Associations between prenatal distress, mitochondrial health, and gestational age: findings from two pregnancy studies in the USA and Turkey

Qiuhan Huang[1], David Shire[1], Fiona Hollis[2], Sameera Abuaish[3], Martin Picard[1,4,5,6], Catherine Monk[1,5,7], Elif Aysimi Duman[8,9], Caroline Trumpff[1]

[1] Division of Behavioral Medicine, Department of Psychiatry, Columbia University Irving Medical Center, New York, NY, USA

[2] Department of Pharmacology, Physiology and Neuroscience, University of South Carolina School of Medicine, Columbia, SC, USA

[3] Department of Basic Sciences, College of Medicine, Princess Nourah Bint Abdulrahman University, Riyadh, Kingdom of Saudi Arabia

[4] Department of Neurology, H. Houston Merritt Center, Neuromuscular Medicine Division, Columbia University Irving Medical Center, New York, NY, USA

[5] New York State Psychiatric Institute, New York, NY, USA

[6] Robert N Butler Columbia Aging Center, Columbia University Mailman School of Public Health, New York, NY, USA

[7] Department of Obstetrics and Gynecology, Columbia University Irving Medical Center, New York, NY, USA.

[8] Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Acibadem University, Istanbul, Turkey.

[9] Institute of Natural and Applied Sciences, Acibadem University, Istanbul, Turkey.

Correspondence: cat2184@cumc.columbia.edu

Abstract (350 words max)

Background: Pregnancy outcomes are influenced by maternal distress but the pathways underlying these effects are still unknown. Mitochondria, crucial for stress adaptation and energy production, may link psychosocial stress to its biological effects, especially during pregnancy when energy demands significantly increase. This study explores two mitochondrial markers-circulating cell-free mitochondrial DNA (cf-mtDNA) and Growth Differentiation Factor-15 (GDF15)-as potential mitochondrial health indicators linking maternal distress to pregnancy outcomes in two longitudinal studies from the USA and Turkey.

Methods: We analyzed biological, demographic, and psychological data from women in two pregnancy studies: EPI (N=187, USA, Mean age=29.6(SD=6.2) and BABIP (N=198, Turkey, Mean age=32.4(SD=4.0)). Data were collected at multiple time points during the perinatal period, including late 2nd and 3rd trimester, with EPI also including additional data at early 2nd trimester and 4-14 months postpartum. Prenatal maternal psychological distress was measured as perceived stress, anxiety, and depressive symptoms. Plasma cf-mtDNA and GDF15 levels were assessed using qPCR and ELISA, respectively. Statistical analyses included Wilcoxon signed-rank tests, Spearman correlations, and Mann-Whitney tests.

Results: Plasma cf-mtDNA levels did not change significantly during pregnancy in either study. Plasma GDF15 levels increased from early to late pregnancy in both studies and significantly decreased postpartum in EPI. Perinatal maternal distress in the late 2^{nd} and 3^{rd} trimesters was not associated with cf-mtDNA or GDF15 in either study. Metabolic distress, measured as higher pre-pregnancy BMI, was negatively correlated with GDF15 in the late 2^{nd} trimester in EPI and showed a similar trend in BABIP. Similarly, higher maternal psychological distress in the early 2^{nd} trimester were associated with lower cf-mtDNA and a trend for lower GDF15 in EPI. Finally, higher pre-pregnancy BMI and maternal distress in late pregnancy were linked to a smaller decline in GDF15 from late pregnancy to postpartum in EPI, suggesting an interaction between metabolic stress, prenatal distress and post-pregnancy physiological recovery.

Conclusions: This study identified distinct patterns of plasma cf-mtDNA and GDF15 levels during the perinatal period across studies from two countries, revealing unique associations between maternal characteristics, prenatal distress, and pregnancy outcomes, suggesting that maternal distress can interact with energy mobilization during pregnancy.

Introduction

Perinatal maternal psychological distress, such as perceived stress, anxiety, and depression, has long been associated with adverse pregnancy outcomes including increased risk of preeclampsia (Kurki et al., 2000; Shamsi et al., 2010), spontaneous abortion, (Nelson et al., 2003; Qu et al., 2017) and shorter gestational age (Hoffman et al., 2016; Lima et al., 2018). However, the biological mechanisms underlying these effects are still largely unknown. Mitochondria produce energy essential for stress adaption (Picard & McEwen, 2018) and mitochondrial biology represents a potential intersection point between psychosocial experiences and their biological embedding ((Boeck, Gumpp, et al., 2018; Boeck, Salinas-Manrique, et al., 2018; Gumpp et al., 2020; Hroudová et al., 2013; Karabatsiakis et al., 2014; Picard et al., 2018; Trumpff et al., 2023; Zvěřová et al., 2019) for a review see (Picard et al., 2019)). Pregnancy is associated with a progressive increase in energy expenditure ((Butte et al., 1999; Butte et al., 2004; Forsum et al., 1992), for a review see (Savard et al., 2021)). The psychological stress response recruits energy-demanding cellular and physiological processes that can compete with growth-related processes, causing energy constraints that may contribute to the biological embedding of stress and adversity across the lifespan (Bobba-Alves et al., 2022). Thus, the energetically demanding period of pregnancy may compound pre-existing vulnerability, making it a time where mothers are particularly sensitive to the detrimental effects of psychological and metabolic stress on mitochondrial biology, potentially influencing pregnancy outcomes.

The mitochondrion is the only mammalian organelle besides the nucleus to contain its own genome. Each mitochondrion contains multiple copies of the 16.6kb-long circular mitochondrial DNA (mtDNA) (Farge & Falkenberg, 2019; Lee & Han, 2017), which is consistently detectable outside of cells in most bodily fluids, including blood, as cell-free mitochondrial DNA (cf-mtDNA) (Michelson et al., 2023). Under conditions of energetic stress, cf-mtDNA can be released into circulation, thereby acting as a biomarker for mitochondrial stress and signaling. cf-mtDNA levels in blood are elevated in several disease conditions, such as sepsis (Dennhardt et al., 2024; Kung et al., 2012; Yamanouchi et al., 2013), cancer (for a review see (Schwarzenbach et al., 2011)), infections (for a review see (Hovhannisyan et al., 2023)), autoimmune disease (Duvvuri & Lood, 2019; Hu & Shu, 2023; Mondelo-Macia et al., 2021), and psychopathology ((Lindqvist et al., 2016; Lindqvist et al., 2018), for a review, see: (Trumpff et al., 2021)). In healthy non-pregnant individuals, levels of circulating cf-mtDNA are elevated following acute psychological stress (Hummel et al., 2018; Trumpff et al., 2019). While nothing is known

about the connection between psychosocial stress, prenatal maternal distress, and cf-mtDNA in pregnancy, abnormal levels of cf-mtDNA have been found in pregnant women with preeclampsia (Bradshaw et al., 2022) and gestational diabetes (McElwain & McCarthy, 2020), suggesting a connection between adverse pregnancy outcomes and energetic stress.

Growth differentiation factor 15 (GDF15) is another emerging marker of energetic stress implicated in pregnancy. GDF15 is a cytokine metabokine from the TGFβ super family that is released to modulate energy metabolism in response to mitochondrial and metabolic stress (Fujita et al., 2015; Wang et al., 2021). Throughout the human body, GDF15 is most highly expressed in placenta tissues (GTEx consortium (Carithers et al., 2015)). The sole known receptor of GDF15, GFRAL, is located in the hindbrain, where the GDF15-GFRAL complex regulates whole-body energy homeostasis (Lockhart et al., 2020; Wang et al., 2021) and supports energy mobilization (Engström Ruud et al., 2024). In pregnancy, circulating levels of serum GDF15 increase up to 200-fold during the 3rd trimester compared to non-pregnant postpartum state (Andersson-Hall, Svedin, et al., 2021). Altered GDF15 levels in pregnancy have been associated with miscarriage (Zeng et al., 2023), preeclampsia (Chen et al., 2016; Cruickshank et al., 2021; Marschalek et al., 2018; Sugulle et al., 2009), gestational diabetes (Sugulle et al., 2009) and recently causally linked to hyperemesis gravidarum (Fejzo et al., 2024). Pregnancy triggers an unparalleled elevation in blood GDF15 signaling onto the brain to alter physiology (Engström Ruud et al., 2024; Worth et al., 2020). No prior studies have investigated the interplay between GDF15 and prenatal maternal distress. Psychiatric disorders such as major depressive disorder have been associated with elevated levels of GDF15 in non-pregnant populations (Frye et al., 2015; Mastrobattista et al., 2023; Pan et al., 2023), and acute psychological stress exposure also increases circulating levels of GDF15 (Huang et al., 2024). These findings position GDF15 as an emerging marker of i) mitochondrial and energetic stress, ii) normal pregnancy physiology, iii) mental stress and psychopathology.

Taken together, cf-mtDNA and GDF15 are two emerging biomarkers that can offer insights into how energetic stress and maternal distress could converge to impact pregnancy outcomes. Here, we examined how levels of cf-mtDNA and GDF15 change across the perinatal period and their interplay with maternal perinatal characteristics, psychological distress (measured by perceived stress, anxiety and depression) and pregnancy outcomes in two pregnancy studies from the USA (EPI study) and Turkey (BABIP study).

