Reconstructing the life-time lead exposure in children using dentine in deciduous teeth

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1. Introduction

The intrinsic importance of teeth as historical records of lead exposure has been recognised, tested and discussed for several decades. From the seminal papers of Rabinowitz et al. (1991; 1993) to the more recent works of Arora et al. (2011; 2006) and Hare et al. (2011) through the wealth of isotope studies by Gulson et al. (1994; 1996), Farmer et al. (2006) and Robbins et al. (2010), research has revealed the value of dental enamel and dentine as biological indicators of exposure. Central to many of these studies has been the aim to develop a blood lead biomarker as an alternative to blood lead measurements (BPb). The latter are still the most reliable indicator of recent lead exposure, both for screening and for bio-monitoring purposes. For periods longer than about 40 days however, the half life of lead in the blood compartment, serial sampling is required. As succinctly stated by Barbosa et al. (2005) 'It appears impossible to differentiate between low-level chronic Pb exposure and a high-level

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short Pb exposure based on a single BPb measurement'. By contrast, deciduous teeth are easy to collect (i.e. require non-invasive procedures), chemically stable and in theory provide a continuous record from *in utero* to several years after birth. Unfortunately, previous work has not had the desired degree of control over the temporal relationship between blood sampling and that part of the tooth corresponding to the exact time of sampling. This failure has been exacerbated by the different analytical tools employed, as a result of which, published tooth data have proven hard to interpret (Grobler et al., 2000). Recent studies (Arora et al., 2006) have sought to resolve this problem using the neonatal line as a fixed point in time. Whilst advancing our understanding, there remain issues to address concerning the processes governing the incorporation of lead into dental tissues and the stability of lead once incorporated.

This paper describes our research on the dentine in a small set of deciduous teeth to reconstruct the childhood history of lead exposure using laser ablation ICP mass spectrometric analysis for lead (LA-ICP-MS) combined with precise age measurements derived from the histological examination of dentine. Until recently, dentine was referred to in the literature as 'hydroxlapatite' and modelled using the stoichiometric composition $Ca_{10}(PO_4)_6(OH)_2$. Biological apatites (in bone and dentine) differ however in several important respects, including non-stoichiometry, small crystal dimensions and a high degree of structural disorder (Boanini et al., 2010). Furthermore, the marked reduction in OH bonds and the substitution of 5-6wt% $(CO_3)^{2-}$ for $(PO_4)^{3-}$ in bioapatites, suggests that dentine should be more correctly described as nanocrystalline 'carbonated apatite' (Pasteris et al., 2004). We welcome this suggestion and encourage its general adoption. Informed by new research on the synthesis of carbonated bioapatites we have also acquired matching data for Zn, Sr and Mg (see section 5. Discussion). These three elements have very different biological roles

from Pb but together help to explain and validate the spatial and temporal patterns we have observed for lead in dentine.

LA-ICP-MS is now an accepted technique for the analysis of dental tissues and with continued improvements in instrument design and the availability of affordable lasers, the elemental analysis of teeth can be performed routinely (Arora et al., 2011; Hare et al., 2011). Though the technique may be regarded as mature, the reproducibility and accuracy of analysis depends upon the type of laser employed and the choice of appropriate standard reference materials. Our work builds upon important advances in laser ablation methodology as reported by Delmdahl and von Oldershausen (2005) and Guillong et al. (2003).

Dentine is described as *coronal* or *root* depending on which part of the tooth is indicated. Within coronal dentine (i.e. the crown of the tooth), *primary dentine* is the first formed and constitutes the bulk of the coronal dentine. It comprises a layer of *mantle dentine*, about 20μm in thickness following the enamel-dentine junction, upon which primary dentine is secreted incrementally; the latter being referred to as *circumpulpal dentine*. After root formation is complete, a thin layer of *secondary dentine* may form in continuity with the primary dentine but distributed more unevenly around the pulp cavity. As discussed later, it is important to realise there is a significant time lag between the completion of primary and onset of secondary dentine. In response to tooth attrition or dental caries, a third type of dentine may form. Known as *tertiary dentine* it forms locally within the circumpulpal dentine and is intended to block the dentinal tubules; reactive in the case of attrition, or reparative in the case of caries (Linde, 1992). In this paper we refer only to primary and secondary dentine and for convenience have used the following abbreviations: EDJ-enamel/dentine junction; DPC-dentine/pulp cavity junction.

2. Samples

The teeth were provided by a tertiary referral dental practice in Teesside, NE England (Queensway Dental Practice) and comprised 15 pairs of extracted deciduous molars (1st and 2nd molars). Ethical approval for the study was granted through the NHS County Durham & Tees Valley 1 Research Ethics Committee and full consent was obtained from both parents and children in all cases. Initially it was intended to compare all 15 pairs but the presence of dental caries and excessive attrition limited the preparation to 22 teeth (Table 1). After analysis, three pairs were subsequently judged unsuitable for histological examination and have been excluded from discussion. Tooth IDs refer to specific teeth in accordance with the two digit Fédération Dentaire Internationale primary dentition nomenclature (Hilson, 1996). Also shown in Table 1 is the age of the child at the time of tooth extraction. In accepting these teeth we had no reason to believe that the children selected for the study had been exposed to anything other than background levels of lead; consistent with an historic industrial region of the UK.

Samples were prepared for analysis as 150µm thick, doubly polished, longitudinal slices of tooth as follows. Using a low speed saw, 500µm thick longitudinal slices were cut from each tooth and mounted onto glass slides using dental wax. These were then ground down to approximately 150µm and polished with 1 µm alumina powder. The slices were then removed, flipped over, remounted and the second surface polished. Small changes in procedure were adopted to prepare the samples for histological examination (e.g. thinning to 100µm) but otherwise the methodologies were broadly similar.

3. Methods of analysis

3.1. LA-ICP-MS Analysis

Trace element analysis of the teeth was performed using a GeoLas 193nm ArF excimer laser coupled to an Agilent 7500c ICP-mass spectrometer at the School of Earth and Environment,

University of Leeds, UK. At 193nm, most materials photoabsorb leading to rupture of the chemical bonds without thermal expansion or damage to the surrounding material (Delmdahl and von Oldershausen, 2005). Guided by histological criteria, a series of 100µm diameter ablation pits were made at intervals of 100-200µm along transects extending from the enamel surface, across the EDJ to the DPC for each tooth. During ablation, the process was monitored via a video camera integrated into the optical array.

Using a spot size of 100µm, a constant energy density of 10J/cm^2 and a pulse rate of 5Hz (Table 2), the aspect ratio (depth/diameter) of each ablation pit never exceeded 1; thus minimising potential element fractionation (Kosler, 2008). Subsequent optical examination of the ablation pits confirmed very flat bottoms, straight vertical sides and no evidence of surface spalling, consistent with controlled excimer excitation.

Ion intensities at isotope masses ^{24}Mg, ^{44}Ca, ^{66}Zn, ^{88}Sr and ^{208}Pb were recorded in time resolved scanning mode and converted into element/calcium ratios using data for standard reference materials analysed before and after each analytical session. Reference material NIST SRM Glass 610 was used for instrument calibration and cross referenced to replicate analyses of NIST SRM Glasses 612 and 614 to establish instrument performance and within-run standard errors. SRM Bone Meal 1486, having a matrix similar in chemical and mineralogical composition to dentine, was used as an external unknown. No apparent isobaric interferences were observed for the above elements. Minimum detection limits were calculated as 3 times the background count rates on the carrier gas blank before ablation. Routine detection limits for Mg, Zn, Sr and Pb were typically 0.03-0.09ppm, 0.15-0.19ppm, 0.002-0.004ppm and 0.01-0.02ppm respectively. The measured values for Sr and Pb were within 2-8% of the certified values for NIST 612 (78.4ppm Sr; 38.57ppm Pb) and NIST 614 (45.8ppm Sr; 2.32ppm Pb), and within 12-17% of the certified values for NIST 1486 (264ppm Sr; 1.33ppm Pb). For Zn and Mg, measured values were within 10-15% of the

certified values for NIST 1486 (147ppm Zn; 4660ppm Mg). Certified data for Zn and Mg in NIST 612 and 614 are not available but the measured values are in close agreement with the working values published by Pearce et al. (1997).

Absolute concentrations of Mg, Sr, Zn and Pb in dentine were calculated by normalizing to 26.5 wt% Ca (Arora et al., 2006). Data processing was performed off-line using SILLS; a software programme specifically written for the signal integration of laboratory laser systems by Murray Allan (University of Leeds) and later modified by Dimitri Meier and Marcel Guillong (Die Eidgenossische Technische Hocchschule, Zurich).

SILLS error estimates for lead analyses of dentine based on data for tooth HT11-85 are shown in Figure 1a. These obey a power law distribution and for a minimum concentration of 0.06ppm Pb the calculated 1σ error is ~40%, decreasing to ~5% for a maximum concentration of 2.0ppm Pb.

