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ARTICLE

Quantified retrospective biomonitoring of fetal and infant elemental exposure using LA-ICP-MS analysis of deciduous dentin in three contrasting human cohorts

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BACKGROUND: Spatial elemental analysis of deciduous tooth dentin combined with odontochronological estimates can provide an early life (*in utero* to ~2 years of age) history of inorganic element exposure and status.
OBJECTIVE: To demonstrate the importance of data normalization to a certified reference material to enable between-study comparisons, using populations with assumed contrasting elemental exposures.
METHODS: We used laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) of dentin to derive a history of elemental composition from three distinct cohort studies: a present day rural cohort, (the New Hampshire Birth Cohort Study (NHBCS; $N = 154$)), an historical cohort from an urban area (1958-1970), (the St. Louis Baby Tooth Study (SLBT; $N = 78$)), and a present-day Nigerian cohort established to study maternal HIV transmission (Dental caries and its association with Oral Microbiomes and HIV in young children-Nigeria (DOMHaIN; $N = 31$)).
RESULTS: We report Li, Al, Mn, Cu, Zn, Sr, Ba and Pb concentrations (µg/g) and qualitatively examine As, Cd and Hg across all three cohorts. Rates of detection were highest, both overall and for each cohort individually, for Zn, Sr, Ba and Li. Zinc was detected in 100% of samples and was stably present in teeth at a concentration range of 64 – 86 µg/g. Mercury, As and Cd detection rates were the lowest, and had high variability within individual ablated spots. We found the highest concentrations of Pb in the pre- and postnatal dentin of the SLBT cohort, consistent with the prevalent use of Pb as an additive to gasoline prior to 1975. The characteristic decline in Mn after the second trimester was observed in all cohorts.
IMPACT:

- Spatially resolved elemental analysis of deciduous teeth combined with methods for estimating crown formation times can be used to reconstruct an early-life history of elemental exposure inaccessible via other biomarkers. Quantification of data into absolute values using an external standard reference material has not been conducted since 2012, preventing comparison between studies, a common and highly informative component of epidemiology. We demonstrate, with three contrasting populations, that absolute quantification produces data with the lowest variability, compares well with available data and recommends that future tooth biomarker studies report data in this way.

KEYWORDS: LA-ICP-MS; deciduous teeth; dentin; elemental analysis; metals

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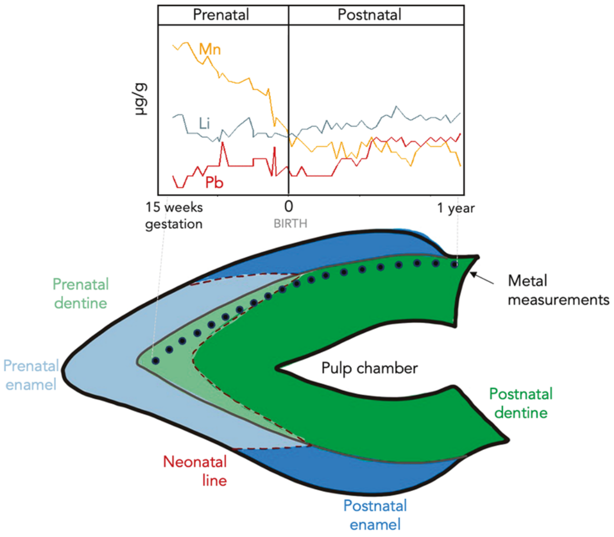
Graphical Abstract



INTRODUCTION

When combined with methods for estimating crown formation times (odontochronology) [1], spatially resolved elemental analysis of deciduous teeth (also known as primary, or baby teeth) can be used to reconstruct an early-life history of elemental exposure [2–6]. Deciduous teeth provide a history of elemental exposure from the second trimester in utero to approximately 2 years of age and can be used to potentially link early life nutrient and toxicant status with health across the lifespan [2]. There are no other easily-obtainable, non-invasive biomarkers that provide an elemental exposure history for this critical developmental window. Deciduous teeth form at about 5-6 weeks gestation, beginning to mineralize in the second trimester, and continuing through the first year of life [7]. Because the mineralization of enamel is slow, and dentin mineralizes to its final state soon after protein matrix deposition, dentin is preferable for reconstructing a historical record of element exposure [8]. About 90% of deciduous teeth have a visible growth line, known as the neonatal line (NL), that forms at birth due to the temporary restriction of tooth growth while feeding transitions from maternal blood to the fetal digestive system. Using the NL as a reference for the day of birth, regions of the tooth that formed before and after birth can be distinguished [7].

Early studies of the spatial distribution of elemental composition in deciduous teeth showed that Ca concentrations in enamel and dentin were uniform [9], leading to the use of Ca as an internal standard in spatial elemental analysis, and the widespread use of Ca ratios as a way of expressing elemental abundances in teeth. Quantification of data into absolute values (use of an external standard reference material (SRM) to express data as micrograms per gram (μg/g) has not been conducted since 2012 (Supplementary Table 1), which prevents comparison between studies, a common and highly informative component of epidemiology. This method accounts for many factors that influence the variability of elemental measurement, including variation in instrumentation and laser conditions (Supplementary Table 1) as well as the variability of dentition in populations.

Environmental exposures and dietary transitions have been linked with spatially resolved concentrations of several elements with +2 cationic form, including lead (Pb) [2, 3, 5, 6, 10–21], barium (Ba) [3, 5, 19–26], manganese [2, 3, 5, 6, 10–21, 27], strontium (Sr) [28, 29], copper (Cu) [8] and zinc (Zn) [8, 21].

We analyzed teeth from three cohorts; the New Hampshire Birth Cohort (NHBCS), an open prospective birth cohort of mother-infant dyads that began in 2009 to study the effects of drinking water arsenic on infant health outcomes; the St. Louis Baby Tooth—Later Life Health Study, a subsample of The Saint Louis Baby Tooth Survey (SLBT), for which children's baby teeth were donated between 1958 and 1970 and the Dental caries and its association with Oral Microbiomes and HIV in young children-Nigeria study (DOMHaIN) [30], a prospective African cohort of HIV-infected (HI), HIV-exposed but uninfected (HEU), and HIV-unexposed and uninfected (HUU) children aged 4 to 11 years recruited from the University of Benin Teaching Hospital (UBTH) in Edo State, Nigeria between May and November 2019. We compared quantified elemental abundances in subsets of teeth from each cohort, using a broader analyte suite than typically reported.

Our hypothesis was that comparing quantified data from three contrasting cohorts, one of which had participants likely to have higher Pb exposure (tooth collection pre-dated the Clean Air Act of 1970), would constitute proof-of-concept for the validity of quantification using an SRM, producing data with lower variability that compares well with available data, and support the recommendation that future tooth biomarker studies report quantified data.

MATERIALS AND METHODS

Study samples

Samples were analyzed between July 2020 and January 2022. We analyzed one naturally exfoliated deciduous tooth per individual for each of cohort. For the DOMHaIN and NHBCS cohorts, all of the teeth analyzed were incisors, whereas for the SLBT cohort, 9 samples (13%) of the specimens were not central incisors.

