**Parity is not associated with multiple measures of biological age:**

**Evidence from NHANES 1999-2010**

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**Abstract**

Understanding factors contributing to variation in ‘biological age’ is essential to understanding variation in susceptibility to disease and functional decline. One factor that could accelerate biological aging in women is reproduction. Pregnancy is characterized by extensive, energetically costly changes across numerous physiological systems. These ‘costs of reproduction’ may accumulate with each pregnancy, accelerating biological aging. Despite evidence for costs of reproduction using molecular and demographic measures, it is unknown whether parity is linked to commonly-used clinical measures of biological aging. We use data collected between 1999-2010 from the National Health and Nutrition Examination Survey *(n*=4,418) to test whether parity (number of live births) predicted four previously-validated composite measures of biological age and system integrity: Levine Method, homeostatic dysregulation, and Klemera-Doubal method biological age, and allostatic load. Parity was not robustly associated with measures of biological aging when controlling for chronological age, lifestyle, health-related, and demographic factors. These findings suggest that composite measures of biological age that integrate indices of function across multiple systems may not be sensitive enough to detect costs of reproduction in women, or that costs of reproduction may be acute rather than chronic. Future work should continue to investigate links between parity and biological age using targeted physiological measures and longitudinal studies.

*Keywords:* biological age; parity; National Health and Nutrition Examination Survey; costs of reproduction; allostatic load

**1. Introduction**

Chronological age is a leading predictor of mortality, morbidity, and functional decline 1,2. Despite the striking association between chronological age, lifespan, and health, individuals vary considerably in their rate of functional decline 3. This variation - attributed to differences in the biological rate of deterioration or repair - is referred to as ‘biological age’, and is thought to reflect the cumulative effect of environmental exposures in combination with underlying genetic variation. Various proximate mechanisms have been proposed to modulate biological age acceleration, including insulin signaling 4, oxidative stress 5, inflammation 6, epigenetic changes 7, and telomere shortening 8. Understanding the environmental, behavioral, and physiological factors that influence biological aging may inform policies and interventions that could help to mitigate their effects, thereby extending the healthspan. Such policies and interventions will become increasingly important as the proportion of the global population over age 60 is expected to increase dramatically over the next 30 years 9.

Environmental factors found to accelerate biological aging and functional decline include smoking 10, obesity 10, socioeconomic status 11, and psychosocial stress 12. Another lifestyle factor that may accelerate biological aging in women specifically is reproduction 13,14. Reproduction in women is an energetically costly process, and is characterized by extensive changes in both form and function across numerous anatomical and physiological systems 15. Pregnancy and breastfeeding are accompanied by shifts in immune function 16–18, energy metabolism and storage 19,20, blood pressure and volume 21,22, and hormone levels and receptor expression 23. Evolutionary theory predicts that these changes should create functional or energetic constraints to somatic maintenance and defense, leading to accelerated biological age - a tradeoff referred to as ‘costs of reproduction’ 24,25.

Consistent with costs of reproduction in women, ever-parity has been linked to mortality from diabetes, cancer of the uterine cervix, gallbladder disease, kidney disease, hypertension, and all-cause mortality 26–29. Similarly, women who give birth to more children are at higher risk of developing obesity, diabetes, hypertension and cardiovascular disease (CVD; Lawlor *et al.*, 2003; Simons *et al.*, 2012), as well as age-corrected all-cause mortality 28,32,33, mortality related to cardiovascular disease 34 and mortality related to kidney disease 35. It is important to note that in the studies with the largest sample sizes (and presumably, the highest statistical power), parity exhibits a U-shaped association with all-cause mortality 32,33 and CVD 34, with highest levels of all-cause mortality and cardiovascular disease observed at lower and higher levels of parity, as compared to what is observed at intermediate levels. The number of children or pregnancies has also been linked to multiple measures of cellular aging, including DNA damage and oxidative stress 36, telomere length 13,14, and DNA methylation age 14,37. While most of these studies examine associations within Western populations, some evidence supporting costs of reproduction is seen in non-Western populations as well 14,36.

Cellular measures of biological age such as telomere length and DNA methylation age may provide insights into the molecular processes linking reproduction to mortality and other health outcomes 7,38, and may eventually serve as early indicators of the costs of reproduction in health and aging. However, such cellular measures often require molecular assays which can be expensive and technically challenging, rendering their implementation difficult in standard clinical contexts and large epidemiological studies. What is unknown is whether it is possible to capture costs of reproduction in women using more easily measured, widely-used clinical measures of biological age.

Four composites of system integrity have been used to operationalize biological age and cumulative system dysregulation within the context of large-scale epidemiological studies in the United States: Homeostatic Dysregulation (HD) 39, Levine Method Biological Age (LM) 40,41, the Klemera-Doubal Method Biological Age (KDM) 40,42, and allostatic load (AL) 43. These measures quantify changes in physiological integrity by combining information from multiple clinical biomarkers that collectively assess the functioning of major organ systems throughout the body. Previous work using a nationally representative sample of adults in the US from the National Health and Nutrition Examination Survey has found that HD, LM, KDM, and AL exhibit robust associations with physical functioning, cognition, hearing and vision, and with self-reports of health and functional disability 11,44. Other population-based studies have found similar links between AL and both objective and subjective markers of physical functioning and general health. Importantly, energetic trade-offs between somatic maintenance and reproduction have been suggested to operate at the system level via the activity of neuroendocrine and sex hormones 45. Thus, in addition to providing more affordable and widely-applicable measures of biological age, composite indices of system-level variables may better approximate costs of reproduction as compared to cellular measures of biological age.

The importance of assessing how parity affects clinical-based measures in addition to cellular-based measures of biological age is further highlighted when considering correlations (or lack thereof) between these two classes of measures. As ‘aging’ may refer to a wide range of processes that may occur at different times or at different speeds, low correlations between different indices of biological age are unsurprising. For example, cellular measures of biological age that examine mitotic (e.g., telomere length) and non-mitotic (e.g., DNA methylation age) processes are not correlated 14. Similarly, HD and KDM show no association with telomere length and DNA methylation age 46, and AL shows little association with cellular-based measures of aging 47,48. It is thus clear that different measures of biological age and cumulative system dysregulation index fundamentally different components of the aging process. As they each index different yet equally important aspects of aging, careful study of a range of such measures is required for a more complete understanding of costs of reproduction in women.

