

Title: Effects of walking in different tree-dominated environments on autonomic response in adults over 50: a randomized crossover trial

NCT07114146

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STATISTICAL ANALYSIS PLAN (SAP)

1. Objective of the analysis plan

This document defines the general approach to the statistical analysis of the study, specifying the methodological principles, the types of models to be used, and the strategies for comparing conditions and measurement time points.

2. Analytical Design

The analysis will be conducted using a crossover design with repeated measures, in which each participant has been evaluated under four experimental conditions (A, B, C, and D).

The dependence between observations within each participant and the longitudinal structure of the data will be considered. Where relevant, the sequence of exposure and the intervention period will be taken into account.

3. Included variables

The following pre-specified variables will be analyzed:

Continuous variables

- RR interval
- RMSSD
- SDNN
- HF power
- LF power

Cardiovascular variables

- Heart rate
- Systolic blood pressure
- Diastolic blood pressure

Psychological variables

- POMS
- Total Mood Disturbance (TMD)

Perceptual variables

- Perceived environmental quality

No fixed hierarchy is established among variables, as different outcomes may be analyzed as primary depending on the specific objective of each analysis.

4. Temporal structure of the data

Continuous variables

Regional variables derived from the continuous recording (RR interval, RMSSD, SDNN, HF power, and LF power) will be analyzed considering the entire duration of the recording, which includes the pre-exercise phase, walking, and recovery.

Discrete variables

Heart rate and blood pressure will be analyzed at the four time points defined in the protocol:

- M1 (baseline)
- M2 (5 min before walking)
- M3 (5 min post-walk)
- M4 (recovery)

Psychological variables

The POMS will be analyzed at two points per session (after M1 and after M3), and the TMD will be calculated based on its scores.

Perceived environmental quality

It will be analyzed as a single measurement per session under conditions A, B, and C.

5. General principles of analysis

1. The analyses will be based on methods appropriate for repeated measures data.
2. Mixed models will be used preferentially, as they allow for the handling of correlated data and incomplete structures.
3. Both the temporal evolution of the variables and the differences between conditions will be analyzed.
4. The selection of the specific model will be adapted to:
 - the nature of the variable,
 - the data distribution,
 - and the available time series structure.
5. Priority will be given to models that allow for a coherent clinical and physiological interpretation.

6. Comparisons between conditions

Comparisons will be made between:

- Outdoor environments (A, B, and C) versus the control condition (D)
- Comparisons between outdoor environments (A vs. B, A vs. C, B vs. C), when relevant

The inclusion of specific comparisons will depend on the objective of the analysis and the variable under consideration.

7. Analysis of continuous variables

Variables derived from the continuous recording (RR interval, RMSSD, SDNN, HF, and LF) will be analyzed using longitudinal models that allow for the evaluation of their evolution over time and their relationship with the experimental condition.

Time may be modeled as a continuous variable and, when necessary, using nonlinear functions to capture complex trajectories.

Differences between conditions will be evaluated both in terms of the mean level and the shape of the temporal evolution.

8. Analysis of heart rate and blood pressure

Heart rate and blood pressure will be analyzed considering the four measurement time points.

The following may be performed:

- overall analyses that include all measurement points (M1–M4),
- comparisons between specific time points, specifically:
 - M2 vs. M3
 - M2 vs. M4

and, where appropriate, other time-based comparisons.

These analyses may be based on:

- absolute values,
- changes relative to a reference point,
- or differences in change between conditions.

9. Analysis of psychological variables (POMS and TMD)

The analysis of POMS and TMD will account for their nature as repeated measures within each session. The following may be evaluated:

- changes between the two measurement points,
- differences between conditions,
- and interactions between condition and time point.

The final strategy can be adapted to the data structure and the specific objective of the analysis.

10. Analysis of Perceived Environmental Quality

Perceived environmental quality will be analyzed by comparing the three outdoor environments (A, B, and C).

The analysis may include:

- descriptive statistics,
- comparison between environments,
- and fitted models where relevant.

11. Covariates

Where appropriate, models may be adjusted for:

- age
- sex
- weight
- height

The inclusion of covariates will depend on their relevance to each variable and the model used.

12. Handling of missing data

Missing data will be treated as such, without systematic imputation.

Methods that allow for the inclusion of all available observations will be used, provided they are compatible with the model's assumptions.

13. Statistical software

Analyses will be performed using appropriate statistical software, preferably R or other equivalent programs that allow for the fitting of models for longitudinal and repeated-measures data.

14. Significance level

A significance level of 0.05 (two-tailed) will be used.

Results will be presented using:

- model estimates,
- 95% confidence intervals,
- and p-values, where applicable.

15. Concluding Remarks

This plan defines the general framework for the study's statistical analysis. The specific specification of the models may be adjusted based on the characteristics of each variable and the objectives of the specific analyses, while always maintaining consistency with the study design and the pre-specified variables.