A limitation we noted when using NIST 1486 as a primary reference material for LA-ICP-MS analysis was its poor response to laser interaction. When ablating a pressed powder pellet of NIST 1486 prepared at 0.6MPa, a high proportion of >1µm particles are generated. As a consequence, there is incomplete vaporization of the larger particles in the plasma resulting in elemental fractionation and lower analytical precision when compared to NIST glasses. Hare et al (2011) reports a similar problem when using NIST 1486 though they attribute their lower precision to sample heterogeneity. Whatever the reason, this material is best used in combination with another SRM. A promising alternative to NIST 1486 is NIST 1400. This is certified bone ash and when sintered at 2GPa and 700^{o}C creates an ultra-dense pellet with excellent ablation performance. Using such material, Balter et al. (2008) reports, for example, a within-run precision for Sr/Ca of 1% by LA-ICP-MS. A more reliable matrix-matched standard for dental tissue analysis is clearly needed. Accuracy in laser ablation however is not solely determined by the matrix but is also a function of the laser-operating conditions

(wavelength, pulse width, pulse energy). Short wavelength excimer lasers have proved very effective in minimising matrix effects since all matrices tend to absorb with similar efficiency (Jackson, 2008). Given that the concentration values obtained for Zn, Mg, Sr and Pb in NIST 1486 (external unknown) were within 10-17% of the certified values, we are confident that our use of NIST glasses for calibration is acceptable.

Although the ArF excimer laser used in this study is capable of producing much smaller ablation spots, a pit diameter of 100µm was selected to yield sufficient counts/sec/ppm Pb for the signal to be significantly in excess (x3) of the background count rate. For the other elements (present at considerably higher concentrations) the spot size could have been reduced to 20µm or less without compromising detection limits.

3.2. Histological Analysis

Histological analysis was performed using an Olympus BX51 microscope with a Q-Imaging Micropublisher 3.3 RTV camera and Imporvision Openlab 5.0.2 image analysis software. The ages sampled by each ablation pit were determined by identifying the neonatal line and measuring the distance from it to the edge of the first ablation pit. This distance was divided by the mean daily rate of dentine secretion as determined by the mean distance between daily growth increments in the same region; the von Ebner lines (Dean, 1995). This gave the number of days and the age from birth to the edge of the pit. In a similar manner, the mean rate of dentine secretion was determined alongside the pit to yield the number of days sampled and the age at the leading edge of the pit. The process was then continued along the transect until the ages sampled by each pit were determined. From these measurements we concluded that a 100µm ablation pit represented on average 42 days of dentine growth. Accordingly, a series of age ranges of 6 weeks duration were constructed allowing the LA-ICP-MS elemental data to be assigned a chronological age. A similar strategy was followed

to determine the ages sampled by each ablation pit in the enamel, using daily cross striations. A detailed description of the histological analyses will be presented elsewhere (Dirks et al. *in prep*).

4. Results

Only the dentine analyses are reported here. Of the 19 teeth analysed, 5 have been excluded from discussion because the longitudinal sections were cut oblique to the ideal plane through the dentine and pulp horns and did not intersect the pulp cavity (see Table 1). To test the extent of within-tooth trace element variation, 3 to 5 ablation transects were made across two pairs of teeth (HT1-54,55 and HT11-64,85), orientated to follow dentine tubules from the EDJ to the pulp cavity, from all regions within the crown (Figure 1b). From these results and associated histological data it was evident that individual transects corresponded to different post-natal time spans. For HT11-85 the time span for dentine ranged 766 to 1018 days after birth. Nevertheless, irrespective of the time span, the overall concentration levels and concentration-time profiles (A, B and D) remained similar and highly correlated for each tooth (Figure 1c). We decided therefore that for the remaining teeth only one transect was required to demonstrate the magnitude and temporal changes in trace element concentration; a conclusion reinforced by the agreement between tooth pairs. Table 3 summarises the maximum and minimum values for Pb, Zn, Sr and Mg in dentine for each tooth or pairs of teeth. Maximum values for secondary dentine, where analysed, are also shown.

Previous researchers (Arora et al., 2006; Humphrey et al., 2008; Kang et al., 2004) when reporting trace element analyses of dentine and enamel by LA-ICP-MS have erred on the side of caution, by normalizing ion intensities to calcium. This avoids the problem of not knowing the absolute Ca concentration and/or within-dentine Ca variation. In this study, for example, the within-dentine ^{44}Ca intensity variation (RSD 6%, n=15) from the EDJ to the DPC for

HT3-55 is too small to account for the corresponding RSD variations in ion intensities for ^{208}Pb (59%) or ^{66}Zn (42%). Moreover, though published Ca values for dentine in deciduous and permanent teeth differ by several wt% (Arnold and Gaengler, 2007; Keinan et al., 2006; Ohmori, 1961), the relative uncertainty is small compared to the within-tooth and between-child differences in trace element concentrations (several orders of magnitude for Pb). We thus consider our measured trace element variation to be robust and to allow easy comparison with previously published data, we have retained the value of 26.5wt% Ca used by Arora et al. (2006). If in future there is consensus that the accepted mean Ca value for dentine is different, then the concentrations presented here can be recalculated accordingly

In Figures 2 to 6 the results are displayed graphically as time-concentration profiles. For clarity, the measurement error for each sample point is not shown. Selection was made to highlight the following deductions.

(i) Agreement for all elements for all pairs of teeth (Figure 2a). This applies to combinations of both 1st and 2nd deciduous molars and is strong evidence to support the contention that position within deciduous dental arch is not an overriding complication in the interpretation of dentine data.

(ii) Profiles for Pb and Zn display a systematic rise in concentration at or close to the DPC (Figure 2b). This is evident in each and every tooth examined, including sections where the pulp cavity was separated from primary dentine by a thin layer of secondary dentine. The only exceptions being those samples for which longitudinal sections did not intersect the DPC (Table 3). For Pb, the rise is demonstrably sharper than for Zn. Values for Pb and Zn in secondary dentine are consistently higher than the corresponding highest values in contiguous primary dentine (Table 3).

(iii) Sr shows either little or no change in concentration with time (excluding HT3). Mg differs from the other elements in displaying a steady and progressive increase in concentration from the EDJ to the DPC soon after birth (Figure 2c).

5. Discussion

To facilitate the discussion and provide a context for interpretation we have used the following simplified time frame of deciduous tooth formation.

After early pre-natal differentiation of enamel and dentine, dentine continues to be laid down by odontoblastic cells that line the pulp cavity. These act as biological channelways in controlling the transfer of mineral ions from the blood plasma to the sites of dentine secretion. Dentine is first secreted as a layer of unmineralized matrix which forms a collagenous framework called predentine. This varies in thickness (10-30µm) and contains dense clusters of apatite nanocrystals (<5nm) (Nanci, 2008; Linde, 1992) which aggregate to form calcospherites. Gradually, by coalescence of these clusters, progressive growth of the apatite crystals and a decrease in collagenous material, the predentine mineralizes into primary dentine in what is known as globular mineralization. Linear mineralization may also occur when the mineralising front appears as a line rather than as a scalloped edge. As apatite maturation proceeds, the odontoblasts migrate progressively inwards leaving a characteristic architecture of open tubules, marking the trace of the odontoblasts, embedded in a matrix of nanocrystalline platelets of apatite (30-50nm) and about 20% organic material. For a typical deciduous tooth there are approximately 24,000 tubules/mm^2 (Schilke et al., 2000). The secretion of dentine can therefore be described as a front of mineralization linked to an inwardly migrating layer of unmineralized predentine. After a period of sustained dentine secretion there is then a hiatus during which root formation takes place followed by the formation of secondary dentine. At no stage does mineralization lead to the occlusion of

dentine tubules ‘dentinal sclerosis’ which implies that the main phase of mineral deposition is completed in close proximity to the predentine layer and does not contribute appreciably to nanocrystal growth elsewhere within the earlier formed dentine. Of the various processes invoked for the localisation of mineralization, the evidence favours cellular control by the odontoblasts (Nanci, 2008; Linde, 1992). The final stage of deciduous tooth development is resorption of the root and shedding of the crown.

From work on the maturation of synthetic bioapatites, Cazalbou et al. (2004b; 2005) conclude that Ostwald ripening (McNaught and Wilkinson, 1997) and dissolution-precipitation mechanisms (excluding the fluoridation of dental enamel) are relatively slow and are not appropriate on a biological timescale. Instead, they propose a model of a structured hydrated layer ‘a non-apatitic environment’, located at the surface of apatite nanocrystals, containing easily exchangeable mobile ionic species. With maturation and growth of the nanocrystals, the non-apatitic domain decreases with respect to the bulk apatitic domain. During this process, ions in the hydrated layer are irreversibly incorporated into the apatite lattice or enriched in the residual hydrated layer. Maturation rates, based on the synthesis of carbonated apatite, lead to a stable end phase within 20-30 days. Boanini and co-workers (2010) have also drawn attention to the fact that not all trace elements in nanocrystalline carbonated apatite are ion substitutions in the apatite lattice but some may be absorbed onto the crystal surface (i.e. doped ions).

