Maternal Diets, Nutritional Status, and Zinc in Contemporary Mexican Infants' Teeth: Implications for Reconstructing Paleodiets

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ABSTRACT Despite attempts to use zinc (Zn) concentrations in hard tissues to comment upon the degree of carnivory in past populations, zinc has yielded inconsistent trophic level effects. The question of what, if anything, zinc in human enamel reveals regarding past diets is the focus of this research. We test whether the zinc content of deciduous tooth enamel from contemporary Mexican infants varies by maternal dietary variables such as zinc intake, proportion of animal products consumed, and dietary components that are known to impact zinc absorption. Deciduous teeth were collected from former participants in a longitudinal study of maternal and infant diet and function in highland Mexico. The Zn/Ca ratios of both prenatal and postnatal regions of 37 anterior teeth representing 26 individuals were assessed via laser ablation–inductively coupled plasma–mass spectrometry. Maternal dietary data collected during lactation were not correlated with zinc levels in the early postnatal enamel of infants' teeth, which were forming at the same time. In the case of prenatal enamel, zinc values were correlated with the consumption of foods known to influence Zn bioavailability, most notably tortillas ($P = 0.008$; $r = 0.510$), but not with meat consumption. Unexpectedly, women who consumed diets with poor zinc bioavailability during pregnancy gave birth to infants whose prenatal enamel demonstrated the highest Zn/Ca ratios, possibly due to enhanced zinc absorption during pregnancy for those mothers suffering most from long-term micronutrient deficiency. These results would suggest that zinc is not a reliable trophic level indicator. Am J Phys Anthropol 000:000–000, 2009. ©2009 Wiley-Liss, Inc.

Although stable isotope analyses of human bones and teeth are central to reconstructions of past diets, trace element techniques, especially those employing nutritionally significant elements such as zinc (Zn), have declined in use. An initial wave of enthusiasm regarding the application of trace element techniques to anthropological questions (Brown, 1973; Gilbert, 1975; Vogel and Van der Merwe, 1977) was quickly subdued when it became clear that interpretation of hard tissue chemistry data would be more complicated than anticipated. Concerns about diagenesis, and the fact that age, sex, and physiological status (i.e., pregnant or lactating) affect the way that trace elements are incorporated into living tissues, lead to considerable uncertainty and skepticism (Buikstra et al., 1989; Sillen et al., 1989; Sandford, 1992; Radosevich, 1993; Ezzo, 1994a,b).

Several of the traditional criticisms of trace element analyses of bones and teeth have been directed specifically at research attempting to use zinc as a paleodietary indicator. Previous successes with modeling the trophic-level discrimination of strontium, whereby high Sr/Ca ratios are indicative of more vegetarian diets, and low Sr/Ca ratios are the result of more carnivorous diets (Brown, 1973), led researchers to investigate the possibility of using Zn/Ca ratios in a similar fashion (Gilbert, 1975). Like strontium, zinc has chemical properties that allow it to be absorbed into bone and dental tissues. Because animal products such as meat, eggs, dairy, and shellfish contribute the greatest amounts of dietary Zn (Cousins, 1996), it seemed plausible to use zinc in bones and teeth as an indicator of the protein or meat content of past diets. The assumption that high Zn concentrations or Zn/Ca ratios measured from skeletal remains can be used to identify those who consumed greater proportions of animal products (especially meat) is still employed by some, despite the fact that little is known about the pathways by which Zn enters the body and is ultimately taken up by hard tissues. Even less is known about how intake and uptake of zinc are related in individuals who are growing, suffering from disease, have extreme demands placed upon their bodies due to their daily workload, or are pregnant or lactating. Because Zn is an essential element whose homeostatic regulation by the body may shift due to any of these common life occurrences, it is not surprising that researchers have faced mixed results in terms of estimating the degree of carnivory of past peoples using zinc.

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Ezzo (1994a,b) considered at length the questionable theoretical underpinnings of models for using zinc as a paleodietary indicator. He has argued that the interpretation of trace elemental data often suffer from a lack of theoretical validity and a number or erroneous assumptions—including the foundational tenet that variation in the zinc concentrations of hard tissues actually represent real variation in dietary intake. The clinical nutrition literature abounds with reports from researchers conducting zinc supplementation studies in the hopes of elucidating the varying relationship between zinc consumption and absorption. Although some researchers have demonstrated higher plasma zinc levels and improved function (e.g., reduced growth stunting in children) due to the addition of zinc to traditional diets (Sazawal et al., 1998), more have found that supplementation of diets with zinc has no impact at all upon function (Caulfield et al., 1999; Tamura et al., 2003; Heinig et al., 2006). Controlled experiments with rats have also shown that zinc intake is not the best determinant of the zinc content of their bones (Weisstaub et al., 2003; Takasugi et al., 2007). Data derived from animal studies and contemporary human groups demonstrate that zinc intake and/or the amount of meat consumed is not a reliable predictor of zinc levels in various tissues, including bone. These findings support Ezzo's argument that zinc cannot be relied upon as an indicator of trophic-level separations. This does not seem surprising given his cautions against the use of essential elements, which are homeostatically regulated by the body to a large extent. However, the fact that zinc is under some homeostatic control has not precluded millions of people worldwide from experiencing the functional impairments associated with zinc deficiency (Prasad, 1983; Cousins, 1996; Hambidge, 2000). Clearly some variation exists in terms of how and why individuals absorb zinc; the question is, then, whether this variation is meaningful in terms of saying something about diet or nutrition in past populations.

