Cell Metabolism

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Letter

The effects of pregnancy, its progression, and its cessation on human (maternal) biological aging

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https://doi.org/10.1016/j.cmet.2024.02.016

It is well established that the state of pregnancy imposes considerable physiological stress within the maternal compartment. Based on this observation, pregnancy has been proposed to serve as a natural challenge that may reveal latent stress-related vulnerabilities of relevance for future disease risk.[1] However, a biomarker of the overall physiological toll imposed by pregnancy is currently lacking.

Poganik and colleagues[2] recently provided compelling evidence that physiological stressors, including pregnancy, are associated with an acceleration of biological aging, as indexed by measures from DNA methylation (DNAm)-based epigenetic clocks. In mice, the authors reported evidence for pregnancy-associated biological aging, with a partial reversal of this effect after the cessation of pregnancy (i.e., in the postpartum). They also observed pregnancy-associated biological aging in humans, with suggestive evidence from a cohort of 14 women that these effects may be partially reversed in the postpartum.

We sought to replicate and extend these novel findings of Poganik and colleagues in a prospective, longitudinal, low-risk human pregnancy cohort at the University of California Irvine Development, Health and Disease Research Program. Longitudinal DNAm data (MethylationEPIC v.1.0, Illumina) were derived from blood samples collected from 119 women in early, mid, and late pregnancy as well as a fourth blood sample at approximately 3 months after delivery in 68 of these women.[3] Using these data, we generated estimates of biological aging by using principal component-based epigenetic clocks (PCHorvath1, PCPhenoAge, PCGrimAge), which are more robust to sources of technical variation than conventional clocks;[4] an updated version of the GrimAge estimator (GrimAge2);[5] and a pace of aging biomarker (PACE).[6]

We used hierarchical generalized additive models[7] to determine whether the stage of pregnancy is associated with accelerated biological aging and if these effects are reversed postpartum. Our models adjusted for maternal age, parity, race and ethnicity, education, household income, and technical factors related to DNAm profiling.

Because certain maternal characteristics may further increase the physiological load imposed by pregnancy,[8] we examined whether maternal pre-pregnancy body mass index (BMI) was associated with measures of biological aging. Sensitivity analyses tested whether changes in measures of maternal biological aging were explained by gestational weight gain or variation in the proportion of different blood cell types across pregnancy and postpartum.[9]

Consistent with Poganik et al.,[2] we found a significant positive association between the stage of pregnancy and biological age (Figures S1A–S1E). Centering parturition as time zero, from early (-26.5 ± 2.2 weeks) to late (-8.8 ± 1.7 weeks) pregnancy, a period of approximately 18 weeks, measures of adjusted maternal biological age increased by 2.39 years for PCPhenoAge ($p < 0.001$, 95% CI [1.75, 3.03]), 1.19 years for PCGrimAge ($p < 0.001$, 95% CI [0.93, 1.46]), 2.52 years for GrimAge2 ($p < 0.001$, 95% CI [2.09, 2.95]), and 0.07 units for PACE ($p < 0.001$, 95% CI [0.05, 0.08]). We also observed a statistically significant "reversal" of biological aging across all epigenetic biomarkers from the late pregnancy to the approximately 3-month postpartum time point.

Maternal pre-pregnancy BMI altered the trajectory of biological aging (lower panels of Figures S1A–S1E). Pre-pregnancy BMI group differences (75^{th} [BMI = 30] versus 25^{th} percentile [BMI = 23]) were most pronounced at the 3 months postpartum time point, with increased biological aging in the high BMI group (PCHorvath1: 1.00 years, $p \approx 0.024$, 95% CI [0.13, 1.87]; PCPhenoAge: 1.42 years, $p \approx 0.037$, 95% CI [0.09, 2.75]; PCGrimAge: 0.66 years, $p \approx 0.007$, 95% CI [0.18, 1.14]; GrimAge2: 1.20 years, $p < 0.001$, 95% CI [0.65, 1.75]; PACE: 0.04 units, $p < 0.001$, 95% CI [0.02, 0.06]). For two epigenetic biomarkers (GrimAge2 and PACE), pre-pregnancy BMI also predicted increased biological aging across pregnancy. A difference-in-difference analysis showed that pre-pregnancy BMI group differences in GrimAge2 and PACE estimates at 3 months postpartum were larger than those observed during pregnancy (see Data S1).