Here, we present nationally-representative estimates of the effect of parity (operationalized as number of live births) on four composites of system integrity indexing biological age and cumulative dysregulation. Using cross-sectional epidemiological data collected in the United States between 1999 and 2010, we test whether parity is associated with HD, KDM, LM, and AL while controlling for a range of covariates (e.g., smoking, obesity) known to modulate biological age to better isolate the unique contribution of parity on biological age. Although each measure utilizes the same panel of biomarkers, differences in scale construction provide a varied, multifactorial approach to the study of costs of reproduction on biological aging. Based on findings from the most highly powered prior studies of all-cause mortality and parity, we hypothesized a U-shaped relationship between parity and biological aging; specifically, that accelerated biological aging would be most apparent in women with the lowest and the highest parity. We also perform exploratory analyses to test whether relationships between parity and biological age are chronic, such that they persist regardless of time since last birth, or acute, such that the effect of parity on biological age decreases as a function of time since last birth. Our findings have significant theoretical implications for our understanding of the relationship between parity and health, and of putative tradeoffs between reproductive and somatic effort.

**2. Materials and Methods**

*Data source*

Data were collected as part of the Centers for Disease Control and Prevention’s National Health and Nutrition Examination Survey (NHANES). NHANES uses multistep cluster sampling, and assigns participants sample weights based on demographic variables such as self-identified race/ethnicity, age, and education; utilization of these sample weights in analyses enables estimation of population-level effects. Continuous sampling for NHANES began in 1999, and data is made publicly available in two-year waves. Details of recruitment procedures and study design are available from the Centers for Disease Control and Prevention 49. Women sampled between 1999 and 2010 are included in the present analyses, as not all the data necessary to construct the biological aging measures (i.e. C-reactive protein) were released for cycles following the 2009-2010 cycle at the time of writing this manuscript. Furthermore, women missing information on any covariate included in analyses were excluded from the sample. A flowchart detailing sample stratification can be found in **Figure 1**, and sample demographic information is presented in **Table 1**.

To assess the representativeness of participants with complete biomarker information, we compared the subset of non-pregnant women aged 18-84 with complete biomarker data (*n* = 5,870) to all non-pregnant women aged 18-84 in NHANES 1999-2010 (*n* = 13,929). The two samples were similar in age, ethnicity, educational attainment, income, smoking status, menopausal status, and number of live births. However, the sample with complete biomarker data was significantly more likely to have ever been pregnant. Comparative demographics and associated tests of difference are reported in **ESM Table I**.

*Ethical approval*

All sampling procedures were approved through the National Center for Health Statistics Ethics Review Board, and all participants provide informed consent before sample collection and interviews.

*Reproductive health and parity data*

Women completed a computer-assisted questionnaire on their reproductive health history. Women reported whether they were currently pregnant, if they have ever been pregnant, how many pregnancies resulted in a live birth (if applicable; NHANES items RHD170 and RHQ171), whether they had regular periods over the last 12 months, and their reason for not having regular periods over the last 12 months (if applicable). As previous work has suggested that current pregnancy modulates certain measures of biological age 14, women who self-reported currently being pregnant were excluded from analyses (NHANES item RIDEXPRG; n = 1,417 out of all women between 18 and 84). Due to the small number of women with complete covariate information who reported 7 or more live births (n = 137), these women were excluded from analyses. The frequency distribution for women included in our analyses is displayed in **Figure 2**. We chose to use number of live births rather than number of pregnancies. NHANES does not collect fine-grained data about pregnancies that do not result in live births, rendering it impossible to estimate the length of each pregnancy, and concomitantly, the physiological cost of each pregnancy. Further, approximately 30% of implantations end in natural miscarriage 50, making number of recognized pregnancies a more imprecise measure of physiological investment in reproduction as compared to number of live births. Women who reported a prior live birth indicated their age at last live birth across all survey cycles. Because responses to this question were bottom-coded at 14 and top-coded at 45 for some cycles, we limited our analysis to women who reported an age of last live birth between 15 and 44. Starting in the 2007-2008 cycle, NHANES added a question on the number of months since last live birth for women who reported up to a two year difference between their current age and age of last birth.

Women were categorized as being pre-menopausal if they reported having regular periods over the last 12 months, if they reported not having regular periods because of a reason other than menopause, or if they were younger than 41. A lower limit of 41 was chosen because the average age of menopause in the US is 51, and perimenopause may last up to 10 years for some women 51. Women were categorized as being post-menopausal if they were older than 61, or if they reported not having regular periods over the last 12 months because of menopause.

*Biological aging measures*

All composite measures of biological aging were constructed using the following 9 biomarkers: albumin, creatinine, glucose, log-transformed C-reactive protein (CRP), lymphocyte percent, mean cell volume, red blood cell distribution width, alkaline phosphatase, and white blood cell count. Where appropriate, female participants from NHANES III, for which data collection ran between 1988 and 1994, were used as the reference sample for the construction of the biological aging measures employed here. Serum creatinine values from NHANES III and NHANES 1999-2004 continuous panels were adjusted according to published recommendations 52.

Homeostatic Dysregulation (HD) is a measure of Mahalanobis distance 53, quantifying the deviation of a participant’s physiology from a young, healthy reference norm. Following previous work 11, we defined our reference population as non-pregnant women from NHANES III aged 20-30 who were not obese (BMI<30) and for whom all biomarkers fell within the clinically normal range for their age and sex (N = 481, see **ESM Tables II-IV**)**.** Biomarker values from the reference population were standardized and used to compute a biomarker variance-covariance matrix (**ESM Table IV**). Biomarker raw means, raw standard deviations, and the standardized-biomarker variance-covariance matrix are implemented within the Mahalanobis distance equation 53 to form the homeostatic dysregulation (HD) algorithm: . Here, *v* is a vector of biomarker values for a participant in the analysis sample; *u* is a vector of biomarker means in the training sample, and *S*is the standardized-biomarker variance-covariance matrix. As HD in the full sample was significantly skewed, natural log-transformed HD was used as the outcome variable in all analyses.

Klemera-Doubal Method (KDM) Biological Age is computed using the Klemera-Doubal equation 42, which extracts information from individual regressions of chronological age onto *m* biomarkers: . Here, *xj* is the value of biomarker *j* measured for an individual in the analytical sample and *CA* is their chronological age. For each biomarker *j*, the parameters *q* (intercept), *k* (slope), and *s* (root mean squared error) are estimated from a regression of chronological age onto the biomarker in the reference population. *sBA* is a scaling factor equal to the square root of the variance in chronological age explained by the biomarker panel in the reference population 40 (Eq. 5). Following previous work 40, we formed our reference population from non-pregnant women in NHANES III aged 30-75 (N = 5,453, see **ESM Tables V and VI**). An individual's KDM Biological Age corresponds to the average chronological age at which their physiology would be observed in

the reference population.

