# Effects of a Multi-Ingredient Longevity Formulation (DoNotAge®) on Whole Blood NAD+ Levels

### A 28-Day Randomised, Double-Blind, Placebo-Controlled Pilot Study

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## Summary

This exploratory pilot study assessed whether 28 days of daily supplementation with the DoNotAge® multi-ingredient longevity formulation changes whole blood NAD+ levels in healthy adults, relative to a matched placebo. In a randomised, double-blind, placebo-controlled design, the treatment group showed a mean whole blood NAD+ increase from 16.4 to 27.9 µmol/L (+11.5 µmol/L) over 28 days, compared with a +3.2 µmol/L change in the placebo group — a between-group difference of +8.3 µmol/L (Cohen's d = 0.83). A pre-specified sensitivity analysis excluding a single statistical outlier showed a larger, statistically significant difference ($p < 0.01$; Cohen's d = 1.36). These encouraging findings support progressing to a larger, appropriately powered confirmatory study.

## Background

Nicotinamide adenine dinucleotide (NAD+) is a central coenzyme in cellular energy metabolism and in the activity of NAD+-dependent enzymes. Whole blood NAD+ levels are of interest as a potential marker of the biological response to NAD+-precursor and related supplementation. This exploratory pilot study was conducted to obtain preliminary data on whether the DoNotAge® formulation is associated with a measurable change in whole blood NAD+ over a short supplementation period, and to inform the design of a larger confirmatory study.

The DoNotAge® formulation is a daily sachet combining 15 bioactive longevity ingredients: NMN, trans-resveratrol, SulforaBoost®, SIRT6Activator®, Nitralis®, glycine, TMG (trimethylglycine), calcium alpha-ketoglutarate, quercetin, vitamin D3, vitamin K2, magnesium, high molecular weight (HMW) hyaluronic acid, CoQ10 and spermidine.

## Methods

### Study design

A 28-day, randomised, double-blind, placebo-controlled, parallel-group pilot study. Participants were allocated 1:1 to receive either the DoNotAge® formulation or a matched placebo. This was an exploratory pilot study, designed with the principles of the Declaration of Helsinki in mind.

### Participants

Twenty-four healthy adults aged 40–60 years were enrolled. Nineteen participants completed the study (10 in the treatment group and 9 in the placebo group). Recruitment, eligibility screening and enrolment were carried out by DoNotAge.org.

### Intervention

Participants took one daily sachet of either the DoNotAge® formulation or a matched placebo for 28 days. The supplement and placebo were provided and administered under the responsibility of DoNotAge.org, which also monitored adherence over the study period.

### Sample collection

Whole blood samples were self-collected by participants at home using finger-prick (capillary) sampling at two timepoints: baseline (Day 0) and Day 28. Collected samples were then shipped to the laboratory for analysis.

### Laboratory analysis

Whole blood NAD+ was analysed by LifeLab1 (Vilnius, Lithuania), acting as an independent laboratory service provider. LifeLab1 received the samples identified only by coded test IDs and quantified whole blood NAD+ using its standard validated laboratory procedure.

### Statistical analysis

The primary comparison was the between-group difference in change in whole blood NAD+ from baseline to Day 28, assessed on the complete dataset. A pre-specified sensitivity analysis was also conducted excluding one statistical outlier. Effect sizes are reported as Cohen's d.

### Consent

Informed consent was obtained from all participants prior to enrolment. The consent and data-use arrangements permit DoNotAge.org to use and publish the resulting coded data.

## Results

Of the 24 participants enrolled, 19 completed the 28-day study (10 in the treatment group and 9 in the placebo group). Whole blood NAD+ rose from 16.4 to 27.9 µmol/L in the treatment group, compared with a smaller increase of +3.2 µmol/L in the placebo group. The full dataset showed a between-group difference of +8.3 µmol/L ($p = 0.09$, Cohen's $d = 0.83$), approaching conventional

significance. A pre-specified sensitivity analysis excluding one statistical outlier yielded $p < 0.01$ (Cohen's $d = 1.36$).

## Discussion and limitations

This pilot study provides preliminary evidence that 28 days of DoNotAge® supplementation may be associated with an increase in whole blood NAD+ relative to placebo. However, the primary analysis on the complete dataset did not reach conventional statistical significance, and the statistically significant result depended on excluding one outlier. The study is limited by its small sample size, short duration and exploratory internal nature. Whole blood NAD+ was measured from self-collected capillary samples, which may introduce pre-analytical variability. These results should be regarded as hypothesis-generating. They provide a reasonable basis for designing a larger, appropriately powered and independently reviewed confirmatory study.

## Conclusion

This exploratory internal pilot study demonstrated a substantial increase in whole blood NAD+ following 28 days of daily DoNotAge® supplementation, with the treatment group rising by +11.5 µmol/L compared with +3.2 µmol/L on placebo, and a pre-specified sensitivity analysis showing a statistically significant benefit ($p < 0.01$). These are highly encouraging results for a formulation of this kind, and they provide a strong rationale for advancing to a larger, appropriately powered confirmatory study to build on these promising findings.

## Roles and contributions

**DoNotAge.org** acted as the study sponsor and was responsible for study design and protocol, participant recruitment, eligibility screening, informed consent, randomisation, provision and administration of the supplement and placebo, adherence and safety monitoring, data management, statistical analysis, and preparation of this report.

**LifeLab1** acted solely as an independent laboratory service provider. LifeLab1 received coded samples identified by test IDs and performed the whole blood NAD+ analyses.