Despite the lack of a strongly supported theoretical model for the relationship between zinc intake and uptake, and the thoughtful critiques of Ezzo and others (Buikstra et al., 1989; Sillen et al., 1989; Sandford, 1992; Burton and Price, 2000), some researchers have continued to argue that high amounts of zinc in bones and/or teeth reflect higher meat consumption (Vuorinen et al., 1996; Szostek and Glab, 2001; Szostek et al., 2003; Dobrovolskaya, 2005; Giorgi et al., 2005; Scarabino et al., 2006). The most convincing of these have demonstrated how their measures of zinc agree with other elemental, isotopic, archaeological, or environmental data to tell a cohesive story about the diets of a given group of people. It is just as common, however, for researchers to interpret zinc data on their own merits and without the benefit of multiple lines of evidence. Given the resiliency of assumptions regarding zinc in hard tissues, the conflicting results of research regarding variation in zinc concentrations and ratios, and the high interpretive stakes of using zinc data to comment on aspects of life in the past, it is necessary to probe further the question of how zinc intake is related to uptake.

This is the first study we are aware of to use modern controlled data from a human sample to test the hypothesis that hard tissue Zn levels reflect the amount and/or proportion of meat consumed in the diet. It uses longitudinal data regarding mothers' diets during pregnancy and lactation to explore variation in the Zn/Ca ratios of their infants' prenatal and early postnatal tooth enamel. This work will investigate whether maternal dietary zinc intake determines the Zn/Ca ratios of infants' enamel, and, if it does not, it will identify what other variables best explain variation in Zn/Ca ratios in this particular group of mild-to-moderately malnourished Mexican mother–infant dyads.

ZINC BIOCHEMISTRY AND BIOAVAILABILITY

Much has been made of the fact that zinc is an essential element and thus is required by humans for the purposes of growth, development, repair, and maintenance of bodily functions. The questions of how regulated Zn absorption from the diet is, what factors affect zinc absorption, and whether this bodes well for reconstructing anything about past diets can best be addressed through the exploration of recent research on zinc biochemistry and bioavailability in contemporary populations.

The zinc atom may easily lose two electrons ($Zn \rightarrow Zn^{2+} + 2e^-$), producing a small ion (74 pm) with a charge of 2^+ (which is also a strong electron acceptor). The ability of zinc atoms to both lose and accept electrons relatively easily allows zinc to be extremely useful in biological systems (Cousins, 1996). Zinc is the only metal that has a role in every class of enzyme, including RNA nucleotide transferases and polymerases. The single Zn atom at the base of a finger protein, for example, determines the protein's entire configuration and enables the initiation of the transcription process and gene expression. Zinc is also integral for proper cellular growth and differentiation, protects against infectious agents, is involved in regulation of synaptic signaling, and may act as a regulator of apoptotic cell death (Vallee and Falchuk, 1993; Cousins, 1996).

An average adult human contains between 1.5 and 2.5 g of zinc, with higher amounts in men than in women by weight (IZiNCG, 2004). Zinc is present in all organs, tissues, fluids and secretions of the body, yet, is mostly highly concentrated in the fat-free body masses. If total body zinc becomes depleted, the loss of zinc is more likely to decline in the bones, liver, testes and plasma, as opposed to the heart, skeletal muscle, and skin (Cousins, 1996).

During the development of bony and dental tissues, Zn^{2+} is incorporated into their hydroxyapatite lattice as an isovalent replacement for Ca^{2+} ions (Cutress, 1983). There is no "store" of zinc that can be released or reserved quickly in response to variations in dietary supply, but under circumstances of bone resorption and tissue catabolism zinc can be released and reutilized (Zlotkin and Cherian, 1988), particularly during pregnancy and lactation (Cross et al., 1995; Prentice, 2003). Bone does not resorb in response to dietary deficiency, but, during normal remodeling, less zinc may be redeposited in bone when an individual's dietary supply is very low (Zhou et al., 1993).

