Variation in Elemental Intensities Among Teeth and Between Pre- and Postnatal Regions of Enamel

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ABSTRACT Microspatial analyses of the trace element composition of dental enamel are made possible using laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS). Fine spatial resolution, multielement capabilities, and minimal sample destruction make this technique particularly well-suited for documenting the distribution of elements in sequentially calcifying layers of enamel. Because deciduous enamel forms from week 13 in utero up to 9 months postnatally (thereafter essentially becoming inert), the application of LA-ICP-MS allows for the retrospective measurement of prenatal and early postnatal trace-element uptake during a critical period of child development. In this study, we compared intra- and intertooth intensities of ^{25}Mg, ^{57}Fe, ^{66}Zn, ^{68}Zn, ^{88}Sr, ^{138}Ba, and ^{208}Pb via LA-ICP-MS of 38 exfoliated deciduous incisors and canines donated by 36 participants in the Solís Valley Mexico Nutrition Collaborative Research Support Program (NCRSP). Pre- and postnatal comparisons within teeth showed significant increases ($P < 0.001$) and greater variation in the abundance of all isotopes in postnatal enamel, with the exception of a decrease in ^{25}Mg ($P < 0.001$) and constant values for ^{88}Sr ($P = 0.681$). Conversely, comparisons by tooth type and mouth quadrant revealed few significant differences between teeth of the same individual. We argue that more variation in the trace element composition of teeth occurs across developmental areas within a tooth than among different teeth of the same person. This study further demonstrates that sequentially calcifying areas of enamel have different chemical concentrations. The results support the use of microspatial analyses of enamel for understanding changes in nutrition, pollution, and residence. Am J Phys Anthropol 128:878–888, 2005.

Prenatal and infant nutritional status greatly influences the trajectory of one's growth and development (Chávez and Martínez, 1982; Allen, 1994; Martorell et al., 1995; Bogin, 2001), morbidity (Keusch, 2003; Scrimshaw, 2003), and overall adaptability throughout the lifespan (Cameron, 1996; Leidy, 1996). Several critical periods of development occur while a child is in utero and breastfeeding and is wholly dependent upon his or her mother for the building blocks of all cells, tissues, and organs (Ulijaszek, 1998). If nutritional requirements are not met during these critical periods, developmental processes may be redirected from their genetic trajectory or differentially cue various stages of development. Therefore, early changes in physiology, function, and metabolism provoked by even mild-to-moderate malnutrition can trigger problems in later life (Barker, 1995; Martorell et al., 1995; Cameron, 1996; O'Donnell, 2001; Cameron and Demerath, 2002).

Given that prenatal and early postnatal life sets the stage for biological and behavioral aspects of later life, it is essential that we refine methods for assessing how and why aspects of these developmental periods may differ if we are to understand biological and behavioral variation in present and past populations. When working with contemporary populations, such variation is observed via dietary recalls of mother and infant diets, documenting the intake of foods with determinable nutrient components, while analyses of soft bodily tissues and fluids (e.g., blood or urine) measure the potential availability of nutrients in the bloodstream or digestive tract. Anthropometric measures provide sensitive yet nonspecific insights into the functional consequences of nutritional uptake by the body, but how is the actual uptake of specific nutrients by the body assessed?

Direct measurements of dietary uptake by the body are limited to samples from which tissues can be readily excised for chemical analysis (e.g., from the skeletal remains of deceased individuals). Paleodietary reconstruction studies use the relative concentration of carbon and nitrogen in bones and teeth to determine the composition of ancient diets (White and Schwarcz, 1989; Schwarcz and Schoeninger, 1991). Determining dietary compositions allows researchers to reconstruct behaviors and/or sociopolitical processes occurring in the past. For example, several researchers investigated the timing of weaning (Katzenberg and Pfeiffer, 1995; Katzenberg et al., 1996; Wright and Schwarcz, 1998; Williams, 2000), social stratification (Cox et al., 2001), and temporal changes in subsistence (White and Schwarcz, 1989; Katzenberg et al., 1995).

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Like studies of nutrition in living populations, paleodietary reconstruction from hard-tissue chemistry has been necessarily limited to explorations of the causes and consequences of postnatal nutrition. With its traditional focus upon data derived from analyses of bone tissues, it is difficult for researchers to identify data from the earliest periods of development. Because bone constantly remodels in response to its environment, it is impossible to reliably capture the chemical signatures of infancy or early childhood (much less the prenatal period) via mature adult remains. Working directly with subadult remains does not so much alleviate the remodeling problem as it introduces other concerns regarding the ability of researchers to reconstruct a representative profile of childhood health from the remains of children who clearly did not survive to adulthood (Wood et al., 1992).

With the present study, we suggest a method for accessing data preserved from the earliest periods of development, including the developmentally crucial prenatal period. Using laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) of deciduous dental enamel, it is possible to retrospectively evaluate an individual's nutritional status (as influenced by dietary intake, nutrient interactions, growth, repair, and activity; Bogin, 2001) from the second trimester to approximately 9 months of age. Deciduous teeth are entirely unique biological tissues in that they permanently document *prenatal* as well as early postnatal environments. Because teeth form incrementally at a known rate, it is possible to identify specific developmental periods (e.g., second and third trimesters) and assess changes in chemical composition along a time axis (Goodman and Rose, 1990). Microspatial analyses of dental tissues made possible using techniques such as LA-ICP-MS allowed us to investigate new questions regarding pre- and early postnatal development.

In this study, we examine the nature of trace-element variation within and among deciduous teeth collected from children living in six rural Mexican communities. We test the hypothesis that trace-element data collected from deciduous enamel will vary consistently, depending on the developmental period examined rather than by tooth type or quadrant of the mouth (i.e., upper, lower, left, and right). That is, variation is expected to occur over the life course of an individual, in particular the neonatal transition, and to be less significant among teeth that develop at the same time. By determining a baseline pattern for trace-element distributions within and among teeth, it will then be possible to explore how variations in trace-element incorporation by enamel relate to variations in early childhood environments.

LA-ICP-MS ANALYSES OF BIOLOGICAL TISSUES

In recent years, there has been a growing interest in the application of LA-ICP-MS to trace-element profiling of biological tissues for several reasons. The minimally destructive nature of LA-ICP-MS analyses allows for the retrospective documentation of relative fluctuations in trace-element absorption over time that are observed as changes in trace-element intensities over space. While LA-ICP-MS analyses were originally employed by researchers in the geological and metallurgical sciences (Gray, 1985), they have since come to reveal information regarding the distribution of trace elements within biological hard tissues such as teeth (Cox et al., 1996; Lee et al., 1999; Lochner et al., 1999; Outridge et al., 2000; Kang et al., 2004), coral (Fallon et al., 2002), shells (Fuge et al.,1993; Belloto and Miekeley, 2000; Toland et al., 2000), fish otoliths (Gemperline et al., 2002), and wood (Watmough et al.,1998). The marriage of laser ablation sample introduction to inductively coupled plasma-mass spectrometry made it possible to carry out in situ trace-element analyses of solid samples (for comprehensive reviews of the benefits and limitations of ICP-MS and LA-ICP-MS, respectively, see Denoyer, 1991; Denoyer et al., 1991).

