Psychobiological regulation of plasma and saliva GDF15 dynamics in health and mitochondrial diseases

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Abstract

GDF15 (growth differentiation factor 15) is a marker of cellular energetic stress linked to physical-mental illness, aging, and mortality. However, questions remain about its dynamic properties and measurability in human biofluids other than blood. Here, we examine the natural dynamics and psychobiological regulation of plasma and saliva GDF15 in four human studies representing 4,749 samples from 188 individuals. We show that GDF15 protein is detectable in saliva (8% of plasma concentration), likely produced by salivary glands secretory duct cells. Plasma and saliva GDF15 levels are not correlated. Using a brief laboratory socio-evaluative stressor paradigm, we find that psychological stress increases plasma (+3.4-5.3%) and saliva GDF15 (+45%) with distinct kinetics, within minutes. Moreover, saliva GDF15 exhibits a robust awakening response, declining by ~42-92% within 30-45 minutes from its peak level at the time of waking up. Clinically, individuals with genetic mitochondrial OxPhos diseases show elevated baseline plasma and saliva GDF15, and post-stress GDF15 levels in both biofluids correlate with multi-system disease severity, exercise intolerance, and the subjective experience of fatigue. Taken together, our data establish the dynamic properties of saliva GDF15, reveal it as a stress-sensitive, and as a clinically relevant marker of mitochondrial diseases. These findings point to a shared psychobiological pathway integrating metabolic and mental stress.

Keywords: growth differentiation factor 15; mitokine; metabokine; stress response; psychobiology; mitochondria; mitochondrial disorders; lactate; mtDNA mutations; MELAS

1. Introduction

The circulating metabokine-cytokine growth differentiation factor 15 (GDF15) is among the most studied circulating proteins in biomedicine. Circulating GDF15 is produced by various somatic tissues and cell types, secreted and transported in the blood, to eventually reach the hindbrain (and possibly other tissues) where it signals energetic stress to alter behavior and whole-body energy expenditure [1-5]. GDF15 fulfills several criteria as a marker of systemic energetic stress: 1) GDF15 is the most robustly elevated circulating protein with aging [6,7], 2) it is induced by some medications targeting mitochondria (e.g., metformin [8]), and 3) it is elevated in several chronic physical and mental illnesses including cardiovascular disease [9], cancer [10], mood disorders [11], Alzheimer's disease [12,13] autoimmune diseases [14], and others. Moreover, 4) GDF15 is the leading candidate biomarker for primary mitochondrial diseases caused by mitochondrial DNA (mtDNA) mutations and deletions causing mitochondrial diseases (MitoD) [15-20]. Mechanistically, mtDNA defects impair energy transformation by oxidative phosphorylation (OxPhos) within mitochondria, triggering intracellular reductive stress (i.e., elevated $NADH/NAD^+$ ratio) that activate the integrated stress response (ISR), leading to systemic GDF15 release [21-24]. Clinically, beyond MitoD, blood GDF15 has emerged as the top discriminatory plasma protein to identify individuals with various current [25] and future clinical illnesses [13]. Moreover, circulating GDF15 is among the best prognostic mortality indicators in clinical populations and older adults [6,26], often predicting death and disability better than well-established disease-specific biomarkers. Thus, resolving GDF15 biology is of high clinical significance.

GDF15 is also secreted in response to various non-disease states. Blood GDF15 increases with nutritional and physiological stressors such as exhaustive exercise [27,28], pregnancy [2,29], fasting [30], and infectious challenges [31]. This secretion pattern aligns with the canonical, energy-mobilizing glucocorticoid and sympathetic hormonal pathways, with which GDF15 interacts [1,32], and may contribute to GDF15-mediated increase in energy expenditure [1,33]. Taken together, the rapidly growing body of literature suggests that GDF15 may play a role in systemic energy homeostasis, resilience, and health, calling for a refined understanding of its natural dynamics and reactivity to challenges that punctuate daily human lives. Moreover, although most GDF15 research has focused on blood, preliminary evidence suggests it is detectable in human saliva [34,35]. Determining if saliva GDF15 is physiologically regulated together with, or independently from, circulating blood levels would expand our understanding of GDF15 biology. It also would establish whether easily accessible saliva can be used to monitor human GDF15 dynamics in health and disease, particularly in MitoD.

Here, we integrate repeated-measures data across four studies (4,749 samples from 188 individuals; see Supplemental Table 1) to define the dynamic properties, natural morning awakening response, and stress-evoked psychobiological regulation of blood and saliva GDF15 in healthy individuals and individuals with rare genetic OxPhos defects causing MitoD.

2. Results

2.1 GDF15 is detectable in human saliva

We first investigated the presence and potential origin of GDF15 in human saliva (Figure 1A). Gene expression of *GDF15* across the human body has yet to be systematically described. Figure 1B shows *GDF15* transcript abundance across 48 organs and tissues from the *Gene Tissue Expression* database [36] (GTEx, n=948 individuals, tissues with >20 participants; see *Materials and Methods* for details), including the minor salivary gland which produces the bulk volume of saliva. This analysis revealed that *GDF15* is widely expressed among most human tissues: highest in the kidney and other glandular and secretory tissues, and lowest in the brain (where its receptor *GFRAL* is expressed), an anatomical expression pattern consistent with its systemic extracellular secretion and role as a body-to-brain signaling protein[3]. The minor salivary gland ranked 17 out of 48 tissues based on *GDF15* expression (Supplemental Table 2).

In the salivary gland analyzed by single-cell transcriptomics, *GDF15* was most highly expressed in salivary duct cells and mucus glandular cells (Figure 1C, Supplemental Table 3; data retrieved from GTEx, n=24 cell types, see *Materials and Methods* for details). Histological analysis of GDF15 protein abundance by immunohistochemistry [37] confirmed its preferential expression in saliva-producing glandular epithelial cells (Figure 1D), providing a basis for its secretion in human saliva. Histologically, salivary duct cells were also enriched for most of the examined mitochondrial proteins (TOMM20 [membrane-associated], TFAM [mtDNA-associated], NDUFS4 [OxPhos]), MCU [calcium import]), although not all (CPT1A [fatty acid oxidation]) (Figure S1). This provided further confidence that mitochondria-rich duct epithelial cells express high levels of the *GDF15* gene and actively produce the GDF15 protein detected in human saliva.

To quantify and compare saliva and plasma GDF15, we simultaneously collected plasma and saliva from 70 healthy individuals aged 18-60 years (69% females), for up to 26 timepoints per individual, over the course of a 2-day hospital visit and a 3-day at-home saliva protocol in Study 1 (*MiSBIE*, see *Materials and Methods* for details; total 2,116 samples) (Figure S2A). Plasma GFD15 levels were on average 404.1pg/mL (median: 378.6, range 148.5-

1,411pg/mL), whereas saliva levels were on average 32.3pg/mL (median: 17.00, range 0.22-683.3pg/mL), or 8.0% of plasma levels (Figure 1E). In this relatively small sample, we found no significant sex difference in either plasma or saliva GDF15 concentration (Figure S2B). Interestingly, plasma and saliva GDF15 levels were not correlated (Spearman r=0.034, p=0.82, Figure 1F). This suggested that unlike systemic stress hormones that are correlated between plasma and saliva, such as cortisol [38], saliva GDF15 may not reflect passive "spillage" of blood into saliva. Saliva GDF15 may therefore be regulated separately from systemic blood levels.

2.2. Saliva GDF15 does not correlate with age

Consistent with its status as an age-related circulating biomarker [6,7], across hundreds of bulk GTEx tissue transcriptomes from individuals aged 20-70 years. *GDF15* expression was positively correlated with age in most human tissues (Figures 1G-H, Supplemental Table 1). As expected, circulating plasma GDF15 in Study 1 also was positively correlated with age (r=0.61, p<0.0001), increasing on average 101.8pg/mL per decade of life. However, *GDF15* expression in some tissues showed no association with age. In fact, of all tissues, the age-to-GDF15 correlation was second lowest and null in the salivary gland (r=-0.040, p=0.61). Accordingly, in Study 1, we found no association between age and saliva GDF15 (Figure 1I).

Together, i) the selective expression of GDF15 in salivary glandular cells, ii) the lack of correlation between GDF15 in plasma and saliva, and iii) the lack of an association with age and either GDF15 gene expression or saliva protein levels converge to demonstrate that saliva GDF15 is likely regulated independently from circulating blood levels.

2.3 Stability and AM-PM dynamic variation

To quantify and compare how much saliva and plasma GDF15 levels change over time, in Study 1, we implemented a repeated-sampling design including 14 saliva collections over a 2-day period (Figure S2A). More frequent saliva sampling relative to blood allows more fully define GDF15 dynamics, clarify the relationship between GDF15 dynamics across both biofluids, and minimize collection artifacts. We found that compared to plasma GDF15 concentrations, which have been shown to exhibit a moderate degree of stability over weeks [39], saliva GDF15 levels presented 10.0-fold higher within-person variations (Figure S2B-C). Thus, saliva GDF15 levels are approximately an order of magnitude more dynamic or changeable than in plasma.

We then examined how much plasma and saliva GDF15 levels vary across consecutive days, and from morning to afternoon within a given day (Figures S2D-E). In Study 1, morning fasting saliva collected under the same conditions on two subsequent days exhibited higher GDF15 levels on the first study visit day (when the hospital environment was novel) and decreased on average by 26% from Day 1 to Day 2 ($p=0.032$, Figures S2D), demonstrating significant within-person variation and possible habituation to the research environment.

We also had the opportunity to investigate changes in GDF15 from morning (9am, fasting) to afternoon (~1-2pm, fed) on the two consecutive days. On Day 1, from morning to afternoon, GDF15 levels decreased on average by -12% ($p<0.0001$) in plasma but tended to increase +6.5% ($p=0.06$) in saliva (Figure S2E). On Day 2 (only saliva collected), saliva GDF15 levels again increased from morning to afternoon by an average of +20% ($p<0.0001$). The magnitude and direction of change in saliva GDF15 between days 1 and 2 were not significantly correlated ($r=0.05$, $p=0.76$, Figure S2F). Thus, the fed state [30] and the time of day might influence blood and saliva levels differently, further highlighting the distinct regulation of saliva GDF15 from circulating blood levels.

2.4 Awakening Response and Day-Night Variation

Waking up from sleep is a psychophysiological challenge accompanied by a return to conscious awareness and elevations in multiple physiological parameters, including core body temperature, energy expenditure, blood pressure, cortisol, and several other metabolically-demanding processes [40,41]. As a result, several hormones detectable in saliva exhibit an "awakening response", meaning that they either spike or decline as one wakes up in the morning; among these is the cortisol awakening response [42,43]. Therefore, we investigated whether saliva GDF15 also exhibits an awakening response.

Study 1 included a 3-day home-based saliva collection protocol optimized to detect awakening responses on three non-consecutive days (Monday, Wednesday, and Friday), including 4 samples per day: (1) awakening, (2) 30 min after awakening, (3) 45 min after awakening, and (4) bedtime (Figure 2A). Remarkably, saliva GDF15 was highest immediately at the time of waking up (i.e., awakening) across the three days. Relative to the awakening timepoint, saliva GDF15 levels fell sharply by an average of -40% at 30 min and -46% at 45 min, reflecting a robust negative awakening response. At bedtime, saliva GDF15 remained on average 47% lower than the awakening value (average group trajectory, Figure 2B; individual participants trajectories, Figure S3A). Observed over the awakening time course, males tended

to exhibit a 39-101% higher median saliva GDF15 levels than females (n.s.), although both exhibited a similar decline by 47-57% after waking up (Figures S3B-C). Thus, saliva GDF15 exhibits rapid and robust variation after waking up.

Intensive daily repeated measures in n=2. Next, we examined the stability and magnitude of the awakening response over multiple weeks. In Study 2, we performed an intensive repeated-sampling protocol for saliva on two male participants, using the same 4-timepoints as in Study 1. Both participants collected saliva for 53 (Participant A) and 60 (Participant B) consecutive days, as described previously [44]. This yielded 199 and 221 GDF15 measures for each participant, shown in Figure 2C. Consistent with Study 1 results, saliva GDF15 levels varied substantially both within and across days. The average combined within-person C.V. was 267%, reflecting substantial day-to-day changes within an individual.

