

Statistical Analysis Plan

Sample size is constrained by the limited number of healthy adults willing to undergo studies involving multiple skin biopsies. Data are also scarce on 7DHC concentration in human skin. Based on data from Moody et al., 1990 (mean 7DHC=44.4 $\mu\text{g/g}$ dry weight; SD=14.8, n=15 and mean 7DHC=35.7 $\mu\text{g/g}$ dry weight; SD=18.7, n=9) [11] and MacLaughlin and Holick, 1985 [10] we calculated that a sample size of n=10 in each age group would be sufficient power to detect an approximate 2-fold difference in baseline skin 7DHC content between old and young subjects (80% power, $\alpha=0.05$).

For serum vitamin D₃, we used data from Osmancevic et al [16], which included vitamin D₃ 24h post-UVR exposure of ~55% BSA, and Libon et al [17], which included vitamin D₃ 5 days post-UVR exposure of ~50% BSA. We extracted these data from published figures in both studies using WebPlotDigitizer version 4.4 [18]. We calculated the SD for the 24h post-UVR vitamin D₃ to be 5 ng/mL (12.9 nmol/L) and for the ~7 day post-UVR to be 8.9 nmol/L (3.4 ng/mL). On this basis, n=10 participants in each age group would yield 80% power to detect a mean difference between the young and old groups of approximately 6.6 ng/mL (17 nmol/L) 24h post-UVR, and 12 nmol/L (4.7 ng/mL) 7 days post-UVR, respectively, each at a 5% significance level.

7DHC data were transformed using $y=1/y$ equation, and all further analyses were carried out with transformed data. Vitamin D₃ results were assessed for normality using the Shapiro-Wilk test and QQ plots. The sets of data were then analysed using a repeated-measures mixed model ANOVA and multiple comparisons (Sidak correction) tests using GraphPad Prism statistical software (version 8.4.3, June 10, 2020).