New Hampshire Birth Cohort Study (NHBCS). The NHBCS, part of the Environmental Influences on Child Health Outcomes consortium [31], was established in 2009 to measure the influence of arsenic and other metals in groundwater on maternal-child health. Participants were recruited through participating prenatal care clinics in New Hampshire. Pregnant women were eligible for the study if they: were aged 18-45 years; were literate in English; used a private drinking water system as their primary drinking water source at their residence; and resided at the same address since their last menstrual period with intention to remain there throughout the pregnancy. Participants were prospectively followed through pregnancy and beyond, including two in-person visits at approximately 12 and 24 gestational weeks.

Tooth collection kits were provided to caregivers for collecting teeth at home when the child begun to exfoliate teeth. Kits consisted of 2 ml or

4 ml cryovials prelabeled with study barcodes, Kimtech^{TM} Delicate Task Wipers for wrapping teeth, envelopes with collection information labels, pre-stamped return mailing envelopes and instructions for collecting and mailing teeth to the study. When samples were returned, study staff logged incoming mail in a Study Tracking System. At the time of this study, 154 teeth samples had been analyzed and were available for inclusion.

St. Louis Baby Teeth Study (SLBT). The St. Louis Baby Tooth—Later Life Health Study is a subsample of The Saint Louis Baby Tooth Survey, originated in 1958. Between 1958 and 1970, approximately 300,000 baby teeth were collected from children largely in the St. Louis, MO, area, but teeth were sent in from all over the US and some internationally. In 2001, approximately 100,000 of the originally collected teeth, from roughly 36,000 unique individuals, were found at Washington University. In 2021, attempts to recontact these 36,000 participants began. At the time of this comparative study, teeth from 78 SLBT participants had been analyzed for metals and were available for inclusion.

Dental Caries and its association with Oral Microbiomes and HIV in Young children-Nigeria (DOMHaIN). DOMHaIN is a prospective study of children aged 4–11 years, recruited from a Special Treatment Clinic (STC) in the University of Benin Teaching Hospital (UBTH) in Edo State, Nigeria. Participants were enrolled in three distinct groups: child HIV infected from maternal exposure (HI), child HIV-exposed from maternal exposure but remained uninfected (HEU), and child HIV-unexposed and uninfected (HUU) between May and November 2019. Additional HI and HEU participants were recruited from HIV/AIDS pediatric clinics or by referral of their mothers attending adult anti-retroviral therapy clinics at UBTH. Participants with HUU status were age-matched and recruited from well-child and pediatric clinics at UBTH [32–34]. At the time of this study, 31 teeth samples had been analyzed and were available for inclusion.

SLBT. This project was approved by the Office of Regulatory Affairs and Research Compliance at the Harvard T.H. Chan School of Public Health under protocol #IRB20-0040. All participants provided informed consent prior to participation.

DOMHaIN. The institutional review board at the University of Maryland Baltimore (HP-00084081), Rutgers State University of New Jersey (Pro2019002047), and the University of Benin Teaching Hospital, Benin City (ADM/E22/A/VOL. VII/14713), Nigeria, approved this study. Co-author Modupe O. Coker is the principal investigator, owner of the collected specimens and knowledge partner of the DOMHaIN study, although this study does not make direct inferences to the people or populations from any of the sites included. Study staff took care to verbally explain (in English and Pidgin English) all study activities, and risks and benefits of voluntary participation to parents/guardians or caregivers. Questions were asked to confirm understanding. Written informed consent was then obtained prior to recruitment.

Sample preparation

Specimens from all three cohorts were prepared for analysis using the same protocol at the Trace Elements Laboratory in Dartmouth College following published methods [5]. Photographs of the labial and lingual aspects of teeth were collected using a USB microscope (Dino-Lite Edge 3.0, Torrance, CA). Teeth with caries or decay were excluded. Teeth were embedded in Epofix^{TM} cold-setting resin (Electron Microscopy Sciences, Hatfield, PA), trimmed with a diamond-coated blade (Buehler) and cut in the bucco-lingual plane approximately 150 µm away from the midline of the tooth. Sections were cut to a thickness of between 200-350 µm, hand-polished with silica carbide papers, sonicating and rinsing with DI water in between each grade, until the neonatal line (NL) could be seen with a binocular microscope, after which they were polished to remove scratches. After polishing, specimens were rinsed in DI water and cleaned in an ultrasonic bath for 30 seconds to remove debris.

ICP-MS analysis

The National Institute of Standards and Technology (NIST) certificate of analysis for SRM 1486 (Bone meal) provides certified values for Zn, Sr and Pb and reference values for Al, Cu, As, Mn and Cd. We used the mean Ba concentration ($N = 3$) reported in the Geological and Environmental Materials (GeoRem) database analysis of NIST 1486 [35] of 256 (± 76) ppm. We used solution mode inductively coupled plasma mass spectrometry (ICP-MS) to quantify the remaining elements in our analytical suite for which reference/certified values were not available, namely Ba, Li and Hg. Triplicate 200 mg aliquots of 1486 were digested in 5 ml HNO_3 via closed vessel, microwave-assisted digestion. The digestion temperature was ramped to 210˚C over 15 minutes, and held for a further 20 minutes. Digested samples were diluted to 50 ml and analyzed by collision cell ICP-MS (Agilent, 8900) operated in helium and oxygen gas modes. Quality control included initial and continuing calibration checks, 3 replicate digestions and laboratory-fortified blank (recoveries 100 ± 10%). We relied on the accuracy and precision of the certified and reference values for NIST 1486 to validate our reference values for the unknowns.

LA-ICP-MS analysis

Methods for spatial elemental analysis of teeth via laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) closely followed those published, with the patterns of ablation following Arora et al. [5, 6]. We analyzed Li7, Al27, Ca44, Mn55, Cu63, As75, Zn66, Sr88, Cd112, Ba137, Hg202 and Pb208. For the NHBCS Li7 was not included as an analyte for the first 40 samples.

We used a New Wave Research 213 nm laser ablation unit (Elemental Scientific Lasers, Bozeman, MT) interfaced with an Agilent 7900 ICP-MS (Santa Clara, CA) operated in no gas mode. The ablation cell was purged and flushed with helium carrier gas for one hour prior to data collection. The LA-ICP-MS was tuned daily using ablation of a raster pattern on the NIST 612 glass standard daily at start-up, maximizing the signal intensity for Li6, Y89, Tl205 and monitoring and recording the Pb/Ca ratio for the optimized tuning parameters. The instrument parameters were recorded daily for instrument performance tracking.

Ablation patterns were 50 µm diameter spots in the dentin, 50 µm away from the enamel-dentine junction (EDJ), spaced 100 µm apart running from the cusp to the root [5, 6]. Patterns were pre-ablated (10 Hz for 1 second) to remove superficial contamination and then ablated at 20 Hz at 75% power for 7 seconds (fluence between 1.2 and 1.4 J cm^{-2}) for data collection.

Primary and secondary standards

A pressed pellet of the National Institute of Standards and Technology (NIST) 1486 (bone meal) SRM was ablated as a primary standard with each specimen. Pellets of SRM powder were pressed with a Specac^{TM} (Orpington, UK) mini pellet press at 2 tons (0.1 MPa) for 10 minutes. A secondary standard consisting of a pressed pellet of Pb-fed goat bone (NY 05-01) courtesy of the Department of Health, Wadsworth Center [36] was analyzed with every fourth sample as a secondary standard.