When considered collectively, the complementary hypotheses of hydrated surface layers and ion doping, as a function of crystal size, offer a possible explanation for changes in the concentration of trace elements in dentine during mineralization. Using these experimental observations, the following four sections detail the variation of Sr, Zn, Pb and Mg in dentine for 18 deciduous molars.

5.1. Strontium

For the majority of teeth there is little apparent variation in Sr from the DEJ to the DPC, including secondary dentine (Table 3). There are however notable differences in the time averaged mean concentrations of Sr between children (Figure 3). Child HT9 maintains a Sr level of 38ppm (±3ppm 1σ) between birth and the cessation of primary dentine formation, whereas child HT2 displays a slightly more elevated, variable level of 65ppm Sr (± 8ppm 1σ), with child HT3 maintaining a very elevated level of 135ppm Sr (±13ppm 1σ). These changes are most likely a response to diet and whether or not the child was breast fed or weaned on proprietary milk products (Humphrey et al., 2008). Lacking such critical information, we are unable to differentiate between the various controls. Overall, the profiles for Sr are in marked contrast to those of Zn, especially the absence of increasing enrichment in proximity to the pulp cavity (see below). Where there are major changes in Sr concentration, they tend to be sharp and completed within a relatively short time interval (e.g. child HT3 shows an increase in Sr from birth to 126 days of 50ppm). One possible explanation for these patterns is the close similarity in ionic radius of Sr (0.132nm) and Ca (0.114nm). By substituting very easily for Ca^{2+}, the Sr^{2+} ion would be strongly and rapidly stabilised in the ordered apatite lattice of the growing nanocrystal. Sr may thus be regarded as controlled primarily by ion substitution and therefore a sensitive indicator of blood plasma levels. Secondary dentine, where present, is not enriched in Sr relative to primary dentine.

5.2. Zinc

Unlike Sr, Zn is homeostatically controlled and one would not expect major postnatal changes in concentration with age. For the majority of tooth profiles this is true. Excluding child HT3, time averaged mean concentrations of Zn vary from ~50-90ppm but increasing to 300-400ppm within 200-300µm of the DPC (Figure 4). In this respect Zn differs substantially from Sr. The smooth, systematic increase in concentration on approaching the pulp cavity occurs irrespective of the age of the final dentine layer or the age of the child at the time of

tooth extraction (Figure 4). For HT12-84 primary dentine was completed at ~514 days, for HT3-55 at ~934 days and HT8-85 at ~1186 days. This strongly suggests that the process controlling Zn incorporation into carbonated apatite is different from that of Sr and is a function of dentine growth. X-ray absorption studies on bone, a close carbonated apatite analogue for dentine, have demonstrated that Zn atoms are localised at the surface of apatite nanocrystals and not in the apatite structure (Bazin et al., 2009). Since the surface area/volume ratio decreases with increasing crystal size, there will be a proportional decrease in the Zn/Ca ratio away from the predentine layer; in good agreement with the observed dentine profiles. We suggest therefore that the concentration of Zn in dentine is primarily a response to homeostatic control but modified by ion doping and absorption processes concomitant with crystal growth. Because ion absorption is a function of the available surface area, rapid changes in the Zn content of dentinal fluid, and by inference blood plasma, are probably smoothed out. A preference for surface absorption as distinct from ion substitution is also in agreement with the smaller ionic radius of Zn (0.088nm) (Boanini et al., 2010). Secondary dentine appears to amplify and continue the apparent enrichment trend described above (Table 3). For example, the Zn concentration in secondary dentine for tooth HT13-84 is more than twice that of the contiguous primary dentine. This could be due to a slower rate of dentine secretion allowing a switch from ion absorption to ion substitution or a decrease in crystal size.

5.3. Lead

Lead shows characteristics of both Sr and Zn. Like Sr, the profiles for Pb are relatively flat for most children and, excluding dentine close to the pulp cavity, show no marked changes in concentration with age. Time averaged means for primary dentine (excluding values close to the DPC) differ by only a factor of 2 to 3 for the whole cohort (max ~0.3ppm; min ~0.1ppm). By contrast, the time averaged Pb concentrations for child HT3 are extremely anomalous and

are 10 times greater than those of the other children, implying a dramatically higher level of environment lead exposure (Figure 5). Furthermore, HT3 displays rapid, reproducible changes in Pb that can be traced in both deciduous molars (HT3-55,65) from birth to the DPC. Of course, we recognise the danger in over extending our interpretation based on such a small cohort and two profiles. Nevertheless, though lacking X-ray spectroscopic verification, we tentatively suggest that Pb, having an ionic radius (0.130nm) similar to Ca (0.114nm) and with profiles that record rapid changes in concentration, is controlled primarily by ion substitution. This does not necessarily preclude some degree of surface absorption since there is a weaker but definite enrichment in Pb on approaching the pulp cavity. Typically, Pb concentrations rise sharply to ~2-6ppm within 100-200µm of the DPC. [N.B. Because the gradient in Pb concentration at the DPC varies with a few 10's of microns, the position of the ablation pit (diameter 100µm) is critical in estimating the maximum concentration.]

5.4. Magnesium

In general, the profiles for Mg differ from those for Sr, Zn and Pb in showing a steady rise in concentration from ~7000ppm at the EDJ to ~10,000ppm at the DPC (Figure 6). The rate of increase is variable and in one case (HT11) the Mg concentration decreased with age after 1 year (from ~7000pppm to ~2000ppm) on all 5 ablation transects. We are unable to find any analytical explanation for this anomalous behaviour and the reversal was not observed on corresponding Pb, Zn or Sr profiles. Lacking published data for changes in plasma Mg levels during early childhood we are unable to comment further. Though not observed for all teeth, there is a tendency for a sharp decrease in Mg immediately adjacent to the DPC (100-200 µm: equivalent to ~1-2 months) suggesting that immature dentine is perhaps Mg-deficient (see Figure 6: HT2-64). Some uncertainty exists as to the exact localization of Mg in biological apatites (Cazalbou et al., 2004a) although the similarity in ionic radius between Mg (0.086nm) and Zn (0.088nm) would tend to favour control via ion absorption. Whether

the temporal changes we observe are a response to plasma Mg concentration, dentine composition or are related to the kinetics and growth mechanisms of nanocrystalline carbonated apatite remains to be proven.

Trace element enrichment close to the pulp cavity has been reported by many other researchers, notably Rabinowitz et al. (1993), Thuy et al. (2003), Kang et al. (2004), Arora et al. (2006), Richter et al. (2011), Arora et al. (2011) and Hare et al. (2011). Arora (2006) in agreement with Rabinowitz (1993) suggested that Pb enrichment was possibly due to the close juxtaposition of odontoblasts and blood vessels, allowing a higher rate of exchange between blood and dentine. This hypothesis was later invoked by (Arora et al., 2011) to account for higher levels of Mn in circumpulpal dentine adjacent to the pulp cavity in naturally shed deciduous incisors. More recently, (Hare et al., 2011) have very elegantly demonstrated, using LA-ICP-MS bio-imaging, an enrichment of Pb and Zn around the margins of the pulp cavity in naturally shed deciduous incisors. Whilst the analysis of fluorine in dentine is not possible by ICP-MS, electron microprobe (Richter et al., 2011) and specific ion electrode (Thuy et al., 2003) studies have shown a major increase in fluorine concentration in close proximity to the pulp cavity. In the case of Thuy and co-workers, the illustrated fluorine enrichment profiles are remarkably similar to those of Zn and Pb in the present study, although the authors make no reference to secondary dentine which would have been well developed in permanent premolars in subjects 13-22 years of age.

This study focussed on the need to acquire data for very low concentrations of Pb; hence the use of 100µm diameter ablation pits. However, as described in section 3.1, given the higher concentrations of Sr, Zn and Mg, satisfactory signal/background ratios could have been obtained using a 20µm diameter laser beam, equivalent to a dentine secretion interval of <10days.

5.5. Dentine as a biomarker of lead exposure history

From the above observations and the excellent reproducibility demonstrated by pairs of deciduous molars, the evidence strongly supports the assertion that dentine carries a record of plasma lead concentrations. As mentioned in section 3.2, to unlock this information, each laser ablation point was assigned a real time interval based on the number of days before and after birth. From this we deduce that:

(i) The steep increase in Pb concentration on approaching the pulp cavity 'pulp proximity enrichment' is not a function of increasing plasma Pb concentration or age of the dentine. The most likely cause is nanocrystal maturation.

(ii) There is a finite time interval 'duration of mineralization' between predentine formation and a stabilized Pb concentration. Thus changes in plasma Pb concentration with time 'history of exposure' can only be inferred from changes in dentine Pb concentration after peak mineralization (i.e. within a stable baseline). There is therefore a finite time delay between the environmental exposure event and its fixed expression in primary dentine. At present we do not have the necessary data to quantify this duration. The large diameter of the laser ablation pits (100µm) combined with the separation between pits (100µm) is inadequate to accurately detect small scale changes in lead concentration close to the pulp cavity. Work is in progress to use a smaller diameter laser beam in conjunction with a higher sensitivity mass spectrometer to detail trace element variation within this critical zone.

