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Tooth Rings: Dental Enamel as a Chronological Biomonitor of Elemental Absorption from Pregnancy to Adolescence

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ABSTRACT

Because the dental hard tissues commence development in the 13th week *in utero* and once formed are essentially inert, they may provide unique windows onto environmental and physiological changes during pregnancy and early life. We review the general potential of teeth as biomonitors and then the development of laser ablation-inductively coupled-plasma mass spectrometry (LA-ICP-MS) to assess elemental absorption over temporal periods from a few days to years.

In studies of exfoliated deciduous incisors of infants from Solis, Mexico (n= 38), we found dramatic increases in lead and zinc from prenatal to postnatal enamel as well as strong correlations (~ 0.5 to 0.9) between prenatal and postnatal enamel intensities of strontium barium, zinc, and lead. Prenatal enamel zinc is negatively correlated with dietary factors inhibiting absorption such as mothers' maize, phytate, and calcium intake during pregnancy. Prenatal enamel lead intensities, possibly from lead-glazed pottery, are inversely correlated with height ($r = -0.53$) and weight ($r = -0.48$) at ~ 5 years. The combination of micro-spatial chemical methods such as LA-ICP-MS with the kymographic development of teeth illustrates the potential of enamel to monitor metal absorption and provide insights into the source, timing, and epidemiological consequences of early life pollution and nutrition.

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INTRODUCTION

> Teeth are storehouses of invaluable information for biological, physiological and medical sciences. ... Routinely analyzed and monitored, teeth can indicate exposure to pollutants and provide a permanent ... record. (Sharon, 1988:124)

In the following paper we report on the use of laser ablation-inductively coupled-plasma mass spectrometry (LA-ICP-MS) to monitor changes in dental enamel elemental intensities over temporal periods from a few days to a few years. Few methods are available to developmental toxicologists, nutritionists, and epidemiologists for assessing changes in the absorption of heavy metals and nutritionally significant elements during early life and especially during pregnancy. Blood elemental concentrations provide a single snapshot of exposure, whereas hair and nails may provide a measure of absorption or excretion averaged over a longer sampling frame (Sharon, 1988). With the exception of the dental hard tissues, all other tissues remodel after initial formation, thus their use as monitors of absorption during specific periods is problematic.

The dental hard tissues, cementum, dentine, and especially enamel, sequentially develop from *in utero* to adolescence, and in the case of dentine and cementum are nearly inert, while enamel is completely inert after calcification (Carlson, 1990). Thus, these tissues may provide permanent source of information on a variety of conditions during early life. Furthermore, teeth offer the possibility of retrospectively gaining information on prior elemental absorption of a cohort of individuals, thus allowing for a "prospective" analysis of the long-term consequences of early life conditions (Sharon, 1988).

The purposes of this paper are to: (1) illustrate the sequential development of enamel and its potential as a chronological biomonitor of environmental conditions and physiological processes; (2) present an overview of the recent development of laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) and its application to dental hard tissues; and (3) present preliminary results from a study of elemental concentrations in prenatal and postnatal enamel of children's teeth from Mexico, specifically maternal diets and prenatal lead and the relationship between prenatal enamel zinc intensities and subsequent growth in height and weight at ~57 months.

THE COUPLING OF TEETH WITH LASER ABLATION-ICP-MS

Teeth as Biomonitors

Teeth are composed of three hard tissues (Figure 1). Cementum consists of a ~20 to 200 μm thick, bone-like covering of the roots of teeth, allowing for the attachment of teeth into their bony sockets. Although difficult to view because of its fragility and thinness, human cementum appears to grow in annual layers (Kagerer and Grupe, 2001). The annual periodicity of cementum in the walrus is better established and Outridge and co-workers (1995) have been tracking walrus migrations and pollution exposure by analysis of yearly cementum layers with LA-ICP-MS.

