Elemental composition of teeth with and without odontoclastic resorption lesions in cats

Patricia A. Colley, DVM, MPVM; Frank J. M. Verstraete, DrMedVet, DAVDC; Philip H. Kass, DVM, PhD, DACVPVM; Peter Schiffman, PhD

Objective—To determine elemental composition of teeth with and without odontoclastic resorption lesions (ORL) in cats.


Procedure—An electron microprobe was used to analyze weight percentages of calcium, phosphorus, fluorine, sodium, magnesium, sulfur, potassium, and iron in enamel, dentin, and cementum.

Results—Calcium and phosphorus were the most abundant elements. Fluorine, sodium, and magnesium combined were <5% and sulfur, potassium, and iron combined were <0.1% of total elemental composition. In enamel of normal teeth, a significant sex-by- jaw location interaction was seen in mean (±SD) phosphorus content, which was higher in mandibular teeth of females (1764 ± 0.41%) but lower in mandibular teeth of males (16.71 ± 0.83%). Mean iron content in dentin of normal teeth was significantly lower (16.71 ± 0.83%) and mean magnesium content was significantly lower (0.54 ± 0.13%) in ORL-affected teeth, compared with normal teeth. In cementum, mean fluorine content was significantly lower (0.017 ± 0.008% vs 0.021 ± 0.005%) in ORL-affected teeth, compared with normal teeth.

Conclusions and Clinical Relevance—Results of our study establish baseline mineral content of enamel, dentin, and cementum for normal teeth in cats. Minimal differences in mineral content of enamel and cementum of normal and ORL-affected teeth were detected.

Odontoclastic resorption lesions (ORL) are a common and painful problem affecting feline teeth. The lesions are not only found in American, European, and Australian domestic cats but have also been observed in feral cats, a captive leopard, and several wild felids. The lesions mainly affect middle-aged and older cats, although lesions have been found in cats as young as 2 years of age. Reported prevalence in hospital populations ranges from 26 to 67%, with no definite sex predilection. However, this range may actually be an underestimation, because early ORL in cats are only detected on radiographs. Surface defects were found at the cemento-enamel junction in 25% of premolars and molars in 1 study, yet radiography revealed that 77% of the teeth were actually affected. In another study, evidence of ORL was radiographically found in 8.7% of cats in which the lesions were missed on clinical examination.

Although it seems that ORL are a recent development, there is evidence that the lesions have been described earlier. A histologic study of resorption lesions in feline teeth was published as early as 1930; in this study, the lesions were found not to be caries, and the odontoclastic activity and inflammatory cells present in ORL were well illustrated. In 1955, Builder published his observations of resorptive lesions of feline teeth but incorrectly speculated that the lesions were a type of dental caries. This supposition was widely held until 1976, when Schneck and Osborn histologic descriptions provided no basis for bacterial invasion consistent with caries.

The pathogenesis of ORL has been described. The lesions are caused by odontoclastic resorption of enamel, dentin, cementum, and alveolar bone. Normally, odontoclasts are found in mammals only during the phase of root resorption prior to the shedding of deciduous teeth. For unknown reasons, odontoclastic activity is initiated in the mature tooth, and resorption begins. The first phase of this process involves active resorption of dental tissues by numerous odontoclasts, followed by proliferation of inflamed gingival epithelium. The second phase is a reparative replacement by bone-like or cementum-like tissue in the defects. Radiographic signs of ORL include areas of increased lucency in the tooth substance, root resorption, root ankylosis, and alveolar bone loss.