Results

We leveraged data and samples from two longitudinal pregnancy studies in the USA (EPI, N=187 (Monk et al., 2016)) and Turkey (BABIP, N = 198 (Duman et al., 2020)). Blood samples and psychological assessments were collected at four timepoints in EPI (from early 2nd trimester to postpartum) and two timepoints in BABIP (from late 2nd trimester to 3rd trimester) (Figure 1A). Therefore, results from early 2nd trimester and postpartum period were only available in EPI. Demographic characteristics of the two studies are summarized in Table 1. On average, compared to Turkish BABIP participants, American EPI participants were significantly younger, had fewer years of education, and had a higher BMI (ps<0.0001).

cf-mtDNA and GDF15 trajectories across pregnancy

In contrast with a prior study (Cushen et al., 2020), we did not find evidence of significant variation in plasma cf-mtDNA across pregnancy in either study (Figures 1B and 1C, left). Across all organs in the human body, GDF15 is most highly expressed in decidual stromal cells of placenta (GTEx consortium ((Carithers et al., 2015), see figure S1) and it can be hypothesized that as the placenta grows, GDF15 levels would increase. In both studies, we found evidence of a continuous increase in plasma GDF15 levels from early to late pregnancy. In EPI, plasma GDF15 levels gradually increased from early to mid-pregnancy (+14.7%), doubled from mid to late pregnancy (+105.8%), and dropped sharply after postpartum (-98.1%) to levels comparable with non-pregnant healthy controls (Figures 1B, right). In BABIP, plasma GDF15 showed similar magnitude of increase from mid to late-pregnancy (+87.2%) (Figure 1C, right).

In EPI, we found a moderate positive correlation in cf-mtDNA measured in the late 2nd trimester and 3rd trimester (Figure S2A) that was not found in BABIP (Figure S2B). For both studies, we observed strong positive correlations in GDF15 levels measured in the late 2nd and 3rd trimesters (rs=0.49-0.65, p<0.0001), confirming the trait nature of this biomarker. No correlation was found between cf-mtDNA and GDF15 measured within the same visit or across different visits (Figures S2C-F).

cf-mtDNA, GDF15 and maternal characteristics

Next, we investigated the association between cf-mtDNA, GDF15, and maternal characteristics. There were no associations between plasma cf-mtDNA, maternal age, and pre-pregnancy BMI in either study (Supplemental Table 1). In EPI, plasma GDF15, which is the most significantly upregulated protein in human aging (Tanaka et al., 2018), showed no association with maternal age during pregnancy (Supplemental Table 2). During postpartum (16-56 weeks), the expected positive correlation between age and circulating GDF15 levels was

observed (Figure S3A, r=0.47, p=0.016). In BABIP, we found a modest negative correlation between 3rd trimester plasma GDF15 levels and maternal age (Figure S3B, r= -0.18, p=0.049). Higher pre-pregnancy BMI was associated with lower levels of plasma GDF15 in the late 2nd (Figure S3C [left], r=-0.18, p=0.035) and 3rd trimesters in EPI (Figure S3C [right], r=-0.15, p=0.070). In BABIP, a similar pattern was found in the late 2nd trimester (Figure S3D [left], r=-0.13, p=0.088), but not in the 3rd trimester (Figure S3D [right]).

cf-mtDNA, GDF15, neonatal characteristics and perinatal complications

In addition to maternal characteristics, we explored whether cf-mtDNA and GDF15 levels differ according to neonatal sex, gestational age at birth, or adverse pregnancy outcomes such as preeclampsia, preterm birth, and gestational diabetes.

Regarding cf-mtDNA, in both studies, we did not find a significant difference in maternal plasma cf-mtDNA levels by neonatal sex (Supplemental Table 3). In either study, no significant association was found between plasma cf-mtDNA levels (at any sampling point) and gestational age at birth. (Figures 2A-B). In EPI, participants who developed preeclampsia (n=6) tended to present lower levels of plasma cf-mtDNA in the late 2nd and 3rd trimesters compared to those without, but the difference did not reach statistical significance (Supplemental Table 4). The sample size of participants with preeclampsia (n=2) was insufficient to investigate this question in BABIP. In EPI, pregnant women who delivered preterm (<37 weeks, n=10) had higher plasma cf-mtDNA levels in the late 2nd trimester (p=0.044) than those that delivered full-term. However, no difference in plasma cf-mtDNA levels was observed between preterm (n=8) and full-term participants in the BABIP study (Supplemental Table 4). Consistent with a previous study that reported elevated circulating cf-mtDNA levels in women with gestational diabetes mellitus (McElwain & McCarthy, 2020), we found higher plasma cf-mtDNA levels in the late 2nd trimester (p=0.022) in EPI and in the 3rd trimester (p=0.013) in BABIP (Supplemental Table 4).

Regarding GDF15, in both studies, no difference was found in maternal plasma GDF15 levels based on neonatal sex (Supplemental Table 3), contrary to a previous study that found elevated serum GDF15 levels in pregnant women carrying a female offspring (Andersson-Hall, Svedin, et al., 2021). A negative correlation was found between gestational age at birth and late 2nd trimester GDF15 levels in EPI (r=-0.24, p=0.0048) but not in BABIP (r=0.055, p=0.53) (Figures 2C-D). In EPI, we observed that participants who developed preeclampsia (n=6) showed lower levels of plasma GDF15 in late 2nd trimester (p=0.017, Supplemental Table 4). The sample size of participants with preeclampsia (n=2) was insufficient to investigate this question in BABIP. Unlike cf-mtDNA, GDF15 did not show any significant difference by maternal

gestational diabetes status in EPI or BABIP (Supplemental Table 4). Further, maternal plasma GDF15 levels did not differ between preterm and full-term pregnancies in EPI (n=10) or BABIP (n=8) (Supplemental Table 4), although results should be considered with caution given that the number of neonates in the preterm group was too low.

cf-mtDNA and GDF15 and maternal prenatal distress

In both studies, no significant associations were found between maternal plasma cf-mtDNA levels and perceived stress, anxiety or depressive symptoms in late 2nd and 3rd trimesters (Supplemental Table 1, for cf-mtDNA levels based on clinical cut-offs see Supplemental Table 5). Early 2nd trimester data was only available in EPI, and we found that higher depressive symptoms (Figure 3A [top], r=-0.56, p=0.032) and higher perceived stress (Figure 3B [top], r =-0.72, p=0.0031) were associated with lower plasma cf-mtDNA levels. In the early 2nd trimester, there was also a trend for a negative association between anxiety symptoms and plasma cf-mtDNA levels (Figure 3C [top], r=-0.51, p=0.055).

Similarly, at early 2nd trimester in EPI study, we found negative trends between maternal GDF15 plasma levels and depressive symptoms (Figure 3A [bottom], r =-0.44, p=0.086), anxiety symptoms (Figure 3B [bottom], r=-0.50, p 0.052), and perceived stress (Figure 3C [bottom], r=-0.35, p=0.18). In both studies, no significant associations were found between maternal GDF15 levels and perceived stress, anxiety or depressive symptoms in late 2nd and 3rd trimesters (Supplemental Table 1, for GDF15 levels based on clinical cut off see Supplemental Table 5), where the placenta-related release of GDF15 might dominate the signal.

Change in GDF15 levels and maternal characteristics during pregnancy

Given the significant changes in GDF15 levels throughout the perinatal period, we investigated whether maternal characteristics and prenatal distress could account for the individual differences in GDF15 trajectories from pregnancy to postpartum, using data from the EPI study which included postpartum time points (Supplemental Table 6). Interestingly, we found that higher pre-pregnancy BMI was associated with lower decline in GDF15 from 3rd trimester to post-partum (Figure 4A, r=0.41, p=0.044), which can be interpreted as an impaired return to pre-pregnancy baseline state. Similarly, higher perceived stress and depressive symptoms in the 3rd trimester were associated with lower decline in GDF15 from the 3rd trimester to post-partum (Figures 4B-C, r=0.43, p=0.042; r=0.59, p=0.004, respectively). Altogether, this suggests that higher prenatal metabolic stress and maternal distress may interact with post-pregnancy physiological recovery.

Discussion

Leveraging longitudinal studies from two countries (USA and Turkey), we investigated the trajectories of perinatal plasma cf-mtDNA and GDF15 levels and their associations with maternal characteristics, prenatal distress, and pregnancy outcomes. We report patterns of both cf-mtDNA and GDF15 across gestation periods and trimester-specific associations with maternal and neonatal characteristics, some of which were consistent across the two studies.

Considering the lack of consistent literature, we first examined how plasma cf-mtDNA and GDF15 changes across the pregnancy. The lack of change in plasma cf-mtDNA levels across pregnancy contrasts with a recent longitudinal study of healthy pregnancies (n=32) that found a 1.7-fold increase in serum cf-mtDNA from early pregnancy (5-8 weeks) to late pregnancy (33-36 weeks)(Cushen et al., 2020). This difference might stem from the different sampling intervals (i.e. having no early first trimester data) used in that study or the use of serum samples versus plasma (Michelson et al., 2023). Regarding GDF15, we observed a significant increase in plasma levels from early to late pregnancy in both studies, followed by an average 98.1% decrease (range across women: 93.4-99.8%) postpartum relative to 3rd trimester levels. Our findings are consistent with previous findings showing up to 100-fold increase in GDF15 levels comparing pregnant and non-pregnant populations (Klein et al., 2023; Moore et al., 2000), and with another study reporting a 200-fold increase in GDF15 levels in the 3rd trimester compared to post-partum (Andersson-Hall, Svedin, et al., 2021). The dramatic decrease from 3rd trimester to postpartum in our results also aligns with recent findings that show late pregnancy GDF15 levels were 172 times higher than those measured early postpartum(Høgh et al., 2024). Our study therefore adds to a robust body of literature demonstrating that GDF15 is not only a marker of aging (Lehallier et al., 2019; Tanaka et al., 2018) and mitochondrial and energetic stress (Fujita et al., 2015; Wang et al., 2021), but is also strongly associated with pregnancy course, rapidly returning to baseline after delivery.

In both studies, women with higher plasma GDF15 levels in the late 2nd trimester also tended to have elevated levels in the 3rd trimester, indicating moderate within-person stability. Plasma cf-mtDNA levels, on the other hand, showed within-person stability for EPI, but not BABIP, participants. No correlation was observed between cf-mtDNA and GDF15 levels, either measured within the same trimester or across different trimesters, suggesting that these two biomarkers may be at least partially regulated independently or influenced by individual-specific factors that have yet to be discovered.

