, Grant/Award Number: UL1TR001881; National Institutes of Health, Grant/Award Number: R01 HD073491

Abstract

Problem: Epigenetic age indices are markers of biological aging determined from DNA methylation patterns. Accelerated epigenetic age predicts morbidity and mortality. Women tend to demonstrate slower blood epigenetic aging compared to men, possibly due to female-specific hormones and reproductive milestones. Pregnancy and the post-partum period are critical reproductive periods that have not been studied yet with respect to epigenetic aging. The purpose of this paper was to examine whether pregnancy itself and an important pregnancy-related variable, changes in body mass index (BMI) between pregnancy and the post-partum period, are associated with epigenetic aging.

Method of Study: A pilot sample of 35 women was recruited as part of the Healthy Babies Before Birth (HB3) project. Whole blood samples were collected at mid-pregnancy and 1 year post-partum. DNA methylation at both time points was assayed using Infinium 450K and EPIC chips. Epigenetic age indices were calculated using an online calculator.

Results: Paired-sample t-tests were used to test differences in epigenetic age indices from pregnancy to 1 year after birth. Over this critical time span, women became younger with respect to *phenotypic epigenetic age*, *GrimAge*, *DNAm PAI-1*, and epigenetic age indices linked to aging-related shifts in immune cell populations, known as *extrinsic epigenetic age*. Post-partum BMI retention, but not prenatal BMI increases, predicted accelerated epigenetic aging.

Conclusion: Women appear to become younger from pregnancy to the post-partum period based on specific epigenetic age indices. Further, BMI at 1 year after birth that reflects weight retention predicted greater epigenetic aging during this period.

KEYWORDS

body mass index, epigenetic age, post-partum period, pregnancy, whole blood

1 | INTRODUCTION

Immune cell-derived epigenetic age indices are indicators of biological aging^{1,2} that are highly correlated with chronological age but capture different processes. Epigenetic age robustly predicts morbidity, such as risk of breast and lung cancer,^{3,4} and earlier mortality.⁵⁻⁷ Sex differences in epigenetic age are also observed, with women showing slower rates of epigenetic aging compared with men.⁸⁻¹¹ Reproductive biology and key reproductive periods, and their hormonal drivers have been proposed as one possible mechanism accounting for these sex differences. For example, earlier onset of menopause, whether naturally occurring or surgically induced, is associated with accelerations in epigenetic aging in women.¹² Also, faster onset and progression of puberty in girls have been associated with epigenetic age accelerations in one study,¹³ though not in another.¹⁴

Pregnancy is a normative reproductive experience with 86% of US women giving birth.¹⁵ The role of pregnancy in biological aging in general, and epigenetic aging in particular, is not well studied. Telomere length, another marker of biological aging, was examined in one study of 81 women followed from mid-pregnancy to 9 weeks post-partum, with results indicating no significant change in telomere length over this time frame.¹⁶ However, the effect of pregnancy on epigenetic age remains relatively unexplored.

Several epigenetic age indices exist that capture different aspects of biological aging. Here, we examined three classes of epigenetic age indices: those based on chronological age, specific to immune cells, and based on clinical or phenotypic indicators of mortality risk. Horvath's DNA methylation age is based on *chronological age* and is calculated from DNA methylation sites strongly associated with chronological age across tissue types.² The association between DNA methylation age and chronological age is imperfect, suggesting a decoupling between chronological age and DNA methylation-derived age. The age acceleration residual captures the difference between chronological age and biological age estimated by Horvath's DNA methylation age.

Immune-specific biological age is determined by both intrinsic, i.e., within-cell biological age, and age-driven changes in immune cell proportions. As such, *immune cell-specific* epigenetic indices also consider cell proportions. Immune cell proportions change as a function of age, with increases in exhausted or senescent CD8+ T cells and decreases in naïve CD8+ T cells over time.^{8,17} Proportions of immune cells in blood can be estimated from whole blood DNA methylation profiles.¹⁸ Intrinsic epigenetic age acceleration (IEAA) captures intrinsic biological age of immune cells, independent of age-related changes in immune cell proportions.¹⁹ In contrast, extrinsic epigenetic age acceleration (EEAA) captures biological age due to both intrinsic immune cell age and age-driven changes in immune cell populations.¹⁹

Finally, *clinical or phenotypic-derived* epigenetic age indices are calculated using DNA methylation sites that are highly correlated with clinical risk factors or outcomes. Phenotypic epigenetic age acceleration (PEAA) was developed as an epigenetic biomarker of "phenotypic age," defined by nine biological markers (albumin, creatinine,

glucose, C-reactive protein, lymphocyte percent, mean cell volume, red cell distribution, alkaline phosphatase, and white blood cell count) and chronological age.²⁰ GrimAge was constructed as a composite marker calculated from epigenetic surrogate markers for 12 plasma proteins (adrenomedullin, β -2-microglobulin, CD56, ceruloplasmin, cystatin C, EGF fibulin-like ECM protein 1, growth differentiation factor 15, leptin, myoglobin, plasminogen activator inhibitor 1, serum paraoxonase/arylesterase 1, and tissue inhibitor metalloproteinases 1) and smoking pack-years, based on self-reported smoking data,²¹ and is strongly predictive of death. DNAm plasminogen activator inhibitor-1 (PAI-1) is the epigenetic surrogate marker for PAI-1,²¹ a glycoprotein involved in suppressing fibrinolysis or the breakdown of blood clots, and which is a risk marker for cardiovascular disease.²² In sum, each epigenetic age index captures different facets of biological aging, as indexed by differences in DNA methylation patterns.

Weight change between pregnancy and the post-partum period could also be factors that affect epigenetic age during the child-bearing years. In healthy adults, obesity and weight gain have been associated with accelerations in epigenetic age.²³ Weight gain during pregnancy, however, is considered normative.²⁴ It might be hypothesized that insufficient or excess weight gain in pregnancy and/or retention of post-partum weight for longer than clinically recommended are associated with more rapid epigenetic aging. However, it is not clear whether variation in weight change over pregnancy and variation in weight loss during the post-partum period are associated with changes in epigenetic age in a manner similar to non-pregnancy populations. The purpose of the present study was to examine changes in epigenetic age variables between the second trimester and 1 year post-birth, and test associations between epigenetic age indices and weight change during pregnancy and the post-partum period.