Analytical data reduction scheme

Initial data reduction was conducted in the Iolite 4 software application [37] using the Trace Elements data reduction scheme, which unifies time data from the laser ablation unit with elemental data from the ICP-MS, averages the ICP-MS response across the flat portion of the signal, conducts baseline subtraction, and ratios to a uniformly present internal standard, presenting data as parts per million (µg/g). We used Ca44, present at 25.4 weight percent in teeth [38], and quantification using the response of the NIST1486 SRM and averaging across laser ablation patterns. Iolite also pools non-certified elemental reference values from the literature to further expand the suite of elements that can be quantified. We routinely trimmed the first and last second of data from each ablated spot to remove the influence of wash-in and wash-out on the averaged data for each spot. Data cleaning steps included flagging and checking anomalously low Ca concentrations (=/- 3Ds of the mean of the sample spots), which can occur for teeth sections that are too thin, where the laser ablates through section.

Detection limits and QC

Limits of detection (LODs) are calculated in Iolite as part of the trace elements data reduction scheme, and the Howell LOD algorithm was used [39]. Due to the nature of LA-ICP-MS analysis in spot mode, LOD calculations use the background signal immediately preceding each spot in the calculation, therefore each ablation pattern has its own LOD value. LOD values are summarized in Supplementary Table 2. Output from the data reduction scheme included quantified µg/g values, analytical standard error and LODs for each spot. Data were reported as <LOD when

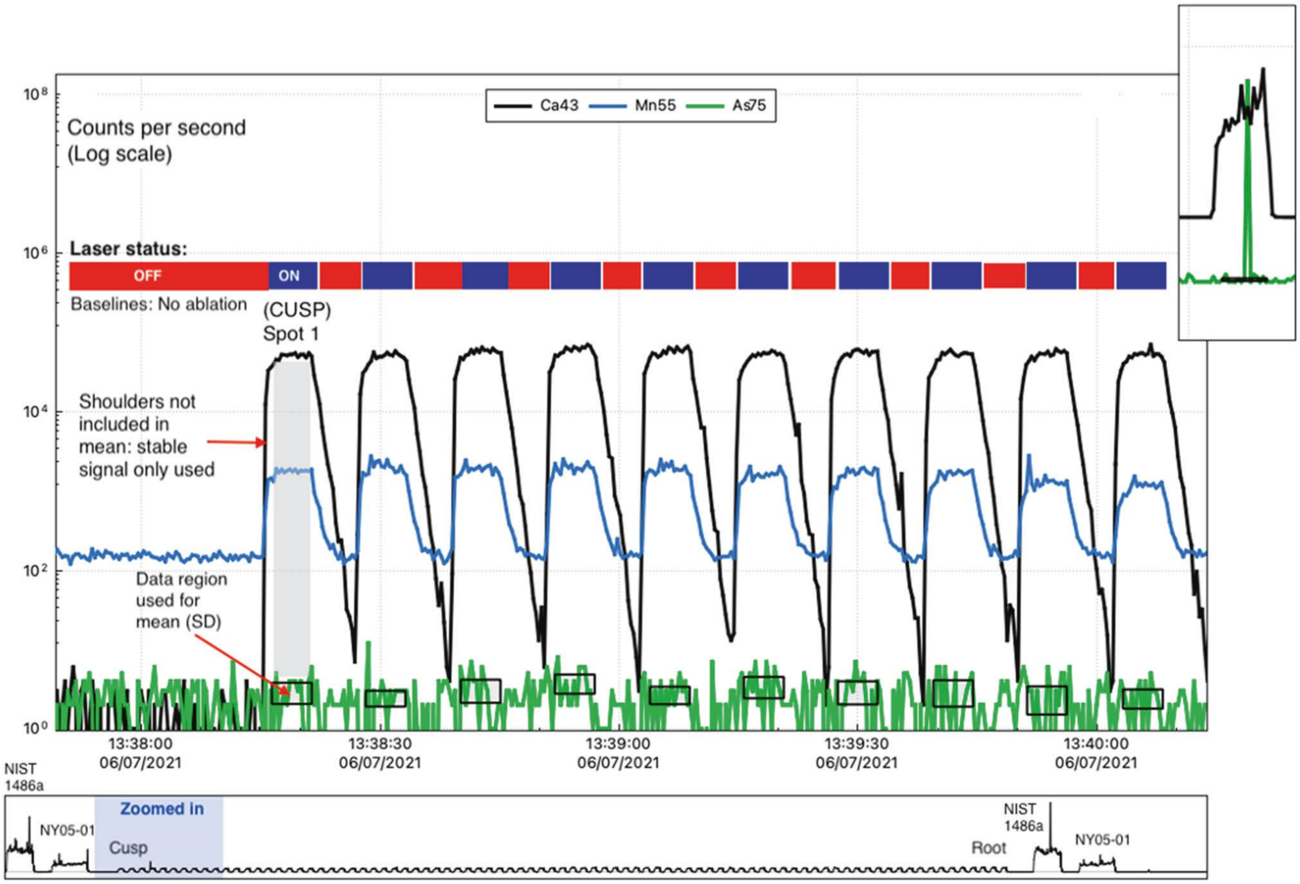


Fig. 1 Ablation patterns of well-detected (calcium, black and manganese, blue) and poorly detected (arsenic, green) elements obtained from primary teeth using LA-ICP-MS. The inset shows an example of transient, anomalous As detection observed sporadically within ablated spots.

either the average µg/g value calculated for each spot was below the LOD, or the mean value minus the standard error was less than zero. This latter criterion was used to remove spots where mean values were not representative across the spot and were inflated by one single anomalously high value (see Fig. 1, inset for an example).

Odontochronology

The date relative to birth (days) for each spot was estimated from measured enamel increment rates [40]. Date estimation uses the length of the prism path between the EDJ and the NL adjacent to each ablated spot. Prism path measurements were made manually with a USB microscope (Dino-Lite Edge 3.0 USB microscope with a polarized light stage and Dino-Capture 2.0 software), calibrated daily at an average magnification of 150X. For spots beyond the NL, days relative to birth were extrapolated using pre-natal growth rates calculated from the measured prism paths, because deciduous teeth growth rates are reportedly stable [1]. We matched the tooth type to the daily increment rate from Birch and Dean [1]. We determined the linear regression for the relationship between calculated days relative to birth and ablated spot number and used this to estimate spots before and after the NL. With dentin increment rates reported at 2.5–4.5 µm/day [40], each 50 µm spot integrates exposure over an 11–20-day period.

Statistical analysis

Variability across element measurements: Between spot variation was calculated by collating data from the secondary standard analyzed with every sample over the period of analysis and calculating the variance (relative standard deviation/mean).

Comparisons across cohorts: We calculated summary statistics and examined distributions of all variables. To visualize element concentrations over time, natural log-transformed (ln) concentrations from all time points were plotted for the three cohorts by days relative to birth. For summary statistics only, element concentrations in dentin for each participant were grouped into the prenatal period (second and third trimesters) and the postnatal period (after birth). We separated tooth element concentrations by prenatal and postnatal periods to investigate nutrient and non-essential element levels between these periods given the change in exposure route and the ability of the placenta to regulate the transfer of elements to the fetus. To compute significance testing, we averaged individual spot element concentrations within each participant for each of the two-time windows and used one measurement per individual to compute ANOVAs across cohorts for each time window. The right-skewed dentin element concentrations were natural log transformed (ln) to reduce the influence of extreme values for data visualization purposes and to conduct ANOVA which requires normal distribution of variables. Among elements with at least 80% detection, µg/g values below the limit of detection were imputed as the LOD/square root [2]. Element µg/g medians and interquartile ranges (IQR) were calculated for both time points across cohorts. Violin plots were created for prenatal and postnatal element µg/g concentrations across the three cohorts for data visualization.