Next, we determined whether our results were explained by maternal gestational weight gain or changes in cell-type proportions, given the dynamic change in leukocytes across pregnancy (Figure S1F). Maternal gestational weight gain did not predict measures of biological aging (all weight gain β coefficients $p > 0.09$). After cell-type adjustment, a pregnancy-associated increase in biological aging followed by a postpartum recovery was observed for all measures of

biological aging except the PCHorvath1 measure (see Data S1). Adjustment for cell types reduced, but did not fully account for, the associations we observed between pre-pregnancy BMI and multiple measures of biological aging at 3 months postpartum (PCPhenoAge, PCGrimAge, GrimAge2, and PACE associations all $p < 0.05$, Figure S1G). Finally, given the established association between breastfeeding and maternal physiology (including maternal postpartum weight loss),[10] we used regression models with robust standard errors (adjusted for relevant covariates) to determine, in the subgroup of women with relevant data (n = 60), whether breastfeeding predicted biological age estimates at 3 months postpartum. Mothers who reported exclusive breastfeeding (versus mixed feeding practices or exclusive formula feeding) had significantly lower PCGrimAge (−0.94 years, $p \approx 0.024$, 95% CI [−1.74, −0.13]) and PACE (−0.05 units, $p \approx 0.032$, 95% CI [−0.09, −0.004]) age estimates independent of pre-pregnancy BMI (Figure S1H).

Using an extended panel of epigenetic biomarkers, our findings replicate those reported by Poganik and colleagues[2] to show that the state and stage of pregnancy are positively associated with biological aging. We now also provide evidence of a postpartum recovery effect. We note that the magnitude of the decrease in maternal biological age from the pregnant to non-pregnant state was about 2 to 3 times more than the increase in biological age from early to late pregnancy, indicating a pronounced reversal of biological aging. In addition, we identify maternal pre-pregnancy BMI and breastfeeding as two factors that may increase or decrease maternal biological aging in the postpartum respectively.

Further work is required to (1) determine whether the reversal of maternal biological age we observed at 3 months postpartum is maintained over time and whether such effects accumulate over successive pregnancies; (2) study the impact of more direct measures of maternal adiposity (rather than maternal BMI) on pregnancy-associated biological aging; (3) examine whether individual differences in pregnancy-associated biological aging predict future maternal cardiometabolic and other health outcomes; and (4) study the effects of interventions in maternal health during pregnancy on trajectories of maternal biological aging.

In conclusion, our main finding that the state of pregnancy and its progression is associated with significantly greater changes in biological aging than would be accounted for by the passage of chronological time (age) provides support for the notion that pregnancy may act as a naturally occurring physiological stressor. This opens the door for further research on the determinants and consequences of this phenomenon and its prognosticating effect on the future health and disease risk of mothers and possibly also of their offspring.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cmet.2024.02.016.

ACKNOWLEDGMENTS

This research was funded by a European Research Area Network (ERA Net) Neuron grant MecTranGen 01EW1407A to C.B. and National Institutes of Health grants R01 HD-060628 to P.D.W. and R01 MD-017387 to P.D.W. and S.E.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Supplemental information