The cause of ORL is still unknown, although numerous risk factors have been cited. Periodontal disease is a risk factor, but ORL can develop without periodontal disease. In 1 study, cats infected with feline immunodeficiency virus (FIV) were found to be at higher risk for ORL; however, several FIV-negative control cats also developed ORL, suggesting that factors other than viral infections contribute to lesion formation. Diet and dietary mineral content have also been studied. Schneck and Osborn reported an association between the lesions and cats fed raw liver. Zetner and Steurer found a significant relationship between the development of ORL and low calcium
content of home-cooked diets but could not find a relationship between the lesions and the pH of commercial cat foods. However, cats without ORL are more likely to be fed diets higher in magnesium, calcium, phosphorus, and potassium. Cats without ORL are more likely to live in a rural area and spend more time outdoors, perhaps supplementing their diet with natural prey. It is possible that a subtle dietary mineral deficiency may be an etiologic factor for ORL. Such a deficiency could cause imbalances in tooth structure, which could be associated with the initiation of the disease process. Also, several studies have suggested that ORL may affect teeth on the basis of location. Lund et al and Harvey reported more lesions in mandibular teeth, van Wessum et al found a slightly higher percentage of lesions in the maxillary teeth, and Coles did not report any significant difference in location of lesions. The objectives of the study presented here were to determine the elemental composition of normal feline teeth, including a comparison of mandibular teeth to maxillary teeth, and of ORL-affected teeth to determine whether differences exist.

Materials and Methods

Animals—Normal teeth from cadavers were obtained from 22 randomly selected apparently healthy adult cats that had been humanely euthanatized at a local county shelter between July 1998 and August 2000. The cats were euthanatized because they were not adopted and not for medical reasons. The ORL-affected teeth were obtained from 21 cats admitted to the University of California Veterinary Medical Teaching Hospital for dental treatment between March 1997 and October 1999.

Preparation of teeth—Cadaver heads were radiographed to confirm the absence of ORL. A maxillary fourth premolar and mandibular first molar were then extracted, without sectioning, from each cadaver specimen for 44 normal teeth. Radiographs were obtained for each cat admitted to the veterinary hospital for dental care as previously described and affected teeth were sectioned and extracted. After extraction, normal and affected teeth were initially stored and dehydrated in 70% isopropyl alcohol. Organic material was dissolved from the teeth by soaking in 5.25% sodium hypochlorite (full-strength household bleach) for 4 hours. After rinsing with water, teeth were again stored in 70% isopropyl alcohol.

Each tooth was examined under a stereomicroscope and evaluated for ORL. Two teeth from each cat admitted to the veterinary hospital for dental care were randomly selected for 42 affected teeth. Cementum was not always seen on all teeth, so only 1 tooth was used from those cats when cementum was present for 15 normal and 15 ORL-affected teeth. Digital images were then obtained to assist in specimen orientation prior to preparation for electron microprobe analysis. Teeth were air-dried and impregnated in epoxy resin. The teeth were then embedded in lucite and ground to expose a sagittal section (mesial-distal plane), using a series of electroplated diamond wheels. The sections were then polished on silk cloth, using 0.05-µm-diameter alumina particles, and coated with approximately 250 Å of carbon.

Sample analysis—Each tooth was analyzed for the following elements: calcium (Ca), phosphorus (P), fluorine (F), sodium (Na), magnesium (Mg), sulfur (S), potassium (K), and iron (Fe). Absolute concentrations were measured in enamel, dentin, and cementum, using an automated electron microprobe equipped with energy-dispersive and wavelength dispersive x-ray microanalysis systems as well as electron backscattered imaging capabilities. Backscattered imaging was used to identify an area of tooth to be analyzed by the microprobe (Fig 1). In backscattered electron images, enamel appeared white as a result of its lower organic content, whereas dentin and cementum appeared gray: cementum was identified by its roughened texture and lack of dentinal tubules. The starting and stopping points (end points) were then visually determined, and the microprobe was programmed to analyze mineral content at 10 points within the given distance, including both end points. The distance measured for ORL-affected teeth ranged from 75 to 800 µm, and the distance measured for normal teeth ranged from 500 to 800 µm. The smaller distance in ORL-affected teeth was the result of sectioning during extraction. Analyses were performed at an accelerating potential of 15 keV, a beam current of 10 nA, and a rastered beam at a magnification of 40,000X (which is equivalent to a beam diameter of approx 2 µm).

The quantitative analyses reported in our study were obtained by wavelength dispersive analysis. Mean atomic number, absorption, and fluorescence matrix corrections were calculated to convert the measured x-ray intensities to weight percentages. The x-ray analyte lines from standards included jadeite (for Na), periclase (for Mg),apatite (for F and P), wollastonite (for Ca), orthoclase (for K), pyrite (for S), and hematite (for Fe).