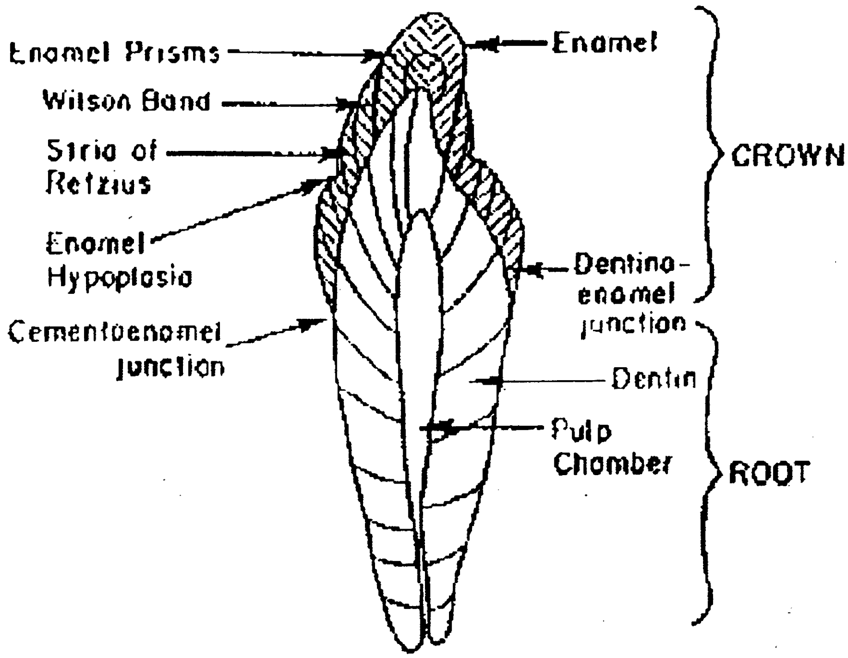

Figure 1. Longitudinal cross-section of a tooth showing tissue locations and the pattern of growth in enamel (stria of Retzius). A Wilson band is an accentuated stria of Retzius that marks the amount of enamel formed at the time of a physiological perturbation.

Dentine forms the bulk of the tooth. The majority of the dentine grows in a regular and ring like fashion starting at the tip of the crown and some dentine is added to specific areas of the tooth after eruption (Carlson, 1990). In the seminal work of Needleman and colleagues (see Needleman and Bellinger, 1991), dentine lead from deciduous teeth was used to link early lead exposure to subsequent behavioral deficiencies.

Enamel, the whitish covering of the tooth crown, is nearly 97% calcified as hydroxyapatite crystal, making it the hardest tissue in the human body. Unlike bone or the other dental tissues, which are derived from mesenchyme, enamel is ectodermal in origin. Like dentine, enamel grows in waves, viewed as stria of Retzius, in a highly regulated ring-like fashion (Figure 1). Ameloblasts, the enamel forming cells, typically secrete human enamel in the form of prisms at a rate of about 4 to 6 µm per day, and in many cases constrictions along prisms indicate a daily rhythm. Once developed, the proteins that formed the structure of enamel are almost completely removed and enamel is subsequently inert. Even after death, enamel almost always maintains its structural and chemical integrity (Goodman and Rose, 1990).

Of the dental tissues, enamel is most used as a biomonitor for a number of reasons suggested above: enamel is well preserved and its surface can be directly observed, it is resistant to changes in life and after death, and it forms in a regular and ring-like fashion. At the macroscopic level, enamel has frequently been used as a recorder of general conditions during its formation. For example, linear enamel hypoplasia (LEH), a ring-like area of decreased enamel thinckness is a nonspecific marker of physiological disruption (or stress), strong enough to temporarily disrupt enamel apposition (Goodman and Rose, 1990). In studies of past populations, they have been used to determine the variation in peak age of onset of early life stress, and in studies of contemporary populations the pattern of LEH has been used to confirm degrees of malnutrition (Goodman and Rose, 1990; Goodman et al., 1992).

