UC Irvine
UC Irvine Previously Published Works

Title

Prospective association between maternal allostatic load during pregnancy and child mitochondrial content and bioenergetic capacity.

Permalink

https://escholarship.org/uc/item/9s09p5tf

Authors

Gyllenhammer, LE
Picard, M
McGill, MA
et al.

Publication Date

2022-10-01

DOI

10.1016/j.psyneuen.2022.105868

Peer reviewed

Prospective association between maternal allostatic load during pregnancy and child mitochondrial content and bioenergetic capacity

Gyllenhammer LE[1,2], Picard M[3,4,5], McGill MA[3], Boyle KE[6,7], Vawter MP[8], Rasmussen JM[1,2], Buss C[1,2,9], Entringer S[1,2,9], Wadhwa PD[1,2,8,10,11]

[1]Development, Health and Disease Research Program, University of California, School of Medicine, Irvine, CA, USA

[2]Department of Pediatrics, University of California, School of Medicine, Irvine, CA, USA.

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

[4]Department of Neurology, Merritt Center, Columbia Translational Neuroscience Initiative, Columbia University Irving Medical Center, New York, NY, USA

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

[6]Department of Pediatrics, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA.

[7]Lifecourse Epidemiology of Adiposity and Diabetes Center, Aurora, Colorado, USA.

[8]Department of Psychiatry and Human Behavior, University of California, School of Medicine, Irvine, CA, USA

[9]Charité-Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health (BIH), Institute of Medical Psychology, Berlin, Germany

[10]Department of Obstetrics and Gynecology, University of California, School of Medicine, Irvine, CA, USA

[11]Department of Epidemiology, University of California, School of Medicine, Irvine, CA, USA

Abstract

Background: Mitochondria are multifunctional energy-producing and signaling organelles that support life and contribute to stress adaptation. There is a growing understanding of the dynamic relationship between stress exposure and mitochondrial biology; however, the influence of stress on key domains of mitochondrial biology during early-life, particularly the earliest phases of intra-uterine/prenatal period remains largely unknown. Thus, the goal of this study was to examine the impact of fetal exposure to stress (modeled as the biological construct allostatic load) upon mitochondrial biology in early childhood.

Corresponding Author: Lauren Gyllenhammer lgyllenh@uci.edu, 714-456-8626.

Methods: In n=30 children (range: 3.5–6 years, 53% male), we quantified mitochondrial content via citrate synthase (CS) activity and mtDNA copy number (mtDNAcn), and measured mitochondrial bioenergetic capacity via respiratory chain enzyme activities (complexes I (CI), II (CII), and IV (CIV)) in platelet-depleted peripheral blood mononuclear cells (PBMCs). In a cohort of healthy pregnant women, maternal allostatic load was operationalized as a latent variable (sum of z-scores) representing an aggregation of early-, mid- and late-gestation measures of neuroendocrine (cortisol), immune (interleukin-6, C-reactive protein), metabolic (homeostasis model assessment of insulin resistance, free fatty acids), and cardiovascular (aggregate systolic and diastolic blood pressure) systems, as well as an anthropometric indicator (pre-pregnancy body mass index [BMI]).

Results: An interquartile increase in maternal allostatic load during pregnancy was associated with higher mitochondrial content (24% and 15% higher CS and mtDNAcn), and a higher mitochondrial bioenergetic capacity (16%, 23%, and 25% higher CI, CII and CIV enzymatic activities) in child leukocytes. The positive association between maternal allostatic load during pregnancy and child mitochondrial content and bioenergetic capacity remained significant after accounting for the effects of key pre- and post-natal maternal and child covariates (p's< 0.05, except CI p=0.073).

Conclusion: We report evidence that prenatal biological stress exposure, modeled as allostatic load, was associated with elevated child mitochondrial content and bioenergetic capacity in early childhood. This higher mitochondrial content and bioenergetic capacity (per leukocyte) may reflect increased energetic demands at the immune or organism level, and thus contribute to wear-and-tear and pathophysiology, and/or programmed pro-inflammatory phenotypes. These findings provide potential mechanistic insight into the cellular processes underlying developmental programming, and support the potential role that changes in mitochondrial content and bioenergetic functional capacity may play in altering life-long susceptibility for health and disease.

1. Introduction:

The importance of mitochondria in health and disease susceptibility is now well recognized.[1,2] Mitochondria, bacterial endosymbionts that contain their own genome (*i.e.*, mtDNA), are intracellular organelles that produce the energy that fuels life, and play a central role in cellular fate, such as programmed cell death,[3] cell cycle regulation,[4] and replicative senescence.[5] Mitochondrial biology determines the limits of an organism's adaptive capacity, and deficits in mitochondrial content and bioenergetic capacity reduce biological adaptability and resilience to a diverse range of physiological and psychological challenges.[6,7] Furthermore, mitochondria are causally implicated in the aging process and the emergence and progression of a range of physical and mental diseases.[2] For these reasons, understanding the determinants of variation in mitochondrial content and bioenergetic capacity assumes critical importance.

The role of stress and stress biology is being increasingly appreciated as an important determinant of variation in mitochondrial biology.[6,8] The majority of studies that have examined the role of stress exposure on mitochondrial biology have been conducted in preclinical models. Furthermore, human data is primarily limited to adults (reviewed here[9]). The effects of early life stress exposure on mitochondrial biology, particularly during the intrauterine period, have not yet been adequately addressed. The importance of this question derives from two considerations. First, the reciprocal relationship between stress and mitochondrial biology makes it difficult to disentangle cause from effect.[8] Mitochondrial biology is a joint function of its initial setting/functional capacity and the cumulative effects of stress-related organismal wear and tear over time.[6] Second, the effects of stress exposure during sensitive developmental periods in early life on health and disease risk are generally larger and longer lasting than those during subsequent life stages.[10–13] In this context, embryonic and fetal life likely represents the most sensitive developmental period because it is during this time that the *initial setting* of phenotypic structure and function of cells, tissues, and organ systems occurs.[14,15] During this period of life before birth, stress exposure originates in the maternal compartment, from where it is transduced via various biological pathways to the developing embryo/fetus. For these reasons, we have hypothesized a critical role for maternal stress-related biological pathways in the process of fetal programming of mitochondrial biology.[16]

Our review of the effects of maternal exposures (including stress) in animals and humans *during pregnancy* on offspring mitochondrial biology lends broad support for the scientific premise underlying our hypothesis and highlights outstanding gaps in knowledge.[16] First, only a very small number of studies have addressed the effect of maternal stress upon offspring mitochondrial biology in humans.[17–20] Second, the mitochondrial outcomes in these few studies have been primarily restricted to those in placental tissue[17–19] and have not been extended to the offspring (child). Third, the mitochondrial measures used in the majority of these studies represent *indirect (proxy) measures* of functional capacity (*e.g.*, mitochondrial DNA copy number; mtDNAcn)[17–19] that do not represent functional capacity. While mtDNA encodes for key components of the electron transport chain and thus is linked to cellular energy (*i.e.*, ATP) production, stand-alone measures of mtDNA quantity (*e.g.*, mtDNAcn) or quality (*e.g.*, mtDNA mutations) do not provide sufficient insight into functional capacity (*e.g.*, while impaired mitochondrial functions can be reflected by a *decrease* in mtDNA quantity, it can also be reflected by an *increase* in mtDNA, which can occur as a secondary/ compensatory response to increase mitochondrial content in the context of impaired mitochondrial functions).[21,22] As discussed elsewhere,[23] decreased mitochondrial energy production capacity can occur due to both a deficit in mitochondrial quantity per cell, and/or due to a decrease in the functional quality of the individual mitochondrion, and we argue here that both of these features could be impacted by early-life exposures. In order to clarify the direction and magnitude of the effects of prenatal stress exposure upon offspring mitochondrial biology in human studies, measurement of mitochondrial biology needs to expand beyond just those of mtDNA. While clinical measurement of mitochondrial biology is necessarily reductionistic, a combination of individual measures, particularly those that represent dynamic or functional measurement support biological interpretability.[24]

