Nature-based therapy for Metabolic Syndrome – Salzburg

Statistical Analysis Plan - Primary Effectiveness Analysis

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Authors:	Christina Pichler, Konstantin Thiel (Biostatistics)
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1 Purpose

This Statistical Analysis Plan (SAP) outlines the statistical methods to be used in the primary NATURE-MET-S effectiveness analysis. The study protocol, registered in 2024, defined repeated assessments of health-related quality of life and allostatic load and prespecified longitudinal comparisons incorporating group effects, time effects, and group-by-time interaction effects.

This SAP provides a detailed description of the planned statistical analyses and supplements the registered study protocol. It prespecifies the analysis populations, outcome definitions, statistical models and reporting procedures to be used in evaluating the study outcomes. The analytical methods were selected to address the longitudinal structure of the data and repeated outcome assessments over time.

2 Trial Overview and Sample Size

The NATURE-MET-S trial was a pragmatic, randomised, controlled, two-arm study of adults aged 40-65 years with metabolic syndrome. Participants were allocated at a ratio of 1:1 to either a ten-week intervention combining moderate-intensity hiking and nature-based mindfulness, or to a control group on a waiting list for usual care. Weeks 1-5 involved a combination of guided and self-guided practice, while weeks 6-10 were entirely self-guided. Participants in the control group were offered the intervention only after completing the final T4 assessment.

Allocation was implemented by an independent biometrician using a minimisation procedure based on Kullback-Leibler divergence to balance age and sex. The study comprised two implementation cohorts, but cohort was not used as an allocation factor. Assessments were scheduled at baseline (T1, day 1), week 5 (T2, approximately day 35), week 10 (T3, approximately day 70) and a follow-up with an exploratory questionnaire at approximately six months (T4, approximately day 180). The primary analysis period covers the interval from T1 to T3.

The planned sample size was 140 participants (70 per group). Sample size calculations were based on the expected changes in SF-12 scores derived from previous studies, as well as simulation-based power analyses for repeated-measures data. It was expected that a sample of 50 evaluable participants per group would provide approximately 87% power to detect the prespecified intervention effect. The recruitment target included an allowance for anticipated attrition during the follow-up period up to T4.

No formal interim efficacy analyses or early stopping rules were planned.

3 Outcomes and analysis population

The primary effectiveness analysis will consider four outcomes. All outcomes were measured at T1, T2 and T3, and will be analysed using the original scales.

Outcome	Definition and direction
SF-12v2 PCS	Norm-based Physical Component Summary (PCS) score; higher values indicate better physical health-related quality of life.
SF-12v2 MCS	Norm-based Mental Component Summary score; higher values indicate better mental health-related quality of life.
Allostatic Load Index 1 (ALI1)	Study-specific allostatic load index based on 12 biomarkers, including blood-derived components; range 0-100, with lower values indicating lower allostatic load.
Allostatic Load Index 2 (ALI2)	Study-specific non-invasive allostatic load index based on eight biomarkers derived from saliva and physiological assessments; range 0-100, with lower values indicating lower allostatic load.

SF-12v2 scores are derived using QualityMetric's norm-based scoring method (with a reference mean of 50 and a standard deviation of 10). The biomarker-scoring documentation and Supplementary Methods describe the identities of the biomarkers, the assay procedures, the directionality, the transformation procedures and the aggregation rules for ALI1 and ALI2. The present SAP does not replace these technical specifications.

The primary analysis population will comprise all participants who were randomised and attended the T1 baseline assessment. Participants will be analysed according to their originally assigned treatment group, irrespective of subsequent adherence to, or exposure to, the intervention. Participants who were randomised but withdrew before the T1 assessment cannot contribute outcome data and will therefore not be included in the longitudinal analyses. All available measurements from T1 to T3 will be included in the analysis of each outcome.

4 Primary Statistical Analysis

Separate linear mixed-effects models (LMMs) will be fitted for PCS, MCS, ALI1 and ALI2. Time will be modelled as a continuous variable (T1 = 0, T2 = 1, T3 = 2). Each model will include the following fixed effects: time; treatment group; and the group-by-time interaction. Baseline age and sex will be included as covariates. A participant-specific random intercept will be included to account for within-subject correlation. Baseline outcome values will be incorporated as part of the repeated outcome measurements and will not be entered as separate covariates.

The models will be fitted using restricted maximum likelihood (REML). Inference for the fixed effects will be based on Kenward-Roger denominator degrees of freedom.

The primary estimand for each outcome will be the difference in change over time between groups, represented by the group-by-time interaction term. Treatment effects will

be reported as estimated mean differences, alongside two-sided 95% confidence intervals on the original outcome scales.

The four primary tests of the group-by-time interaction (PCS, MCS, ALI1 and ALI2) will be considered a single family of confirmatory tests. Multiplicity will be controlled using Holm's procedure. Statistical significance will be concluded for Holm-adjusted two-sided p-values below 0.05. Reported confidence intervals will not be adjusted for multiplicity.

5 Missing data and sensitivity analyses

The primary analyses will be based on all available outcome measurements, with no data imputation. Linear mixed-effects models will be estimated using likelihood-based methods, which provide valid inferences under the assumption that data are missing at random, given the observed data and the model's covariates.

The extent of missing outcome data and participant withdrawal will be summarised by treatment group and assessment time point.

As a sensitivity analysis, complete-case analyses will be performed for each outcome, including only participants with observations at T1, T2 and T3. The results of the sensitivity analyses will be compared with those of the primary analyses to assess the robustness of the findings.

Model assumptions will be evaluated using standard diagnostic procedures.

6 Exploratory and safety analyses

The T4 follow-up assessment will be exploratory and limited to PCS and MCS outcomes. Due to the unequal time intervals between assessments, the exploratory analyses will treat time as a categorical variable and estimate the differences between groups in change from baseline at each follow-up assessment.

Further exploratory analyses of selected secondary and ancillary outcomes may be performed using extensions of the primary modelling framework or appropriate correlation analyses. The results of these analyses will be interpreted as exploratory and hypothesis-generating.

Intervention exposure, acceptability, and adverse events will be summarised descriptively.

7 Relationship to the registered protocol

This SAP aligns with the objectives and outcome domains outlined in the registered study protocol (version 4, 27 May 2024). It provides detailed specifications of the statistical methods to be used for the primary effectiveness analyses.