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Saliva cell-free mitochondrial DNA (cf-mtDNA) as a dynamic biomarker of stress and emotion in daily life: Evidence from two independent repeated-measures studies

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ABSTRACT

The present study analyzes the impact of naturalistic stress and emotions on saliva cell-free mitochondrial DNA (cf-mtDNA) in daily life across two independent cohorts with different temporal resolutions. Study 1 examined the interaction between daily stress and major depressive disorder (MDD) on cf-mtDNA in young adults (n = 18, 8 MDD, 10 controls). Across four days, participants provided 4 saliva samples and nightly stress inventories. Among individuals with MDD, cf-mtDNA concentrations were 68% lower on days with reported stress exposure compared with days without reported stress exposure. Among stress-exposure days, a greater number and higher severity of stressors were associated with 24–27% lower cf-mtDNA concentrations. Study 2 extended this framework by implementing a finer temporal resolution in healthy young adults (n = 25), measuring saliva and momentary affective states with hourly ecological momentary assessments, up to 20 times per day for 2 days. Higher positive affect was associated with higher cf-mtDNA; however, this relationship weakened as negative affect increased. Exploratory analyses of individual emotions showed that cf-mtDNA tended to be higher during positive emotional states and lower during negative emotional states; however, person-specific models indicated that these associations, particularly for happiness and stress, varied substantially across participants. Overall, cf-mtDNA did not exhibit a uniform stress response in daily life; rather, the dynamics were dependent on diagnostic status and stress exposure timing in Study 1, and on momentary emotional context in Study 2. Accordingly, cf-mtDNA should be conceptualized as a dynamic biobehavioral signal rather than a static indicator of between-person differences.

1. Introduction

Stress is among the most potent predictors of poor mental and physical health outcomes, conferring an increased risk of depression, obesity, diabetes, and cancer (Epel et al., 2018). In everyday life, stress can arise from relatively minor yet frequent events, such as tight work deadlines and interpersonal conflicts. In a national daily diary study of U.S. adults, participants reported at least one such stressor on approximately 40% of interview days, though rates of exposure likely vary across socioeconomic and other contextual conditions (Almeida et al., 2002). Although it is well established that repeated activation of the stress response is a major contributor to accelerated physiological wear and tear (McEwen, 1998), the rapid biological cascades that follow naturally occurring stressors have not been fully characterized. Advances in stress assessment methods, particularly ecological momentary assessment (EMA) and daily diary approaches, have enabled detailed

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characterization of these stress responses as they unfold in real time (Almeida et al., 2002; Shiffman et al., 2008). However, measuring relevant biomarkers at comparable frequencies has proven difficult, severely limiting our understanding of the immediate biological effects of naturalistic stressors. Addressing this gap will require the use of biomarkers that can be sampled frequently in daily life, aligning with the temporal granularity of existing psychological assessments.

1.1. Cell-free mitochondrial DNA as a candidate biomarker of acute stress processes in daily life

Cell-free mitochondrial DNA (cf-mtDNA) is a compelling candidate to address this gap. While cortisol is one of the most commonly studied stress biomarkers (Hellhammer et al., 2009), it only reflects the activity of a single arm (i.e., the hypothalamic-pituitary-adrenal axis) of a much broader stress-response network (Picard and McEwen, 2018). As central regulators of cellular energy and adaptation, mitochondria represent another essential arm, or potential downstream target of this system (Picard and McEwen, 2018). Following stress, mitochondria release mitochondrial-derived molecules, like cf-mtDNA, into circulation, which has systemic implications. MtDNA shares characteristics (e.g., circularity and size) with bacterial DNA that can trigger an inflammatory response when released into circulation (Collins et al., 2004; West and Shadel, 2017).

This immunological activity has positioned cf-mtDNA as a pro-inflammatory biomarker of disease (de Miranda et al., 2024; Harrington et al., 2019; Meng et al., 2019; Sayal et al., 2023; Kananen et al., 2020). Elevated levels are consistently associated with poor health outcomes in severe illnesses like cancer and sepsis (de Miranda et al., 2024; Harrington et al., 2019; Meng et al., 2019; Sayal et al., 2023; Kananen et al., 2020). However, emerging evidence suggests cf-mtDNA is not merely a marker of acute pathology. It fluctuates dynamically, exhibiting diurnal variation and rapid reactivity to psychosocial stress (Limberg et al., 2025; Trumpff et al., 2019, 2022, 2025; Behnke et al., 2025). In controlled experiments, serum cf-mtDNA increases ~1.7–3-fold within 20–30 min of socio-evaluative stress (Hummel et al., 2018), and saliva cf-mtDNA levels rise by ~2.8-fold on average (with some participants showing >10-fold elevations) within just 10 min (Trumpff et al., 2025). These findings suggest that cf-mtDNA may function as a rapid stress-responsive signal, rather than solely as a downstream correlate of disease. Determining whether cf-mtDNA exhibits comparable responsiveness to real-world stressors is critical for clarifying its role in the biological embedding of stress (Trumpff et al., 2019).

1.2. Psychiatric conditions and cf-mtDNA

Chronic stress disrupts saliva cf-mtDNA dynamics, predicting flattened cf-mtDNA rhythms and reduced morning surges 1–4 h after awakening (Behnke et al., 2025). Altered stress responsivity is a hallmark of many psychopathologies, and decades of research on cortisol show that both reactivity and recovery differ systematically among these individuals (Burke et al., 2005; Zorn et al., 2017). Among psychiatric disorders, major depressive disorder (MDD) stands out as a particularly relevant context in which to study potential alterations to cf-mtDNA-stress dynamics (Burke et al., 2005; Zorn et al., 2017). MDD is characterized by persistent low mood, altered reactivity to daily stress, impairments in energy metabolism, and increased oxidative stress (Gimenez-Palomo et al., 2021). Accordingly, individuals with MDD may exhibit distinctive cf-mtDNA release patterns, particularly following acute stress. Studies comparing cf-mtDNA concentrations between groups have yielded mixed results, underscoring the need for within-person designs (Daniels et al., 2024; Lindqvist et al., 2016, 2018; Melamud et al., 2023; Park et al., 2022; Behnke et al., 2023; Trumpff et al., 2021). Some studies have reported elevated cf-mtDNA concentrations among individuals with depression and other psychiatric conditions (Lindqvist et al., 2016; 2018), whereas others have found weaker, absent, or context-dependent associations (Melamud et al., 2023; Park et al., 2022). Similarly, acute laboratory stressors have been associated with rapid increases in cf-mtDNA (Trumpff et al., 2019; 2025; Hummel et al., 2018), yet little is known about how cf-mtDNA behaves in response to naturally occurring stressors, particularly in individuals with psychopathologies. Importantly, most prior studies have relied on between-person designs or responses to experimentally induced stress, whereas comparatively few have examined within-person fluctuations in naturally occurring stress and emotion. As a result, it remains unclear whether cf-mtDNA is more informative as a stable marker of psychiatric status or as a dynamic indicator of ongoing psychobiological processes. Intensive longitudinal designs are particularly well suited to address this question because they allow stress, affect, and cf-mtDNA to be measured repeatedly as they unfold in everyday life.