The zinc content of the body is maintained via a balance between absorption of dietary zinc (~5 mg/day) and endogenous excretion through an adaptive regulation stimulated by the supply of dietary zinc (Cousins, 1996; Hambidge, 2003). Released from food during digestion, free zinc ions are primarily taken up by the duodenum and proximal jejunum (Hsieh et al., 1983). The amount of intestinal absorption usually ranges from 20 to 30% but can also reach 60%, with the amount being determined by the previous dietary intake. Low zinc intake

TABLE 1. Dietary factors affecting Zn bioavailability and effects of zinc deficiency

Dietary sources	Inhibitors	Promoters	Functional consequences of deficiency
High	Phytate (+Ca)	Vitamin C	Growth stunting
Meat (beef > chicken)	Bran	(ascorbic acid)	Depressed appetite
Shellfish	Cereals	Vitamin A	Impaired cognition
Eggs, breast milk		Protein	Impaired reproduction
Moderate	Legumes		Impaired immune function
Green leafy vegetables	Soy protein		
Low	Tea, coffee		
Fats, oils, sugar, alcohol			

stimulates an increased efficacy of absorption and also a decrease in secretion, primarily by means of fecal excretion, of endogenous zinc (Hsieh et al., 1983; Hambidge, 2003). Even after several weeks of declining total zinc absorption, the activity of zinc-dependant processes may be maintained with substantial reductions in zinc losses (Hsieh et al., 1983).

Zinc uptake by hard tissues is determined not only by dietary factors, themselves influenced by socioeconomic status and cultural norms, but also by physiological variables such as the body's absorptive needs for maintenance, growth, and the overall rate of zinc loss. It is further influenced by one's sex, age, and reproductive status. For growing children and pregnant and lactating women, the zinc required to meet new tissue needs or breast milk production is added to the endogenous losses when calculating their overall physiologic requirements for zinc absorption (Moser-Veillon, 1995; King, 2000b). Full-term infants' absorption of zinc from hepatic reserves accumulated during gestation may also modify their needs for absorbed dietary zinc (Zlotkin and Cherian, 1988).

It is also necessary to be aware of competitive interactions between zinc and other ions with similar physio-chemical properties, such as calcium and iron. The results of animal trials and studies of contemporary human groups show that the ability of these elements to inhibit Zn absorption is contextual, with the general consensus being that their effects are only felt when the concentrations of Ca or Fe in the diet are very high relative to that of zinc (Vallee and Falchuk, 1993; Cousins, 1996; WHO, 1996).

To determine the proportion of zinc in the diet that is actually being absorbed by the intestine, it is also necessary to consider the overall dietary pattern. This requires an understanding of the sources of dietary zinc and of the physio-chemical interactions among dietary components that may affect its bioavailability. The term *bioavailability* refers to the availability of micronutrients for use by the body as a result of the interplay of dietary intake, elemental interactions, inhibitors, and enhancers.

Zinc occurs in a variety of foods, but its highest concentrations are found in animal-source foods such as the organs and tissues of cattle, chicken, fish and shellfish, with lesser amounts in eggs and dairy products (see Table 1). Concentrations are also relatively high in nuts, legumes, seeds, whole-grain cereals, and relatively low in tubers, refined cereals, fruits, and vegetables. Dietary factors that are known to alter the proportion of zinc available for absorption include phytate and dietary calcium (inhibit absorption) and protein (enhance absorption) (Smith et al., 1983; Lönnerdal, 2000). Phytic acid molecules and the salts that they form are generally referred to as "phytates"; strong chelators of zinc with significant inhibitory effects on zinc absorption (IZiNCG, 2004). Phytates are common in cereal grains, nuts, and legumes, with lower contents in other plant foods such as fruits, leaves, and other vegetables. Phytates cannot be digested or absorbed in the human intestinal tract, so the mineral to which they bind also passes through the intestine without being absorbed (Cousins, 1996; Gharib et al., 2006). High phytate:zinc molar ratios are thus indicative of foods with low zinc bioavailability (IZiNCG, 2004; Gharib et al., 2006). Given that nutrient intake, metabolic status, and overall dietary pattern may significantly alter the bioavailability of zinc, it is reasonable to expect that it would be difficult to consistently demonstrate a linkage between meat consumption and the zinc content of hard tissues. Possible connections between both the quantity of zinc consumed and the quality of dietary patterns to the zinc content of teeth will be investigated further here.

THE SOLÍS NCRSP

The individuals who donated teeth for this research live in six rural communities located in the Solís Valley of Mexico. This long and narrow valley, situated ~170 km northwest of Mexico City in the *altiplano*, is home to some 50 villages in total. Locals practice a form of peasant agriculture that relies upon a combination of small-scale maize agriculture, food gathering, food purchasing, wage labor, and male migration to Mexico City, the United States, and Canada. Fertility rates are high, with women giving birth to 8–12 children on average. The population has experienced greater life expectancies in recent decades, resulting in rapid population growth in a region characterized by a limited availability of land and economic opportunity. Common diseases such as respiratory and gastrointestinal infections are exacerbated by endemic malnutrition and a lack of infrastructural investment in combating environmental contamination (Saucedo et al., 1998).