RESULTS

Between-cohort calcium (Ca) variability was low (Supplementary Fig. 1), confirming its suitability as an internal standard. To illustrate the extent of daily variation we encountered, we collated data from the secondary standard (RM NY05-01; [36]) analyzed with each sample and calculated the variance (relative standard deviation/mean) (Table 1). Variability in elemental detection is shown for counts per second (CPS); the raw, non-normalized counts by the ICP-MS, Ca-normalized CPS, and quantified data obtained from the data reduction scheme, expressed as µg/g.

Table 1. Variability (%) in elemental data from reference material (RM) NY05-01 [42] collected on a per-sample basis over an 18-month period.

Measure	Variability in Secondary RM NY05-01 (%)			
	Zn	Sr	Ba	Pb
Counts Per Second (CPS)	68	66	72	90
Calcium Normalized CPS	55	36	61	104
Quantified data (ppm)	24	6	34	32

Variability in raw CPS was as high as 90% for some elements, shown for Pb isotope 208, which relates to differences in instrument sensitivity from sample and laser variability. Normalization to the Ca signal accounted for (normalized to) the mass of material ablated and reduced variation substantially in some, but not all cases, because the varying ICP-MS relative element sensitivity response across time negates the effect of normalizing to a uniformly-distributed element. Using a data reduction scheme also further normalizes data to a SRM produces data with the lowest variability.

Solution-mode ICP-MS analysis

Recoveries for certified values of Fe, Zn, and Pb were 130 ± 2%, 95 ± 3%, 97 ± 6% and for reference elements Mn, Cu, and Cd were 124 ± 3%, 93 ± 13%, 102 ± 23%. We suspect that Fe and Mn high recoveries could be due to Ca and P polyatomic interferences relative to the aqueous calibration. The reference value for Cd is 0.003 mg/kg and is close to our method detection limit (0.001) which explains the relatively high variability of the three replicates, although the recovery is excellent. We determined a value of 1.14 (± 0.04) and 0.02 (± 0.01) ppm for Li and Hg respectively. Mercury levels in NIST 1486 are low and at the detection limits of our method, hence our analysis of Hg trends should be viewed as qualitative.

Elemental detection characteristics

Typical ablation patterns of well-detected (>95% detection) and poorly detected (<30%) elements are shown (Fig. 1), plotted on a unified, logarithmic Y axis are shown for a tooth from the NHBCS, in which the household drinking water As concentration was 40.5 µg/L, and the infant was primarily fed formula reconstituted with household tap water, i.e., an individual where we might expect to see an As signal in the tooth given known exposure. Calcium (black) and manganese (Mn) (blue) are plotted with arsenic (As) (green), expressed as counts per second. Baseline data on the left of the time-resolved analysis is collected when the laser is not firing.

Element responses for well-detected elements (namely Ca and Mn) were flat-topped peaks, with low standard errors, corresponding to the consistent detection of that element in the ablated material. Black boxes indicate the portion of the data used to determine the mean and standard deviation for each spot and included approximately 13 ICP-MS measurements of the element signal during ablation at each spot, excluding datapoints at the shoulders to prevent 'wash-in/wash-out' effects on the data. Data for As do not differ between periods where the laser is off and when it is firing. However, we periodically observed transient high As responses inconsistently across a single spot (Fig. 1, inset).

Polyatomic interferences

Iron (Fe) abundance was determined by monitoring atomic mass 57 to determine Fe abundance in the tooth specimens, anticipating a potential argon-oxygen polyatomic interference at mass 56. We found that the Fe57 signal was high and very closely followed Ca. Since the isotopic abundance of Fe57 is 2.2%, we suspected it was also a polyatomic interference. Analyzing Fe at both mass 56 and 57 (the abundance of Fe56 is fifty times higher than Fe57) we saw that Fe56 gave a very low, close-to-background signal, confirming that the signal at mass 57 was not Fe. Fe56 was generally poorly detected in deciduous dentin.

Odontochronology

For all cohorts collectively, 18% of teeth had no visible NL, slightly higher than 10% reported previously [41]. Lack of a visible NL occurs when teeth are not developing at the time of birth, resulting from either early or delayed tooth development. For this study, data from teeth without an NL were excluded from statistical analysis, although LA-ICP-MS data was collected.

Rates of detection

Elements commonly measured in teeth (Mn, Zn, Sr, Pb, Ba) and less common elements (Li, Al, Cu, As, Cd and Hg) were measured in all three cohorts. Rates of detection (expressed as % of ablation patterns) were highest, both overall and for each cohort individually, for Zn, Sr and Ba, with Zn and Sr at 100% detection throughout, and Ba at 99% (Table 2). For the three cohorts collectively, Cu, Mn and Li were detected in 95-98% of ablation patterns. Rates of detection varied by cohort. Pb was detected in 99% of ablation patterns in the SLBT, followed by DOMHaIN at 97% and NHBCS at 90%, which also reflects the abundance of Pb detected in these cohorts. For all cohorts, toxic metalloids Hg, Cd and As consistently had the lowest detection rates. Overall detection rates were 18%, 2% and 13% respectively, but for the individual cohorts, SLBT had the highest As detection rate of 27%, the NHBCS cohort the lowest at 9% and only 7% of DOMHaIN ablation patterns had detectable As. The SLBT cohort had the highest detection rate for Hg and Cd: 61% of patterns had Hg µg/g values above the LOD, whereas this was 2% for DOMHaIN and 3% for NHBCS. SLBT had a 5% Cd detection rate, whereas this was 2% for NHBCS and 0% for DOMHaIN.

MULTI-COHORT COMPARISON

Elemental trends across time were consistent between cohorts, including a decrease in Mn from the second trimester onward, increases in Ba and Al after birth, and an increase in postnatal Pb around day 150 (Fig. 2). We found significant differences ($p < 0.05$) between cohorts and all element concentrations in both pre- and postnatal time periods using averaged ln-µg/g concentrations (Table 3) and show distributions of elements measured in the prenatal or postnatal periods across cohorts using violin plots (Fig. 3). In particular, the DOMHaIN cohort teeth had much higher median Sr concentrations than SLBT or NHBCS; in prenatal dentin the median Sr concentration was three times higher than NHBCS (114.8 vs. 36.4 µg/g), and just over double the Sr of SLBT (114.8 vs.

Table 2. Rates of detection expressed as % of ablation patterns.

Analyte	Nhbcs	Slbt	Domhain	All
Li	95	99	81	95
Al	67	93	91	77
Mn	94	98	98	96
Cu	98	99	95	98
Zn	100	100	100	100
As	9	27	7	13
Sr	100	100	100	100
Cd	2	5	0	2
Ba	99	99	100	99
Hg	3	61	2	18
Pb	90	99	97	93

51.2 μg/g). This difference was not as pronounced for postnatal dentin (Fig. 3; Table 3; postnatal Sr μg/g for DOMHaIN, SLBT, NHBCS: 90.0, 62.0, 36.3 μg/g). Across all cohorts, zinc abundance was high in dentin (between 65-87 μg/g). Micronutrients Mn and Cu were consistent, with Mn appreciably higher in prenatal dentin than in postnatal dentin (Figs. 2 & 3; Table 3). Al was higher in both pre- and postnatal dentin in the DOMHaIN cohort (prenatal Al DOMHaIN, NHBCS, SLBT: 0.5, 0.2, and 0.04 μg/g; postnatal Al: 1.1, 0.6, 0.1 μg/g).