At the histological level, one can observe finer changes in the developmental disruption of enamel as "Wilson bands", or accentuated stria of Retzius (Figure 1). Wilson bands are histological manifestations of the same processes that led to a LEH; however, they may often be observed where a LEH could not be.

The chemistry of enamel also has a long history of study. Early studies (~1960 to 1995) most often assayed whole tooth or enamel concentrations thought to be related to tooth calcification and caries resistance via the removal and digestion of whole teeth or a chunk of enamel (Curzon and Cutress, 1983). Driven by epidemiological interests, others have biopsied layers or areas of enamel (see Brundevold et al., 1975). These studies hinted at the potential of enamel to record changing patterns of elemental absorption.

Laser Ablation-Inductively Coupled Plasma-Mass Spectroscopy (LA-ICP-MS)

The analytical advantages of LA-ICP-MS result from the marriage of the high-sensitivity, simultaneous multielement technique with low detection limits, wide linear dynamic range, and high sample throughput capability of ICP-MS (Montaser and Golightly, 1992) with the fine spatial resolution (~10 μm) of laser ablation. In LA-ICP-MS, a high-energy pulsed laser beam is directed onto a longitudinally sliced tooth or other solid sample surface. The interaction of the laser beam energy with the solid sample produces a volitalized ablated sample that is transported with a stream of argon gas into the plasma region where predominately singly charged ions are produced and extracted by the mass spectrometer for detection. The high sensitivity, low detection limits, semiquantitative screening, isotopic analysis capabilities, depth profiling, and area scanning capabilities make LA-ICP-MS an attractive technique for analysis of sequentially calcifying tissues. In addition, because of minimal sample preparation, throughput is improved and contamination is minimized. The coupling of LA with ICP-MS merges the strengths of each instrument: the quantitative and multielement capabilities of ICP-MS with LA's surface sampling abilities.

Gray (1985) first reported on the development and application of ICP-MS. More recently, LA-ICP-MS has been used increasingly to study biological materials, including teeth. As noted above, Outridge et al.'s (1995) study of walrus cementum was the first to incorporate LA-ICP-MS. Cox et al. (1996) subsequently reported on LA-ICP-MS scans of Pb/Ca concentrations in contemporary and archaeological human tooth enamel. Budd et al. (1998) have further tested the utility of LA-ICP-MS for studies of lead in archeological and contemporary human enamel and also dentine. They found sharp rises in Pb/Ca in the surface enamel (outer 30 μm) of all teeth and stable, low lead concentrations in the central/core enamel region, followed by moderately elevated lead intensities in dentine.

In summary, the combination of laser ablation with ICP-MS provides an ideal technique for studying spatial variation in a wide range of elemental concentrations in teeth and with a knowledge of tooth development, one can translate areas of enamel to an individual's age at the formation. In the following, we provide a first report on a study of elemental concentrations in prenatal and postnatal enamel of mild-to-moderately malnourished children.

METHODS AND MATERIALS

From 1984 to 1986 a cohort study was completed in the Solis Valley of Highland Mexico to better understand the functional consequences of mild-to-moderate malnutrition (Allen et al., 1992). Micronutrient malnutrition was common, in large part due to low dietary diversity, specifically low consumption of animal products, fruits, and vegetables and high consumption of dietary fiber/phytate via maize. Low zinc and iron bioavailability is particularly prevalent and related to growth (Allen, 1993; Murphy et al., 1992).

One cohort consisted of pregnant women, who were studied from the beginning of the second trimester of pregnancy through the birth of their infants, who were then studied with them to ~6 to 9 months post-natal. Since the calcification of deciduous enamel almost perfectly overlaps the period of study, we collected previously exfoliated teeth to evaluate the relationship between enamel histology and chemistry and factors that may relate to histological changes and variation in elemental absorption.