For these reasons, we conducted a study of the prospective association of maternal stress during pregnancy on child mitochondrial biology, hypothesizing that maternal stress biology would associate with child mitochondrial content and bioenergetic capacity. We took advantage of a convenience sample of [N=30] mother-child dyads in whom we had previously collected serial maternal biosamples over the course of pregnancy and child biosamples in early childhood (3.5–6 yrs age). We operationalized maternal stress using a composite biological measure of chronic maternal stress, *i.e.*, allostatic load. Allostatic load represents a multi-system composite biological indicator of chronic and cumulative stress exposure,[25] and a large number studies have demonstrated its prediction of morbidity and mortality-related outcomes across the lifespan,[25–29] and its association with of adverse gestational and birth outcomes in the context of pregnancy.[27–32] Because immune cells are prominently implicated in a range of common age-related disorders including those in which mitochondrial dysfunction has been implicated, we assessed child mitochondrial biology in (platelet-depleted) peripheral blood mononuclear cells (PBMCs) obtained from venous blood. We operationalized child mitochondrial biology using measurements of mitochondrial content (mtDNAcn and citrate synthase [CS]) and essential mitochondrial enzymes from the electron transport chain (ETC) to characterize mitochondrial bioenergetic capacity (from complex I [CI], complexes II [CII] and complexes IV [CIV]). Furthermore, we combined mitochondrial content and bioenergetic capacity measures to reflect individual complex and global energy production capacity on a per-mitochondrion basis (e.g., mitochondrial health index [MHI]).[23,33] We performed these assessments in early childhood, as cord blood was not available in this cohort, and this is the first age, after childbirth, to feasibly and consistently gather sufficient blood volumes/cell number through venipuncture to perform our comprehensive mitochondrial studies. Finally, in order to isolate the effects of maternal biological stress during pregnancy and to improve model precision estimates, we controlled (by study design or statistically) for the effects of other key prenatal, birth and postnatal factors on child cellular function, including maternal socio-economic status (SES), maternal race and ethnicity, infant sex, postnatal psychosocial stress exposure (*i.e.*, maternal depressive symptomatology), postnatal breastfeeding exposure, and offspring blood leukocyte populations. In particular, we modeled and controlled for maternal reports of *postnatal* psychological state and other offspring postnatal factors to isolate and examine the fetal period as a critical window of exposure upon mitochondrial biology.

2. Methods:

2.1 Participants

The study population was comprised of mother–child dyads recruited from a population-based prospective cohort study at the University of California, Irvine, Development, Health, and Disease Research Program. A population of healthy women with a singleton, intrauterine pregnancy was recruited in their first trimester of pregnancy. Maternal exclusionary criteria included uterine anomalies, preexisting major medical comorbidities (hypertension, infection, or type 1 or 2 diabetes), antenatal systemic corticosteroid administration, maternal use of psychotropic medications, or illicit drug use. Once enrolled, women were invited to attend three prenatal visits for the study, which corresponded to early-, mid- and late-gestation, at a mean ± standard deviation gestational age of

13.1±1.8 weeks, 20.5±1.4 weeks and 30.5±1.4 weeks, respectively. Newborn exclusionary criteria included congenital malformations, chromosomal abnormalities, major perinatal complications, or preterm birth <34 weeks. Children were invited to attend a study visit in early childhood (4.5±0.6 yrs, range 3.5–6 yrs), at which time a fasting blood draw was collected. 55 children attended this visit, of which, 41 children consented to a blood draw, and sufficient PBMC counts (>10^6) were obtained in 30 children for the measurement of mitochondrial content and enzymatic. This group of 30 mother-child dyads did not significantly differ from the larger group in terms of any of the sociodemographic characteristics. Measures of maternal psychological state were collected serially from birth until the early childhood visit when the children were approximately ~6 mo, 12 mo, 24 mo, and 3.5–6 yrs old, and offspring birth breastfeeding status was collected in the first 6 months of life. The University of California, Irvine, Institutional Review Board approved the protocol, and written, informed consent was obtained from all mothers.

2.2 Maternal allostatic load.

At each of the 3 pregnancy visits in early, mid and late gestation, maternal antecubital (venous) blood samples were collected in the morning between 7:30am and 9:00am following an overnight fast in serum (BD Vacutainer) and plasma tubes (EDTA BD Vacutainer). The plasma tubes were centrifuged for 15 min at 1200 g and plasma aliquots were stored at −80°C until analysis. The serum tubes were allowed to clot for 30 min at room temperature, then centrifuged at 4 °C at 1500× g, and serum aliquots were stored at −80 °C until analysis.

Immediately following each of the 3 pregnancy visits, subjects collected saliva samples at home for 4 consecutive days using a Salivette sampling device (Salimetrics, Carlsbad, CA) immediately and 30 minutes post awakening, and at 1200 hours, 1600 hours, and 2000 hours following a protocol that has previously been described.[34]

2.2.1 Cortisol: Saliva samples were assayed to determine cortisol concentrations using a high-sensitivity enzyme immunoassay (Salimetrics). Ten percent of the samples were run in duplicates. The assay has a lower limit of sensitivity of 0.007 μg/dL, a standard curve range from 0.012 μg/dL to 3.0 μg/dL, an average intra-assay coefficient of variation of 5.42%, and an average inter-assay coefficient of variation <10%. Exact timing of cortisol sampling was determined using a Medication Event Monitoring System® (Aardex Group, Union City, CA). For each sampling day, total cortisol output was estimated using the area under the curve (AUC) with respect to ground and was adjusted by residualization for awakening time, providing a summary measure of cumulative cortisol exposure in pregnancy shown to associate with child outcomes.[34]

2.2.2 Interluekin-6 (IL-6) and c-reactive protein (CRP): Serum IL-6 concentration was determined using a commercial high sensitivity ELISA (eBioscience) with a sensitivity of 0.03 pg/ml. The intra- and inter-assay coefficients of variability for IL-6 measurements were 10% and 14%, respectively. CRP concentrations were determined using a commercial Roche, COBAS Cardiac C-Reactive Protein (Latex) High-Sensitivity Test with a sensitivity

of 0.15 mg/L, and intra- and inter-assay CVs of 1.6% and 8.4%, respectively. IL-6 and CRP were $\log_2$ transformed to bring outliers closer to the mean and normalize the distribution.

2.2.3   Free fatty acids (FFA): Metabolomics analysis of fasting plasma samples and handling/ summarizing of metabolomics data have been previously described in detail.[35] 21 FFAs which included 7 saturated (C11:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0), 5 monounsaturated (C14:1, C16:1, C17:1, C18:1, C20:1) and 9 polyunsaturated (C18:2, C18:3, C20:2, C20:3, C20:4, C20:5, C22:4, C22:5, C22:6) FFAs were quantified. Mean values across pregnancy were computed for each NEFA and of the 21 FFAs, a subset of 18 FFAs were available in 90% of women (C11:0, C20:3, C22:6 <90%). The individual 18 FFAs were combined into a total FFA score by computing a z-scored mean for each stage of gestation of the 18 FFAs, using median replacement for any remaining missing FFA values.

2.2.4   Homeostatic model of insulin resistance (HOMA-IR): Serum glucose was quantitatively determined enzymatically using reagents from Vital Diagnostics (Lincoln, RI). Samples were incubated with the reagent at 37 °C and the absorbance was read at 340/380 nm. Serum insulin was measured using a radioimmune assay procedure developed by EMD Millipore (St Charles, MO). HOMA-IR was computed using the formula: (glucose in mg/dL × insulin in μU/mL)/405,[36] and provides a score that combines information from both fasting glucose and insulin concentrations. HOMA-IR was $\log_{10}$ transformed, transforming the skewed distribution to determine a stronger linear correlation with gold-standard clamp-derived values of insulin resistance and glucose disposal.[37]

2.2.5   Maternal anthropometry and blood pressure: Maternal pre-pregnancy body mass index (BMI; weight kg/height m^2) was computed based on pre-pregnancy weight abstracted from the medical record, and maternal height was measured at the research laboratory during the first pregnancy visit. Maternal blood pressure was measured in duplicate at each pregnancy visit.

2.2.6   Calculation of maternal allostatic load: Based on published guidelines,[25] our study focuses on maternal allostatic load indexed by *neuroendocrine* (cortisol), *immune* (IL-6 and CRP), *metabolic* (HOMA-IR, FFA), and *cardiovascular* (aggregate systolic and diastolic blood pressure) systems, and *anthropometric* (pre-pregnancy BMI) biomarkers, because these factors *a)* are responsive to chronic maternal stress;[38–41] *b)* are known to play obligatory roles in the initiation, maintenance and progression of gestation, fetal development, and birth; and *c)* they mediate the effects of various gestational perturbations including stress on fetal physiology,[42] by either directly crossing the placenta,[43–45] or by triggering placental production of stress-related ligands that are released into the fetal compartment.[46]. Allostatic load scores based on these markers have been used in studies with pregnant women to predict pregnancy complications and birth outcomes.[27,32] Over 11 different algorithmic computations of allostatic load have been cited in the literature.[25] Many of the components conventionally included in the allostatic index change across pregnancy, and there are no commonly accepted "high-risk" cut-offs for the components in pregnant populations. Thus, gestational timing needs to be considered and (pregnancy) population specific scoring needs to be employed. Therefore, we chose to utilize the z-score

method, because the weight of each component is standardized to the (*pregnant) population* and to the *gestation specific* mean. Before computation of the allostatic load, values for each biomarker were standardized to each stage of gestation (*i.e.*, z-score) and then averaged across pregnancy (except the time-invariant pre-pregnancy BMI value). An allostatic load index for each woman was then computed as the sum of z-scores, consistent with previous pregnancy research.[27]

2.3 Child blood collection and PBMC isolation

Child antecubital venous blood samples were collected by venipuncture at the early childhood visit between 7:30am and 9:00am, following an overnight fast in sodium citrate and EDTA tubes (BD Vacutainer). The sodium citrate tubes were used for platelet-depleted PBMC isolation, performed within a two-hour window under sterile conditions. The platelet-depleted PBMCs were isolated using SepMate tubes (StemCell), and differential centrifugation with an additional wash carried out at $200 \times g$ for 10min to maximally deplete platelets. Cell number was quantified using the Countess Automated Cell Counter (Invitrogen) in a 1:1 ratio of cells to trypan blue. Cells were resuspended cells in freezing medium (1mL / 1×10^7 cells (10% DMSO [Sigma-Aldrich, D2438-5X10ML] in FBS [HyClone, SH3007102])) and stored in liquid nitrogen until processed. The EDTA tube was used to quantify absolute and percent count of leukocyte populations (complete blood count with leukocyte differential).