Although GDF15 is strongly associated with aging (Lehallier et al., 2019; Tanaka et al., 2018), we found no link between maternal age and GDF15 levels during pregnancy. In contrast, the anticipated positive association was found during the postpartum period. The lack of GDF15's association with age during pregnancy might come from its role in pregnancy-specific metabolic and physiological adaptations (Crespi, 2024), such as pregnancy-induced insulin resistance (Andersson-Hall, Joelsson, et al., 2021) and placental invasion (Zeng et al., 2023). Interestingly, recent work suggests that epigenetic aging is accelerated during pregnancy and reversed during post-partum (Pham et al., 2024). Future work is needed to understand the interplay between plasma GDF15 levels and epigenetic aging trajectories during pregnancy.

Our results also link GDF15 to fetal development. Our finding showing that plasma GDF15 levels in mid pregnancy are negatively correlated with gestational age at birth in EPI highlights a potentially significant role of GDF15 in prenatal development and pregnancy outcomes. Interestingly, a recent study in preterm infants also found an inverse relationship between gestational age at birth and serum GDF15 levels at birth (Almudares et al., 2023). Taken together, elevated GDF15 levels in mid-pregnancy could indicate pregnancy-related energetic stress, potentially leading to earlier delivery.

In pregnant populations, previous studies found a negative correlation between GDF15 levels and BMI (Petry et al., 2018), and lower GDF15 increase in pregnancy in obese participants compared to normal weight participants (Andersson-Hall, Joelsson, et al., 2021; Hjort et al., 2023). In line with these findings, we found that pre-pregnancy BMI and plasma GDF15 levels were inversely correlated during pregnancy, particularly in mid-pregnancy. Since higher BMI is often associated with metabolic stress (Manna & Jain, 2015), the inverse relationship between BMI and GDF15 in pregnancy may result from increased metabolic stress impairing GDF15 upregulation in pregnancy necessary for energy mobilization and metabolic adaptations (Parrettini et al., 2020). Additionally, we also found that higher pre-pregnancy BMI is associated with a lower decrease in GDF15 levels from 3rd trimester to 4-14 months postpartum. This is in line with previous findings showing that the magnitude of the reversal of epigenetic aging observed from pregnancy to post-partum is lower in pregnant women with higher BMI (Pham et al., 2024). Taken together, these findings suggest that metabolic stress might increase the physiological load of pregnancy and alter post-pregnancy recovery.

A growing body of literature suggests that psychological stress and mood affect mitochondria biology (Boeck, Salinas-Manrique, et al., 2018; Gumpp et al., 2021), including cf-mtDNA (Trumpff et al., 2021) and GDF15 levels (Huang et al., 2024) in non-pregnant adults. In

this study, we found that higher perceived stress, depression, and anxiety symptoms are associated with lower cf-mtDNA levels in the early 2nd trimester of pregnancy. While some studies have found cf-mtDNA levels to be elevated in depression (Lindqvist et al., 2016), our findings are in line with a study showing cf-mtDNA levels to be lower in patients with major depressive disorder (Fernström et al., 2021). However, the time-specific effect of prenatal distress on cf-mtDNA should be interpreted cautiously due to the small sample size.

In parallel, we found that women with higher levels of depressive and anxiety symptoms during early 2nd trimester tended to have lower levels of plasma GDF15. This contrasts with previous studies indicating that GDF15 levels are elevated in psychopathological conditions in non-pregnant older adults (Mastrobattista et al., 2023). Early 2nd trimester is a critical period for hormonal and physiological changes during pregnancy that may be supported partly by a GDF15 increase (Newbern & Freemark, 2011; Soma-Pillay et al., 2016). Our results suggest that the presence of psychological distress might counteract this elevation early in pregnancy, resulting in the observed lower levels of these markers in distressed pregnant women. If normal placental development is associated with increasing GDF15 release (independent of stress), then pregnancy-rise of GDF15 may be interpreted as a marker of normal progress and healthy pregnancy. Our observation that perceived stress and depressive symptoms were linked to a blunted post-partum decrease in GDF15 suggests that prenatal distress may interfere with post-pregnancy physiological recovery, perhaps through mechanisms or sources of GDF15 other than the placenta. Taken together, these findings uncover a novel potential link between prenatal distress and biomarkers of mitochondrial health and call for future studies investigating this relationship in larger studies.

This study is not without limitations. As discussed in detail in the methods, we used the R&D ELISA kits that has been shown to underestimate GDF15 levels in individuals carrying the H202D variant of the GDF15 gene (Karusheva et al., 2022). This limitation of the kit could have affected the results comparing absolute GDF15 levels between participants, but not the findings related to within-person. Despite the repeated-measures design, EPI had a small sample size in the early 2nd trimester and postpartum. A key strength of this study is its unique cross-cultural approach. Although both studies are not directly comparable in terms of design and timepoints, the data from populations in the United States and Turkey reveals similar biological patterns across cultural contexts. This consistency enhances the robustness and broad relevance of mitochondrial markers like cf-mtDNA and GDF15 in relation to prenatal distress and pregnancy outcomes. However, this cross-cultural approach also raises challenges since the two studies

involved different populations, sampling times, and assessments for depression and anxiety symptoms, which may affect the comparability of these measures. Future research should aim for more standardized assessments across diverse populations to further validate such findings.

Conclusion

Using studies from two different countries, we describe distinct patterns in circulating plasma cf-mtDNA and GDF15 during pregnancy and their associations with maternal distress and pregnancy outcomes. Plasma cf-mtDNA levels showed no significant variation in either study, while GDF15 levels increased from early to late pregnancy and were negatively correlated with pre-pregnancy BMI in the late 2nd trimester. Higher perceived stress and depressive symptoms were linked to lower cf-mtDNA levels during early pregnancy, indicating a potential early impact of maternal distress on mitochondrial markers. Maternal distress also influenced GDF15 trajectories, suggesting an interaction with post-pregnancy recovery. While they remain to be confirmed by larger studies, our findings from studies in two culturally distinct countries underscore cf-mtDNA and GDF15 as potential biomarkers for mitochondrial and psychosocial stress during pregnancy that can shed light on the biological mechanisms connecting maternal distress to adverse pregnancy outcomes.

Methods

1. Participants

1.1. EPI (USA)

Healthy pregnant women (N=187, ages 20–45; Mage=29.64, SDage=6.24) were recruited as part of the "Prenatal stress: the epigenetic bases of maternal and perinatal effects" (EPI) study during the years 2011–2016 through the Department of Obstetrics and Gynecology at Columbia University Medical Center as described previously (Monk et al., 2016). Exclusion criteria were multiparity, medication use, and tobacco or recreational drug use. Participants provided written informed consent prior to participating in the study. Participants completed their first visit either in the early (13-18 weeks) or late 2nd trimester (19-30 weeks), depending on the time of recruitment, with subsequent visits occurring during the 3rd trimester (31-39 weeks) and postpartum (16-56 weeks). 13 participants completed their late 2nd trimester visit with gestational age greater than 28 weeks and were excluded from trimester-related analyses.

During the study visit, oral and written consents were obtained by trained graduate assistants. Afterwards, participants completed questionnaires and a blood sample was collected by the study phlebotomist (Figure 1A, left). All procedures were approved by the Institutional Review Board of the New York State Psychiatric Institute/Columbia University Medical Center and all methods were performed in accordance with relevant guidelines and regulations.

1.2. BABIP (Turkey)

Healthy pregnant women (N=198, ages 23-44; Mage=32.42, SDage=4.00) were recruited during the years 2018-2022 through doctors' offices, flyers and online advertisements from Istanbul, Turkey as part of the "Bogazici Mother Baby Relationship Project" (BABIP) birth cohort as described previously (Duman et al., 2020). Exclusion criteria were multiparity and severe pregnancy complications. During lab visits, participants provided oral and written consents, completed questionnaires, and had blood samples collected by nurses. All procedures were approved by the Institutional Review Board of Bogazici University, where the study was initiated. Participants completed the first visit during their late 2nd trimester (20-30 weeks) and the second visit during their 3rd trimester (27-38 weeks). 2 participants completed their first visit with gestational age greater than 28 weeks and were excluded from trimester-related analyses. Information about pregnancy outcomes, such as gestational age, neonatal sex and perinatal complications were collected at one month after birth via online questionnaires (Figure 1A, right).

2. Psychosocial assessment and blood collection

2.1. EPI (USA)

Prenatal distress in participants was evaluated at each visit using the Hamilton Depression Rating Scale (HAM-D)(Hamilton, 1960), the Hamilton Anxiety Rating Scale (HAM-A)(Hamilton, 1959), and the Perceived Stress Scale (PSS)(Cohen et al., 1983). Blood was collected at each visit by the study phlebotomist using EDTA coated tubes. Plasma was isolated immediately after collection by centrifugation and was stored at -80°C until further processing.

2.2. BABIP (Turkey)

Prenatal distress was evaluated at two prenatal visits during the late 2nd trimester and 3rd trimester using the Beck's Depression Inventory-II (BDI-II)(Beck et al., 1961), the Center for Epidemiological Studies Depression (CESD)(Radloff, 1977) , the State-Trait Anxiety Inventory-State (STAI-S)(Spielberger et al., 1983), and the Perceived Stress Scale (PSS)(Cohen et al., 1983). At each visit, blood samples were collected by nurses using EDTA coated tubes. Plasma

was isolated immediately after collection by centrifugation and aliquots were stored at -80°C until they were transferred on dry ice to Columbia University Irving Medical Center for analysis.

3. Maternal characteristics and pregnancy outcomes

3.1. EPI (USA)

Detailed information was collected during labor to comprehensively document neonatal and maternal outcomes. Recorded parameters included neonatal sex, gestational age at birth, and perinatal complications such as preeclampsia, preterm birth, and gestational diabetes.

