Statistical Analysis Plan (SAP)

Study Title	Effects of Heart Rate Oscillations During Meditation on Plasma Biomarkers of Alzheimer's Disease
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1. Objectives and hypotheses
   The aim of the study was to examine how two types of meditation practices (with or without slow breathing) affect plasma Aβ levels of compared with a no-intervention control group. In addition, we examined the effects of the two types of meditation practices on emotional well-being relative to the control group.

   We predicted that meditation with slow breathing would increase heart rate oscillations during practice, leading to greater reductions in plasma Aβ levels relative to the other conditions. In addition, we hypothesized that both meditation practices would improve emotional well-being compared with the control condition.

2. Sample Size & Power consideration
   For plasma Aβ, we estimated reliability using correlations between pre- and post-intervention time points in the HRV-ER trial. Effect sizes were derived from the pre-post difference in the younger adult slow-paced breathing group of HRV-ER. We powered the study to detect small-to-medium effect sizes ($f = 0.2$) with a 0.459 correlation among repeated measures, aiming for 80% power at $\alpha = .05$. Based on these parameters, a total sample size of $N = 72$ (with two assessments) was required. Based on the HRV-ER study, we estimated a 12% dropout rate ($72 \times 12\% \approx 9$). Thus, we planned on a total enrollment of $N = 81$ (72+9) to achieve a final $N = 72$ for analyses. Ultimately, 89 participants completed the study.

3. Outcome Measures and Analyses
   We examined condition differences in intervention-related change in the following outcome measures. The average time between pre- and post-intervention assessments was one week.

   Primary outcome measures
   - **Change in plasma amyloid beta levels:** We computed an aggregate score based on plasma Aβ40 and Aβ42 levels (pg/mL), calculated as the average of their Z-scores. Higher values on this composite measure are associated with greater risk of Alzheimer's disease. We conducted a 2 (time: pre, post) × 3 (condition: meditation with slow breathing, meditation without slow breathing, no intervention control) ANOVA to test for a time × condition interaction in plasma Aβ levels, assessing group differences in change over time.

   - **Change in plasma Ab42/40 ratio**: We conducted a time X condition ANOVA with plasma Ab42/40 ratio scores as the dependent variable (to assess group differences in change). The ratio was calculated by dividing the plasma Ab42 concentration (pg/ml) by the plasma Ab40 concentration (pg/ml).

   Secondary outcome measures
   - **Change in plasma pTau-181/tau ratio:** We conducted a time X condition ANOVA with plasma pTau-181/total Tau ratio scores as the dependent variable (to assess

group differences in change). The ratio was calculated by dividing the plasma pTau-181 concentration (pg/ml) by the plasma total Tau concentration (pg/ml).

- **Change in emotional well-being:** We conducted a time X condition ANOVA with emotional well-being as the dependent variable (to assess group differences in change). Emotional well-being was measured by the Profile of Mood States (POMS), which consists of 40 items that are rated on a 5-point scale ranging from "0=not at all" to"4=extremely. Total Mood Disturbance (TMD) was calculated by summing the totals for the negative items and then subtracting the totals for the positive items. A constant (i.e., 100) was added to the TMD formula to eliminate negative scores. Higher scores indicate more negative mood states. The scores range from 56 and 216.

Safety outcomes
Three non-serious adverse events were reported and subsequently resolved.

4. Populations and subgroups to be analyzed
   Population analyzed is composed by all randomized subjects who completed assessments and whose data quality was sufficient for data analysis.