

### Alcohol Consumption and the Diversity of the Oral Microbiome in Postmenopausal Women

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**Objectives:** Alcohol has been shown to reduce neutrophil function and decrease salivary flow, which could affect the composition of the oral microbiome. We hypothesized that the  $\alpha$ - and  $\beta$ -diversity of the oral microbiome would differ by frequency of alcohol consumption.

**Methods:** A food frequency questionnaire was used to assess the frequency of consumption of beer, wine, and liquor in a sample of 1,179 postmenopausal women in an ancillary study of periodontal disease (OsteoPerio) of the Women's Health Initiative Observational Study. Frequency of alcohol consumption was converted to grams of alcohol consumed per day (g/d), and the following categories were created: non-drinkers and tertiles of alcohol consumption in g/d among drinkers. The oral microbiome was assessed from subgingival plaque samples using 16S rRNA amplicon sequencing. PERMANOVA was used to examine  $\beta$ -diversity (between-sample diversity) and ANOVA was used to examine  $\alpha$ -diversity (within-sample diversity) across alcohol intake categories. The Shannon index (species evenness), Chao1 index (species

richness), and observed operational taxonomic unit (OTU) count were used to assess  $\alpha$ -diversity. Models were adjusted for age, race, education, neighborhood socioeconomic status, smoking, menopausal hormone use, non-alcohol total energy, body mass index, antibiotic use, and dental care habits. Sensitivity analyses were conducted by removing those who currently smoke ( $n = 32$ ) and those that took antibiotics in the past 30 days ( $n = 123$ ).

**Results:** Over half of the participants (66%) consumed alcohol, with 12% reporting  $\geq 1$  drinks/day.  $\beta$ -diversity across categories of alcohol consumption was statistically significantly different (PERMANOVA  $P = 0.016$ ).  $\alpha$ -diversity was significantly higher in the highest tertile of alcohol consumption compared to non-drinkers for all  $\alpha$ -diversity measures. The adjusted means (SE) in the highest tertile of alcohol intake compared to non-drinkers were 5.17 (0.09) vs. 4.96 (0.09) for Shannon Index, 155.37 (4.24) vs. 146.00 (3.97) for Chao1 Index, and 124.94 (3.81) vs. 116.35 (3.57) for observed OTU count. Sensitivity analyses showed similar results.

**Conclusions:** Alcohol consumption was associated with subgingival bacterial diversity.

**Funding Sources:** National Heart, Lung, and Blood Institute, National Institute of Dental and Craniofacial Research, and Department of Defense.