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Tracing fetal and childhood exposure to lead using isotope analysis of deciduous teeth

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ABSTRACT

We report progress in using the isotopic composition and concentration of Pb in the dentine and enamel of deciduous teeth to provide a high resolution time frame of exposure to Pb during fetal development and early childhood. Isotope measurements (total Pb and $^{208}Pb/^{206}Pb$, $^{207}Pb/^{206}Pb$ ratios) were acquired by laser ablation inductively coupled mass spectrometry at contiguous 100 micron intervals across thin sections of the teeth; from the outer enamel surface to the pulp cavity. Teeth samples ($n=10$) were selected from two cohorts of children, aged 5–8 years, living in NE England. By integrating the isotope data with histological analysis of the teeth, using the daily incremental lines in dentine, we were able to assign true estimated ages to each ablation point (first 2–3 years for molars, first 1–2 years for incisors+pre-natal growth). Significant differences were observed in the isotope composition and concentration of Pb between children, reflecting differences in the timing and sources of exposure during early childhood. Those born in 2000, after the withdrawal of leaded petrol in 1999, have the lowest dentine Pb levels (<0.2 μg Pb/g) with $^{208}Pb/^{206}Pb$ (mean $\pm 2\sigma$: 2.126–2.079) $^{208}Pb/^{206}Pb$ (mean $\pm 2\sigma$: 0.879–0.856) ratios that correlate very closely with modern day Western European industrial aerosols (PM_{10}, $PM_{2.5}$) suggesting that diffuse airborne pollution was probably the primary source and exposure pathway. Legacy lead, if present, is insignificant. For those born in 1997, dentine lead levels are typically higher (>0.4 μgPb/g) with $^{208}Pb/^{206}Pb$ (mean $\pm 2\sigma$: 2.145–2.117) $^{208}Pb/^{206}Pb$ (mean $\pm 2\sigma$: 0.898–0.882) ratios that can be modelled as a binary mix between industrial aerosols and leaded petrol emissions. Short duration, high intensity exposure events (1–2 months) were readily identified, together with evidence that dentine provides a good proxy for childhood changes in the isotope composition of blood Pb. Our pilot study confirms that laser ablation Pb isotope analysis of deciduous teeth, when carried out in conjunction with histological analysis, permits a reconstruction of the timing, duration and source of exposure to Pb during early childhood. With further development, this approach has the potential to study larger cohorts and appraise environments where the levels of exposure to Pb are much higher.

1. Introduction

This paper describes our progress in reconstructing detailed chronologies of pre- and post-natal childhood exposure to Pb using the stable Pb isotope composition of dentine and enamel in deciduous teeth. The primary aim was to measure age-related changes of the biomarker that shed light on sources of exo- and endogenous Pb from '*in utero*' to several years after birth.

Subsequent to the publications of Gulson and Wilson (1994), Gulson (1996) and Farmer et al. (1994) documenting Pb exposure using the isotopic composition of Pb in deciduous teeth, comparatively few, more recent studies (Grobler et al., 2000; Gulson et al., 2004; Farmer et al., 2006; Robbins et al., 2010) have addressed the issue of variation in exposure source. With the exception of Grobler et al. (2000), these have used either large (mg) sub-samples of whole tooth (dentine+enamel) or transverse sections (mm slices) of dentine-free enamel. Enamel has tended to be the preferred tissue because it develops over a relatively short

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period of time and ceases to form once the tooth has erupted into the oral cavity. Neither of these types of sample is optimal for resolving fine, time-scale chemical variation accompanying pre- and post-natal tooth growth. Advances in instrumental analysis, most notably laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), now permit the acquisition of elemental and isotope data at high spatial resolution (less than 100 μm) without the need for sample digestion. Of those papers detailing the concentration of Pb in dental tissues by LA-ICP-MS, we refer to Arora et al. (2004, 2006, 2014), Dolphin et al. (2005), Hare et al. (2011), Humphrey et al. (2008a), Kang et al. (2004) and Shepherd et al. (2012). These papers raise the interesting question "If the micro-technology exists to measure the isotopic abundance of elements in very small samples, why are there so few publications relating to the isotope composition of Pb in children's teeth?" We argue that progress has been constrained by three main issues: lowered perceptions of health risk of Pb, analytical challenges and insufficient use of dental histology for chronological sampling.

With regard to the first issue, the phasing out of leaded petrol in Western Europe in the 1980–90's and abatement in the use of leaded paints and solders, environmental levels of Pb have fallen dramatically. Pb in air, for example, has decreased from 0.31 μg/m^3 prior to 1990 to 0.045 μg/m^3 in 2007 (Bierkens et al., 2011). Over the same period, blood Pb levels in European children have continued to decline. In Sweden for example, Strömberg et al. (2008) report a decrease from 5.8 μg/dL in 1978–1982, 3.4 μg/dL in 1989 to less than 1.5 μg/dL in 2005 for children living in an urban environment. This has led to a perception of lowering risk about the chronic health effects of exogenous Pb on children at low levels of exposure (Lanphear, 2007). Challenging this complacency, there is now a wealth of clinical evidence that documents the irreversible damage to cognitive development (Canfield et al., 2003; Lanphear et al., 2005; Chandramouli et al., 2009) and delayed neurodevelopment in infants (Jedrychowski et al., 2008) with blood Pb values significantly lower than 10 μg/dL. Of increasing concern is the prenatal exposure to Pb through the placental transfer of endogenous blood Pb from mother to child during pregnancy, which can result in poor birth outcomes (Hu and Hernandez-Avila, 2002; Xie et al., 2013). Using a combination of blood Pb concentrations and Pb isotope ratios Manton et al. (2003) and Gulson et al. (2015) have demonstrated very convincingly the importance of maternal bone restructuring during pregnancy and lactation on the release of Pb from skeletal reservoirs and its transfer to the infant. Thus the intensity, timing and duration of low level exposure to Pb, especially during the first few years of life, are factors as important now as they were before the introduction of the major public health interventions.

