Kim Catherine (Orcid ID: 0000-0001-9237-0532)

Gestational diabetes mellitus, epigenetic age, and offspring metabolism

Short title: GDM, epigenetic age, and offspring metabolism

Catherine Kim,[1] Kylie K. Harrall,[2] Deborah H. Glueck,[3] Belinda L. Needham,[4] Dana Dabelea, [5]

[1] Departments of Medicine, Obstetrics & Gynecology, and Epidemiology, University of Michigan, Ann Arbor, MI, ORCID 0000-0001-9237-0532

[2]Lifecourse Epidemiology of Adiposity and Diabetes (LEAD) Center, University of Colorado Aurora, CO, ORCID 0000-0003-4467-2282

[3]LEAD Center and Department of Pediatrics, University of Colorado School of Medicine, Aurora, CO, ORCID 0000-0003-0047-8939

[4]Department of Epidemiology, University of Michigan, Ann Arbor, MI, ORCID 0000-0001-5939-2027

[5]LEAD Center, Department of Pediatrics, Department of Epidemiology, University of Colorado, CO, ORCID 0000-0001-9514-8929

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Corresponding author:

Catherine Kim M.D. M.P.H.

2800 Plymouth Road

Building 16, Room 405E

Ann Arbor, MI 48109

Telephone: (734) 936-5216

Fax (734) 936-8944

Email: cathkim@umich.edu

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Novelty statement:

What is known: Offspring exposed to GDM in-utero have greater insulin resistance than unexposed offspring, but mechanisms are not known.

What this study found: In a cohort of racially and ethnically diverse children, children exposed to GDM in-utero have faster epigenetic aging, and faster epigenetic aging is associated with greater insulin resistance and secretion.

Implications: Epigenetic modification, particularly pathways associated with epigenetic aging, may influence or be affected by offspring insulin metabolism.

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design of the study; the collection, analysis, and interpretation of the data; the writing of the report; and did not impose any restrictions regarding the publication of the report.

STRUCTURED ABSTRACT

AIMS No reports examine the relationships between in-utero exposure to GDM, offspring epigenetic age acceleration (EAA), and offspring insulin sensitivity.

METHODS Using data from a cohort study, we examined associations between gestational diabetes mellitus (GDM) exposure in-utero and offspring EAA at approximately 10 years of age, using separateregression models adjusting for offspring chronologic age and sex. We also examined associations between EAA with updated homeostasis model assessment of insulin sensitivity and secretion (HOMA2-S and HOMA2-β) measured at approximately 10 and 16 years of age, using mixed linear regression models accounting for repeated measures after adjustment for offspring chronologic age and sex.

RESULTS Compared to unexposed offspring (n=91), offspring exposed to GDM (n=88) had greater EAA or older extrinsic age compared to chronologic age (beta-coefficient 2.00, 95% confidence interval [0.71, 3.28], p=0.0025), but not greater intrinsic EAA (beta-coefficient -0.07, 95% CI [-0.71, 0.57], p=0.93). Extrinsic EAA was associated with lower insulin sensitivity (beta-coefficient -0.018, 95% CI [-0.035, -0.002], p=0.03) and greater insulin secretion (beta-coefficient 0.018, 95% CI [0.006, 0.03], p=0.003), and these associations persisted after further adjustment for measures of maternal and child adiposity. No associations were observed between intrinsic EAA and insulin sensitivity and secretion, before or after adjustment for measures of maternal and child adiposity.

CONCLUSIONS In this study, children exposed to GDM experience greater extrinsic EAA, which is associated with lower insulin sensitivity and greater insulin secretion. Further studies are needed to determine directionality of these associations.

Key words: gestational diabetes; epigenetics; insulin resistance

Previous studies have linked in-utero exposure to gestational diabetes mellitus (GDM) with altered offspring DNA methylation patterns in epigenome-wide association studies (EWAS) [1-4]. Different studies have identified different epigenomic regions and specific cytosine-phosphate-guanine sites (CpGs), complicating interpretations of clinical significance. One potential framework for interpreting such differences is that of epigenetic aging. Epigenetic age acceleration (EAA) is the residual from a regression of DNA methylated age on chronologic age. A positive value of EAA indicates faster than expected epigenetic aging, whereas a negative value indicates slower than expected epigenetic aging. EAA may be estimated using any of several epigenetic clocks [5-7]. Although such clocks rely on different CpG sites, overlapping transcriptional pathways are targeted, potentially affecting shared cellular functions [8]. In one 2021 report examining Chinese offspring aged approximately six years, exposure to GDM in-utero was associated with faster EAA calculated from the Horvath ("intrinsic") and Hannum ("extrinsic") epigenetic clocks [9].

