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Official Study Title: Achieving Nutritional Adequacy Of Vitamin E With An Egg/Plant-Based Food Pairing

ClinicalTrials.gov ID: NCT04287816

Document Date: 10/01/2025

Statistical Analysis Plan

Power Analysis & Sample Size Determination. Plasma deuterium labeled (d_x) α -tocopherol (α -T) area under the curve from 0 to 72 hours (AUC_{0-72}) was the primary outcome and formed the basis for power analysis. Sample size estimates were derived from a previously published study demonstrating a dose-dependent increase in d_6 - α -T bioavailability with 0–11 g of fat from cream cheese.¹ Using the smallest observed difference in d_6 - α -T AUC_{0-72} —between the 0 g and 2.4 g fat trials—power analysis indicated that a sample size of 5 participants would be sufficient to detect statistically significant differences (90% power, $\alpha = 0.05$; PS Power and Sample Size).

All data (means \pm SE) were analyzed using GraphPad Prism (version 10, GraphPad Software, LLC). Variance was assessed using the Brown–Forsythe test, and data were log-transformed when necessary to meet assumptions of equal variance prior to statistical testing.

Predefined between-treatment comparisons included:

1. Dose-dependent effect of eggs on d_x - α -T pharmacokinetics: SPINACH, SPINACH + 1 EGG_{0 h}, SPINACH + 2 EGGS_{0 h} and SPINACH + 3 EGGS_{0 h}
2. Food matrix effects on d_x - α -T pharmacokinetics: SPINACH + 2 EGGS_{0 h}, SPINACH + WHITE and SPINACH + OIL
3. Meal timing effects on d_x - α -T pharmacokinetics: SPINACH, SPINACH + 1 EGG_{0 h}, SPINACH + EGG_{3 h}, and SPINACH + EGGS_{0 & 3 h}.

Dose-dependent and matrix effect comparisons, which formed the primary objective of the study, were analyzed together using one-way repeated measures analysis of variance (ANOVA), or linear mixed-effects models (LMM) in cases of missing data, followed by Holm–Šidák post hoc tests when a significant main effect was observed ($p < 0.05$).

The meal timing effect, which was the exploratory objective, was analyzed separately using repeated measures ANOVA or LMM in cases of missing data, followed by Fisher’s least significant difference (LSD) test when a main effect was significant ($p < 0.05$).

Reference

1. Bruno, R. S., Leonard, S. W., Park, S.-i., Zhao, Y. & Traber, M. G. Human vitamin E requirements assessed with the use of apples fortified with deuterium-labeled α -tocopheryl acetate. *The American Journal of Clinical Nutrition* **83**, 299-304 (2006). <https://doi.org/10.1093/ajcn/83.2.299>