Mitochondrial Stress, Brain Imaging, and Epigenetics (MiSBIE)

Statistical Analysis Plan

NCT04831424
October 6, 2023

Outcome Measures

Primary Outcome Measure:

1. ***Average TSST-induced elevation in cortisol***
   This is designed to measure cortisol reactivity to the trier social stress test (TSST), quantified from salivary cortisol (LC-MS) over an 8-timepoints timecourse. The elevation will be measured as the area under the curve (AUC) for the cortisol time course.
   [Time Frame: Day 1 post challenge (approximately 2 hours)]

SAP and Results: The AUC analysis for salivary cortisol reactivity to the TSST utilized two metrics: AUCg, where the ground is set at zero, and AUCi, where the ground is set at the baseline level. Given the non-Gaussian distribution of both groups, we applied the Mann-Whitney U test to compare the Control and MitoD groups. For AUCg, the MitoD group's cortisol level was 31.9% higher than the Control (64.49 ng/mL vs. 48.97 ng/mL), but this difference was not statistically significant ($p = 0.40$). The AUCi showed a significant 86.8% higher level in the MitoD group compared to the Control (20.91 ng/mL vs. 11.21 ng/mL, $p = 0.049$).

2. ***Average allostatic load index***
   Groups will be compared on a quantitative allostatic load (AL) index integrating baseline fasting measures of neuroendocrine, immune and metabolic systems, urinary catecholamines, hematological measures, and hair/diurnal cortisol levels.
   [Time Frame: Blood collected on Day 1]

SAP and Results: We calculated allostatic load using the standard count-based index (Juster et al., 2024). In short, for each biomarker, we calculated the 12.5th, 25th, 75th, and 87.5th percentile according to sex-specific reference ranges. We used these percentiles to score each variable as either dysregulated (1) or not dysregulated (0) and summed each variable's score to produce the final allostatic load value. Using the non-parametric Mann-Whitney test, we find that allostatic load is significantly higher in participants with mitochondrial disease than in controls ($p = 0.018$), with a medium effect size (Hedge's $g = 0.52$).

Secondary Outcome Measures:

3. ***Average TSST-induced elevation in heart rate***
   Groups will be compared on heart rate (HR) as a measure of cardiovascular reactivity to stress, monitored using a continuous 3-lead ECG. The elevation will be computed from the baseline HR to the peak HR reached during the TSST.
   [Time Frame: Day 1 post challenge (approximately 2 hours)]

SAP and Results: Given the non-Gaussian distribution of this data, we conducted the Kruskal-Wallis test to examine differences in heart rate elevation among four clinical groups.

Given the non-Gaussian distribution of this data, we conducted a Mann-Whitney U test to examine differences in heart rate elevation between the control group and combined mitochondrial disease groups (m.3243A>G, MELAS, and deletion). The Mann-Whitney U test indicated that there were no statistically significant differences in heart rate elevation among the groups ($p = 0.42$, $d = 0.27$).

4. ***Correlation between anxiety and mitochondrial respiration***
   The association between mitochondrial respiration using extracellular flux analysis (Seahorse) on blood lymphocytes, and anxiety symptoms measured using the state and trait anxiety inventory (STAI), will be quantified by a linear regression across all study participants.
   [Time Frame: Day 1]

SAP and Results: Mitochondrial respiration in isolated lymphocytes was measured on a XFe96Seahorse extracellular flux analyzer. In brief, 0.25M live lymphocytes were seeded in an extracellular flux cell culture plate, and a MitoStressTest was performed following the manufacturer's protocol. Baseline oxygen consumption rate (OCR) was corrected for non-mitochondrial respiration (OCR after rotenone/antimycinA injection) to quantify basal mitochondrial respiration. Linear regression analysis across all participants revealed that higher values of mitochondrial respiration in isolated lymphocytes are associated with lower *state* anxiety ($R^2 = 0.05$, $p = 0.04$). However, the relationship between mitochondrial respiration and *trait* anxiety was not significant ($R^2 = 0.01$, $p = 0.49$).

5. ***Average neuropsychological function***
   Groups will be compared on the fluency/initiation domain of executive functioning assessed using the Delis-Kaplan Executive Function System (D-KEFS) test.
   [Time Frame: Day 2 neuropsychological session]

SAP and Results: To calculate a standardized score (z-score), we first adjusted raw neuropsychological scores for age using data from the reference group (control group). Predicted values from this model, representing age-adjusted scores, were used to calculate z-scores (Andrade, 2021). Next, we conducted a Mann-Whitney U test to examine differences in z-scores for D-KEFS letter fluency, a measure of fluency and initiation, between two groups: the control group and combined mitochondrial disease groups (m.3243A>G, MELAS, and deletion). The Mann-Whitney U test results indicated that there were no statistically significant differences in the fluency/initiation domain among the groups ($d = 0.11$, $p = 0.61$).

References:

Andrade, C. (2021). Z Scores, Standard Scores, and Composite Test Scores Explained. *Indian J Psychol Med*, *43*(6), 555-557. https://doi.org/10.1177/02537176211046525

Juster, R.-P., Cipriani, E., Kerr, P., & DuBois, L. Z. (2024). Configurations, comparisons, and challenges calculating allostatic load. *Psychoneuroendocrinology*, *160*, 106787. https://doi.org/https://doi.org/10.1016/j.psyneuen.2023.106787