The majority of epigenetic clocks were originally derived from EWAS primarily in adults and are believed to reflect aging across a range of tissues [6]. In adults, more rapid "ticking" on epigenetic clocks is associated with greater risk for metabolic syndrome [10] and diabetes [11], along with mortality and other chronic diseases typically observed in later life [11]. Thus, EAA

in adults is viewed as an adverse outcome that is either cause or consequence of poorer metabolic indices. In contrast, the implications of more rapid epigenetic aging in youth are not understood. In general, childhood is characterized by rapid turnover in aging markers and development as opposed to senescence. In one report of Australian youth (average age of 17 years) [12], greater EAA was associated with lower insulin sensitivity. Whether more rapid epigenetic aging is associated with indicators of insulin sensitivity or insulin secretion at even younger ages (late childhood as opposed to adolescence) is not known.

The Exploring Perinatal Outcomes in Children (EPOCH) study is a longitudinal cohort study of youth in Colorado, enriched in offspring exposed to maternal GDM in-utero [13]. We have previously reported that such exposure to maternal GDM predicted greater offspring adiposity [14] as well as reduced insulin sensitivity [15]. In the present study, we examined whether GDM predicted EAA in late childhood (average age 10 years) and whether EAA, in turn, predicted glucose-insulin metabolism in late childhood as well as in later adolescence (average age 17 years). We hypothesized that exposure to GDM would be associated with more rapid EAA in offspring, before and after adjustment for chronologic age and sex. Based upon studies in adults, we hypothesized that greater EAA would be linked with lower insulin sensitivity in adolescent offspring, before and after adjustment for age and sex. We hypothesized that these associations would persist but be attenuated after adjustment for maternal and child adiposity measures.

Methods

Participants

The design, methods, and baseline characteristics of EPOCH participants have been previously described [16]. EPOCH is an observational historical prospective study that recruited healthy 6- to 13-yr old children who were offspring of singleton pregnancies, born at a single hospital in Denver between 1992 and 2002, whose biological mothers were members of Kaiser Permanente of Colorado (KPCO). The study population was sampled to reflect similar racial and ethnic distributions of Colorado. We enrolled children exposed to maternal diabetes in utero and a random sample of children not exposed to maternal diabetes. The first research visit (visit 1) occurred at a mean (sd) age among the offspring of 10.4 (1.5) years and the second visit occurred during the period 2010 to 2015 (mean follow-up 6.3 years), at which time the offspring had a mean (sd) age of 16.7 (1.2) years. The present analysis focuses upon the 179 children who underwent EWAS at visit 1 as part of a prior discovery study of the examination of the impact of fetal nutrition upon offspring epigenetic signatures, for a total of 88 children exposed to GDM and 91 not exposed to GDM who were of similar age and sex. All participants provided informed consent. The study was approved by the Colorado Multiple Institutional Review Board.

Epigenetic age

The procedures assessing genome-wide DNA methylation in EPOCH have been previously described and were conducted using standard methods using the Illumina's Infinium Human Methylation 450k BeadChip on bisulfite-treated samples [2]. We chose to examine two measures of EAA based upon the previous report by Shiau et al that found associations between in-utero exposure and offspring EAA [9], as well as other reports finding correlations between these measures and with glucose, insulin resistance, and diabetes in adults [5, 10]. These epigenetic age estimates were developed by regressing chronological age on individual CpG sites

using supervised machine learning algorithms to select the most informative set to predict chronological age [6, 7].

Intrinsic EAA can be calculated as the residual from a multivariable regression of Horvath EAA and blood cell count estimates on chronological age, and thus is independent of age-related changes in blood cell composition [6, 17]. To account for cell composition variability we estimated the proportions of CD4 + T lymphocytes, CD8 + T lymphocytes, B lymphocytes, natural killer cells, monocytes, and granulocytes using the Houseman *et al*. method [18]. Extrinsic EAA can be calculated by first combining Hannum EAA with three imputed blood cell components (naïve cytotoxic T cells, exhausted cytotoxic T cells, and plasmablasts estimated using the approach described by Klemera and Doubal[19]) to form an aggregate measure and then regressing this measure onto chronologic age. Thus, this measure captures both intrinsic epigenetic age as well as the weighted average of age-related characteristic changes in blood cell composition such as decreases in naive CD8+ T cells and increases in memory or exhausted CD8+ T cells.