1.3. The present study

In the present study, we examine whether saliva cf-mtDNA responds to day- and momentary-level psychological stress under ecologically valid, real-world conditions, using data from two independent cohorts with intensive repeated-measures designs. Across both studies, we test whether real-world psychosocial dynamics map onto cf-mtDNA in the same direction as laboratory stress reactivity, or whether timing and psychological context produce distinct signatures. Study 1 investigates whether exposure to daily stress influences saliva cf-mtDNA concentrations among healthy adults and individuals with MDD across four consecutive days. Study 2 uses hourly sampling with EMA to examine how acute fluctuations in stress and emotion affect concurrent saliva cf-mtDNA concentrations. The primary outcome of the study is cf-mtDNA; however, cell-free nuclear DNA (cf-nDNA) is also quantified as a control. Divergence of these markers would suggest a more specific regulatory mechanism outside of cell death. Additionally, while stress is the primary focus of this paper, affect represents a broader, multidimensional construct that both shapes and is shaped by stress. Negative affect can arise as a consequence of stress, yet heightened negative affective states may also amplify the perception of stress (Feldman et al., 1999). Because positive affect can influence physiological processes and may buffer stress responses (van Steenbergen et al., 2021), we conducted exploratory analyses testing whether cf-mtDNA is sensitive not only to stress and negative affect individually, but also to their interplay with positive affect.

2. Methods

2.1. Study 1: daily stress, cf-mtDNA, and major depressive disorder

2.1.1. Participants and procedures

This study was approved by The Pennsylvania State University Institutional Review Board (IRB #9617) and registered with the Food and Drug Administration (IND #125994). The full protocol has been previously described in detail (Greaney et al., 2020; study design in Fig. 1A). Participants (n = 18, 8 MDD, 10 healthy controls [HC]; 10 females; age: $M = 23 \pm 4$ years; BMI: $M = 24.95$, $SD = 4.76$ kg/m^2) were recruited from the Pennsylvania State University and the surrounding area. To determine eligibility, individuals underwent the Mini-International Neuropsychiatric Interview (MINI), which identifies current or past major psychiatric disorders according to the criteria in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM–5). The MINI diagnostic algorithm identified 8 participants who met criteria for MDD (4 female) and 10 healthy controls (6 female) who demonstrated no history of major psychiatric illness (Sheehan et al., 1998). Exclusion criteria included the presence of any comorbid major psychiatric condition (e.g., bipolar disorder, schizophrenia, etc.), the current or recent (within 8 weeks) use of psychoactive or psychopharmacological drugs (including antidepressants and anxiolytics), and

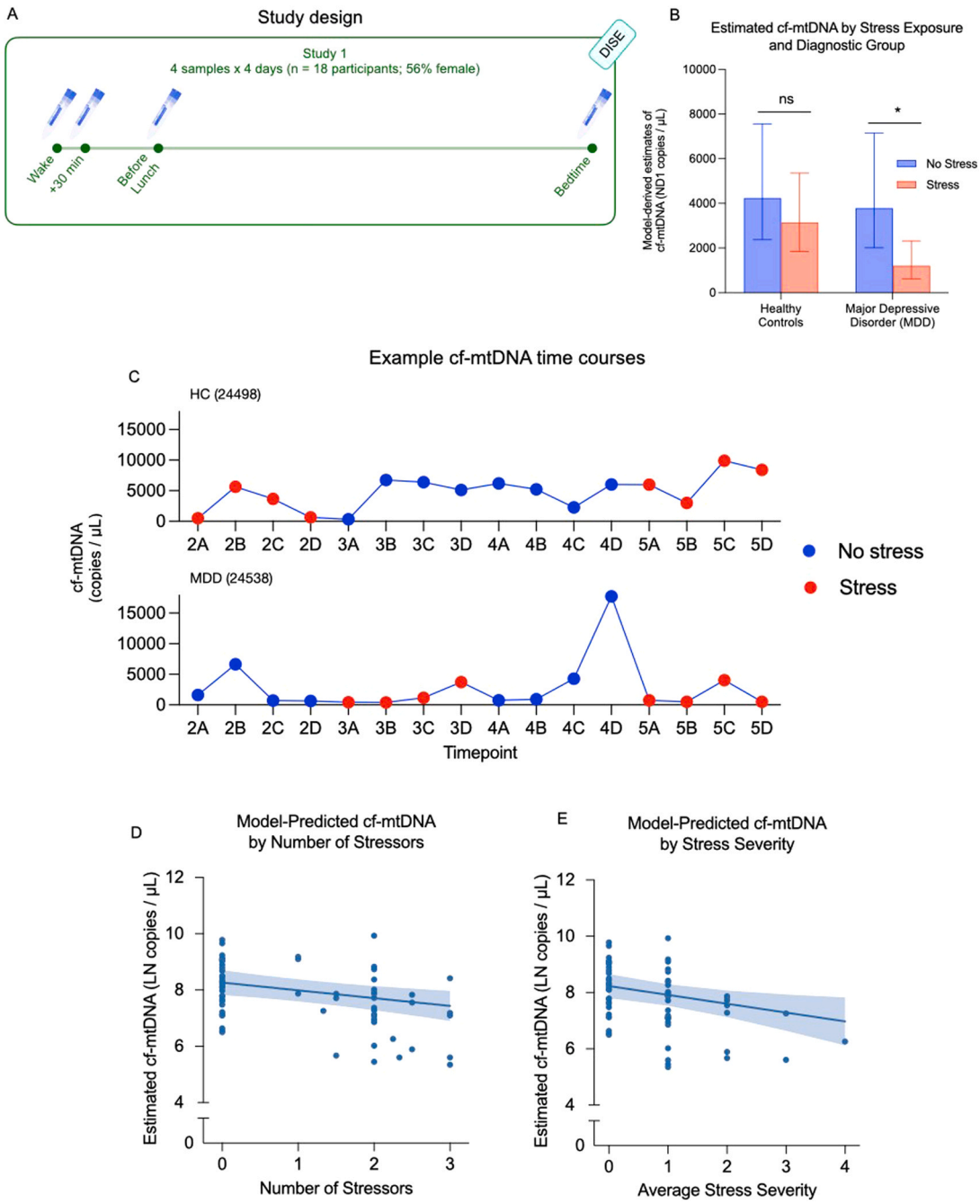

Fig. 1. Study 1: Cell-free mitochondrial DNA (cf-mtDNA) dynamics by day-level stress. **(A)** Protocol schematic depicting four saliva samples per day across four consecutive days with nightly Daily Inventory of Stressful Events (DISE) interviews (n = 18, MDD = 8, HC = 10, 260 saliva samples). **(B)** The interaction between cf-mtDNA and stress exposure for healthy controls and participants with Major Depressive Disorder (MDD). The effect of stress exposure was significant for participants with MDD (68% reduction), but not for healthy controls. **(C)** Example plot of cf-mtDNA concentrations across all timepoints and days for one participant with MDD and one healthy control to illustrate the repeated-measures structure of the data. On the x-axis, the numeric value represents the day, and the letter represents the time point within the day. **(D)** Model-predicted values of ln(cf-mtDNA) as a function of the number of stressors are presented. Each one-unit increase in the number of stressors was associated with a 27% lower concentration on the response scale, calculated from the exponentiated GLMM coefficient (log-b = –0.31, p = .01). **(E)** Model-predicted ln(cf-mtDNA) values as a function of average daily stressor severity are presented. Each one-unit increase in stress severity was associated with 24% lower cf-mtDNA concentration on the response scale, derived from the exponentiated GLMM coefficient.

active suicidal ideation with intent or plans. Additional details regarding diagnostic classification and clinical screening procedures can be found in previous publications (Darling et al., 2024; Greaney et al., 2019, 2022).