Participant A (male, age 34, sedentary, stress score average: 0.90 out of 5 on the modified Differential Emotional Scale) saliva GDF15 levels ranged from undetectable to 50.4pg/mL (median: 4.3pg/ml, Figure 2D). Saliva GDF15 exhibited an average -77.8% decline from awakening to 30 min, and -81.3% at 45 min. At bedtime, saliva GDF15 levels were, on average -74.9% lower than at awakening (Figure 2F).

Participant B (male, age 35, competitive runner, stress score average: 2.57 out of 5 on the modified Differential Emotional Scale) saliva GDF15 levels ranged from undetectable to 4,565pg/mL (median: 9.1pg/ml, Figure 2E). His awakening response was on average -88% at 30 min, -92% at 45 min, and -72% at bedtime (Figure 2G). During the data collection period, Participant B was actively training for a marathon (race on day 27, red arrow), possibly accounting for the high GDF15 values and the overall higher mean levels than Participant A. The highest GDF15 value (4,565.1pg/mL) was recorded at the 45 min timepoint the day after the marathon (day 28), consistent with prior reports linking exhaustive exercise with *blood* GDF15 [45], but here observed punctually in *saliva*, at a specific timepoint, *the day after* the thorough effort.

Within each participant, the three morning time points were significantly correlated with one another ($r=0.36-0.60$, all $p<0.01$, Figure S3E). This result is consistent with robust day-to-day variation, marked by some low GDF15 mornings and some high GDF15 mornings. Morning and evening timepoints were not correlated, possibly pointing to different drivers of saliva GDF15 levels between morning and evening.

Day-night variation in blood GDF15. The awakening responses observed in Studies 1 and 2 were observed in most individuals and were remarkably large effect sizes, suggesting potential

night-day GDF15 changes. Unlike blood, which can be drawn through an intravenous catheter throughout the sleep-wake transition, saliva cannot be collected easily during sleep. To explore if the saliva GDF15 awakening response is also apparent in plasma, Study 3 (n=6 participants), included collection of blood via indwelling catheter and intravenous (IV) line every 30 minutes overnight during sleep, and in the morning after waking up, on two separate visits (see *Study 3 Materials and Methods*). Some participants exhibited up to 25.6% variation in serum GDF15 levels between day and nighttime, consistent with a previous report [46]. However, in this small sample, we found no systematic diurnal variation across participants (Figure S4). Thus, unlike the robust saliva GDF15 awakening response, blood diurnal variation was either too small in magnitude or too idiosyncratic in its timing to be detected in this sample.

2.5 Psychobiological regulation of plasma and saliva GDF15

The human mind is connected to every organ such that physiology is rapidly altered by real or perceived threats [47]. For example, simple stressful thoughts increase heart rate and blood pressure. In mice, evoked stress was recently shown to elevate blood lactate levels, a marker of reductive stress (high $NADH/NAD^+$ ratio), which mechanistically functions upstream of the ISR-GDF15 cascade [19,48]. Extending this observation to humans, we therefore examined whether plasma and saliva GDF15 are affected by acute mental stress in humans.

We evoked mental stress in healthy human participants using a validated socio-evaluative speech test (modified from the Trier Social Stress Test (TSST) [49], see *Methods* for details). During the speech task, participants defend themselves against an alleged transgression while facing a white coat-wearing evaluator, a mirror, and a camera. Unlike physical exercise, which increases whole-body energy expenditure several-fold [41], the socio-evaluative speech task does not elicit a large increase in metabolic rate. It does however trigger a coordinated multi-system response, including elevations in heart rate and blood pressure, cortisol, cell-free mtDNA, inflammatory cytokines, and other stress mediators [43,50-52]. In Study 1, participants performed the speech task while both plasma and saliva were concurrently collected (they had a salivette in the mouth while blood was drawn through an intravenous catheter) at eight timepoints: 5 min before and 5, 10, 20, 30, 60, 90, 120 min after the onset of the stressor (Figure 3A), enabling to capture both the reactivity and recovery phases of stress responses.

We first confirmed that, similar to what was observed in mice [48], evoked mental stress elevated circulating lactate in humans. Lactate is produced by increasing the intracellular

$NADH/NAD^+$ ratio (i.e., reductive stress), which pushes the lactate dehydrogenase reaction towards lactate production. In our cohort, the brief TSST mental stress increased plasma lactate by an average of 14.5% (p=0.0054, range: -39 to +151%, Figure 3B) from baseline to 5 min. This result documents systemic reductive stress from a brief mental stressor in humans (see Figures S5A-B for full time course).

Concurrently, the speech task significantly increased plasma GDF15 by 5.9% at 10min (Hedges' g=0.59, p=0.032) before declining by 6.8% from 10 to 30 min (g=-0.49, p=0.0010) after the stressor (Figures 3C-D). This represents, to our knowledge, the first evidence that mental stress alters blood GDF15 concentration within minutes.

Saliva GDF15 also exhibited significant change over the stress reactivity-recovery time course. Acute mental stress increased saliva GDF15 by a striking 42.8% at 5 min (g=0.84, p=0.0014). This response was approximately 7 times larger than the one observed in plasma. Saliva GDF15 returned to baseline levels 5 min later, 10 min after the onset of the stressor, demonstrating a rapid increase and decrease in saliva GDF15 concentration (Figures 3E-F). There was no significant correlation between plasma and saliva GDF15 responses within each person, further highlighting their independent (psycho)biological regulation (Figure S5C).

In relating GDF15 and lactate, we found a modest positive trend between the saliva GDF15 response and the acute rise in blood lactate (r=0.21, p=0.058; Figure S5D), consistent with a potential link between systemic reductive stress and saliva GDF15 reactivity. However, resting blood lactate levels explained no more than 5-15% of the variance in inter-individual differences in saliva and plasma GDF15 or plasma GDF15 reactivity (Figure S5E-G).

To validate the speech task-induced changes in plasma GDF15, we analyzed samples from the *Mitochondria and Psychological Stress Study (*see *Study 4 – MaPS Materials and Methods* for details) where 70 healthy individuals (mean age 30.6 years, 51.2% female) underwent two conditions, randomized in order between a speech task and control (no stressor other than the blood draw and laboratory setting), in random order. As in Study 1, plasma GDF15 was measured at 8 timepoints (5 min prior and 5, 10, 20, 30, 60, 90, and 120min after the onset of the speech task). In Study 4, saliva was not collected and blood was drawn without the participant's awareness via an obscured IV line running through the wall behind the participant. This protocol variation minimizes psychological interference after the induction of the stressor. However, eliminating interpersonal interactions for 2 hours after the stressor, which can produce anxiety.

Study 4 validated the direction and magnitude of results from Study 1 (Figures 3G-I and S5H). Compared to the control visit, mental stress increased plasma GDF15 by +3.5% at the 5 min timepoint in the stress visit (paired t-test: p=0.0085). On both control and speech task visits, GDF15 gradually increased over the 2-hour post-stressor recovery period, reaching a maximum at 120min (+9.0% in the stress visit, +8.1% in the control visit, relative to baseline). Notably, participants randomized to the stress condition on their first visit exhibited higher baseline plasma GDF15 concentrations than those randomized to control on their first visit, consistent with a potential influence of novelty and anticipatory stress on GDF15 levels (Figure S5I). Interestingly, and perhaps consistent with this hypothesis, across the cohort, two participants who did not return for their second visits (lost to follow-up) presented with the two highest baseline GDF15 levels (Figures S5J-L). Combined with our Study 1 results, these findings establish that psychosocial stress raises human plasma GDF15 levels within minutes.

2.6 Saliva GDF15 in mitochondrial diseases

To examine plasma and saliva GDF15 dynamics in a relevant clinical context, we leveraged Study 1 healthy control and patient populations. Healthy controls in Study 1 are defined as individuals who do not have a diagnosis of MitoD (i.e., have normal mitochondrial OxPhos capacity). The MitoD patient population includes three groups: (1) single, large-scale mtDNA deletions (n=15) [53], (2) mtDNA mutation 3243A>G who present without stroke-like episodes (n=20) [54], and (3) mtDNA mutation 3243A>G who present with stroke-like episodes and more severe overall disease severity (MELAS; n=5) [55].

Compared to the control group, plasma GDF15 levels were 4.3-fold higher in the mtDNA deletion group (average 2,155 pg/mL, range 583-5,292 pg/mL), 1.6-fold higher in individuals with the 3243A>G mutation (average 1,035 pg/mL, range 130-3,725 pg/mL), and 1.1-fold higher in those with 3243A>G with MELAS (average 862 pg/mL, range 685-1,796 pg/mL) (Figures 4B, left), confirming that plasma GDF15 is elevated in primary OxPhos defects [15-18].

We then tested whether OxPhos defects also increased saliva GDF15. Indeed, compared to healthy controls, saliva GDF15 was 1.7-fold higher in deletion patients (average 87.2 pg/mL, range 1.0-1,677 pg/mL), 0.95-fold higher in the 3243A>G mutation group (average 63.5 pg/mL, range 0.22-873 pg/mL), and 3.3-fold higher in the MELAS group (average 116.2 pg/mL, range 10.8-331 pg/mL) (Figure 4B, right).

Similar to healthy controls, there was no correlation between plasma and saliva GDF15 levels in MitoD (Figure S6B). The AM-PM and day-to-day stability of blood and saliva GDF15 in

MitoD is shown in Figures S6C-SE, revealing a similar degree of variation (i.e., low stability) as in controls. In contrast to the healthy controls, where plasma GDF15 correlated with age, there were no associations between plasma or saliva GDF15 and age in our MitoD groups (Figure 4C), confirming that mitochondrial OxPhos defects disproportionally increase GDF15 beyond the more modest, progressive effect of aging.

2.7 Saliva GDF15 dynamics in OxPhos defects

The presence of the GDF15 protein in saliva and its elevated levels in MitoD offered the opportunity to examine whether the dynamic properties and psychobiological regulation of saliva GDF15 are influenced or preserved among individuals with OxPhos defects.

MitoD patients exhibit a normal awakening response. We first investigated whether patients with MitoD also exhibited a negative saliva GDF15 awakening response. As in healthy controls, saliva GDF15 levels were highest at awakening. In all three MitoD groups, awakening saliva GDF15 levels were markedly higher than controls: 2.3-fold higher for deletion ($p=0.0041$), 1.0-fold higher for 3243A>G ($p=0.0021$), 1.6-fold higher in MELAS ($p=0.0035$) (Figure 4D). All MitoD groups exhibited robust awakening responses, whereby saliva GDF15 levels decreased by 71.5% in deletion, 56.5% in mutation, and 38.8% in MELAS at 45min ($p<0.05$ for deletion and mutation). Bedtime saliva GDF15 levels were also lower than the awakening levels in all disease groups, validating the results in the control group of Study 1 and the intensely sampled participants of Study 2.

Stress-evoked regulation of GDF15 in MitoD. As for baseline differences between controls and MitoD individuals with OxPhos defects, plasma GDF15 levels were elevated across the entire speech task session. Moreover, MitoD groups showed a relatively distinct reactivity pattern in response to stress when each participant's trajectory is projected relative to their baseline levels (Figures 4E and 4F). In the deletion group, the already elevated plasma GDF15 did not increase further; it decreased by 3.6% at 30 min. In the mutation group, plasma GDF15 peaked at 11.6% at 120 min, and peaked 2.1% at 5 min in the MELAS group.

Saliva GDF15 was also elevated in MitoD throughout the speech task and exhibited a similar trajectory as controls. In MitoD, acute socio-evaluative stress increased the already elevated saliva GDF15 at the 5min timepoint by 46% in Deletion, 23% in non-MELAS mutation, and 55% in MELAS. However, the effect was only statistically significant in the more robustly powered non-MELAS mutation group (Figures 4G and 4H). As in controls, saliva GDF15

declined by 42% from 5min to 120min (g=0.72, p=0.025) in the mutation group but remained marginally elevated in the deletion group.

The GDF15 concentration is already elevated at baseline in MitoD groups, so the stress-induced relative % change from baseline underestimates the absolute release of GDF15. Examined as absolute values (delta pg/ml), socio-evaluative stress produced plasma and saliva GDF15 elevations that were either similar or exaggerated by up to 6.3-fold (MELAS group, p<0.01) in MitoD groups compared to controls.