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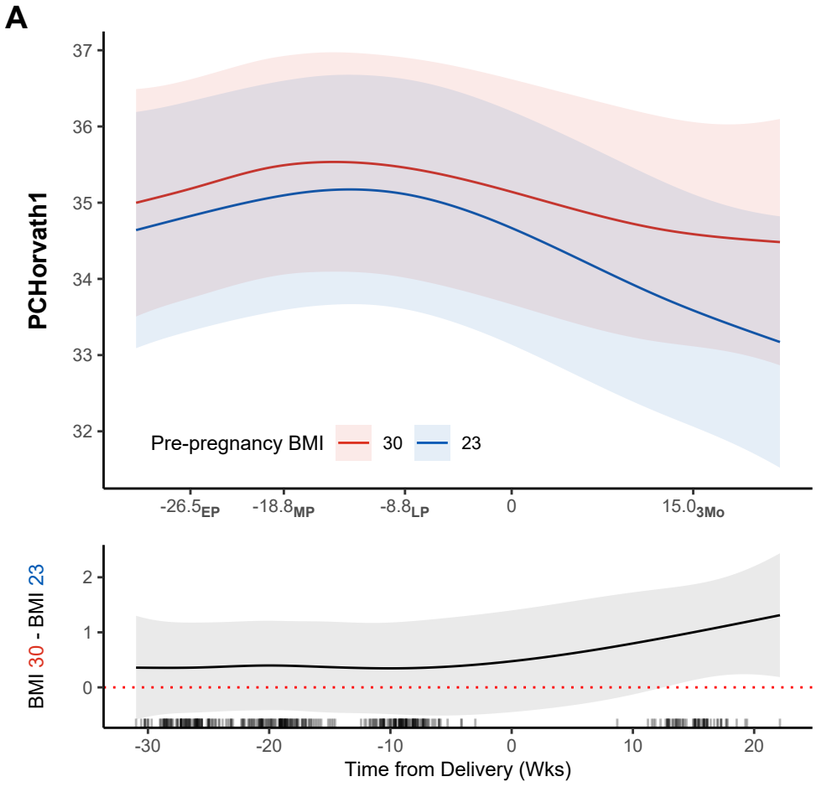
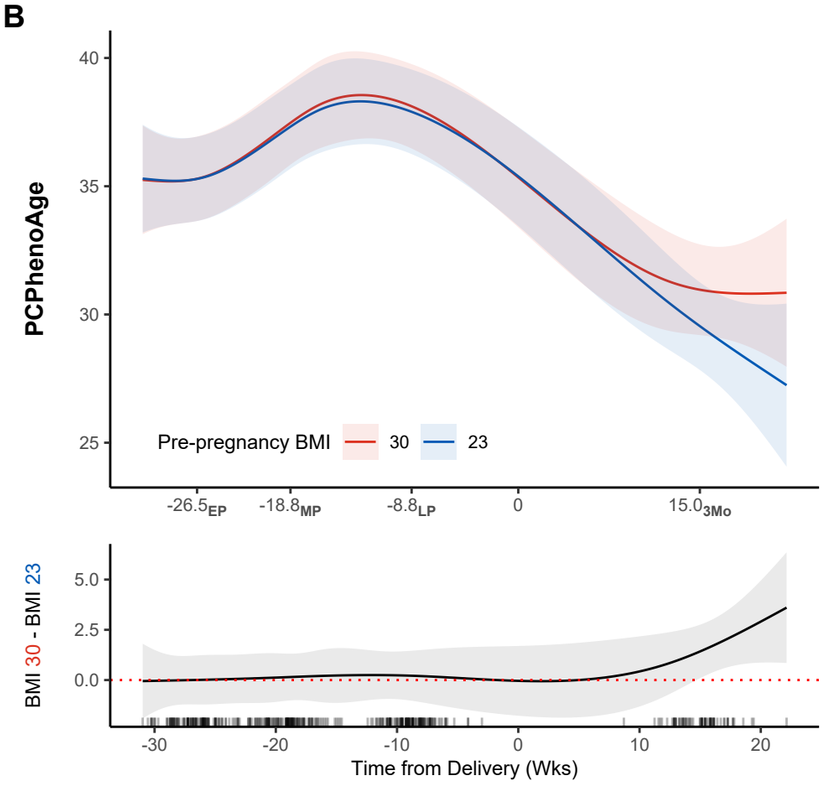
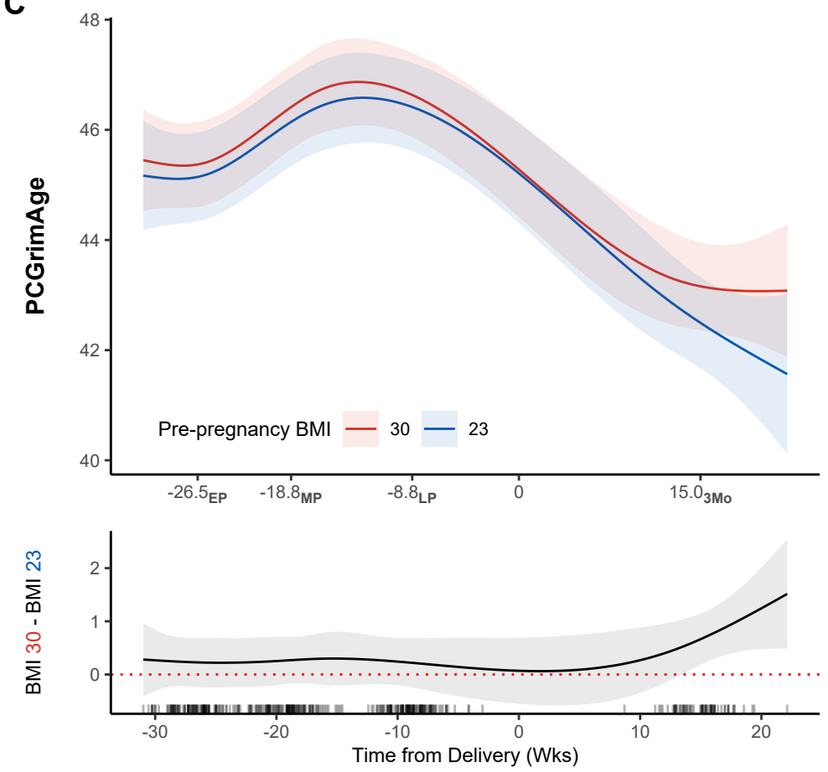
A