The second issue concerns the analytical sensitivity and precision needed to identify individual sources of environmental Pb. Meaningful application of stable Pb isotopes to exposure studies depends on there being measurable differences in the isotopic composition of a limited number of anthropogenic and/or geogenic sources (Gulson et al., 2004). In Western Europe the markedly different isotopic composition of leaded petrol compared to other anthropogenic sources made it relatively easy to calculate its contribution to total body burdens (Campbell and Delves, 1989; Delves and Campbell, 1993). However, excluding base metal mining/smelter environments, the current situation in a post-leaded petrol era is very different. Sources are often little above elevated background concentrations and broadly similar in isotopic composition (Ayrault et al., 2012). If teeth are to be routinely used as reliable biomarkers of low level exposure, there is a need for better isotope discrimination.

The third issue relates to the temporal relationship between point analyses as performed by laser ablation micro-sampling and the development history of the tooth. As demonstrated by Humphrey et al. (2008b) and Shepherd et al. (2012), to extract temporal information on trace elements in dental tissue it is essential to assign an estimated age to the point of analysis as well as having an understanding of the processes controlling their incorporation into the tooth matrix.

Our study sought to assess these issues by acquiring Pb concentration and Pb isotope data for dentine and enamel in deciduous teeth for which we could apply histological control on the timing of tissue growth. In doing so we acknowledge the constraints on interpretation imposed by the small number of samples analysed and the analytical limitations of LA-ICP-MS.

2. Material and methods

2.1. Materials

We analysed 6 deciduous incisors and 4 deciduous molars donated by children living in NE England for which ethical approval had previously been granted. On completion of the original projects, the teeth were entered into the Newcastle University Faculty of Medical Sciences Biobank and anonymised according to standard ethical procedures. The Biobank records include the name of the person who collected the tissues but do not permit identification of the donor. We therefore do not have postcode data for the sample but do know the general area in which the children who donated the teeth lived at the time of collection.

The incisors were a sub-sample of a larger collection of naturally exfoliated deciduous teeth acquired during the 2005 Newcastle 'Tooth Fairy' Study; a joint project between the University of Newcastle, Public Health England and Newcastle City Council with the aim of examining the relationship between parental socio-economic status, place of residence and Pb in children's teeth as a measure of environmental Pb exposure (Hodgson et al., 2015; County Durham and Darlington Local Research Ethics Committee: Ref. no. 05/00904/10). The cohort comprised 69 children, aged 5–8 years, living in the city and inner urban areas of Newcastle upon Tyne. Though little evidence now remains of the city's once industrial past, its environs carry a legacy of environmental heavy metal pollution. By contrast, the molars had been surgically extracted and were a sub-sample of 15 children, aged 6–8 years, attending a dental clinic in Billingham, Teesside, in 2009 for treatment (Shepherd et al., 2012; County Durham & Tees Valley Research Ethics Committee: Ref. no. 09/H0905/42). Though we lack residential postcode data for this cohort, they are inferred to reside primarily in the immediate urban and rural areas. Prior to the present study the teeth from both cohorts had been analysed by LA-ICP-MS and provisional data were acquired for the concentration of Pb in enamel and dentine (Shepherd et al., 2012; Hodgson et al., 2015). Comparison with published data indicated that the mean dentine Pb for the Newcastle cohort (0.26 ± 0.16 μg/g; $n=69$) and Billingham cohort (0.18 ± 0.07 μg/g; $n=15$) were significantly lower than the mean value of 2.23 ± 1.32 μg/g for deciduous teeth of children living in non-polluted areas of South Africa (Grobler et al., 2000) and the overall means reported for primary school children in Taipei and Boston of 4.4 ± 3.5 μg/g and 3.3 ± 2.5 μg/g respectively (Rabinowitz et al., 1991). From these comparisons we concluded that our cohorts are representative of low exposure populations. Samples were selected from the upper quartile of teeth having the higher dentine Pb levels. For the incisors this corresponded to a range 0.18–0.95 μg Pb/g ($n=6$); for the molars 0.05–0.22 μg Pb/g ($n=4$), excluding a maximum outlier of 0.69 μg Pb/g. Time frames of childhood exposure also differed (Newcastle 1997–2005; Billingham 2001–2009) (see Table 1). This disparity, though precluding absolute time comparisons, still permits between-cohort comparisons and a critical assessment of the