2.1.2. *Saliva collection*

Participants collected saliva four times each day (i.e., immediately upon awakening, 30 min after awakening, before lunch, and at bedtime) for four consecutive days. Participants were provided with detailed instructions to collect samples independently at home using Sarstedt Salivettes (Cat# 51.1534.500). Participants recorded saliva collection times in daily diaries. Samples were stored in participants' home freezers at approximately –20°C. The samples were transported on ice to the Mitochondrial Psychobiology Laboratory at Columbia University for processing. Nearly all participants returned complete sample sets (16 samples each), totaling 284 samples or 72 complete days of data. Six

days (24 samples) were excluded due to missing stress data, and one additional day (4 samples) was removed for being outside the interquartile range for cf-mtDNA. After exclusions, 260 samples or 65 days of data (29 non-stressor and 36 stressor) were included in the final analysis.

2.1.3. Assessment of daily stress

Daily stress was measured using the Daily Inventory of Stressful Events (DISE), a validated 24-hour retrospective checklist of six common stressors: arguments, argument avoidance, stressful events at work or school, stressful events at home, discrimination, network stress, and any other stressors (Almeida et al., 2002). A day was classified as a stressor day if at least one DISE-validated event was reported, and as a non-stressor day if no events occurred. Participants rated the perceived severity of each stressor on a 0–4 scale, which was averaged to yield a daily mean severity score. Additionally, the total number of stressors reported was summed to capture the frequency of daily stressors. Saliva concentrations were averaged within day to align biomarker timescales with the DISE, yielding day-level cf-mtDNA and cf-nDNA values.

2.2. Study 2: hourly affect and cf-mtDNA

2.2.1. Participants and procedures

This study was approved by the New York State Psychiatric Institute Institutional Review Board (IRB #8149). Adults (n = 25, 13 females, age: $M = 31 \pm 8$ years; BMI: $M = 23.9$, $SD = 3.1$ kg/m^2) were recruited from the Columbia University Irving Medical Center (CUIMC) regional area (Behnke et al., 2025). Participants completed two days of intensive hourly saliva and EMA sampling according to established procedures (Kirschbaum and Hellhammer, 1994; see study design in Fig. 2A).

2.2.2. Saliva collection

On each study day, participants collected three saliva samples within the first hour of awakening (immediately upon awakening, 30 min post-awakening, and 45 min post-awakening), followed by hourly samples for the remainder of the day, yielding up to 20 samples per day. To minimize contamination, participants were instructed to refrain from eating, brushing their teeth, and drinking for at least 30 min before collection. Samples were collected using Sarstedt Salivettes (Cat# 51.1534.500) and stored in home freezers at approximately –20°C. Participants shipped the samples to the Mitochondrial Psychobiology Laboratory at Columbia University with the provided ice packs. All samples arrived frozen. On average, participants returned 16.5 (SD = 3.68) saliva samples per day. A single outlier with a cf-mtDNA value 16 SDs above the mean was removed.

2.2.3. Assessment of emotional states

During each saliva collection, participants completed a brief survey on a mobile device through REDCap, rating their current emotional state. The EMA included ratings of eight emotions selected to capture both positive (i.e., happy, energetic, in control, and calm) and negative (i.e., stressed, anxious, fatigued, and frustrated) affective states. Responses were recorded on a 5-point scale (0 = "not at all" to 4 = "extremely"). On average, participants completed 13.86 (SD = 3.17) EMA measurements per day.

2.2.4. Saliva processing for Study 1 and Study 2

For both studies, saliva samples were stored at –80 °C and processed in batches to minimize inter-assay variability. During processing, samples underwent a maximum of two freeze-thaw cycles. Salivettes were thawed at room temperature and centrifuged at 1000 g for five minutes. The clear saliva fraction was carefully aspirated from the top of the tube using a pipette, leaving the cellular debris at the bottom of the tube. This fraction was transferred to 1.5 mL microcentrifuge tubes and spun again at 5000 g for 10 min. The resulting supernatants were transferred to new 1.5 mL tubes. Storage conditions, duration, and freeze-thaw history were applied uniformly across all samples.

2.2.5. Cf-mtDNA and cf-nDNA quantification

Cf-mtDNA and cf-nDNA were quantified from the saliva supernatant using a modified version of the MitoQuicLy protocol (Michelson et al., 2023). Supernatants were thermolyzed in duplicate overnight. The resulting lysates were analyzed in triplicate using real-time quantitative polymerase chain reactions (qPCR) with TaqMan chemistry, targeting the mitochondrial gene ND1 and the nuclear gene B2M simultaneously. For each target, triplicate cycle threshold (Ct) values were compared against a DNA standard curve to calculate absolute copy numbers. Triplicate Ct values were inspected, and the two most concordant replicates were averaged to obtain the final value. Between-plate reliability of duplicates was tested using intraclass correlation coefficients (ICCs) with the irr package in R (Gamer et al., 2019; version 4.4.2; R Core Team., 2024).

2.3. Analytical approach

2.3.1. Study 1

Prior to conducting the primary analysis, we ensured the number and severity of stressors did not differ by diagnostic group (MDD vs. HC). To test this, we used a Poisson generalized linear mixed-effects model with a log link for the number and a linear mixed-effects model for severity. Additionally, we assessed the relationship between log-transformed cf-mtDNA and cf-nDNA concentrations using repeated measures correlations. To examine the influence of stressor days, diagnostic group, and their interaction on saliva cf-mtDNA levels, generalized linear mixed-effects models (GLMMs) with a gamma error distribution, log link, and random intercepts to capture individual differences in baseline cf-mtDNA were computed using the glmmTMB package in R (Brooks et al., 2017). The primary model was specified as:

$$\log(\mu_{ij}) = \beta_0 + \beta_1(\text{Stress}_{ij}) + \beta_2(\text{Group}_j) + \beta_3(\text{Stress}_{ij} \times \text{Group}_j) + u_{0j}$$

where μ_{ij} represents the expected cf-mtDNA concentration for participant j on day i, β_0 is the fixed intercept, and $u_{0j} \sim N(0, \sigma_u^2)$ captures the between-person variability in baseline cf-mtDNA levels (no stress day, number of stressors = 0, or stress severity of 0). β_2 estimates the differences in cf-mtDNA levels between diagnostic groups, while β_3 captures the interaction effect, indicating whether stress-related changes in cf-mtDNA differ between MDD and HC participants. The second model incorporated time-invariant covariates (age, sex, and BMI) to control for potential confounders:

$$\log(\mu_{ij}) = \beta_0 + \beta_1(\text{Stress}_{ij}) + \beta_2(\text{Group}_j) + \beta_3(\text{Stress}_{ij} \times \text{Group}_j) + \beta_4(\text{Age}_j) + \beta_5(\text{Sex}_j) + \beta_6(\text{BMI}_j) + u_{0j}$$

where β_4, β_5, and β_6 estimate the effects of age, sex, and BMI, respectively. Additionally, both models were performed with cf-nDNA as the outcome. These models served as an additional check to determine whether the observed cf-mtDNA relationship was specific to mtDNA or reflected broader changes (e.g., cell death). A significant effect of stress on cf-mtDNA, but not cf-nDNA, would suggest mitochondrial specificity.