Levine Method (LM) Biological Age is computed from a multivariate analysis of mortality hazards using NHANES III data 40,41. Herein, a multivariate Gompertz model of mortality hazard is fit to the selected biomarkers and chronological age to form a predicted hazard of mortality called a “mortality score”. This mortality score is converted to a biological age value using a second univariate Gompertz regression of the mortality hazard onto chronological age. In this manner, the LM biological age is interpretable as the chronological age at which an individual’s physiology-based risk for mortality would be approximately normal in the reference population. We applied published parameters from Liu and colleagues’ original work 41 to compute LM biological age for participants in our sample.

Allostatic Load (AL) is computed as the proportion of biomarker values for which a participant is at risk. In accordance with recommendations from a review of AL implementation in NHANES 54, we defined risk as residing within the highest quartile of a given biomarker’s distribution within the sample of nonpregnant women aged 18-84 with complete biological age biomarker data, excepting albumin for which risk was defined as residing in the lowest quartile (N = 5,870; **ESM Table VII**). In this manner, the number of biomarkers for which a participant is at risk is divided by the total number of biomarkers in the panel to calculate a final allostatic load score with values ranging from 0-1.

All four biological aging measures were computed using the same panel of 9 biomarkers. These biomarkers were selected based upon their inclusion in the LM biological age algorithm, which utilized machine-learning analysis to select the most parsimonious panel of biomarkers for mortality prediction. The use of common biomarkers ensures the different measures are indexing the same physiological processes. Differences in the analytical approach and statistical operations leading to the final composite measure reflects different approaches toward the conceptualization of biological age. For HD, biological age is conceptualized as deviation from an ideal physiological state attained in one’s 20s. For KDM, biological age is conceptualized as the average change in physiology that occurs with increasing chronological age. Building upon this, LM captures the increased risk in mortality that accompanies physiological changes occurring with age. Finally, AL conceptualizes aging as the accumulation of changes that become impactful only once they reach a critical threshold. Biomarker and biological age summary statistics for the final analytical sample (*n* = 4,418) are provided in **ESM Table VIII.**

Univariate distributions, bivariate distributions, and Pearson correlations coefficients for age, LM, log-transformed HD, and KDM are displayed in **Figure 3**. Expectedly, all four measures of biological age were significantly correlated with chronological age, and all four measures of biological age were significantly correlated with each other.

*Covariates*

Self-reported race/ethnicity 55, socioeconomic status (SES) 56,57, and smoking 10 moderate the relationship between chronological age and biological aging. Self-reported race/ethnicity was categorized as non-Hispanic (NH) white, NH black, Hispanic, and ‘other’ (NHANES item RIDRETH1). SES was indexed by educational attainment (NHANES item DMDEDUC2) and federal income-to-poverty ratio (FIPR; NHANES item INDFMPIR as calculated per Department of Health and Human Services guidelines). Height and weight were measured by an NHANES examiner, and BMI was calculated as weight (kg) divided by height (meters squared; NHANES item BMXBMI). As prior work has shown that BMI exhibits a U-shaped curve with negative health outcomes 58, our models included both linear and quadratic terms for BMI. On the basis of responses to a computer-assisted questionnaire on smoking habits, women were classified as never, past, or current smokers. To better isolate the effect of parity and biological age, our primary models controlled for the aforementioned covariates.

*Statistical analyses*

All analyses were performed in R using the *survey* package, which supports functionality for analyzing data from complex survey designs. To facilitate accessibility of our methods, we also performed all analyses in Stata version 16.1. R scripts, Stata scripts, and data files have been uploaded online and can be found at <https://osf.io/b2jft/>.

We followed all NHCS guidelines for the analysis of NHANES data 59. As the survey weights relevant to the smallest sample subpopulation for which all data are available should be used, we used mobile examination center (MEC) weights to adjust for complex survey design, oversampling, non-coverage, day of the week, and survey nonresponse to compute nationally representative estimates 60,61. Per NHANES analytical guidelines for combining data across cycles, 12-year MEC weights were calculated using the NHANES-provided variables WTMEC4YR and WTMEC2YR as follows:

Because we estimated four regressions (one per outcome measure) for each set of analyses, statistical significance was set to *p* < 0.0125 (0.05/4) 62.

We estimated multiple linear regression models to examine the association of number of live births on biological age when controlling for chronological age, self-reported race/ethnicity, educational attainment, FIPR, BMI, and smoking. To focus on biological aging, we conducted analyses using versions of each biological age measure after adjustment for chronological age, computed as the residuals of regressions of chronological age onto each measure. Following adjustment, biological aging measures were no longer correlated with chronological age (**ESM Table IX**). Separate models were estimated for LM, log-transformed HD, KDM, and AL.

We estimated both linear and quadratic terms for number of live births, as it has been previously suggested that the number of live births may exert quadratic, rather than linear, effects on morbidity and mortality 32–34. As higher values correspond to more advanced biological age across all biological aging measures, a positive linear effect suggests a higher number of live births is associated with a higher biological age. A positive quadratic effect would suggest a convex (or U-shaped) shape to the fitted curve, while a negative quadratic effect would suggest a concave shape to the fitted curve. As some prior work suggests that costs of reproduction should be the most apparent after menopause 63, we also included model terms for the main effect of menopause status and the interaction between menopause status and number of live births 16. Equations for each regression are provided in **ESM Text 1**. In cases where interactions between menopause status and live births were statistically significant, we then ran regressions stratified by menopause status to clarify these interactions.

Figure 3 was generated using Stata through post-estimation marginal standardization postestimation commands in Stata for regressions adjusting for the distribution of other covariates 64. The y-axes in these figures represent the extent to which chronological age deviates from biological age. For each measure, this presents the difference between actual chronological age and chronological age predicted by biological age (i.e., the residual of chronological age regressed onto the biological aging measure). In all four cases, positive values indicate aging deceleration (chronological age > biological age) while negative values indicate age acceleration (chronological age < biological age).

*Sensitivity analyses*

We conducted a series of follow-up regressions to probe the robustness of our primary analyses. First, we repeated the multiple linear regressions exactly as described above, including only chronological age as a covariate. This was done to ensure the relationship between variables included in our primary analyses and biological age were so strong as to masking putative relationships between parity and biological age. For example, in our sample BMI was significantly, positively correlated with LM and KDM (*r* = 0.29 and 0.28, respectively; *p* < 0.001).

