

**STATISTICAL ANALYSIS PLAN
UNIVERSITY OF MISSOURI**

Project Title: Estrogen receptor alpha signaling in endothelial cells exacerbates arterial stiffening via upregulation of ENaC in insulin resistant females

IRB Number: 2012990

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Principal Investigator: Camila Manrique

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Clinical Trial Phase: *Phase II*

Clinicaltrials.gov Number: NCT03837626

Study Drug/Study Device: *Amiloride*

Statistical analysis plan – primary outcome

Linear mixed-effects models were used to evaluate changes in continuous outcome variables. Each outcome was analyzed in a separate model using the **lmer** function in the *lme4* package (R Foundation for Statistical Computing). Fixed effects included time, group, and their interaction (time \times group). A random intercept for participant ID was included to account for within-participant correlation from repeated measurements. Models were estimated using restricted maximum likelihood (REML).

Model assumptions were examined and satisfied, including assessment of normality of residuals, homogeneity of variance, multicollinearity, and evaluation for singular fit. Missing data were handled by the default behavior of **lmer()**, which excludes observations containing NA values; no imputation was performed.

Continuous data were visualized using GraphPad Prism (version 10.0) and are presented as mean values with 95% confidence intervals. Because only two groups were compared, no correction for multiple comparisons was applied.

Significance as set as $p \leq 0.05$.