Model fit was assessed using Akaike Information Criterion and Bayesian Information Criterion (AIC/BIC). Lower relative AIC and BIC values indicate a better fit, and a reduction of greater than 2 points was interpreted as a meaningful improvement (Burnham and Anderson, 2004). Estimated marginal means and contrast estimates were calculated with the emmeans package to obtain back-transformed effects on the original response scale (Lenth, 2025).

2.3.2. Study 2

A confirmatory factor analysis (CFA) was performed to reduce the eight psychological states into two latent affective dimensions, positive affect (PA; calm, in control, energetic, and happy) and negative affect (NA; stressed, anxious, fatigued, and frustrated). These scores were used

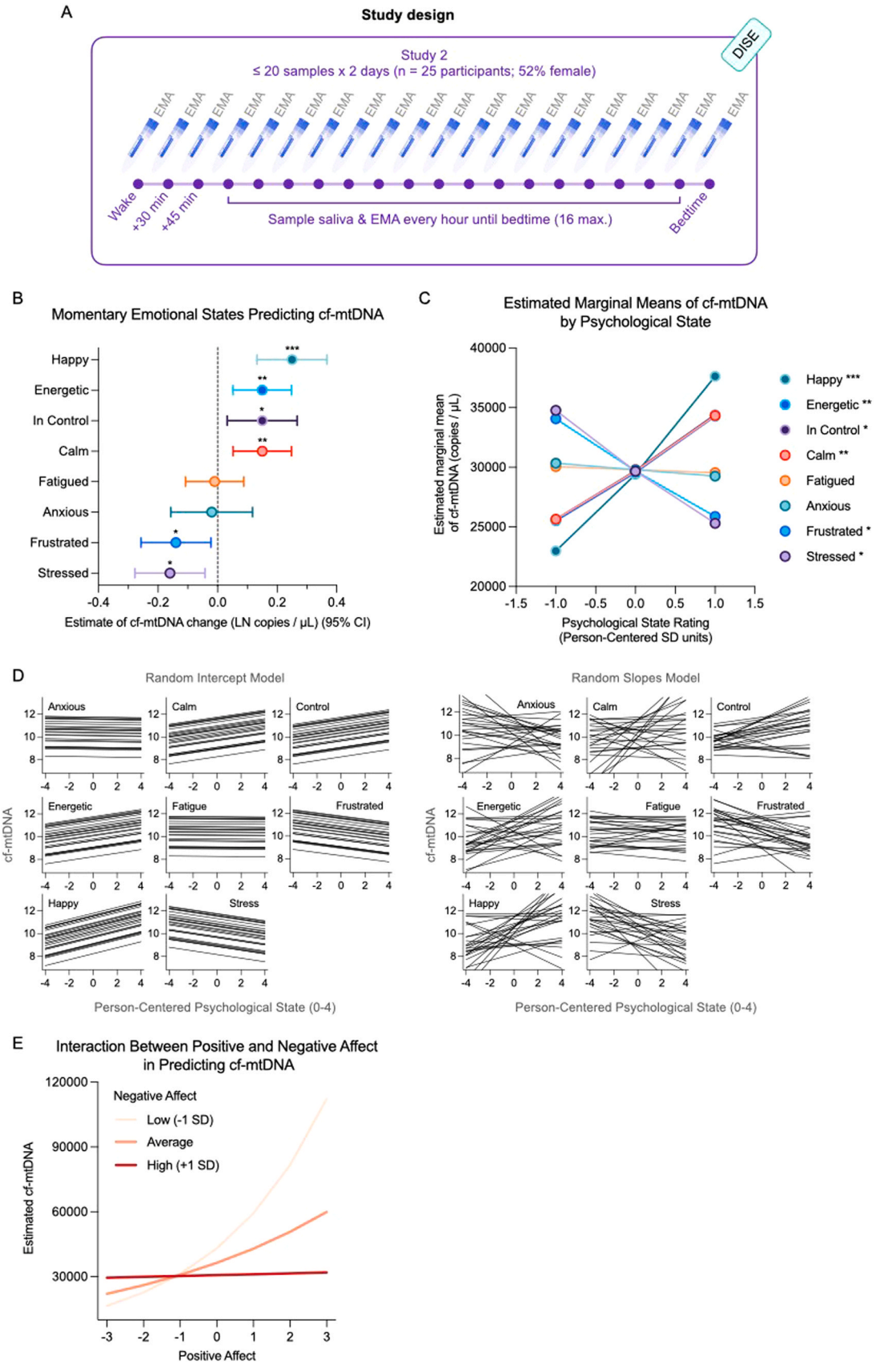

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Fig. 2. Study 2: Cell-free mitochondrial DNA (cf-mtDNA) dynamics by momentary emotional states. **(A)** Protocol schematic depicting two days of hourly saliva collection with simultaneous EMAs (n = 25, 826 saliva samples). **(B)** Forest plot of fixed effects from gamma generalized linear mixed models (random intercepts; each emotion was person-centered). Asterisks indicate significant effects. **(C)** Estimated marginal means of cf-mtDNA levels on the response scale (copies/mL) plotted by psychological state (+/– 1 SD), error bars = 95% CI. **(D)** Models with fixed and random slopes demonstrate the person-specific heterogeneity of effects, with asterisks indicating significant effects for happiness (–49% to + 262%) and stress (–68–80%). Only 6 participants had negative slopes for happiness, while 4 participants had positive slopes for stress. **(E)** Positive Affect (PA) × Negative Affect (NA) interaction using CFA-derived latent factors. There was a significant interaction effect between PA and NA on cf-mtDNA (log-b = –0.22, SE = 0.10, p = .02). At the mean level of NA, higher PA was associated with higher cf-mtDNA (log-b = 0.33, SE = 0.12, p = .005), whereas NA showed no main effect at the mean level of PA (log-b = –0.02, SE = 0.09, p = .83). No significant main effects or interaction were observed for cf-nDNA (log-b = –0.15, SE = 0.11, p = .16). Thus, NA attenuated the positive association between PA and cf-mtDNA.

to test the effect of PA, NA, and their interaction on cf-mtDNA and cf-nDNA. The model was estimated using full-information maximum likelihood (FIML). Modification indices were examined to improve model fit, and changes were implemented iteratively. Residual covariances between energetic and happy, as well as between fatigued and energetic, were added based on these indices. Additionally, a cross-loading of fatigue onto both PA and NA was introduced. All modifications were made one at a time and retained only if they improved model fit without undermining theoretical interpretability. The final model demonstrated a good fit (χ^2 (16) = 92.48, p < .001; CFI = 0.98; RMSEA = 0.07; SRMR = 0.03). All factor loadings were statistically significant ($ps \leq .006$). Loadings ranged from 0.13 to 0.69 for NA and from 0.85 to 1.21 for PA, with fatigued cross-loading negatively onto PA. Factor scores derived from the CFA were grand-mean centered. To isolate within-person effects, factor scores were person-centered by subtracting each participant's mean from their time-specific value. These within-person deviations were entered into a GLMM using the following equation:

$$\log(\mu_{ij}) = \beta_0 + \beta_1(\mathrm{NA}_{ij}) + \beta_2(\mathrm{PA}_{ij}) + \beta_3(\mathrm{NA}_{ij} \times \mathrm{PA}_{ij}) + u_{0j}$$

.