GDM; insulin sensitivity; insulin secretion

Demographic information was collected via self-report. Race/ethnicity was collected using 2000 U.S. Census-based questions and categorized as Hispanic (any race), non-Hispanic white, non-Hispanic African-American, and non-Hispanic other, which were further categorized as white vs. non-white. Exposure to maternal GDM and maternal pre-pregnancy body mass index (BMI) were obtained from KPCO medical records. All pregnant women at KPCO were routinely screened for gestational diabetes (GDM) at 24–28 weeks using the two-step standard protocol [20]; GDM was diagnosed if glucose values exceeded two or more thresholds set by the

National Diabetes Data Group on the 3-h, 100-g oral glucose tolerance test [21]. At visits 1 and 2, after an overnight fast, children underwent fasting venous blood measurement of glucose and insulin and whole blood draw. The computer-based homeostatic model was used to calculate insulin resistance [updated homeostatic model assessment of insulin resistance (HOMA2-IS)] and β-cell function [updated homeostatic model assessment of β-cell function (HOMA2-β)] (https://www.dtu.ox.ac.uk/homacalculator).

Covariates

In EPOCH, offspring diet in childhood and adolescence was assessed with the Block Kids Food Frequency Questionnaire,[22] the data from which was in turn used to calculate Healthy Eating Index 2010, a diet quality index ranging from 1–100.[23] Offspring physical activity in childhood and adolescence was assessed at both research visits with the 3-day Physical Activity Recall.[24] Participants recalled prior day activities in 30-minute blocks, along with intensity level (light, moderate, hard, very hard) as appropriate. We calculated the average daily number of 30-minute blocks of physical activities with metabolic equivalents (METs) of 6 or greater.[23] Pubertal development was self-assessed using a diagrammatic representation of Tanner staging adapted from Marshall and Tanner;[25] for the purpose of the analysis, youth were categorized as prepubertal (Tanner <2) and pubertal (Tanner 2–5).[13]

Statistical analysis

Baseline characteristics were described using numbers (percentages) for categorical variables and means (standard deviations) and medians (interquartile ranges) for quantitative

variables with normal and skewed distributions, respectively (Table 1). Baseline characteristics were compared between groups with and without GDM. Continuous variables were compared using Satterthwaite t-tests, and categorical variables were compared using chi-square tests. HOMA2-S and HOMA2-β were log transformed for comparison testing.

First, we fit separate general linear regression models to examine the association between GDM with EAA. The outcome was EAA and the main predictor was GDM. Models were adjusted for offspring chronologic age and sex. Next, general linear mixed models were fit to assess whether intrinsic or extrinsic EAA at visit 1 were associated with repeated measurements of indicators for insulin sensitivity (HOMA2-S) and secretion (HOMA2-β) at visits 1 and 2. (Table 2). The outcome was repeated measurements of HOMA2, and the main predictor was EAA. Models examining the association between EAA and offspring insulin sensitivity and secretion were adjusted for offspring chronological age and sex. A random intercept was fit to account for within-participant correlation. HOMA2 measures were log-transformed to meet assumptions of normality. We also examined the pattern of associations between EAA at visit 1 with visit 1 insulin secretion and sensitivity measures (adjusting for chronologic age at visit 1), and between EAA at visit 1 with visit 2 insulin secretion and sensitivity measures (adjusting for chronologic age at visit 1) (Table 3). Models were adjusted for offspring chronological age and sex. A random intercept was fit to account for within-participant correlation, and a random slope was fit for age using unstructured covariance. Along similar lines, when models adjusted for measures of adiposity, random slopes were fit when there were repeated measures. HOMA2 measures were log-transformed to meet assumptions of normality. We also examined the pattern of associations between EAA at visit 1 with visit 1 insulin secretion and sensitivity measures

(adjusting for chronologic age at visit 1), and between EAA at visit 1 with visit 2 insulin secretion and sensitivity measures (adjusting for chronologic age at visit 1) (Table 3).

A series of sensitivity analyses were performed. First, models were re-run using estimates of EAA that were not adjusted for cell counts. Second, models were adjusted for race/ethnicity. Interactions with sex were assessed.

We also examined whether associations persisted after adjustment for maternal and child adiposity measures. When offspring insulin secretion and insulin resistance were the dependent variables, both maternal and offspring adiposity measures were used as covariates. These adiposity measures included maternal pre-pregnancy BMI; childhood BMI; childhood waist-to-height ratio, and childhood visceral adiposity (Table 2). Along similar lines, we examined whether the pattern of associations changed when we examined fetal nutrition, defined as the presence of maternal overweight (pre-pregnancy BMI $\geq$ 25 kg/m^2) OR GDM, was the independent variable. Analyses were performed using the Statistical Analysis Software (SAS) version 9.4 (SAS Institute).

