Aluminum concentration in deciduous teeth is dependent on tooth type and dental status

Arne Halling, Owe Löfman, Ali-Reza Nosratabadi, Christer Tagesson and Britt Öster

Unit of Community Dentistry, Unit of Community and Environmental Medicine, Department of Environmental and Occupational Medicine, Center for Public Health Sciences, and Faculty of Health Sciences, Department of Health and Environmental, University of Linköping, Sweden

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Aluminum (Al) concentration was assessed in deciduous teeth in relation to sex, year of birth, tooth type, and the presence of caries and roots. Three hundred and twenty-three deciduous teeth from children born during the period 1952–93 in a county in southeast Sweden were sampled, and the Al content determined by graphite furnace atomic absorption spectrophotometry. The arithmetic mean of the Al concentration was 0.58 ± 0.64 ppm dry weight (mean ± standard deviation) and differed significantly between incisors (1.05 ± 1.04 ppm) and canines (0.48 ± 0.50 ppm) and between incisors and molars (0.53 ± 0.55 ppm). A significant difference was found between teeth with and without caries. No significant differences were found between sexes. The Al concentration correlated significantly with tooth weight for incisors ($r = -0.47$) and canines ($r = -0.45$) but not for molars ($r = 0.03$). No significant change in Al concentration was found over time. Caries-free deciduous molars are suggested as the most useful teeth for biological monitoring of aluminum. □ *Aluminum; assessment; atomic absorption spectrophotometry; biomonitoring; deciduous teeth*

Arne Halling, Unit of Community Dentistry, Center for Public Health Sciences, SE-581 85 Linköping, Sweden. Tel: +46 13 22 88 79, fax: +46 13 14 19 18, e-mail: Arne.Halling@lio.se

Environmental aluminum (Al) is predominantly insoluble and is found in only very small amounts in living organisms (1). Acid rain increases the solubility of Al and also enhances the biological availability (2). Many scientific reports have pointed out Al as a potentially toxic agent with adverse skeletal and neurotoxic health effects (3, 4), even though the toxic mechanisms are still unclear and under debate (1, 2, 5–7).

Exposure to Al occurs through ingestion of Al-containing pharmaceuticals, foods, and water and through inhalation (8). Ingested or inhaled Al is absorbed to a certain extent, and elimination takes place mainly via urine (9). Al is firmly bound and forms complex ions with a wide range of compounds—for example, amino acids, collagen, and glycosaminoglycans (1) in mineralized tissues such as the teeth and bones (10). The main deposition in deciduous teeth takes place during the mineralization process, extending from the 4th month in utero to the age of 3 to 4 years (11). Al is a minor inorganic constituent of human teeth and is uniformly distributed throughout enamel and dentin (12). Each tooth has its own timetable of development, and the time span for tooth formation is longer than that of any other organ system (11). Human deciduous teeth have earlier been used as biological indicators of exposure to several heavy metals, such as cadmium, copper, lead, mercury, iron, and zinc (13–15). Recently, Bjertness et al. (16) found that the Al content of deciduous teeth was correlated to the Al concentration in drinking water and suggested that Al in deciduous teeth may serve as an indicator of environmental Al exposure. To our knowledge, no studies on Al concentrations related to sex, year of birth, and type of tooth have been published, and we therefore decided to investigate this and how the presence of caries and roots influences the concentration of Al in deciduous teeth.

Materials and methods

Subjects

The study included 327 shed or extracted deciduous teeth without fillings, collected between May 1993 and June 1996 from children born in 1952–93 and living in the county of Östergötland, Sweden. To sample teeth from the whole geographic area, a general invitation to participate in the investigation was directed towards local public dental clinics, children, and parents. This was enhanced by information given by media and other information channels.

Four teeth could not be classified owing to extensive caries and were excluded. Thus a total of 323 deciduous teeth were studied. The following variables were used: Sex; Year of birth; Type of tooth: medial and lateral incisors, canines, first and second molars; Dental status: a) dentin caries (yes/no): teeth were recorded as carious when, on visual inspection, the tooth surface was broken by loss of carious tooth surface; b) root-status: with roots = whole or partially resorbed roots; without roots = totally resorbed roots; Fillings (yes/no): presence of any fillings in the mouth of the donors at the time of exfoliation or extraction.