2.8 Blood and saliva GDF15 correlate with MitoD symptom severity

Sensitivity and specificity to MitoD diagnosis. Using all plasma and saliva timepoints from Study 1, we systematically compared MitoD patients and controls, computing effect sizes at each timepoint (Figures S6F-I). The standardized effect sizes in plasma and saliva GDF15 tended to be lowest across the three MitoD groups when sampled in the morning fasting state. In contrast, the stress task moderately increased group differences (Figure 5A). In the afternoon of Day 2 in Study 1, participants performed cognitively demanding tasks during a 2-hour magnetic resonance imaging (MRI) of the brain, during which saliva was collected at 3 timepoints. Compared to the morning fasting sampling, the control-MitoD differences in saliva GDF15 were largest during the afternoon MRI (Figure 5B), indicating that stress stressful/demanding challenge may reveal group differences otherwise minimized at rest.

We then examined the ability of blood and saliva GDF15 levels to distinguish or diagnose MitoD groups (all three groups combined) from controls. In a receiver operating characteristic (ROC) curve analysis for morning fasting plasma GDF15, the area under the curve combining sensitivity and specificity was 0.85 (95% C.I. 0.75-0.96, cut-off value = 689.6pg/mL, Figure 5C), similar to previous studies [18]. The ROC curve was similar at the 20min post-TSST timepoint, an AUC of 0.86 (95% C.I. 0.75-0.97, cut-off value at 621.3pg/mL). Using saliva GDF15 at the same time as the morning fasting blood timepoint (Figure 5D), the AUC was 0.75 (95% C.I. 0.63-0.86, cut-off value of 20.9pg/mL), indicating moderately lower sensitivity/specificity characteristics in saliva compared to plasma. However, when saliva GDF15 was sampled in the afternoon after the MRI, the AUC was 0.81 (95% C.I. 0.71-0.91), comparable to plasma. Thus, in this MitoD sample, saliva GDF15 (particularly when measured in the afternoon after demanding tasks) provides similar sensitivity/specificity characteristics as plasma GDF15.

Blood and stress-induced saliva GDF15 correlate with MitoD disease severity. Finally, to examine whether GDF15 levels across biofluids reflect the severity of disease, we correlated the independently regulated plasma and saliva GDF15 levels with disease severity indices. Disease severity was measures using five independent approaches: 1) *Clinician-assessed, symptoms-based multi-system involvement* (Newcastle Mitochondrial Disease Assessment Sale – NMDAS) [56]; 2) *Autonomic dysregulation* (Composite Autonomic Symptom Score – COMPASS 31) [57], 3) *neurological symptoms* (Columbia Neurological Scale – CNS) [58], 4) *Functional capacity* (30-second sit-to-stand test) [59]; 5) *Perceived fatigue* and its impact on physical, cognitive, and psychosocial functioning (Fatigue Impact Scale – FIS)[60] (Figures 5E-F).

Plasma GDF15 from the fasting morning blood draw was expectedly correlated with multi-system disease severity index by the NMDAS score ($r=0.69$, $p<0.0001$), as in previous studies [19]. However, the GDF15-symptom correlations were stronger when measured on blood collected in the afternoon at the 20 min post stress-task timepoint ($r=0.80$, $p<0.0001$), suggesting that the mental stress challenge may elicit functionally and clinically significant GDF15 release that is more strongly indicative of disease severity (Figure 5G). Similar patterns were observed for other disease severity indices: the GDF15-symptoms correlations in MitoD patients were stronger for plasma sampled at the 20 min post-stress. These analyses repeated with age-adjusted GDF15 values yielded similar results for all symptoms.

Interestingly, MitoD severity also correlated with saliva GDF15 under certain sampling conditions. Morning fasting saliva GDF15 levels were only moderately associated with symptoms, albeit all in the same direction as plasma ($rs=0.13$-0.27, $ps=0.16$-0.73). However, afternoon MRI saliva GDF15 (after patients experience the stress and discomfort of the study/clinical procedures and report more fatigue) was more strongly and significantly correlated with clinician-rated MitoD disease severity, objective neurological signs and symptoms, functional capacity, and fatigue ($rs=0.39$-0.62, $ps=0.03$-0.0006, Figure 5H). Thus, MitoD manifestations in mitochondrial OxPhos defects correlate with both plasma and afternoon saliva GDF15.

3. Discussion

As GDF15 becomes one of the most studied proteins in the biomedical sciences, maximizing its utility and potential as a biomarker requires understanding its dynamic nature, including its (psycho)biological regulation and whether it matters when and under what conditions we sample biofluids. Here we report the expression of *GDF15* across most human

tissues, including salivary gland epithelial secretory cells, which are associated with the presence and dynamic variation of the GDF15 protein in human saliva. Using both standardized laboratory stressors and ecological sampling afforded by non-invasive saliva collection, we establish the directionality and magnitude of GDF15 variation within and across days in healthy females and males, and among individuals with rare mitochondrial OxPhos defects causing MitoD. Our findings reveal previously unappreciated dynamic properties of saliva GDF15 in humans, including i) a robust decline within the first hour upon awakening in the morning, ii) a rapid increase within 5-10min of experiencing socio-evaluative stress, and iii) a mild morning-to-afternoon diurnal variation. Finally, we confirm elevated GDF15 in both plasma and saliva in individuals with MitoD and we document the correlation of strategically sampled GDF15 measures with multiple indices of disease severity and symptomatology.

One of the most remarkable and robust findings from the studies described above is the reactivity of GDF15 – both in plasma and saliva – in response to a simple and relatively mild 5-minute mental stressor. The GDF15 response among groups replicated across studies but relatively small in plasma (3-6% across Studies 1 and 4), whereas saliva GDF15 reactivity was ~8-10-times larger (42-71%). Furthermore, GDF15 levels increased and decreased remarkably quickly – within minutes – in both plasma and saliva. We note that the group averages (required to perform statistical analyses) obscure the true individual-level dynamics of human GDF15 biology, which can be appreciated from the individual participant trajectories (for example, see Figure 3D). In some participants, saliva GDF15 increased >300-400% over 10 min and declined/returned to normal levels within 5min. The magnitude and pace of these responses appear consistent with pulsatile release, as for several stress hormones and neuromodulators, including cortisol [61] and catecholamines [62]. This insight afforded by repeated sampling at the scale of minutes suggests future avenues of research to elucidate the mechanisms of GDF15 release from different tissues and cell types.

Several indirect elements of our results suggest that psychosocial factors generally modulate plasma GDF15 levels. First, in the control condition of the MaPS Study 4, participants visited a novel laboratory environment, were instrumented with an IV line causing some discomfort, isolated in a quiet room without access to their personal communication device, and monitored remotely for a 2-hour. Under these conditions, we observed a gradual elevation in plasma GDF15 from 60 to 120 min. This pattern was not or more mildly observed in MiSBIE Study 1 participants, where interactions with the study staff occurred at each measurement timepoint (8 times over the 2 hours, for blood and saliva collections). The basis for this discrepancy in GDF15 dynamics requires further research. Second, we note that two MaPS

Study 4 participants randomized to the TSST on their first visit did not return for the second visit. Curiously, both participants exhibited abnormally high GDF15 levels throughout the protocol – the highest average GDF15 observed across our control cohorts (see Figure S4G-I). Given that high GDF15 levels signaling on the brainstem causes aversive responses and visceral malaise (e.g., nausea [4,63]), we speculate that their decision not to return for the second visit could have been influenced by the particularly aversive experience of their first visit, possibly either enhanced or provoked by abnormally high plasma GDF15 levels. Third, the saliva GDF15 awakening response, measured across three days, was highest on the first day (Monday) and progressively declined as the psychological novelty of the daily exercise subsided. This observation is not definitive, but also aligns with the dynamic psychological modulation of saliva GDF15 levels.

Our results also align with three lines of evidence linking GDF15 to the psychobiology of stress responses. First, in the same manner that immune cytokines trigger sickness behavior and associated energy conservation responses by acting on the brain [64], GDF15 signaling on the brain may exist to suppress non-vital physiological functions and the desire to move (e.g., [65]). By triggering malaise and inactivity, GDF15 from peripheral tissues would free organismal energetic resources to support stress-related energy demands, promoting resilience to stressors [66]. Second, human cortisol deficiency has been shown to elevate GDF15 levels, suggesting a functional interaction between the social stress-responsive hypothalamic-pituitary-adrenal (HPA) axis and the ISR-GDF15 axis [67]. Consistent with the physiological management of energetic resources, the primary effect of HPA axis glucocorticoids is to mobilize energy substrates (the "gluc" in glucocorticoids[68]) to enhance the probability of survival during a threat. Accordingly, in mice GDF15 signaling triggers hypothalamic corticotropin-releasing hormone (CRH) release [69], which activates the HPA axis, leading to corticosterone secretion [32]. Determining whether GDF15 elevation after mental stress in humans contribute to subsequent cortisol elevation (known to peak around 20 min after stress) requires further research. Third, plasma GDF15 is elevated in some psychiatric conditions (Frye et al., 2015; Kumar et al., 2017; Li et al., 2023; Mastrobattista et al., 2023). This suggests that GDF15 may be a biomarker of sustained mental stress or other altered psychological states. This notion, supported by our stress reactivity results in Studies 1 and 4, would broaden the popular idea that GDF15 specifically marks metabolic stress and physical (i.e., biological) illness [11]. The convergence of OxPhos defects and acute mental stress as triggers of blood and saliva GDF15 motivates further studies examining the cellular and molecular intersection point(s) for cognitive and subcellular energetic stress.

Finally, our work extends prior knowledge regarding the utility of GDF15 as a biomarker of MitoD [15-18,20] by documenting its elevation in saliva. In our small sample of MitoD participants with rare homogenous genetic lesions causing OxPhos defects, we show for the first time that *saliva* GDF15 is robustly elevated by 1.0-3.5-fold. However, the effect sizes for saliva are smaller than in blood. These results call for further studies to examine the clinical utility of highly accessible saliva GDF15 measurement as a prognostic marker of different MitoD, including direct comparisons with other chronic illnesses to establish the sensitivity and specificity of saliva GDF15 as MitoD biomarker. We show that saliva and plasma GDF15 correlate robustly with multi-system symptoms (up to r=0.8, GDF15 accounting for >60% of variance in symptoms), particularly when sampled in the afternoon after moderately challenging or psychologically stressful medical procedures (MRI). Furthermore, our home-based sampling protocol where participants easily collect saliva on their own, store salivettes in their freezer, and subsequently ship samples by regular mail, provides a portable opportunity to perform intensive longitudinal, repeated-measures studies or to explore the clinical utility of saliva GDF15 in hard-to-reach study populations, at reasonable costs.

The lack of correlation between saliva and plasma GDF15 levels opens some exciting questions around the specificity and generalizability of GDF15 dynamics in different biofluids. Plasma GDF15 concentration is roughly an order of magnitude higher than saliva. Plasma GDF15 is a marker of aging, saliva GDF15 is not. Following mental stress, saliva GDF15 increases more strongly and quickly than plasma GDF15. Together, these observations point towards a fairly independent regulation of GDF15 secretion between salivary glands and blood. Nevertheless, despite not being significantly correlated at any of the examined timepoints, both plasma and saliva GDF15 are elevated in MitoD and correlate with objective clinical signs and symptoms, functional capacity, and perceived fatigue.

In summary, this work identifies saliva GDF15 as a dynamic marker of mitochondrial OxPhos defects that is also rapidly regulated by psychosocial stress. Our strategic repeated-measures sampling across laboratory and home-based studies demonstrates general dynamic principles for the diurnal and psychobiological regulation of both blood and saliva GDF15, extending our understanding of GDF15 biology in humans beyond diseases. These results in humans also point towards a shared psychobiological pathway integrating metabolic and mental stress implicating GDF15, opening questions around its role in human health and resilience across the lifespan.