DISCUSSION

Normalization to an SRM

In this study, we quantified elemental concentrations in deciduous teeth using an SRM, which allowed for data comparisons across three distinct cohorts and over multiple analytical sessions. Normalizing the LA-ICP-MS response within and across analytical sessions is essential whether by concentration or known (or calculated) isotope ratio of a standard. A survey of reported instrumentation and parameters in LA-ICP-MS analysis of human and non-human primate deciduous teeth (Supplementary Table S1) shows the use of different laser wavelengths (specifically 213 nm and 193 nm), ICP-MS instrumentation, and laser parameters such as spot size, laser frequency and fluence, such that comparisons between published studies using element: Ca ratios in dentin is not currently possible. Normalization can also be conducted by normalizing to a known 'true' value of each element ratio in NIST 1486 and that normalization factor applied to all element ratios [26, 42]. However, we anticipated that normalization to μg/g would be a more accurate approach for a number of reasons. First, concentrations in NIST 1486 are certified for many elements, while element ratios to Ca are not; second, missing certified or reference concentrations can be determined in-house by conventional digestion and ICP-MS analysis and validated against standard quality control practices, while validation of isotopic ratios is much more difficult. Third, different studies have used different Ca isotopes for normalization (Ca43, Ca44) (Supplementary Table 1) making it impossible to compare ratios between studies, and fourth, concentration values are more meaningful for the end users of the data [2, 4]. Additionally, specimens were analyzed between July 2020 – July 2022. To compare data collected over multiple analytical sessions conducted over many months, it was particularly important to normalize data. We use the element: Ca ratio response in our data reduction scheme, with the assumption that Ca is homogenously distributed within a tooth and across an individual's teeth, but then further converted this ratio to a concentration using the calculated response factor (ratio/concentration) and the certified concentration value for NIST 1486.

Comparison of measured elements with available literature

Despite the current limitations on quantification in LA-ICP-MS analysis, our study found concentrations and distributions in agreement with published data. Further, we quantified values for Li and Mn, for which no quantified values in deciduous teeth were available for comparison. In the present study, similarities between cohorts included the distinct trend of dentin Mn decline across early life [20], increases in Al and Li after birth, and increases in Pb around 6 months of age [43, 44], when mobility increases.

Zinc

Analysis of dentin from the three cohorts found significant differences between the concentration of all elements, with the exception of Zn. Zinc had the highest detection rate (100% throughout) and was stably present in deciduous teeth at median concentrations between 64 and 86 μg/g in pre- and postnatal dentin. Bulk Zn concentrations of 133 (±SD 36) μg/g were measured via particle induced X-ray emission (PIXE) in the deciduous dentin of 125 children aged 6–7 and 9–10 from three cities in Finland [8]. Zn concentrations varied from ~50–90 μg/g furthest away from the dentin/pulp cavity junction, increasing to 300–400 μg/g closer to the pulp cavity [45]. LA-ICP-MS 2D elemental mapping of three whole deciduous incisor ground sections from children living in a rural Australian community found that dentin 50 μm away from the EDJ on the labial side of the tooth (the region measured in this study) had Zn concentrations within the range of 100–150 μg/g. Zinc is homeostatically regulated, and it has been inferred that the physical processes governing Zn absorption into dentin during hydroxyapatite crystal growth effectively smooth out any rapid biological changes in dentinal fluid and (by inference) blood plasma [45]. The stability of the observed Zn concentrations across three diverse populations in this study suggests that Zn could be a useful standard for confirming the quantitative accuracy of deciduous tooth analysis.

Strontium

Strontium abundance and distribution in teeth has provided a history of feeding; distinguishing breast feeding and formula feeding, and dietary transitions [28, 29, 46, 47]. Strontium is non-essential and not actively transported across the mammary gland [48], and breastfed infants tend to have lower concentrations of postnatal Sr. Tooth regions with higher Sr concentrations that coincide with the NL often indicate introduction of formula. A particularly wide range of Sr concentrations have been measured in deciduous teeth; from 50–318 μg/g [49]. The DOMHaIN cohort had the highest concentrations of Sr (a median of 114 μg/g in prenatal and 92 in postnatal dentin): roughly double that of SLBT, and triple that of NHBCS. Concentrations measured here also broadly agree with those reported in Hare et al. [4], with reported maximum values of 120, 80 and 40 μg/g Sr for three mapped specimens. The heterogeneity in Sr distribution in teeth has been attributed to Sr-rich food [50] and it is conceivable that heterogeneity in tooth Sr reflects consumption patterns.

Barium

Barium is also non-essential and like Sr, sharp changes in Ba concentration in deciduous teeth can also indicate dietary transitions, such as weaning in both humans and primates [6, 26]. Because Ba transfer across the placenta is restricted, enrichment typically occurs immediately after birth from feeding either human milk or infant formula. Additionally, introduction of solid foods influence dentin Ba concentrations because Ba bioavailability in plants and animals is high [26]. The source of milk in the first year of life can also be inferred from spatial analysis of Ba in deciduous teeth [20]: exclusively breastfed infants had 39% less Ba in dentin than exclusively formula-fed infants in a Massachusetts, USA, population pilot study. Median Ba concentrations ranged from 1.27–5.17 μg/g in deciduous dentin, with concentrations being significantly higher in the SLBT cohort, and higher in postnatal dentin. The SLBT cohort had the most dramatic change in median Ba concentrations between prenatal and postnatal time periods (2.66 and 5.17 μg/g), while little differences were observed in the present-day cohorts in New Hampshire (1.27 and 1.50 μg/g) and Nigeria (1.97 and 2.04 μg/g). This observed difference may be indicative of the historical influence of dramatically lower breastfeeding rates in the 1950s–1970s [51].

Lead

The use of deciduous teeth as biomarkers of Pb exposure is well established [2, 3, 5, 6, 10–21]. The similarity between the Pb and Ca ionic radii in the +2 cationic form means that active sites within proteins readily accommodate Pb in place of Ca, making teeth particularly good biomarkers for this element. Further, the pathophysiology of Pb-induced health effects involves direct and indirect interference with Ca metabolism and homeostasis [52].