(iii) In the absence of major temporal changes in dentine Pb, time averaged means can be used as a comparative measure of the relative degree of exposure for a given child. The higher the background environmental lead, the higher the time averaged dentine lead. Since the concentration of Pb close to the DPC is similarly a reflection of plasma

Pb, these values if used judiciously might also provide comparisons of relative exposure.

Point (iii) poses the important question: Can dentine lead be used as an empirical measure of cumulative childhood lead exposure? Being non-invasive, it has potential merit and compares favourable with *in vivo* bone lead studies (Ambrose et al., 2000; Hu, 1998). Unlike bone however, there is no evidence to indicate a turnover of dentine lead (Gulson, 1996). Nor do teeth store sufficient amounts of lead as to pose a longer term health risk.

Secondary dentine remains an unresolved problem. As shown in Table 3, it is anomalously enriched in Pb compared to primary dentine and in permanent teeth, where it is better developed, displays an incremental layered structure. Zn also shows a modest enrichment (30-50%). For Mg and Sr, secondary dentine is indistinguishable from primary dentine and according to Nanci (2008) has the same ratio of mineral to organic material. Due to the hiatus in dentine formation during the period of root formation, secondary dentine cannot be considered a simple continuation of primary dentine secretion. Odontoblast activity, in controlling the transfer of mineral ions to the predentine layer, is temporarily suspended. On resumption of activity the incorporation of trace elements into the carbonated apatite is evidently different. Unfortunately, lacking data for the timing of secondary dentine formation or its nanocrystallinity, we can only speculate that the enrichment of Pb (and Zn) is due either to slower growth kinetics or crystal size. There is however a third factor to consider; namely root resorption prior to tooth shedding. The natural process of root dissolution releases previously stored lead (Arora et al., 2004) directly into the blood plasma. By analogy with primary dentine, such additional lead would be rapidly incorporated into secondary dentine. Comparison of the Pb levels in primary and secondary with respect to

exposure history is therefore difficult to interpret and further high spatial resolution analysis across the primary-secondary dentine interface is clearly warranted.

The ability to resolve time differences of ~ 42 days permits an exciting insight into patterns of pre- and post-natal lead exposure. As demonstrated by Arora et al.(2006), using the neonatal line as a fixed point in time, it is possible to distinguish between plasma lead levels corresponding to the mother's blood lead during term and the child's ambient exposure to lead immediately after birth. A sense of change can be seen in child HT3 where a pre-natal dentine Pb concentration of ~0.6ppm increases to ~0.9ppm within 42 days of birth. Although these values are low, the ability to detect measurable differences indicates the potential of LA-ICP-MS to provide information on lead exposure during and after foetal development. Whilst this paper does not address health issues linked to lead exposure, one of the most important goals of our long term research programme is to validate the use of dentine lead as a proxy for BPb. However, we recognise that this will require teeth from children living in more polluted areas and access to serial blood samples. Several studies have used dental tissue data to infer BPb levels but, as emphasised by Grobler et al. (2000), the results are often difficult to compare because different parts of the tooth were analysed and the tests lacked crucial information that would allow unambiguous temporal correlation with blood samples. By successfully using histological criteria to determine an age for each dentine micro-analysis, we believe our study brings the development of a retrospective BPb biomarker one step closer. Finally, although we have emphasised the reconstruction of exposure history, a true time frame also facilitates a more reliable estimate of cumulative exposure.

7. Conclusions

Lead analyses for 19 deciduous molars combined with detailed histological work to establish the age of the dentine confirm that primary circumpulpal dentine can be confidently used to reconstruct the history of early childhood lead exposure. Using a laser beam of 100µm diameter, the time span of each ablation point corresponded to an average of 42 days of dentine growth. Complementary data acquired for Zn, Mg and Sr have proved very informative in identifying the mechanisms controlling the uptake of Pb into dentine, and suggest that these controls are linked to the special physical and chemical properties of nanocrystals and nanocrystal growth. For elements with higher dentine concentrations than Pb, a spatial resolution of 10µm is well within the analytical sensitivity of quadrupole mass spectrometry.

Between-tooth differences for paired deciduous molars were found to be small and, although within-tooth dentine variation can be detected, robust and reproducible differences in exposure to Pb can be quantified using a single, well positioned, laser ablation transect on longitudinal sections. Interpretation must however distinguish between primary and secondary dentine and allowance made for a possible time difference (small but as yet unquantified) between pre-dentine formation and establishment of a stabilised Pb dentine signal. Unlike enamel which ceases to form before the tooth has erupted into the oral cavity, dentine formation in deciduous teeth is more continuous and affords an unbroken record of first few years of post-natal history.

To achieve the longer term goal of using dentine as a proxy for BPb it is important that experimental designs incorporate procedures for determining the age of the dentine, to permit unambiguous temporal correlation with blood samples. Moreover, since blood Pb has a short half life and that close to the pulp cavity Pb displays a very high concentration-time gradient, small errors in assigning an age to the dentine can lead to large errors in estimating BPb

levels. We contend that in the absence of a true time frame, serious miscalculations may arise in using Pb analyses for dental tissues as proxies for BPb.

Whilst this study focussed on exposure to Pb, the multi-element capability of LA-ICP-MS opens up potential avenues of research in epigenetics where changes in both toxic and essential elements during early childhood can have important health outcomes later in life. This study also signals future challenges for histological research. Of particular interest would a better understanding of the time gap between the cessation of primary dentine formation and secretion of secondary dentine. Use could then be made of the information stored in secondary dentine and thus further extending the historical record of childhood exposure.

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References

Ambrose TM, Al-Lozi M, Scott MG. Bone Lead Concentrations Assessed by in Vivo X-Ray Fluorescence. Clin Chem 2000; 46: 1171-1178.

Arnold WH, Gaengler P. Quantitative analysis of the calcium and phosphorus content of developing and permanent human teeth. Annals of Anatomy - Anatomischer Anzeiger 2007; 189: 183-190.

Arora M, Hare D, Austin C, Smith DR, Doble P. Spatial distribution of manganese in enamel and coronal dentine of human primary teeth. Science of The Total Environment 2011; 409: 1315-1319.

Arora M, Kennedy BJ, Elhlou S, Pearson NJ, Walker DM, Bayl P, et al. Spatial distribution of lead in human primary teeth as a biomarker of pre- and neonatal lead exposure. Science of The Total Environment 2006; 371: 55-62.

Arora M, Y. Chan SW, Kennedy BJ, Sharma A, Crisante D, Murray Walker D. Spatial distribution of lead in the roots of human primary teeth. Journal of Trace Elements in Medicine and Biology 2004; 18: 135-139.

Balter V, Telouk P, Reynard B, Braga J, Thackeray F, Albarède F. Analysis of coupled Sr/Ca and 87Sr/86Sr variations in enamel using laser-ablation tandem quadrupole-multicollector ICPMS. Geochimica et Cosmochimica Acta 2008; 72: 3980-3990.

Barbosa F, Jr., Tanus-Santos JE, Gerlach RF, Parsons PJ. A critical review of biomarkers used for monitoring human exposure to lead: advantages, limitations, and future needs. Environ Health Perspect 2005; 113: 1669-74.

Bazin D, Carpentier X, Brocheriou I, Dorfmuller P, Aubert S, Chappard C, et al. Revisiting the localisation of Zn2+ cations sorbed on pathological apatite calcifications made through X-ray absorption spectroscopy. Biochimie 2009; 91: 1294-1300.

Boanini E, Gazzano M, Bigi A. Ionic substitutions in calcium phosphates synthesized at low temperature. Acta Biomaterialia 2010; 6: 1882-1894.

Cazalbou S, Combes C, Eichert D, Rey C. Adaptative physico-chemistry of bio-related calcium phosphates. Journal of Materials Chemistry 2004a; 14: 2148-2153.

Cazalbou S, Combes C, Eichert D, Rey C, Glimcher MJ. Poorly crystalline apatites: evolution and maturation in vitro and in vivo. Journal of Bone and Mineral Metabolism 2004b; 22: 310-317.

Cazalbou S, Eichert D, Ranz X, Drouet C, Combes C, Harmand MF, et al. Ion exchanges in apatites for biomedical application. Journal of Materials Science: Materials in Medicine 2005; 16: 405-409.

Delmdahl R, von Oldershausen G. Quantitative solid sample analysis by ArF excimer laser ablation. Journal of Molecular Structure 2005; 744-747: 255-258.

Dean MC. The nature and periodicity of incremental lines in primate dentine and their relationship to periradicular bands in OH16 (Homo habilis). In: Moggi-Cecchi, J. editor. Aspects of Dental Biology: Palaeontology, Anthropology and Evolution. International Institute for the Study of Man, Florence; 1995. p. 239-265.

Farmer JG, MacKenzie AB, Moody GH. Human teeth as historical biomonitors of environmental and dietary lead: some lessons from isotopic studies of 19th and 20th century archival material. Environmental Geochemistry and Health 2006; 28: 421-430.