An exploratory analysis of the eight momentary person-centered psychological states was performed to assess their correspondence with cf-mtDNA and cf-nDNA concentrations. Momentary emotion ratings were person-centered, so coefficients reflected changes in cf-mtDNA for a 1-unit deviation from each individual's average. The effects of each of the EMA psychological variables on cf-mtDNA and cf-nDNA were analyzed using GLMMs with gamma error distributions, log links, and random intercepts to capture individual differences in baseline cf-DNA. The models assessed the relationship of person-centered psychological states and cf-mtDNA while allowing for between-person variability in baseline cf-mtDNA through random intercepts. Random slopes were introduced to assess the variability in individual responses to each emotion. Because eight separate psychological-state models were estimated, p-values for these exploratory analyses were adjusted for multiple comparisons using the Benjamini-Hochberg false discovery rate (FDR) procedure. Adjusted p-values are reported alongside unadjusted results.

$$\log(\mu_{ij}) = \beta_0 + \beta_1(\mathrm{PsychologicalState}_{ij}) + u_{0j}$$

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3. Results

3.1. Study 1: daily stress, cf-mtDNA, and major depressive disorder

3.1.1. Sample characteristics and data quality

On stressor days, participants reported approximately one stressor (M = 1.39, SD = 0.73, range 0–4) with an average severity of 1.89 (SD = 0.78; range 0–3). Notably, participants with MDD did not differ from healthy controls in the number (log-b = 1.13, 95% CI [0.50, 2.31], p = .73) or severity (log-b = –0.08, 95% CI [–0.83, 0.65], p=.83) of stressors reported.

Between-plate reliability was excellent for cf-mtDNA (ICC =.95, p < .001) and cf-nDNA (ICC =.93, p < .001). Within persons, cf-mtDNA and cf-nDNA were positively correlated (r_{rm} =.59, 95% CI [0.49, 0.67], p < .001; Supplemental Figure S1A), reflecting the expected moderate concordance between the two markers.

3.1.2. Stress exposure is associated with reduced cf-mtDNA

There were no significant main effects of diagnostic group or daily stress exposure on cf-mtDNA concentrations; however, there was a significant group × stress exposure interaction. Daily stress exposure predicted lower cf-mtDNA in MDD, suggesting stress-contingent dysregulation rather than a baseline group difference (log-b = –0.85, 95% CI [–1.65, –0.05], p = .03; Table 1 Model 1; Fig. 1B). Post hoc comparisons showed that in healthy controls, cf-mtDNA levels were approximately 26% lower on stressor days (~3145 copies/μL) versus non-stressor days (~4238 copies/μL), although this difference was not significant (1.35-fold; log-contrast = 0.30, SE = 0.25, p=.22). In individuals with MDD, cf-mtDNA concentrations were 68% lower on stressor days (~1206 copies/μL) versus non-stressor days (~3791 copies/μL; log-contrast = 1.15, SE = 0.32, p<.001). When a statistical outlier, or one non-stressor day with cf-mtDNA concentrations 3.67 times higher than the next highest measure, was included, these effects remained significant (Supplemental Table S1). Example trajectories for MDD and HC participants are shown in Fig. 1C.

The conditional R^2 suggests this model explained approximately 71% of the variance, with the marginal R^2 (fixed effects) accounting for 20.4%, indicating substantial inter-individual variability in cf-mtDNA. A sensitivity analysis adjusting for BMI, age, and sex did not change the pattern of results (Table 1, Model 2). These covariates collectively added ~10% more explained variance through fixed effects, but none were individually significant. Although cf-nDNA correlated with cf-mtDNA

Table 1
Study 1: Day-level stress exposure predicting saliva cf-mtDNA in GLMMs with random intercepts.

	Model 1		Model 2	
Intercept	8.35 (0.30)***		5.93 (1.55)***	
Stress Exposure	–0.30 (0.25)		–0.35 (0.25)	
Group (MDD)	–0.11 (0.44)		–0.15 (0.42)	
Stress × Group	–0.85 (0.40)*		–0.79 (0.40)*	
Age	—		0.06 (0.05)	
BMI	—		0.04 (0.04)	
Sex	—		0.50 (0.37)	
Intercept variance (ID)	0.54 (0.74)		0.44 (0.67)	
AIC	1180		1184	
BIC	1194		1203	
Estimated Marginal Means (SE)	Non-stressor Day	Stressor Day	Non-stressor Day	Stressor Day
Healthy Control	4238 (1250)	3145 (855)	4409 (1250)	3123 (799)
Major Depressive Disorder	3791 (1230)	1206 (401)	3806 (1160)	1221 (387)

Note. Results from gamma generalized linear mixed models with a log link predicting cell-free mitochondrial DNA (cf-mtDNA) as a function of day-level stress exposure. Both models included random intercepts to account for repeated measures. Model fit indices include AIC and BIC, where lower values indicate a better fit. Estimated marginal means are reported on the response scale (copies/μL) on non-stressor and stressor days. Coefficients are presented as Estimate (SE). * p < .05, ** p < .01, *** p < .001.

within persons, there was no evidence of a relationship between stress exposure and cf-nDNA (*ps* > .25; Supplemental Figures S2A–S2C, Supplemental Table S2), suggesting that the effect of stress was specific to cf-mtDNA.

3.1.3. A higher number and severity of stressors were associated with lower cf-mtDNA

An exploratory analysis of the dose-response effect of stress showed that the number (Fig. 1D) and severity (Fig. 1E; Supplemental Table S3) of stressors were each associated with lower cf-mtDNA. Each additional stressor experienced was associated with a 27% reduction (log-*b* = –0.31, *SE* = 0.12, *p* = .01) in cf-mtDNA at the day level. Similarly, a one-unit increase in stressor severity was associated with a 24% decrease (log-*b* = –0.28, *SE* = 0.10, *p* = .004) in cf-mtDNA. Although these models demonstrate a dose-response effect of stress on cf-mtDNA, we consider these results preliminary due to the limited number of high-stress scores in this cohort.