3.2. BABIP (Turkey)

Participants provided detailed information about perinatal complications, such as preeclampsia, preterm birth, and gestational diabetes**,** during the two prenatal visits as well as in the 1-month postpartum assessment. Pregnancy outcomes, such as gestational age at birth and neonatal sex were also recorded in the postpartum assessment.

4. GDF15 assays

For both studies, plasma GDF15 levels were quantified using a high-sensitivity ELISA kit (R&D Systems, DGD150) following the manufacturer's instructions. Plasma samples were diluted with assay diluent (1:64 ratio for pregnancy samples, 1:4 ratio for postpartum samples) to maximize the number of samples within the dynamic range of the assay. Absorbance was gauged at 450nm, and concentrations were computed utilizing the Four Parameter Logistic Curve (4PL) model. Samples were run in duplicates on separate plates and the concentration for each sample was computed from the average of the duplicates. Samples with C.V.s larger than 15% were re-run. Samples with concentration above the dynamic range of the assay were rerun with 1:256 dilution with assay diluent. Standard curve (5 samples per plate) and plasma reference samples (3 samples per plate) were run with each individual assay and the inter-assay C.V. was monitored. All standard curves and references were overlaid on top of each other to monitor failed runs. Data-preprocessing and quality control measures was done using the R Software (version 4.2.2).

5. cf-mtDNA Assays

Mitochondrial and nuclear DNA in cell-free plasma were quantified using previously described methods (Michelson et al., 2023) with a few modifications. Briefly, plasma samples were thawed from storage at -80°C and centrifuged (5,000 x g, 10 minutes, 4 °C; Eppendorf 5427R with rotor FA-45-48-11; Eppendorf, Enfield, CT). Supernatants were transferred to 96-

well plates and stored at -80°C until analysis. After thawing plates, samples were thermolyzed overnight on replicate 96-well plates. Replicate lysates were analyzed in triplicates on 384-well plates using TaqMan chemistry-based real time quantitative polymerase chain reactions (qPCR) targeting mitochondrial gene ND1 and nuclear gene B2M. The medians of triplicate cycle threshold (C_T) values of samples were compared to those of serial dilutions of DNA standards to determine absolute copy numbers of target genes. Average PCR efficiencies for ND1 and B2M were 96.1% and 94.5%, respectively. The average coefficients of variation of natural log transformed ND1 and B2M copy number between replicates were 2.6% and 6.8%. Copy numbers were adjusted by plate-specific correction factors calculated from measurements of reference standards to correct for batch effects. Detailed information about these methods is available in the supplemental information.

6. Statistical analysis

Statistical analyses were conducted using GraphPad Prism (version 9.4.1) and R Software (version 4.2.2 and 4.3.0). The change in GDF15 between pairs of visits was calculated by subtracting the GDF15 levels measured during the former visit from those measured during the later visit. Non-parametric signed-rank Wilcoxon paired t-test was used to compare levels between visits. In EPI, due to the limited sample size (none of the participants had complete data for all 4 visits), comparisons were restricted to pairs of visits. Spearman rank correlations were used to assess continuous associations. Non-parametric Mann-Whitney t-test was used to assess group difference.

Acknowledgments

This work was supported by the NIMH grant R01 MH092580 C.M., the Wharton Fund to C.T., the Bogazici University Research Foundation Grant #11662 awarded to E.A.D.

Author contributions

C.T. and E.A.D. conceived and supervised this research project. C.M. designed the EPI study and supervised data collection. E.A.D. designed the BABIP study and supervised data collection. Q.H., S.A., and D.S. performed the GDF15 and cf-mtDNA assays. Q.H. performed statistical analyses and prepared the figures. C.T. and Q.H. drafted the manuscript. E.A.D., M.P.

441 and F.H. advised on manuscript and figure preparation. All authors reviewed, commented and

442 edited the final version of the manuscript.

Reference list

Almudares, F., Hagan, J., Chen, X., Devaraj, S., Moorthy, B., & Lingappan, K. (2023). Growth and differentiation factor 15 (GDF15) levels predict adverse respiratory outcomes in premature neonates. *Pediatr Pulmonol*, *58*(1), 271-278. https://doi.org/10.1002/ppul.26197

Andersson-Hall, U., Joelsson, L., Svedin, P., Mallard, C., & Holmang, A. (2021). Growth-differentiation-factor 15 levels in obese and healthy pregnancies: Relation to insulin resistance and insulin secretory function. *Clin Endocrinol (Oxf)*, *95*(1), 92-100. https://doi.org/10.1111/cen.14433

Andersson-Hall, U., Svedin, P., Mallard, C., Blennow, K., Zetterberg, H., & Holmang, A. (2021). Growth differentiation factor 15 increases in both cerebrospinal fluid and serum during pregnancy. *PLoS One*, *16*(5), e0248980. https://doi.org/10.1371/journal.pone.0248980

Beck, A. T., Erbaugh, J., Ward, C. H., Mock, J., & Mendelsohn, M. (1961). An Inventory for Measuring Depression. *Archives of General Psychiatry*, *4*(6), 561-+. https://doi.org/DOI 10.1001/archpsyc.1961.01710120031004

Bobba-Alves, N., Juster, R. P., & Picard, M. (2022). The energetic cost of allostasis and allostatic load. *Psychoneuroendocrinology*, *146*, 105951. https://doi.org/10.1016/j.psyneuen.2022.105951

Boeck, C., Gumpp, A. M., Calzia, E., Radermacher, P., Waller, C., Karabatsiakis, A., & Kolassa, I.-T. (2018). The association between cortisol, oxytocin and immune cell mitochondrial oxygen consumption in postpartum women with childhood maltreatment. *Psychoneuroendocrinology*.

Boeck, C., Salinas-Manrique, J., Calzia, E., Radermacher, P., von Arnim, C. A., Dietrich, D. E., Kolassa, I.-T., & Karabatsiakis, A. (2018). Targeting the association between telomere length and immuno-cellular bioenergetics in female patients with Major Depressive Disorder. *Scientific reports*, *8*(1), 9419.

Bradshaw, J. L., Cushen, S. C., Phillips, N. R., & Goulopoulou, S. (2022). Circulating Cell-Free Mitochondrial DNA in Pregnancy. *Physiology (Bethesda)*, *37*(4), 0. https://doi.org/10.1152/physiol.00037.2021

Butte, N. F., Hopkinson, J. M., Mehta, N., Moon, J. K., & Smith, E. O. (1999). Adjustments in energy expenditure and substrate utilization during late pregnancy and lactation. *Am J Clin Nutr*, *69*(2), 299-307. https://doi.org/10.1093/ajcn/69.2.299

Butte, N. F., Wong, W. W., Treuth, M. S., Ellis, K. J., & O'Brian Smith, E. (2004). Energy requirements during pregnancy based on total energy expenditure and energy deposition. *Am J Clin Nutr*, *79*(6), 1078-1087. https://doi.org/10.1093/ajcn/79.6.1078

Carithers, L. J., Ardlie, K., Barcus, M., Branton, P. A., Britton, A., Buia, S. A., Compton, C. C., DeLuca, D. S., Peter-Demchok, J., Gelfand, E. T., Guan, P., Korzeniewski, G. E., Lockhart, N. C., Rabiner, C. A., Rao, A. K., Robinson, K. L., Roche, N. V., Sawyer, S. J., Segre, A. V., Shive, C. E., Smith, A. M., Sobin, L. H., Undale, A. H., Valentino, K. M., Vaught, J., Young, T. R., Moore, H. M., & Consortium, G. T. (2015). A Novel Approach to High-Quality Postmortem Tissue Procurement: The GTEx Project. *Biopreserv Biobank*, *13*(5), 311-319. https://doi.org/10.1089/bio.2015.0032

Chen, Q., Wang, Y., Zhao, M., Hyett, J., da Silva Costa, F., & Nie, G. (2016). Serum levels of GDF15 are reduced in preeclampsia and the reduction is more profound in late-onset than early-onset cases. *Cytokine*, *83*, 226-230. https://doi.org/10.1016/j.cyto.2016.05.002

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A global measure of perceived stress. *J Health Soc Behav*, *24*(4), 385-396. https://www.ncbi.nlm.nih.gov/pubmed/6668417

Crespi, B. J. (2024). Nausea, vomiting and conflict in pregnancy: The adaptive significance of Growth-Differentiation Factor 15. *Evol Med Public Health*, *12*(1), 75-81. https://doi.org/10.1093/emph/eoae008

Cruickshank, T., MacDonald, T. M., Walker, S. P., Keenan, E., Dane, K., Middleton, A., Kyritsis, V., Myers, J., Cluver, C., Hastie, R., Bergman, L., Garcha, D., Cannon, P., Murray, E., Nguyen, T. V., Hiscock, R., Pritchard, N., Hannan, N. J., Tong, S., & Kaitu'u-Lino, T. J. (2021). Circulating Growth Differentiation Factor 15 Is Increased Preceding Preeclampsia Diagnosis: Implications as a Disease Biomarker. *J Am Heart Assoc*, *10*(16), e020302. https://doi.org/10.1161/JAHA.120.020302

Cushen, S. C., Sprouse, M. L., Blessing, A., Sun, J., Jarvis, S. S., Okada, Y., Fu, Q., Romero, S. A., Phillips, N. R., & Goulopoulou, S. (2020). Cell-free mitochondrial DNA increases in

maternal circulation during healthy pregnancy: a prospective, longitudinal study. *Am J Physiol Regul Integr Comp Physiol*, *318*(2), R445-R452. https://doi.org/10.1152/ajpregu.00324.2019

