

Vitamin D3 Supplementation Alters Gut Microbiome Composition and Increases Stability in Healthy Individuals

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ABSTRACT

Early onset colorectal cancer incidence has increased significantly. Evidence links serum 25(OH)D levels and the gut microbiome to colon carcinogenesis, with vitamin D pivotal in modulating the immune system and intestinal epithelial function. The effect of supplemental vitamin D₃ on the gut microbiome in healthy adults is unclear. **PURPOSE:** Investigate the effect of a 12-week oral dose of 4000 IU vitamin D₃ on acute and long-term gut microbiome changes in healthy adults. **METHODS:** Forty-three participants, aged 18-53, were randomized based on baseline serum 25(OH)D levels into treatment ($n=21$) and placebo groups ($n=22$). Fecal samples and dietary intake were collected at 15-time points during the 78-day study (daily collection for the first 14 days and one sample on day 78), and 16S rRNA sequencing was performed on samples (days 1,7,14, and 78). Bioinformatics tools (QIIME) and statistical methods (repeated measures ANOVA, PERMANOVA) were employed to assess microbial α -diversity, β -diversity, and microbiome stability. Participant characteristics were compared using student t-tests and chi-square. **RESULTS:** Vitamin D₃ supplementation significantly increased serum 25(OH)D levels in the treatment group (baseline 39.94 ± 15.22 ng/mL, post-intervention 72.60 ± 27.87 ng/mL, $p<0.001$). While no changes in α -diversity were observed, there were significant shifts in β -diversity within the treatment group ($p=2.22e-16$, Paired Wilcoxon Test). Microbial stability correlated positively with serum 25(OH)D levels up to a 60 ng/mL increase from baseline with no further changes beyond this threshold. The treatment group showed a significant increase in beneficial bacteria, including *Bifidobacterium*, *Anaerostipes*, and *Eubacterium eligens*, and a significant decrease in opportunistic pathogens, *Bilophila wadsworthia*, and *Escherichia-Shigella* post-intervention. **CONCLUSION:** Vitamin D₃ supplementation significantly modifies the microbiome composition and influences microbiome stability in healthy adults. These data suggest that moderate dose Vitamin D₃ supplementation may reduce the risk of colorectal cancer, in part, through improving the gut microbiome composition and stability.