After longitudinally sectioning teeth for histological analysis, we ablated teeth using a CETAC LSX-100 laser ablation system attached to a Perkin Elmer Elan 6000a ICP-MS. A 75 μM ablation lines paralleled the neonatal line (Figure 2). The instrument was optimized by using National Institute for Standards and Technology (NIST) 612 glass reference standard. Using GLITTER software, we corrected for drift and background intensities and finally normalized intensities relative to ^{43}Ca. Expressed values are semi quantitative intensities of analyte counts relative to counts of ^{43}Ca. Statistical analyses were performed using SPSS 11.0.

We first present prenatal and postnatal concentrations for four isotopes that readily substitute for calcium in hydroxyapatite crystal: ^{138}Ba, ^{88}Sr, ^{208}Pb, and ^{66}Zn. Zinc is a key essential nutrient that is limited in the Solis diets, whereas strontium and barium are not essential and not bioregulated, thus they provide a more direct reflection of exposure.

RESULTS

Prenatal and Postnatal Enamel Isotopic Intensities

A wide variation in isotopic intensities was found among individuals with a strong positive deviance. The standard deviations are near to or higher than the mean (Table 1). Barium and strontium, nonessential divalent cations of the same alkaline

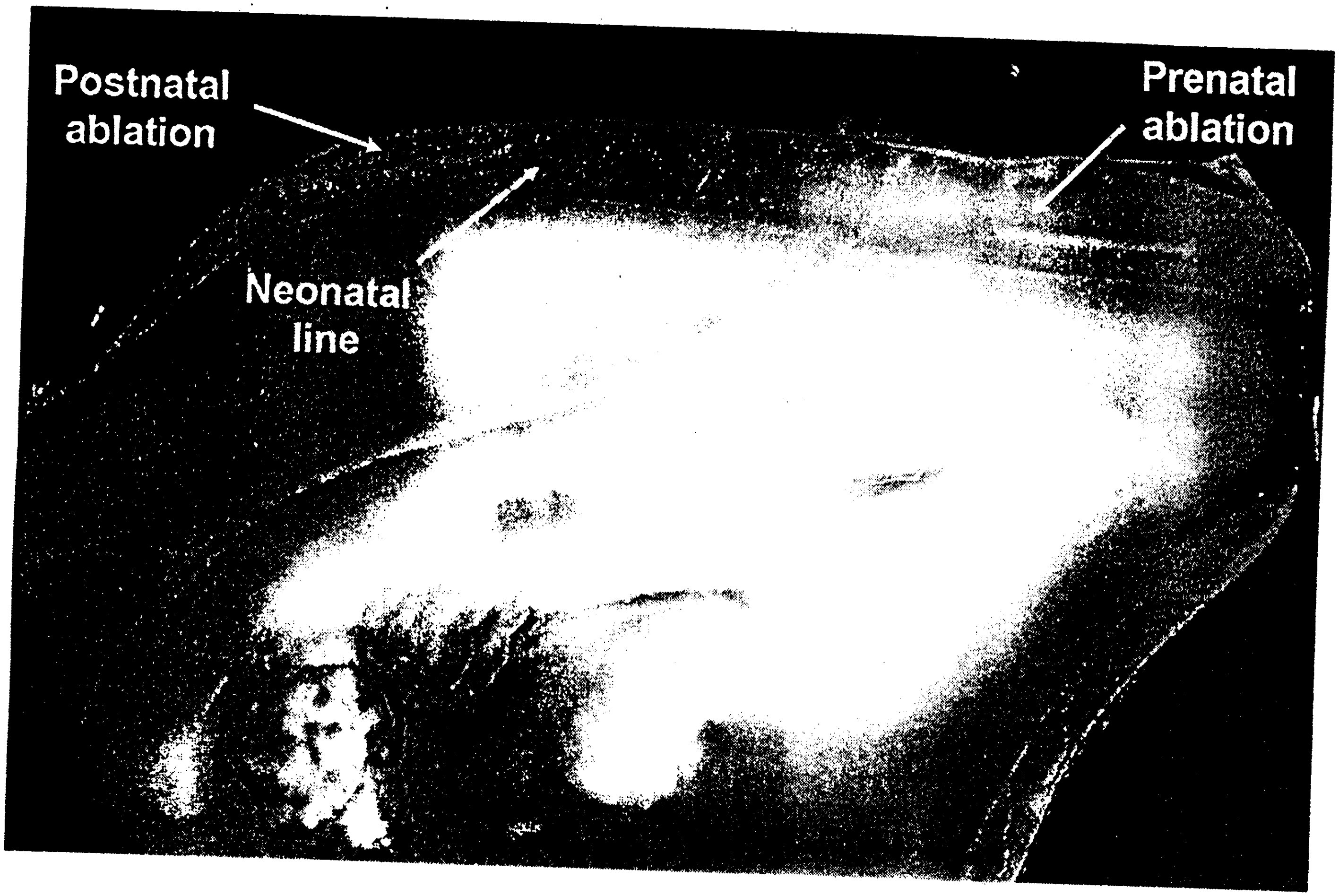

Figure 2. Longitudinal cross-section of a deciduous incisor tooth from Solis, Mexico, with labeled prenatal and postnatal laser ablation lines (~75 μm in diameter) and the neonatal line.

Table 1. **Descriptive statistics (mean and standard deviation) for prenatal (Pre) and postnatal (Pst) Barium (Ba), Zinc (Zn), Strontium (Sr) and Lead (Pb) Ca normalized intensities and correlation between pre- and postnatal intensities (N = 38).**