PCHorvath1
Pre-pregnancy BMI 30 23
-26.5EP -18.8MP -8.8LP 0 15.03Mo
BMI 30 - BMI 23
Time from Delivery (Wks)

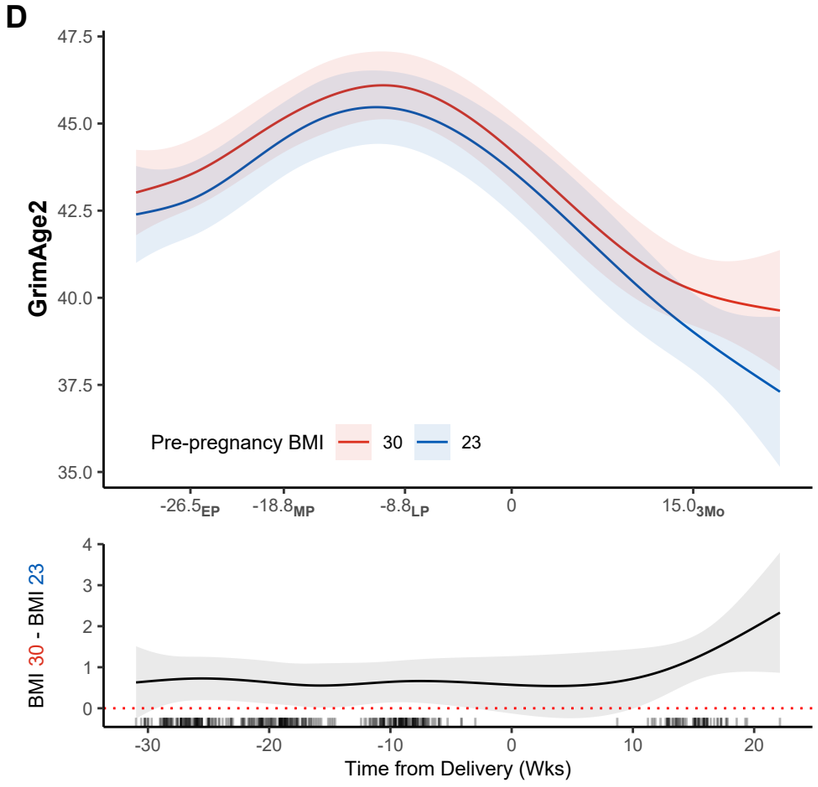
B


PCPhenoAge
Pre-pregnancy BMI 30 23
-26.5EP -18.8MP -8.8LP 0 15.03Mo
BMI 30 - BMI 23
Time from Delivery (Wks)

C


PCGrimAge
Pre-pregnancy BMI 30 23
-26.5EP -18.8MP -8.8LP 0 15.03Mo
BMI 30 - BMI 23
Time from Delivery (Wks)