Grobler SR, Theunissen FS, Kotze TJvW. The relation between lead concentrations in human dental tissues and in blood. Archives of Oral Biology 2000; 45: 607-609.

Guillong M, Horn I, Gunther D. A comparison of 266 nm, 213 nm and 193 nm produced from a single solid state Nd:YAG laser for laser ablation ICP-MS. Journal of Analytical Atomic Spectrometry 2003; 18: 1224-1230.

Gulson B, Wilson D. History of lead exposure in children revealed from isotopic analyses of teeth. Arch Environ Health 1994; 49: 279-83.

Gulson BL. Tooth analyses of sources and intensity of lead exposure in children. Environ Health Perspect 1996; 104: 306-12.

Hare D, Austin C, Doble P, Arora M. Elemental bio-imaging of trace elements in teeth using laser ablation-inductively coupled plasma-mass spectrometry. Journal of Dentistry 2011; 39: 397-403.

Hu H. Bone lead as a new biologic marker of lead dose: recent findings and implications for public health. Environ Health Perspect 1998; 106.

Humphrey LT, Dean MC, Jeffries TE, Penn M. Unlocking evidence of early diet from tooth enamel. Proceedings of the National Academy of Sciences 2008; 105: 6834-6839.

Jackson SE. Calibration strategies for elemental analysis by LA-ICP-MS. Mineral. Assoc. Can. Short Course Series 40: Laser Ablation ICP-MS in the Earth Sciences 2008: 169-188.

Kang D, Amarasiriwardena D, Goodman A. Application of laser ablation inductively coupled plasma-mass spectrometry (LA-ICP-MS) to investigate trace metal spatial distributions in human tooth enamel and dentine growth layers and pulp. Analytical and Bioanalytical Chemistry 2004; 378: 1608-1615.

Keinan D, Smith P, Zilberman U. Microstructure and chemical composition of primary teeth in children with Down syndrome and cerebral palsy. Archives of Oral Biology 2006; 51: 836-843.

Kosler J. Laser ablation sampling strategies for concentration and isotope ratio analyses by ICP-MS. Mineral. Assoc. Can. Short Course Series 40: Laser Ablation ICP-MS in the Earth Sciences 2008: 79-92.

Linde A. Structure and calcification of dentin. In: Bonucci E, editor. Calcification in biological systems. Boca Raton: CRC Press: 1992. p. 269-311.

McNaught A, Wilkinson A. IUPAC. Compendium of Chemical Terminology (the "Gold Book"). Oxford: Blackwell Scientific Publications, 1997.

Nanci A. Ten Cate's Oral Histology: Development, Structure and Function: Elsevier, 2008.

Ohmori I. Biochemical studies on deciduous tooth substances. Part 1 Application of silver nitrate. Bull Tokyo Med Dent Univ 1961; 8: 83-95.

Pasteris JD, Wopenka B, Freeman JJ, Rogers K, Valsami-Jones E, van der Houwen JAM, et al. Lack of OH in nanocrystalline apatite as a function of degree of atomic order: implications for bone and biomaterials. Biomaterials 2004; 25: 229-238.

Pearce N, WT Perkins W, Westgate J, Gorton M, ackson S, Neal C, et al. A compilation of new and published major and trace element data for NIST SRM 610 and NIST SRM 612 glass reference materials. Geostandards Newsletter 1997; 21: 115-144.

Rabinowitz MB, Bellinger D, Leviton A, Wang JD. Lead levels among various deciduous tooth types. Bulletin of Environmental Contamination and Toxicology 1991; 47: 602-608.

Rabinowitz MB, Leviton A, Bellinger D. Relationships between serial blood lead levels and exfoliated tooth dentin lead levels: Models of tooth lead kinetics. Calcified Tissue International 1993; 53: 338-341.

Richter H, Kierdorf U, Richards A, Melcher F, Kierdorf H. Fluoride concentration in dentine as a biomarker of fluoride intake in European roe deer (Capreolus capreolus) - An electron-microprobe study. Archives of Oral Biology 2011; In Press, Corrected Proof.

Robbins N, Zhang Z-F, Sun J, Ketterer ME, Lalumandier JA, Shulze RA. Childhood lead exposure and uptake in teeth in the Cleveland area during the era of leaded gasoline. Science of The Total Environment 2010; 408: 4118-4127.

Schilke R, Lisson JA, Bauß O, Geurtsen W. Comparison of the number and diameter of dentinal tubules in human and bovine dentine by scanning electron microscopic investigation. Archives of Oral Biology 2000; 45: 355-361.

Thuy TT, Nakagaki H, Thanh Ha NT, Morita I, Tatematsu M, Anh Lan H, et al. Fluoride profiles in premolars after different durations of water fluoridation in Ho Chi Minh City, Vietnam. Archives of Oral Biology 2003; 48: 369-376.

Table 1

Deciduous teeth selected for analysis

Child ID	Tooth ID		Age of Child (years)
	Tooth 1	Tooth 2	
HT1	55	54	6
HT2	54	64	7
HT3	55	65	7
HT5	65	54	8
HT6	*(65)*	*(74)*	8
HT8	*(84)*	85	7
HT9	55*	65*	6
HT11	85	64	6
HT12	85*	84	8
HT13	75*	84	8
HT14	74	84*	7

(N.B. Samples marked with an asterisk did not expose the pulp cavity in longitudinal section.)

Table 2

Laser and ICP-MS specifications and operating conditions

Laser model	Geolas ArF eximer
Laser wavelength	193nm
Laser energy	10J/cm^2
Laser pulse rate	5Hz
Pulses per ablation analysis	200
Beam diameter	100µm
ICP-MS model	Agilent 7500c quadrupole MS
Scanning mode	Peak jumping
Background acquisition time	~20secs
Signal Acquisition time	~40secs
Dwell times	20ms ^{208}Pb
	10ms ^{24}Mg, ^{44}Ca, ^{66}Zn, ^{88}Sr

Table

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Table 3

Data summary showing the minimum and maximum values for lead, zinc, strontium and magnesium in circumpulpal primary dentine

Tooth ID	Pb ppm		Zn ppm		Sr ppm		Mg ppm	
	min	max	min	max	min	max	min	max
HT 1-54 mean	0.06	1.79(3.00)	54	303(373)	40	75	1851	6855
HT 1-55 mean	0.06	1.67(2.21)	51	292(344)	40	79	7249	11185
HT 2-54	0.07	1.63	67	379	57	89	7338	10937
HT 2-64	0.08	3.35	55	377	52	83	7633	12024
HT 3-55	0.63	10.83	102	328	105	157	6679	11030
HT 3-65	0.59	15.20	108	413	106	147	6962	11440
HT 5-54	0.07	0.64	57	182	40	50	6840	10238
HT 5-65	0.13	0.48	54	140	38	49	7515	10168
HT 8-85	0.16	5.81	59	372	36	55	7007	11056
HT 9-55*	0.06	0.23	63	89	34	41	7114	9230
HT 9-65*	0.04	0.87	56	187	35	47	3065	6021
HT 11-64 mean	0.06	1.97(3.37)	65	333(481)	49	66	6249	10096
HT 11-85 mean	0.05	1.69(3.36)	57	290(404)	42	54	1156	8372
HT 12-84	0.06	1.50	50	425	44	193	1772	4372
HT 12-85*	0.08	0.27	63	100	38	50	7589	8696
HT 13-75*	0.10	1.10	71	143	118	156	8775	9470
HT 13-84	0.13	1.24(5.79)	75	139(314)	119	156	7597	10876
HT 14-74	0.14	0.95	79	276	47	60	7484	9554

Mean values are for multiple laser transects on the same sample

*Refers to sections that did not cut the pulp cavity

Values in brackets are for secondary dentine

Table captions

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(N.B. Samples marked with an asterisk did not expose the pulp cavity in longitudinal section.)