	Pre ^{138}Ba	Pst ^{138}Ba	Pre ^{88}Sr	Post ^{88}Sr	Pre ^{66}Zn	Pst ^{66}Zn	Pre ^{208}Pb	Post ^{208}Pb
Mean	10.73	17.70	144.36	141.73	5.10	17.55	3.30	19.86
S. D.	11.95	14.28	76.62	78.81	7.78	21.35	5.17	38.88
Pre-Post Difference (T-value)	4.22***		.373		4.95***		2.96**	
Pre-Post correlation	0.54**		.090**		.081**		0.62**	

** = p< .01

*** = p <.001

earth groups (ZA) as calcium, differ from each other in that strontium is more concentrated overall, as it is in the environment, while barium significantly increases from prenatal to postnatal concentrations, whereas strontium does not. The prenatal and postnatal normalized intensities of both elements are highly correlated within individuals, as is true for the other isotopes. Lead and zinc increase significantly from prenatal to postnatal enamel (Table 1).

In addition to the high pre- to postnatal of the same isotope within individuals, isotopes of different elements are highly correlated within individuals. For example, barium and strontium normalized intensities are intercorrelated of different elements (prenatal r = 0.62**, postnatal r = 0.42**).

Prenatal Zinc Intensities and Maternal Prenatal Diets

Previous work in the Solis Valley has shown that growth appears to be related to poor bioavalability of zinc, assumed to be related to high intakes of maize, mainly in the form of tortillas, which contains phytates that bind zinc (Allen et al., 1992). As expected, but heretofore never empirically demonstrated, prenatal enamel zinc is negatively associated with dietary components posited to negatively affect absorption and bioavalability for a subsample (n = 22; Table 2). Prenatal ^{66}zinc is negatively

Table 2. **Correlations between prenatal enamel 66 zinc normalized intensities and intake of calcium, tortillas and phytate during pregnancy (n =22 total, 11 high dairy, 11 low diary).**