D


GrimAge2
Pre-pregnancy BMI 30 23
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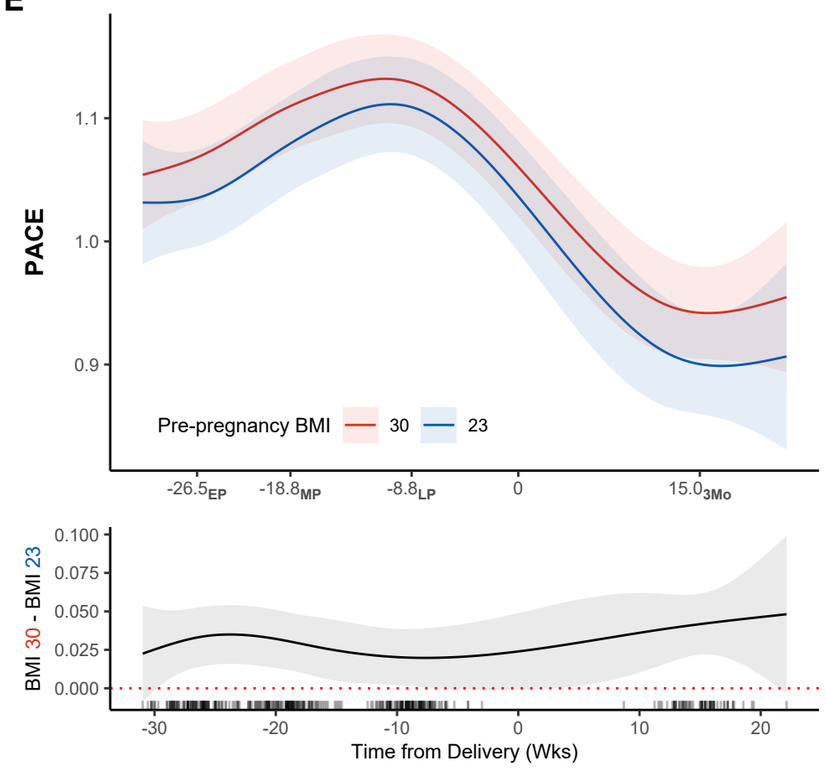
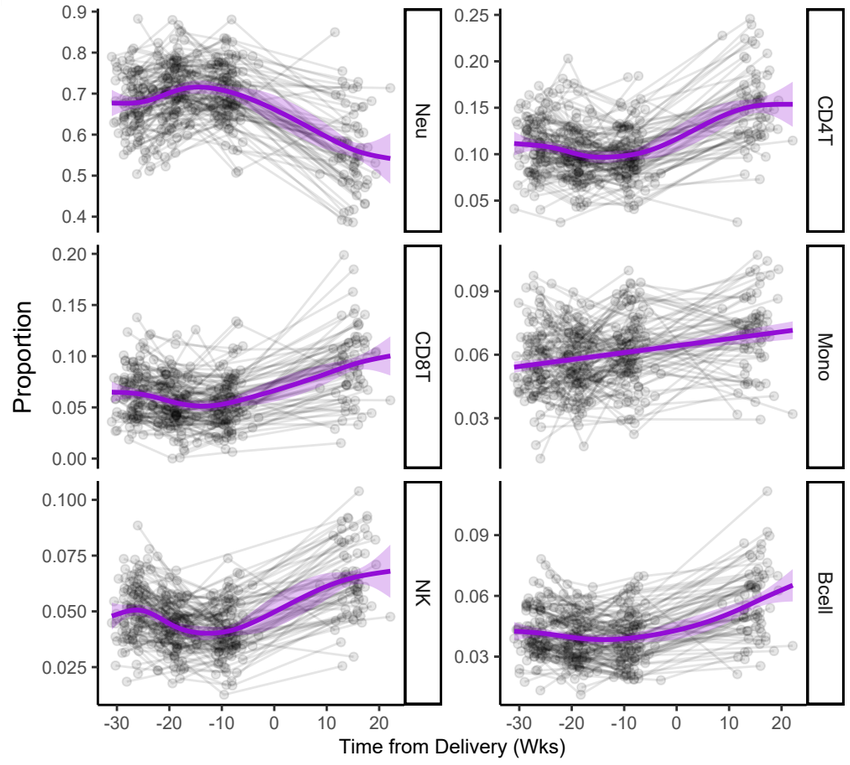
BMI 30 - BMI 23
Time from Delivery (Wks)