Previously, the preferred option for gathering trace-element data from tissue samples was total digestion followed by traditional solution analyses (Fuge et al., 1993). Total digestion not only results in destruction of the sample, but it homogenizes the fluctuations in trace-element absorption so that the time-specific data locked within each layer of tissue are obscured. With LA-ICP-MS, it is possible to ablate a solid sample directly and, with a resolution of $\sim$10 μm, it provides an ideal means for extracting material from differing locations within enamel that developed at different times. Another benefit of LA-ICP-MS is that, unlike electron or proton microprobes with X-ray emission detectors, it is possible to reach detection limits conservatively, approaching $<$1 ppm for much of the periodic table (Outridge et al., 1995).

LA-ICP-MS analyses are limited, however, by their semiquantitative nature. Their determinations have an accuracy of only $\pm$30–50% (Amarasiriwardena et al., 1997) due to the lack of appropriate elemental standards for quantitative analysis. Data are measured as intensities (counts per second) as opposed to concentrations (ppm). The quantification of trace-element concentrations from LA-ICP-MS is difficult for several reasons, including uneven ablation of samples, differences in the efficiency of transporting material to the plasma, and nonrepresentative subsampling (Bellotto and Miekeley, 2000). Perhaps the greatest problem faced by researchers attempting to convert time-resolved trace-element intensities into quantifiable concentrations is the lack of solid standard reference materials (SRMs) that are matrix-matched for calibration purposes (Outridge et al., 1995; Bellotto and Miekeley, 2000). Currently, SRMs may be tailored to a particular biological tissue (e.g., enamel, coral, or wood) through the production of pressed pellets made from powdered samples of the tissues to be used in the analysis. Alternatively, because Ca exists naturally in the sample matrix, exhibits a chemical behavior similar to that of Sr, Ba, Mg (alkaline earth elements), Fe, and Zn, is relatively interference-free, and is monitored simultaneously with the analyte atoms, we chose to use ^{43}Ca as an internal standard (Cox et al., 1996; Lee et al., 1999; Outridge et al., 1995). Calcium thus serves as a baseline against which fluctuations in trace-element intensities due to ablation variations can be corrected. And so, although LA-ICP-MS is limited in its precision, once data are appropriately calibrated, it is possible to identify useful patterns in chemical compositions within samples (Outridge et al., 1995).

The few published studies applying LA-ICP-MS to human teeth showed the utility of the technique for documenting the incorporation of trace elements into enamel and dentine in archaeological and contemporary samples. The majority of these studies focused on determining baseline patterns of elemental distributions within human teeth, and particularly within enamel. In Lochner et al. (1999), the LA-ICP-MS analysis of 10 deciduous human teeth focused on fluctuations in trace-element levels of enamel before and after birth. It was found, from a subsample of six teeth, that the concentrations of all 14

elements examined generally rose after birth, including Fe, Pb, and Zn. In a related study by Lee et al. (1999), the teeth of rats injected with lead were assessed to determine if the resultant physiological disruption could be identified using trace-element analyses of teeth. They found that this was indeed the case, lending support to the argument that other events/periods of physiological change (i.e., the neonatal line) could be used to delineate periods of differential physiology in human teeth. Extending their discussion to a horizontal ablation across the cusps of two human deciduous molars, Lee et al. (1999) found that Zn and Pb levels rose as the laser sampled postnatal enamel, reaching especially high values in the surface enamel. Elevated elemental intensities at the enamel surface are potentially due to a process of de- and remineralization resulting from the interaction between saliva and teeth (Reitznerová et al., 2000). Budd et al. (1998, p. 121) applied LA-ICP-MS techniques in an attempt to "establish baseline data relating to exposure among pre-industrial or even prehistoric populations" by assessing the distribution of Pb in six archaeological and three modern teeth from the UK. While this study did not expressly endeavor to examine pre- and postnatal enamel differences, Pb values were again found to rise as ablations approached later-forming enamel and the tooth surface. Further research by Budd et al. (2000) applied new findings regarding Pb levels in the seven teeth of four Neolithic individuals to the question of changing patterns of Pb exposure in Britain. Each of these published studies focused on identifying baseline distributions of trace elements in enamel via analyses of modern and archaeological teeth, discussing the uptake of Pb and issues of environmental pollution in particular. Our research used larger sample sizes from contemporary individuals to explore the dynamic ways in which nutritionally significant elements (e.g., Zn and Fe) in enamel reflect environmental influences on maternal health and nutrition (Dolphin et al., 2002; Goodman et al., 2003). Because diets were studied during deciduous tooth formation, we were afforded the possibility of studying the relationship between dietary intake and hard-tissue concentrations. Here, however, we focused only on variation by developmental time period (prenatal vs. postnatal) and tooth type.

THE SETTING: SOLÍS, MEXICO

The dental samples used in this study were collected from children living in the Solís Valley, located 170 km northwest of Mexico City, where the municipalities of Temascalcingo, Contepec, and Tepuxtepec converge in the northwest corner of the state of Mexico. This rural agricultural area is home to 50 communities with populations ranging from approximately 800–1,900 individuals (Ryan and Martinez, 1996). Diets are not diverse in the valley, with only about 25 food items, and 60–70% of energy coming from maize in the form of tortillas (Allen et al., 1992). Tortilla consumption was highest among less wealthy, illiterate households, where aspirations for children's futures were not high. In high tortilla consumption families, animal protein contributed a smaller portion of the diet, while those eating fewer tortillas generally ate more animal protein. All adults, including pregnant and lactating mothers, had very similar dietary patterns (Allen et al., 1992). Although a slight increase in consumption by mothers began in late pregnancy and carried on throughout lactation, this increase did not change mothers' overall dietary patterns. Thus, while the proportion of tortillas and animal products in the diet remained unchanged, maternal and fetal demands for nutrients increased, potentially resulting in micronutrient deficiencies (Allen et al., 1992) that may be documented in the prenatal enamel of their children.

TABLE 1. Instrument operating conditions

Laser ablation operating parameters	
Laser type	Nd:YAG
Laser mode	Frequency quadrupled 266-nm UV, Q-switched mode
Repetition rate/Hz	10
Laser energy/mJ	1.53
Sampling scheme	Scanning
Scanning speed/$\mu m \cdot s^{-1}$	20
ICP-MS operating parameters	
Forward power/kW	1
Ar gas flow rates/min^{-1}	
Coolant	15
Auxiliary	1.2
Nebulizer gas	1.125
Measurement conditions	
Dwell time/msc	10–30
Resolution	high
Reading/replicates	200
Isotopes measured	^{25}Mg, ^{43}Ca, ^{57}Fe, ^{66}Zn, ^{68}Zn, ^{88}Sr, ^{138}Ba, ^{208}Pb
Internal standard	^{43}Ca

The Solís Valley was the site of several international and national human development projects. The largest of these, the Nutrition Collaborative Research Support Program (NCRSP), started in 1982 with the aim of testing the functional consequences of mild-to-moderate nutrition (Allen et al., 1992). Children and their mothers participated in the NCRSP project from the recognized time of conception to 2 years of age, with follow-up studies being conducted at several intervals up to age 6 years. Many of these individuals still participate in ongoing research within the valley. Approximately 600–700 variables were observed, spanning a breadth of potential socioeconomic, biological, and psychosocial factors related to childhood health. Starting in 1991, exfoliated deciduous teeth were collected from NCRSP children.