Dennhardt, S., Ceanga, I. A., Baumbach, P., Amiratashani, M., Kroller, S., & Coldewey, S. M. (2024). Cell-free DNA in patients with sepsis: long term trajectory and association with 28-day mortality and sepsis-associated acute kidney injury. *Front Immunol*, *15*, 1382003. https://doi.org/10.3389/fimmu.2024.1382003

Duman, E. A., Atesyakar, N., & Ecevitoglu, A. (2020). Multilevel Impact of Prenatal Risk and Protective Factors on Stress Biology and Infant Development: Study protocol of BABIP prospective birth cohort from Turkey. *Brain Behav Immun Health*, *1*, 100005. https://doi.org/10.1016/j.bbih.2019.100005

Duvvuri, B., & Lood, C. (2019). Cell-Free DNA as a Biomarker in Autoimmune Rheumatic Diseases. *Front Immunol*, *10*, 502. https://doi.org/10.3389/fimmu.2019.00502

Engström Ruud, L., Font-Gironès, F., Zajdel, J., Kern, L., Teixidor-Deulofeu, J., Mannerås-Holm, L., Carreras, A., Becattini, B., Björefeldt, A., Hanse, E., Fenselau, H., Solinas, G., Brüning, J. C., Wunderlich, T. F., Bäckhed, F., & Ruud, J. (2024). Activation of GFRAL^{+} neurons induces hypothermia and glucoregulatory responses associated with nausea and torpor. *Cell Reports*, *43*(4). https://doi.org/10.1016/j.celrep.2024.113960

Farge, G., & Falkenberg, M. (2019). Organization of DNA in mammalian mitochondria. *International journal of molecular sciences*, *20*(11), 2770.

Fejzo, M., Rocha, N., Cimino, I., Lockhart, S. M., Petry, C. J., Kay, R. G., Burling, K., Barker, P., George, A. L., Yasara, N., Premawardhena, A., Gong, S., Cook, E., Rimmington, D., Rainbow, K., Withers, D. J., Cortessis, V., Mullin, P. M., MacGibbon, K. W., Jin, E., Kam, A., Campbell, A., Polasek, O., Tzoneva, G., Gribble, F. M., Yeo, G. S. H., Lam, B. Y. H., Saudek, V., Hughes, I. A., Ong, K. K., Perry, J. R. B., Sutton Cole, A., Baumgarten, M., Welsh, P., Sattar, N., Smith, G. C. S., Charnock-Jones, D. S., Coll, A. P., Meek, C. L., Mettananda, S., Hayward, C., Mancuso, N., & O'Rahilly, S. (2024). GDF15 linked to maternal risk of nausea and vomiting during pregnancy. *Nature*, *625*(7996), 760-767. https://doi.org/10.1038/s41586-023-06921-9

Fernström, J., Ohlsson, L., Asp, M., Lavant, E., Holck, A., Grudet, C., Westrin, Å., & Lindqvist, D. (2021). Plasma circulating cell-free mitochondrial DNA in depressive disorders. *PloS one*, *16*(11), e0259591.

Forsum, E., Kabir, N., Sadurskis, A., & Westerterp, K. (1992). Total energy expenditure of healthy Swedish women during pregnancy and lactation. *Am J Clin Nutr*, *56*(2), 334-342. https://doi.org/10.1093/ajcn/56.2.334

Frye, M. A., Nassan, M., Jenkins, G. D., Kung, S., Veldic, M., Palmer, B. A., Feeder, S. E., Tye, S. J., Choi, D. S., & Biernacka, J. M. (2015). Feasibility of investigating differential proteomic expression in depression: implications for biomarker development in mood disorders. *Transl Psychiatry*, *5*(12), e689. https://doi.org/10.1038/tp.2015.185

Fujita, Y., Ito, M., Kojima, T., Yatsuga, S., Koga, Y., & Tanaka, M. (2015). GDF15 is a novel biomarker to evaluate efficacy of pyruvate therapy for mitochondrial diseases. *Mitochondrion*, *20*, 34-42. https://doi.org/10.1016/j.mito.2014.10.006

Gumpp, A. M., Behnke, A., Bach, A. M., Piller, S., Boeck, C., Rojas, R., & Kolassa, I. T. (2021). Mitochondrial bioenergetics in leukocytes and oxidative stress in blood serum of mild to moderately depressed women. *Mitochondrion*, *58*, 14-23. https://doi.org/10.1016/j.mito.2020.12.009

Gumpp, A. M., Boeck, C., Behnke, A., Bach, A. M., Ramo-Fernández, L., Welz, T., Gündel, H., Kolassa, I.-T., & Karabatsiakis, A. (2020). Childhood maltreatment is associated with changes in mitochondrial bioenergetics in maternal, but not in neonatal immune cells. *Proceedings of the National Academy of Sciences*, *117*(40), 24778-24784.

Hamilton, M. (1959). The assessment of anxiety states by rating. *Br J Med Psychol*, *32*(1), 50-55. https://doi.org/10.1111/j.2044-8341.1959.tb00467.x

Hamilton, M. (1960). A Rating Scale for Depression. *Journal of Neurology Neurosurgery and Psychiatry*, *23*(1), 56-62. https://doi.org/DOI 10.1136/jnnp.23.1.56

Hjort, L., Wewer Albrechtsen, N. J., Minja, D., Rasmussen, C., Moller, S. L., Lusingu, J., Theander, T., Bygbjerg, I. C., Schmiegelow, C., & Grunnet, L. G. (2023). Cord Blood FGF-21 and GDF-15 Levels Are Affected by Maternal Exposure to Moderate to Severe

559 Anemia and Malaria. *J Endocr Soc*, *7*(10), bvad120.
560 https://doi.org/10.1210/jendso/bvad120

561 Hoffman, M. C., Mazzoni, S. E., Wagner, B. D., Laudenslager, M. L., & Ross, R. G. (2016).
562 Measures of maternal stress and mood in relation to preterm birth. *Obstetrics &*
563 *Gynecology*, *127*(3), 545-552.

564 Høgh, S., Borgsted, C., Hegaard, H. K., Renault, K. M., Ekelund, K., Bruzzone, S. E. P.,
565 Clemmensen, C., Klein, A. B., & Frokjaer, V. G. (2024). Growth Differentiation Factor 15
566 during pregnancy and postpartum as captured in blood, cerebrospinal fluid and placenta:
567 a cohort study on associations with maternal mental health. *Psychoneuroendocrinology*.
568 https://doi.org/10.1016/j.psyneuen.2024.107212

569 Hovhannisyan, G., Harutyunyan, T., Aroutiounian, R., & Liehr, T. (2023). The Diagnostic,
570 Prognostic, and Therapeutic Potential of Cell-Free DNA with a Special Focus on COVID-
571 19 and Other Viral Infections. *Int J Mol Sci*, *24*(18).
572 https://doi.org/10.3390/ijms241814163

573 Hroudová, J., Fišar, Z., Kitzlerová, E., Zvěřová, M., & Raboch, J. (2013). Mitochondrial
574 respiration in blood platelets of depressive patients. *Mitochondrion*, *13*(6), 795-800.

575 Hu, M. M., & Shu, H. B. (2023). Mitochondrial DNA-triggered innate immune response:
576 mechanisms and diseases. *Cell Mol Immunol*, *20*(12), 1403-1412.
577 https://doi.org/10.1038/s41423-023-01086-x

578 Huang, Q., Trumpff, C., Monzel, A. S., Rausser, S., Haahr, R., Devine, J., Liu, C. C., Kelly, C.,
579 Thompson, E., & Kurade, M. (2024). Psychobiological regulation of plasma and saliva
580 GDF15 dynamics in health and mitochondrial diseases. *bioRxiv*.

581 Hummel, E. M., Hessas, E., Muller, S., Beiter, T., Fisch, M., Eibl, A., Wolf, O. T., Giebel, B.,
582 Platen, P., Kumsta, R., & Moser, D. A. (2018). Cell-free DNA release under psychosocial
583 and physical stress conditions. *Transl Psychiatry*, *8*(1), 236.
584 https://doi.org/10.1038/s41398-018-0264-x

585 Karabatsiakis, A., Bock, C., Salinas-Manrique, J., Kolassa, S., Calzia, E., Dietrich, D. E., &
586 Kolassa, I. T. (2014). Mitochondrial respiration in peripheral blood mononuclear cells

correlates with depressive subsymptoms and severity of major depression. *Transl Psychiatry*, *4*, e397. http://www.ncbi.nlm.nih.gov/pubmed/26126180

Karusheva, Y., Ratcliff, M., Morseburg, A., Barker, P., Melvin, A., Sattar, N., Burling, K., Backmark, A., Roth, R., Jermutus, L., Guiu-Jurado, E., Bluher, M., Welsh, P., Hyvonen, M., & O'Rahilly, S. (2022). The Common H202D Variant in GDF-15 Does Not Affect Its Bioactivity but Can Significantly Interfere with Measurement of Its Circulating Levels. *J Appl Lab Med*, *7*(6), 1388-1400. https://doi.org/10.1093/jalm/jfac055

Klein, A. B., Ranea-Robles, P., Nicolaisen, T. S., Gil, C., Johann, K., Quesada, J. P., Pistolevij, N., Hviid, K. V. R., Fich, L., Offersen, S. M., Helge, J. W., Nielsen, H. S., Bakker, J., Kleinert, M., & Clemmensen, C. (2023). Cross-species comparison of pregnancy-induced GDF15. *Am J Physiol Endocrinol Metab*, *325*(4), E303-E309. https://doi.org/10.1152/ajpendo.00134.2023

Kung, C. T., Hsiao, S. Y., Tsai, T. C., Su, C. M., Chang, W. N., Huang, C. R., Wang, H. C., Lin, W. C., Chang, H. W., Lin, Y. J., Cheng, B. C., Su, B. Y., Tsai, N. W., & Lu, C. H. (2012). Plasma nuclear and mitochondrial DNA levels as predictors of outcome in severe sepsis patients in the emergency room. *J Transl Med*, *10*, 130. https://doi.org/10.1186/1479-5876-10-130

Kurki, T., Hiilesmaa, V., Raitasalo, R., Mattila, H., & Ylikorkala, O. (2000). Depression and anxiety in early pregnancy and risk for preeclampsia. *Obstet Gynecol*, *95*(4), 487-490. https://doi.org/10.1016/s0029-7844(99)00602-x

Lee, S. R., & Han, J. (2017). Mitochondrial nucleoid: shield and switch of the mitochondrial genome. *Oxidative Medicine and Cellular Longevity*, *2017*.