2 | METHODS

2.1 | Participants

A sample of 35 women was studied who were recruited into the Healthy Babies Before Birth (HB3) project, which is a longitudinal study designed to test the impact of antenatal maternal mood on pregnancy and post-partum outcome. Inclusion criteria were 18 years of age or older and singleton pregnancies up to 12 weeks gestation at time of recruitment. Exclusion criteria were current substance abuse, HIV-positive status, current smoking, or medications that could affect inflammatory processes, for example, glucocorticoids at the time of recruitment. The current sample focused on women recruited at only one of the two study sites (Los Angeles, CA), who had whole blood samples collected at study entry in early pregnancy and again at 1 year after birth. Sample characteristics are presented in Table 1. The majority of participants were White (43%), married (91%), and primiparous (57%). Few adverse pregnancy outcomes were reported for the current sample, with only two participants (6%) giving birth preterm (<37 weeks gestation), and one (3%) giving birth to a low birthweight baby (< 2500 g). Less than half

TABLE 1 Sample characteristics (n = 35)

Variable	Mean \pm SD or % (N)	Range
Age (years)	33.6 \pm 5.29	23.0-45.0
Marital status (married)	91% (32)	
Per Capita Household income (\$1000)	46.2 \pm 29.8	4.06-125
Education (y)	16.9 \pm 2.90	12.0-26.0
Race/ethnicity		
White	43% (15)	
Black	14% (5)	
Latina	26% (9)	
Asian	11% (4)	
Multi-Race	6% (2)	
BMI (kg/m ²)		
Pre-pregnancy BMI	25.0 \pm 5.82	16.8-36.4
Late pregnancy change in BMI (T3-T1)	3.02 \pm 1.15	0.930-5.15
Post-partum change in BMI (P3-P1)	-1.26 \pm 2.50	-10.3-2.51
Total change in BMI (P3-Pre-pregnancy)	0.433 \pm 2.64	-10.7-5.15
Parity (Primiparous)	57% (20)	
Baby sex (female)	34% (12)	
Breastfeeding at 12 mo post-partum	49% (17)	
GA at first assessment (weeks)	16.3 \pm 2.78	13.3 - 24.4
Months post-partum at P3 (months)	11.5 \pm 0.505	11.0-12.0
Time between assessments (mo)	16.9 \pm 0.938	15.0-19.0
Assay run (April - 450K)	54% (19)	

(43%) reported at least one obstetric risk, that is, serious infection, hypertension, diabetes, or anemia, either during this or a previous pregnancy. Study data were collected and managed using REDCap electronic data capture tool.²⁵

2.2 | Protocol

Women completed a maximum of six assessments, three over pregnancy (8-16 weeks gestation, 20-26 weeks gestation, and 30-36 weeks gestation) and three over the post-partum period (6 weeks, 6 months, and 1 year post-birth). Women were included in the current analyses if they had information available on all variables. Demographics and previous pregnancy information were obtained at study entry. Height and weight were taken at each assessment. Whole blood samples were collected at the first or second pregnancy visit (16.3 \pm 2.78 weeks gestation; 8 -26 weeks gestation) and at the 1-year post-birth final study visit (11.5 \pm 0.505 months). On average, there were 16.9 \pm 0.938 months between the two assessments.

2.3 | DNA methylation

DNA was extracted from whole blood and assayed for DNA methylation by the UCLA Neurosciences Genomics Core. The first wave of participants (n = 19) was batched together using the Illumina Infinium

HumanMethylation450 BeadChip (Illumina, Inc; 485,577 CpG sites). The second wave of participants (n = 16) was run a year later using a different chip, the Infinium MethylationEPIC BeadChip Kit (Illumina, Inc; 868,464 CpG sites). A change in chip used was necessary because Illumina had discontinued the previous chip between the two runs. Approximately 90% of the CpG sites on the 450K chip are also included on the 850K chip. Both pregnancy and post-partum samples from a given participant were run on the same chip and in the same batch.

DNA methylation data were pre-processed as per standard protocols.^{2,26} Raw data were normalized using Noob in the minify package in R.²⁷ CpG sites that were missing on the 450K or EPIC chips were added, and processed data were uploaded into the epigenetic clock online calculator (<https://dnamage.genetics.ucla.edu/>). The online calculator also produces quality control checks. All samples were correctly identified as being female in origin, with tissue sources of either whole blood or blood PBMCs, and had sample and gold standard correlations $>.80$ (mean $r = .96$).

2.4 | Epigenetic age variables

2.4.1 | DNA methylation age and age acceleration residual

The epigenetic age of each blood sample was estimated using several well-defined algorithms available through an online DNA

methylation calculator.² DNA methylation age (DNAm age; years), or biological age, was calculated using the Horvath method,² which uses the weighted average of regression coefficients obtained from 353 CpG “epigenetic clock” sites. The age acceleration residual represents the difference between chronological and biological age, which is calculated by taking the residual from the linear regression model of biological age regressed onto chronological age. Again, positive values indicate accelerated biological aging.

2.4.2 | Estimates of immune cell proportions

Additional age-adjusted estimates of proportions of immune cells in circulation and biological age estimates specific to blood were obtained using the advanced analysis option in the online epigenetic clock calculator.² Proportions of plasmablast cells, exhausted or senescent CD8+ T cells (CD8+ CD28-CD45FA- T cells), naïve CD8+ T cells, and naïve CD4+ T cells were calculated using Houseman's estimation technique, which is based on DNA methylation signatures derived from purified samples of leukocytes,¹⁸ and then adjusted for chronological age.

2.4.3 | Intrinsic and extrinsic epigenetic age acceleration

IEAA is estimated using the 353 CpG sites from the Horvath method² to calculate the residual of biological age regressed onto chronological age, adjusting for imputed measures of blood cell counts known to change with age, specifically CD8+ naïve T cells, senescent CD8+ T cells, and plasmablasts.¹⁹ EEAA is estimated using the 71 CpG sites used by the Hannum method of calculating DNAm Age⁹ and is then enhanced using static weighted averages of blood cell counts that vary with age, that is, CD8+ naïve T cells, senescent CD8+ T cells, and plasmablasts.²⁸ The weights are calculated from the correlation between chronological age and each individual variable.²⁸

2.4.4 | Phenotypic epigenetic age acceleration

Phenotypic epigenetic age acceleration was calculated by Morgan Levine using R syntax as described elsewhere.²⁰ PEAA is calculated from 513 CpG sites that were selected based on ability to predict both chronological age and phenotypic indicators of aging. PEAA is associated with all-cause mortality, cancers, physical function, and Alzheimer's disease.²⁰

2.4.5 | GrimAge and DNAm PAI-1

GrimAge and DNAm PAI-1 are epigenetic age markers enriched for DNA methylation sites that are surrogate biomarkers for blood

plasma proteins related to morbidity and mortality and cigarette smoking (packs per year).²¹ DNAm PAI-1 is one of seven surrogate DNA methylation indices validated by identifying the CpG sites most associated with blood plasma protein concentrations. DNAm PAI-1 emerged as the blood plasma protein surrogate index most associated with risk for cardiovascular disease and physical functioning. GrimAge is a composite biomarker calculated from the DNAm-based surrogate epigenetic indices determined for seven blood plasma proteins and the epigenetic index capturing smoking pack-years. Both GrimAge and DNAm PAI-1 are powerful predictors of morbidity and mortality.²¹

2.4.6 | Assay reliability

A total of 16 mid-pregnancy samples were assayed on both the 450K and 850K DNA methylation chips, allowing for a test of reliability of epigenetic age variables produced by the two assays. Estimates of DNA methylation age, EEAA, PEAA, GrimAge, DNAm PAI-1, and age-adjusted CD8+ Naïve cells were fairly reliable or consistent between the two chips, r 's > .60. Age acceleration residuals and IEAA, however, were only moderately reliable, r 's = .40-.50, and age-adjusted proportion of CD8pCD28nCD45RAn were not consistent between runs, r = -.01. To account for potential between-person differences generated by assay, a covariate capturing assay batch was included in all linear regression models. (Within-person comparisons would not be affected by between-chip differences because samples from the same participant were always assayed on the same chip).