Statistical analysis—An ANOVA with 1 repeated factor (2 replicate teeth) and 2 grouping factors (sex, presence, or absence of ORL) was used to compare mean mineral percentages in enamel and dentin. Repeated-measures ANOVA with 1 repeated factor (jaw) and 1 grouping factor (sex) was used to compare mean mineral percentage between maxillary and mandibular teeth. Two-factor ANOVA (sex, presence or absence of ORL) was used to compare mean mineral percentages in cementum, because only 1 tooth was used. An arc-sine square root transformation was used on the percentages prior to statistical analysis. A P value < 0.05 was considered significant.

Results

Study population—Of the 22 cadavers, 12 (54.5%) were males, 1 of which was neutered, and 10 (45.5%) were females, 1 of which had steel sutures in
the linea alba. Of the 21 ORL-affected cats, 15 (71.4%) were males, 14 of which where neutered, and 6 (28.6%) were spayed females. Of the 22 cadavers, 20 (90.9%) were domestic short-, medium-, or long-haired cats, and 2 were Siamese mix, whereas 14 of the 21 (66.7%) ORL-affected cats were domestic, and 7 (33.3%) were purebred. The mean age of the ORL-affected cats was 9.5 years (range, 2 to 21 years). It was not possible to determine the exact ages of the cadavers, so age was estimated on the basis of dentition and physical appearance. All the cadavers appeared to be between 2 and 7 years of age and appeared to have been physically healthy before death.

**Elemental composition of normal teeth**—Baseline elemental composition of normal feline teeth was established, with Ca and P being the most abundant elements in enamel, dentin and cementum. Fluorine, Na, and Mg combined were < 5%, whereas S, K, and Fe combined were < 0.1% of the total elemental composition in all 3 dental tissues (Table 1). In the comparison of normal mandibular teeth to normal maxillary teeth, a significant interaction between sex and jaw location was observed for mean P content of enamel (P = 0.014), mean Fe content was higher in mandibular teeth than maxillary teeth (17.64 ± 0.41% vs 17.53 ± 0.26%), whereas in males, mean phosphorus content was lower in mandibular teeth than maxillary teeth (16.71 ± 0.83% vs 17.42 ± 0.48%). Mean Fe content of dentin in normal teeth was significantly lower in mandibular teeth than maxillary teeth (0.014 ± 0.005% vs 0.023 ± 0.019%, P = 0.009), irrespective of sex.

**Comparison of normal and ORL-affected teeth**—In enamel, mean Na content was higher in ORL-affected teeth (0.77 ± 0.046% vs 0.74 ± 0.025%; P = 0.014), and mean Fe content was lower in ORL-affected teeth (0.017 ± 0.008% vs 0.021 ± 0.005%; P = 0.046), compared with normal teeth. No significant differences were observed in dentin. In cementum, mean Fe content was lower (2.98 ± 0.27% vs 2.99 ± 0.20%; P = 0.008), and mean Mg content was lower (0.54 ± 0.13% vs 0.60 ± 0.13%; P = 0.029) in ORL-affected teeth, compared with normal teeth.

**Discussion**

In our study, electron microprobe analysis was used to determine the content of 8 individual elements in feline enamel, dentin, and cementum. Many techniques are available to measure elemental composition of mineralized tissues and range in capability from a single element analysis to analysis of multiple elements simultaneously. Spark source mass spectrometry is 1 method of evaluating multiple elements and has been used to study elemental composition of human enamel. Microhardness testing has been used in leopard and lion teeth as well as in the enamel and dentin of feline premolar teeth. However, microhardness measurements only reflect the degree of mineralization, not the individual elemental percentages. Electron microprobe analysis measures the concentration of individual elements simultaneously within a given material and has been used to accurately evaluate elemental composition of human enamel and dentin, fish enameloid, and urinary calculi.