2.4 Child mitochondrial content and bioenergetic capacity measures

Mitochondrial content and bioenergetic capacity were assessed in platelet-depleted PBMCs following previously-published procedures.[23,33] In brief, enzymatic activities were quantified spectrophotometrically for citrate synthase (CS), NADH-Ubiquinone Oxidoreductase (CI), succinate dehydrogenase (SDH, cCII), and cytochrome c oxidase (COX, CIV), and expressed as nmol/min/10^6 cells (reflective of *maximal* enzymatic activity).[47] The mitochondrial ETC system consists of four multimeric complexes (CI-IV), which work in concert to generate a transmembrane potential (like charging a battery), which in turn is used to drive multiple functions including the aerobic synthesis of ATP by CV. Thus, our measurement of maximal enzyme activity (i.e., CI, CII, and CIV) provides a characterization of mitochondrial bioenergetic capacity. In parallel, mtDNA and nuclear DNA abundance were quantified by TaqMan-based duplex quantitative real-time polymerase chain reaction (qPCR) to normalize the enzymatic activities to cell number and to calculate mtDNA copy number (mtDNAcn), as previously described.[33] Briefly, two mitochondrial and nuclear amplicons pairs were quantified (primer set 1:ND1 (mtDNA)/B2M (nDNA); primer set 2: COX1 (mtDNA)/RNaseP (nDNA)), using TaqMan Universal Master mix fast (life technologies #4444964), and 300 nM of custom design primers and 100 nM probes, and the average mtDNAcn value across the two primer sets was used in analysis. Both mtDNAcn and CS enzymatic activity[48] served as estimates of mitochondrial content, with CS recognized as the more direct measure.[48,49] The CS data were also used as a proxy of mitochondrial content/mass and used to normalize the enzymatic activities to derive specific ETC activity. Finally, a composite measure of both mitochondrial content and respiratory chain capacity, the Mitochondrial Health Index (MHI)[23] was assessed by mathematically integrating normalized (mean-centered) enzymatic activities and mtDNAcn

[(complex I + II + IV)/ (mtDNAcn + CS)], reflecting global energy production capacity on a per-mitochondrion basis.[33] The age of the child at the early childhood visit was negatively inversely associated with all mitochondrial measures, therefore, to account for effects of ages at assessment all mitochondrial measures were residualized for age at blood draw when assessing relationship between mitochondrial measures, or age was included as a covariate in all linear regression models.

2.5 Covariates

A priori selection of the covariates was based on theoretical considerations and empirical evidence of possible associations with either mitochondrial outcomes or allostatic load (to account for potential confounding) or with only the mitochondrial outcomes (to improve model precision). The following covariates were considered in statistical analyses: maternal socio-economic status (SES), maternal race and ethnicity, infant sex, child age at blood draw, postnatal stress exposure (*i.e.*, maternal depressive symptomatology), postnatal breastfeeding exposure, and offspring blood leukocyte populations.

Maternal socioeconomic status (SES) was defined as a combination (mean) of maternal educational level (originally assessed in categories from *less than high school* to *advanced degree* and then recoded into values from 1 to 5) and household income (originally assessed in categories from $15,000 to $100,000 and then recoded into values from 1 to 5). Maternal race and ethnicity of the mother was assessed by self-report and participants were coded into two groups, non-Hispanic and Hispanic. Infant sex was abstracted from delivery medical records.

To control for stress exposure in the postnatal environment of the child, we collected maternal report of psychological state, specifically depressive symptomatology, across the early-life of child (at 6-, 12-, 24- months old and at the 4–6yr visit). At each visit, this was measured using the validated and standardized Center for Epidemiological Studies-Depression (CES-D), 20-item scale,[50] and a mean was computed across the postnatal period.

Infant feeding method (breastmilk or formula) was assessed via monthly maternal interviews from the newborn to 6-month child assessments, as breastfeeding status is a postnatal factor that has been previously associated with leukocyte mitochondrial measures.[51] To control for infant breastfeeding status over the early 6-month postnatal period, a composite variable was computed indexing whether the mother primarily breastfed (>75% time), formula fed (>75% time) or provided mix feeding for her child until 6 months of age.

Whole blood leukocyte populations were assessed via a standardized complete blood count (CBC) with differential, as mitochondrial content and bioenergetic capacity is known to vary across leukocyte populations.[33,52] PBMCs include lymphocytes (T cells, B cells, and NK cells), monocytes, and dendritic cells, with lymphocytes accounting for 70–90% and monocytes accounting for 10–20% of the population; thus CBC measurement allowed for adjustment for lymphocytes and monocyte counts as needed.

2.6 Statistical analysis.

The primary outcome variables included the child's mitochondrial enzymatic activity (complexes I, II and IV), mitochondrial content measures (CS and mtDNAcn), and a composite mitochondrial index, MHI.

First, bivariate associations were examined between mitochondrial bioenergetic capacity and content measures and leukocyte cell subset counts (i.e., monocyte and lymphocyte count). Next, the linear relationships were modeled between maternal allostatic load and child mitochondrial measures, with and without the *a priori* covariates included in the model. We also examined the relationship between maternal allostatic load and child mitochondrial bioenergetic capacity after adjusting enzymatic activity for mitochondrial content (*i.e.*, enzymatic activity per mitochondrial content). Robust variance estimates of the regression coefficient estimates were obtained to produce unbiased variance estimates of the regression coefficients and unbiased inferences of the regression coefficient estimates.

In order to estimate the magnitude of associations between maternal allostatic load and the child mitochondrial measures, Pearson's correlations were calculated; additionally, high and low maternal allostatic load groups (median split) were created, and the group mean and standard deviation were used to calculate Cohen's D effect size. Further, to interpret the regression beta coefficients, % difference in child mitochondrial measures were calculated by log transformation in the response variable and the following formula $100(e^{\beta c} - 1)\%$.[53]

Statistical analyses were performed with SAS® Software Version 9.4, and a Type 3 analysis using Wald test was performed when *a priori* covariates were included in the model. Results were considered statistically significant at the level of $p < 0.05$.

3. Results:

Characteristics of the 30 mothers and their children are reported in Table 1. The socio-demographic characteristics were similar for children who did and did not provide a PBMC cell sample for the described mitochondrial analyses (data not shown).

3.1 Stability of the maternal allostatic load score across pregnancy:

We examined the within-individual stability of the allostatic load score across pregnancy using the scores computed for each subject at her early, mid and late pregnancy visits. The allostatic load (AL) scores were highly correlated (all p's<0.001; early- to mid-AL r=0.89, early- to late-AL r=0.77, mid- to late-AL r=0.80), and exhibited high within-individual stability across pregnancy (intra-class correlation (ICC)= 0.85 with inclusion of time invariant pre-pregnancy BMI, and ICC= 0.76 without time-invariant pre-pregnancy BMI).[54] Accordingly, we utilized an allostatic load score averaged across pregnancy for the proposed analyses.

We then examined the relationship between the individual components of the AL score and the overall combined AL score. All of the components were significantly and positively related to the combined AL score and to one another, with the exception of cortisol (see Supplemental Table 1).

3.2   Association between measures of mitochondrial content and bioenergetic capacity.

The measures of PBMC mitochondrial content and bioenergetic capacity were positively correlated in our study population, however these relationships were attenuated when the mitochondrial enzymatic activity is expressed per mitochondrion (rather than per cell) (see Table 2). Each cell contains large numbers of mitochondria, and mitochondrial enzymatic activity can be normalized to cell count (*e.g.*, complex I activity per leukocyte), or it can be normalized to mitochondrial content (*e.g.*, complex I activity per "mitochondrion"). In our data, when mitochondrial enzymatic activity is normalized per cell, it is tightly correlated with mitochondrial content. Specifically, all measures of child mitochondrial content (indexed by CS and mtDNAcn) were significantly and positively associated with mitochondrial bioenergetic capacity (*i.e.*, complex I, II, IV enzymatic activity per cell) (r's> 0.83), with at least 69% (R^2>0.69) of the variance in enzymatic activity explained by cellular mitochondrial content. But when mitochondrial enzymatic activity is normalized to the most direct marker of mitochondrial content (*i.e.*, CS), the relationship between enzymatic activity and mtDNAcn, the more indirect measure of cellular mitochondrial content[49], is attenuated.