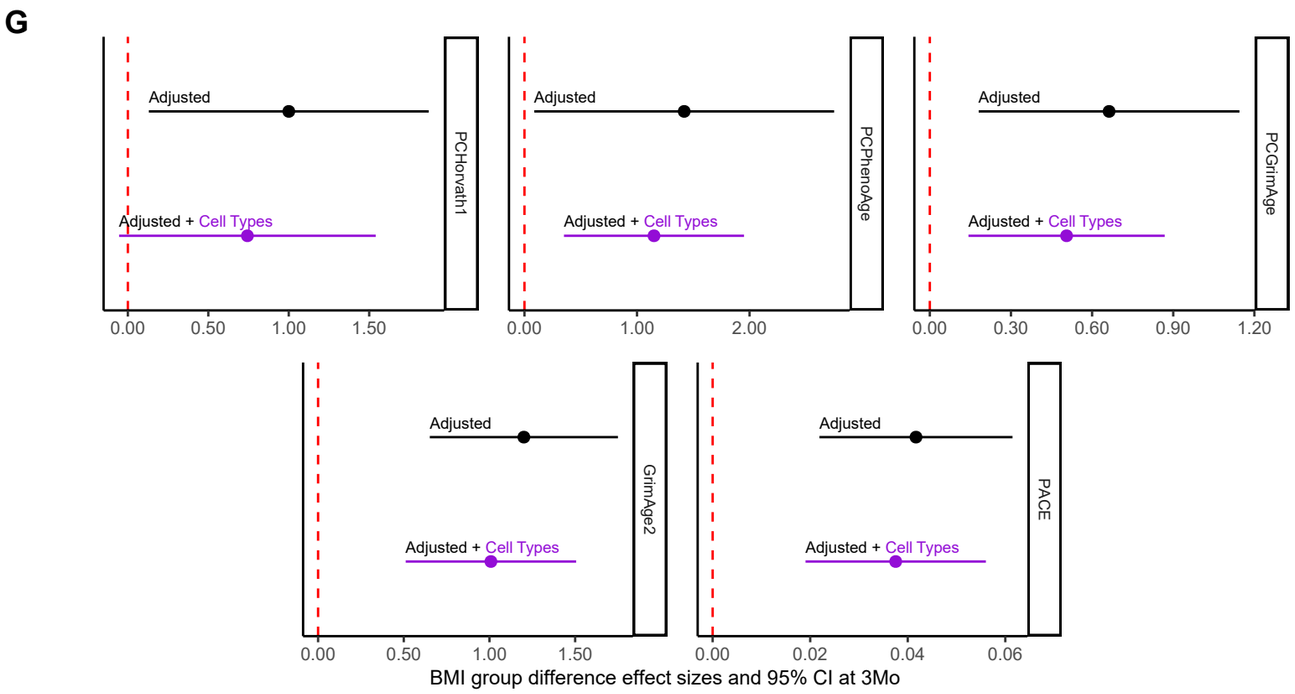
E


PACE
Pre-pregnancy BMI 30 23
-26.5EP -18.8MP -8.8LP 0 15.03Mo
BMI 30 - BMI 23
Time from Delivery (Wks)

F


Neu
CD4T
CD8T
Mono
NK
Bcell
Proportion
Time from Delivery (Wks)

G


PCHorvath1
PCPhenoAge
PCGrimAge
GrimAge2
PACE
Adjusted
Adjusted + Cell Types
BMI group difference effect sizes and 95% CI at 3Mo