Lehallier, B., Gate, D., Schaum, N., Nanasi, T., Lee, S. E., Yousef, H., Moran Losada, P., Berdnik, D., Keller, A., Verghese, J., Sathyan, S., Franceschi, C., Milman, S., Barzilai, N., & Wyss-Coray, T. (2019). Undulating changes in human plasma proteome profiles across the lifespan. *Nat Med*, *25*(12), 1843-1850. https://doi.org/10.1038/s41591-019-0673-2

Lima, S. A. M., El Dib, R. P., Rodrigues, M. R. K., Ferraz, G. A. R., Molina, A. C., Neto, C. A. P., De Lima, M. A. F., & Rudge, M. V. C. (2018). Is the risk of low birth weight or preterm

labor greater when maternal stress is experienced during pregnancy? A systematic review and meta-analysis of cohort studies. *PLoS One*, *13*(7), e0200594.

Lindqvist, D., Fernström, J., Grudet, C., Ljunggren, L., Träskman-Bendz, L., Ohlsson, L., & Westrin, Å. (2016). Increased plasma levels of circulating cell-free mitochondrial DNA in suicide attempters: associations with HPA-axis hyperactivity. *Translational psychiatry*, *6*(12), e971.

Lindqvist, D., Wolkowitz, O. M., Picard, M., Ohlsson, L., Bersani, F. S., Fernström, J., Westrin, Å., Hough, C. M., Lin, J., & Reus, V. I. (2018). Circulating cell-free mitochondrial DNA, but not leukocyte mitochondrial DNA copy number, is elevated in major depressive disorder. *Neuropsychopharmacology*, *43*(7), 1557-1564.

Lockhart, S. M., Saudek, V., & O'Rahilly, S. (2020). GDF15: A Hormone Conveying Somatic Distress to the Brain. *Endocr Rev*, *41*(4). https://doi.org/10.1210/endrev/bnaa007

Manna, P., & Jain, S. K. (2015). Obesity, Oxidative Stress, Adipose Tissue Dysfunction, and the Associated Health Risks: Causes and Therapeutic Strategies. *Metab Syndr Relat Disord*, *13*(10), 423-444. https://doi.org/10.1089/met.2015.0095

Marschalek, J., Wohlrab, P., Ott, J., Wojta, J., Speidl, W., Klein, K. U., Kiss, H., Pateisky, P., Zeisler, H., & Kuessel, L. (2018). Maternal serum mitochondrial DNA (mtDNA) levels are elevated in preeclampsia - A matched case-control study. *Pregnancy Hypertens*, *14*, 195-199. https://doi.org/10.1016/j.preghy.2018.10.003

Mastrobattista, E., Lenze, E. J., Reynolds, C. F., Mulsant, B. H., Wetherell, J., Wu, G. F., Blumberger, D. M., Karp, J. F., Butters, M. A., Mendes-Silva, A. P., Vieira, E. L., Tseng, G., & Diniz, B. S. (2023). Late-Life Depression is Associated With Increased Levels of GDF-15, a Pro-Aging Mitokine. *Am J Geriatr Psychiatry*, *31*(1), 1-9. https://doi.org/10.1016/j.jagp.2022.08.003

McElwain, C., & McCarthy, C. M. (2020). Investigating mitochondrial dysfunction in gestational diabetes mellitus and elucidating if BMI is a causative mediator. *Eur J Obstet Gynecol Reprod Biol*, *251*, 60-65. https://doi.org/10.1016/j.ejogrb.2020.04.037

643 Michelson, J., Rausser, S., Peng, A., Yu, T., Sturm, G., Trumpff, C., Kaufman, B. A., Rai, A. J., &
644 Picard, M. (2023). MitoQuicLy: a high-throughput method for quantifying cell-free DNA
645 from human plasma, serum, and saliva. *Mitochondrion*, *71*, 26-39.

646 Mondelo-Macia, P., Castro-Santos, P., Castillo-Garcia, A., Muinelo-Romay, L., & Diaz-Pena, R.
647 (2021). Circulating Free DNA and Its Emerging Role in Autoimmune Diseases. *J Pers*
648 *Med*, *11*(2). https://doi.org/10.3390/jpm11020151

649 Monk, C., Feng, T., Lee, S., Krupska, I., Champagne, F. A., & Tycko, B. (2016). Distress During
650 Pregnancy: Epigenetic Regulation of Placenta Glucocorticoid-Related Genes and Fetal
651 Neurobehavior. *Am J Psychiatry*, *173*(7), 705-713.
652 https://doi.org/10.1176/appi.ajp.2015.15091171

653 Moore, A. G., Brown, D. A., Fairlie, W. D., Bauskin, A. R., Brown, P. K., Munier, M. L., Russell, P.
654 K., Salamonsen, L. A., Wallace, E. M., & Breit, S. N. (2000). The transforming growth
655 factor-ss superfamily cytokine macrophage inhibitory cytokine-1 is present in high
656 concentrations in the serum of pregnant women. *J Clin Endocrinol Metab*, *85*(12), 4781-
657 4788. https://doi.org/10.1210/jcem.85.12.7007

658 Nelson, D. B., Grisso, J. A., Joffe, M. M., Brensinger, C., Shaw, L., & Datner, E. (2003). Does
659 stress influence early pregnancy loss? *Ann Epidemiol*, *13*(4), 223-229.
660 https://doi.org/10.1016/s1047-2797(02)00419-2

661 Newbern, D., & Freemark, M. (2011). Placental hormones and the control of maternal
662 metabolism and fetal growth. *Curr Opin Endocrinol Diabetes Obes*, *18*(6), 409-416.
663 https://doi.org/10.1097/MED.0b013e32834c800d

664 Pan, L. A., Naviaux, J. C., Wang, L., Li, K., Monk, J. M., Lingampelly, S. S., Segreti, A. M.,
665 Bloom, K., Vockley, J., Tarnopolsky, M. A., Finegold, D. N., Peters, D. G., & Naviaux, R.
666 K. (2023). Metabolic features of treatment-refractory major depressive disorder with
667 suicidal ideation. *Transl Psychiatry*, *13*(1), 393. https://doi.org/10.1038/s41398-023-
668 02696-9

669 Parrettini, S., Caroli, A., & Torlone, E. (2020). Nutrition and Metabolic Adaptations in
670 Physiological and Complicated Pregnancy: Focus on Obesity and Gestational Diabetes.
671 *Front Endocrinol (Lausanne)*, *11*, 611929. https://doi.org/10.3389/fendo.2020.611929

672 Petry, C. J., Ong, K. K., Burling, K. A., Barker, P., Goodburn, S. F., Perry, J. R. B., Acerini, C. L.,
673 Hughes, I. A., Painter, R. C., Afink, G. B., Dunger, D. B., & O'Rahilly, S. (2018).
674 Associations of vomiting and antiemetic use in pregnancy with levels of circulating
675 GDF15 early in the second trimester: A nested case-control study. *Wellcome Open Res*,
676 *3*, 123. https://doi.org/10.12688/wellcomeopenres.14818.1

677 Pham, H., Thompson-Felix, T., Czamara, D., Rasmussen, J. M., Lombroso, A., Entringer, S.,
678 Binder, E. B., Wadhwa, P. D., Buss, C., & ODonnell, K. J. (2024). The effects of
679 pregnancy, its progression, and its cessation on human (maternal) biological aging. *Cell*
680 *Metabolism*, *36*(5), 877-878. https://doi.org/10.1016/j.cmet.2024.02.016

681 Picard, M., & McEwen, B. S. (2018). Psychological Stress and Mitochondria: A Conceptual
682 Framework. *Psychosom Med*, *80*(2), 126-140.
683 https://doi.org/10.1097/PSY.0000000000000544

684 Picard, M., Prather, A. A., Puterman, E., Cuillerier, A., Coccia, M., Aschbacher, K., Burelle, Y., &
685 Epel, E. S. (2018). A mitochondrial health index sensitive to mood and caregiving stress.
686 *Biological psychiatry*.

687 Picard, M., Trumpff, C., & Burelle, Y. (2019). Mitochondrial Psychobiology: Foundations and
688 Applications. *Curr Opin Behav Sci*, *28*, 142-151.
689 https://doi.org/10.1016/j.cobeha.2019.04.015

690 Qu, F., Wu, Y., Zhu, Y. H., Barry, J., Ding, T., Baio, G., Muscat, R., Todd, B. K., Wang, F. F., &
691 Hardiman, P. J. (2017). The association between psychological stress and miscarriage: A
692 systematic review and meta-analysis. *Sci Rep*, *7*(1), 1731.
693 https://doi.org/10.1038/s41598-017-01792-3

694 Radloff, L. S. (1977). The CES-D scale: A self-report depression scale for research in the
695 general population. *Applied psychological measurement*, *1*(3), 385-401.