Table 1. Basic material. Aluminum concentration (ppm dry weight of tooth tissue) and age by sex, caries, fillings, roots, and tooth type

		Age (years)		Aluminum concentration		
Subject	No.	Mean	*s*	Mean	*s*	*P*
All	323	11.1	2.9	0.58	0.64	
From boys	190	10.8	2.8	0.57	0.65	0.86
From girls	133	11.6	2.9	0.58	0.63	
With caries	44	10.5	2.7	0.61	0.38	<0.05
Without caries	278	11.2	2.9	0.57	0.68	
With filling(s)*	152	11.7	2.7	0.57	0.63	0.91
Without filling(s)	154	10.7	3.0	0.58	0.66	
With root(s)	109	10.7	3.3	0.50	0.51	0.19
Without root(s)	214	11.4	2.6	0.62	0.70	
Incisors	40	6.8	1.9	1.05†	1.04	See footnotes
Canines	102	12.0	2.4	0.48‡	0.50	
Molars	181	11.6	2.4	0.53‡	0.55	

* One or more fillings in any other tooth in the mouth than the analyzed one.
† Significantly higher value than for canines and molars; $P < 0.0001$.
‡ Non-significant difference between mean values compared for canines and molars; $P = 0.53$.

The number of subjects is presented in Table 1 on the basis of sex, caries, fillings, and root status.

Chemical analysis

The teeth were kept dry and mailed in plastic tubes to the laboratory for analysis. When present, all soft caries debris was removed with a stainless steel excavator until a hard tooth surface could be felt. After that, each tooth was cleaned in 3 mL 0.5 M (mol/L) HCl for 10 min and then rinsed four times with ultrapure water (Milli-Q_{plus}, Molsheim, France) for 10 min each time and dried in air over a clean filter paper at room temperature overnight. The tooth was then accurately weighed (23.8–1321.5 mg) and thereafter transferred to a 16 × 80 mm (10 mL) centrifuge tube (Nalgene, USA) and dissolved in 2 M HCl (1–14 mL depending on the tooth weight) at 80°C for 2 days. Two centrifuge tubes with 2 M HCl alone were prepared under the same conditions to test for acid purity and for any accidental contamination. All chemicals were of highest available purity, and all materials were carefully washed. Before use, all laboratory utensils and chemicals were randomly checked for contamination.

The Al concentration was determined by graphite furnace atomic absorption spectrophotometry with polarized Zeeman correction, using a Hitachi Z-8270 atomic absorption spectrophotometer with an SSC-300 autosampler (Hitachi Ltd, Tokyo, Japan). Each series contained 36 samples (18 tooth samples in duplicate), 2 acid blanks, and 7 control samples, which were included in each series. Samples (10 μL) were injected into a graphite tube type (SGL-Carbon Group, Bonn, Germany), and the signal height measured. Each sample was analyzed in duplicate, and when the two values differed by more than 10%, two further measurements were made. Al concentrations were calculated as ppm dry weight.

The mean coefficient of variation within series (calculated from 323 deciduous teeth measured twice) was 8%. The validity of the technique for measuring Al in biological materials was ascertained by attending an external quality control (Institut für Standardisierung und Dokumentation im medizinischen Laboratorium e.V., Düsseldorf, Germany). To check the reliability of the method, a control sample of 20 dissolved deciduous teeth was used and included teeth from all series (see above). The results from measurements of these control samples were used to calculate the coefficient of variation within series (8%) and between series (14%).

Statistical methods

The Kolmogorov–Smirnov test for both Al concentration and tooth weight showed significant deviations from normal distribution. These variables were therefore transformed to their 10 logarithms. After variable transformation both variables conformed to a Gaussian distribution. Transformed data were used in the regression statistics, ANOVA, and multiple group comparisons. The comparison of arithmetic mean values between groups, if more than two, was performed by using analysis of variance with the Bonferroni correction and significance level set at $P = 0.01$; otherwise the Student *t* test was used.

To show the development of Al concentration over time, the original variable in units of ppm dry weight were transformed to their logarithms with the base of 10. To enable comparison between teeth of different types and both sexes, the transformed variables were then converted to their respective *z* scores and pooled in the time series analysis.

Simple linear regression was used to show the relationship between continuous variables, and the Pearson and determination coefficients calculated. A *P* value <0.05 was considered significant. Data analyses were performed using SPSS Version 9.0 (SPSS Inc., Chicago, Ill., USA).

Results

Al concentration in relation to tooth type and dental status

For the teeth analyzed together, the Al concentration was 0.58 ± 0.64 ppm dry weight (Table 1). Of the teeth, 89.9% had Al values ≤1.0 ppm dry weight. The Al concentration was higher in incisors than in canines and molars, whereas there were no significant differences between canines and molars, between medial and lateral incisors, or between first and second molars, upper and lower jaws, or left and right sides. By contrast, there was a significant difference between teeth with and without caries. No significant differences were found between boys and girls or between teeth with and without roots or

Table 2. Regression factors for ^{10}log Al concentration (ppm*) over ^{10}log weight (mg) in the different tooth types

Tooth type	*n*	Intercept	*β*-coeff.	95% CI† Lower	95% CI† Upper	*r*	*P* value
Incisors	40	1.112	−0.599	−0.972	−0.225	−0.47	<0.005
Canines	102	1.036	−0.622	−0.867	−0.377	−0.45	<0.005
Molars	181	−0.526	0.044	−0.147	−0.234	0.03	0.651
All	323	0.260	−0.257	−0.364	−0.150	−0.26	<0.001

* ppm: parts per million dry weight of tooth tissue.
† CI = confidence interval.

between groups with and without fillings in other teeth than the ones analyzed.