Results

Table 1 shows participant characteristics by GDM status. In these unadjusted comparisons, compared to offspring without GDM, offspring who were exposed to GDM had mothers with higher pre-pregnancy BMI. Offspring who were exposed to GDM had greater waist-to-height ratio than offspring not exposed to GDM, although offspring BMI and visceral adiposity were similar by GDM exposure. Offspring with exposure to GDM had higher extrinsic EAA, while intrinsic EAA was similar by GDM status. Offspring with exposure to GDM also

had similar estimates of insulin sensitivity and secretion from visits 1 and 2 when compared to offspring not exposed to GDM.

In models examining the association between in-utero exposure to GDM (independent variable) and offspring EAA, GDM was associated with higher extrinsic EAA after adjustment for offspring age and sex (beta-coefficient 2.00, 95% CI [0.71, 3.28], p=0.0025). We have previously reported that fetal overnutrition, defined as GDM or in-utero exposure to maternal overweight/obesity (pre-pregnancy BMI > 25 kg/m^2), was associated with offspring insulin sensitivity.[15] Therefore, we examined whether fetal overnutrition was associated with epigenetic aging. In these models, fetal overnutrition was linked to faster extrinsic EAA (beta-coefficient 2.14, 95% CI [0.84, 3.44], p=0.0014). Exposure to maternal overweight/obesity had a weaker and non-statistically significant association with extrinsic EAA (beta-coefficient, 1.42 (-0.23, 3.07), p=0.09) than the association between GDM and epigenetic aging. Taken together, these analyses suggest that exposure to maternal GDM, rather than maternal overweight, informed the association between fetal overnutrition and extrinsic EAA.

GDM was not associated with higher intrinsic EAA after adjustment for offspring age and sex (beta-coefficient -0.03, 95% CI [-0.71, 0.65], p=0.93). Fetal overnutrition was also not associated with intrinsic EAA after adjustment for age and sex (beta-coefficient -0.064 95% CI [-0.76, 0.63], p=0.86) were observed. Associations between GDM and intrinsic EAA remained non-significant after additional adjustment for maternal BMI (beta-coefficient -0.24, 95% CI -1.15, 0.68, p=0.61).

Table 2 shows the associations between EAA assessed at visit 1 and measures of insulin sensitivity and secretion assessed at visit 1 and visit 2. Higher extrinsic EAA was associated with lower HOMA2-S and higher HOMA2-β. However, intrinsic EAA was not associated with higher

HOMA2-S or HOMA2-β. The associations between extrinsic EAA and log HOMA2- β remained significant after adjustment for maternal BMI and measures of offspring adiposity, although the association between extrinsic EAA and log HOMA2S was attenuated. (Table 2). Associations were primarily driven by associations between EAA at visit 1 and insulin secretion and resistance at visit 1, since associations between EAA at visit 1 and insulin secretion and resistance at visit 2 were not significant (Table 3).

In sensitivity analyses, we investigated whether the association between extrinsic EAA at visit 1 and insulin resistance and secretion at visit 1 were altered by adjustment for adjustment for additional covariates at visit 1 in addition to chronologic age and sex. These covariates included HEI score; METS per day; and pre-pubertal stage vs. pubertal stage. After adjustment for these covariates, the patterns of associations remained similar (Ancillary Table).

In sensitivity analyses, the pattern of associations was similar when we examined estimates of EAA that were not adjusted for cell counts, so we present only conventional intrinsic and extrinsic EAA measures. The pattern of associations was also similar when we adjusted for race/ethnicity, with the exception that the association between extrinsic EAA and log HOMA2-S slightly decreased (p=0.055). Given the small number of persons who were black (n=8) or other race/ethnicity (n=7), we present analyses that do not adjust for these variables, Interactions with sex were not significant at $p<0.10$ and thus non-stratified analyses are presented.

Discussion

Although in-utero exposure to GDM is recognized to have adverse effects on offspring metabolism, the role that epigenetic modification plays is not completely understood [2, 4, 26].

Interpreting EWAS through the framework of epigenetic aging may be useful in integrating the disparate findings from several cohorts, particularly since EAA is associated with glucose metabolism in adults [5, 10, 27]. Such associations are important to understand given the high prevalence of GDM and subsequent increased risk of glucose intolerance in offspring [28, 29]. Using data from a well-characterized cohort of children, we found that in-utero exposure to GDM was associated with more rapid epigenetic aging by one measure of EAA. More rapid epigenetic aging was associated with lower insulin sensitivity and higher insulin secretion when children were aged 10 to 17 years of age, a time of rapid maturation. These associations persisted after adjustment for measures of maternal and childhood adiposity, although cross-sectional associations were stronger than prospective associations. Other reports have not examined whether epigenetic aging is associated with glucose and insulin metabolism in youth.

