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## **Transient Elevation of Pyridinoline in Gingival Crevicular Fluid During Periodontitis**

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Pyridinolines are collagen cross links in connective tissue and bone. Gingival inflammation which causes connective tissue and bone dissolution may increase concentration of pyridinoline at periodontal disease sites or in circulation. Previous studies have reported increased pyridinolines in gingival crevicular fluid (GCF) during ligature induced periodontitis (Shibutani, J Periodontol 68:385, 1997). This study describes a method to measure pyridinoline in GCF and analysis of GCF pyridinoline concentrations during a study of natural progression of periodontitis in dogs. A chemiluminescent competitive immunoassay (Assay Designs, Inc.; Ann Arbor, MI) was adapted to measure pyridinoline in GCF. Serum and GCF were collected at three month intervals for 18 months at 39 disease sites in 5 dogs with natural periodontitis. Analysis of pyridinoline in GCF samples showed that 75% of samples (203/275) were above the assay detection limit (0.0002 pMoles/ $\mu$ L). Pyridinoline concentrations ranged from 0.0015 to 0.0276 pMoles/ $\mu$ L in serum and from 0.020 to 1.90 pMoles/ $\mu$ L in GCF. Pyridinoline concentrations in GCF samples were 10-500 times higher than serum levels at same time point. Elevated concentrations of pyridinoline in GCF are similar to levels of pyridinoline reported in human synovial fluid from rheumatoid arthritis. Transient elevations of GCF pyridinoline concentrations exceeding 2.5 times median values occurred in 70% of sites (68-71% CI) suggesting episodic resorption of connective tissue and bone. **The majority of periodontitis sites showed transient elevations of pyridinoline concentrations in GCF which may identify sites experiencing episodic connective tissue or bone destruction that occur prior to changes in clinical probing or radiographic measures.**

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## **Bioavailability and Bacterial Uptake of Xylitol from Dentifrice**

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Changes in plaque pH and *Streptococcus mutans* levels have been observed following long term consumption of foods or chewing gums containing xylitol. Similar effects have not been definitively shown for dentifrices containing xylitol perhaps as the result of insufficient dosage, inadequate delivery from the dentifrice matrix and/or limited exposure to plaque bacteria during relatively short brushing periods. Although xylitol is readily soluble in an aqueous solution, the carbohydrate may interact with the dentifrice matrix limiting its availability and/or inhibiting uptake by plaque bacteria. We have developed an *in vitro* bioassay which allows measurement of availability, bacterial uptake and utilization of xylitol from a dentifrice matrix. Briefly, the assay uses *Enterococcus* (formerly *Streptococcus*) *avium* 14025 which is capable of xylitol metabolism. Standardized cultures of *E. avium* are exposed to either aqueous xylitol solutions or dilute xylitol dentifrice slurry supernatants in Phenol Red Broth (PRB). This enables evaluation of carbohydrate utilization without promoting culture growth. Consumption of xylitol or production of acid metabolites are measured using ion exchange HPLC. Viability of the organisms is maintained in the presence of dentifrice solutions and xylitol is consumed in a dose dependent manner. Equimolar concentrations of acetate and formate are produced per mole of xylitol consumed. The kinetics of aqueous xylitol consumption was calculated to be 54.4 nmoles/L/min. Consumption of xylitol from two different experimental dentifrices were 28.3 and 5.5 nmoles/L/min respectively. **These results suggest dentifrice formulations can effect the availability of xylitol and/or its uptake by bacteria.**