	Enamel Zinc	**Calcium Intake**	**Tortilla Intake**	**Phytate Intake**
Enamel Zinc				
• Total	---	-0.32	-0.28	-0.28
• High Dairy	---	-0.17	-0.16	-0.22
• Low Dairy	---	-0.57	-0.50	-0.49
Calcium Intake				
• Total	-0.32	---	0.96**	0.92**
• High Dairy	-0.17	---	0.96**	0.91**
• Low Dairy	-0.57	---	0.98**	0.95**
Tortilla Intake				
• Total	-028	0.96**	---	0.93**
• High Dairy	-0.16	0.96**	---	0.91**
• Low Dairy	-0.50	0.98**	---	0.98**
Phytate Intake				
• Total	-0.28	0.92**	0.93**	---
• High Dairy	-0.22	0.91**	0.91**	---
• Low Dairy	-0.49	0.95**	0.98**	---

** $p < .01$

correlated with calcium ($r = -0.32$), tortilla ($r = -0.28$), and phytate ($r = -0.28$) intakes during pregnancy. We medially divided the total group into high and low dairy intake subgroups in order to demonstrate the potential affect of a better dietary quality and calcium source. In the high dairy group, the three dietary factors are less strongly correlated with enamel zinc, whereas in the low dairy group, with nearly all the calcium coming from alkali processed maize, the dietary components are nearly perfectly intercorrelated and their correlations with enamel zinc are more strongly negative.

Prenatal Lead and Growth

In addition to studies of the potential determination of elemental intensities, as presented above, enamel elemental intensities may be used as a measure of prior exposure/absorption to predict for subsequent development. At the time of collection of exfoliated teeth, we also assessed the growth of children at follow-up (~57 months of age), and here we relate prenatal enamel lead and zinc intensities to follow-up heights and weights. Prenatal lead intensities are inversely correlated with weight ($r = -0.48$; $p = .031$, $n = 20$) and height at around five years ($r = -0.53$; $p = 0.016$, $n = 20$).

A scatterplot further shows the inverse association between prenatal enamel lead intensities and height at ~57 months (Figure 3). Interestingly, individuals in the highest quartiles of prenatal enamel zinc also tend to have better growth status and lower enamel lead intensities. In multiple regression analyses with height-for-age as the dependent variable, prenatal enamel lead intensities explain more variation than any dietary variables, including measures of zinc status.

DISCUSSION

With exfoliated teeth of children, we related their mothers' diets during pregnancy and breastfeeding to elemental intensities measured by LA-ICP-MS to determine: (1) relative ^{43}Ca normalized elemental concentrations in prenatally and postnatally formed enamel, (2) the correlation among elements and isotopes, (3) a statistical association between prenatal enamel lead and zinc and diet and subsequent growth.

With the exception of strontium, all other elements increased from prenatal to postnatal enamel. A possible explanation might be surface enrichment; however, we avoided ablating near tooth enamel outer surfaces and checked for any unusual variation in scans that might suggest contamination or surface enrichment. More likely, the differences between prenatal and postnatal concentrations are reflective of variation in the way that elements make their way into fetal vs. newborn blood supplies.

Even with great increases from prenatal to postnatal enamel, all prenatal/postnatal elements intensities strongly correlated within individuals, suggesting continuity in environments and physiological processes.