Only one other report has examined whether in-utero exposure to GDM predicts EAA in offspring. Our findings regarding the association between GDM exposure and extrinsic EAA are similar to those reported in the Tianjin GDM Observational Study, which found that six year-old children exposed to GDM had more rapid EAA [30]. Our findings expand on that study in that we examined a cohort with a different racial/ethnic composition and we also examined children who were approximately four years older, which may be important in that childhood is a time of rapid growth as well as greater discrepancies between epigenetic age and chronologic age [31]. Unlike that study, we did not find that GDM was associated with greater intrinsic EAA, which may reflect the smaller size of our study, our examination of different ethnicities, or the older age of children in EPOCH compared to the Tianjin GDM study. Other studies have reported inconsistent associations between GDM and methylation of particular CpGs; a meta-analysis of seven pregnancy cohorts in the Pregnancy and Childhood Epigenetics (PACE) consortium

(which also included our EPOCH cohort) noted that exposure to GDM was not associated with specific CpGs identified through EWAS of cord blood, although exposure to GDM was associated with differentially methylated regions including the promotor of a gene associated with autism spectrum disorder and of the gene body of CYP2E1, which is upregulated in type 1 and type 2 diabetes [4]. The clinical significance of this association is still uncertain.

No reports examine the relationship between EAA measures and glucose and insulin metabolism in pre-pubertal children, although one report did examine associations between EAA and insulin sensitivity at 17 years of age. In this Australian cohort, EAA measures were also associated with lower insulin sensitivity estimated from fasting insulin and glucose measures [12]. In the Tianjin cohort, greater EAA was associated with several anthropometric measures, including weight for age Z score, BMI for age Z score, body fat percentage, skin fold measurements and blood pressure [30]. Associations with glucose and insulin were not reported. In older persons, greater EAA is associated with adverse indicators of glucose metabolism in cross-sectional and longitudinal analyses. Among mid-life adults, estimates of EAA are associated with metabolic syndrome, and intrinsic EAA predicted incident metabolic syndrome [10]. In the same cohort, epigenetic age estimates using another epigenetic clock was associated with incident diabetes [32]. In a cohort of hypertensive African-Americans approximately 60 years of age, higher extrinsic EAA was associated with higher fasting insulin [33].

The extent to which insulin and glucose affect EAA or vice-versa is not established. In one report, bariatric surgery led to substantial decreases in BMI and also small, but significant, improvements in EAA over one year, suggesting that insulin and glucose might affect EAA even though EAA measures predict incident metabolic derangement [34]. Our finding that cross-

sectional associations were stronger than longitudinal associations suggest that insulin resistance and secretion might influence EAA in children rather than vice-versa.

We found a more consistent and significant pattern of associations using extrinsic EAA rather than intrinsic EAA. This may be due to the fact that EPOCH estimates of EAA were derived from blood, and the Hannum calculator (the basis of extrinsic EAA) was trained using venous samples, whereas the Horvath calculator (the basis of intrinsic EAA) was trained using a range of tissue types. Despite these differences in associations, and the fact that these clocks share only 6 CpG sites of the 71 CpG sites in the Hannum clock [5] and the 353 CpG sites in the Horvath clock [6, 7], intrinsic and extrinsic EAA estimates are highly correlated (r=0.76) with each other and with mortality [17]. An analysis of microarray expression data from monocytes note that these different clocks do share several overlapping transcriptional profiles, namely involving epidermal growth factor receptor signaling, mitochondrial translation and function, and oxidative phosphorylation, whereas transcriptional profiles unique to each clock are hypothesized to reflect tissue differences reflecting how each clock was developed [8].

The strengths of this report include a diverse longitudinal cohort assessing youth during the pubertal transition and assessment of insulin sensitivity and secretion. However, there are several limitations. It is unclear whether the association between GDM and EAA was determined by dysglycemia vs. other in-utero factors other than adiposity, and examinations are needed which are powered to distinguish the effects of treatment and the degree of glucose elevations. In addition, as mentioned above, EPOCH only assessed epigenome-wide DNA methylation at one point in time, and repeated measures would be useful in determining directionality of associations. Finally, the study of epigenetic aging in relation to outcomes other than mortality is recent, and no consensus exists on optimal choice of epigenetic clock particularly in younger

populations. Although we chose epigenetic measures that have been examined in adults with respect to glucose and insulin metabolism, these measures have not been previously validated using glucose and insulin or chronologic age in youth. Our analyses suggest that depending on how epigenetic age was derived in a particular cohort (from blood only or a range of tissue types) may influence selection of the optimal epigenetic age measure.

We conclude that GDM is associated with offspring epigenetic EAA as estimated with at least one epigenetic clock. We also conclude that EAA, insulin secretion and resistance are also associated. Additional examinations are needed to determine whether the relationship between GDM and EAA is due to dysglycemia or other in-utero exposures, whether offspring metabolism affects EAA in children, and to what extent this relationship is similar across other measures of aging, such as telomere length. Such studies would ideally involve aging measures assessed at several points in time.