We then estimated a second set of sensitivity analyses, with time since last birth used to create additional model terms. We chose these as sensitivity analyses rather than primary analyses for two reasons. First, models including time since last birth by default eliminate all nulliparous women, rendering us unable to calculate estimates for the effect of parity for nulliparous women. Second, data on time since last birth were missing for a significant portion of our sample. In these models, we assessed the extent to which effects of parity may be chronic and accumulate over time, or acute and only present in the postnatal period. To assess potential chronic effects, years since last birth was calculated for women across all survey cycles as age of last live birth subtracted from current chronological age. To assess potential acute effects data on months since last birth was available for women sampled in the 2007-2008 and 2009-2010 cycles. We estimated one set of regressions exactly as described above for our primary analyses, and added terms for the main effect of years since last birth and interactions between years since last birth and parity. We then estimated a second set of regressions exactly as described above for our primary analyses and added terms for the main effect of months since last birth and interactions between months since last birth and parity; however, since data on months since last birth were not available for any postmenopausal women, main and interactive effects of menopausal status were not included.

**3. Results**

The linear effect of number of live births, quadratic effect of live births, and main effect of menopause status was not significant in any primary model (see **Table 2; Figure 4**). The interaction between menopause status and live births did not significantly predict acceleration in LM, KDM, or AL; however, the interaction between menopause status and both the linear and quadratic term for live births significantly predicted HD.

*Sensitivity analyses*

Sample sizes for our sensitivity analyses controlling for chronological age only were slightly larger (*n* = 5,184), as less participants were excluded due to missing covariate information. Similar to our primary analyses, the main effects of live births (both linear and quadratic terms) were not significant across all measures of biological age (**Table 2**). Menopause status positively predicted KDM (estimate = 3.34, 95% CI = 0.86, 5.82, *p* = 0.009) and AL (estimate = 0.05, 95% CI = 0.01, 0.09, *p* = 0.010), but not LM and HD.

Of the 4,418 women in our primary analyses, data on years since last live birth were available for 3,587. The average years since last live birth was 23.8 (SE = 0.28). After correcting for multiple comparisons, the main effect of years since last live birth was not significant in any model, nor were any of the interaction terms between years since last live birth and parity (**Table 2**).

Our sample size for analyses including months since last live birth (*n* = 107) was significantly limited by the fact that this subsample excluded all postmenopausal women, and excluded women sampled prior to this question being added in the 2007-2008 cycle. Because of this limited sample size, these results should be interpreted as exploratory only. On average, women with valid responses to this question gave birth 10.7 months ago (SE = 0.63). After correcting for multiple comparisons, the main effects of months since last live birth and parity was not significant in any model, nor were any of the interaction terms between months since last live birth and parity (**Table 2**).

While the main effect of months since last live birth, as well as the interactions between months since last live birth and the linear and quadratic terms for last live birth, were significant in predicting LM, none of these effects were statistically significant in predicting log-transformed HD and KDM (**Table 2**).

*Stratified Analyses*

Follow-regressions stratified by menopause status revealed trends in significant linear and quadratic effects for live births for LM, HD, and AL measures of biological aging in postmenopausal women (**Table 3**). The linear effect of live births was also associated with KDM, but did not remain significant after control for multiple comparisons. Similar patterns were observed in sensitivity analyses controlling for only chronological age. These models revealed consistent trends curve between parity and biological aging in postmenopausal women. Specifically, each association was characterized by a negative linear term for the effect of parity on biological aging coupled with a positive quadratic term, giving rise to the anticipated U-shape for the overall relationship (shown in grey on **Figure 4)**. By contrast, neither the linear nor the quadratic effects of live births were significantly associated with any measure of biological aging in stratified analyses of premenopausal women (**Table 4**).

**4. Discussion**

Our primary aim was to examine putative physiological costs of reproduction, as indexed by four validated measures of biological age and system integrity among a nationally-representative sample of US women of reproductive and post-reproductive age. Based on prior work, we hypothesized a U-shaped relationship between parity and biological age. When controlling for lifestyle, health-related, and demographic factors, the main effect of parity (defined as number of live births) was not significantly associated biological aging examined. However, the association between parity and biological age was markedly different between pre and postmenopausal women. For premenopausal women, the association between parity and biological aging was nonsignificant and negligible. By contrast, analyses in postmenopausal women revealed the hypothesized U-shape between parity and biological age, with biological age acceleration reaching a minimum at 2-3 live births and more pronounced aging at either extreme. Notably, this pattern was observed for all four measures, although effects did not remain significant for KDM after controlling for multiple comparisons. To our knowledge, our study represents the first application of biological age composites indexing system integrity (LM, HD, KDM, AL) to quantify costs of reproduction in women. In what follows, we situate our results in what we view as the most tenable hypotheses relating parity to physiological dysregulation and biological aging.

First, it is possible that reproduction may exert significant physiological effects, but that the proxies used in LM, HD, KDM, and AL are imprecise measures of the effects they aim to index. For example, NHANES white blood cell count data reflects the total number of white blood cells, and does not distinguish between different cell types. Total white blood cell count is significantly increased during pregnancy, but returns to baseline within two years postpartum 65. By contrast, higher proportions of CD4+ effector memory cells and CD8+ lymphocytes 65, higher lymphocyte-monocyte ratios 16, and lower platelet-lymphocyte ratios 16 are retained in parous women as compared to nulliparous women. This differential composition of white blood cells types as a function of parity suggests that while reproduction does alter immune function, total white blood cell count may be too coarse of a measure to detect shifts in immune function in this context. Similarly, while alkaline phosphatase levels are correlated with bone mineral density 66, work in mice suggests that parity exhibits a dose-response relationship with bone mineral density in absence of significant changes in alkaline phosphatase levels 67.

The inclusion of proxies in LM, HD, KDM, and AL composites that do not accurately capture reproduction-induced changes across the systems they are hypothesized to reflect may in turn dilute the effects of better proxies that are indeed associated with parity. For example, low grade albuminuria risk increases with parity 68, and it is possible that relationships between parity and albumin (included in our composites) were obscured by the inclusion of more coarse proxies, as detailed above.