METHODS

Thirty-eight teeth from 36 individuals were randomly chosen for LA-ICP-MS analysis from a larger sample of exfoliated deciduous teeth collected from children living in six communities of the Solís Valley, Mexico. These same children were participants in a cohort study of mother-child dyads from 1984–1986 that aimed to better understand the functional consequences of mild-to-moderate malnutrition (Allen et al., 1992; Murphy et al., 1992).

Each individual was represented by at least one unworn, caries-free deciduous incisor or canine. In total, 27 incisors and 11 canines were included. Each tooth was cleaned and embedded in resin, according to protocols outlined in Goodman and Rose (1990). Preparation of embedded tooth sections for LA-ICP-MS analysis required further cleaning, polishing, and etching of the exposed enamel surfaces. Once rinsed in DDI (deionized- distilled) water, each exposed surface was polished using a new Buehler polishing cloth mounted on a high-speed wheel. Polishing was lubricated using a Beuhler 0.3-μm alpha

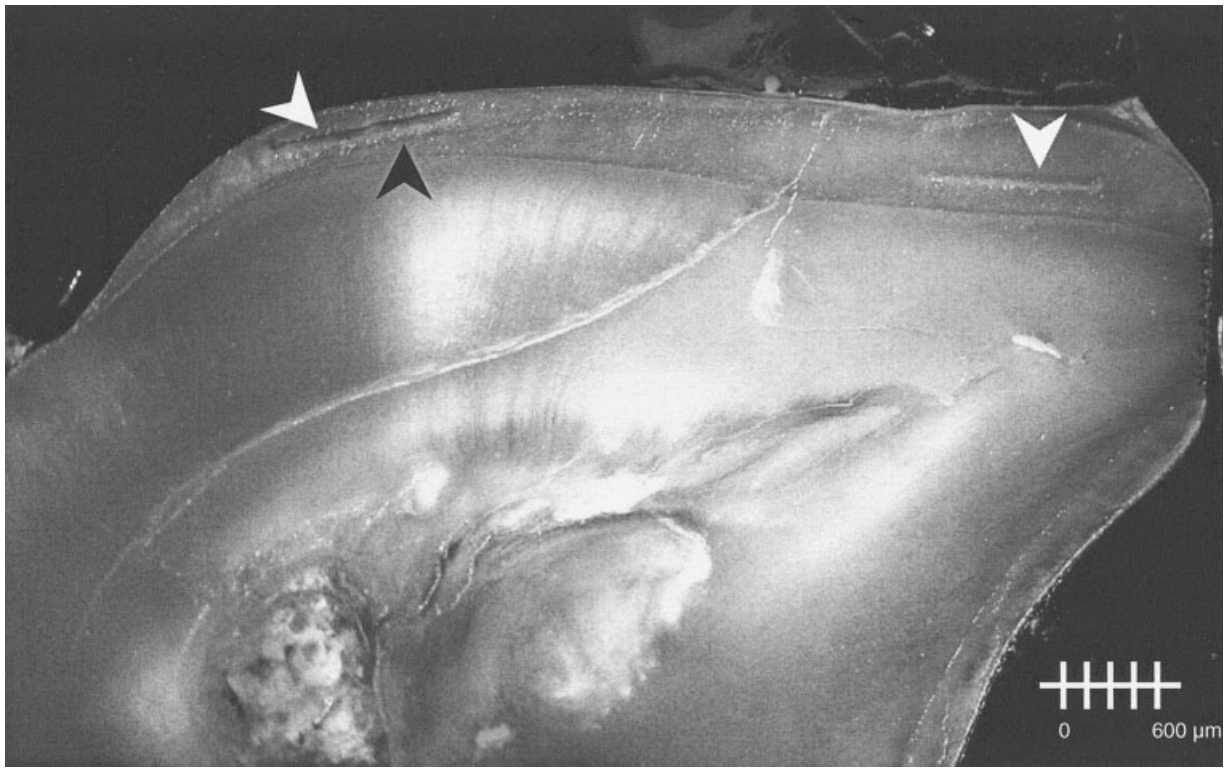

Fig. 1. Location of neonatal line (black arrowhead) and of ablations in prenatal and postnatal regions of enamel (white arrowheads) in deciduous Solís tooth.

TABLE 2. Paired-samples t*-tests of log-transformed prenatal vs. postnatal mean intensity ratios for incisors and canines combined*

	$^{25}Mg/^{43}Ca$	$^{57}Fe/^{43}Ca$	$^{66}Zn/^{43}Ca$	$^{68}Zn/^{43}Ca$	$^{88}Sr/^{43}Ca$	$^{138}Ba/^{43}Ca$	$^{208}Pb/^{43}Ca$
Prenatal (n = 36)							
Mean	1.96	−0.82	0.51	0.48	2.13	0.90	0.26
SD	0.12	0.29	0.30	0.31	0.21	0.28	0.43
Postnatal (n = 36)							
Mean	1.86	−0.44	1.11	1.05	2.12	1.16	0.82
SD	0.12	0.63	0.38	0.38	0.23	0.33	0.67
Paired-samples *t*–test							
t	5.53	−3.86	−10.02	−9.88	0.41	−6.13	−5.98
P	0.000*	0.000*	0.000*	0.000*	0.681	0.000*	0.000*

* Significant at $P \leq 0.01$.

alumina micropolish mixed with DDI water. Polishing of the enamel surface not only removes saw marks but also enhances the transmittance of light through the block and the likelihood of identifying specific periods of development during ablation.

Trace-element analysis was performed using a 266-nm Nd:YAG Cetac LSX-100 Laser Ablation System (Cetac Technologies, Omaha, NE), as coupled to the Perkin Elmer ELAN 6000 ICP-MS (Perkin Elmer Instruments, Shelton, CT). Instrument operating conditions are presented in Table 1. Each embedded tooth was placed on the stage, with the enamel surface exposed to the laser above. Once enclosed within the sample cell of the laser module, the tooth was magnified and viewed in a video monitor via a CCD (charge-coupled device) camera fitted with a zoom lens. Each tooth was mounted and oriented on the stage of the laser ablation cell so as to best view the labial enamel and the neonatal line. Daily, prior to the analysis, ^{88}Sr was used to optimize the nebulizer gas flow rate and tune the LA-ICP-MS. Once the locations of pre- and postnatal enamel were identified, the tooth was oriented so that a sample was taken from each region (Fig. 1). Care was taken to avoid the enamel surface, where questions regarding surface enrichment have been raised (Budd et al., 1998; Reitznerová et al., 2000). A National Institute of Standards and Technology (NIST612) glass standard was ablated throughout the analysis so as to monitor the instrument drift during data analysis.