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The authors declare no conflicts of interest.

References

1. Chen P, Piaggi P, Traurig M, boardus C, Knowler W, Baier L*, et al.* Differential methylation of genes in individuals exposed to maternal diabetes in utero. *Diabetologia* 2017; **60**:645-655.
2. Yang I, Zhang W, Davidson E, Fingerlin T, Kechris K, Dabelea D. Epigenetic marks of in utero exposure to gestational diabetes and childhood adiposity outcomes: the EPOCH study. *Diabet Med* 2018; **35**:612-620.
3. Hjort L, Martino D, Grunnet L, Naeem H, Maksimovic J, Olsson A*, et al.* Gestational diabetes and maternal obesity are associated with epigenome-wide methylation changes in children. *JCI Insight* 2018; **3**:pii122572.
4. Howe C, Cox B, Fore R, Jungius J, Kvist T, Lent S*, et al.* Maternal gestational diabetes mellitus and newborn DNA methylation: findings from the pregnancy and childhood epigenetics consortium. *Diabetes Care* 2020; **43**:98-105.
5. Hannum G, Guinney J, Zhao L, Zhang L, Hughes G, Sadda S*, et al.* Genome-wide methylation profiles reveal quantitative views of human aging rates. *Mol Cell* 2013; **49**:359-367.
6. Horvath S. DNA methylation age of human tissues and cell types. *Genome Biol* 2013; **14**:R115.
7. Horvath S. Erratum to: DNA methylation age of human tissues and cell types. *Genome Biol* 2015; **14**:R115.
8. Liu Z, Leung D, Thrush K, Zhao W, Ratliff S, Tanaka T*, et al.* Underlying features of epigenetic aging clocks in vivo and in vitro. *Aging Cell* 2020; **19**:e13229.
9. Shiau S, Wang L, Liu H, Zheng Y, Drong A, Joyce B*, et al.* Prenatal gestational diabetes mellitus exposure and accelerated offspring DNA methylation age in early childhood. *Epigenetics* 2021; **16**:186-195.
10. Nannini D, Joyce B, Zheng Y, Gao T, Liu L, Yoon G*, et al.* Epigenetic age acceleration and metabolic syndrome in the Coronary Artery Risk Development in Young Adults Study. *Clin Epigenetics* 2019; **11**:160.
11. Hillary R, Stevenson A, McCartney D, Campbell A, Walker R, Howard D*, et al.* Epigenetic measures of ageing predict the prevalence and incidence of leading causes of death and disease burden. *Clin Epigenetics* 2020; **12**:115.
12. Huang R, Lillycrop K, Beilin L, Godfrey K, Anderson D, Mori T*, et al.* Epigenetic age acceleration in adolescence associates with BMI, inflammation, and risk score for middle age cardiovascular disease. *J Clin Endocrinol Metab* 2019; **104**:3012-3024.
13. Kim C, Harrall K, Glueck D, Shumer D, Dabelea D. Childhood adiposity and adolescent sex steroids in the Exploring Perinatal Outcomes among Children study. *Clin Endocrinol (Oxf)* 2019; **91**:525-533.
14. Hockett C, Bedrick E, Zeitler P, Crume T, Daniels S, Dabelea D. Exposure to diabetes in utero is associated with earlier pubertal timing and faster pubertal growth in the offspring: the EPOCH study. *J Pediatr* 2019; **206**:105-112.
15. Sauder K, Hockett C, Ringham B, Glueck D, Dabelea D. Fetal overnutrition and offspring insulin resistance and beta-cell function: the Exploring Perinatal Outcomes among Children (EPOCH) Study. *Diabet Med* 2017; **34**:1392-1399.
16. Brumbaugh D, Crume T, Nadeau K, Scherzinger A, Dabelea D. Intramyocellular lipid is associated with visceral adiposity, markers of insulin resistance, and cardiovascular risk in prepubertal children: the EPOCH study. *J Clin Endocrinol Metab* 2012; **97**:E1099-1105.
17. Chen B, Marioni R, Colicino E, Peters M, Ward-Caviness C, Tsai P*, et al.* DNA methylation-based measures of biological age: meta-analysis predicting time to death. *Aging (Albany NY)* 2016; **8**:1844-1865.
18. Houseman E, Accomando W, Koestler D, Christensen B, Marsit C, Nelson H*, et al.* DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC Bioinformatics* 2012; **86**:doi: 10.1186/1471-2105-1113-1186.