3.2. Study 2: hourly affective states and cf-mtDNA

3.2.1. Sample characteristics and data quality

A total of 826 saliva samples were returned. Data for seven emotions (i.e., happy, energetic, calm, in control, anxious, fatigued, and frustrated) were available for 807 samples, while 762 samples had valid stress ratings. Among positive emotions, calm was reported most often (present on 88.7% of assessments, *M* = 2.24, *SD* = 1.25), followed by feeling in control (85.7%, *M* = 2.11, *SD* = 1.31), happy (81.2%, *M* = 1.75, *SD* = 1.15), and energetic (74.3%, *M* = 1.47, *SD* = 1.19). Negative emotions were reported less frequently, with fatigue present on 54.4% of assessments (*M* = 0.88, *SD* = 1.05), followed by stress (41.5%, *M* = 0.56, *SD* = 0.79), anxiety (36.0%, *M* = 0.49, *SD* = 0.75), and frustration (23.5%, *M* = 0.33, *SD* = 0.69).

Between-plate reliability was high for both cf-mtDNA (ICC =.99, *p* < .001) and cf-nDNA (ICC =.99, *p* < .001). Repeated measures correlation tests revealed that cf-mtDNA and cf-nDNA were strongly associated (r_{rm} =.81, *p* < .001; Supplemental Figure S1B). At the hourly level, cf-mtDNA and cf-nDNA also fluctuated substantially.

3.2.2. Interaction between positive and negative affect on cf-mtDNA

The model revealed a significant PA × NA interaction (log-*b* = –0.22, *SE* = 0.10, *p* = .02; Fig. 2E), indicating that higher NA attenuated the positive association between PA and cf-mtDNA. Increased PA was associated with higher cf-mtDNA (log-*b* = 0.33, *SE* = 0.12, *p* = .005). NA showed no main effect at the mean level of PA (log-*b* = –0.02, *SE* = 0.09, *p* = .83). Participant trajectories for cf-mtDNA and cf-nDNA across time by hourly NA and PA levels are shown in Supplemental Figures S3A–B and S5A–B.

3.2.3. Cf-mtDNA increases with positive emotions and decreases with negative emotions

GLMMs with a gamma error distribution, a log link function, and random subject intercepts revealed divergent associations between person-centered affect ratings and contemporary cf-mtDNA concentrations (Table 2; Fig. 2B–2 C). Each of the four positive emotions was associated with higher cf-mtDNA levels. Every one unit increase in happiness corresponded to a 28% increase in momentary cf-mtDNA (log-*b* = 0.25, *SE* = 0.06, *p* < .001, *p*FDR < .001). Additional units in calmness (log-*b* = 0.14; *SE* = 0.05, *p* = .008, *p*FDR =.03), energy (log-*b* = 0.15, *SE* = 0.05, *p* = .002, *p*FDR =.02), and feeling in control (log-*b* = 0.15, *SE* = 0.06, *p* = .01, *p*FDR =.03) were each associated with ~16% increases in saliva cf-mtDNA levels.

Conversely, higher stress and frustration ratings were linked to lower saliva cf-mtDNA concentrations. Every one unit increase in stress corresponded to a 15% decrease in cf-mtDNA (log-*b* = –0.16, *SE* = 0.06, *p*=.01, *p*FDR =.03), and every one unit increase in frustration corresponded to a 13% decrease in cf-mtDNA (log-*b* = –0.14, *SE* = 0.06, *p* = .03, *p*FDR =.03). Neither anxiety (*p* = .80,) nor fatigue (*p* = .86) was significantly associated with momentary cf-mtDNA concentrations. Effects persisted after adjustment for age, sex, and BMI (Supplemental Table S4) and were robust to the inclusion of a statistical outlier (Supplemental Table S5).

3.2.4. Person-specific heterogeneity in affect–cf-mtDNA coupling

Models including random slopes revealed between-person heterogeneity in the relationship of cf-mtDNA with happiness and stress perception (Fig. 2D; Supplemental Table S6). In uncorrected models, momentary cf-mtDNA levels remained positively associated with happiness ratings (log-*b* = 0.31, *SE* = 0.12, *p* = .009; *p*FDR = .08) and negatively associated with stress (log-*b* = –0.28, *SE* = 0.12, *p* = .02, *p*FDR = .09). However, neither association remained significant after correction for multiple comparisons across the eight random slopes models. Despite this attenuation, substantial variability in within-person slopes was observed across individuals (–49% to +262% for happiness, –68% to +80% for stress), highlighting substantial inter-individual differences in these psychobiological associations.

The variance explained was considerably lower in Study 2 compared to Study 1. Across the univariate models, marginal R^2 values (fixed effects) accounted for approximately 1–3% of the variance. Allowing random slopes for happiness and stress modestly increased the explained variance from 1.5% to 2.2% and 1.3–1.4%, respectively, with the total variance explained remaining low (2.6% and 1.6%). The model with the greatest explanatory power was the one that tested the PA × NA interaction, which increased the fixed-effects R^2 to 3% and the total variance to 3.6%.

Table 2
Study 2: Person-centered psychological states predicting cf-mtDNA in GLMMs with random intercepts.

	Anxious	Calm	In Control	Energetic	Fatigued	Frustrated	Happy	Stressed
Fixed Effects								
Intercept	10.30 (0.20)***	10.30 (0.20)***	10.30 (0.20)***	10.29 (0.20)***	10.30 (0.20)***	10.30 (0.20)***	10.29 (0.20)***	10.30 (0.21)***
Variable	–0.02 (0.07)	0.14 (0.05)*	0.15 (0.06)*	0.15 (0.05)**	–0.01 (0.05)	–0.14 (0.06)*	0.25 (0.06)***	–0.16 (0.06)*
Random Effects								
Intercept	1.00 (1.00)	0.98 (0.99)	0.98 (0.99)	0.99 (0.99)	1.00 (1.00)	1.00 (1.00)	0.97 (0.98)	1.05 (1.02)
Model Fit Indices								
AIC	18,318	18,311	18,312	18,309	18,318	18,314	18,299	17,290
BIC	18,337	18,330	18,330	18,328	18,337	18,332	18,318	17,309
Estimated Marginal Means (SE)								
Low(–1)	30,339	25,639	25,637	25,500	30,056	34,066	22,978	34,769
Average (0)	29,796	29,666	29,702	29,562	29,797	29,685	29,400	29,660
High (+1)	29,263	34,327	34,410	34,272	29,540	25,868	37,616	25,301

Note. Results from gamma generalized linear mixed models with a log link predicting cell-free mitochondrial DNA (cf-mtDNA) as a function of person-centered emotions. All models included random intercepts to account for repeated measures. Model fit indices include AIC and BIC, where lower values indicate a better fit. Estimated marginal means are reported on the response scale (copies/μL) at low, average, and high levels of the psychological state. Coefficients are presented as Estimate (SE). Benjamini-Hochberg FDR-adjusted significance is indicated by asterisks. * $p < .05$, ** $p < .01$, *** $p < .001$.