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Figure 1a

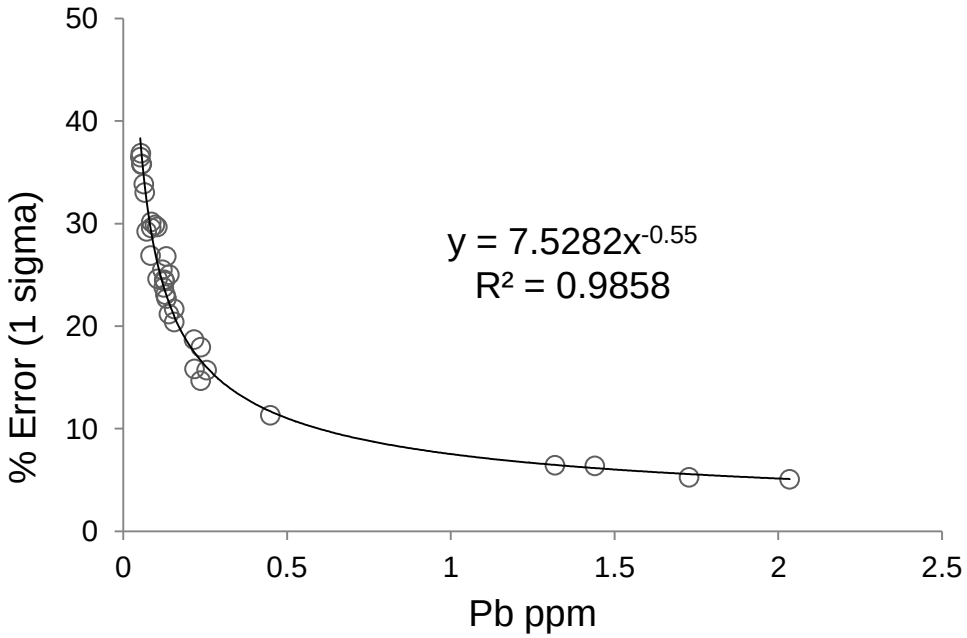

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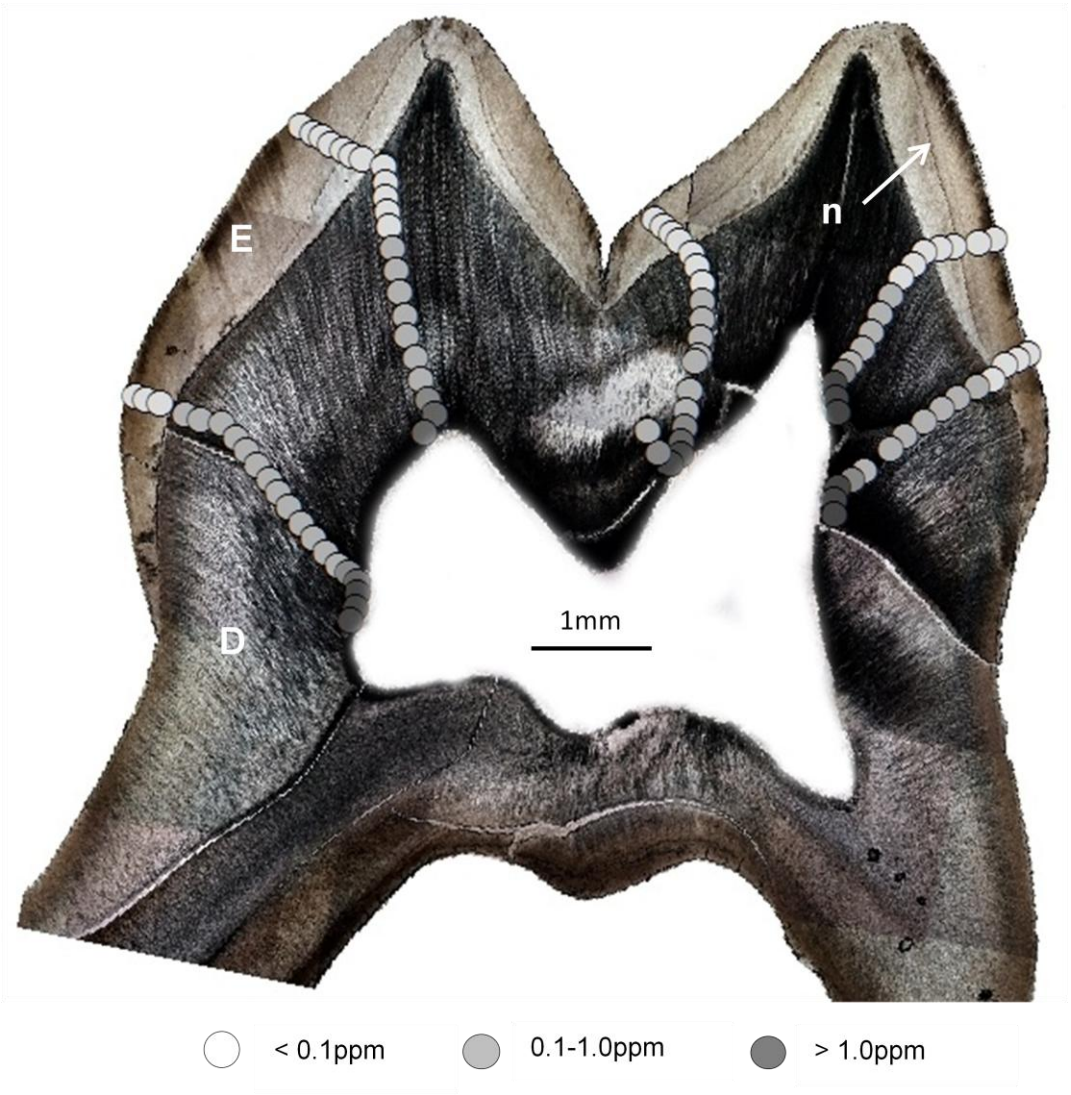
Figure 1b



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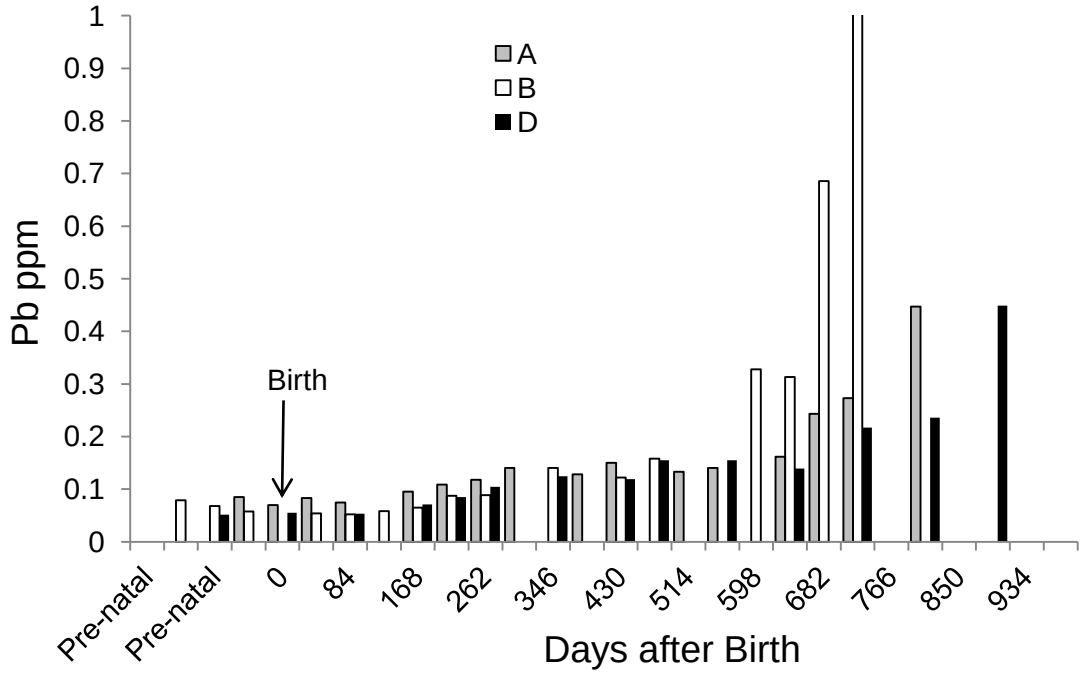

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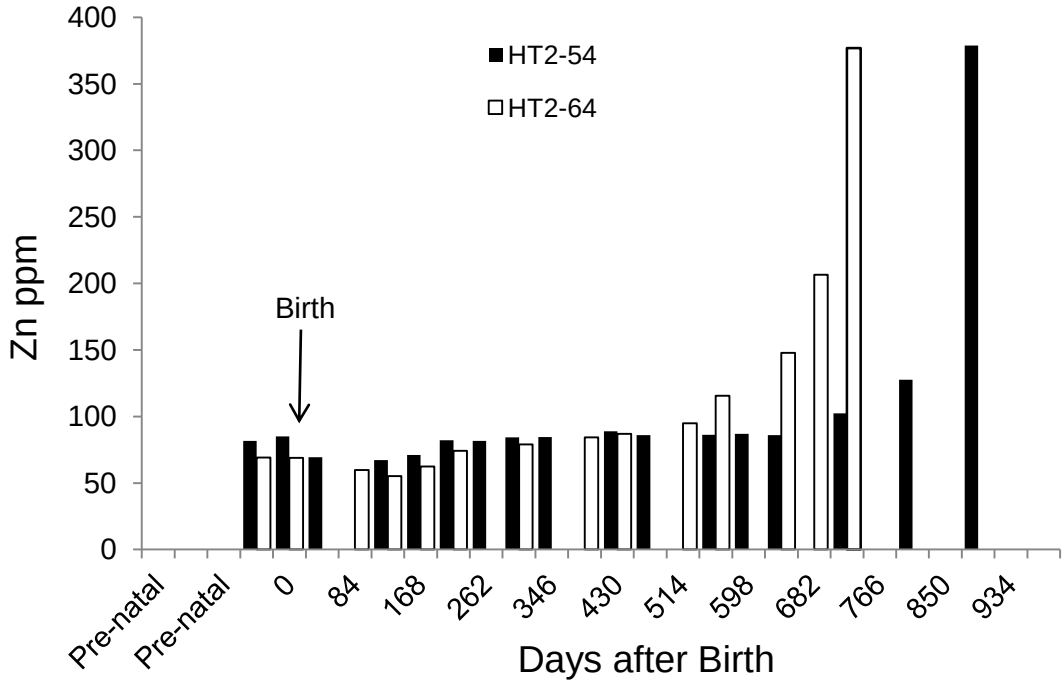