696 Savard, C., Lebrun, A., O'Connor, S., Fontaine-Bisson, B., Haman, F., & Morisset, A. S. (2021).
697 Energy expenditure during pregnancy: a systematic review. *Nutr Rev*, *79*(4), 394-409.
698 https://doi.org/10.1093/nutrit/nuaa093

699 Schwarzenbach, H., Hoon, D. S., & Pantel, K. (2011). Cell-free nucleic acids as biomarkers in
700 cancer patients. *Nat Rev Cancer*, *11*(6), 426-437. https://doi.org/10.1038/nrc3066

Shamsi, U., Hatcher, J., Shamsi, A., Zuberi, N., Qadri, Z., & Saleem, S. (2010). A multicentre matched case control study of risk factors for preeclampsia in healthy women in Pakistan. *BMC Womens Health*, *10*, 14. https://doi.org/10.1186/1472-6874-10-14

Soma-Pillay, P., Nelson-Piercy, C., Tolppanen, H., & Mebazaa, A. (2016). Physiological changes in pregnancy. *Cardiovasc J Afr*, *27*(2), 89-94. https://doi.org/10.5830/CVJA-2016-021

Spielberger, C., Gorsuch, R., Lushene, R., Vagg, P. R., & Jacobs, G. (1983). *Manual for the State-Trait Anxiety Inventory (Form Y1 – Y2)* (Vol. IV).

Sugulle, M., Dechend, R., Herse, F., Weedon-Fekjaer, M. S., Johnsen, G. M., Brosnihan, K. B., Anton, L., Luft, F. C., Wollert, K. C., Kempf, T., & Staff, A. C. (2009). Circulating and placental growth-differentiation factor 15 in preeclampsia and in pregnancy complicated by diabetes mellitus. *Hypertension*, *54*(1), 106-112. https://doi.org/10.1161/HYPERTENSIONAHA.109.130583

Tanaka, T., Biancotto, A., Moaddel, R., Moore, A. Z., Gonzalez-Freire, M., Aon, M. A., Candia, J., Zhang, P., Cheung, F., Fantoni, G., consortium, C. H. I., Semba, R. D., & Ferrucci, L. (2018). Plasma proteomic signature of age in healthy humans. *Aging Cell*, *17*(5), e12799. https://doi.org/10.1111/acel.12799

Trumpff, C., Marsland, A. L., Basualto-Alarcon, C., Martin, J. L., Carroll, J. E., Sturm, G., Vincent, A. E., Mosharov, E. V., Gu, Z., Kaufman, B. A., & Picard, M. (2019). Acute psychological stress increases serum circulating cell-free mitochondrial DNA. *Psychoneuroendocrinology*, *106*, 268-276. https://doi.org/10.1016/j.psyneuen.2019.03.026

Trumpff, C., Michelson, J., Lagranha, C. J., Taleon, V., Karan, K. R., Sturm, G., Lindqvist, D., Fernstrom, J., Moser, D., Kaufman, B. A., & Picard, M. (2021). Stress and circulating cell-free mitochondrial DNA: A systematic review of human studies, physiological considerations, and technical recommendations. *Mitochondrion*, *59*, 225-245. https://doi.org/10.1016/j.mito.2021.04.002

Trumpff, C., Monzel, A., Sandi, C., Menon, V., Klein, H.-U., Fujita, M., Lee, A. J., Petyuk, V. A., Hurst, C., Duong, D. A., Seyfried, N., Wingo, A., Wingo, T. S., Wang, Y., Thambisetty, M., Ferrucci, L., Bennett, D. A., Jager, P. D., & Picard, M. (2023). Psychosocial experiences

are associated with human brain mitochondrial biology. *bioRxiv*, 2023.2010.2006.559575. https://doi.org/10.1101/2023.10.06.559575

Wang, D., Day, E. A., Townsend, L. K., Djordjevic, D., Jorgensen, S. B., & Steinberg, G. R. (2021). GDF15: emerging biology and therapeutic applications for obesity and cardiometabolic disease. *Nat Rev Endocrinol*, *17*(10), 592-607. https://doi.org/10.1038/s41574-021-00529-7

Worth, A. A., Shoop, R., Tye, K., Feetham, C. H., D'Agostino, G., Dodd, G. T., Reimann, F., Gribble, F. M., Beebe, E. C., Dunbar, J. D., Alexander-Chacko, J. T., Sindelar, D. K., Coskun, T., Emmerson, P. J., & Luckman, S. M. (2020). The cytokine GDF15 signals through a population of brainstem cholecystokinin neurons to mediate anorectic signalling. *Elife*, *9*. https://doi.org/10.7554/eLife.55164

Yamanouchi, S., Kudo, D., Yamada, M., Miyagawa, N., Furukawa, H., & Kushimoto, S. (2013). Plasma mitochondrial DNA levels in patients with trauma and severe sepsis: time course and the association with clinical status. *J Crit Care*, *28*(6), 1027-1031. https://doi.org/10.1016/j.jcrc.2013.05.006

Zeng, Y. T., Liu, W. F., Zheng, P. S., & Li, S. (2023). GDF15 deficiency hinders human trophoblast invasion to mediate pregnancy loss through downregulating Smad1/5 phosphorylation. *iScience*, *26*(10), 107902. https://doi.org/10.1016/j.isci.2023.107902

Zvěřová, M., Hroudová, J., Fišar, Z., Hansíková, H., Kališová, L., Kitzlerová, E., Lambertová, A., & Raboch, J. (2019). Disturbances of mitochondrial parameters to distinguish patients with depressive episode of bipolar disorder and major depressive disorder. *Neuropsychiatric disease and treatment*, 233-240.

Table 1: Participants characteristics of women in EPI (US) and BABIP (Turkey) cohorts

	EPI	BABIP	p
Number of Participants	187	198	
Age* (years)	29.6 (6.2)	32.4 (4.0)	<0.0001†
Race‡, No. (%)			
American Indian or Alaskan Native	28 (15.0)		

Asian Indian	57 (30.5)		
Black or African American	3 (1.6)		
Japanese	9 (4.8)		
Guamanian or Chamorro	17 (9.1)		
Hispanic or Latino	58 (31.0)		
White	73 (39.0)		
Turkish[§]		198 (100.0)	
Yearly household income[‖], No. (%)			
< $15,000	27 (14.4)		
$16,000 – $25,000	36 (19.3)		
$26,000 – $50,000	41 (21.9)		
$51,000 – $100,000	47 (25.1)		
$101,000 – $250,000	29 (15.5)		
> $250,000	7 (3.7)		
< □100,000		41 (20.7)	
□100,000 – □150,000		42 (21.2)	
□151,000 – □200,000		32 (16.2)	
□201,000 – □250,000		21 (10.6)	
□251,000 – □300,000		17 (8.6)	
> □301,000		28 (14.1)	
N/A [¶]		17 (8.6)	
Education (years) [*]	14.9 (3.1)	16.6 (2.2)	<0.0001[†]
Pre-pregnancy self-reported weight (pounds) [*]	153.4 (36.4)	137.3 (26.5)	<0.0001[†]
Pre-pregnancy Body Mass Index [*]	26.1 (5.8)	23.1 (4.1)	<0.0001[†]
Perinatal Distress, averaged across all visits [*]			
Hamilton Depression Rating Scale (HAM-D)	6.7 (5.5)		
Hamilton Anxiety Rating Scale (HAM-A)	7.1 (5.8)		
Perceived Stress Scale (PSS)	21.8 (7.6)	14.4 (5.8)	<0.0001[†]
Beck's Depression Inventory-II (BDI-II)		8.4 (6.5)	
State-Trait Anxiety Inventory-State (STAI-S)		45.8 (1.9)	
Center for Epidemiological Studies Depression (CESD)		11.8 (8.9)	

754 * Data shown as mean and standard deviation (SD).

[†] P-value from Welch's t-test.

[‡] Participants may report more than one race.

[§] All participants in the BABIP study self-identified as Turkish, a demographic that generally aligns with the "White" category but does not directly correspond to the "White" classification used in the United States.

[‖] $ for US dollars, □ for Turkish lira.

[¶] N/A codes for missing data

Age: 19-45 (Mean=29.64, SD=6.24)

Age: 23-44 (Mean=32.42, SD=4.00)

A

B

C

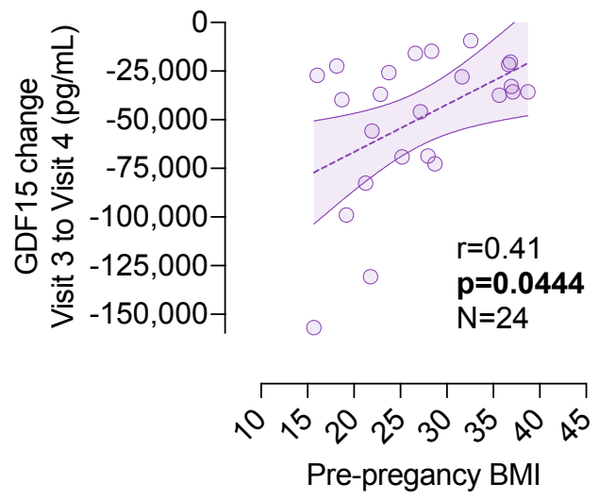
r=0.17
p=0.49
N=20

D

Figure 2. Association between maternal plasma cf-mtDNA and GDF15 levels and gestational age at birth. Associations between gestational age at birth and cf-mtDNA at each visit in EPI (**A**) and BABIP (**B**). Associations between gestational age at birth and GDF15 at each visit in (**C**) EPI and (**D**) BABIP. 13 EPI participants and 2 BABIP participants were removed from the analysis and graphs since they completed their late 2nd trimester visit in their 3rd trimester. See Supplemental Figure S4 for cf-mtDNA and GDF15 data shown in raw values. P-values and effect sizes from Spearman's rank correlation. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure 3. Associations between maternal plasma cf-mtDNA and GDF15 levels and maternal psychological distress in early pregnancy (available only in EPI study). Association between plasma cf-mtDNA (top) and GDF15 (bottom) levels in the early 2nd trimester (13-18 weeks) and (**A**) depressive symptoms assessed by the Hamilton depression rating scale (HAM-D), (**B**) psychological stress assessed by perceived stress scale (PSS), and (**C**) anxiety symptoms assessed by the Hamilton anxiety rating scale (HAM-A) in EPI. P-values and effect sizes from Spearman's rank correlation. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