A second and not mutually exclusive hypothesis is that reproduction exerts significant effects, but that these effects differ in whether they are acute or chronic in nature. In addition to transient changes in global white blood cell counts, other markers of immune function, such as IL-6, TNF-α, and CRP (included in our composites) increase across pregnancy, but return to pre-pregnancy levels within four months postpartum 69,70. Other indices may change across pregnancy and return to pre-pregnancy levels even faster, such as glomerular filtration rate (an indicator of kidney function) which returns to baseline levels within one week postpartum 71, or systolic blood pressure which returns to baseline levels shortly after birth 72. Yet, other indices of cardiovascular function such as ventricular volumes and cardiac output that change across pregnancy continue to exhibit differences from baseline values at one year postpartum 73. Roughly half of women who develop gestational diabetes continue to be diabetic after pregnancy 74, suggesting a chronic effect of pregnancy on glucose metabolism. Measures of cellular aging exhibit both chronic 13,36 as well as acute 14 responses to pregnancy. Taken together, pregnancy or lactation may be associated with both acute and chronic changes across different systems, and that even within the same physiological system, costs may be both acute and chronic depending on the measure. As a result, the measures included in our biological age composites could be acutely, but not chronically, affected by reproduction, if they are affected at all. Though our sensitivity analyses did not consistently suggest a statistically significant effect of time since last live birth on measures of biological age (suggesting a lack of acute effects), data at finer timescales is needed to better understand changes potential transient changes in biological age markers perinatally.

A related possibility suggests the negative effects of reproduction may not manifest until after menopause. In this model, the protective forces acting to ensure survival during the reproductive stage of the lifespan are diminished in the post-menopausal periodREF. This hypothesis is supported by the following evidence. First, menopausal status was independently associated with increased biological age acceleration as measured by KDM and AL, replicating previous findings with epigenetic clock measures of biological ageREF. Second, when the sample was stratified by menopausal status, both the linear and quadratic effects of parity were associated with biological age acceleration registered in all four measures, but only for postmenopausal women. For premenopausal women no significant effects were observed across all models and measures. Associations between parity and female longevity once obscured also emerge when pre and postmenopausal women are considered independently63.

A final hypothesis is that the measures included in our biological aging composites do in fact accurately index the integrity of systems they represent, and that reproduction is not associated with any chronic costs in these systems. While this hypothesis is supported by studies finding no link between parity and all-cause mortality 75, it contradicts others that do find a link 28,32,33, and further contradicts other research linking parity with other health outcomes, such as type II diabetes and CVD 31.

*Limitations*

The fact that NHANES is cross-sectional rather than longitudinal in design contributes to two significant limitations in our study. First, its cross-sectional nature does not allow us to draw conclusions about causal relationships (or lack thereof); thus, it is crucial that future work follow women as they transition from nulliparity to parity, and as they continue to reproduce, to best evaluate causal relationships between reproduction and biological age. However, should a causal relationship between chronic effects of parity and biological age exist, this should have been apparent in our cross-sectional data, especially given the low levels of error or bias in reporting the number of live births. Second, we are only able to examine relatively chronic, rather than acute, effects of reproduction on biological age given the current study design. Longitudinal studies, ideally with dense sampling schedules, would better enable us to assess the time scales at which costs of reproduction may be apparent. Frequently sampling women before, during, and after pregnancy would allow for the investigation of putative acute and chronic changes in markers and composites of biological age. In the absence of dense longitudinal sampling, we cannot be certain that biomarkers measured in this cross-sectional sample are not also representative of acute states unrelated to parity or reproduction. For example, it is possible that some participants could have been experiencing mild infections during MEC examinations, leading to altered clinical measures of immune function. Though this could contribute to imprecision in our biological aging measures, such imprecision would not be systematic and thus we would not expect it to significantly affect the present study’s findings. Women’s prenatal health also predicts both pregnancy outcomes 76,77, as well as postnatal health risks. As such, longitudinal studies are necessary to understand what factors moderate reproduction-related changes in biological age across women.

Another limitation is that BMI is an important contributor to observed differences in biological age 78. Changes in body mass and adiposity are central to the physiological changes occurring with pregnancy as women begin “metabolizing for two” 79. Parity is associated with increased central adiposity 80, and pregnancy-related weight gain can mediate associations between obesity and long-term morbidity 81. These risks might be reduced by breast-feeding, which acts to mobilize accumulated fat and reset maternal metabolism 82. As NHANES does not include fine-grained data on lactation practices, we were unable to examine the additive effects of parity and lactation on biological age. Both cross-sectional and longitudinal future studies should aim to more fully quantify pre- and post-natal factors indexing reproduction-related energetic investment.

Finally, because data were collected in the United States, it is unknown whether similar patterns would be observed outside the context of WEIRD (Western, Educated, Industrialized, Rich, and Democratic) 83 samples. WEIRD and non-WEIRD countries are characterized by significantly different activity patterns, nutrition, infectious disease ecology, and morbidity and mortality 84, all of which could shape how reproduction affects women’s health and hence, costs of reproduction. Non-WEIRD countries are also characterized by higher parity 85. It is possible that the parity in our sample was too restricted in range to detect extant parity-biological age associations, and based on our sample, we cannot make estimations about the nature of these associations in women who report more than six previous live births. Whereas some studies have indeed examined links between parity and aging in non-Western settings 14,86, more research is necessary to better catalogue and understand cross-cultural variation in costs of reproduction in women.

*Conclusions*

We analyzed links between parity and different measures of biological aging using a large, nationally-representative epidemiological sample of pre- and post-menopausal women in the United States. Our results do not suggest a linear or quadratic relationship between parity and accelerated biological age in women, when biological age is measured using coarse clinic- and lab-based measures of physiological function. Future work should employ longitudinal designs, include a broader range of measures, collect more detailed data on variables quantifying energetic investment in reproduction, and utilize system-specific measures of biological age to more fully elucidate costs of reproduction and the time scales in which they are apparent.

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**Author Contributions**

TNS, WJH, and CPR contributed to the study conceptualization, data analysis, data interpretation, and manuscript writing. AYR contributed to data analysis, data interpretation, and manuscript writing. All authors have approved of the submitted manuscript.

**Competing Interests:** The authors have declared that no conflicts of interest exist.

**Supplementary Information:** All script and data files that accompany this paper can be found at <https://osf.io/b2jft/> (DOI: 10.17605/OSF.IO/B2JFT).