2.5 | Weight changes over pregnancy and the post-partum period

Participant height in inches was assessed at study intake, and participants self-reported their last known pre-pregnancy weight. At each assessment, weight in pounds was measured by study personnel using a balance beam scale. Body mass index (BMI; kg/m²) was calculated by converting height and weight to metric units, then dividing weight (kg) by height squared (m²) for reported pre-pregnancy weight and weight at each time point.

Pregnancy BMI change was calculated by subtracting first pregnancy assessment of BMI from last pregnancy assessment of BMI. Post-partum BMI change was calculated by subtracting first post-partum assessment of BMI from the last post-partum assessment of BMI. Total BMI change was calculated by subtracting pre-pregnancy BMI from last post-partum assessment BMI.

2.6 | Covariates

Assay batch (54% [19] on Illumina 450K; 46% [16] on Illumina EPIC) was included in all models. Given the small sample size, only

covariates significantly associated with epigenetic age variables were included in analyses, specifically race/ethnicity (coded as White or not White), years of education, parity (coded as primiparity or multiparity), marital status (married and/or cohabiting or not), gestational age at pregnancy blood sampling (weeks), breastfeeding (stopped breastfeeding before 1 year post-partum or still breastfeeding at 1 year post-partum), and pre-pregnancy BMI. Note that gestational age at pregnancy blood sampling was only included in models examining pregnancy-to-post-partum change in epigenetic age indices.

Per capita household income was also considered as a covariate, but was not significantly associated with epigenetic age indices. Given power considerations, it was not included as a covariate in analyses.

2.7 | Analytic strategy

All analyses were run using SPSS v. 24.²⁹ Data were inspected for outliers and normality prior to analyses. Outliers were defined as values $> \pm 3$ standard deviations from the respective means. First, trends in epigenetic age variables between pregnancy and the post-partum period were inspected. Bivariate correlations were used to test associations between chronological age and DNA methylation age at the pregnancy and post-partum period assessments. Paired-sample t-tests were used to determine whether there were significant changes in the epigenetic age variables from mid-pregnancy to 1 year post-partum.

Next, associations between BMI changes between pregnancy to 1 year post-birth and epigenetic age variables in pregnancy, and change over the follow-up, as appropriate, were assessed. Change in epigenetic age variables was calculated by subtracting pregnancy values from 1-year post-birth values. Bivariate correlations were inspected to identify significant associations and were followed by

linear regression analyses controlling for assay batch and covariates as noted above. Linear regression models predicting change in epigenetic age variables also co-varied for baseline or mid-pregnancy epigenetic values.^{30,31} Given the small sample size ($N = 35$), both significant ($P < .05$) and marginally significant ($P < .10$) effects were reported. Bivariate associations significant at $P < .10$ were followed by linear regression models.

3 | RESULTS

3.1 | Change in epigenetic age from pregnancy to the post-partum period*

Mean epigenetic age indices at mid-pregnancy and 1 year after birth are reported in Table 2. At study entry, women were on average 33.6 ± 5.29 years old chronologically, but 36.3 ± 5.52 years old with respect to DNA methylation age.

Strength of associations between chronological age and DNA methylation age was compared for pregnancy and post-birth assessments (Figure 1). Consistent with studies of general adult populations, chronological age and DNA methylation were highly correlated (r 's = .758 and .770, at each time point). Strength of associations between the two periods was not statistically different, $z = -0.120$, $P = .905$, suggesting that the relation between chronological age and DNA methylation age remains constant from pregnancy to the post-partum.

Paired-sample t-tests were used to determine whether change in epigenetic age variables occurred between mid-pregnancy and 1 year post-birth (Table 2). As expected, DNA methylation age increased significantly between the two time points, $t(34) = 2.34$, $p = .0025$, $M = 1.21$ years, $SD = 3.05$ years. Considerable variation was present, however, with changes in DNA methylation age ranging from increases of 5.51 years to decreases of 3.07 years (Figure 2A).

TABLE 2 Epigenetic age variable descriptive statistics ($n = 35$)

Variable	Mid-pregnancy		1 y post-partum		Difference (PP - Preg)		P
	Mn \pm SD	Range	Mn \pm SD	Range	Mn \pm SD	Range	
Chronological age (y)	33.6 \pm 5.29	23.0-45.0	35.1 \pm 5.19	24.0-46.0	1.46 \pm 0.505	1.0-2.00	<.001
DNA methylation age (y)	36.3 \pm 5.52	30.0-42.6	37.5 \pm 5.20	30.4-45.3	1.21 \pm 3.05	-3.07-5.51	.025
Age acceleration residual	0.014 \pm 3.51	-3.82-3.37	0.181 \pm 3.12	-3.99-6.59	0.167 \pm 3.04	-3.68-4.30	.747
EEAA (BioAge4HASStaticAdjAge)	1.06 \pm 3.77	-2.79-7.33	-1.97 \pm 4.28	-5.79-5.97	-3.03 \pm 3.78	-7.76-8.00	<.001
IEAA (AAHOAdjCellCounts)	0.158 \pm 3.35	-2.68-2.89	-0.043 \pm 2.92	-4.09-3.16	-0.201 \pm 3.01	-4.23-3.63	.695
Senescent CD8+ T cells	0.775 \pm 2.35	-1.76-4.13	-1.70 \pm 2.87	-4.99-2.74	-2.48 \pm 2.54	-5.86-2.75	<.001
Naïve CD8+ T cells	-5.69 \pm 37.4	-45.2-49.9	23.8 \pm 46.7	-35.2-89.8	29.5 \pm 29.1	-23.7-62.8	<.001
PEAA	1.32 \pm 5.96	-8.65-11.0	-3.18 \pm 5.41	-10.1-8.09	-4.50 \pm 4.98	-13.4-3.31	<.001
DNAm PAI-1	663 \pm 1970	-2430-5777	-387 \pm 2580	-5411-7501	-1049 \pm 1584	-6086-1723	<.001
GrimAge	1.08 \pm 3.38	-4.88-11.3	-1.56 \pm 3.35	-9.34-7.06	-2.65 \pm 1.50	-6.22-0.45	<.001

Abbreviations: EEAA, extrinsic epigenetic age acceleration (ie, not adjusted for cell populations, combination of age intrinsic to cells and due to age-related changes in cell populations); IEAA, intrinsic epigenetic age acceleration (ie, adjusted for cell populations, intrinsic to immune cells); PEAA, phenotypic epigenetic age acceleration.