19. Klemera P, Doubal S. A new approach to the concept and computation of biological age. *Mech Ageing Dev* 2006; **127**:240-248.
20. American Diabetes Association. Classification and diagnosis of diabetes: standards of medical care in diabetes-2020. *Diabetes Care* 2020; **43**:S14-31.
21. National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes* 1979; **18**:1039-1057.
22. Cullen K, Watson K, Zakeri I. Relative reliability and validity of the Block Kids Questionnaire among youth aged 10 to 17 years. *J Am Diet Assoc* 2008; **108**:862-866.
23. Sauder K, Bekelman T, Harrall K, Glueck D, Dabelea D. Gestational diabetes exposure and adiposity outcomes in childhood and adolescence: an analysis of effect modification by breastfeeding, diet quality, and physical activity in the EPOCH study. *Pediatr Obes* 2019; **doi: 10.1111/ijpo.12562**.
24. Dollman J, Stanley R, Wilson A. The Concurrent Validity of the 3-Day Physical Activity Recall in Australian Youth. *Pediatr Exerc Sci* 2015; **27**:262-267.
25. Marshall W, Tanner J. Growth and physiological development during adolescence. *Annu Rev Med* 1968; **19**:283-300.
26. Moen G, Sommer C, Prasad R, Sletner L, Groop L, Qvigstad E*, et al.* Mechanisms in endocrinology: epigenetic modifications and gestational diabetes: a systematic review of published literature. *Eur J Endocrinol* 2017; **176**:R247-267.
27. Levine M, Lu A, Quach A, Chen B, Assimes T, Bandinelli S*, et al.* An epigenetic biomarker of aging for lifespan and healthspan. *Aging (Albany NY)* 2018; **10**:573-591.
28. Pettitt D, Aleck K, Baird H, Carraher M, Bennett P, Knowler W. Congenital subsceptibility to NIDDM. Role of intrauterine environment. *Diabetes* 1988; **37**:622-628.
29. Pedersen J. Weight and length at birth in infants of diabetic mothers. *Acta Endocrinol* 1954; **16**:330-342.
30. Shiau S, Wang L, Liu H, Zheng Y, Drong A, Joyce B*, et al.* Prenatal gestational diabetes mellitus exposure and accelerated offspring DNA methylation age in early childhood. *Epigenetics* 2020; **doi: 10.1080/15592294.2020.1790924**.
31. Simpkin A, Howe L, Tilling K, Gaunt T, Lyttleton O, McArdle W*, et al.* The epigenetic clock and physical development during childhood and adolescence: longitudinal analysis from a UK birth cohort. *Int J Epidemiol* 2017; **46**:549-558.
32. Kim K, Joyce B, Zheng Y, Schreiner P, Jacobs Jr.. D, Catov J*, et al.* DNA methylation GrimAge and incident diabetes; the Coronary Artery Risk Development in Young Adults (CARDIA) Study. *Diabetes* 2021; **doi: 10.2337/db20-1167**.
33. Ammous F, Zhao W, Ratliff S, Mosley T, Bielak L, Zhou X*, et al.* Epigenetic age acceleration is associated with cardiometabolic risk factors and clinical cardiovascular disease risk scores in African-Americans. *Clin Epigenetics* 2021; **13**:55.
34. Fraszczyk E, Luijten M, Spijkerman A, Snieder H, Wackers P, Bloks V*, et al.* The effects of bariatric surgery on clinical profile, DNA methylation, and ageing in severely obese patients. *Clin Epigenetics* 2020; **12**:14.

Table 1. Visit 1 characteristics of offspring who underwent EWAS in EPOCH. Means (standard deviations), median (interquartile range), or n (percent) shown.

	Exposed to GDM (n=88)	Not exposed to GDM (n=91)	p-value
Age (years)	9.5 (1.7)	10.0 (1.4)	0.07
Girls (n, %)	43 (48.9)	46 (50.6)	0.82
Race/ethnicity (n, %)			0.99
Non-Hispanic White	56 (61.5)	54 (61.4)	
Hispanic	27 (29.7)	27 (30.7)	
Non-Hispanic Black	4 (4.4)	4 (4.6)	
Other	4 (4.4)	3 (3.4)	
Maternal pre-pregnancy BMI (kg/m^2)	27.8 (6.4)	24.2 (5.5)	**0.001**
Offspring BMI (kg/m^2) at visit 1	18.6 (3.8)	17.9 (3.7)	0.23
Visceral Adiposity (cm^2) at visit 1	23.3 (18.7)	21.7 (15.8)	0.61
Waist-to-height ratio at visit 1	0.47 (0.07)	0.43 (0.05)	**0.0006**
Healthy Eating Index score at visit 1	50.2 (9.2)	49.3 (9.6)	0.51
METS per day at visit 1	66.3 (11.7)	68.6 (9.7)	0.17
Tanner stage 1 (pre-pubertal), (n, %)	41 (62)	31 (46)	0.067
Extrinsic EAA at visit 1	0.95 (6.02)	-0.92 (5.43)	**0.03**
Intrinsic EAA at visit 1	0.05 (4.05)	-0.06 (3.02)	0.85
HOMA2-β at visit 1	118.7 (99.0)	107.5 (64.1)	0.11
HOMA2-β at visit 2	160.0 (60.7)	148.5 (54.2)	0.92
HOMA2-S at visit 1	103.5 (134.9)	95.7 (131.8)	0.69
HOMA2-S at visit 2	53.8 (26.5)	58.1 (34.2)	0.17
Fasting glucose levels at visit 1 (mmol/l, DCCT units)	4.5 (1.4) (2.6, 2.3)	4.8 (0.5) (6.5, 2.2)	0.08