3.2.5. Sensitivity analysis: Cf-nDNA is related to frustration and happiness

Results from the PA × NA interaction model were similar to those of the cf-mtDNA model (log-b = –0.17, SE = 0.07, p = .01; Supplemental Figure S3C), suggesting that elevated negative emotions may dampen the release of both nuclear and mitochondrial DNA at this time scale. In sensitivity analyses using cf-nDNA, 6 of the 8 emotions had no relation to momentary cf-nDNA. Happiness was associated with higher levels of cf-nDNA and remained significant after Benjamini-Hochberg FDR correction across the eight emotion-specific models (log-b = 0.21, SE = 0.07, p = .003, pFDR =.02). Frustration was associated with lower levels of cf-nDNA; however, this relationship did not survive correction for multiple comparisons (log-b = –0.16, SE = 0.07, p = .02, pFDR =.08; Supplemental Table S7). No other emotion was significantly associated with cf-nDNA. These associations were similar after covariate adjustment (Supplemental Table S8). Participant trajectories by positive and negative affect levels are provided in the supplement for both cf-mtDNA (Supplemental Figure S4A and S4B) and cf-nDNA (Supplemental Figure S5A and S5B).

4. Discussion

This study used both within-person and between-group analyses to evaluate the relationship between saliva cf-mtDNA and naturally occurring stressors and emotions in daily life. Overall, we found converging evidence that saliva cf-mtDNA is sensitive to stress, affect, and MDD diagnosis. At both the day and hourly level, naturalistic stressors were associated with reduced saliva cf-mtDNA concentrations. At the day level, this effect was specific to individuals with MDD and to cf-mtDNA, as it was not replicated with cf-nDNA. Preliminary findings suggest the existence of a dose-response relationship between the number or severity of daily stressors and cf-mtDNA levels. Study 2 replicated and extended the protocol of Study 1, revealing stress-induced attenuation of cf-mtDNA at the hourly level, with significant between-person heterogeneity.

Although prior work on cf-mtDNA has primarily examined psychological stress in isolation, the present study extends this literature by situating stress within a broader affective context. Person-centered analyses demonstrated that higher-than-average positive emotions were associated with higher cf-mtDNA levels, while higher-than-average stress and frustration were associated with lower cf-mtDNA levels. As elevated cf-mtDNA levels have been repeatedly associated with poor health outcomes such as worse illness severity in sepsis (de Miranda et al., 2024; Harrington et al., 2019), reduced survival in cancer (Meng et al., 2019; Sayal et al., 2023), and a higher risk of all-cause mortality in population-based cohorts (Kananen et al., 2020), it is unlikely that positive emotions directly increase cf-mtDNA concentrations per se. Rather, negative psychosocial experiences may limit the normal fluctuations of cf-mtDNA. The emerging picture is one in which daily stressors restrict the range over which saliva cf-mtDNA can fluctuate on short time scales. PA (in the absence of NA) may not increase cf-mtDNA, but permit more flexibility, which may be interpreted as an indication of adaptive capacity.

It should be noted that the direction of the associations observed in the present study diverged from those found in the laboratory (Limberg et al., 2025; Trumpff et al., 2019). This is not entirely unexpected, as real-world stressors are considerably different from those administered in controlled settings (Limberg et al., 2025; Trumpff et al., 2019). Prior laboratory studies have generally captured short-term cf-mtDNA increases following discrete, time-locked stressors, whereas the present studies sampled naturally occurring stressors whose onset, duration, and resolution were less precisely defined. Thus, discrepant directions of association may reflect differences in stressor timing and sampling density rather than a direct contradiction across studies. Everyday stressors often co-occur, persist for extended periods, and lack clear resolution, decreasing the likelihood of observing discrete peaks in cf-mtDNA. More studies using ambulatory designs are needed, as the stressors that contribute to disease unfold in the diffuse, ongoing conditions of daily life, rather than in brief, well-defined laboratory paradigms. Moreover, recent evidence indicates that saliva cf-mtDNA levels rise rapidly (within 10 min) following acute psychosocial stress (Trumpff et al., 2025). Therefore, the probability of obtaining saliva within that narrow post-stressor window with a standardized sampling schedule is low. If daily-life stress produces a similarly brief cf-mtDNA spike followed by a longer trough, then standardized hourly schedules will under-sample peaks and over-sample recovery. This offers a parsimonious explanation for the lower cf-mtDNA concentrations observed on stressor days, motivating event-contingent sampling to map peak-to-nadir trajectories in daily life.

In Study 1, MDD diagnosis alone was not associated with cf-mtDNA, and group differences emerged only in the context of stress exposure. This pattern may help reconcile inconsistencies in the emerging psychiatric cf-mtDNA literature. Although several studies have reported altered circulating cf-mtDNA concentrations in individuals with depression and other psychiatric disorders (Lindqvist et al., 2016; 2018; Park et al., 2022), the present findings suggest that stress-related changes in cf-mtDNA may be more apparent under conditions of environmental challenge than as stable group differences. Depressed individuals tend to experience stronger and more persistent emotional responses to everyday challenges, along with slower recovery and greater physiological reactivity (Peeters et al., 2003), which may impact their cf-mtDNA response. A stress-contingent dynamic aligns with diathesis-stress models in which latent vulnerabilities manifest under environmental challenge (Monroe and Simons, 1991). Similar results have been reported in studies of individuals with early-life adversity, in which group-by-gene expression differences only emerge under acute stress (Etzel et al., 2024). Rather than a stable biomarker used to compare groups, cf-mtDNA may be best conceptualized as a dynamic marker of stress-related vulnerability or resilience, more in line with traditional biomarkers of stress, like cortisol, where reactivity to stress, depending on the condition, is increased or decreased (Morris et al., 2014). Cf-mtDNA may also be more similar to heart rate variability, in which reduced variability is associated with poor health and increased variability is associated with better health. This reframing helps explain mixed cross-sectional findings and motivates future work to probe potential mitochondrial stress-recovery phenotypes in psychiatric populations (Melamud et al., 2023; Park et al., 2022).

In Study 2, where saliva was sampled up to 40 times per participant across two days, models incorporating random slopes revealed that the direction and magnitude of the effects were not universal. A minority of participants showed lower cf-mtDNA concentrations with higher levels of happiness, while others showed higher cf-mtDNA with increased stress. These between-person differences may reflect methodological factors. It is possible that these individuals are fundamentally different from their peers in some way. Prior work suggests that situational physiological states and individual differences in blood pressure, affect, and other parameters explain some of the variance in blood cf-mtDNA reactivity (Trumpff et al., 2019), a relationship that has not yet been studied in saliva. Alternatively, participants with positive slopes may have been sampled closer to a discrete stress exposure, which would increase the likelihood of capturing the post-stress influx of cf-mtDNA. To disentangle timing artifacts from true individual differences, future studies should employ designs that trigger saliva collection immediately after stress exposure and estimate person-specific slopes using mixed-effects models, an approach well established in EMA research for capturing rapid, within-person processes in daily life (Dawood et al., 2020).

A key takeaway from this study is that the physiological effects of stress and emotions should not be studied in isolation, and univariate models can oversimplify complex cf-mtDNA dynamics. Examination of Fig. 2E demonstrates that the PA-cf-mtDNA relationship was contingent upon the level of NA. The danger of testing the effect of negative emotions on cf-mtDNA without considering the broader affective context is

the risk of an incomplete (or even inaccurate) conclusion that particular emotions work singularly on cf-mtDNA. Humans are capable of feeling more than one emotion simultaneously, and it is important not to oversimplify that inner complexity. As this study highlights, there may be nuanced effects that calibrate mitochondrial signaling in nonlinear ways.