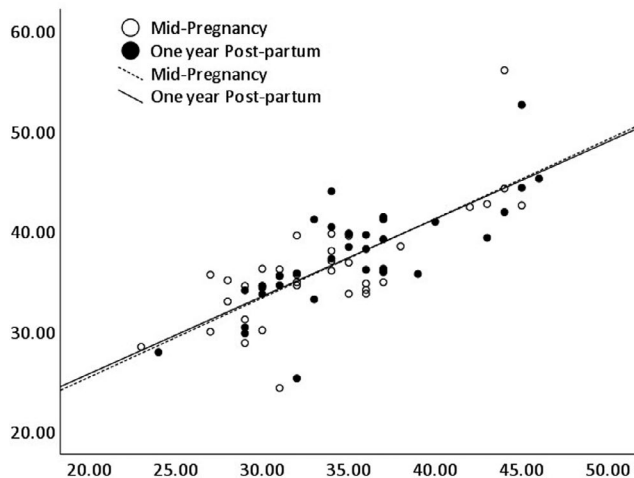


FIGURE 1 Scatterplot showing the association between chronological age and DNA methylation age during pregnancy and at 1 y post-partum. At the mid-pregnancy assessment, DNA methylation age and chronological age are correlated, $r = .758$, and at the 1 y post-partum assessment, $r = .770$

On average, there were no significant changes in rate of DNA age acceleration from pregnancy to the post-partum period, $t(34) = 0.326$, $P = .747$, indicating that rate of epigenetic aging did not change over this period (Figure 2B).

Significant changes were observed for PEAA, GrimAge, and DNAm PAI-1. In contrast to studies of non-pregnant adults, significant decreases in PEAA, $t(34) = -5.34$, $P < .001$, $M = -4.50$, $SD = 4.98$ (Figure 2C), GrimAge, $t(34) = -10.5$, $P < .001$, $M = -2.65$, $SD = 1.50$ (Figure 2D), and DNAm PAI-1, $t(34) = -3.92$, $P < .001$, $M = -1050$, $SD = 1584$ (Figure 2E), were observed between mid-pregnancy and 1 year post-birth. This suggests that women become on average younger with respect to PEAA, GrimAge, and DNAm PAI-1 between pregnancy and the post-partum period.

Immune-specific epigenetic age indices also evidenced significant changes over time. Specifically, there were significant decreases in EEAA observed from mid-pregnancy to 1 year post-partum, $t(34) = -4.73$, $P < .001$, $M = -3.03$, $SD = 3.78$ (Figure 2F), but IEAA did not significantly change over the follow-up, $t(34) = -0.396$, $P = .695$ (Figure 2G). IEAA captures epigenetic aging that is independent of age-related shifts in immune cell populations, whereas EEAA is an indicator of immune epigenetic age enriched for age-related shifts in immune cell populations. Given that decreases in only EEAA were observed, this suggests that women become younger with respect to age-related shifts in immune cell populations specifically. Consistent with this pattern, changes were also detected in age-adjusted proportions of immune cells. Again, contrary to what is expected based on observations in non-pregnant adults, proportion of senescent CD8+ T cells significantly decreased from pregnancy to the post-partum period, $t(34) = -5.78$, $P < .001$, $M = -2.48$, $SD = 2.54$ (Figure 2H), and naïve CD8+ T cells increased, $t(34) = 5.99$, $P < .001$, $M = 29.5$, $SD = 29.1$ (Figure 2I).

3.2 | Body mass index (BMI) change and post-partum epigenetic variables[†]

Participants on average gained $3.02 \pm 1.15 \text{ kg/m}^2$ during pregnancy, lost $1.26 \pm 2.50 \text{ kg/m}^2$ during the year after birth, and gained $0.433 \pm 2.64 \text{ kg/m}^2$ from before pregnancy to 1 year after birth. In bivariate correlations, increases in BMI during pregnancy were associated with lower post-birth DNA methylation age, $r = -.329$, $P = .053$, lower GrimAge, $r = -.389$, $P = .021$, and lower DNAm PAI-1, $r = -.351$, $P = .039$.[‡] After covariates were added to models, increases in BMI during late pregnancy only predicted lower post-birth GrimAge, $b = -1.35$, $SE = 0.507$, $P = .013$ (Table 3).

Having no decrease in BMI during the year post-birth (reflecting weight retention) was associated with higher post-birth DNA methylation age, $r = .395$, $P = .021$, higher age acceleration residual, $r = .362$, $P = .035$, higher IEAA, $r = .401$, $P = .019$, higher PEAA, $r = .489$, $P = .003$, higher GrimAge, $r = .639$, $P < .001$, higher DNAm PAI-1, $r = .409$, $P = .016$, smaller decreases in GrimAge from pregnancy to 1 year after birth, $r = .446$, $P = .008$, and smaller decreases in DNAm PAI-1, $r = .491$, $P = .003$. Each of these associations persisted after adjusting for covariates, p 's $< .028$ (Table 3; Figure 3).

Next, change in BMI during pregnancy and during the first year post-birth were both entered into linear regression models with covariates. Having no decrease in BMI by 1 year post-birth continued to predict higher 1-year age acceleration residuals, IEAA, PEAA, GrimAge, change in GrimAge, and change in DNAm PAI-1, p 's $< .041$. In contrast, BMI change during pregnancy was not independently associated with any epigenetic age indices, p 's $> .217$.

Finally, bivariate correlations between total BMI change from before pregnancy to the post-birth and epigenetic age indices were computed. Greater total increases in BMI from preconception to 1 year post-birth were associated with higher post-birth PEAA, $r = .331$, $P = .052$, GrimAge, $r = .430$, $P = .010$, and higher DNAm PAI-1, $r = .351$, $P = .038$; smaller decreases in PEAA, $r = .440$, $P = .08$, smaller decreases in GrimAge, $r = .451$, $P = .007$, and smaller decreases in DNAm PAI-1, $r = .496$, $P = .002$, from pregnancy to 1 year post-birth; and large increases in IEAA from pregnancy to 1 year post-birth, $r = .316$, $P = .064$. After adjusting for covariates, greater total increases in BMI from preconception to 1 year post-birth continued to be associated with increases in IEAA, $b = 0.504$, $SE = 0.216$, $P = .028$, and smaller decreases in PEAA, $b = 0.700$, $SE = 0.326$, $P = .042$, GrimAge, $b = .0439$, $SE = 0.098$, $P < .001$, and DNAm PAI-1, $b = 336$, $SE = 130$, $P = .017$ (Table 3).