H

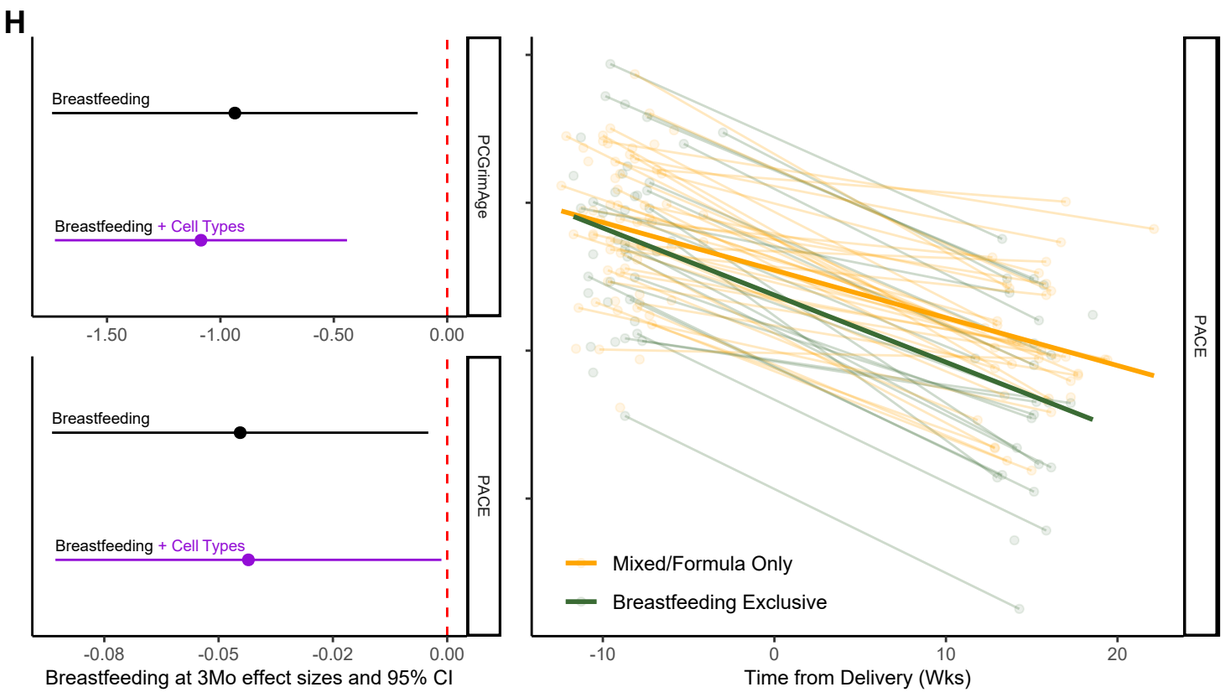

PCGrimAge
PACE
Breastfeeding
Breastfeeding + Cell Types
Breastfeeding at 3Mo effect sizes and 95% CI
Mixed/Formula Only
Breastfeeding Exclusive
Time from Delivery (Wks)

Figure S1: Pregnancy-associated changes in maternal biological age. Adjusted biological age estimates across pregnancy (solid lines) and associated 95% confidence intervals (95% CI: shaded ribbons) are shown for epigenetic biomarkers of aging (A-E). The upper panel of each plot illustrates the biological age trajectories of the high and low pre-pregnancy body mass index (BMI) groups across pregnancy and the postpartum (High BMI=30; red line; Low BMI=23; blue line) (A-E). The bottom panel of each plot describes the difference in biological age between the BMI pre-pregnancy groups (A-E) over the same period. A pregnancy-associated increase and a postpartum reversal of biological age are observed for all measures of maternal biological age. The bottom panels show that pre-pregnancy BMI modifies this postpartum recovery effect with statistically significant (i.e., 95% CI does not include zero) lower recovery at 3 months postpartum (3Mo) for all biomarkers. The estimated proportion of six cell types across pregnancy and 3Mo are displayed (F). The purple lines and shaded ribbons are smoothed estimated mean trends over time. Cell types were estimated, transformed with the rcomp() function of the {compositions} package, decomposed using principal components analysis and the first three principal components were considered for cell type adjusted models (G, H). Pre-pregnancy BMI group differences in biological age at 3Mo are not fully explained by individual differences in cell type proportions (G and see Supplement). Exclusive breastfeeding is associated with lower PCGrimAge and PACE at 3Mo (H, left panel) with unadjusted group differences in PACE estimates displayed over time (H, right panel). EP=early pregnancy; MP=mid pregnancy; LP=late pregnancy; 3Mo=approximately 3-months postpartum.