Compared to Study 1, where fixed effects explained up to 30.8% of the total variance of cf-mtDNA, the explanatory power of momentary affect for cf-mtDNA in Study 2 was smaller. This likely reflects both the finer temporal resolution and greater volatility of within-day fluctuations, which reduce the proportion of variance attributable to single predictors when averaged at the group level. Despite the relatively small proportion of average variance explained by the fixed effects in Study 2, these values are consistent with those found in other intensive longitudinal EMA studies (McNeish et al., 2021). Hour-to-hour assessments introduce substantial biological and contextual noise as unmeasured variables (e.g., sleep, diet, illness, or physical activity), which likely influence cf-mtDNA levels. In this context, even small percentages of explained variance can reflect a meaningful biological signal. By contrast, Study 1 yielded larger fixed-effects R^2 values, likely because day-level averaging reduced noise. Despite the explanatory power of momentary affect being smaller at the hourly level, these effects are not necessarily inconsequential. Instead, they highlight the sensitivity of cf-mtDNA to psychological states even against a backdrop of high within-person volatility. While these findings indicate that cf-mtDNA shows detectable associations with psychological stress at the day level, denser sampling is likely necessary to fully characterize the underlying temporal dynamics.

To contextualize these patterns, we compared cf-mtDNA with cf-nDNA. Cf-mtDNA consistently demonstrated greater sensitivity to psychological states than cf-nDNA, corroborating findings from previous studies (Behnke et al., 2025). In Study 1, cf-nDNA showed no relationship with stress exposure or the number or severity of stressors. This divergence supports the idea that a portion of cf-mtDNA is released through an active mechanism independent of cell death. Although cf-mtDNA and cf-nDNA were moderately correlated in this sample, only cf-mtDNA was associated with stress, suggesting differential biological sensitivity despite shared variance. In Study 2, cf-mtDNA again demonstrated broader emotional reactivity, correlating with six of the eight momentary affective states, while cf-nDNA was associated with only two affective states. Notably, cf-mtDNA and cf-nDNA were strongly correlated in Study 2, yet their associations with emotional states diverged, highlighting how cf-mtDNA captured unique variance.

While this remains to be confirmed by additional studies, the observed decrease in cf-mtDNA during stress may reflect an adaptive suppression of mitochondrial signaling. From a systems perspective, this could serve as a stabilizing strategy that helps conserve energy, dampen excessive signaling cascades, and maintain homeostasis when an organism is threatened (Bobba-Alves, Juster and Picard, 2022). Resilience often involves the selective silencing or attenuation of biological reactivity when sustained activation would be metabolically costly or maladaptive. This energy conservation model aligns well with theories such as allostatic load and our emerging understanding of the aging process (Shaulson, Cohen and Picard, 2024). This interpretation remains speculative and requires direct testing to confirm.

4.1. Limitations

By conventional standards, both cohorts were modest in size; however, each study employed intensive repeated-measures designs, which increased the precision and reliability of within-person estimates. It should be noted that this design does not eliminate limitations related to between-subject sample size or generalizability. With the feasibility of intensive saliva cf-mtDNA established, these associations should be tested in larger and more diverse cohorts. A notable limitation of Study 1 is that, although participants were screened to minimize psychiatric comorbidity and psychotropic medication use, other factors associated with MDD may have contributed to the observed group differences. Variables such as medical conditions, sleep disturbances, and major life stressors were not comprehensively assessed and may influence cf-mtDNA concentrations. Future studies should incorporate broader assessments of health and lifestyle factors to better isolate the specific contribution of depression to alterations in cf-mtDNA.

Although Study 2 utilized frequent measurement of saliva cf-mtDNA, the temporal resolution may still have been too coarse to capture these mechanisms as they occur in daily life. Data regarding the precise timing of stressors relative to saliva sampling was unavailable, which may explain the absence of short-lag cf-mtDNA spikes. To better understand the true mitochondrial dynamics that follow naturalistic stressors, future research should integrate event-contingent saliva sampling protocols to reveal for whom and under what circumstances cf-mtDNA is released or suppressed. Such approaches will likely enhance our understanding of the psychological and contextual factors shaping mitochondrial signaling and clarify its role in stress reactivity and resilience.

The present findings provide mixed evidence regarding mitochondrial specificity. In Study 1, daily stress exposure was associated with cf-mtDNA but not cf-nDNA, supporting the interpretation that the observed effects were not solely driven by generalized cellular release or passive cell death. In Study 2, happiness was associated with both cf-mtDNA and cf-nDNA, indicating overlap in the biological processes influencing these biomarkers on an hourly timescale. However, the pattern of associations was considerably broader for cf-mtDNA than for cf-nDNA. After correction for multiple comparisons, six affective states were associated with cf-mtDNA, whereas only happiness remained associated with cf-nDNA. These findings suggest that cf-mtDNA may be more sensitive to momentary affective experience than cf-nDNA, while also sharing some underlying regulatory mechanisms.

Finally, Study 2 presumes that the average level of each emotion reported across a two-day period reflects one's true affective baseline. The person-centered values were derived using data from only two days, and variation from these values indicated meaningful fluctuations in affect. However, a baseline derived from two days of data may not accurately capture one's normative state, underscoring the need for longer sampling periods. Despite their limitations, differences in sampling intensity, and cohort characteristics, both studies showed effects in the same direction.

4.2. Conclusion

Here, we show that cf-mtDNA measured in daily life does not exhibit a uniform or directionally consistent association with psychological states. Across two intensive longitudinal studies, cf-mtDNA levels varied as a function of temporal context, affective state, and individual differences, rather than showing a simple stress-reactive increase. These findings do not contradict prior laboratory work. Rather, they suggest that real-world mitochondrial signaling unfolds on time scales and under conditions that are not captured by coarse or fixed sampling schedules. This study contributes to a developing area of research demonstrating cf-mtDNA as a dynamic, context-dependent biomarker that is highly sensitive to timing and measurement design.

CRediT authorship contribution statement

Lauren Petri: Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Martin Picard:** Writing – review & editing, Supervision, Resources. **Caroline Trumpff:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Formal analysis, Conceptualization. **Samantha Leonard:** Writing – review & editing. **Sun Ah Lee:** Writing – review & editing, Data curation. **David Shire:** Writing – review & editing, Visualization, Investigation. **Idan Shalev:** Writing – review & editing. **Lacy Alexander:** Writing – review & editing. **Almeida David:** Writing – review & editing,

Supervision, Conceptualization. **Alexander Behnke:** Writing – review & editing. **Jody Greaney:** Writing – review & editing, Resources.

Ethics declaration

Written informed consent to take part in the study and to publish the article has been obtained from all participants or their legal representatives. The privacy rights of participants have been observed.

This study included organ or tissue donors. This study includes human biological material and consent was obtained by donors, or their next of kin or legal representatives, for use in this study and for publication of the article. The samples used in this research were not sourced from executed prisoners or prisoners of conscience.

This study was performed in compliance with relevant laws, regulatory frameworks and guidelines where the research took place. This study was approved by the The Pennsylvania State University Institutional Review Board. (Approval No. IRB #9617).

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2026.107952.

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