Ion intensities were measured in counts per second (cps) for the following elements: ^{25}Mg, ^{43}Ca, ^{57}Fe, ^{66}Zn,, ^{68}Zn, ^{88}Sr, ^{138}Ba, and ^{208}Pb. The resulting data were background-subtracted and refined using the GEMOC GLITTER! 4.0 data reduction program (GEMOC Laser ICPMS Total Trace Element Reduction Package, New Wave Research/Merchantek Products, Fremont, CA). Complications in determining absolute quantification of elemental

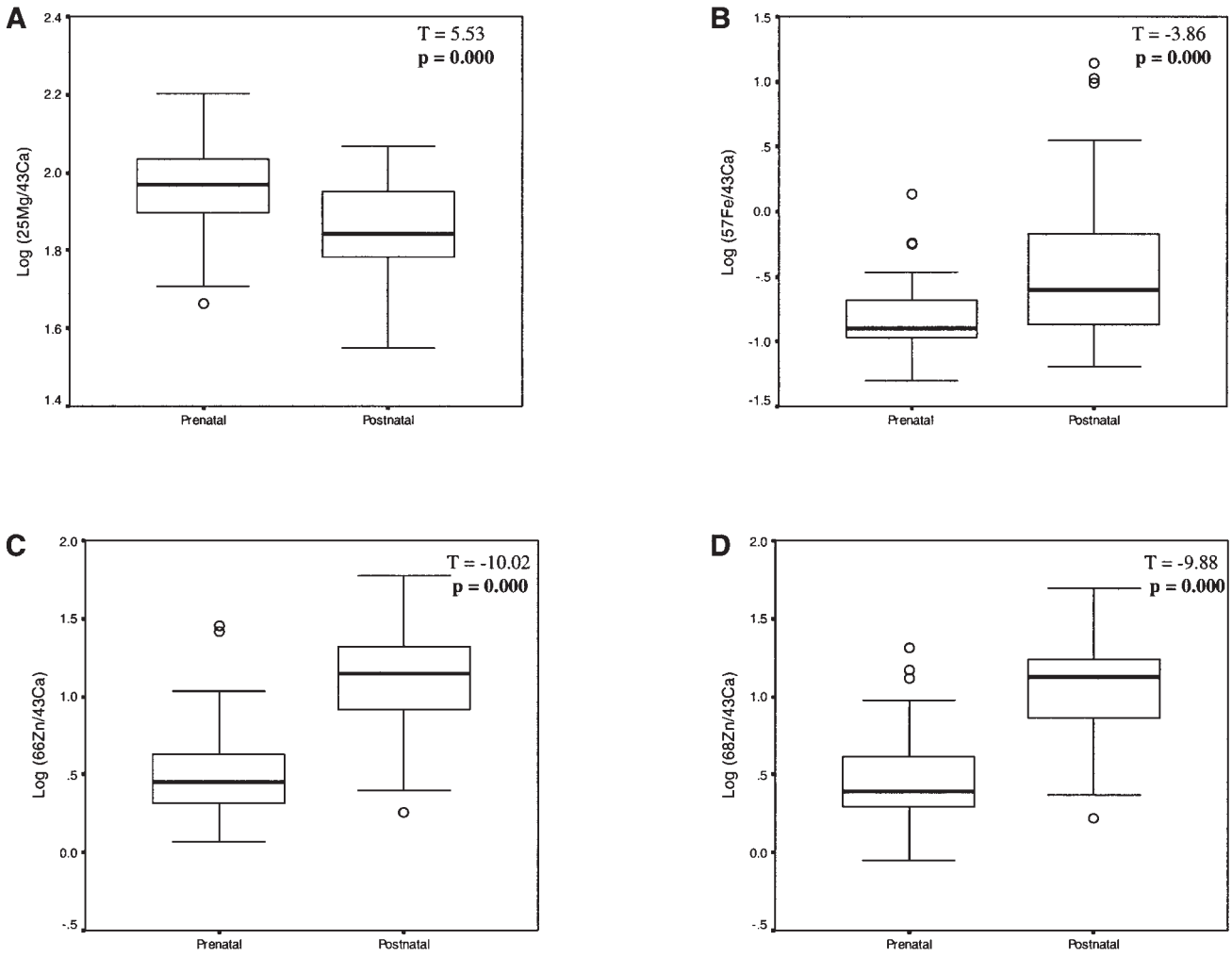

Fig. 2. (See legend page 883)

concentrations arise with solid sampling via the laser (e.g., fluctuations in laser energy); thus all data were normalized to an internal standard (^{43}Ca) and adjusted for instrument drift, in order to facilitate comparisons between ablations. The magnitude of calcium signals was uniform throughout all regions of enamel, and thus do not appear to differ significantly due to fluctuations in the mineralization process.

Prior to statistical analysis, data were log-transformed so as to approach a normal distribution. The results of paired *t*-test comparisons between log-transformed pre- and postnatal elemental concentrations are presented in Table 2, as are a series of boxplots further illustrating the nature of differences between developmental periods (Fig. 2). Independent-samples *t*-tests and Levene's test for equality of variances were used to compare incisors vs. canines and left vs. right and upper vs. lower mouth quadrants in Tables 3–5.

RESULTS

Significant differences were found for almost all elements when comparing prenatal and postnatal intensity ratios using paired-samples *t*-tests (Table 2). The group median and variation in pre- and postnatal ^{43}Ca-normalized intensities for ^{25}Mg, ^{57}Fe, ^{66}Zn, ^{68}Zn, ^{88}Sr, ^{138}Ba, and ^{208}Pb are presented as boxplots (Fig. 2). There was a significant decrease in ^{25}Mg from the prenatal to postnatal enamel ($P < 0.001$), while ^{57}Fe, ^{66}Zn, ^{68}Zn, ^{138}Ba, and ^{208}Pb intensity ratios increased during the postnatal developmental period ($P < 0.001$), and ^{88}Sr intensity ratios remained constant throughout the enamel ($P = 0.681$). For ^{57}Fe, ^{66}Zn, ^{68}Zn, ^{138}Ba, and ^{208}Pb, the postnatal period showed a greater and positively skewed variation among individuals. As expected, both ^{66}Zn and ^{68}Zn shared the same trend. Although barium and strontium are both nonessential divalent cations of the same alkaline earth elements group as calcium, they differ in that ^{88}Sr is more concentrated than ^{138}Ba overall, and that ^{138}Ba intensity ratios rise in postnatal enamel while ^{88}Sr values remain constant. As discussed below, this may be related to the greater trophic discrimination of barium (Burton et al., 1999).