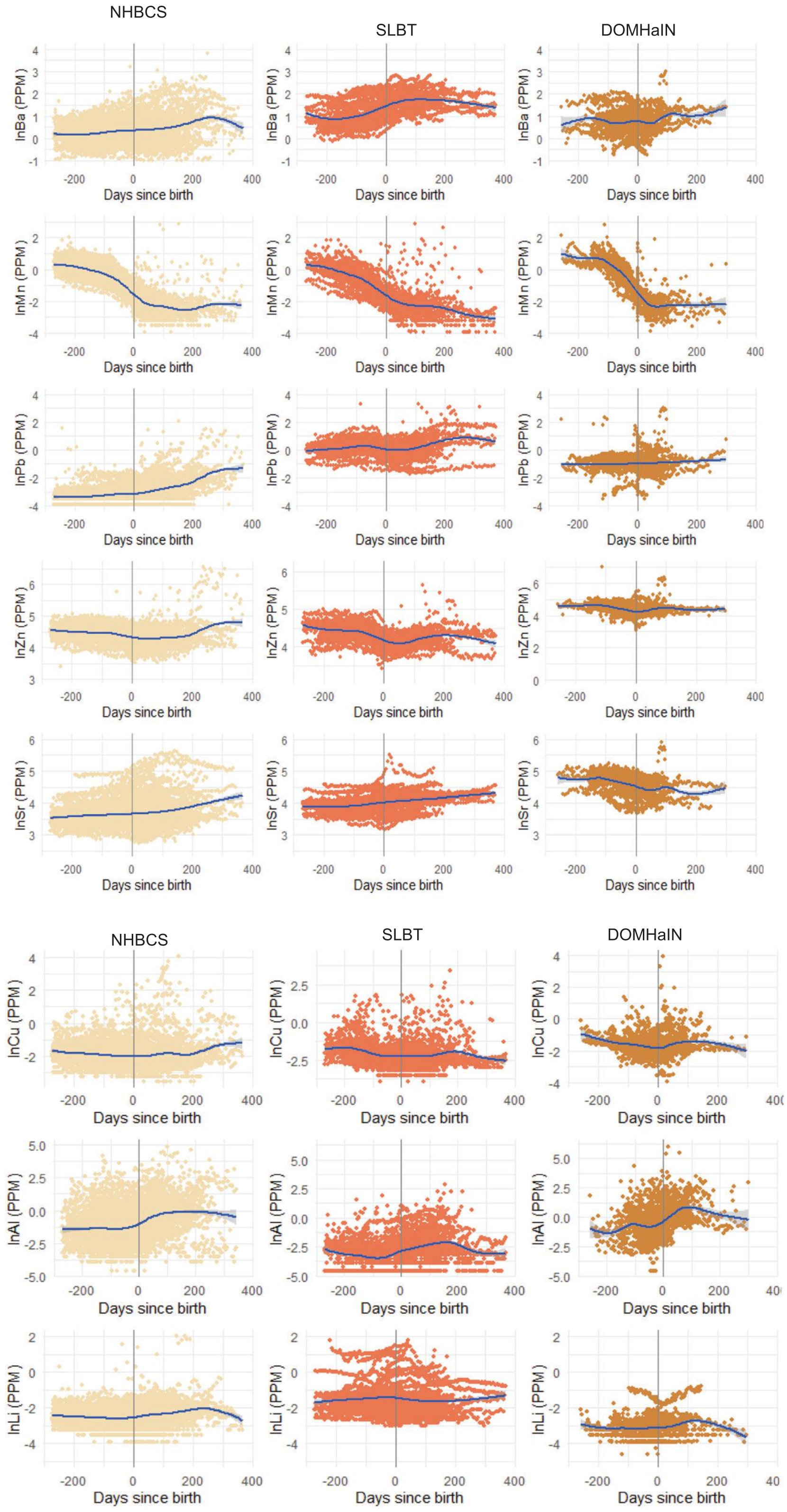


Fig. 2 **Elemental concentrations in deciduous dentin.** Dentin element concentrations (as ln-transformed ppm) commonly found in teeth (Ba, Mn, Pb, Sr, Zn) and others (Cu, Al, Li) from the early second trimester until 1 year of age, by three cohorts (cream: NHBCS, coral: SLBT, brown: DOMHaIN). Dots represent individual dentin element measurements. The vertical line at day 0 indicates birth. The line represents Loess smoother.

Table 3. Medians (25th percentile, 75th percentile) of elements (μg/g) measured in tooth dentin using LA-ICP-MS in three cohorts (NHBCS, SLBT, DOMHaIN).

Prenatal Period (2T - Birth)				
Element	**NHBCS median (1Q, 3Q)**	**SLBT median (1Q, 3Q)**	**DOMHaIN median (1Q, 3Q)**	***p*-value from ANOVA using ln-ppm**
Ba	1.3 (0.9, 1.8)	2.6 (2.0, 4.2)	2.0 (1.4, 3.0)	<0.001
Mn	0.7 (0.4, 1.1)	0.6 (0.4, 1.0)	0.9 (0.4, 1.5)	<0.001
Pb	0.04 (0.02, 0.05)	1.2 (0.8, 1.7)	0.4 (0.3, 0.5)	<0.001
Sr	36.4 (31.1, 43.0)	51.2 (43.8, 57.3)	114.8 (86.8, 131.2)	0.001
Zn	86.3 (75.6, 98.0)	81.0 (69.6, 95.7)	87.3 (70.4, 103.2)	0.03
Cu	0.14 (0.1, 0.2)	0.1 (0.1, 0.2)	0.2 (0.1, 0.3)	<0.001
Al	0.2 (0.1, 0.6)	0.04 (0.0, 0.1)	0.5 (0.2, 1.0)	<0.001
Li	0.08 (0.06, 0.10)	0.2 (0.1, 0.3)	0.04 (0.0, 0.1)	<0.001
Postnatal Period (Birth - 1 Year)				
Ba	1.5 (1.0, 2.3)	5.3 (3.9, 6.9)	2.2 (1.5, 3.4)	<0.001
Mn	0.1 (0.1, 0.2)	0.11 (0.07, 0.2)	0.14 (0.1, 0.2)	0.004
Pb	0.05 (0.0, 0.1)	1.3 (0.8, 2.0)	0.4 (0.2, 0.6)	<0.001
Sr	36.3 (28.9, 56.9)	62.0 (54.1, 72.3)	90.0 (70.2, 103.4)	<0.001
Zn	74.1 (63.8, 86.5)	65.8 (54.2, 76.3)	73.2 (58.4, 87.8)	0.03
Cu	0.13 (0.1, 0.2)	0.10 (0.08, 0.13)	0.2 (0.1, 0.3)	<0.001
Al	0.6 (0.3, 1.6)	0.1 (0.0, 0.2)	1.1 (0.6, 2.6)	<0.001
Li	0.09 (0.07, 0.14)	0.19 (0.11, 0.34)	0.04 (0.03, 0.06)	<0.001

P values from ANOVAs using ln-transformed elements concentrations.

Our findings of higher Pb in the historic, urban SLBT cohort add further support to the use of deciduous teeth as biomarkers of Pb exposure. The average prenatal dentin Pb concentration in SLBT teeth was 30 times higher than NHBCS, and four times higher than DOMHaIN teeth, at 1.22 (±0.86) μg/g. Likewise, postnatal dentin Pb in SLBT was 1.31 (±1.28) μg/g, 26 times that of NHBCS and 4 times higher than DOMHaIN. Our hypothesis in including SLBT was that environmental exposure to Pb would have been significantly higher for participants of this cohort, given that timing of specimen collection (1958–1970) occurred before the US Environmental Protection Agency's Clean Air Act (1970), which began the phase-out of Pb as an additive to gasoline and banned lead-based paint for houses in 1978 [53]. The participants and their mothers included in the SLBT would have been exposed to considerably higher environmental Pb concentrations than those in the present-day NHBCS, where dentin Pb was very low. The dentin Pb concentrations from SLBT compare well with those reported by Arora et al. [2] in Pb-exposed Australian children living in a rural mining town aged 6–7.5 years in the 1990's, which ranged from 0.23–1.39 μg/g prenatal dentin Pb, and 0.26–4.54 μg/g postnatal Pb. They also compare well with the concentrations reported in roots of deciduous maxillary teeth from Australian children from the 1990's in an urban setting (1.7 (± 1.43) μg/g; [12]), and in incisors from indigenous Canadian children (1.5 (± 3.2) μg/g; [54]). The lowest pre- and postnatal Pb concentrations of this study were seen in the NHBCS, a rural cohort in a region of New Hampshire where exposures occur primarily through house paint, drinking water and diet (median prenatal and postnatal dentin Pb = 0.04 and 0.05 μg/g, respectively). Upward trends in postnatal dentin Pb were observed across all three cohorts between 150 and 200 days after birth, which is consistent with Pb biomarker literature that as infant diet diversity and mobility increase, so does their Pb body burden [43, 44].