4 | DISCUSSION

The purpose of this study was to evaluate changes in immune cell epigenetic age indices between pregnancy and the post-partum period and explore associations with important pregnancy-related factors, namely weight change from mid-pregnancy to 1 year post-partum. The results suggest that some epigenetic age variables changed in

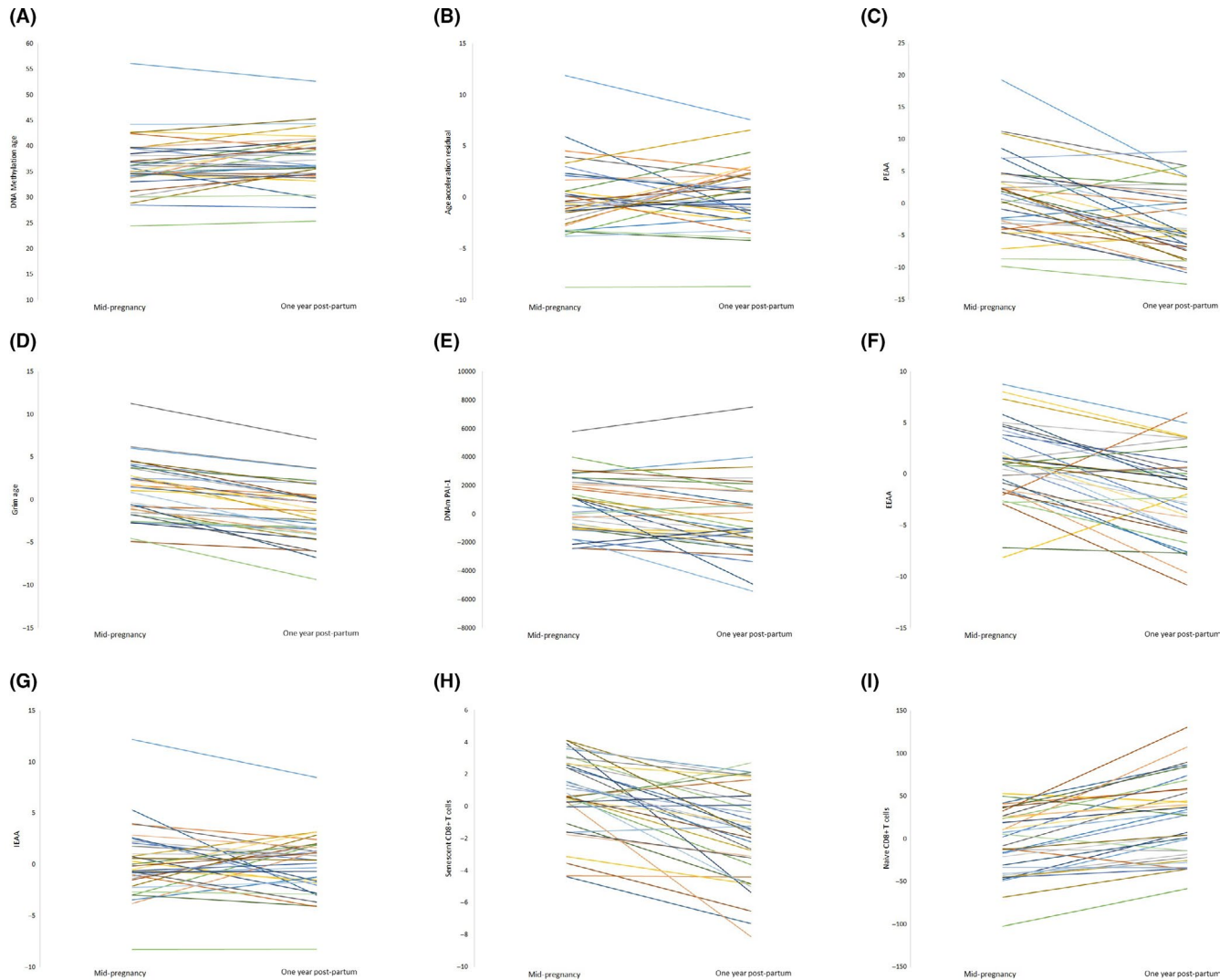


FIGURE 2 Spaghetti plots of change between mid-pregnancy and 1 y post-partum for (A) DNA methylation age, (B) age acceleration residual, (C) phenotypic epigenetic age acceleration (PEAA), (D) GrimAge, (E) DNAm PAI-1, (F) extrinsic epigenetic age acceleration (EEAA), (G) intrinsic epigenetic age acceleration (IEAA), (H) senescent CD8+ T cells, and (I) naïve CD8+ T cells. DNA methylation age and naïve CD8+ T cells significantly increased (p 's < .025), PEAA, GrimAge, DNAm PAI-1, EEAA, and senescent CD8+ T cells significantly decreased (p 's < .001), and age acceleration residual and IEAA did not significantly change (p 's > .695) between mid-pregnancy and 1 y post-partum

counterintuitive ways between mid-pregnancy and 1 year post-partum, with women appearing to become biologically younger with respect to PEAA, GrimAge, DNAm PAI-1, and immune cell population epigenetic age indices (EEAA, age-adjusted proportion of senescent CD8+ T cells and naïve CD8+ T cells). There is also evidence that epigenetic age between mid-pregnancy and 1 year post-partum could be affected by weight change during pregnancy and the post-partum period. In particular, weight retention over the post-partum period was predictive of epigenetic age acceleration from pregnancy to the post-partum. Collectively, these preliminary findings shed light on how epigenetic aging processes operate during pregnancy and the post-partum period.

To the best of our knowledge, this is the first study to assess changes in immune cell proportions during pregnancy and the post-partum period, and results suggest that pregnancy physiology could influence proportion of immune cells, with implications

for understanding immune activity during pregnancy. In non-pregnant adults, EEAA and age-adjusted proportions of senescent CD8+ T cells increase over time, and age-adjusted proportion of naïve CD8+ T cells decrease over time.^{8,17,32} We observed the opposite from pregnancy to the post-partum period. EEAA and age-adjusted proportion of senescent CD8+ T cells *decreased*, and age-adjusted proportion of naïve CD8+ T cells *increased* from pregnancy to the post-partum period. This pattern suggests a regeneration of T cells from pregnancy to the post-partum period, potentially indicating that, following major changes in the maternal immune system during normal pregnancy and recovery from labor and delivery,³³⁻³⁵ there is a partial post-partum rejuvenation. In addition, PEAA, GrimAge, and DNAm PAI-1, which are all enriched for DNA methylation sites associated with clinical indicators of morbidity and mortality risk, and which also increase with biological age in non-pregnant adults, decreased between pregnancy and the post-partum period. This

Predictor	Outcome	B	SE	β	P
Preg BMI change	PP DNA methylation age	1.23-	0.952	-.271	.207
	PP GrimAge	-1.35	0.507	-.464	.013
	PP DNAm PAI-1	-680	376	-.302	.082
PP BMI change	PP DNA methylation age	1.02	0.435	.484	.028
	PP Age accel resid	0.730	0.253	.577	.008
	PP IEAA	0.795	0.229	.671	.002
	PP PEAA	1.30	0.393	.592	.003
	PP GrimAge	0.874	0.217	.645	<.001
	PP DNAm PAI-1	497	179	.476	.010
	Change GrimAge ^a	0.450	0.118	.744	.001
	Change DNAm PAI-1 ^a	323	140	.519	.030
Total BMI change	PP PEAA	0.728	0.426	.356	.099
	PP GrimAge	0.472	0.253	.373	.074
	PP DNAm PAI-1	277	189	.284	.154
	Change in IEAA ^a	0.504	0.216	.443	.028
	Change in PEAA ^a	0.700	0.326	.372	.042
	Change in GrimAge ^a	0.439	0.098	.212	<.001
	Change in DNAm PAI-1 ^a	336	130	.561	.017

TABLE 3 Linear regression models predicting epigenetic age variables from pregnancy-related variables

Abbreviations: Age accel resid, age acceleration residual; BMI, body mass index; EEAA, extrinsic epigenetic age acceleration; IEAA, intrinsic epigenetic age acceleration; PEAA, phenotypic epigenetic age acceleration; PP, post-partum; Preg, pregnancy.