A



B

r=0.43
p=0.0420
N=23

C

r=0.59
p=0.0040
N=22

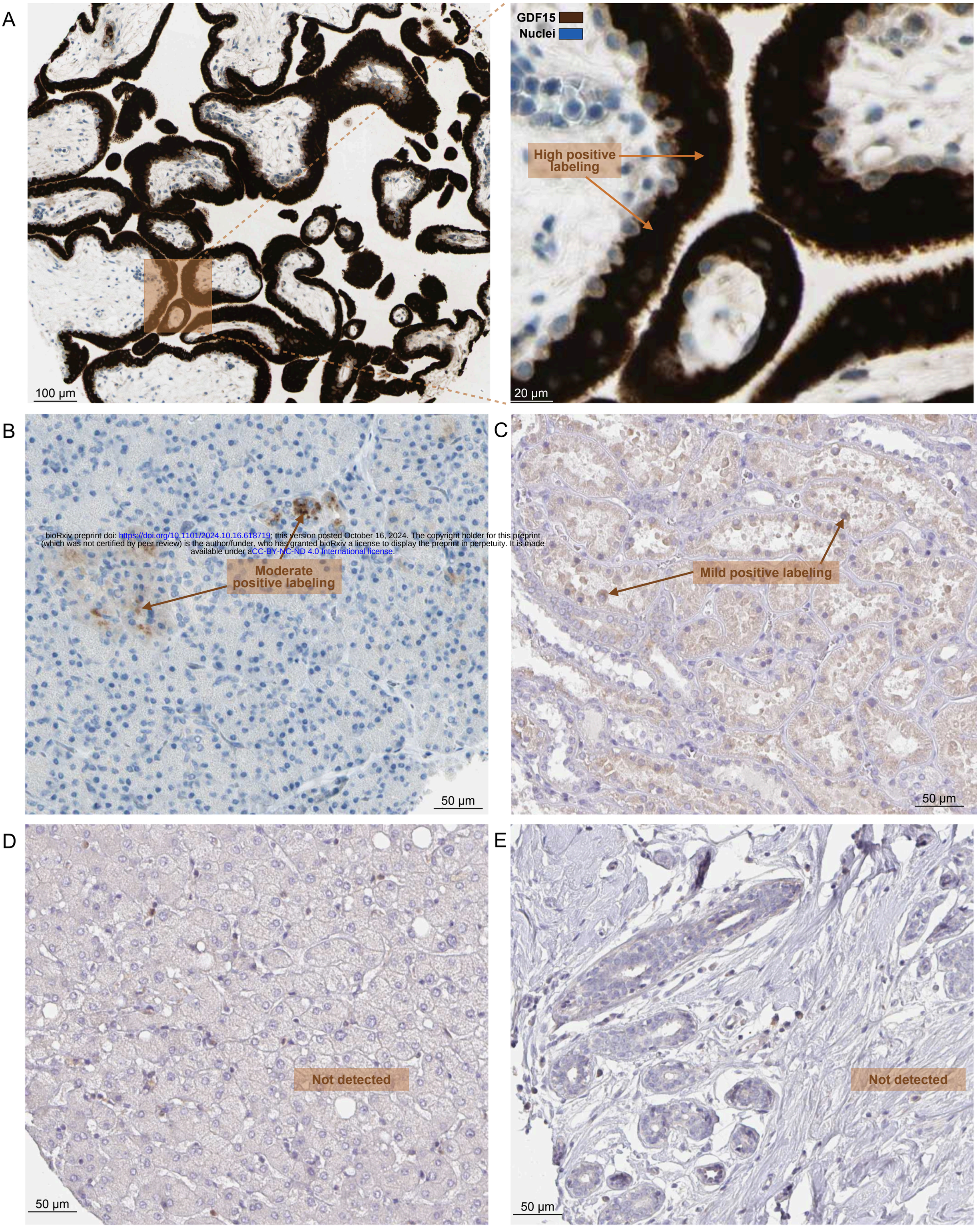


Figure S1. GDF15 protein immunohistochemistry in different tissues. Human Protein Atlas (HPA) immunohistochemistry of GDF15 protein in the (**A**) placenta, (**B**) pancreas, (**C**) kidney, and (**D**) liver, (**E**) breast. Subject IDs are #2169 (19-year-old, female) for A, #2032 (35-year-old, female) for B, #2530 (41-year-old, female) for C, #3402 (54-year-old, female) for D, #3856 (27-year-old, female) for E based on sample availability.

Figure S2. Association between maternal cf-mtDNA and GDF15 plasma levels across study timepoints. Association between levels measured at different visits for cf-mtDNA (left) and GDF15 (right) from (**A**) EPI and (**B**) BABIP. Association between cf-mtDNA and GDF15 levels measured in the same visits from (**C**) EPI and (**D**) BABIP. Association between GDF15 and cf-mtDNA levels measured at different visits from (**E**) EPI and (**F**) BABIP. P-values and effect sizes from Spearman's rank correlation. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure S3. Associations between GDF15 levels and maternal characteristics. Associations between maternal age and GDF15 levels at postpartum (**A,** EPI) and late-pregnancy (**B,** BABIP). Associations between pre-pregnancy BMI and GDF15 levels at mid- and late-pregnancy from EPI (**C**) and BABIP (**D**), 13 EPI participants and 2 BABIP participants were removed from the analysis and graphs since they completed their late 2nd trimester visit in their 3rd trimester. P-values and effect sizes from Spearman's rank correlation. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

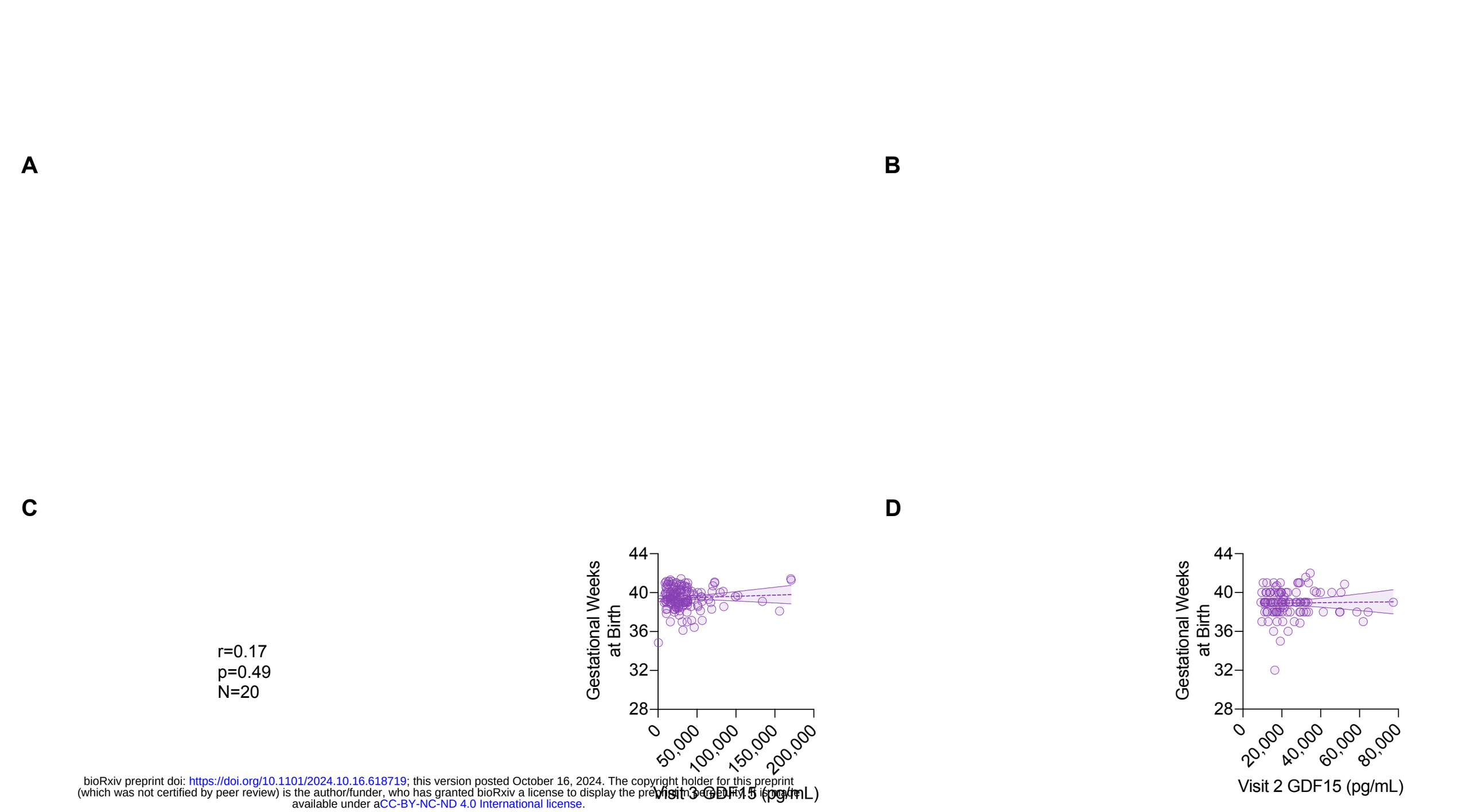


Figure S4. Association between maternal plasma cf-mtDNA and GDF15 levels and gestational age at birth (raw values). Associations between gestational age at birth and cf-mtDNA at each visit in (**A**) EPI and (**B**) BABIP. Associations between gestational age at birth and GDF15 at each visit in (**C**) EPI and (**D**) BABIP. 13 EPI participants and 2 BABIP participants were removed from the analysis and graphs since they completed their late 2nd trimester visit in their 3rd trimester. P-values and effect sizes from Spearman's rank correlation. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.