Figure 3 shows the range and direction of variation in elemental intensities across developmental periods for each individual sampled. Prenatal and postnatal values vary among individuals, as does the degree of pre- vs. postnatal difference, yet the direction of individual transitions is relatively constant. The decrease from pre-to-postnatal enamel for ^{25}Mg, and the increased postnatal values

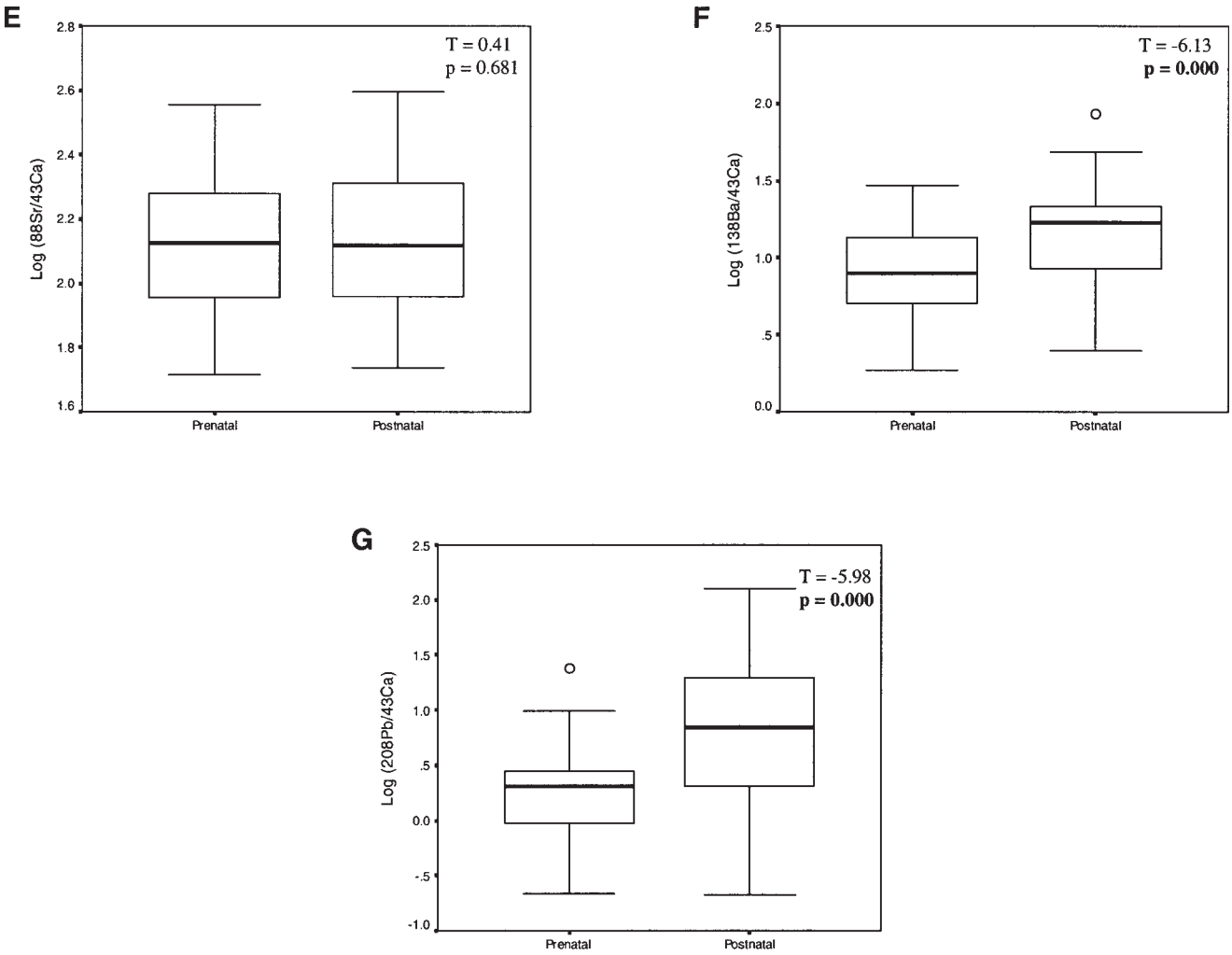

Fig. 2. A–H: Boxplots show median prenatal and postnatal intensities for several trace elements. Bold line at center of each box depicts median intensity value, while boxes surrounding it mark 50% interquartile range. Whiskers indicate boundaries of lowest and highest quartiles. Open circles represent outliers. Significant paired Student's *t*-test results are in bold.

for ^{66}Zn, ^{68}Zn, and ^{138}Ba, are the most consistent. In the case of ^{88}Sr, however, a clearly biomodal distribution is observable. For most individuals represented, it appears that those in the higher ^{88}Sr group have postnatal values either equal to or greater than their prenatal values. Individuals in the lower ^{88}Sr group have lower postnatal rather than prenatal levels.

Whereas many elements varied within teeth from prenatal to postnatal enamel, there are very few significant differences when one compares teeth (Tables 3–5). There were no significant differences in the elemental composition of incisors and canines when developmental periods were controlled for (Table 3).

Comparisons between left and right incisor elemental intensity ratios for prenatal and postnatal enamel are presented in Table 4. No significant differences were found within prenatal or postnatal enamel for any of the elements studied, except for prenatal ^{68}Zn ($P = 0.029$).

Table 5 presents the results of comparisons of elemental intensities in upper and lower incisors for both the prenatal and postnatal portions of enamel. Intensities of prenatal ^{66}Zn ($P = 0.018$) and ^{68}Zn ($P = 0.005$), and postnatal ^{66}Zn ($P = 0.009$) and ^{68}Zn ($P = 0.014$), were significantly higher in lower incisors, while ^{25}Mg ($P = 0.031$) and ^{138}Ba ($P = 0.041$) were significantly lower. For all other elements, there were no significant differences between upper and lower incisors.

Due to the small sample size of canines (n = 8) with accompanying information regarding the quadrant of the mouth from which they originated, comparisons of this tooth type by quadrant are not presented. However, we found no significant differences or patterns by which canines in one quadrant or another had more or less of an element.

DISCUSSION

Variation between prenatal and postnatal enamel

The overarching objectives of this study were to determine 1) whether variations in the chemical composition of enamel reflect changing environments during the time of their development; and 2) whether interpretation of such variation might be confounded by tooth type or mouth quadrant.