4. Methods

4.1 Study 1: MiSBIE cohort

4.1.1 Participants

Participants were enrolled in the Mitochondrial Stress, Brain Imaging, and Epigenetics (MiSBIE) study in adherence to the directives outlined by the New York State Psychiatric Institute IRB protocol #7424, clinicaltrials.gov #NCT04831424. Recruitment was conducted both within our local clinic at the Columbia University Irving Medical Center, and nationally throughout the United States and Canada. All enrolled participants provided written informed consent, authorizing their participation in the investigative procedures and the potential dissemination of data. Recruitment occurred from June 2018 to May 2023. A total of 110 participants ages 18 to 60 years were enrolled, including 70 healthy controls (females n=48, males n=22) and 40 individuals with genetically defined mitochondrial diseases (MitoD, females n=28, males n=12). This manuscript specifically reports on MiSBIE GDF15 dynamics in blood and saliva. Sex was determined by self-report. This single-step method is limited, does not distinguish between gender and sex, and can exclude both transgender and intersex people; current best practices include a two-step [70] measurement and broadening the available answer options.

Our inclusion criteria for controls included healthy adults willing to provide saliva samples and have blood collected using an intravenous catheter during the hospital visits. Exclusion criteria included the presence of pronounced cognitive impairment precluding informed consent, concurrent neoplastic disease, recent occurrences of flu or other temporally pertinent infections within the four-week window preceding study participation, Raynaud's syndrome, engagement in ongoing therapeutic or exercise trials registered on ClinicalTrials.gov, and the existence of metallic elements within or on the body, alongside claustrophobia posing an impediment to magnetic resonance imaging (MRI). In-person laboratory procedures were performed over 2-days (Tuesday and Wednesday), followed by a 1-week home-based saliva collection protocol.

For the MitoD groups, eligibility criteria for inclusion included the presence of a genetically ascertained diagnosis of symptomatic MitoD, which encompassed either (i) the m.3243A>G pathogenic variant, with [55] or without [54] the clinical manifestations of mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS), or (ii) a single, large-scale mitochondrial DNA deletion-associated presentation of chronic progressive external ophthalmoplegia (CPEO), Kearns-Sayre syndrome (KSS) [53], or other syndromic variant. A standardized questionnaire was administered to all participants, collecting essential information including demographic attributes, age, gender, ethnicity, prevailing health status, socioeconomic

status, and ongoing medication regimens. Exclusion criteria were similar to those of healthy controls, with the additional criteria excluding patients who used steroid therapy (including oral dexamethasone, prednisone, or analogous agents).

Disease severity and symptomatic profiles were quantified using five different approaches. 1) The Newcastle Mitochondrial Disease Adult Scale (NMDAS) [56], a validated metric administered by a qualified clinician, which assesses symptomatic and functional limitations across several domains of life; 2) *Autonomic dysregulation* (Composite Autonomic Symptom Score – COMPASS 31) [57], which assesses autonomic symptoms in six domains; 3) *neurological symptoms* (Columbia Neurological Scale – CNS) [58], which assesses central nervous system symptoms; 4) *Functional capacity* (30-second sit-to-stand test) [59], 5) *Perceived fatigue* and its impact on physical, cognitive, and psychosocial functioning (Fatigue Impact Scale – FIS) [60]

Non-stress plasma and saliva samples were collected both in the morning, under fasting conditions around 9:30 AM, or after lunch around 1:00 PM. Both the breakfast and lunch meals were selected from a restricted MiSBIE study menu to avoid large differences in meal types between participants.

4.1.2 Stress reactivity

A validated socio-evaluative speech stress task was conducted on all MiSBIE participants on Day 1, as described previously [71,72]. The 5-min test included 2 min of preparation and 3 min of delivering a simulated speech to a court judge where participants defend themselves against an alleged shoplifting transgression. The speech was delivered in front of a confederate evaluator wearing a white lab coat (adding gravity to the situation), a mirror where participants can see themselves, and a (non-operational) video camera. To minimize participant discomfort during the task and avoid venipuncture-related stress responses during the task, an intravenous catheter was placed in the right arm >45 min before the initiation of the speech stress task and participants sat quietly, undisturbed for 30 minutes before receiving instructions for the speech task. Blood and saliva samples were collected at -5, +5, 10, 20, 30, 60, 90, and 120 min relative to the beginning of the TSST. Concurrently, during each blood collection, saliva samples were collected using salivettes (Starstedt Cat# 51.1534.500). On day 2, while in the MRI scanner, to avoid habituation to the speech task, participants were informed that their performance on the previous day was “below average compared to other participants” and asked to prepare a speech to a court judge in defense of another alleged transgression (running a stop sign). Saliva samples were collected using salivettes at 3 timepoints: ~30 minutes before

the MRI session, 33 minutes into the session (~9 minutes before beginning the speech task), and 75 minutes into the session (~18 minutes after the beginning of the speech task).

4.1.3 Plasma and saliva collection

The blood and saliva collection protocols are illustrated in Supplemental Figure S2A. Plasma was collected via a central venous catheter at 9 time points: 1) day 1 morning fasting sample, 2) 8 afternoon samples collected during the stress reactivity protocol. A total of 16-20mL of whole blood was collected at the morning fasting timepoint in two 10 mL K_2EDTA blood collection tubes (BD #366643). A total of 5mL of whole blood was collected at each afternoon time point both before and after stress in 6mL K_2EDTA blood collection tubes (BD #367899).

Saliva was collected at 26 timepoints: 1) day 1 morning fasting sample, 2) 8 afternoon samples collected during the stress reactivity protocol, 3) 1 sample after a cold pressor test, 4) day 2 morning fasting sample, 5) 3 afternoon stress samples before and during the MRI, 6) 12 at home samples collected across 3 days: (i) immediately upon awakening, (ii) 30 min after waking up, (iii) 45 min after waking up, and (iv) at bedtime. A total of 1-2 mL of saliva was collected at each sample timepoint using a salivette (Starstedt # 51.1534.500) following the Biomarker Network recommended procedure [73]. Participants were directed to position the cotton swab at the center of their tongue within their mouth for 2 to 5 min. It was emphasized that they should refrain from biting down on the cotton and ensure the swab did not come into contact with their cheeks. Afterward, participants reinserted the cotton swab into the salivette recipient tube. Samples were collected on visit days 1 and 2, the salivettes were placed on ice (4°C) in a styrofoam box for transportation and further processing at the laboratory. For home-based sample collection, salivettes were frozen immediately after collection in a home freezer (typically -20°C). During the at-home morning collections, individuals were advised to ideally delay tooth brushing and eating until after the third sample (+45 minutes), while the nighttime sample was to be taken prior to bedtime tooth brushing. If participants needed to, they were advised to eat breakfast within the 30min break of awakening sampling and note the time and content. Participants were instructed to avoid consuming water or any other liquids within 10 minutes of each saliva sample collection. This was monitored and controlled for the laboratory samples. Within 2 weeks of collection, participants either transported (~10%) or shipped them (~90%) to the laboratory in a non-temperature controlled, pre-stamped USPS priority shipping.

4.1.4 Biofluids (Plasma and Saliva) processing and storage

Whole blood tubes for morning fasting plasma samples were immediately inverted 10-12 times and centrifuged at 1000xg for 5 minutes at room temperature after collection. Samples

were placed on ice (4°C) in a styrofoam box and transported to the laboratory for further processing. When samples reached the laboratory, they were immediately centrifuged at 2000xg for 10 minutes at 4°C. To avoid platelets and other cellular debris, approximately 80% of the plasma was collected from the upper portion of each tube, transferred and pooled into a fresh 15 mL conical tube. To further eliminate cellular components, pooled plasma tube was centrifuged at 2000xg for 10 minutes at 4°C. Around 90% of the resulting plasma supernatant was transferred to fresh 15 mL conical tube and mixed through inversion. The resulting cell-free plasma was aliquoted into 0.5-1.5 mL aliquots and stored at -80°C before being used for stress reactivity GDF15 measurements by ELISA.

Stress reactivity time points include 8 tubes for each timepoint, starting at -5 minutes to 120 minutes. Whole blood tubes for afternoon stress plasma samples were immediately inverted 10-12 times and centrifuged at 2000xg for 3.5 minutes at room temperature after collection. Samples were placed on ice (4°C) in a styrofoam box, and transported to the laboratory together for further processing. When samples reached the laboratory, they were immediately centrifuged at 2000xg for 10 minutes at 4°C. To avoid platelets and other cellular debris, approximately 80% of the plasma was collected from the upper portion of each tube and transferred into fresh 8*15 mL conical tubes. To further eliminate cellular components, plasma tubes were centrifuged at 2000xg for 10 minutes at 4°C. Around 90% of the resulting plasma supernatant was transferred to fresh 8*15 mL conical tubes and mixed through inversion. The resulting cell-free plasma was aliquoted into 0.5-1.5 mL aliquots for each timepoint and stored at -80°C before being used for stress reactivity GDF15 measurements by ELISA.

When saliva samples reached the laboratory, Salivettes were centrifuged at 1000xg for 5 min in a refrigerated centrifuge at 4°C. To avoid cellular contamination with leukocytes or epithelial buccal cells[74], supernatant was carefully removed from the top of the tube, transferred to cryovials, and stored immediately at -80°C. Before use, saliva was thawed and centrifuged at 5000xg for 10 min to further eliminate cellular components. The resulting supernatant was collected as cell-free saliva and was used for subsequent GDF15 assessment.

4.2. Study 2: Intensive repeated-measures n=2 study

4.2.1 Participants and procedures

To evaluate the intra-individual fluctuations of salivary GDF15, repeated daily measurements were taken over periods of 53 and 60 days from two Caucasian male participants: author M.P., 34 years old (Participant A) and author G.G., 35 years old (Participant

B, see [44]). Study investigators served as participants to mitigate the excessive demand on volunteer participants required to collect numerous daily samples over multiple consecutive weeks. The study was approved by the New York State Psychiatric Institute (Protocol #7748). Participants provided written informed consent for participating and publishing the results. As for the at-home collection protocol in the Study 1 MiSBIE cohort, participants collected saliva immediately upon awakening, 30min and 45min after waking up, and at bedtime. Participants delayed consuming liquid and tooth brushing until after the third sample (45 min), and the nighttime sample was taken prior to bedtime tooth brushing. On each morning and evening, participants completed a morning diary assessing positive and negative affective states, using items adapted from the modified Differential Emotional Scale (MDES), including ratings of stress levels on a 5-point Likert scale: 1 (Not at all), 2 (A little bit), 3 (Somewhat), 4 (Moderately) to 5 (Extremely) [75].

4.2.1. Saliva processing and storage

Salivette samples were stored in home freezer (-20°C) before being transported or shipped frozen to the laboratory. Upon arrival salivettes were thawed at room temperature and centrifuged at 1000 x g for 5 min in a refrigerated centrifuge operating at 4°C to extract saliva from the cotton swab. 200uL of saliva supernatant was taken from the top, transferred to a 1.5 ml Eppendorf tube and stored at −80°C. Prior to analysis, Eppendorf tubes was further centrifuged at 2000 x g for 10 min at 4°C to remove cellular debris, and the resulting supernatant was collected as cell-free saliva and was used for GDF15 measurements by ELISA. To control for the effect of saliva protein concentration on GDF15 results, total protein concentration was measured using the Bicinchoninic acid (BCA) assay as described previously [44].

4.3. Study 3: Overnight meal alignment study

4.3.1 Participants and procedures

Healthy females and males were recruited to participate in a randomized crossover study testing the impact of varying mealtimes, relative to wake time, on energy balance regulation (clinicaltrials.gov #NCT03663530). Participants interested in the study underwent a multi-stage screening process. Inclusion criteria included being 20-49 years of age at the time of screening, having a body mass index between 20-34.9 kg/m^2, healthy habitual sleep patterns, and habitually consuming the first meal of the day within 1 h of waking. Individuals who were pregnant or lactating, smoked (currently or <3 years since stopping), reported excessive

caffeine or alcohol intake, had extreme morning or evening chronotype, or who were actively engaging in intentional weight change or had a recent weight change >5% in the past 3 months were excluded. In addition, prospective participants with cancer; chronic cardiovascular, metabolic, autoimmune, neurologic, psychiatric, or sleep-related disorders/conditions; or who or take medications or supplements known to affect sleep or energy balance were not included cancer were not included. Individuals meeting all eligibility criteria competed a two-week outpatient screening period, which included daily assessment of sleep via wrist accelerometer (Actigraph GT3X+, Actigraph Corp., Pensacola, FL.) and daily sleep diaries along with assessment of daily meal timing/eating patterns. Those with average nightly total sleep time ≥7 h and average daily eating start times within 1 h of awakening were invited to participate in the study. All procedures were approved by the Columbia University Irving Medical Center Institutional Review Board, and all participants provided informed consent form prior to participating in the study.