The PBMC cell mixture is composed of varied leukocyte populations that have different mitochondrial content and ETC enzymatic activities, and thus, we next, we examined association of inter-individual differences in leukocyte populations with mitochondrial measures.[33,52] As shown in Table 2, there was no association between the monocyte cell count and mitochondrial markers, however, there was a significant association between lymphocyte count and all the mitochondrial measures, with the exception of the CS measure of mitochondrial content. Therefore, in subsequent analyses we controlled for lymphocyte count. We passively depleted platelets by differential centrifugation from the PBMCs; however, this procedure does not fully deplete all platelets. Therefore, as a manipulation check we examined and confirmed there was no association between platelet count (from whole blood measurement) and PBMC mitochondrial measures.[55] If platelet contamination was biasing our PBMC mitochondrial measures, we would expected to see higher mitochondrial content and enzymatic activity in children with higher whole blood platelets counts, but this relationship did not exist (all p>0.10), indicating that potential residual platelet contamination is not confounding our mitochondrial data.[55]

3.3   Maternal allostatic load and mitochondrial content.

As shown in Figure 1, maternal allostatic load was prospectively, significantly associated with PBMC mitochondrial content (CS: β= 5.3, p=0.0042; adjusted β=5.9, p=0.0049; Supplemental Table 2a), and mtDNAcn (β= 13.0, p=0.0284; adjusted β=13.1, p=0.0355; Supplemental Table 2b), before and after adjusting for key maternal and child covariates. Computing standardized effects sizes for each mitochondrial measures indicates that for each additional interquartile difference in maternal allostatic load, offspring leukocyte mitochondrial content were higher by 25% (95% C.I. 8–44%) for CS, and by 15% (95% C.I. 1–31%) for mtDNAcn. The positive relationship between child mitochondrial content and maternal allostatic load and is consistent across gestation (i.e., maternal allostatic load index measured in early-, mid- and late-gestation; see Supplemental Table 3a–b).

3.4   Maternal allostatic load and mitochondrial bioenergetic capacity.

As shown in Figure 2, maternal allostatic load was prospectively and significantly associated with PBMC mitochondrial enzymatic capacity detected per cell. Specifically, maternal allostatic load was positively associated with complex I, II and IV enzymatic activities (CI: β=0.35, p=0.0261; adjusted β=0.32, p=0.0642; CII: β=3.31, p=0.0374; adjusted β=2.92, p=0.0241; CIV: β=0.76, p=0.0101; adjusted β=0.79, p=0.0135; Figure 2 a–c; Supplemental Table 2c–e). For each additional interquartile difference in maternal allostatic load, offspring leukocyte mitochondrial outcomes were higher by 16% (95% C.I. −1–37%) for CI, 23% (95% C.I. 1–51%) for CII, and 25% (95% C.I. 5–48%) for CIV, with the relationship between gestational stress and offspring CI enzymatic activity attenuated to a trend after adjustment for key maternal and child covariates. The positive relationship between child mitochondrial bioenergetic capacity and maternal allostatic load is consistent across the different stages of gestation (see Supplement 3c–e).

In contrast, maternal allostatic load was not associated with the quality or the energy production capacity of mitochondria, derived from mitochondrial enzymatic relative to mitochondrial content (CI: β=−0.0002, p>0.10; adjusted β=−0.0011, p=0.0809; CII: β=0.0046, p>0.10; adjusted β=−0.0011, p>0.10; CIV: β=0.0005, p>0.10; adjusted β=−0.0001, p>0.10, Figure 2 d–f). Moreover, when complex I was expressed on a per-mitochondrion basis and adjusted for other covariates, there was a trend towards an inverse association between maternal allostatic load and Complex I enzymatic activity (adjusted β=−0.0011, p=0.0809). Consistent with the above-described findings with the individual measures of mitochondrial enzymatic activity adjusted for total mitochondrial content, there was no association between maternal allostatic load and MHI, a summary measure of mitochondria-specific bioenergetic function measure (p>0.10).

4.   Discussion:

To the best of our knowledge, this study represents the first report in humans of the prospective association between maternal allostatic load (quantified during pregnancy) and offspring leukocyte mitochondrial content and bioenergetic capacity. The findings support our fetal programming hypothesis, in that key features of offspring mitochondrial biology appear to exhibit variation as a function of gestational exposure,[16] and adds to the small literature specific to gestational stress exposure in humans.[17,19,56] This study also extends the considerable literature on stress biology and fetal programming down to the level of intracellular phenotypic outcomes that reflect bioenergetic processes and constitute a major pathway to future health and disease risk. Specifically, our findings indicate than an interquartile increase in maternal allostatic load during pregnancy was significantly associated with a 25% and 15% higher difference in child leukocyte CS and mtDNAcn measures of mitochondrial content, respectively, and with a 16%, 23%, and a 25% higher difference in complex I, II and IV enzymatic measures of mitochondrial bioenergetic capacity, respectively. Based on the Cohen's d measure of effect size (as shown in Table 3), these differences translate to moderate to large effects. Given the paucity of data on maternal biological stress and child mitochondrial biology, it is, however, difficult to place the magnitude of this effect into any direct context.

Our findings of the association between maternal stress biology and child mitochondrial content are consistent with that of studies that have assessed the effect of *postnatal* early life stress[20,57] (reviewed here[58]). Specifically, adults exposed postnatally to early life stress have been reported to exhibit higher leukocyte mitochondrial content (measured by CS)[20] and bioenergetic function (measured by respirometry)[20,57] than non-exposed adults. Additionally, Gumpp et al.[20] examined the intergenerational (mom-to-child) transmission of maternal early life stress upon child mitochondrial content and bioenergetic function. Similar to our study, early life maternal stress associated with higher child mitochondrial content, but this finding was a trend association, and the associations with bioenergetic function were null. These diminished relationships, may, in part, be due to two main factors. First, there are differences in stress exposure measurement between the two studies. While *early life stress* can induce chronic long-term changes in stress physiology, this exposure measurement is distal to the index pregnancy, and *pregnancy-specific stress* was not measured in this study. Our measurement of proximal biological stress (*i.e.*, allostatic load index) during the developmental window of interest (*i.e.*, intrauterine period) likely strengthened our power to detect an association with child mitochondrial biology. Second, the methodology used by Gumpp et al. is respirometry in live cells, which reflects either basal energy consumption by the leukocytes (basal or routine respiration), or the maximal capacity to respire (uncoupled respiration), which depends on the integrated function of several complexes in the oxidative phosphorylation system. In contrast, while more crude and less integrative, our measures of individual enzymatic activities reflect each component's maximal activity or capacity. We know of only one other previous human study (by Brunst *et al.*[19]) of prenatal (*pregnancy specific)* psychological stress and offspring mitochondrial leukocyte content (assessed indirectly by a single measure of mtDNAcn). Unlike our study, the study by Brunst et al. did not observe a significant effect. It is possible that the absence of an association in this study may, in part, reflect methodological limitations, as no steps were reported in that study to remove platelets from the leukocyte sample, which can induce large variation and noise in leukocyte mtDNAcn measurement.[55] As noted previously, steps were taken in our study to deplete platelets and we also quantified two independent measures of mitochondrial content, CS and a less direct measure, mtDNAcn.[49] The clinical significance of this finding is currently unknown, as there are no studies that yet that have examined the link between newborn or child measures of leukocyte mitochondrial content with subsequent child or adult health outcomes; however, studies have shown that mitochondrial biology in adults is directly and indirectly involved in the pathogenesis of aging and a broad range of diseases.[2]

Our findings indicate the observed association between maternal allostatic load and child mitochondrial bioenergetic capacity (*i.e.*, complex I, II, IV enzymatic activity) was no longer significant after adjusting for mitochondrial content (with the individual complexes or using the combined MHI score). This suggests that the effects of prenatal biological stress exposure on mitochondrial biology may be specific to mitochondrial content (mitochondrial density per cell) as opposed to qualitative mitochondrial alterations, such as ETC capacity per unit mitochondrion. While this observation requires confirmation in future studies, our data in this study on the associations between measures of mitochondrial content and bioenergetic capacity may be informative. As described earlier, approximately

70% of the variance in mitochondrial bioenergetic capacity per cell is explained by its mitochondrial content (*i.e.*, cellular mitochondrial density), suggesting that the number of mitochondria per cell may be the major determinant of overall cellular mitochondrial bioenergetic capacity. Consistent with this observation, we note that when Gumpp *et al.*[20] normalized their measures of mitochondrial bioenergetic capacity (measured by flux respirometry) to mitochondrial content (*i.e.*, CS), the magnitude of the effect in adults of postnatal stress exposure on mitochondrial respiration was attenuated. Taken together, these findings in the context of the immune cells examined in the present study may suggest a larger impact of early life stress on mitochondrial biogenesis than bioenergetic capacity per mitochondrion. This observation remains to be confirmed in future larger studies that incorporate measures of mitochondrial content as well as bioenergetic capacity and compute composite indices of mitochondrial bioenergetic capacity per mitochondrion.[24] Additionally, the mitochondrial enzymatic activity assays used in this study are reflective of maximal capacity and should not be interpreted as physiological activation.[47] Future studies should explore these relationships with mitochondrial bioenergetic capacity using tools such as flux respirometry experiments in live intact cells, which provide increased physiological relevance.[59] Furthermore, we note that while the predominant biological function of mitochondria is the generation of cellular energy (i.e., ATP production by oxidative phosphorylation), additional critical mitochondrial functions include (but are not limited to) the generation and detoxification of reactive oxygen species (ROS), synthesis and catabolism of metabolites, regulation of cytoplasmic and mitochondrial calcium, and of regulation of cellular apoptosis.[59] Dysregulation among any of these processes can represent mitochondrial allostatic load (MAL), and attempts to quantify additional domains of mitochondrial biology should be considered in future studies.