The Al concentration correlated negatively with tooth weight ($r = -0.26$, $P < 0.001$). Looking at specific tooth types, Al concentration in incisors and canines correlated negatively with tooth weight, with Pearson correlation coefficients of −0.47 and −0.45, respectively, in both cases ($P < 0.01$). This relation was significant for incisors and canines ($P < 0.01$). The Al concentration in molars, in contrast, did not vary with tooth weight ($r = 0.03$) (Table 2).

Al concentration in relation to year of birth

The relative levels of Al concentration converted from units of ppm dry weight to Z scores of $_{10}$log-transformed values and including all observations sampled during the study are shown in Fig. 1. The Al concentration did not change significantly over time, although incisors had a tendency toward decreasing values ($r = 0.026$; $\beta = -0.01224$; $P = 0.87$), and canines inversely a tendency towards increasing values ($r = 0.089$; $\beta = 0.0312$; $P = 0.38$). Molars showed a trend identical to all teeth pooled together with no significant change ($r = 0.062$; $\beta = 0.0133$; $P = 0.409$).

When all types of teeth were pooled and aggregated in birth cohorts in accordance with age groups, as shown in Table 3, analysis of variance (arithmetical mean) showed a significant increase over time. Analyzed by tooth type, however, the ANOVA failed to show that the Al concentration correlated with the year of birth.

Discussion

Our findings indicate that Al concentration in deciduous teeth is influenced by the tooth type and dental caries. We found significant differences in Al concentration between incisors and canines and between incisors and molars.

The true exposure of deciduous teeth to aluminum is an integrated function of the total exposure burden and individual predisposing factors pre- and postpartum up to the time of exfoliation. The transportation and exchange of constituents to and from dentin takes place via the blood pool through pulpal tissue and from the saliva, with

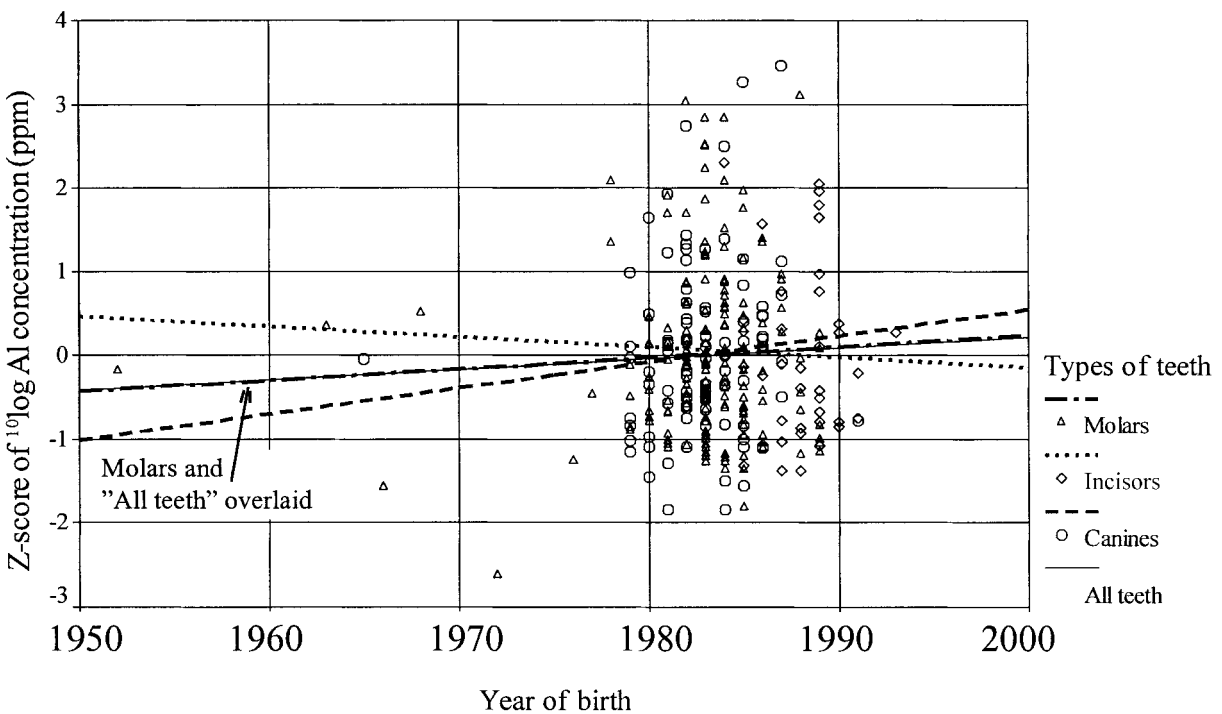

Fig. 1. Change in Z-score of ^{10}log aluminum concentration over the study period for all teeth together and for the respective tooth types in both sexes.