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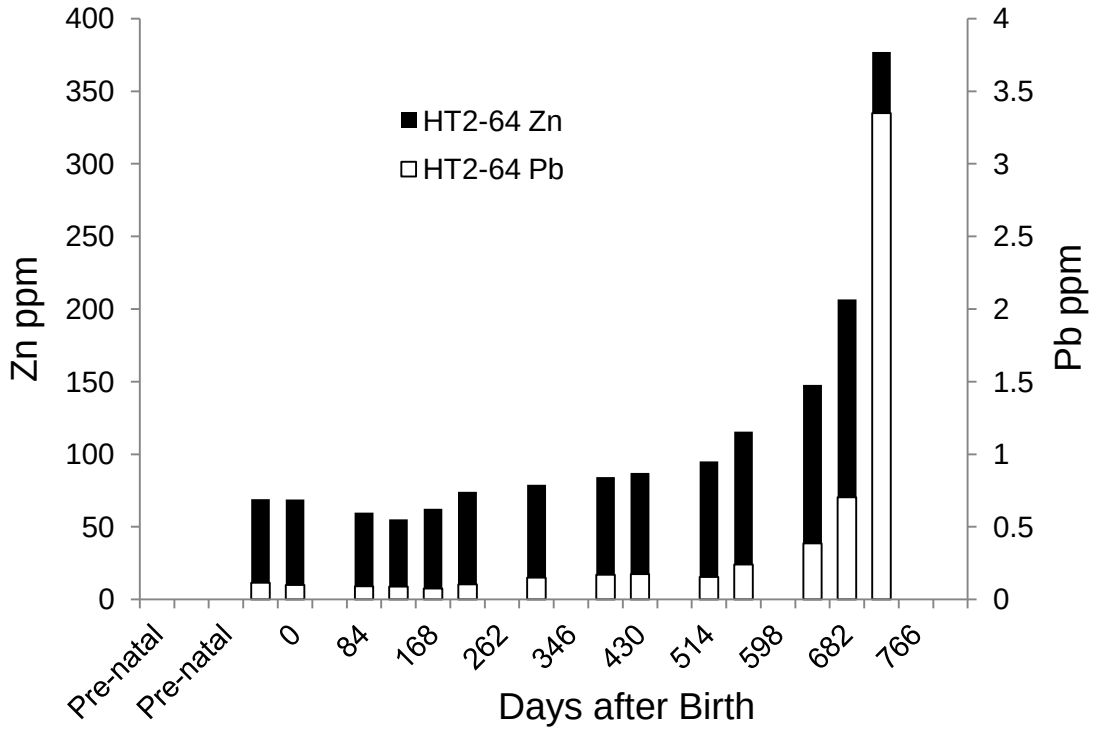

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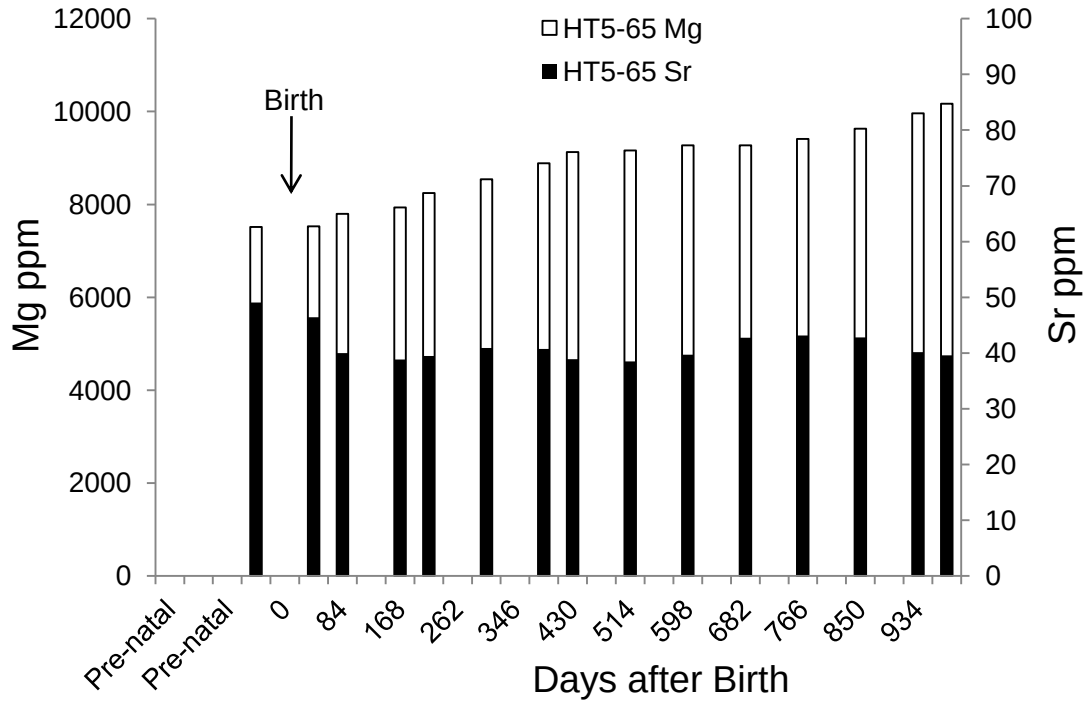

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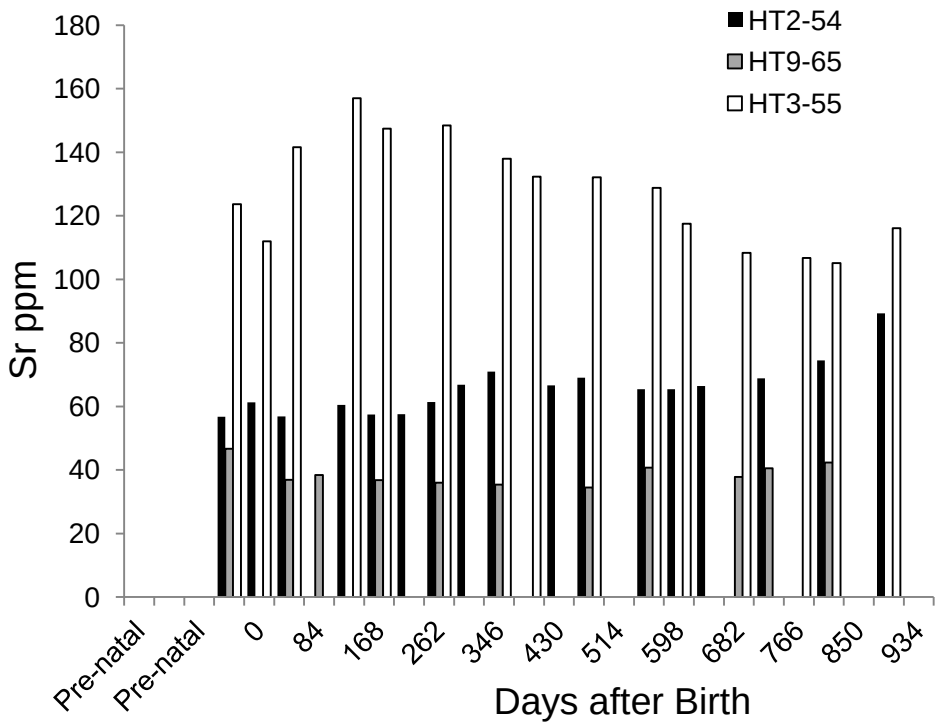