Although the specific mechanisms regulating mitochondrial content, particularly during development, are poorly understood, it seems reasonable to speculate and suggest that upregulated mitochondrial biogenesis subsequent to biological stress exposure may reflect a secondary, compensatory adaptive response to stress mediators such as glucocorticoids and pro-inflammatory cytokines[6] (that are a component of the maternal allostatic load measure). Indeed, glucocorticoids[60] and inflammatory mediators[61] have been shown to induce the expression or activity of coactivators and transcription factors that regulate mitochondrial biogenesis. A compensatory increase in early-life mitochondrial biogenesis in response to the biological stress context may be adaptive from an evolutionary perspective, but at the individual level it may reflect a trade-off favoring short-term survival and reproductive fitness at the long-term cost of increased disease susceptibility, particularly for complex, common disorders.[16] While such recalibrations and compensatory increases can be adaptive, they may not be entirely benign. Chronically, these adaptations could contribute to increased wear-and-tear and pathophysiology.[24] For example, upregulation of cellular bioenergetic activity (*i.e.*, oxidative respiration) consequent to mitochondrial biogenesis can result in increased ROS production[62] to potentiate intracellular inflammatory processes. A recent experimental study illustrates this point well. Here, findings demonstrate that a substantial proportion of proinflammatory cytokine production in human leukocytes in response to *ex vivo* antigen stimulation was determined by leukocyte mitochondrial respiratory capacity (10–30% of the inter-individual variance).[63] Such mitochondrial modulation of

pro-inflammatory responsivity might be further accentuated by an upregulation in the oxidative stress exposure. Although the generation of a ROS is a normal part of the oxidative phosphorylation, generation of excess superoxide can come at the expense of ATP production,[64] and can, in turn, damage mtDNA, nuclear DNA, and other cellular components.[65] Accordingly, the incorporation in future studies of cellular oxidative stress measures in conjunction with measures of mitochondrial content and bioenergetic capacity would be illustrative in providing a better understanding of the impact of early life stress on cellular and mitochondrial health.

The elevation of mitochondrial content in response to biological stress could also be explained as a response to increased energetic demands in the developing fetus. For example, chronic glucocorticoid signaling triggers allostatic responses in cultured human cells *in vitro*, producing allostatic load that is associated with an elevated energetic cost of living and increased mtDNAcn.[66] Stressed cells consume more energy to stay alive, and consequently exhibit increased mitochondrial biogenesis (gene expression program to synthesize additional mitochondria) and increased ETC capacity measured using respirometry. If maternal allostatic load similarly imposed additional energetic costs and increased the cost of living on the developing fetus and child, the optimal response would be to increase mitochondrial energy production capacity – as observed in our study. This allostatic response would be consistent with studies of children exposed to high levels of pathogens, a type of stressor that increases the energetic cost of living, and comes – in a tradeoff manner – at the expense of growth.[67,68] It is also noteworthy that children age 1–5 consume the greatest amount of energy of any ages across the human lifespan, possibly further enhancing their vulnerability to early-life stressors that require additional energy to overcome.[69] Thus, the elevation in mitochondrial content, along with proportional elevation in their energy production capacity in child leukocytes may reflect increased energetic demands among immune cells, or possibly at the whole-body level. Further studies are required to refute this hypothesis and to examine other potential mechanisms for our results.

Although maternal allostatic load broadly associated with an increase in offspring mitochondrial content and bioenergetic capacity (at the cellular level), when bioenergetic capacity was analyzed on a per-mitochondrion basis, maternal allostatic load was negatively associated with one complex, specifically CI. Isolated CI deficiency is the most frequently-encountered defect of the mitochondrial energy metabolism.[70] CI may be particularly vulnerable to bioenergetic defects due to its large size and complexity, but also due to the relatively large number of CI subunits encoded by mtDNA, which is more susceptible to oxidative damage than nuclear DNA. As discussed above, elevations in mitochondrial content and bioenergetic capacity (at the cellular level) may lead to increased wear and tear pathophysiology, and the trend negative association between maternal allostatic load and child CI bioenergetic capacity (per mitochondrion) may be an early indicator of this decline. This relationship should be further explored in future studies, and measurement of mtDNA mutation and deletion load (i.e., heteroplasmy, particularly in mtDNA encoded CI subunits) may further support our understanding of fetal programming of mitochondrial biology by elucidating these complex-specific effects.

In terms of strengths and limitations, our study is strengthened by the well-characterized and comprehensive assessment of our primary exposure of interest - biological stress during gestation – and of our primary outcomes of interest - layered and dynamic measures of child mitochondrial content and bioenergetic capacity. Other methodological strengths include addressing common methodical confounds in mitochondrial measurement (*e.g.*, platelet contamination, effects of leukocyte subpopulations) to increase measurement precision.[33,55] Although the direct measurement of mitochondrial bioenergetic capacity (complexes I, II, IV enzymatic activity) in combination with mitochondrial content (through composite indices) is an important and unique empirical contribution of our study to our understanding of early life influences on mitochondrial biology, it is important to appreciate that mitochondrial health extends beyond the measures of bioenergetic function assessed here. It is critical to conceptually recognize that mitochondria are dynamic living organisms whose complex behavior can be disrupted in different ways,[24] and as discussed above, future studies should measure layered features of mitochondrial biology in combination to further develop this field. Although our study is limited by the relatively modest sample size, our sample was adequate given the large effect size. Our capacity to detect this large effect was likely supported by the steps taken for precise mitochondrial measurement, which should be considered in future studies. We also make note that although CS has proven a reliable marker of mitochondrial content in human skeletal muscle,[48] this has not been demonstrated in human leukocytes, and both CS and mtDNAcn are indirect markers of mitochondrial content.[49,71] Finally, our measures of mitochondrial biology in leukocytes may only partially extrapolate to those in other tissues of interest (*e.g.*, skeletal muscle, brain).[72,73] Previous work suggests that mtDNAcn and other mitochondrial parameters may vary in a tissue- and cell type-dependent manner (reviewed in[49]). Future work should address the cross-tissue generalizability issue further.

In summary, our study adds to the growing literature on stress and fetal programming by extending the targets of programming to intracellular energetic processes, specifically mitochondria. We show that prenatal exposure to maternal allostatic load is prospectively associated with higher leukocyte mitochondrial content and bioenergetic capacity in the offspring. Although the described association implicates higher mitochondrial content and bioenergetic capacity (per leukocyte), this may reflect increased energetic demands at the immune or organism level, and thus contribute to wear-and-tear and pathophysiology, and/or programmed pro-inflammatory phenotypes. Our data further supports the hypothesis that mitochondrial biology is sensitive and responsive to prenatal exposures, and that mitochondrial stress transduction represent a key common cellular pathway in the intergenerational transmission of the effects of maternal conditions, states and exposures during early and sensitive periods of fetal development.[16] Furthermore, these finding highlight the potential role that mitochondrial content and bioenergetic capacity may serve in programmed susceptibility for health and disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Funding:

References:

1. Picard M, Wallace DC, Burelle Y. The rise of mitochondria in medicine. Mitochondrion, 2016; 30:105–16. [PubMed: 27423788]

2. Javadov S, Kozlov AV, Camara AKS. Mitochondria in Health and Diseases. Cells, 2020; 9(5).

3. Rasola A, Bernardi P. The mitochondrial permeability transition pore and its involvement in cell death and in disease pathogenesis. Apoptosis, 2007; 12(5):815–33. [PubMed: 17294078]

4. Owusu-Ansah E, Yavari A, Mandal S, Banerjee U. Distinct mitochondrial retrograde signals control the G1-S cell cycle checkpoint. Nat Genet, 2008; 40(3):356–61. [PubMed: 18246068]

5. Qian W, Choi S, Gibson GA, et al. Mitochondrial hyperfusion induced by loss of the fission protein Drp1 causes ATM-dependent G2/M arrest and aneuploidy through DNA replication stress. J Cell Sci, 2012; 125(Pt 23):5745–57. [PubMed: 23015593]