^aindicates models for which baseline (pregnancy) epigenetic age indices and gestational age at pregnancy assessment were additionally included as covariates.

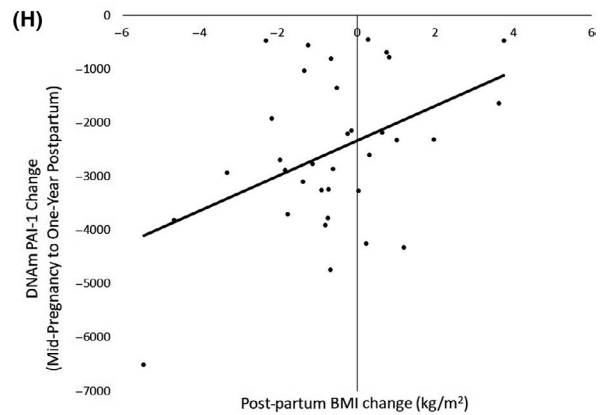
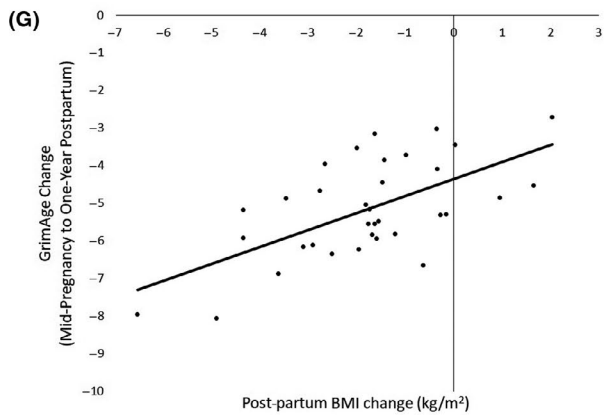
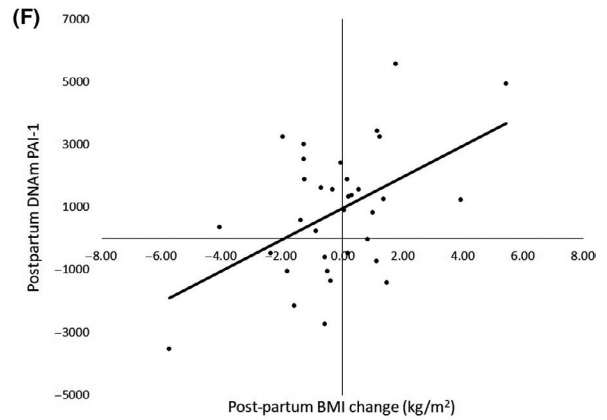
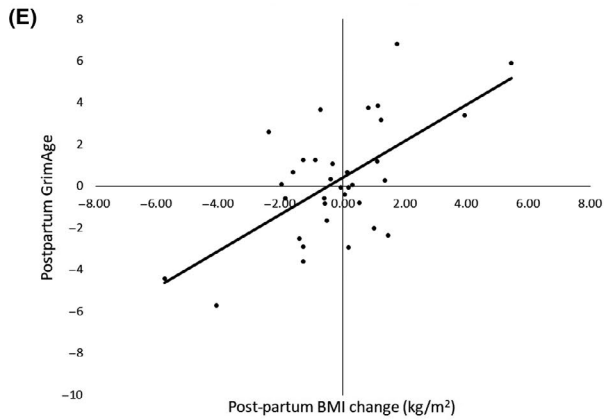
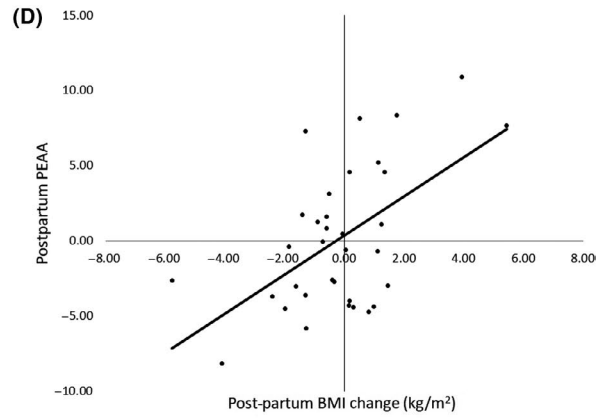
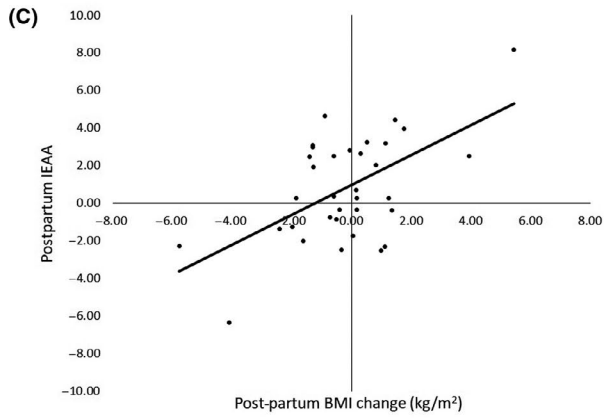
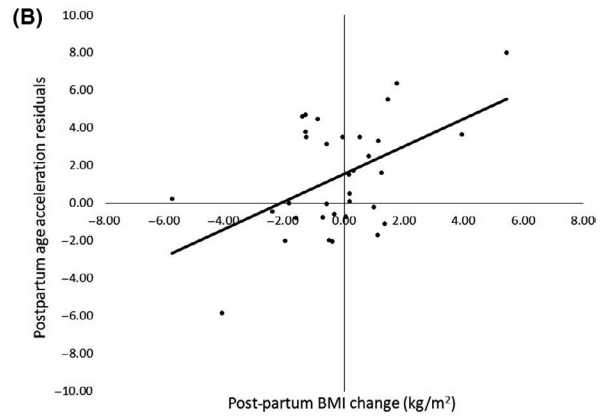
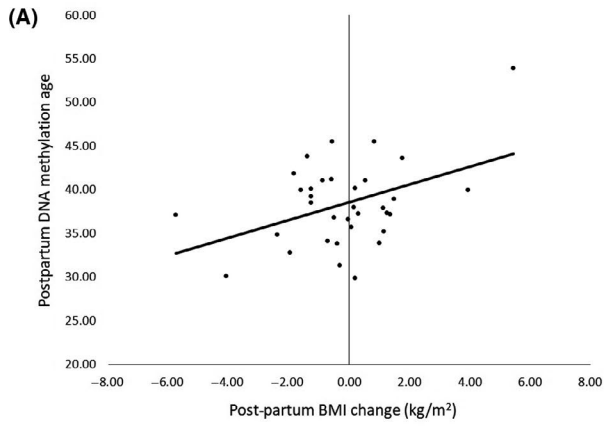
suggests that overall pregnancy may slow some aspects of aging. It is possible that this is driven by the unique neuroendocrine activity that characterizes pregnancy and the post-partum period, particularly for growth and thyroid hormones,²⁴ although additional research is needed to explore this possibility. Collectively, then, our findings suggest that in some ways, women's immune indicators of biological age become younger between mid-pregnancy and 1 year post-partum, consistent with a protective health effect of parity³⁶⁻⁴⁰ and slower biological aging in women compared to men.⁸⁻¹¹