The main study included two experimental conditions, circadian alignment and circadian misalignment, followed for 6 weeks each. During the circadian alignment condition, participants were instructed to consume their first meal within 1 h of awakening. Two subsequent meals and an evening snack were to be consumed at 5, 10, and 11 hours post-awakening, respectively. During the circadian misalignment condition, meals were delayed by 4 hours relative to the aligned condition. For the present analyses, only samples collected during the first 2 weeks of the aligned condition were used. This corresponded to a period during which all meals and snacks were provided to participants based on weight maintenance energy needs, determined using the Mifflin-St. Jeor equation [76]. Participants were required to consume all of the foods provided each day and to refrain from consuming other foods or beverages. On days 3-4 and 14-15 of this controlled feeding period, participants underwent cardiometabolic and energy expenditure assessments in a whole-room indirect calorimeter. During these 24-h visits, blood samples were collected every 30 min, from 7 PM to 8:30 AM, via an indwelling catheter connected to an IV line that ran through a port to the outside of the room. Throughout the wake period, participants remained in a semi-recumbent position in dim light, wearing blue-blocking lenses. They were instructed to consume the meals and snack provided at the assigned times, and to turn the lights out at 11:30 PM, to initiate sleep, and were awoken via phone call at 8 AM and instructed to turn the lights back on.

4.3.2. Serum processing and storage

Whole blood was collected into 4mL BD vacutainer serum tubes (cat# 367812). Blood remained upright in the tube until clotted, after which it was centrifuged for 20 minutes at 3000

RPM and 4°C. Resultant serum was aliquoted into cryovials and stored at -80°C until GDF15 measurements by ELISA.

4.4. Study 4: The Mitochondria and Psychological Stress (MaPS) study

4.4.1 Participants

Generally healthy community volunteers were recruited to participate in the MaPS study (clinicaltrials.gov #NCT04078035). Eligibility criteria were (1) 20-50 years of age (equal numbers within each decade of age); (2) for females, regular menstrual cycles over past 12 months, not lactating or pregnant; (3) generally physically healthy (no reported history of chronic systemic immune, metabolic or mitochondrial diseases, or chronic diseases that influence the central nervous, autonomic nervous or neuroendocrine systems); (4) not taking medications that influence the central nervous, autonomic nervous or neuroendocrine systems; (5) no reported history of psychotic illness or mood disorder; (6) resting blood pressure<140/90mmHg; (7) not a current smoker or user of illicit drugs; (8) weight>110lbs and BMI<30; and (9) fluent in English (used everyday in speaking and reading for at least 10 years). All enrolled participants provided written informed consent as approved by the University of Pittsburgh IRB. Recruitment occurred between July 2020- December 2022. A total of 72 participants were enrolled (n=34 self-identified as female; 38 as male). Participants were rescheduled if (1) they endorsed having an infection, cold or flu, taking antibiotics or glucocorticoids, or having received a vaccination or tattoo in the prior two weeks; (2) presented with symptoms in a range consistent with possible infection on the day of the visit; or (3) reported having taken glucocorticoids in the past 24 hours on the day of the visit.

4.4.2. Stress Reactivity

Participants attended two afternoon laboratory sessions scheduled at least one month apart at the Behavioral Physiology Laboratory, University of Pittsburgh. Both sessions started between 1:00-3:00 PM. Participants were asked to avoid eating or drinking after 12 pm prior to each session. The order of sessions was counterbalanced in randomized starting order across participants. At one session, they completed the same 5-minute socio-evaluative speech task that was used in Study 1, MiSBIE (see above). At the other session they rested quietly for the 5-minute task period and were not exposed to the stressor. On both occasions, an intravenous catheter was inserted and participants sat quietly for a 30-minute baseline (habituation) period before completing the task. Following the task period, participants watched a wildlife documentary for a 120-minute recovery period. Participants were left alone as much as possible,

except when the study nurse had to re-insert or reposition the catheter, during all periods except when performing the speech task. Blood samples were collected 5 minutes before and 5, 10, 20, 30, 45, 60, 75, 90, and 120min after the beginning of the task period. Saliva samples were not collected in this study.

4.5. GDF15 assays

Plasma, serum and saliva GDF15 levels were quantified using a high-sensitivity ELISA kit (R&D Systems, DGD150, SGD150) following the manufacturer's instructions. Different lot numbers were used, and the average coefficient of variation (C.V.) between lot numbers was determined by reference samples for quality control. Plasma and serum samples were diluted with assay diluent provide in the kit (1:4 ratio), saliva samples were not diluted. Absorbance was gauged at 450nm, and concentrations were computed utilizing the Four Parameter Logistic Curve (4PL) model. Samples were run in duplicate plates when possible, concentration for each sample was computed from the average between duplicates. Data below minimum detection level are excluded for graphing and statistical analysis. We are aware that 15-30% of the population carries the common H202D variant in GDF15 [77]. Compared to the Roche Elecsys assay, the R&D ELISA kits tend to reports GDF15 concentrations that are 36% lower in individuals carrying one D allele or 61% lower in those that carry two D alleles at position 202 of the pro-peptide (or position 6 of the mature peptide) [78]. Our group comparison results might be affected by this issue, but our findings related to stress reactivity and within-person dynamics are likely to remain unaffected.

Standard curve (5 samples per plate) and plasma reference samples (2-3 samples per plate, same sample per batch) were run with each individual assay and the inter-assay C.V. was monitored. All standard curves and references were overlaid on top of each other to monitor failed runs. Samples were run in duplicate plates when possible and those with C.V. larger than 15% were rerun. When it was not possible to rerun (e.g.. no sample left), sample sets with a C.V. >15% between the duplicates were excluded for sensitivity analysis and did not change the results. For the MiSBIE study, samples were run in 3 batches over 2 years, multiple quality control measures were applied to monitor batch-to-batch and within batch variability. For a full experimental setup, see Supplementary Table 4. Data-preprocessing and quality control measures was done using R Software (version 4.2.2) and are available as supplemental files.

To analyze *GDF15 gene* expression, we utilized RNA sequencing data from the Genotype-Tissue Expression (GTEx) Project v8 dataset encompassing 948 donors and a total of 17,382

samples. GTEx v8 is available for download at https://gtexportal.org/home/downloads/adult-gtex/bulk_tissue_expression. Gene read counts were normalized using the trimmed mean of M-values (TMM) normalization method with the edgeR package [79]. The age of donors is part of the protected access data and was obtained through the dbGAP accession #phs000424.v8.p2 under the project #27813 (Defining conserved age-related gene expression trajectories). We extracted donor's age, *GDF15 gene* expression data sequenced all 54 human tissues. Single cell data sequenced in the minor salivary gland and immunohistochemistry analysis of GDF15 protein abundance in various tissues were extracted from the Human Protein Atlas and presented in this paper. The Human Protein Atlas data is available for download at https://www.proteinatlas.org/about/download. Histology graphs used in this paper are: GDF15 (https://www.proteinatlas.org/ENSG00000130513-GDF15/tissue/salivary+gland), TOMM20 (https://www.proteinatlas.org/ENSG00000173726-TOMM20/tissue/salivary+gland), CPT1A (https://www.proteinatlas.org/ENSG00000110090-CPT1A/tissue/salivary+gland), TFAM (https://www.proteinatlas.org/ENSG00000108064-TFAM/tissue/salivary+gland), NDUFS4 (https://www.proteinatlas.org/ENSG00000164258-NDUFS4/tissue/salivary+gland), MCU (https://www.proteinatlas.org/ENSG00000156026-MCU/tissue/salivary+gland)

4.6. Statistical analyses

The association between age and plasma/salivary GDF15 level at morning, fasting sample (baseline) was determined using Spearman rank correlation. Group differences (e.g. sex difference, difference between the controls, 3243 and deletion groups) were assessed using non-parametric Mann Whitney test and Tukey multiple comparisons post hoc test where appropriate. Differences across two timepoints in the same individual (e.g. Morning to afternoon, day 1 to day 2 fasting) were assessed using paired non-parametric signed-rank Wilcoxon test. Diurnal pattern was assessed using Tukey multiple comparison post hoc test. Stress reactivity was operationalized as % change from baseline at each time point, and a mixed effect models were used to test whether acute stress affected plasma and saliva GDF15 levels over time. The magnitude of GDF15 stress reactivity and comparisons of GDF15 levels between MitoD groups and the control group were quantified as effect sizes (Hedge's g) which calculates differences of the means divided by the pooled standard deviations. To capture longitudinal stress reactivity and awakening response over the time range of 8 time points spanning from 5 minutes before TSST to 120 min after TSST, we used area under the curve (AUC). Specifically, for stress reactivity, AUC of GDF15 levels over the 8 time points was calculated as the area under the longitudinal GDP15 measures, where missing GDF15 values were imputed using mean GDF15

of all available time points if missing happened at last few consecutive time points, or using weighted average of two adjacent available GDF15 measures weighted by fraction of time intervals, with bigger weights for closer measures, if the missing value happened elsewhere. Only participants with pre-stress baseline and at least 4 stress timepoints were included in the AUC analysis. For awakening response analysis from immediate upon awakening to 45 min, we averaged GDF15 measures collected at the same timepoint during a day from three days (Mon, Weds, and Fri). Awakening response AUC was calculated similarly using GDF15 at 3 timepoints. We also examined the ability of blood and saliva GDF15 levels to distinguish (i.e., diagnose) all three MitoD groups combined from controls using simple logistic models, where results are presented using receiver operating characteristic (ROC) curves. The optimum GDF15 cutoff values were determined using Youden index. Statistical analyses were conducted using GraphPad Prism (version 9.4.1) and R Software (version 4.2.2 and 4.3.0)

5. Code availability statement

The R scripts for data analysis are available on GitHub at https://github.com/mitopsychobio/2024_GDF15_Dynamics_Huang

SUPPLEMENTAL INFORMATION

Supplemental Table S1. Number of samples processed by study and sample types, with proportion of missing data.

Supplemental Table S2. GDF15 gene expression in human tissues.

Supplemental Table S3. Single cell RNA sequencing based GDF15 expression in minor salivary gland.

Supplemental Table S4. Sample size for plasma and saliva GDF15, by group, at different timepoints.

Financial competing interests

The authors have no competing interests to declare.

Author contributions

M.P., M.H., C.T., R.P.S., V.L., R.P.J. developed Study 1 (MiSBIE). M.P., G.G., S.R., C.T. developed Study 2 (intensively sampled n=2). M.P.S.O. developed Study 3 (24-hour meal alignment). A.L.M., B.A.K., M.P. developed Study 4 (MaPS). C.K. and K.E. recruited MiSBIE participants. C.K. and S.R. enrolled participants, collected study visits data and samples. M.K. processed samples. S.L. and M.H. performed the medical examinations. J.D. and A.S.M. analyzed gene expression data. Q.H., R.H., S.R., A.S.M., C.C.L. performed GDF15 assays. E.T. performed the lactate assays in collaboration with J.M. Q.H., A.S.M. and C.C.L. performed statistical analyses. Q.H. and C.T. prepared the figures. S.W. and T.W. advised on statistical analyses. C.T., Q.H., M.P. drafted the manuscript. M.P. and C.T. conceived and supervised the overall project. All authors reviewed the final version of the manuscript.

Acknowledgements

Work of the authors is supported by R01MH122706, R01AG066828 and Baszucki Brain Research Fund to M.P., the Wharton Fund to M.P. and C.T., R01MH119336 to A.L.M., B.A.K., and M.P., R01HL142648, R35HL155670 and UL1TR001873 to M.P.S.O., the New York Obesity Nutrition Research Center grant P30DK026687-39, the Chercheur-boursier Junior 2 salary award from the Fonds de recherche du Québec en santé (FRQS-297877) to G.G., the Fonds de Recherche Santé Québec and the Fondation de l'Institut universitaire en santé mentale de Montréal to R.P.J.