Table 1. Visit 1 characteristics of offspring who underwent EWAS in EPOCH. Means (standard deviations), median (interquartile range), or n (percent) shown.

Fasting glucose levels at visit 2 (mmol/l, DCCT units)	5.4 (2.5) (2.6, 2.4)	4.9 (0.5) (2.6, 2.2)	0.20

Table 2. Association between epigenetic age acceleration (EAA) (independent variable) at visit 1 and repeated measures of insulin sensitivity and secretion (dependent variables) at visits 1 and 2, assuming no visit by predictor interaction. Models were adjusted for offspring chronologic age (years) and sex. For covariates of offspring age and measures of adiposity, random slopes were fit, essentially accounting for repeated measures at visit 1 and 2 and the correlation between individuals. Beta-coefficients and 95% confidence intervals shown.

	log HOMA2-S	log HOMA2-β
Model 1: adjusted for age and sex		
Extrinsic EAA	**-0.018 (-0.035, -0.002) p=0.03**	**0.018 (0.006, 0.030) p=0.003**
Intrinsic EAA	0.013 (-0.024, 0.049) p=0.49	0.003 (-0.024, 0.030) p=0.83
Model 2: adjusted for age, sex, and maternal pre-pregnancy BMI		
Extrinsic EAA	-0.02 (-0.03, 0.001) p = 0.06	**0.02 (0.01, 0.03) p = 0.004**
Intrinsic EAA	0.01 (-0.03, 0.05) p = 0.72	0.01 (-0.02, 0.04) p = 0.61
Model 3: adjusted for age, sex, and childhood BMI		
Extrinsic EAA	-0.01 (-0.03, 0.005) p = 0.17	**0.01 (0.003, 0.03) p = 0.012**
Intrinsic EAA	0.01 (-0.02, 0.05) p = 0.40	0.002 (-0.02, 0.03) p = 0.87
Model 4: adjusted for age, sex, and childhood waist-to-height ratio		
Extrinsic EAA	-0.01 (-0.02, 0.01) p = 0.24	**0.01 (0.002, 0.02) p = 0.02**
Intrinsic EAA	0.02 (-0.02, 0.05) p = 0.32	0.001 (-0.02, 0.03) p = 0.94
Model 4: adjusted for age, sex, and childhood visceral adiposity		
Extrinsic EAA	-0.01 (-0.03, 0.004) p = 0.13	**0.02 (0.01, 0.03) p = 0.006**
Intrinsic EAA	0.01 (-0.02, 0.04) p = 0.65	0.01 (-0.02, 0.03) p = 0.54

Table 3. Separate single timepoint models for the association between epigenetic age acceleration (EAA) (independent variable) at visit 1 and measures of insulin sensitivity and secretion (dependent variables), at each visit. Models were adjusted for offspring chronologic age (years) at visit 1 and sex. Beta-coefficients and 95% confidence intervals shown.

	log HOMA2-S at visit 1	log HOMA2-β at visit 1
Model 1: adjusted for age and sex		
Extrinsic EAA at visit 1	**-0.02 (-0.04, -0.001) p = 0.04**	**0.02 (0.01, 0.04) p = 0.0023**
Intrinsic EAA at visit 1	-0.01 (-0.06, 0.03) p = 0.54	0.02 (-0.02, 0.05) p = 0.33
Model 1: adjusted for age and sex		
	log HOMA2-S at visit 2	log HOMA2-β at visit 2
Extrinsic EAA at visit 1	-0.005 (-0.03, 0.02) p = 0.67	0.01 (-0.01, 0.02) p = 0.37
Intrinsic EAA at visit 1	0.04 (-0.01, 0.09) p = 0.08	-0.01 (-0.05, 0.02) p = 0.49