Post-partum weight change emerged as a strong predictor of epigenetic age indices. In non-pregnant adult samples, obesity and higher body weight are associated with accelerated epigenetic age.²³ In the current sample, weight gain during pregnancy was not consistently associated with post-partum epigenetic age indices. It is possible that processes linking weight gain and biological aging are interrupted during pregnancy in an adaptive manner, or that no association was found because weight gain during pregnancy can be driven by other factors, for example, fluid retention,

and placenta and fetal growth. This finding along with the others requires replication. In contrast, weight retention during the post-partum period was associated with accelerations in DNA methylation age and higher PEAA at 12 months post-partum, and this was independent of weight change during pregnancy. These findings are important in the context of prior work suggesting that post-partum weight gain and weight retention increases risk for later health issues.⁴¹⁻⁴⁴

There are several limitations to consider, and foremost is that this was a pilot study with a relatively small sample size. It is not possible to determine whether null results indicate a lack of association or a lack of power to detect associations. Nevertheless, meaningful and potentially important patterns were detected, supporting the usefulness of pursuing these questions in future research. Second, although a clear strength of the study is use of data collected in a prospective design, the time period from mid-pregnancy to 1 year post-partum does not capture the full length of pregnancy, which would be of interest to do. Future

FIGURE 3 Associations between post-birth change in BMI with (A) post-partum DNA methylation age, (B) post-partum age-adjusted acceleration residuals, (C) post-partum IEAA (intrinsic epigenetic age acceleration), (D) post-partum PEAA (phenotypic epigenetic age acceleration), (E) post-partum GrimAge, (F), post-partum DNAm PAI-1, (G) change in GrimAge from mid-pregnancy to 1 y post-partum, and (H) change in DNAm PAI-1 from mid-pregnancy to 1 y post-partum, adjusting for covariates. Baseline or mid-pregnancy values were also included as covariates for models of change



work should consider tracking epigenetic aging from preconception through pregnancy and possibly longer into the post-birth period and to test additional factors that are associated with rates of aging. Third, there was a change in DNA methylation chips mid-way through our pilot project (450K vs EPIC), and indications of systematic between-person differences in epigenetic age variables were detected by chip. Within-person differences however were not due to chip differences because samples drawn for each participant from the two different time points were always included on the same chip. Thus, observed change in epigenetic age could not be due to batch. Likewise, batch was adjusted for in our models. Optimally, future research should replicate these findings using a single chip to reduce batch effects. Fourth, it is possible that adverse pregnancy outcomes, such as preterm birth, or obstetric risk factors, such as hypertension, diabetes, serious infections, or anemia, could affect epigenetic age indices in immune cells during pregnancy. Due to the small sample size and low frequency of specific pregnancy complications, we were not able to test associations between pregnancy complications and change in epigenetic age indices between pregnancy and a year post-partum. Future work should consider how epigenetic aging during pregnancy and the post-partum is affected by pregnancy complications and adverse outcomes.

5 | CONCLUSIONS

From pregnancy to post-partum, women's epigenetic age, as indexed by the EEAA, PEAA, GrimAge, and DNAm PAI-1, decreased from pregnancy to the post-partum, indicating deceleration of biological aging. This is the reverse of what has been observed in non-pregnant adults. Importantly, not all women exhibited deceleration. When examining predictors of rates of epigenetic aging, post-partum weight gain was associated with increases in epigenetic aging. These findings shed light on the unique biological states that constitute pregnancy and the post-partum period and highlight a possible mechanism through which pregnancy and post-partum associated factors could affect maternal health over the long-term.

ACKNOWLEDGMENTS

All protocols and methods were reviewed and approved by the University of California—Los Angeles, Cedars-Sinai Medical Centre institutional review boards, and participants provided written informed consent. All authors have reviewed this manuscript and consent to its publication. Data will be deposited following completion of all data analyses, but are available upon request from J. Carroll and C. Dunkel Schetter. The authors report no competing interests. Healthy Babies Before Birth (HB3) Study was supported by funding from NIH (R01 HD073491; MPI: Coussons-Read & Dunkel Schetter). Additional epigenetic assays were funded by the NIH/National Center for Advancing Translational Science UCLA CTSI Grant (UL1TR001881). REDCap is supported by the Colorado Clinical

& Translational Sciences Institute (CTSII) with the Development and Informatics Service Center (DISC) grant support [NIH/NCRR Colorado CTSI Grant Number UL1 RR025780] and by the UCLA Cousins Center for Psychoneuroimmunology. K. Ross conducted analyses and led writing the manuscript, J. Carroll advised on direction of analyses and manuscript, S. Horvath advised on epigenetic clock and related analyses, and C. Dunkel Schetter and M. Cousins Read oversaw the larger study design, data collection, and advised on the manuscript. The authors thank the participants and the staff especially Dr Roberta Mancuso, Project Coordinator in Denver and Susan Jackman, RN, Coordinator of the Cedars Sinai Health Center site.

ORCID

Kharah M. Ross  <https://orcid.org/0000-0002-1472-5630>

ENDNOTES

- * Pregnancy and post-partum epigenetic age indices were adjusted for gestational age at pregnancy at time of blood sampling using repeated measure ANCOVAs. Magnitude of change over the follow-up was not affected by gestational age at sampling, and the same pattern of results emerged.
- † Pattern of results was similar and consistent when timing of weight measurement (pregnancy gestational age or weeks post-partum) and timing between weight measurements were included in models as covariates.
- ‡ Given that there is no theoretical reason to assume that mid-pregnancy epigenetic age values would drive changes in weight over the follow-up, pregnancy epigenetic age variables were not considered.

REFERENCES

1. Mitteldorf J. How does the body know how old it is? Introducing the epigenetic clock hypothesis. *Interdiscip Top Gerontol*. 2015;40:49-62.
2. Horvath S. DNA methylation age of human tissues and cell types. *Genome Biol*. 2013;14(10):R115.
3. Ambatipudi S, Horvath S, Perrier F, et al. DNA methylome analysis identifies accelerated epigenetic ageing associated with postmenopausal breast cancer susceptibility. *Eur J Cancer*. 2017;75:299-307.
4. Levine ME, Hosgood HD, Chen B, Absher D, Assimes T, Horvath S. DNA methylation age of blood predicts future onset of lung cancer in the women's health initiative. *Aging*. 2015;7:690-700.
5. Marioni RE, Shah S, McRae AF, et al. DNA methylation age of blood predicts all-cause mortality in later life. *Genome Biol*. 2015;16:25.
6. Horvath S, Pirazzini C, Giula Bacalini M, et al. Decreased epigenetic age of PBMCs from Italian semisupercentenarians and their offspring. *Aging*. 2015;7:1159-1170.
7. Christiansen L, Lenart A, Tan QH, et al. DNA methylation age is associated with mortality in a longitudinal Danish twin study. *Aging Cell*. 2016;15(1):149-154.
8. Kananen L, Marttila S, Nevalainen T, et al. The trajectory of the blood DNA methylome ageing rate is largely set before adulthood: evidence from two longitudinal studies. *Age (Dordr)*. 2016;38(3):65.
9. Hannum G, Guinney J, Zhao L, et al. Genome-wide methylation profiles reveal quantitative views of human aging rates. *Mol Cell*. 2013;49(2):359-367.
10. Weidner CI, Lin Q, Koch CM, et al. Aging of blood can be tracked by DNA methylation changes at just three CpG sites. *Genome Biol*. 2014;15:R24.