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Figure legends

Figure 1. *GDF15* is expressed in human salivary gland and GDF15 protein is detectable in saliva, where it does not correlate with age.

(**A**) Study design where GDF15 is concurrently measured in human plasma and saliva.

(**B**) *GDF15* gene expression (RNA transcript levels) across human tissues from the GTEx dataset.

(**C**) *GDF15* gene expression in single cells from the minor salivary gland, retrieved from the Human Protein Atlas.

(**D**) Immunohistochemistry for GDF15 protein in the minor salivary gland of a 62-year-old male (HPA subject ID 2367). For additional mitochondrial proteins, see Supplemental Figure S1.

(**E**) Study 1 GDF15 measured in plasma and saliva (all timepoints, plasma: N=571 observations, 59 missing due to failed blood draw; saliva: N=1,476 observations, 315 missing due to low saliva volume, 29 below the minimum detection limit).

(**F**) Correlation between morning fasting plasma and saliva GDF15 levels in Study 1 (N=47, 66% female).

(**G**) Correlations (Spearman's r) between *GDF15* expression and age in all GTEx tissues showing largely positive correlations. The bladder has the smallest sample size (N=21) and includes several outliers that invite caution in interpreting this datapoint.

(**H**) Correlations between age and *GDF15* gene expression in thyroid (N=948, strongest correlation) and minor salivary gland (N=937) where each datapoint represents a GTEx participant.

(**I**) Correlations between Study 1 participant age and morning fasting GDF 15 levels in plasma (N=68, 68% female) and saliva (N=48, 67% female).

GTEx data (B,G,H) excludes cultured cells and tissues with N<20; see Supplemental Table 2 for full dataset used. Data from Human Protein Atlas (C,D) is detailed in Supplemental Table 3. Data from healthy participants aged 18-60 years old in Study 1 (N=70, 69% female). Effect sizes and p-values from (E) Mann-Whitney t-test, (F,H,I) Spearman's rank correlation. Data below minimum detectable concentration excluded for graphs and analyses (E,F,I). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure 2. Saliva GDF15 exhibits a robust negative awakening response and large within-person variation over days to weeks.

(**A**) Schematic of experimental design measuring GDF15 awakening response from human saliva samples. Saliva was sampled at awakening, 30 and 45 min after awakening, and at bedtime.

(**B**) Saliva GDF15 diurnal variation. Data from the Study 1 healthy adults on three non-consecutive days four timepoints each day (N=710 observations, 68% female).

(**C**) Study 2 saliva GDF15 concentrations (*left*, log10 scale; *right*, median on linear scale) in Participants A and B.

(**D,E**) Intensive repeated sampling of saliva GDF15 levels showing day-to-day variation Participant A (N=199 observations, repeated measures for 53 days) and Participant B (N=221 observations, repeated measures for 60 days). The 4-day yellow highlight (Days 27-31) were

relatively anomalous and showed no cortisol awakening response and no cell-free mtDNA awakening response[44].

(**F,G**) Diurnal profiles for saliva GDF15 in Participant A and B. *Top*, individual days; bottom, average trajectory. One outlier timepoint, Day 28, 45 min, was removed from analyses. See figure S3D for median (**A,B**).

Data are mean ± SEM unless otherwise noted. Effect sizes and P-values from (B,F,G) Mixed-effect model and Tukey multiple comparison test corrected for multiple testing. All data were included in graph, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure 3. Socio-evaluative stress acutely increases plasma and saliva GDF15.

(**A**) Study 1 experimental design measuring psychological response in GDF15 after socio-evaluative stress elicited by a modified version of the Trier Social Stress Test (TSST) speech task.

(**B**) Plasma lactate levels 5 min before and 5min after the stressful speech task shown as individual trajectories (*left*) and percent change from baseline (*right*). N=47, 72% female, 94 observations. See Supplemental Figure S5A-B for the full time course.

(**C,D**) Plasma GDF15 levels before and after psychological stress shown as (**C**) individual trajectories and (**D**) percent change from baseline (-5 min). N=65, 68% female, 512 observations.

(**E,F**) Same as in (**C,D**) for Saliva GDF15. N=57, 65% female, 442 observations.

(**G**) Study 4 experimental design including two visits randomized to either socio-evaluative speech task (same as in Study 1) or a control session without stress exposure. Plasma GDF15 was measured at 8 timepoints before and after stress, following the same schedule as Study 1.

(**H,I**) Plasma GDF 15 levels for control and stress visit shown as (**H**) individual trajectories and (**I**) percent change from baseline (-5min). N=70, 48% female, 916 observations. See Supplemental Figure S5 for additional data.

Data shown as mean ± SEM. P-values from Wilcoxon paired t-test (B) or mixed-effect model followed by Tukey multiple comparison test, corrected for multiple testing (D,F,I). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure 4. Mitochondrial OxPhos defects increase plasma and saliva GDF15, and mostly preserve GDF15 dynamics.

(**A**) Study 1 control and MitoD groups including the m.3243A>G point mutation without (Mutation) or with MELAS, and single, large-scale mitochondrial DNA deletion (Deletion).

(**B**) Mean plasma and saliva GDF15 concentrations in MitoD participants (N=40, 70% female) aged 20-60 years old. See Supplemental Figure S6A for log 10 transformed distribution with individual datapoints.

(**C**) Correlations between age and plasma GDF15 levels. Data from morning fasting timepoints. 3243A>G mutation carriers and MELAS groups aggregated as Mutation.

(**D**) Saliva GDF15 diurnal variation in mitochondrial diseases at awakening, 30 and 45 min after awakening, and bedtime shown as individual trajectory (left) and group average.

(**E**) individual trajectories and (**F**) percent change from baseline (-5 min). The magnitude (% change) in GDF15 were compared using Hedge's g for selected timepoints.

(**G,H**) Same as in (**E,F**) for Salivary GDF15 levels.

Data shown as mean ± SEM. The magnitude (% change) in GDF15 were compared using Hedge's g for selected timepoints. Effect sizes and P-values from (B) Mann-Whitney t-test and (C) Spearman's rank correlation, (D,F,H) mixed-effect model and Tukey multiple comparison tests corrected for multiple testing. Patients with MitoD plasma timepoints: Deletion N=15, 73% female, 102 observations; 3243A>G mutation N=20, 75% female, 160 observations; MELAS N=5, 40% female, 28 observations. Patients with MitoD saliva timepoints: Deletion N=15, 73% female, 302 observations; 3243A>G N=20, 74% female, 453 observations; MELAS N=5, 940% female, 5 observations. See Supplemental Table 4 for sample size at each timepoint. *p<0.05, **p<0.01, **p<0.001, ****p<0.0001.

Figure 5. Plasma and post-stress saliva GDF15 classify individuals with mitochondrial OxPhos defects and correlate with disease severity.

(**A**) Standardized effect sizes (Hedge's g) for the difference in plasma GDF15 between control and MitoD participants in Study 1, using measurements performed at different timepoints: Day 1 morning fasting, Day 2 morning fasting, stress reactivity quantified by area under the curve relative to ground, pre-MRI session, mid-2-hour MRI session, awakening sample collected at home. Bars highlight the higher effect size relative to the morning fasting timepoint for 5 of the 6 group comparisons. Hedges g>0.8 is considered a large effect size. Control N=48-69, MitoD N=31-37.

(**B**) Same as (A) for saliva GDF15.

(**C,D**) Receiver Operating Curve (ROC) curve analysis for plasma (C) or saliva (D) GDF15 in distinguishing Controls (N=48-68) from MitoD groups combined (N=30-33). For ROC curve analysis separately for Deletion and Mutation groups, see Supplemental Figures S5J-K.

(**E,F**) Correlations between disease severity indices (*rows*) and plasma or saliva GDF15 (*columns*) measured from at all Study 1 timepoints. *NMDAS*: Newcastle Mitochondrial Diseases Assessment Score; *COMPASS*: Composite Autonomic Symptom Score-31, *CNS*: Columbia Neurological Score, *FIS*: total Fatigue Impact Scale. For the CNS and 30 seconds sit-stand test, higher scores indicate fewer symptoms and better functional capacity.

(**G**) Spearman's rank correlations between plasma GDF15 measured from Day 1 morning fasting (*top*), 20 min post stress (*bottom*). N=28-32 MitoD.

(**H**) Same as (G) for Saliva GDF15 measured from Day 1 morning fasting (top) and sample taken at the end of 2-hour MRI session (bottom). N=26-30 MitoD.

Total sample sizes: Patients with MitoD plasma timepoints: Deletion N=15, 73% female, 102 observations; 3243A>G mutation N=20, 75% female, 160 observations; MELAS N=5, 40% female, 28 observations. Patients with MitoD saliva timepoints: Deletion N=15, 73% female, 302 observations; 3243A>G N=20, 74% female, 453 observations; MELAS N=5, 940% female, 5 observations. Only participants with pre-stress baseline and more than five stress timepoints were included in the AUC analysis. See Supplemental Table 4 for sample size at each timepoint. *p<0.05, **p<0.01, **p<0.001, ****p<0.0001.