Several trace elements vary in their distribution within deciduous enamel along a temporal axis, specifically between prenatally and postnatally formed enamel. Each element has its own characteristic distribution through-

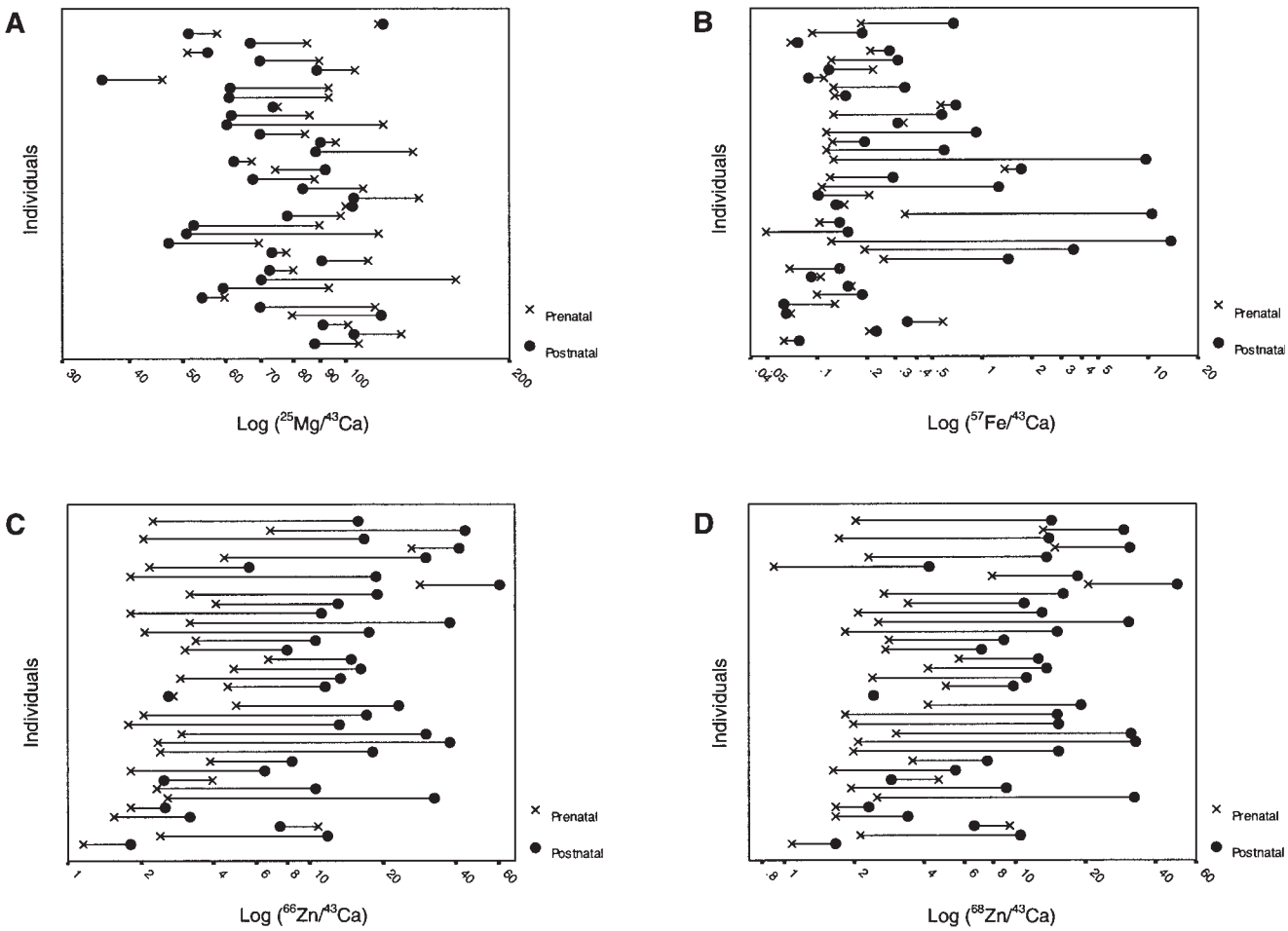

Fig. 3. (See legend page 885)

out the deciduous tooth. The greater intensity values for ^{57}Fe, ^{66}Zn, ^{68}Zn, ^{138}Ba, and ^{208}Pb in postnatal enamel, as well as the greater variation among individuals postnatally, are likely related to the birth transition from life in utero to postnatal environments. While individuals' elemental intensities varied somewhat within developmental periods, most individuals followed a shared pattern of increase or decrease over time. Only ^{88}Sr showed a dual directionality of variation among individuals. The overall tendency for a pre- to postnatal shift may have to do with reduced regulation of an infant's trace-element exposure during breastfeeding as opposed to placental exchange or transport of trace elements. As most Solís children are exclusively breastfed for 3–6 months (Allen et al., 1992), we assume that most of the nutrient uptake into postnatal incisor enamel occurs via lactating mothers. The trace-element uptake of prenatal and early postnatal enamel draws upon the stores and overall nutritional status of the mother and the buffered environment of the placenta. Once born, a breastfeeding infant continues to reap the benefits of maternal buffering, but once weaning begins, children, like their mothers, are vulnerable to environmental differences impacting their nutrient uptake (e.g., socioeconomic status, disease, diet, pollution, or household size).

The distribution of ^{88}Sr often varied across developmental periods within individuals. Although ^{88}Sr values may vary by one's geographic location during tissue development (Price et al., 2000; Hodell et al., 2004), this is not the case for the teeth discussed here. All tooth donors were native to, and continued to live in, the Solís Valley throughout their childhood. Figure 3 clearly illustrates the presence of two distinct groups of individuals in this sample: "low" ^{88}Sr individuals and "high" ^{88}Sr individuals. Because Sr/Ca ratios are indicative of diet, varying by trophic level (with lower Sr/Ca ratios indicating those higher in protein and higher Sr/Ca ratios indicating those lower in protein; Sillen and Kavanagh, 1982; Sandford, 1993), it would seem that the Solís Valley diet diverges significantly between the two groups of individuals. More interestingly, the two groups further differ in the distribution of ^{88}Sr across the pre-to-postnatal transition. For the "high" ^{88}Sr group, values tend to start out low during the prenatal period (in utero) and to climb during the postnatal period. This trend to higher Sr values is to be expected when an individual drops to a lower trophic level (e.g., placenta to breast/weaning foods). For the "low" ^{88}Sr group, however, a very different and surprising pattern emerges. Individuals from the "low" ^{88}Sr group tend to show a decrease in values after birth, directly conflicting with the trophic-level explanation for the behavior of Sr in biological tissues. The chemical data presented here, and the dietary data reported elsewhere (Allen et al., 1992), indicate some differences in dietary quantity and quality in the Solís Valley. Those individuals with greater access to resources eat better-