11. Simpkin AJ, Hemani G, Suderman M, et al. Prenatal and early life influences on epigenetic age in children: a study of mother-offspring pairs from two cohort studies. *Hum Mol Genet.* 2016;25(1):191-201.
12. Levine ME, Lu AT, Chen BH, et al. Menopause accelerates biological aging. *Proc Natl Acad Sci USA.* 2016;113(33):9327-9332.
13. Binder AM, Corvalan C, Mericq V, et al. Faster ticking rate of the epigenetic clock is associated with faster pubertal development in girls. *Epigenetics.* 2018;13(1):85-94.
14. Simpkin AJ, Howe LD, Tilling K, et al. The epigenetic clock and physical development during childhood and adolescence: longitudinal analysis from a UK birth cohort. *Int J Epidemiol.* 2017;46(2):549-558.
15. Pew Research Center. They're Waiting Longer, but U.S. Women Today More Likely to Have Children Than a Decade Ago; 2018.
16. Mitchell AM, Kowalsky JM, Epel ES, Lin J, Christian LM. Childhood adversity, social support, and telomere length among perinatal women. *Psychoneuroendocrinology.* 2018;87:43-52.
17. Marttila S, Kananen L, Hayrynen S, et al. Ageing-associated changes in the human DNA methylome: genomic locations and effects on gene expression. *BMC Genom.* 2015;16:179.
18. Houseman EA, Accomando WP, Koestler DC, et al. DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC Bioinformatics.* 2012;13:86.
19. Carroll JE, Irwin MR, Levine M, et al. Epigenetic aging and immune senescence in women with insomnia symptoms: findings from the women's health initiative study. *Biol Psychiatry.* 2017;81(2):136-144.
20. Levine ME, Lu AT, Quach A, et al. An epigenetic biomarker of aging for lifespan and healthspan. *Aging (Albany NY).* 2018;10(4):573-591.
21. Lu AT, Quach A, Wilson JG, et al. DNA methylation GrimAge strongly predicts lifespan and healthspan. *Aging (Albany NY).* 2019;11(2):303-327.
22. Kohler HP, Grant PJ. Plasminogen-activator inhibitor type 1 and coronary artery disease. *N Engl J Med.* 2000;342(24):1792-1801.
23. Horvath S, Erhart W, Brosch M, et al. Obesity accelerates epigenetic aging of human liver. *Proc Natl Acad Sci USA.* 2014;111(43):15538-15543.
24. Cunningham GF, Leveno KJ, Bloom SL, Hauth JC, Rouse DJ, Song CY. *Williams Obstetrics*, 24 ed. New York: McGraw Hill Professional; 2014.
25. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap) - A metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform.* 2009;42:377-381.
26. Triche TJ Jr, Weisenberger DJ, Van Den Berg D, Laird PW, Siegmund KD. Low-level processing of Illumina Infinium DNA methylation beadArrays. *Nucleic Acids Res.* 2013;41(7):e90.
27. minfi: Analyze Illumina Infinium DNA methylation arrays [computer program]. Version 1.32.02019.
28. Klemera P, Doubal S. A new approach to the concept and computation of biological age. *Mech Ageing Dev.* 2006;127(3):240-248.
29. *IBM SPSS Statistics for Windows. Statistics for Windows, Version 24.0 [computer program].* Armonk, NY: IBM Corp; 2016.
30. Dalecki M, Willits FK. Examining change using regression analysis: Three approaches compared. *Sociol Spectr.* 1991;11(2):127-145.
31. Gillespie DF, Streeter CL. Fitting regression-models to research questions for analyzing change in nonexperimental research. *Soc Work Res.* 1994;18(4):239-245.
32. Pawelec G, Larbi A, Derhovanessian E. Senescence of the human immune system. *J Comp Pathol.* 2010;142(Suppl 1):S39-S44.
33. Mor G, Cardenas I. The immune system in pregnancy: a unique complexity. *Am J Reprod Immunol.* 2010;63(6):425-433.
34. Ross KM, Miller G, Culhane J, et al. Patterns of peripheral cytokine expression during pregnancy in two cohorts and associations with inflammatory markers in cord blood. *Am J Reprod Immunol.* 2016;76(5):406-414.
35. Christian LM, Porter K. Longitudinal changes in serum proinflammatory markers across pregnancy and postpartum: effects of maternal body mass index. *Cytokine.* 2014;70:134-140.
36. Grundy E, Tomassini C. Fertility history and health in later life: a record linkage study in England and Wales. *Soc Sci Med.* 2005;61(1):217-228.
37. Jaffe DH, Neumark YD, Eisenbach Z, Manor O. Parity-related mortality: shape of association among middle-aged and elderly men and women. *Eur J Epidemiol.* 2009;24(1):9-16.
38. Manor O, Eisenbach Z, Israeli A, Friedlander Y. Mortality differentials among women: the Israel Longitudinal Mortality Study. *Soc Sci Med.* 2000;51(8):1175-1188.
39. Kvale G, Heuch I, Nilssen S. Parity in relation to mortality and cancer incidence: a prospective study of Norwegian women. *Int J Epidemiol.* 1994;23(4):691-699.
40. Lund E. Number of children and death from hormone-dependent cancers. *Int J Cancer.* 1990;46(6):998-1000.
41. Shao Y, Qiu J, Huang H, et al. Pre-pregnancy BMI, gestational weight gain and risk of preeclampsia: a birth cohort study in Lanzhou, China. *BMC Pregnancy Childbirth.* 2017;17(1):400.
42. Olson CM. Achieving a healthy weight gain during pregnancy. *Annu Rev Nutr.* 2008;28:411-423.
43. Hedderson MM, Gunderson EP, Ferrara A. Gestational weight gain and risk of gestational diabetes mellitus. *Obstet Gynecol.* 2010;115(3):597-604.
44. Endres LK, Straub H, McKinney C, et al. Postpartum weight retention risk factors and relationship to obesity at 1 year. *Obstet Gynecol.* 2015;125(1):144-152.

How to cite this article: Ross KM, Carroll J, Horvath S, Hobel CJ, Coussons-Read ME, Dunkel Schetter C. Immune epigenetic age in pregnancy and 1 year after birth: Associations with weight change. *Am J Reprod Immunol.* 2020;83:e13229. <https://doi.org/10.1111/aji.13229>