Figure 1

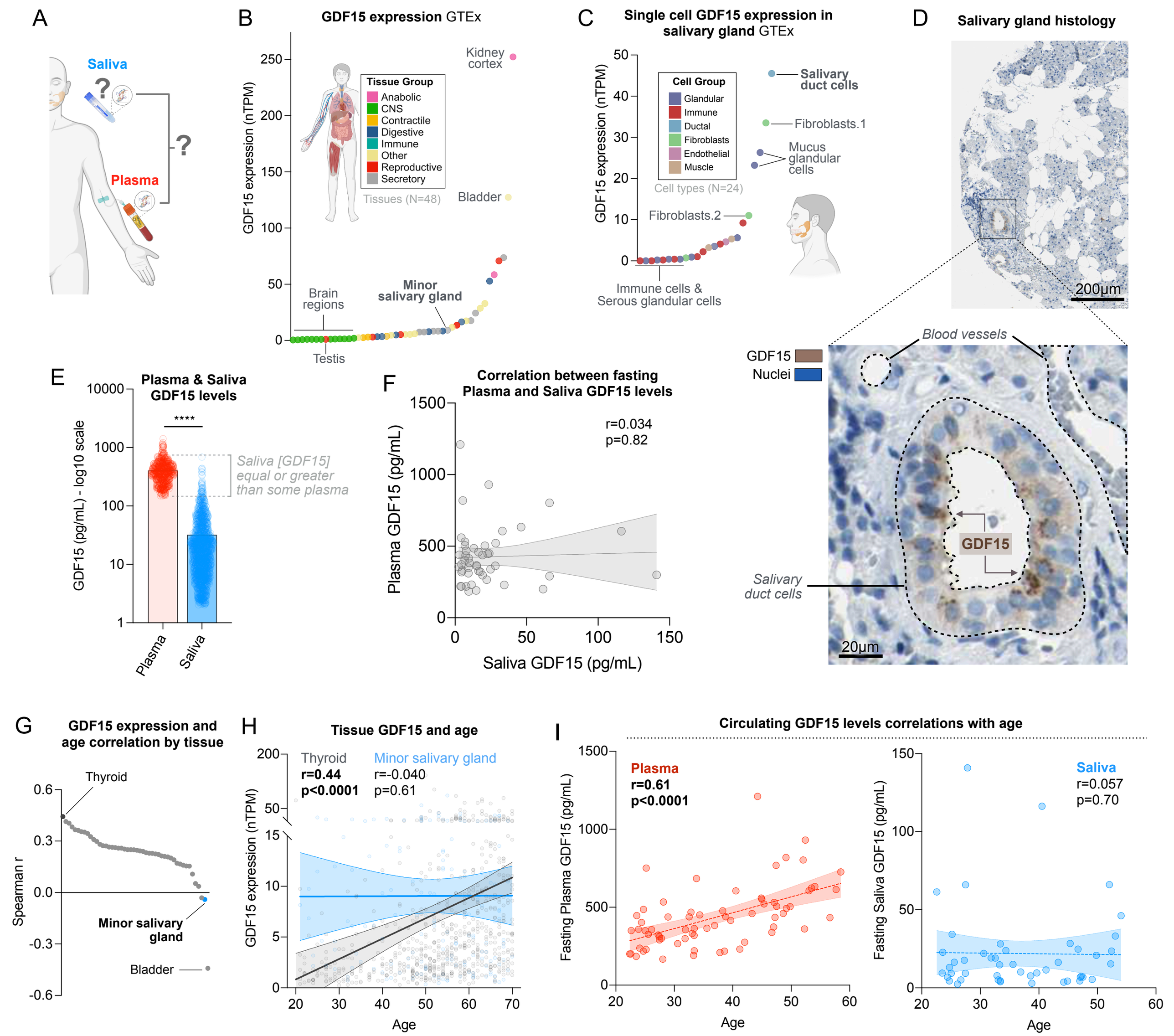

A
Saliva
?
?
Plasma
B
GDF15 expression GTEx
GDF15 expression (nTPM)
Tissue Group
Anabolic
CNS
Contractile
Digestive
Immune
Other
Reproductive
Secretory
Tissues (N=48)
Kidney cortex
Bladder
Brain regions
Minor salivary gland
Testis
C
Single cell GDF15 expression in salivary gland GTEx
GDF15 expression (nTPM)
Cell Group
Glandular
Immune
Ductal
Fibroblasts
Endothelial
Muscle
Cell types (N=24)
Salivary duct cells
Fibroblasts.1
Mucus glandular cells
Fibroblasts.2
Immune cells & Serous glandular cells
D
Salivary gland histology
200µm
Blood vessels
GDF15
Nuclei
GDF15
Salivary duct cells
20µm
E
Plasma & Saliva GDF15 levels
GDF15 (pg/mL) - log10 scale
****
Saliva [GDF15] equal or greater than some plasma
Plasma
Saliva
F
Correlation between fasting Plasma and Saliva GDF15 levels
r=0.034
p=0.82
Plasma GDF15 (pg/mL)
Saliva GDF15 (pg/mL)
G
GDF15 expression and age correlation by tissue
Thyroid
Spearman r
Minor salivary gland
Bladder
H
Tissue GDF15 and age
Thyroid
r=0.44
p<0.0001
Minor salivary gland
r=-0.040
p=0.61
GDF15 expression (nTPM)
Age
I
Circulating GDF15 levels correlations with age
Plasma
r=0.61
p<0.0001
Fasting Plasma GDF15 (pg/mL)
Age
Saliva
r=0.057
p=0.70
Fasting Saliva GDF15 (pg/mL)
Age

Figure 2

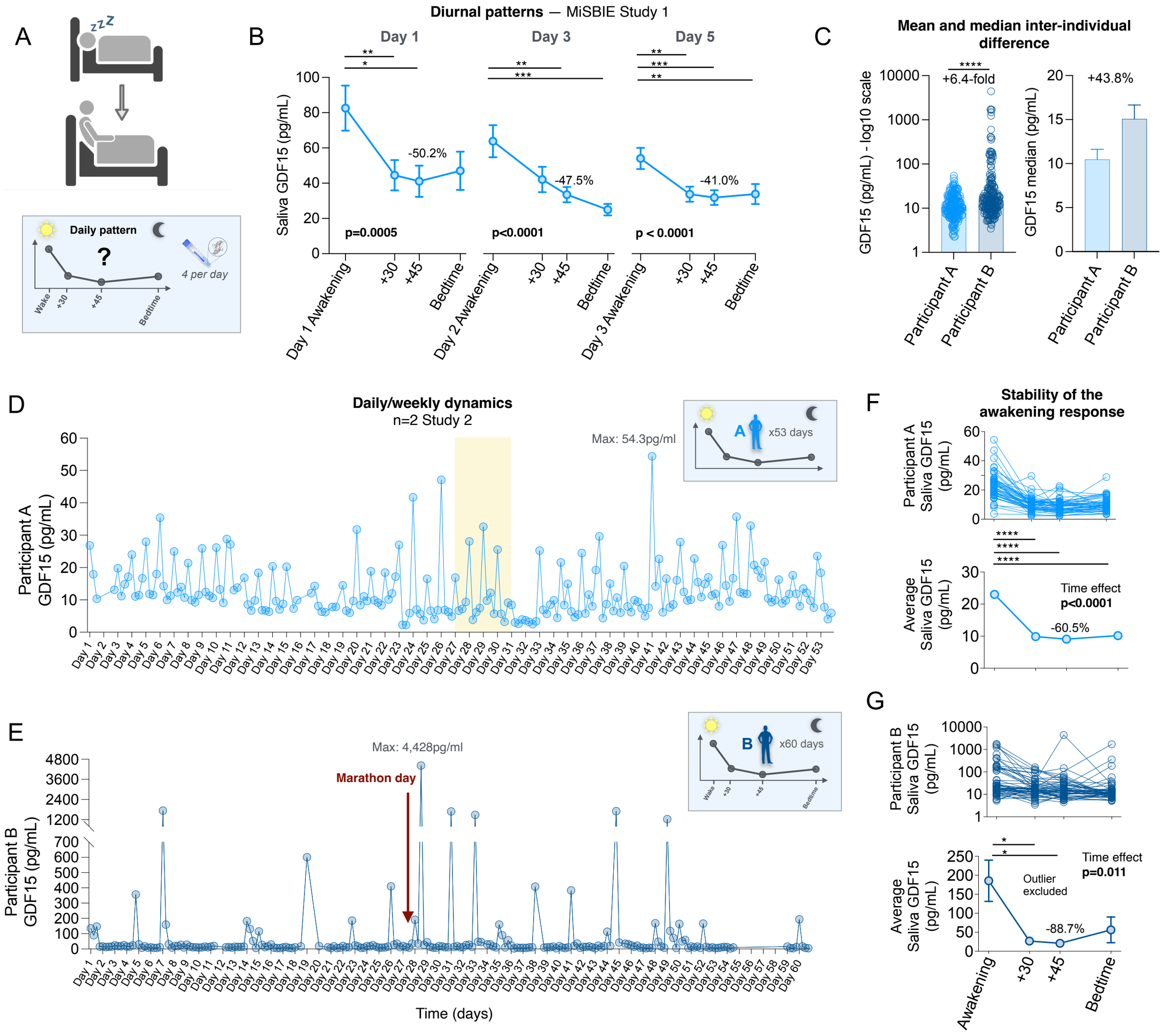

A
Daily pattern
?
4 per day
Wake
+30
+45
Bedtime
B
Diurnal patterns — MiSBIE Study 1
Day 1
Day 3
Day 5
Saliva GDF15 (pg/mL)
-50.2%
-47.5%
-41.0%
p=0.0005
p<0.0001
p < 0.0001
Day 1 Awakening
Day 2 Awakening
Day 3 Awakening
C
Mean and median inter-individual difference
GDF15 (pg/mL) - log10 scale
+6.4-fold
GDF15 median (pg/mL)
+43.8%
Participant A
Participant B
D
Daily/weekly dynamics
n=2 Study 2
Participant A GDF15 (pg/mL)
Max: 54.3pg/ml
x53 days
E
Max: 4,428pg/ml
Marathon day
Participant B GDF15 (pg/mL)
x60 days
Time (days)
F
Stability of the awakening response
Participant A Saliva GDF15 (pg/mL)
Average Saliva GDF15 (pg/mL)
Time effect
p<0.0001
-60.5%
G
Participant B Saliva GDF15 (pg/mL)
Average Saliva GDF15 (pg/mL)
Time effect
p=0.011
Outlier excluded
-88.7%
Awakening
+30
+45
Bedtime

Figure 3

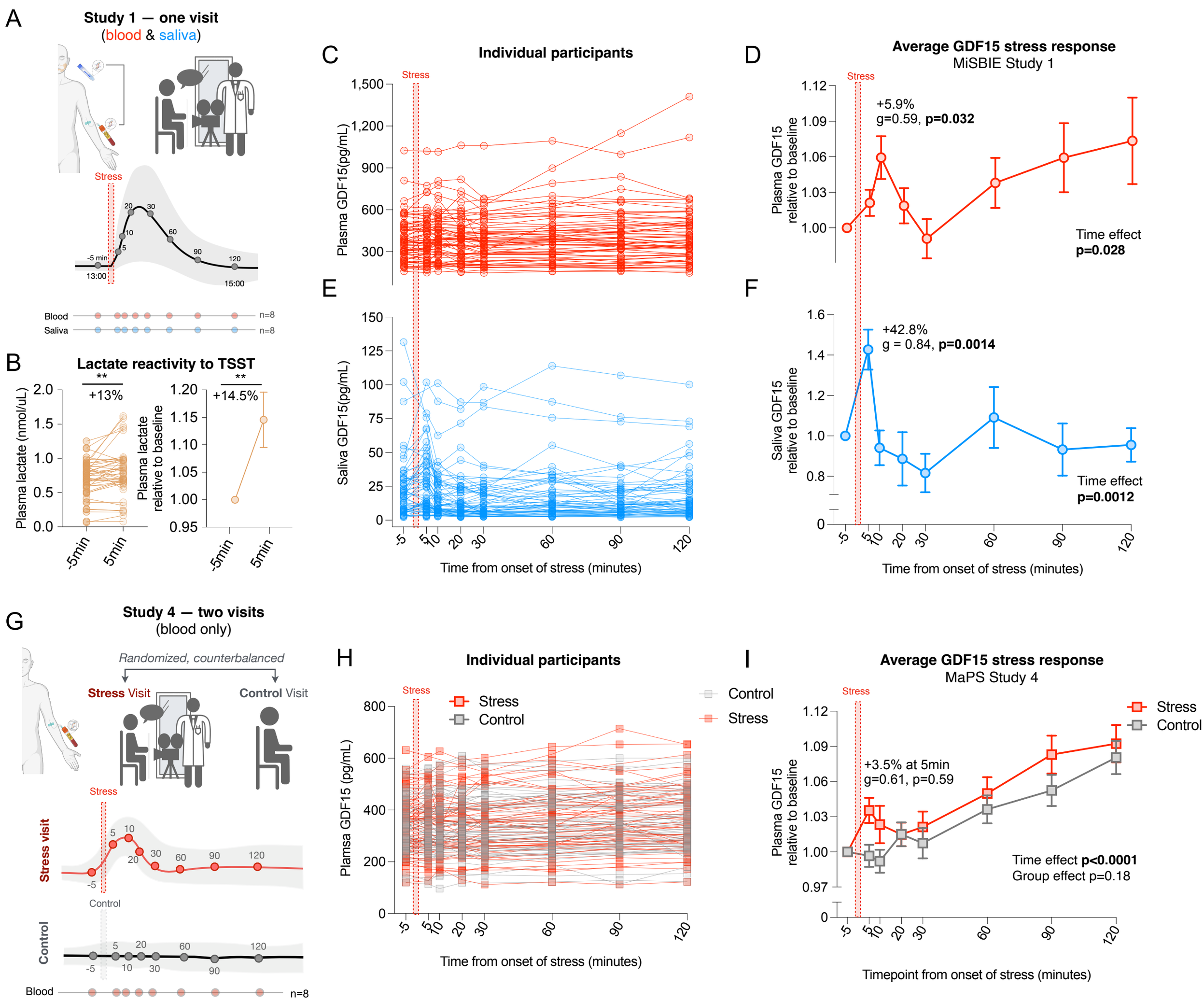

A
Study 1 — one visit
(blood & saliva)
Stress
-5 min
13:00
15:00
Blood
Saliva
n=8
B
Lactate reactivity to TSST
Plasma lactate (nmol/uL)
+13%
Plasma lactate relative to baseline
+14.5%
-5min
5min
C
Individual participants
Plasma GDF15(pg/mL)
D
Average GDF15 stress response
MiSBIE Study 1
Plasma GDF15 relative to baseline
+5.9%
g=0.59, p=0.032
Time effect
p=0.028
E
Saliva GDF15(pg/mL)
Time from onset of stress (minutes)
F
Saliva GDF15 relative to baseline
+42.8%
g = 0.84, p=0.0014
Time effect
p=0.0012
Time from onset of stress (minutes)
G
Study 4 — two visits
(blood only)
Randomized, counterbalanced
Stress Visit
Control Visit
Stress visit
Control
Blood
n=8
H
Individual participants
Stress
Control
Plamsa GDF15 (pg/mL)
Time from onset of stress (minutes)
I
Average GDF15 stress response
MaPS Study 4
Plasma GDF15 relative to baseline
+3.5% at 5min
g=0.61, p=0.59
Time effect p<0.0001
Group effect p=0.18
Timepoint from onset of stress (minutes)