**Figure Legends**

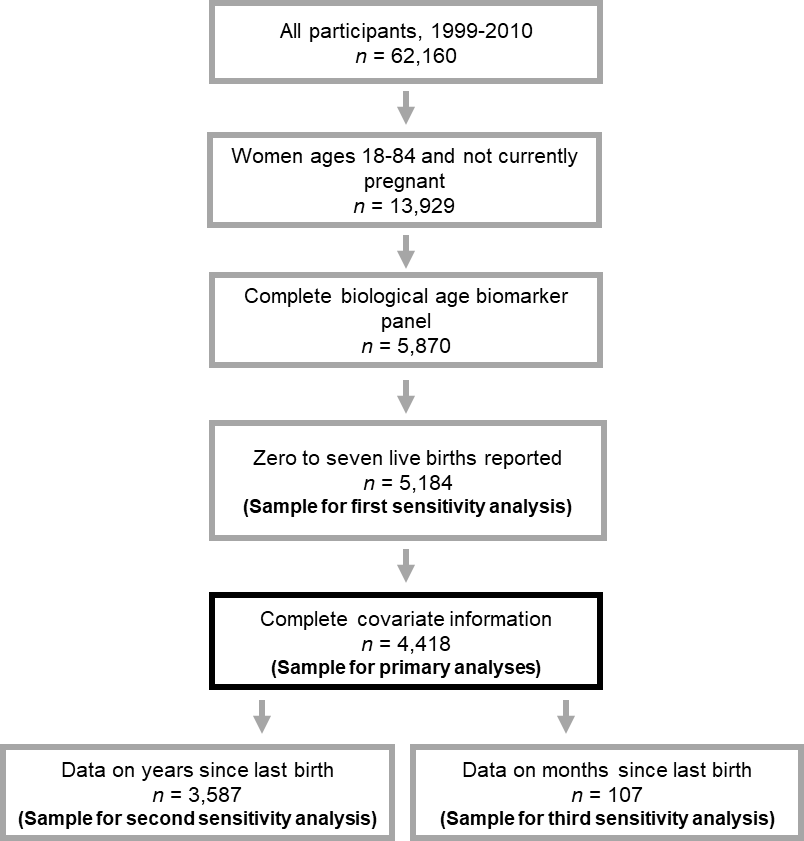
**Figure 1.** Flow chart illustrating sample stratification.

**Figure 2.** Distribution of live births for premenopausal (black bars; *n* = 2,166) and postmenopausal (gray bars; *n* = 2,252).

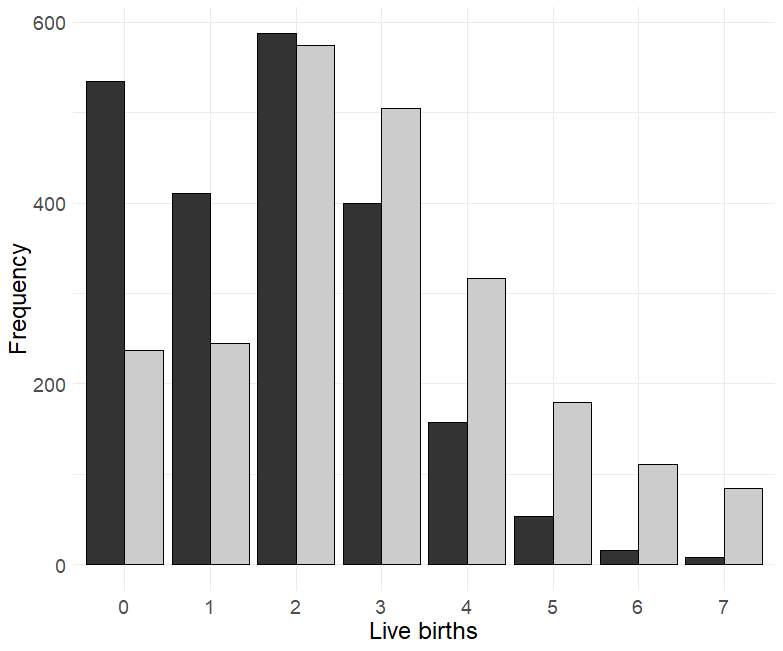
**Figure 3.** Associations between measures of chronological and biological age employed in the present study, National Health and Nutrition Examination Survey 1999-2010 (*n* = 4,418). Numbers represent Pearson correlation coefficients. *Note*: \*\*\* p < 0.001

**Figure 4.** Predicted values and 95% confidence intervals derived from primary models for LM age acceleration (panel A), HD acceleration (panel B), KDM age acceleration (panel C), and AL age acceleration (panel D) among premenopausal women (black lines) and postmenopausal women (grey lines), National Health and Nutrition Examination Survey (*n* = 4,418). *Note:* Figure generated using marginal standardization adjusted for the distribution of age, BMI, FIPR, smoking, education, and race/ethnicity.