Manganese

The range of Mn concentrations measured was narrow: 0.1–0.83 μg/g, consistent with the role of Mn as an essential, homeostatically regulated micronutrient [55]. There were differences in dentin Mn concentration between the three cohorts, with higher dentin Mn concentrations in the DOMHaIN cohort. We saw a consistent, characteristic decline in Mn concentrations in late prenatal and postnatal dentin, with 0.56–0.83 μg/g in prenatal dentin and 0.10 – 0.14 μg/g in postnatal dentin. This observation agrees with known physiologic requirements of Mn during fetal development, when maternal Mn absorption is upregulated during the prenatal period and actively transferred across the placenta [56, 57]. Absolute μg/g concentrations of Mn in teeth from LA-ICP-MS analysis have not been reported in the literature (Literature Survey, Table S1), however bulk ICP-MS analysis conducted on tooth fragments that had also been analyzed via LA-ICP-MS report a median concentration of 0.49 μg/g for prenatal dentin and 0.14 μg/g for postnatal dentin [5], in good agreement with our measures.

Lithium

Lithium concentrations overall ranged from 0.04–0.2 μg/g, with significantly higher concentrations in the SLBT cohort. Absolute concentrations of Li in deciduous dentin have not previously been reported, although Li was measured by Friedman et al. [20] from a suburban pilot study outside of Boston, MA, USA, Li:Ca ratios were reported in supplemental material. Li:Ca Ratios were not related to infant feeding, but were higher among female children [20]. The high detection rate we observed for Li (99%) suggests that teeth may be a robust biomarker for this element. Teeth may be a useful non-invasive biomarker for monitoring the environmental body burden of increased environmental Li. Several sources of exposure to environmental Li exist including water and air contamination. Recent work from the US Geological Service reported that 37% of public drinking water wells from 33 principal aquifers across the US had Li concentrations above the Health Based Screening Level [58]. In the US arid regions had higher water Li which are increasing due to mining currently taking place as a result of the demand for Li for electric vehicle batteries. The most common source of Li exposure is from spent Li batteries, which have been historically disposed of in regular household waste, prior to

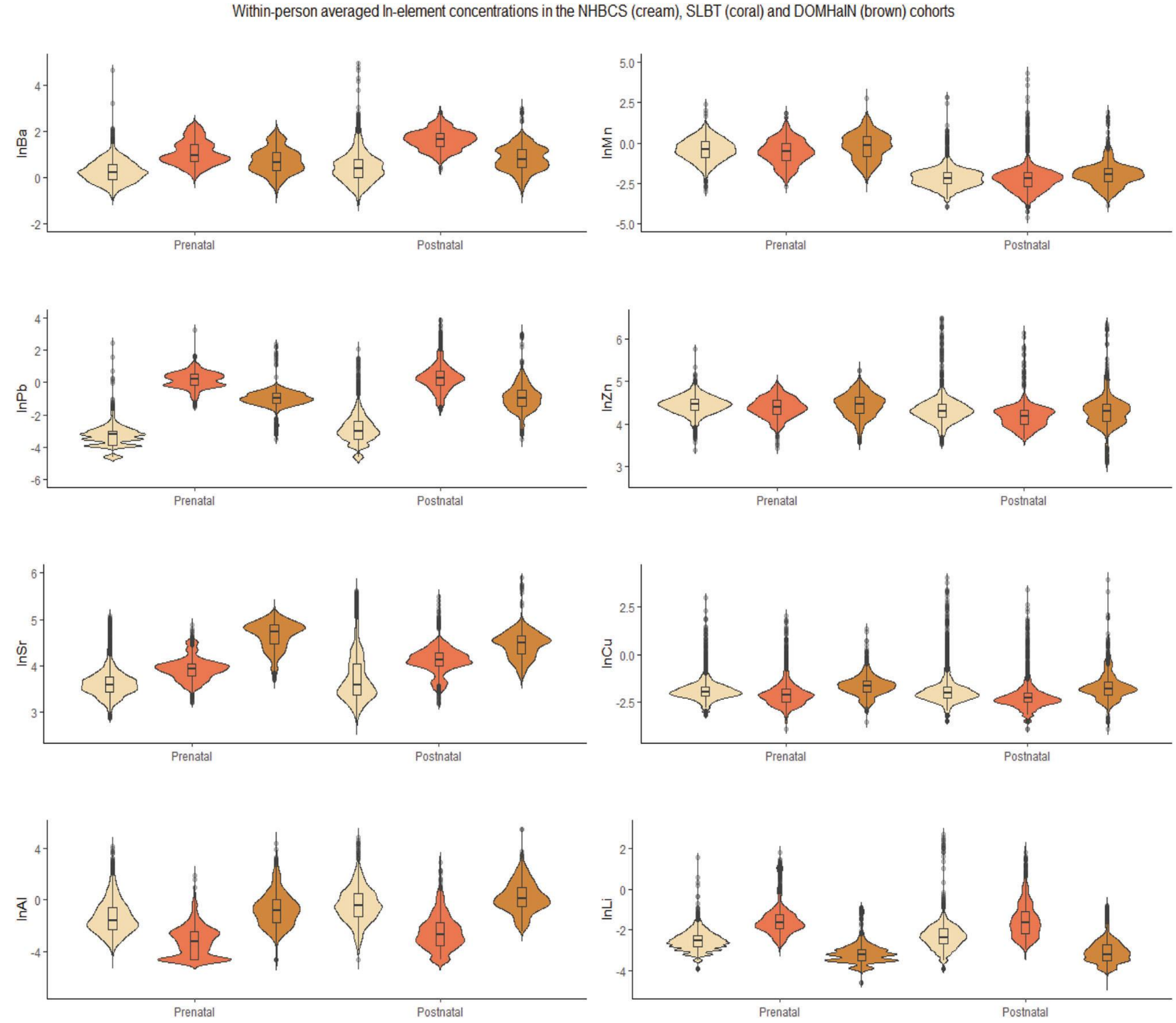


Fig. 3 Distribution of log transformed elemental concentrations in prenatal and postnatal dentin. Violin plots of dentin elements (ln-transformed, ppm) distributions for each cohort (cream: NHBCS, coral: SLBT, brown: DOMHaIN) by prenatal and postnatal time periods. Boxplots within violin plots represent the 25th percentile, median and 75th percentile.

concerted efforts to implement organized collection of Li batteries for recycling. Lithium is also used in psychotropic medicines [59], particularly the carbonate salt (Li_2CO_3) and acetate ($LiCH_3COO$), are used in the treatment of manic-depressive disorder. Medicinal use of Li for the past 50 years has been primarily in the treatment of bipolar disorder and other psychiatric conditions [60], but Li was banned from use for the treatment of mania in the US in 1949 because of its excessive toxicity, and did not gain approval from the FDA until 1970 [60]. This would suggest that the higher dentin Li concentrations observed in the SLBT are not a result of maternal medication.