Figure 4

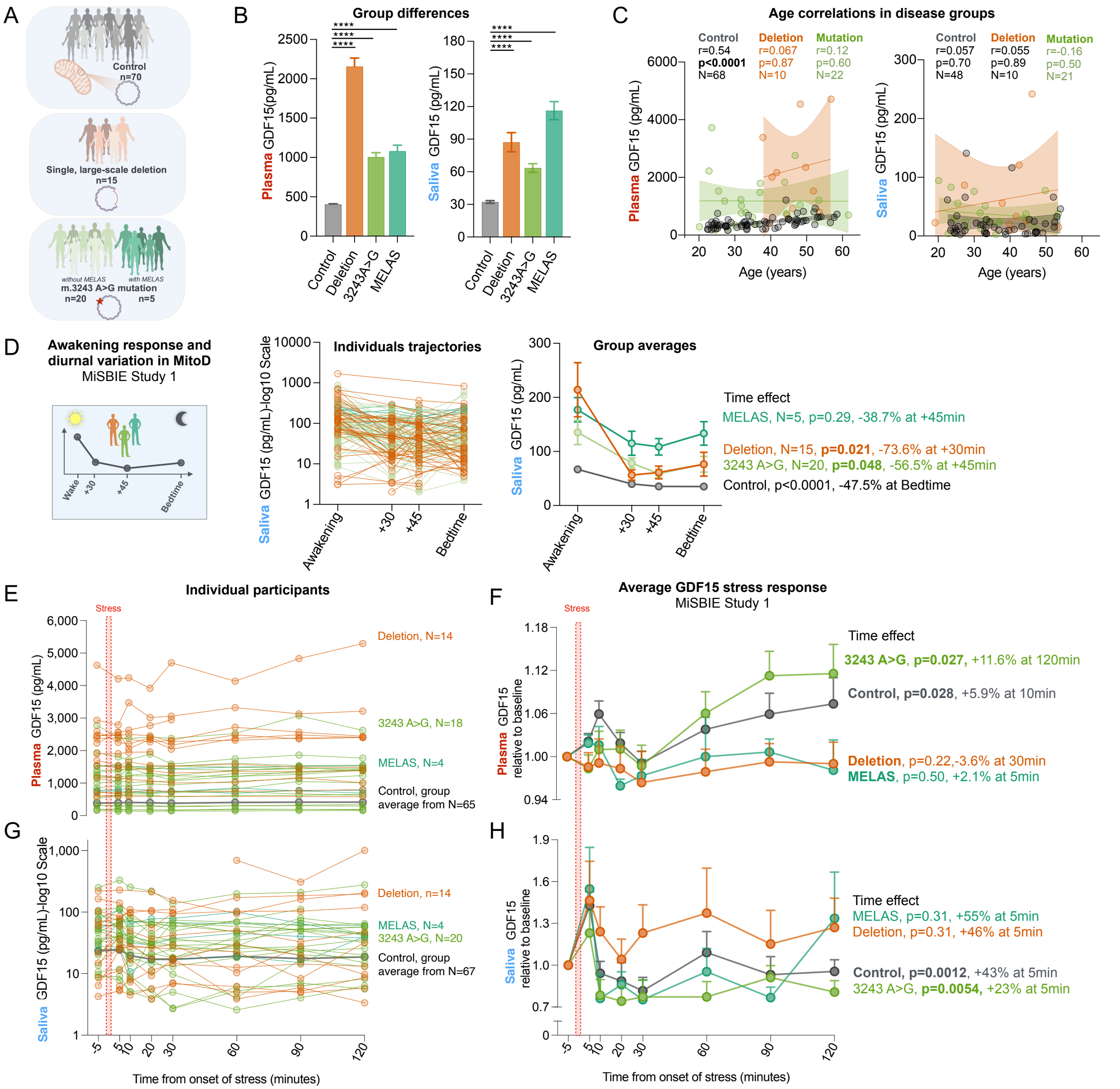
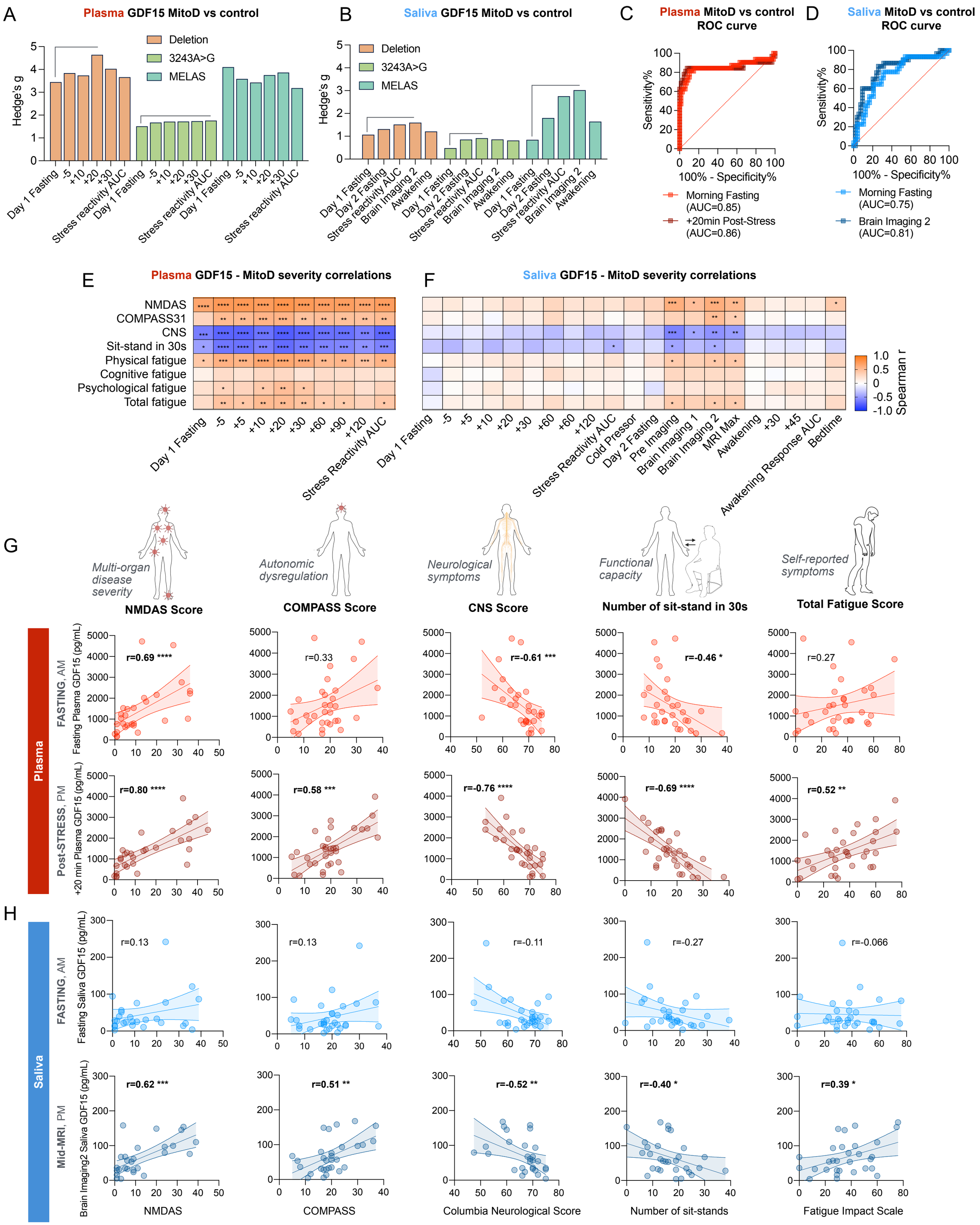

A
Control
n=70
Single, large-scale deletion
n=15
without MELAS
with MELAS
m.3243 A>G mutation
n=20
n=5
B
Group differences
Plasma GDF15(pg/mL)
Saliva GDF15 (pg/mL)
Control
Deletion
3243A>G
MELAS
C
Age correlations in disease groups
Control
r=0.54
p<0.0001
N=68
Deletion
r=0.067
p=0.87
N=10
Mutation
r=0.12
p=0.60
N=22
Control
r=0.057
p=0.70
N=48
Deletion
r=0.055
p=0.89
N=10
Mutation
r=-0.16
p=0.50
N=21
Plasma GDF15 (pg/mL)
Saliva GDF15 (pg/mL)
Age (years)
D
Awakening response and diurnal variation in MitoD
MiSBIE Study 1
Wake
+30
+45
Bedtime
Individuals trajectories
Saliva GDF15 (pg/mL)-log10 Scale
Awakening
+30
+45
Bedtime
Group averages
Saliva GDF15 (pg/mL)
Time effect
MELAS, N=5, p=0.29, -38.7% at +45min
Deletion, N=15, p=0.021, -73.6% at +30min
3243 A>G, N=20, p=0.048, -56.5% at +45min
Control, p<0.0001, -47.5% at Bedtime
E
Individual participants
Stress
Plasma GDF15 (pg/mL)
Deletion, N=14
3243 A>G, N=18
MELAS, N=4
Control, group average from N=65
G
Saliva GDF15 (pg/mL)-log10 Scale
Deletion, n=14
MELAS, N=4
3243 A>G, N=20
Control, group average from N=67
Time from onset of stress (minutes)
F
Average GDF15 stress response
MiSBIE Study 1
Stress
Plasma GDF15 relative to baseline
Time effect
3243 A>G, p=0.027, +11.6% at 120min
Control, p=0.028, +5.9% at 10min
Deletion, p=0.22,-3.6% at 30min
MELAS, p=0.50, +2.1% at 5min
H
Saliva GDF15 relative to baseline
Time effect
MELAS, p=0.31, +55% at 5min
Deletion, p=0.31, +46% at 5min
Control, p=0.0012, +43% at 5min
3243 A>G, p=0.0054, +23% at 5min
Time from onset of stress (minutes)

Figure 5


A
Plasma GDF15 MitoD vs control
Deletion
3243A>G
MELAS
Hedge's g
Day 1 Fasting
-5
+10
+20
+30
Stress reactivity AUC
B
Saliva GDF15 MitoD vs control
Day 2 Fasting
Brain Imaging 2
Awakening
C
Plasma MitoD vs control ROC curve
Sensitivity%
100% - Specificity%
Morning Fasting (AUC=0.85)
+20min Post-Stress (AUC=0.86)
D
Saliva MitoD vs control ROC curve
Morning Fasting (AUC=0.75)
Brain Imaging 2 (AUC=0.81)
E
Plasma GDF15 - MitoD severity correlations
NMDAS
COMPASS31
CNS
Sit-stand in 30s
Physical fatigue
Cognitive fatigue
Psychological fatigue
Total fatigue
Stress Reactivity AUC
F
Saliva GDF15 - MitoD severity correlations
Cold Pressor
Pre Imaging
MRI Max
Awakening Response AUC
Bedtime
Spearman r
G
Multi-organ disease severity
NMDAS Score
Autonomic dysregulation
COMPASS Score
Neurological symptoms
CNS Score
Functional capacity
Number of sit-stand in 30s
Self-reported symptoms
Total Fatigue Score
Plasma
FASTING, AM
Fasting Plasma GDF15 (pg/mL)
r=0.69 ****
r=0.33
r=-0.61 ***
r=-0.46 *
r=0.27
Post-STRESS, PM
+20 min Plasma GDF15 (pg/mL)
r=0.80 ****
r=0.58 ***
r=-0.76 ****
r=-0.69 ****
r=0.52 **
H
Saliva
FASTING, AM
Fasting Saliva GDF15 (pg/mL)
r=0.13
r=0.13
r=-0.11
r=-0.27
r=-0.066
Mid-MRI, PM
Brain Imaging2 Saliva GDF15 (pg/mL)
r=0.62 ***
r=0.51 **
r=-0.52 **
r=-0.40 *
r=0.39 *
NMDAS
COMPASS
Columbia Neurological Score
Number of sit-stands
Fatigue Impact Scale