6. Picard M, Juster RP, McEwen BS. Mitochondrial allostatic load puts the 'gluc' back in glucocorticoids. Nat Rev Endocrinol, 2014; 10(5):303–10. [PubMed: 24663223]

7. Morava E, Kozicz T. Mitochondria and the economy of stress (mal)adaptation. Neurosci Biobehav Rev, 2013; 37(4):668–80. [PubMed: 23415702]

8. Picard M, McEwen BS. Psychological Stress and Mitochondria: A Conceptual Framework. Psychosom Med, 2018; 80(2):126–140. [PubMed: 29389735]

9. Picard M, McEwen BS. Psychological Stress and Mitochondria: A Systematic Review. Psychosom Med, 2018; 80(2):141–153. [PubMed: 29389736]

10. Entringer S, Buss C, Wadhwa PD. Prenatal stress, development, health and disease risk: A psychobiological perspective-2015 Curt Richter Award Paper. Psychoneuroendocrinology, 2015; 62:366–75. [PubMed: 26372770]

11. Fleming TP, Watkins AJ, Velazquez MA, et al. Origins of lifetime health around the time of conception: causes and consequences. Lancet, 2018; 391(10132):1842–1852. [PubMed: 29673874]

12. Reynolds RM. Glucocorticoid excess and the developmental origins of disease: two decades of testing the hypothesis--2012 Curt Richter Award Winner. Psychoneuroendocrinology, 2013; 38(1):1–11. [PubMed: 22998948]

13. Gee DG, Casey BJ. The Impact of Developmental Timing for Stress and Recovery. Neurobiol Stress, 2015; 1:184–194. [PubMed: 25798454]

14. Wadhwa PD, Buss C, Entringer S, Swanson JM. Developmental origins of health and disease: brief history of the approach and current focus on epigenetic mechanisms. Semin Reprod Med, 2009; 27(5):358–68. [PubMed: 19711246]

15. Hanson M, Godfrey KM, Lillycrop KA, Burdge GC, Gluckman PD. Developmental plasticity and developmental origins of non-communicable disease: theoretical considerations and epigenetic mechanisms. Prog Biophys Mol Biol, 2011; 106(1):272–80. [PubMed: 21219925]

16. Gyllenhammer LE, Entringer S, Buss C, Wadhwa PD. Developmental programming of mitochondrial biology: a conceptual framework and review. Proc Biol Sci, 2020; 287(1926):20192713. [PubMed: 32345161]

17. Brunst KJ, Sanchez Guerra M, Gennings C, et al. Maternal Lifetime Stress and Prenatal Psychological Functioning and Decreased Placental Mitochondrial DNA Copy Number in the PRISM Study. Am J Epidemiol, 2017; 186(11):1227–1236. [PubMed: 28595325]

18. Brunst KJ, Zhang L, Zhang X, et al. Associations Between Maternal Lifetime Stress and Placental Mitochondrial DNA Mutations in an Urban Multiethnic Cohort. Biol Psychiatry, 2021; 89(6):570–578. [PubMed: 33229036]

19. Brunst KJ, Sanchez-Guerra M, Chiu YM, et al. Prenatal particulate matter exposure and mitochondrial dysfunction at the maternal-fetal interface: Effect modification by maternal lifetime trauma and child sex. Environ Int, 2018; 112:49–58. [PubMed: 29248865]

20. Gumpp AM, Boeck C, Behnke A, et al. Childhood maltreatment is associated with changes in mitochondrial bioenergetics in maternal, but not in neonatal immune cells. Proc Natl Acad Sci U S A, 2020; 117(40):24778–24784. [PubMed: 33004627]

21. Giordano C, Iommarini L, Giordano L, et al. Efficient mitochondrial biogenesis drives incomplete penetrance in Leber's hereditary optic neuropathy. Brain, 2014; 137(Pt 2):335–53. [PubMed: 24369379]

22. Jeng JY, Yeh TS, Lee JW, et al. Maintenance of mitochondrial DNA copy number and expression are essential for preservation of mitochondrial function and cell growth. J Cell Biochem, 2008; 103(2):347–57. [PubMed: 18072287]

23. Picard M, Prather AA, Puterman E, et al. A Mitochondrial Health Index Sensitive to Mood and Caregiving Stress. Biol Psychiatry, 2018; 84(1):9–17. [PubMed: 29525040]

24. Picard M, Trumpff C, Burelle Y. Mitochondrial Psychobiology: Foundations and Applications. Curr Opin Behav Sci, 2019; 28:142–151. [PubMed: 32637466]

25. Juster RP, McEwen BS, Lupien SJ. Allostatic load biomarkers of chronic stress and impact on health and cognition. Neurosci Biobehav Rev, 2010; 35(1):2–16. [PubMed: 19822172]

26. Guidi J, Lucente M, Sonino N, Fava GA. Allostatic Load and Its Impact on Health: A Systematic Review. Psychother Psychosom, 2021; 90(1):11–27. [PubMed: 32799204]

27. Wallace ME, Harville EW. Allostatic load and birth outcomes among white and black women in New Orleans. Matern Child Health J, 2013; 17(6):1025–9. [PubMed: 22833335]

28. Olson DM, Severson EM, Verstraeten BS, et al. Allostatic Load and Preterm Birth. Int J Mol Sci, 2015; 16(12):29856–74. [PubMed: 26694355]

29. Jack-Roberts C, Maples P, Kalkan B, et al. Gestational diabetes status and dietary intake modify maternal and cord blood allostatic load markers. BMJ Open Diabetes Res Care, 2020; 8(1).

30. Barrett ES, Vitek W, Mbowe O, et al. Allostatic load, a measure of chronic physiological stress, is associated with pregnancy outcomes, but not fertility, among women with unexplained infertility. Hum Reprod, 2018; 33(9):1757–1766. [PubMed: 30085177]

31. Accortt EE, Mirocha J, Dunkel Schetter C, Hobel CJ. Adverse Perinatal Outcomes and Postpartum Multi-Systemic Dysregulation: Adding Vitamin D Deficiency to the Allostatic Load Index. Matern Child Health J, 2017; 21(3):398–406. [PubMed: 28120286]

32. Hux VJ, Roberts JM. A potential role for allostatic load in preeclampsia. Matern Child Health J, 2015; 19(3):591–7. [PubMed: 24939173]

33. Rausser S, Trumpff C, McGill MA, et al. Mitochondrial phenotypes in purified human immune cell subtypes and cell mixtures. Elife, 2021; 10.

34. Entringer S, Buss C, Rasmussen JM, et al. Maternal Cortisol During Pregnancy and Infant Adiposity: A Prospective Investigation. J Clin Endocrinol Metab, 2017; 102(4):1366–1374. [PubMed: 28009530]

35. Lindsay KL, Hellmuth C, Uhl O, et al. Longitudinal Metabolomic Profiling of Amino Acids and Lipids across Healthy Pregnancy. PLoS One, 2015; 10(12):e0145794. [PubMed: 26716698]

36. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia, 1985; 28(7):412–9. [PubMed: 3899825]

37. Otten J, Ahren B, Olsson T. Surrogate measures of insulin sensitivity vs the hyperinsulinaemic-euglycaemic clamp: a meta-analysis. Diabetologia, 2014; 57(9):1781–8. [PubMed: 24891021]

38. Keshavarzi F, Farnia V, Yazdchi K, et al. Effect of maternal anxiety on maternal serum and fetal cord blood cortisol. Asia Pac Psychiatry, 2014; 6(4):435–9. [PubMed: 24664930]

39. Wadhwa PD, Dunkel-Schetter C, Chicz-DeMet A, Porto M, Sandman CA. Prenatal psychosocial factors and the neuroendocrine axis in human pregnancy. Psychosom Med, 1996; 58(5):432–46. [PubMed: 8902895]

40. Hantsoo L, Kornfield S, Anguera MC, Epperson CN. Inflammation: A Proposed Intermediary Between Maternal Stress and Offspring Neuropsychiatric Risk. Biol Psychiatry, 2019; 85(2):97–106. [PubMed: 30314641]

41. Nagl M, Steinig J, Klinitzke G, Stepan H, Kersting A. Childhood maltreatment and pre-pregnancy obesity: a comparison of obese, overweight, and normal weight pregnant women. Arch Womens Ment Health, 2016; 19(2):355–65. [PubMed: 26386682]

42. Entringer S, Buss C, Wadhwa PD. Prenatal stress and developmental programming of human health and disease risk: concepts and integration of empirical findings. Curr Opin Endocrinol Diabetes Obes, 2010; 17(6):507–16. [PubMed: 20962631]

43. Chapman K, Holmes M, Seckl J. 11beta-hydroxysteroid dehydrogenases: intracellular gate-keepers of tissue glucocorticoid action. Physiol Rev, 2013; 93(3):1139–206. [PubMed: 23899562]