Mercury

Poorly detected elements such as Hg, As and Cd received different statistical treatment than well-detected elements, with descriptive statistics limited to a detection rate, rather than a median and IQR. Mercury was notable because detection rates differed widely between the three cohorts (Table 3). Overall, Hg detection rates were 18%, the majority of which came from the SLBT, which had a Hg detection rate of 61%. This is compared to 3% Hg detection in NHBCS participants and 2% in DOMHaIN. Mercury, like Pb, is an element whose presence in the environment has been positively impacted by environmental legislation and the implementation of air quality standards [61, 62]. The major anthropogenic contributors to air- and water-born Hg contamination are emissions from coal-fired power plants, waste incinerators, and artisanal gold mining activity [61]. While the environmental burden of Hg is a current global concern [63], over recent decades, several laws have reduced environmental Hg release in the US including the implementation of emissions control technology in coal-fired power plants and waste incinerators, reduction in the number of active coal-fired power plants [64], phase out of common Hg-containing commercial products such as Hg-containing thermometers, and Hg-entrapment technology in dental offices using dental amalgams fillings (Code of Federal Regulations, Title 40, Chapter 1, Subchapter N, Part 441).

Arsenic and Cadmium

Detection rates for As and Cd were 13 and 2% overall respectively. SLBT had higher As detection rates (27%), with NHBCS – a cohort in which groundwater contamination with geogenic As is an exposure route [65, 66] – having only 9% detection. Elevated exposure to As in children enrolled in the NHBC has been shown using urine and nail biomarkers [67–74], with exposures including household drinking water from unregulated private wells [75, 76], and food containing rice [70]. The crystallographic structure of hydroxyapatite allows incorporation of a wide range of elements including As [77], but biological pathways overshadow crystal-chemical effects in trace element incorporation in bioapatite [77]. Assuming that As deposition in deciduous dentin occurs primarily via the blood supply to the tooth during active growth and development, the rapid clearance rate of As from blood via the kidney from short, low-dose exposure events [78] may significantly reduce the amount of As reaching the dentin, making teeth a poor biomarker of low-level, transient As exposures. Of the few reported measurements of As in teeth, exposures were high and acute. A study of an urban population impacted by a decommissioned Pb smelter in southeast Los Angeles County (California, US), closed for hazardous waste violations, found 20 of the 43 child participants had detectable prenatal As in their teeth, and 17 had detectable post-natal As (the detection limit for As was 1 µg/g) [18]. Levels were reported as counts ratioed with Ca and cannot be compared between studies. Fifteen participants had As in both pre- and post-natal tooth material. Of the 152 NHBCS teeth included in the present study, 112 had As concentrations that exceeded detection limits in prenatal dentin, and 108 had arsenic concentrations that exceeded detection limits in post-natal dentin.

Our observations with the ultra-trace non-essential metalloids (noted in Fig. 1) are that spot analysis does not reliably capture the heterogeneous distribution of these elements. Arsenic was not homogenously distributed in deciduous dentin. Figure 1 is representative of As data collected across cohorts and in addition to the high within-spot variation, indicates that As concentrations in the dentin of deciduous teeth were below detection limits of the current instrumentation and should be explored using a more sensitive technique. Both As and Cd may bind preferentially with enamel, which was not analyzed in this study. Two dimensional elemental mapping could be a better approach and is being conducted in follow-up studies on specimens selected from these cohorts.

LIMITATIONS OF THIS STUDY

Normalization to the NIST SRM is still not ideal [79]. The NIST 1486 SRM is certified only for bulk (solution mode) analysis: concentrations are not validated at a 50 µm scale. As a solid standard, NIST recommend that ≥250 mg of sample be used to ensure representativeness with the certified value; orders of magnitude more than is contained a typical plume of ablated particulates. Our calibration is also based on a single point using the certified values of NIST 1486; therefore µg/g values are considered semi-quantitative. Matrix-matched standards for LA-ICP-MS are not available for the majority of biological specimens. The physical interaction between the laser and the sample differs between solid dentin and a pressed pellet of bone powder, which will affect the abundance of particulates generated. For quantification, consistency in the choice of SRMs in the literature (Supplementary Table 1) has been helpful in comparing results across studies, but this is not routinely done. Shepherd et al. [45] point out that ablation of NIST 1486 pressed pellets prepared at 0.6 MPa generate a high proportion of larger particles in the plasma, resulting in elemental fractionation and lower analytical precision, compared to the NIST glasses, suggesting that NIST 1400 (bone ash) may be a viable alternative, indicating there is room for development of matrix-matched standards.

General conclusions

Our work highlights the utility of using quantified µg/g values when measuring elements in the dentin of deciduous teeth, demonstrated here with three geographically and historically distinct human cohorts. This research supports the findings of previous studies that spatial analysis of teeth is informative of elemental exposures and highlights the importance of innovative solutions to overcome methodological shortfalls, which makes absolute quantification of data particularly important. By normalizing data to a certified SRM, studies can be compared, regardless of the ablation parameters, laser wavelength and instrumentation, and they will continue to be informative as new instrumentation appears.

Overall, consensus within the field is critical to reusability of data. We suggest that, given the potential health information obtainable with LA-ICP-MS analysis of deciduous teeth, consensus SRMs, standardized pellet preparation procedures and ablation characteristics be used, and that in the longer term funding be made available for production of a true matrix-matched certified multi-point calibration series of reference materials: currently commercially available biological CRMs made from tooth material still do not exist [79]. Matrix-matched multi-point calibration series are becoming available for spatial analysis of soft tissue [80]; hopefully this will inspire possibilities for the production of a wider range of SRMs for harder biological tissues.

DATA AVAILABILITY

Use of the data may be possible under certain conditions by contacting the New Hampshire Birth Cohort Study Principal Investigator: Margaret R. Karagas. (Margaret.r.karagas@dartmouth.edu), the Saint Louis Baby Teeth Study Principal Investigator, Marc G. Weisskopf (mweissko@hsph.harvard.edu) and the Dental Caries and its association with Oral Microbiomes and HIV in young children-Nigeria Principal Investigator, Modupe O. Coker (mc2190@sdm.rutgers.edu).

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AUTHOR CONTRIBUTIONS

T. Punshon: Investigation, Data curation, Writing - Original Draft preparation, Writing – Review and Editing. J.A. Bauer: Formal Analysis, Writing – Original draft preparation, Writing – Review and Editing, Visualization. Margaret R. Karagas: Funding acquisition, Data curation, Supervision, Project Administration, Writing – Review and Editing. Modupe O. Coker: Funding acquisition, Data curation, Supervision, Project Administration, Writing – Review and Editing. Marc G. Weisskopf: Funding acquisition, Data curation, Supervision, Project Administration, Writing – Review and Editing. Joseph J. Mangano: Project Administration. Felicitas B. Bidlack: Methodology, Supervision, Writing – Review and Editing. Matthew N. Barr: Investigation. Brian P. Jackson: Conceptualization, Investigation, Writing – Review and Editing.

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COMPETING INTERESTS

The authors declare no competing interests.

ETHICAL APPROVAL

NHBCS: Participants received a detailed description of the study procedures before consenting to participate. Study materials and protocols for NHBCS were approved by the Committee for the Protection of Human Subjects at Dartmouth College. Data from the first analyzed NHBCS teeth ($n = 154$) are included in this study.

ADDITIONAL INFORMATION

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