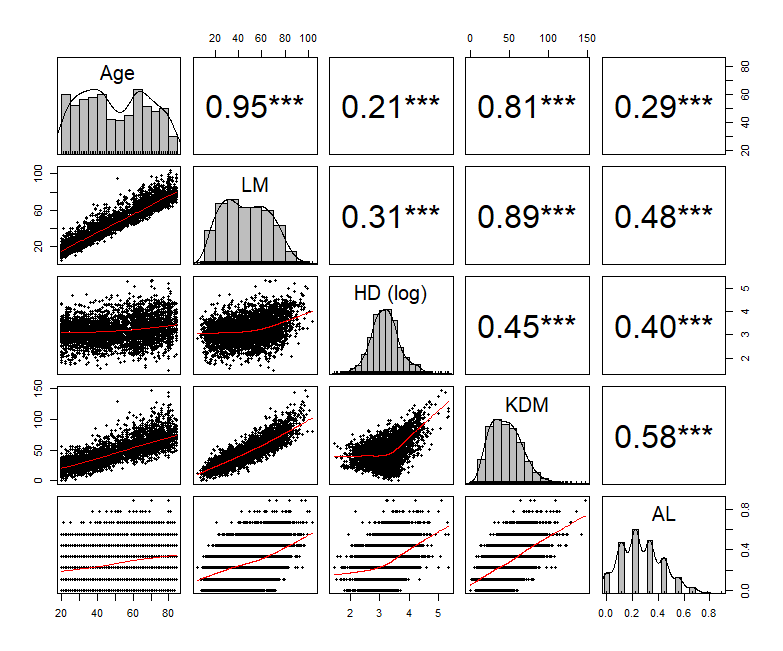
**Figure 1.**



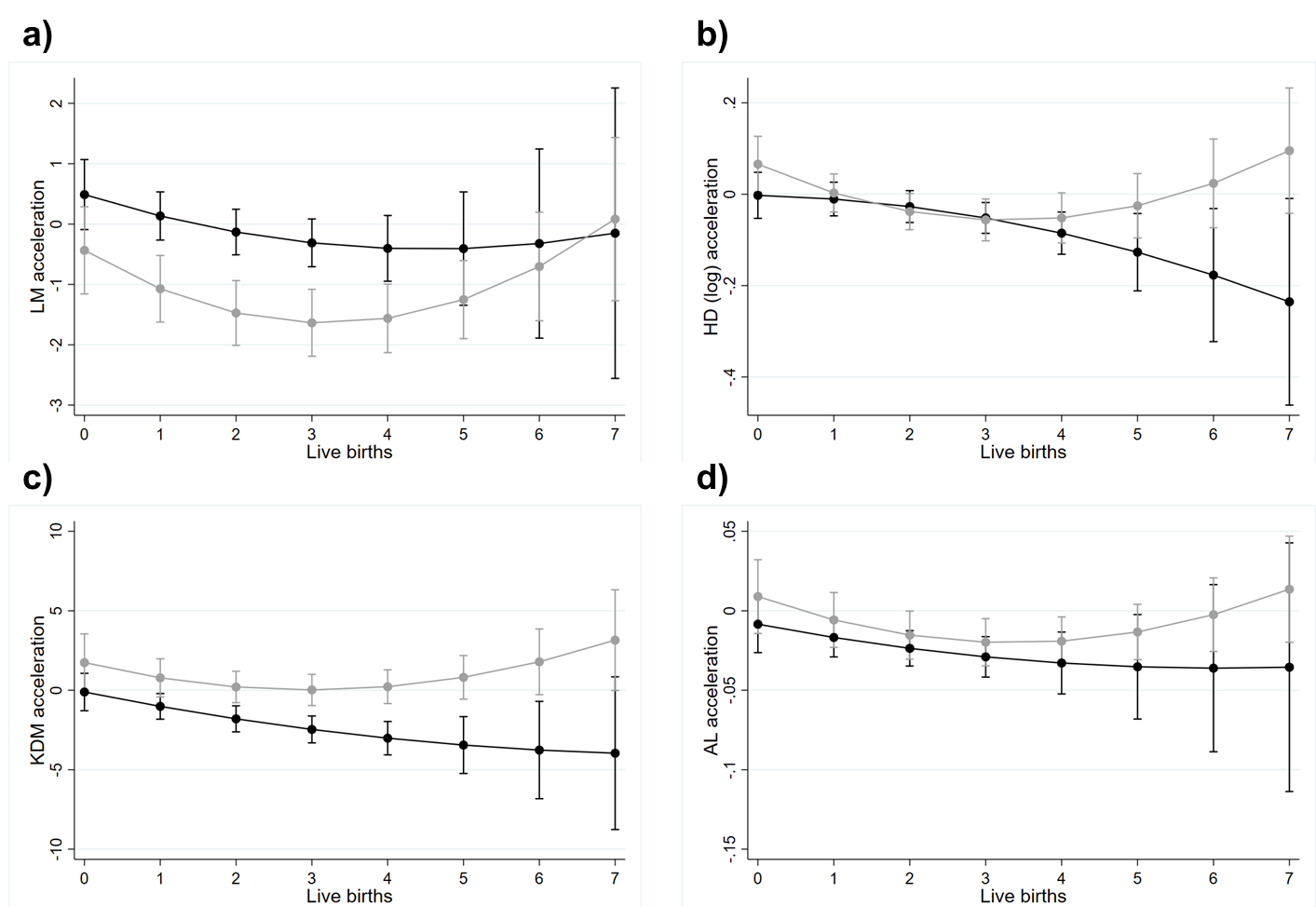
**Figure 2.**



**Figure 3.**



**Figure 4.**

****

**Table 1.** Sample demographic characteristics (*n* = 4,418), National Health and Nutrition Examination Survey, 1999-2010. Means, standard errors (SE), and percentages represent nationally-representative estimates based on adjustment for complex survey design, survey nonresponse, non-coverage, and complex survey design.

|  |  |
| --- | --- |
| Mean age (SE, range) | 47.51 (0.39, 20-84) |
| Mean BMI (SE, range) | 28.56 (0.13, 14.7-71.3) |
| Mean FIPR (SE, range) | 2.93 (0.04, 0-5) |
| Smoking (n, %) |  |
| Never | 2645 (56.3%) |
| Past | 949 (22.8%) |
| Current | 824 (20.8%) |
| Education (n, %) |  |
| Less than high school | 1171 (24.0%) |
| High school or equivalent | 1082 (17.6%) |
| Some college or AA degree | 1337 (25.4%) |
| College graduate or above | 828 (33.0%) |
| Race/ethnicity (n, %) |  |
| Non-Hispanic white | 2316 (73.9%) |
| Non-Hispanic black | 841 (10.6%) |
| Hispanic | 1114 (11.4%) |
| Other | 147 (4.15%) |
| Menopause status (n, %) |  |
| Premenopausal | 2166 (55.9%) |
| Postmenopausal | 2252 (44.1%) |
| Mean number of live births (SE, range) | 2.03 (0.04, 0-7) |
| Ever-parity (n, %) |  |
| Nulliparous | 771 (20.3%) |
| Parous | 3647 (79.7%) |