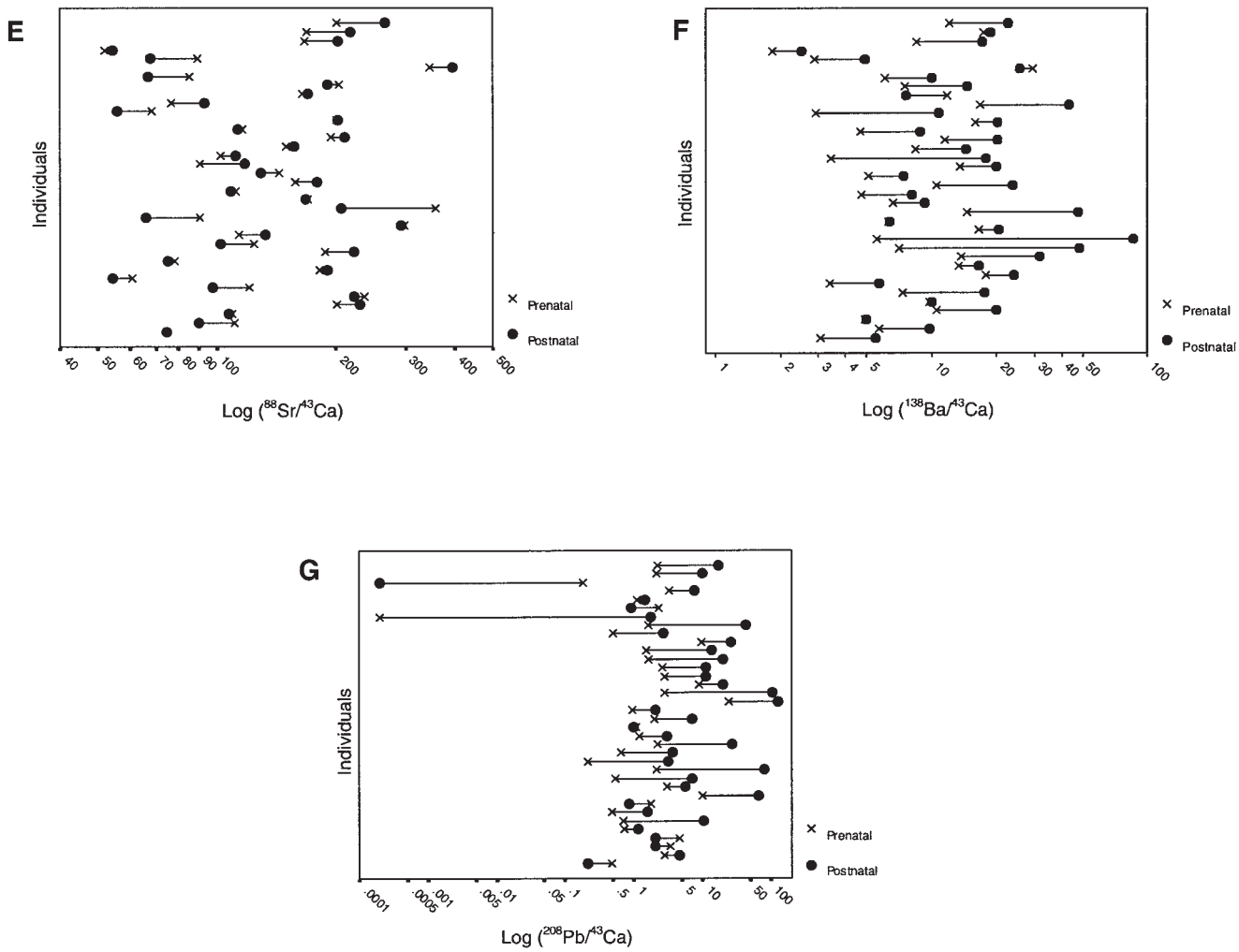

Fig. 3. A–H: Log-transformed prenatal and postnatal elemental intensities for each individual sampled (N = 36).

quality diets (e.g., more animal protein), as opposed to those eating poorer-quality diets (more tortillas) (Allen et al., 1992). This differentiation may be used to explain why there are "low" (better-quality diet) and "high" (poorer-quality diet) ^{88}Sr groups of the population. But this trophic-level effect on Sr values falls short of explaining why, despite essentially becoming less carnivorous after birth, children who are already in the "low" ^{88}Sr group actually decline in their uptake of Sr: the opposite of what we expected. Weaning behaviors are similar among Solís mothers, even if dietary quality may not be. Perhaps mothers and their children in differing nutritional circumstances uptake Sr differentially, in a way that cannot be explained away completely by trophic-level hypotheses. We plan to further explore this unusual distribution of ^{88}Sr values within individuals' teeth in the light of the many variables known to us from the Mexico NCRSP project (e.g., socioeconomic status, dietary intake, morbidity, and cognitive development). However, such work is beyond the scope of this paper, and it is enough to document this unusual behavior of Sr uptake by developing tissues.

Barium did not follow the bimodal pattern of strontium. It did, however, demonstrate a shift to higher ^{138}Ba values in postnatal as opposed to prenatal enamel. Burton et al. (1999) suggested that Ba is more sensitive to trophic shifts than Sr, thus potentially explaining the more dramatic shift for Ba.

The distribution of enamel Pb values documented in this study provides insight into the extent of in utero regulation of trace metals via the placenta in developing fetuses from environmental pollutants. It appears that prenatal maternal buffering is more effective than postnatal buffering via the breast. This would explain the dramatic rise in postnatal Pb values (Fig. 2), despite the consistent and regular use of lead-glazed ceramics in the Solís Valley (Tunstall and Amarasiriwardena, 2002), including their use by expectant mothers. Despite the apparent improved prenatal vs. postnatal maternal buffering of Pb uptake by developing enamel, Goodman et al. (2003) showed that prenatal enamel Pb levels are still high enough to be correlated with reduced growth in height and weight by about 5 years of age.

As with the variation in Pb values among Solís individuals, postnatal Fe and Zn levels likely reflect real differences in individuals' interactions with their environment. Both Fe and Zn are nutritionally significant trace elements implicated in childhood growth and development, morbidity, and cognition (Hsief et al., 1983; Cousins, 1996; Yip and Dallman, 1996; Hambridge, 2000; Failla,

TABLE 3. Independent-samples t*-tests of log-transformed incisor vs. canine mean intensity ratios for prenatal and postnatal enamel, respectively*

	$^{25}Mg/^{43}Ca$		$^{57}Fe/^{43}Ca$		$^{66}Zn/^{43}Ca$		$^{68}Zn/^{43}Ca$		$^{88}Sr/^{43}Ca$		$^{138}Ba/^{43}$		$^{208}Pb/^{43}Ca$	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Incisors (n = 27)														
Mean	1.94	1.85	−0.79	−0.49	−0.56	1.11	0.51	1.03	2.11	2.12	0.86	1.13	0.30	0.69
SD	0.12	0.12	0.31	0.57	0.33	0.40	0.34	0.37	0.19	0.23	0.30	0.37	0.44	0.70
Canines (n = 11)														
Mean	2.00	1.83	−0.89	−0.54	0.46	1.00	0.43	0.96	2.16	2.13	0.95	1.13	0.10	0.78
SD	0.10	0.97	0.22	0.86	0.24	0.41	0.24	0.41	0.24	0.24	0.24	0.31	0.54	0.81
Independent-samples *t*-test														
t	−1.647	0.490	1.050	0.231	0.865	0.746	0.733	0.526	−0.706	−0.252	−0.926	−0.019	1.154	−0.356
P	0.108	0.627	0.301	0.819	0.393	0.461	0.468	0.602	0.485	0.803	0.360	0.985	0.256	0.724

No significant differences at $P \leq 0.05$.