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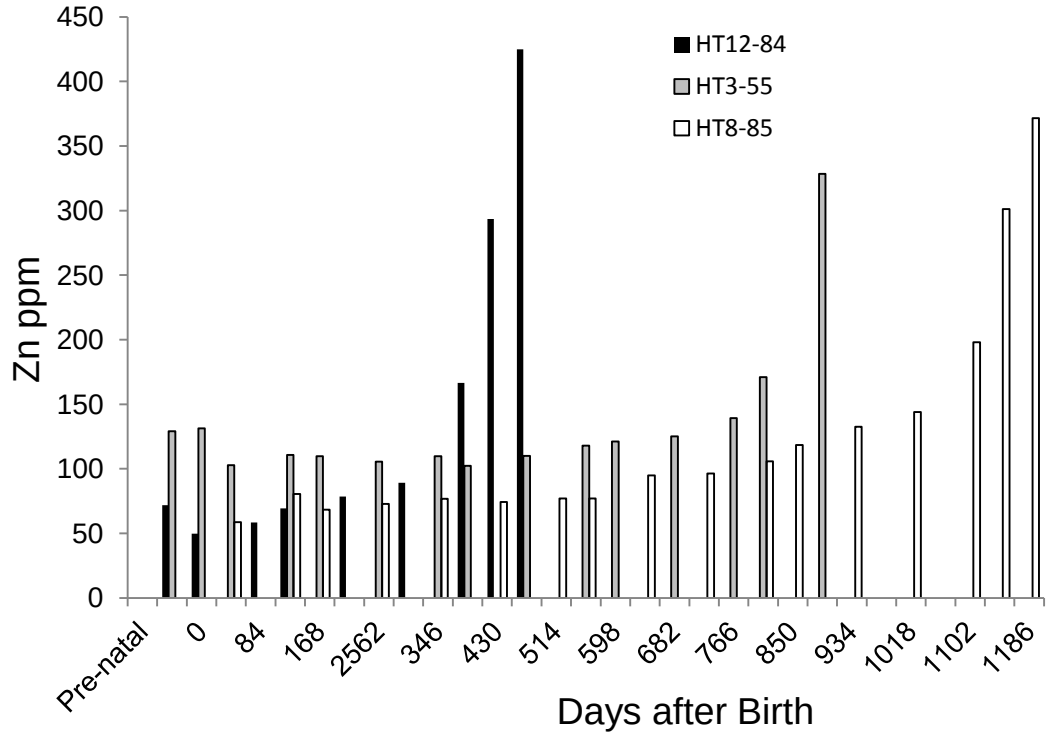
Figure 4



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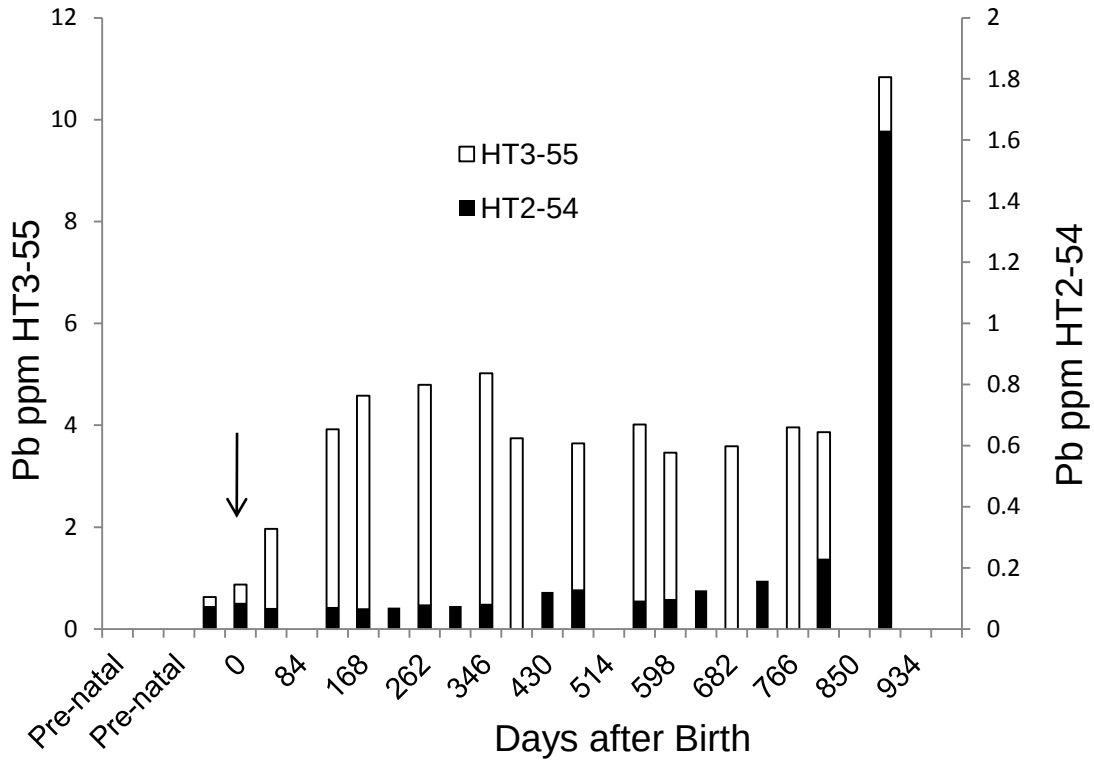

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Figure 6

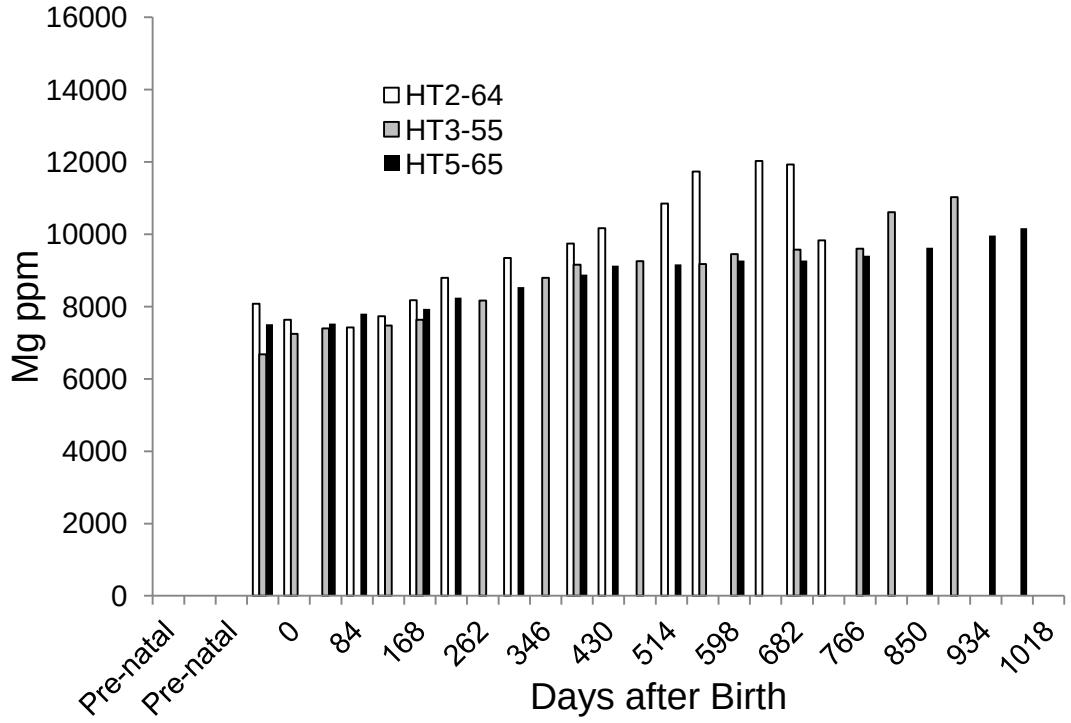

Figure captions

Figure 1a

Graph summarising the estimated % within-run errors (1 sigma) on lead measurements in dentine from 0.06ppm to 2.0ppm Pb. The regression equation was based on data for HT11-85 but is also valid for higher lead concentrations.

Figure 1b

Illustration to show the distribution of multiple laser ablation transects in the crown of tooth HT1-55. Each transect begins at the enamel surface, crosses the enamel-dentine junction and follows the line of the dentine tubules inwards to the pulp cavity. On reaching the pulp cavity each transect was extended laterally along the edge of the dentine to replicate the youngest layers of dentine. The symbols used for the ablation point have been enlarged to emphasise the line of the transects. Greyscale shading for individual ablation points generalises the overall lead concentration (ppm).

Key: D-dentine; E-enamel; n-neonatal line in enamel (neonatal line in dentine not shown).

Figure 1c

Transects A, B and D for tooth HT11-85 showing the close agreement between individual time-adjusted lead concentration profiles in dentine.

Figure 2a

Graph illustrating the excellent agreement for Zn concentrations in dentine for paired molars (child HT2). The time adjusted concentration profiles are very similar and independent of the age of final dentine secretion. Along the laser ablation transect for HT2-54, the final dentine layer was secreted approximately 160 days after the corresponding layer in HT2-64.

Figure 2b

Graph showing the sharper increase in Pb concentration relative to Zn on approaching the pulp cavity for ablations along the same transect. All data are time adjusted.

Figure 2c

Graph demonstrating the contrasting behaviour of Mg and Sr during the secretion of dentine over a period of 970 days after birth for child HT5 (see text for details).

Figure 3

Contrasting time adjusted Sr concentration profiles for three individual children illustrating the potential of laser ablation analysis to assess differences and changes in nutrition and diet over the first few years of early childhood.

Figure 4

Time adjusted Zn concentration profiles for 3 individual children showing the distinctive rise in concentration on approaching the pulp cavity. Note that the mean and maximum values in dentine differ very little between children, presumably in response to strong homeostatic control.

Figure 5

Graph showing highly contrasting levels of total lead in dentine and corresponding time adjusted concentration profiles. The profile for HT3-55 is closely replicated in deciduous molar HT3-65 (not shown). Child HT3 also shows anomalously high levels of Sr compared to other children (see Figure 2c).

Figure 6

Graph illustrating the monotonous rise in Mg concentration from birth to the cessation of primary dentine secretion.