44. Dahlgren J, Samuelsson AM, Jansson T, Holmang A. Interleukin-6 in the maternal circulation reaches the rat fetus in mid-gestation. Pediatr Res, 2006; 60(2):147–51. [PubMed: 16864694]

45. Zaretsky MV, Alexander JM, Byrd W, Bawdon RE. Transfer of inflammatory cytokines across the placenta. Obstet Gynecol, 2004; 103(3):546–50. [PubMed: 14990420]

46. Hsiao EY, Patterson PH. Activation of the maternal immune system induces endocrine changes in the placenta via IL-6. Brain Behav Immun, 2011; 25(4):604–15. [PubMed: 21195166]

47. Janssen RC, Boyle KE. Microplate Assays for Spectrophotometric Measurement of Mitochondrial Enzyme Activity. Methods Mol Biol, 2019; 1978:355–368. [PubMed: 31119674]

48. Larsen S, Nielsen J, Hansen CN, et al. Biomarkers of mitochondrial content in skeletal muscle of healthy young human subjects. J Physiol, 2012; 590(14):3349–60. [PubMed: 22586215]

49. Picard M Blood mitochondrial DNA copy number: What are we counting? Mitochondrion, 2021; 60:1–11. [PubMed: 34157430]

50. Radloff LS. The CES-D Scale: A Self-Report Depression Scale for Research in the General Population. Applied Psychological Measurement, 1977; 1(3):385–401.

51. Cosemans C, Nawrot TS, Janssen BG, et al. Breastfeeding predicts blood mitochondrial DNA content in adolescents. Sci Rep, 2020; 10(1):387. [PubMed: 31941967]

52. Chacko BK, Kramer PA, Ravi S, et al. Methods for defining distinct bioenergetic profiles in platelets, lymphocytes, monocytes, and neutrophils, and the oxidative burst from human blood. Lab Invest, 2013; 93(6):690–700. [PubMed: 23528848]

53. Barrera-Gomez J, Basagana X. Models with transformed variables: interpretation and software. Epidemiology, 2015; 26(2):e16–7. [PubMed: 25643111]

54. Koo TK, Li MY. A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. J Chiropr Med, 2016; 15(2):155–63. [PubMed: 27330520]

55. Urata M, Koga-Wada Y, Kayamori Y, Kang D. Platelet contamination causes large variation as well as overestimation of mitochondrial DNA content of peripheral blood mononuclear cells. Ann Clin Biochem, 2008; 45(Pt 5):513–4. [PubMed: 18753426]

56. Lambertini L, Chen J, Nomura Y. Mitochondrial Gene Expression Profiles Are Associated with Maternal Psychosocial Stress in Pregnancy and Infant Temperament. PLoS One, 2015; 10(9):e0138929. [PubMed: 26418562]

57. Boeck C, Koenig AM, Schury K, et al. Inflammation in adult women with a history of child maltreatment: The involvement of mitochondrial alterations and oxidative stress. Mitochondrion, 2016; 30:197–207. [PubMed: 27530300]

58. Zitkovsky EK, Daniels TE, Tyrka AR. Mitochondria and early-life adversity. Mitochondrion, 2021; 57:213–221. [PubMed: 33484871]

59. Brand MD, Nicholls DG. Assessing mitochondrial dysfunction in cells. Biochem J, 2011; 435(2):297–312. [PubMed: 21726199]

60. Psarra AM, Sekeris CE. Glucocorticoid receptors and other nuclear transcription factors in mitochondria and possible functions. Biochim Biophys Acta, 2009; 1787(5):431–6. [PubMed: 19100710]

61. Cherry AD, Piantadosi CA. Regulation of mitochondrial biogenesis and its intersection with inflammatory responses. Antioxid Redox Signal, 2015; 22(12):965–76. [PubMed: 25556935]

62. Korshunov SS, Skulachev VP, Starkov AA. High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria. FEBS Lett, 1997; 416(1):15–8. [PubMed: 9369223]

63. Karan KR, Trumpff C, McGill MA, et al. Mitochondrial respiratory capacity modulates LPS-induced inflammatory signatures in human blood. Brain Behav Immun Health, 2020; 5.

64. Pitkanen S, Robinson BH. Mitochondrial complex I deficiency leads to increased production of superoxide radicals and induction of superoxide dismutase. J Clin Invest, 1996; 98(2):345–51. [PubMed: 8755643]

65. Shokolenko I, Venediktova N, Bochkareva A, Wilson GL, Alexeyev MF. Oxidative stress induces degradation of mitochondrial DNA. Nucleic Acids Res, 2009; 37(8):2539–48. [PubMed: 19264794]

66. Bobba-Alves N, Sturm G, Lin J, et al. Chronic Glucocorticoid Stress Reveals Increased Energy Expenditure and Accelerated Aging as Cellular Features of Allostatic Load. bioRxiv, 2022:2022.02.22.481548.

67. Urlacher SS, Ellison PT, Sugiyama LS, et al. Tradeoffs between immune function and childhood growth among Amazonian forager-horticulturalists. Proc Natl Acad Sci U S A, 2018; 115(17):E3914–E3921. [PubMed: 29632170]

68. Urlacher SS, Snodgrass JJ, Dugas LR, et al. Constraint and trade-offs regulate energy expenditure during childhood. Sci Adv, 2019; 5(12):eaax1065. [PubMed: 32064311]

69. Pontzer H, Yamada Y, Sagayama H, et al. Daily energy expenditure through the human life course. Science, 2021; 373(6556):808–812. [PubMed: 34385400]

70. Smeitink J, van den Heuvel L, DiMauro S. The genetics and pathology of oxidative phosphorylation. Nat Rev Genet, 2001; 2(5):342–52. [PubMed: 11331900]

71. McLaughlin KL, Hagen JT, Coalson HS, et al. Novel approach to quantify mitochondrial content and intrinsic bioenergetic efficiency across organs. Scientific Reports, 2020; 10(1):17599. [PubMed: 33077793]

72. Yoshino H, Nakagawa-Hattori Y, Kondo T, Mizuno Y. Mitochondrial complex I and II activities of lymphocytes and platelets in Parkinson's disease. J Neural Transm Park Dis Dement Sect, 1992; 4(1):27–34. [PubMed: 1347219]

73. Mosconi L, Berti V, Quinn C, et al. Perimenopause and emergence of an Alzheimer's bioenergetic phenotype in brain and periphery. PLoS One, 2017; 12(10):e0185926. [PubMed: 29016679]
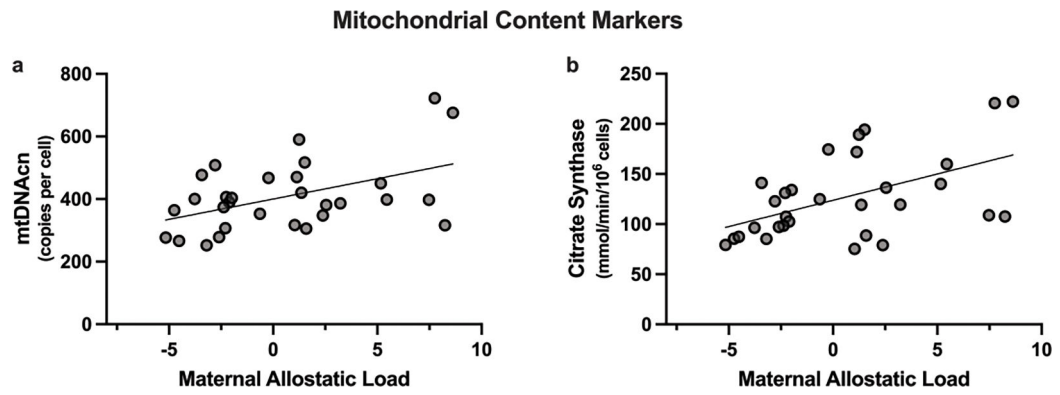
Figure 1. Maternal allostatic load is associated with child mitochondrial content
Maternal allostatic load is positively associated with markers of mitochondrial content in offspring leukocytes (A), mitochondria DNA copy number (mtDNAcn; β=13.1, p=0.0355) and (B) citrate synthase (CS) enzymatic activity (β=5.9, p=0.0049).
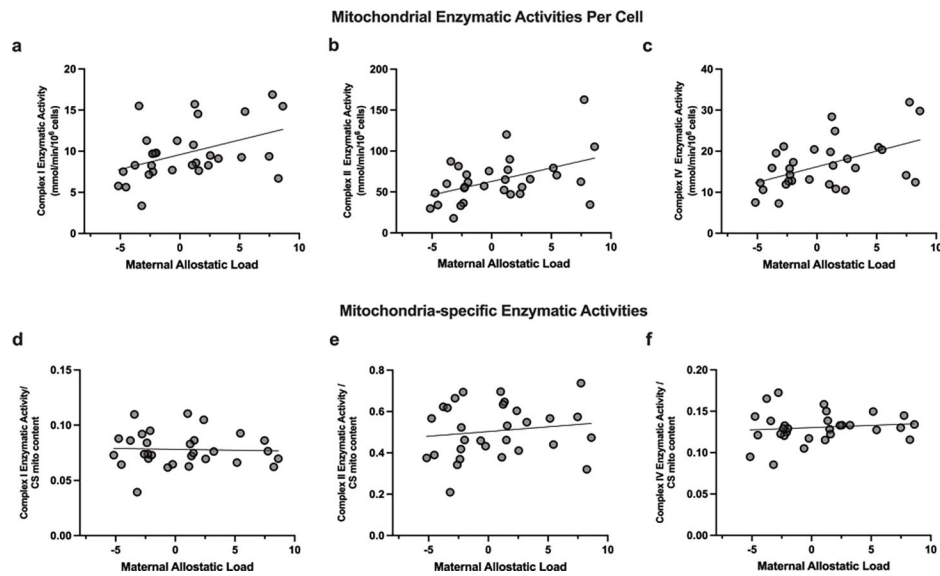
Figure 2. Maternal allostatic load is associated with child mitochondrial enzymatic activity (per cell).