Table 3. Al concentration (ppm dry weight of tooth tissue) in different tooth types in relation to year of birth (mean ± standard deviation number of analyzed teeth within parentheses)

Tooth type	Year of birth			
	Before 1979	1979–83	1984–88	1989–93
Incisors	–	0.46 – (1)	0.92 ± 1.02 (19)	1.19 ± 1.09 (20)
Canines	0.36 – (1)	0.43 ± 0.32 (64)	0.57 ± 0.72 (36)	0.22 – (1)
Molars	0.51 ± 0.51 (10)	0.52 ± 0.57 (95)	0.56 ± 0.57 (70)	0.27 ± 0.13 (6)
All*	0.50 ± 0.48 (11)	0.49 ± 0.48 (160)	0.62 ± 0.70 (125)	0.95 ± 1.02 (27)

* $P < 0.005$; before 1979 versus 1989–93: $P < 0.05$; 1979–83 versus 1989–93: $P < 0.001$; 1984–88 versus 1989–93: $P < 0.05$.

contributions from breast feeding, milk surrogates, or other nutritional constituents (17) but also antacids (18), fluorides (19, 20), and citrates (21). The differences in Al concentration between tooth types may be explained by differences in chronology, mineralization, and maturation of the tooth tissues. They may also be the result of different quantities, distributions, and proportions of tissue constitution in the respective tooth type and the degree of tissue-specific mineral density. Al concentration in enamel is reported to be higher than in dentin (22–24), although this finding is not entirely consistent (25). Using only studies in which the same analysis techniques have been used for enamel and dentin provides stronger evidence for a higher concentration of aluminum in enamel than in dentin. This may in our study explain the higher Al concentrations in incisors and canines than in molars and also the higher average concentrations in teeth without roots than in teeth with roots except for molars. Accepting this evidence also explains why the Al concentration in incisors and canines, in contrast to molars, correlated negatively with tooth weight.

There was a significantly higher concentration of Al in teeth with caries than in teeth without caries. The higher average Al values seen in teeth with caries concerned all types of teeth, although, when tested by tooth type, only molars reached a significant difference, which may be due to the small numbers of carious canines and incisors in the material. The higher concentration of Al in the carious teeth may be explained by a higher potential for metals to accumulate in demineralized dental tissues, as has been shown for lead by Bercovitz & Laufer (26) and Gil et al. (27). Although only teeth without fillings were to be included in this study, it is difficult to determine retrospectively with certainty whether a decayed tooth has previously contained a restoration.

The significant change in Al content found over time when all types of teeth were pooled and analyzed in arbitrarily defined birth cohorts (Table 3) can be ascribed to the skewed distribution of tooth types over time, since a proportionally larger number of incisors with their higher amounts of Al influence the later years of the study period. Molars did summarize the average development of all teeth well, which might strengthen the proposal to use molars in biomonitoring of Al exposure.

Some difficulties existed in collecting deciduous teeth, which was most notable for incisors and canines. To obtain a sufficient number of tooth samples representing the geographic area of the county, the collection was supported by a campaign in the media and public dental clinics, which might, to a certain extent, have violated the principles of random sampling. Most of the teeth were collected prospectively over a period of approximately 3 years, but a minor part had been exfoliated at an earlier time. The background information of these retrospectively collected samples may be biased by recalling errors, which also includes the question of general tooth status and other fillings in the mouth before and at the time of exfoliation.

For further research it would be desirable to ascertain the significance of different sources of external and internal aluminum exposure. Such studies would preferably include not only dental sources such as glass ionomer cement used for restorations, cementation of orthodontic appliances, and temporary aluminum crowns but also the assessments of aluminum exposure from the air, ingested food and drinks, and other objects put in the mouth. One should also consider the interaction between these background factors and the occurrence of other geochemical determinants like geographic variations in pH and Al concentration in soils, lakes, and tap water.

Conclusions

We present a method for determination of the Al content in deciduous teeth by graphite furnace atomic absorption spectrophotometry. Wide variations of Al content were found between different tooth types, of which molars appeared to be the most stable. We conclude that it is important to use a homogeneous tooth material for the biological monitoring of aluminum in humans and propose caries-free deciduous molars to be the best choice for this purpose. To what extent the Al concentration in deciduous teeth mirrors external exposure has to be further elucidated.

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