TABLE 4. Independent-samples t*-tests of log-transformed left vs. right incisor mean intensity ratios for prenatal and postnatal enamel, respectively*

	$^{25}Mg/^{43}Ca$		$^{57}Fe/^{43}Ca$		$^{66}Zn/^{43}Ca$		$^{68}Zn/^{43}Ca$		$^{88}Sr/^{43}Ca$		$^{138}Ba/^{43}Ca$		$^{208}Pb/^{43}Ca$	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Left incisors (n = 10)														
Mean	1.97	1.90	−0.74	−0.61	0.44	0.99	0.33	0.89	2.08	2.10	0.91	1.15	0.28	0.66
SD	0.20	0.086	0.44	0.48	0.19	0.44	0.22	0.41	0.21	0.24	0.34	0.31	0.69	0.94
Right incisors (n = 9)														
Mean	1.87	1.79	−0.79	−0.35	0.67	1.31	0.70	1.26	2.08	2.08	0.81	1.18	0.30	0.96
SD	0.13	0.15	0.27	0.70	0.48	0.41	0.43	0.36	0.21	0.24	0.30	0.44	0.27	0.56
Independent-samples *t*-test														
t	1.603	1.892	0.325	−0.943	−1.366	−1.698	−2.433	−2.061	0.003	0.183	0.633	−0.182	−0.079	−0.789
p	0.127	0.076	0.749	0.359	0.197	0.108	0.029*	0.055	0.997	0.857	0.535	0.858	0.939	0.447

* Significant at $P < 0.05$.

TABLE 5. Independent-samples t*-tests of log-transformed upper vs. lower incisor mean intensity ratios for prenatal and postnatal enamel, respectively*

	$^{25}Mg/^{43}Ca$		$^{57}Fe/^{43}Ca$		$^{66}Zn/^{43}Ca$		$^{68}Zn/^{43}Ca$		$^{88}Sr/^{43}Ca$		$^{138}Ba/^{43}Ca$		$^{208}Pb/^{43}Ca$	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Upper incisors (n = 14)														
Mean	1.97	1.90	−0.89	−0.45	0.42	0.91	0.35	0.85	2.10	2.11	0.86	1.24	0.22	0.60
SD	0.10	0.10	0.27	0.75	0.22	0.36	0.72	0.35	0.18	2.21	0.29	0.37	0.48	0.73
Lower incisors (n = 14)														
Mean	1.87	1.81	−0.71	−0.60	0.75	1.29	0.72	1.19	2.09	2.06	0.81	0.96	0.37	0.79
SD	0.12	0.14	0.35	0.31	0.39	0.34	0.36	0.33	0.19	0.23	0.34	0.33	0.42	0.63
Independent samples *t*-test														
T	2.289	1.842	−1.526	0.696	−2.600	−2.862	−3.192	−2.647	0.176	0.640	0.460	2.155	−0.867	−0.740
P	0.031*	0.077	0.139	0.496	0.018*	0.009**	0.005**	0.014*	0.862	0.528	0.650	0.041*	0.395	0.466

* Significant at $P < 0.05$.
** Significant at $P < 0.01$.

2003; Krebs, 2003; Rosado, 2003), and their bioavailability is directly tied to environmental variables. In the case of zinc, we previously established an association between one's prenatal enamel zinc levels and the proportion of tortilla-to-animal products consumed. Because a household's tortilla consumption is determined by its socioeconomic status, with low-income families eating proportionately more tortillas and fewer animal products, and since tortilla consumption inhibits Zn bioavailability, low-income families were significantly correlated with lower levels of enamel Zn when compared to their wealthier counterparts (Dolphin et al., 2002). Although essential elements such as Zn and Fe are subject to some physiological regulation, there are many instances when homeostatic controls (e.g., body stores) are not sufficient to counteract the effects of micronutrient deficiencies such as those common to some individuals in the Solís Valley. The fact that the chemical composition of enamel varies predictably with environmental context has significant consequences for the biomonitoring of contemporary populations at risk for micronutrient deficiency and its resultant developmental impairments. Understanding the variation of nutritionally significant trace elements within a specified context can also broaden our framework for interpreting the influences upon, and the consequences of, differential access to resources in past populations.

Consistency by tooth type

The second goal of this research was to determine whether the type of tooth ablated influences variation in the trace-element content of enamel. Despite a few significant differences, we found an overall pattern of remarkable similarity in trace-element values by tooth type and

by quadrant. The few significant differences (7 of 42 comparisons) do not fit a pattern, and appear to be random occurrences (Tables 3–5).

With this preliminary work, we demonstrated that variation in the trace-element composition of deciduous enamel is determined primarily by the timing of its formation. We argue that interindividual variation results from differences in the incorporation of elements into developing tissues, as caused by environmental factors particular to each individual's interaction with the mother's and their own environment. These results have consequences for both the conduct of palaeodietary research utilizing tooth chemistry and for the biomonitoring of contemporary populations. Although focussing only on the trace-element composition of deciduous teeth, the fine temporal variations in enamel chemistry documented here can inform the stable isotope methods at the core of paleodietary reconstructions that traditionally rely on total dissolution techniques, and as a result, tend to homogenize data, missing insights into how diets and nutrition change across developmental periods.

CONCLUSIONS

We employed LA-ICP-MS to study changes in the elemental composition of deciduous incisor teeth, and found that trace-element composition of deciduous enamel varies according to the developmental period examined rather than by tooth type or mouth quadrant. This finding has implications for the application of tooth chemistry to issues of palaeodietary reconstruction and the use of teeth as biomonitors of contemporary human populations. By confirming that the chemical composition of enamel changes significantly along a temporal axis, our findings suggest that microspatial analyses of teeth will allow researchers to access a new set of retrospective questions about variations in diet, growth and development, and morbidity. In particular, our data indicate that microspatial analyses of deciduous enamel can provide an entirely unique window into individuals' earliest development, including the critical prenatal period.

By documenting the fact that chemical variation in enamel is tied to one's changing environment over time, we provide a baseline for the distribution of several elements common to the literature on palaeodiets, migration studies, and environmental pollution (e.g., Sr and Pb), and for nutritionally significant elements (e.g., Fe and Zn) more commonly addressed in nutritional studies of contemporary populations. Determination of such a baseline sets the scene for future research using the Solís Valley NCRSP data set to understand how variation in maternal environments impacts the uptake of trace elements by developing enamel. This work will also explore the relationship between the prenatal and early-postnatal composition of enamel and later childhood health. Individuals' life histories will be used to paint a portrait of differing individual responses to their biosocial environment, ultimately shedding light on the distribution of variation within the overall population. Such research will involve a larger sample size, the testing of new calibration techniques (including the use of pressed pellet apatite standards), comparisons of multiple teeth from single individuals, and comparisons of teeth from siblings. Also, a cross-cultural comparison of LA-ICP-MS elemental data will utilize deciduous teeth from the sister project (in Kalama, Egypt) of that conducted in the Solís Valley.

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