Maternal allostatic load is positively associated with mitochondrial enzymatic activity (per cell) in offspring leukocytes (A), complex I (B) complex II, and (C) complex IV enzymatic activity. However, maternal allostatic load was not associated with the energy production capacity of mitochondria, derived from mitochondrial enzymatic activity relative to mitochondrial content (D-F).

Table 1:

Maternal and Child Characteristics

	Mean ± SD, or n (%)	25% (Q1)	75% (Q3)	IQR
Maternal Variables:				
Age (yrs)	28.4 ± 4.7	26.0	31.0	5.0
SES index	3.22 ± 0.56	2.67	3.67	1.00
Ethnicity, n (%Hispanic)	12 (43%)	-	-	-
Allostatic Load Composite Score	0.56 ± 4.08	−2.59	2.55	5.14
Pre-pregnancy BMI (kg/m^2)	28.9 ± 6.9	22.8	33.7	11.0
Log HOMA-IR	0.42 ± 0.22	0.28	0.56	0.28
FFA (mean18)	14.9 ± 3.7	12.2	17.3	5.1
CRP (mg/L)	10.4 ± 9.7	3.4	14.2	10.8
IL-6 (pg/ml)	1.03 ± 1.07	0.44	1.31	0.87
Cortisol AUC (µg/dL)	3.36 ± 1.31	2.83	3.71	0.88
Blood Pressure (diastolic + systolic)	172 ± 17	160	180	20
Child Variables:				
Offspring Sex, n (%Male)	16 (53%)	-	-	-
Offspring Age (yrs)	4.5 ± 0.6	3.9	5.1	1.2
Lymphocyte Count (thos/µL)	3.27 ± 0.96	2.50	3.90	1.40
Monocyte Count (thos/µL)	0.50 ± 0.24	0.35	0.60	0.25
Mitochondrial measures:				
Complex I (mmol/min/10^6 cells)	9.78 ± 3.35	7.64	11.28	3.64
Complex II (mmol/min/10^6 cells)	64.7 ± 29.2	47.7	77.0	29.4
Complex IV (mmol/min/10^6 cells)	16.6 ± 6.2	12.3	20.4	8.1
CS (mmol/min/10^6 cells)	126.7 ± 41.7	96.3	141.2	44.8
mtDNAcn (copies per cell)	407.4 ± 112.5	317.2	467.6	150.4

The maternal allostatic load score and the components are mean pregnancy values (mean across early-, mid- and late- gestation visits). SES= socioeconomic status; BMI= body mass index; Log HOMA-IR= logarithmic base 10 of the homeostatic model of insulin resistance; FFA= free fatty acid; CRP= c-reactive protein; IL-6= interleukin 6; AUC= Area under the curve; CS= citrate synthase; mtDNAcn= mitochondrial DNA copy number; all reported enzymatic activity levels are normalized to nuclear DNA copies (i.e., per cell).

Table 2:

Association between measures of mitochondrial content, mitochondrial bioenergetic capacity and leukocyte populations

		mitochondrial bioenergetic capacity (per cell)			mitochondria-specific bioenergetic capacity			mitochondrial content		leukocyte populations	
		CI activity	CII activity	CIV activity	CI activity/ per mito	CII activity/ per mito	CIV activity/ per mito	CS activity	mtDNAcn	Lymphocyte count (thos/ μL)	Monocyte count (thos/ μL)
CI activity	r	1	0.88	0.91	0.33	0.44	0.41	0.86	0.87	0.42	0.09
	p		<.0001	<.0001	0.0725	**0.0157**	**0.0238**	<.0001	<.0001	**0.0232**	0.6447
CII activity	r	0.88	1	0.92	0.18	0.62	0.49	0.83	0.94	0.57	0.01
	p	<.0001		<.0001	0.3313	**0.0003**	**0.0064**	<.0001	<.0001	**0.0011**	0.9695
CIV activity	r	0.91	0.92	1	0.03	0.36	0.45	0.94	0.95	0.43	0.11
	p	<.0001	<.0001		0.8838	**0.0475**	**0.0125**	<.0001	<.0001	**0.0215**	0.5947
CI activity/ per mito	r	0.33	0.18	0.03	1	0.68	0.59	−0.17	0.09	0.35	0.1
	p	0.0725	0.3313	0.8838		**<.0001**	**0.0006**	0.3699	0.6221	0.0641	0.6105
CII activity/ per mito	r	0.44	0.62	0.36	0.68	1	0.76	0.12	0.48	0.58	0.01
	p	**0.0157**	**0.0003**	**0.0475**	**<.0001**		**<.0001**	0.5223	**0.0075**	**0.0009**	0.975
CIV activity/ per mito	r	0.41	0.49	0.45	0.59	0.76	1	0.14	0.46	0.51	0.18
	p	**0.0238**	**0.0064**	**0.0125**	**0.0006**	**<.0001**		0.4497	**0.0105**	**0.005**	0.3518
CS activity	r	0.86	0.83	0.94	−0.17	0.12	0.14	1	0.87	0.25	0.05
	p	<.0001	<.0001	<.0001	0.3699	0.5223	0.4497		<.0001	0.184	0.7825
mtDNAcn	r	0.87	0.94	0.95	0.09	0.48	0.46	0.87	1	0.49	0.04
	p	<.0001	<.0001	<.0001	0.6221	**0.0075**	**0.0105**	<.0001		**0.0065**	0.8592
Lymphocyte count (thos/ μL)	r	0.42	0.57	0.43	0.35	0.58	0.51	0.25	0.49	1	0.23
	p	**0.0232**	**0.0011**	**0.0215**	0.0641	**0.0009**	**0.005**	0.184	**0.0065**		0.1522
Monocyte count (thos/ μL)	r	0.09	0.01	0.11	0.1	0.01	0.18	0.05	0.04	0.23	1
	p	0.6447	0.9695	0.5947	0.6105	0.975	0.3518	0.7825	0.8592	0.1522	

Pearson correlation (r) with p-value to examine the linear relationship between mitochondrial content, mitochondrial bioenergetic capacity (enzymatic activity indexed per cell), mitochondria-specific bioenergetic capacity (enzymatic activity indexed per mitochondrion (i.e., divided by CS)) and leukocyte populations. CI= complex I; CII= complex II; CIV= complex IV; CS= citrate synthase; mtDNAcn= mitochondrial DNA copy number; mito= mitochondria. P values unadjusted for multiple testing are shown.

Table 3.

Effect size estimation

Child Mitochondrial Measures	Low Maternal Allostatic Load			High Maternal Allostatic Load			Cohen's D effect size (median AL split)	Correlation with Maternal Allostatic Load r (p-value)
	Mean	SD	N	Mean	SD	N		
CS	111.2	26.4	15	142.2	48.9	15	**0.789**	0.47 (0.009)
mtDNAcn	368.4	80.1	15	446.4	128.6	15	**0.729**	0.44 (0.014)
Complex I	8.6	2.9	15	11	3.4	15	**0.766**	0.43 (0.019)
Complex II	53.7	20.3	15	75.7	33.1	15	**0.802**	0.46 (0.011)
Complex IV	14.2	4.2	15	19.1	7	15	**0.854**	0.48 (0.008)

Maternal allostatic load was divided into a "high" and "low" allostatic load (by a median split), and a Cohen's D effect size was calculated to examine the magnitude of the relationship between high vs. low maternal allostatic load and offspring mitochondrial content and mitochondrial bioenergetic capacity (enzymatic activity indexed per cell). Additionally, the Pearson correlation (r) with p-value was calculated to further examine the magnitude of the linear association between maternal allostatic load and mitochondrial content, and mitochondrial bioenergetic capacity (enzymatic activity indexed per cell). AL= maternal allostatic load; CS= citrate synthase; mtDNAcn= mitochondrial DNA copy number; CI= complex I; CII= complex II; CIV= complex IV.