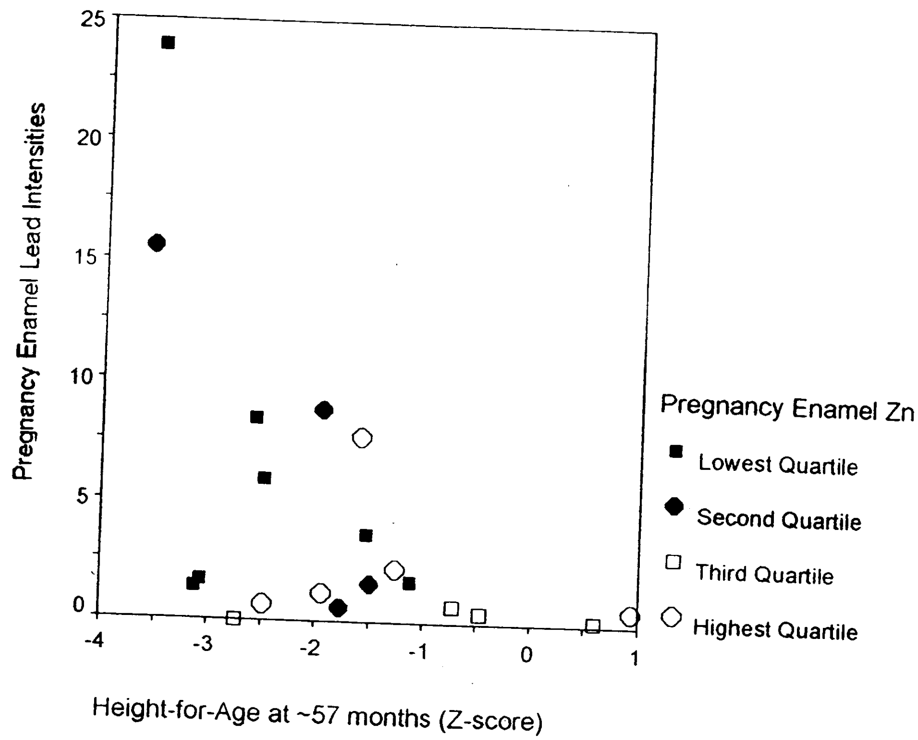

Figure 3. Scatterplot of 20 individual values for enamel lead intensities (relative to ^{43}Ca) during pregnancy (y axis) and height-for-age Z-scores at ~57 months (x axis). Individual values are coded by prenatal enamel zinc quartiles.

We have shown for the first time that enamel zinc intensities may be related to dietary components that affect zinc bioavailability: intake of phytate, tortillas, and calcium. The relationship between zinc and calcium provides a cautionary tale. Both zinc and calcium are key essential nutrients. However, excess intake of calcium inhibits zinc absorption (Murphy et al., 1992). Thus, low zinc status may be related as much to low zinc intake as it is to high calcium intake. The data presented above point to the inhibitory effects of calcium are least when calcium comes from dairy products rather than high phytate tortillas.

Lead pollution remains as one of the most significant challenges to public health in many industrialized areas of the U.S. and throughout less-developed countries. Salsa dishes, cooking pots, and other lead glazed ceramics are used throughout the Solis Valley and Tunstall and Amarasiriwardena (2002) have shown that lead isotope ratios of a few Solis teeth are a close match to the lead isotope ratios of these ceramics. This study has shown that higher prenatal enamel lead intensities are also associated with reduced growth in height and weight at around 5 years of age. The relationship is only suggestive because of small sample size and many intervening variables. Consistent with the cautionary note on zinc, a wide variety of other factors may explain or confound this relationship between prenatal lead and subsequent development. For example, more traditional families tend to use lead glazed ceramics and also tend to be poorer than less traditional families; their diets are less diverse and more likely to be nutritionally deficient. On the other hand, although calcium intake, particularly via alkali processed tortillas, might inhibit zinc intake, it may protect against lead absorption.

The above points emphasize the importance of tracking interactions among and between nutrients and pollutants. Interactions occur at the social level, at the level of food and nutrient intake, movement across the gut, and the absorption of cations into enamel apatite. Fortunately, LA-ICP-MS provides a time-specific assessment of multiple elements. Finally, we have demonstrated the ability to distinguish pre- and postnatal elemental concentrations.

In the quotation at the start of this paper, Sharon (1988) suggested that teeth are biomonitors and later on calls for the development of a "tooth tissue bank" for epidemiological and ecological monitoring. Fifteen years hence, the application of LA-ICP-MS is beginning to affirm his suggestion. In both an international and domestic context, teeth may be used to track the importance of pollutants and nutrients during different critical periods of life and to further explicate the links between environments, nutrition, and babies at risk due to social inequalities.

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