**Table 2.** Multiple linear regression examining the chronic and acute effects of number of live births on biological age, National Health and Nutrition Examination Survey 1999-2010. Values represent coefficient estimates and 95% confidence intervals. *Notes:* \* *p* < 0.05, \*\* *p* < 0.01, \*\*\* *p* < 0.001; values in **bold** represent effects significant after multiple comparison correction.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **LM** | **HD (log)** | **KDM** | **AL** |
| **Primary model (*n* = 4,418)** † | | | | |
| Live births (linear) | -0.40 (-0.84, 0.04) | -0.004 (-0.04, 0.04) | -0.96 (-1.94, 0.02) | -0.01 (-0.02, 0.003) |
| Live births (quadratic) | 0.04 (-0.05, 0.14) | -0.004 (-0.01, 0.01) | 0.06 (-0.15, 0.27) | 0.001 (-0.002, 0.004) |
| Menopause status | -0.92 (-1.98, 0.13) | 0.07 (-0.02, 0.16) | 1.85 (-0.60, 4.30) | 0.02 (-0.02, 0.003) |
| Live births (linear) x menopause status | -0.36 (-1.02, 0.31) | **-0.07 (-0.12, -0.02) \*\*** | -0.19 (-1.55, 1.17) | -0.01 (-0.03, 0.01) |
| Live births (quadratic) x menopause status | 0.08 (-0.05, 0.20) | **0.02 (0.01, 0.03) \*\*** | 0.14 (-0.12, 0.39) | 0.002 (-0.002, 0.01) |
| **Sensitivity analysis 1 (n = 5,184)** †† | | | | |
| Live births (linear) | -0.09 (-0.54, 0.35) | -0.001 (-0.04, 0.03) | -0.52 (-1.36, 0.31) | -0.004 (-0.02, 0.01) |
| Live births (quadratic) | 0.08 (-0.03, 0.18) | -0.001 (-0.01, 0.01) | 0.09 (-0.09, 0.26) | 0.002 (-0.001, 0.01) |
| Menopause status | 0.25 (-0.83, 1.34) | 0.08 (-0.01, 0.20) | **3.34 (0.86, 5.82) \*\*** | **0.05 (0.01, 0.09) \*** |
| Live births (linear) x menopause status | -0.70 (-1.38, -0.03) \* | **-0.07 (-0.12, -0.02) \*\*** | -0.57 (-1.86, 0.72) | -0.02 (-0.03, 0.002) |
| Live births (quadratic) x menopause status | 0.10 (-0.03, 0.21) | **0.01 (0.004, 0.02) \*\*** | 0.15 (-0.08, 0.38) | 0.002 (-0.002, 0.01) |
| **Sensitivity analysis 2 (n = 3,587)** † | | | | |
| Live births (linear) | -0.01 (-1.13, 1.11) | 0.04 (-0.07, 0.14) | -2.13 (-4.09, -0.18) \* | -0.03 (-0.06, -0.01) \* |
| Live births (quadratic) | -0.01 (-0.20, 0.18) | -0.01 (-0.03, 0.004) | 0.26 (-0.07, 0.58) | 0.004 (-0.0001, 0.001) |
| Years since last live birth | 0.01 (-0.08, 0.09) | -0.01 (-0.02, 0.003) | -0.06 (-0.28, 0.16) | -0.001 (-0.004, 0.002) |
| Live births (linear) x years since last live birth | 0.02 (-0.05, 0.09) | 0.004 (-0.001. 0.01) | 0.10 (-0.04, 0.23) | 0.001 (-0.001, 0.003) |
| Live births (quadratic) x years since last live birth | -0.003 (-0.01, 0.01) | -0.0001 (-0.001, 0.0001) | -0.02 (-0.04, 0.01) | -0.0002 (-0.0004, 0.0001) |
| Menopause status | -0.91 (-3.17, 1.34) | 0.31 (0.07, 0.55) | 3.52 (-2.35, 9.39) | 0.02 (-0.06, 0.11) |
| Live births (linear) x menopause status | -0.67 (-2.56, 1.22) | **-0.24 (-0.40, -0.07) \*\*** | -1.60 (-5.57, 2.38) | -0.02 (-0.07, 0.04) |
| Live births (quadratic) x menopause status | 0.15 (-0.16, 0.45) | **0.04 (0.01, 0.06) \*\*** | 0.35 (-0.26, 0.96) | 0.003 (-0.004, 0.01) |
| **Sensitivity analysis 3 (n = 107)** † | | | | |
| Live births (linear) | -6.63 (-13.19, -0.07) \* | 0.25 (-0.39, 0.90) | -2.70 (-14.83, 9.43) | -0.06 (-0.21, 0.08) |
| Live births (quadratic) | 1.15 (0.14, 2.17) \* | -0.02 (-0.12, 0.08) | 0.66 (-1.49, 2.82) | 0.02 (-0.01, 0.04) |
| Months since last live birth | -1.07 (-1.81, -0.34) \* | 0.05 (-0.04, 0.14) | -0.71 (-2.06, 0.65) | -0.01 (-0.02, 0.01) |
| Live births (linear) x months since last live birth | 0.60 (0.14, 1.05) \* | -0.04 (-0.10, 0.02) | 0.23 (-0.72, 1.17) | -0.001 (-0.01, 0.01) |
| Live births (quadratic) x months since last live birth | -0.09 (-0.15, -0.02) \* | 0.01 (-0.003, 0.014) | -0.03 (-0.18, 0.11) | 0.0003 (-0.001, 0.002) |

† Models were adjusted for the following variables: chronological age, body mass index, federal income-to-poverty ratio, smoking, education, and self-identified race/ethnicity.

†† Model was adjusted for chronological age only.

**Table 3.** Multiple linear regression examining the chronic and acute effects of number of live births on biological age for post-menopausal women only, National Health and Nutrition Examination Survey 1999-2010. Values represent coefficient estimates and 95% confidence intervals. *Notes:* \* *p* < 0.05, \*\* *p* < 0.01, \*\*\* *p* < 0.001; values in **bold** represent effects significant after multiple comparison correction.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **LM** | **HD (log)** | **KDM** | **AL** |
| **Primary model (*n* = 2,252)** † | | | | |
| Live births (linear) | **-0.68 (-1.11, -0.25)\*\*** | **-0.07 (-0.11, -0.04)\*\*\*** | -1.07 (-2.12, -0.02)\* | **-0.02 (-0.03, -0.01)\*\*** |
| Live births (quadratic) | **0.10 (0.03, 0.17)\*\*** | **0.010 (0.004, 0.016)\*\*** | 0.16 (-0.08, 0.04) | **0.002 (0.001, 0.004)\*** |
| **Sensitivity analysis 1 (n = 2,498)** †† | | | | |
| Live births (linear) | **-0.80 (-1.30, -0.30)\*\*** | **-0.08 (-0.12, -0.04)\*\*\*** | -1.10 (-2.10, -0.10)\* | **-0.02 (-0.02, -0.01)\*\*** |
| Live births (quadratic) | **0.17 (0.09, 0.25)\*\*\*** | **0.013 (0.007, 0.019)\*\*\*** | **0.23 (0.08, 0.39)\*\*** | **0.004 (0.002, 0.01)\*\*\*** |

**Table 4.** Multiple linear regression examining the chronic and acute effects of number of live births on biological age for pre-menopausal women only, National Health and Nutrition Examination Survey 1999-2010. Values represent coefficient estimates and 95% confidence intervals. *Notes:* \* *p* < 0.05, \*\* *p* < 0.01, \*\*\* *p* < 0.001; values in **bold** represent effects significant after multiple comparison correction.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **LM** | **HD (log)** | **KDM** | **AL** |
| **Primary model (*n* = 2,166)** † | | | | |
| Live births (linear) | -0.24 (-0.70, 0.22) | 0.02 (-0.03, 0.06) | -0.51 (-1.53, 0.51) | -0.01 (-0.02, 0.01) |
| Live births (quadratic) | 0.04 (-0.06, 0.13) | -0.01 (-0.02, 0.004) | 0.03 (-0.18, 0.24) | 0.001 (-0.002, 0.003) |
| **Sensitivity analysis 1 (n = 2,686)** †† | | | | |
| Live births (linear) | -0.03 (-0.46, 0.52) | 0.01 (-0.03, 0.05) | -0.24 (-1.15, 0.67) | -0.004 (-0.02, 0.01) |
| Live births (quadratic) | 0.06 (-0.04, 0.17) | -0.003 (-0.01, 0.01) | 0.05 (-0.13, 0.24) | 0.002 (-0.001, 0.01) |