Findings in our study establish baseline elemental compositions of enamel, dentin, and cementum for normal feline teeth. Elemental content of Ca and P in feline enamel and dentin is similar to human enamel and dentin. Calcium content of human enamel is 34.6 to 38.2%, compared with 36.3 ± 0.41% in feline enamel; Ca content of human dentin is 24.7 to 31.9%, compared with 29.4 ± 0.55% in feline dentin. Phosphorus content of human enamel (16.3 to 19.2%) is similar to feline enamel (17.3 ± 0.48%), and phosphorus content of human dentin (12.3 to 14.0%) is similar to feline dentin (14.1 ± 0.28%).

The small differences observed in enamel composition of normal mandibular and maxillary teeth were significant, but it is unknown whether they are clinically relevant. Results of mean P content on the basis of jaw location were different for females and males. Mean P content of normal enamel was lower in males than females by only 0.5%, so it is doubtful that this difference is clinically important. In dentin, a significant difference was seen in mean Fe content, which was lower in normal mandibular teeth, compared with maxillary teeth, but it is doubtful that this difference is clinically relevant, because mean Fe content of dentin in normal teeth is < 0.03% of total elemental composition. Minor variations related to age and jaw location have also been observed in 1 study in rats. Sex is not consistently considered a risk factor in the cause of ORL. No sex differences were observed by Scarlett et al and Coles, but Lund et al reported more affected females, whereas van Wessum et al reported more affected males. It is also difficult to determine from previous studies whether jaw location is a potential risk factor for ORL development. Lund et al and Harvey reported more lesions in mandibular teeth, van Wessum et al found a slightly higher percentage of lesions in the maxillary teeth, and Coles did not report any significant difference in location of lesions.

Compared with normal teeth, significant differences were observed in mean enamel content of Na, which was higher in ORL-affected teeth, mean enamel content of Fe, which was lower in ORL-affected teeth, and mean cementum content of F and Mg, which were both lower in ORL-affected teeth. In humans, Na has not been shown to influence the integrity or susceptibility of enamel to caries, erosions, or other defects. However, several studies have described Fe as an effective cariostatic agent. Ferric chloride and ferrous sulfate added to the diet or drinking water of hamsters reduced the amount of experimental caries. Iron inter-
fere with the metabolism of glucose and reduces acid formation by cariogenic bacteria in plaque. It is important to emphasize that feline ORL are not caries; the lesions are not demineralized and do not contain bacteria.\textsuperscript{7,11,19} Therefore, the differences in Fe and F content are unlikely to be clinically important in the pathogenesis of feline ORL. It is unknown whether the difference in mean Mg content of cementum in normal teeth and ORL-affected teeth, which in our study was 0.06%, is clinically relevant. In humans, the concentration of Mg in dentin is related to the concentration of Mg in the soil, which is explained by the consumption of locally produced food.\textsuperscript{31} The concentration of Mg in the enamel, on the other hand, was not related to concentration in the soil but was found to increase with age.\textsuperscript{39} Magnesium concentration in human dentin is slightly lower in females then in males and also increases with age.\textsuperscript{39} Because a previous study\textsuperscript{1} reported that cats without ORL were more likely to be fed diets higher in Ca, P, Mg, and K, further studies are indicated to focus on the relation between diet, elemental composition of teeth, and the development of ORL.

In our study, accurate medical information was known for all of the clinically affected cats that included age, sex or neuter status, FeLV and FIV status, and results of CBC and serum biochemical analysis. It was not possible to control for age, sex or neuter status, FeLV and FIV status, or prior health status of the cadavers, which were used to obtain reference range values. The age of the cadavers appeared to be between 2 and 7 years of age, but ORL affect middle-aged to older cats.\textsuperscript{2} However, in humans, no significant differences were observed in Ca and P in relation to age, and minimal variations were observed in other elements.\textsuperscript{39-41} There is little information on the influence of other factors such as overall health status and infectious diseases on variations in elemental composition of teeth in humans; it is, therefore, unknown whether any confounding factors where present in the cadaver specimens.\textsuperscript{31,33,42-44}

References

34. Rosalen PL, Pearson SK, Bowen WH. Effects of copper, iron