Because the communities of the Solís Valley are typical in many ways of other poor rural Mexican villages, several communities within the valley were selected as sites of international and national development projects designed to study their health. A major research project, the Nutrition Collaborative Research Support Program (NCRSP), began in 1982 with the aim of "test[ing] whether chronic mild-to-moderate malnutrition affects functional outcomes of individuals" (Allen et al., 1992:1) in the six participating communities. The NCRSP longitudinal study examined the relationship between dietary intake and several functional outcomes such as growth, cognitive development, and morbidity. Several target groups (e.g., mother–child dyads, preschoolers, school-aged children) were followed for the duration of the project (1982–1984), with some individuals participating in follow-up research in 1987. A concerted effort was made to measure the daily food intake (via 24 h-dietary recalls and observation of meals, ingredients in recipes, and amounts consumed three times per month), heights, weights, skinfold thicknesses, health and socioeconomic status of all households, with special attention being focused upon pregnant and breastfeeding women (Allen et al., 1992). Many of the participants continued to be involved in follow-up research in the Solís Valley. One such project, directed by Alan Goodman in 1991–1993, involved the collection of exfoliated deciduous teeth from the NCRSP children (NIH R15 DEO9863).

Inhabitants of the Solís Valley in the 1980s ate a predominantly traditional Central Mexican diet based on maize, beans, chiles, and wild foods such as *nopales* (cactus leaves) (Backstrand, 1990). Despite documentation by NCRSP researchers of the consumption of over 300 different food items, only a few were eaten regularly. Tortillas were the most commonly consumed food in all Solís households, contributing an average of 60–70% of dietary energy, while only 6–7% of daily energy intake was derived from animal products such as eggs, chicken, and cow's milk (Backstrand, 1990; Allen et al., 1992). In the poorest households, tortillas provided up to 90% of the daily intake of calories and the consumption of animal products was almost nonexistent, as opposed to wealthier households where members consumed more animal products and fewer tortillas. No changes in the overall dietary pattern were demonstrated by pregnant women, although they did consume slightly more than their nonpregnant/nonlactating peers. Data collected from the NCRSP study indicated that Solís mothers (pregnant and lactating) consumed sufficient calories, yet suffered from micronutrient deficiency and mild-to-moderate malnutrition (Allen et al., 1992).

Given the ubiquity and diverse functions of zinc, it stands to reason that several aspects of human biology, such as physical growth (Prasad, 1985; Neggers et al., 1990; Hambidge, 2000), immune function (Bahl et al., 1998; Black, 1998; Fraker et al., 2000; Failla, 2003; Ibs and Rink, 2003), and cognitive/behavioral development (Bahl et al., 1998; Bhatnagar and Taneja, 2001; Krebs, 2003) would be affected by one's zinc status. Although recommended daily allowances (RDAs) of Zn are 5 mg/day for infants, and 15 mg/day for pregnant or lactating women (Picciano, 1996), the zinc intakes of pregnant Solís women were 12.6 $\pm$ 3.08 mg/day and 13.7 $\pm$ 3.57 mg/day for lactating women (Allen et al., 1992). Not only do the Solís mean intakes not meet RDA values, but also the actual uptake of Zn by mothers and their infants would have been further compromised by the high phytate and calcium content of their diets.

Being entirely reliant upon their mothers for the energy and nutrients they need for growth while in utero, and prior to the onset of weaning, infants' absorption of zinc in their deciduous enamel should theoretically reflect factors affecting their mothers' nutritional environments. Pregnant women's diets provided sufficient energy (quantity) and protein, yet at the same time suffered from poor nutrient bioavailability (quality) (Allen et al., 1992). Mother's consumption of the region's major food staple, tortillas, contributed from 51.3% to 75.9% of dietary energy during pregnancy and 35.5%–79.4% during lactation for the subsample of participants included in this present study. Because of the high household consumption of tortillas in Solís, the pregnant and lactating mothers sampled here consumed diets with phytate:zinc molar ratios ranging from 33.1 to 39.8. According to the guidelines of the Food and Nutrition Board/Institute of Medicine (FOB/IOM, 2002), a phytate:zinc molar ratio >15 indicates a diet with seriously impaired bioavailability. Thus, with ratios more than double the value used to indicate groups with impaired nutrient bioavailability, Solís women were clearly at risk of having little zinc available to pass on to their growing offspring.

MATERIALS AND METHODS

The trace element content of 37 anterior deciduous teeth representing 26 of the Solís NCRSP participants was assessed using laser ablation–inductively coupled plasma–mass spectrometry (LA-ICP-MS). When one individual provided multiple deciduous teeth, mean prenatal and mean postnatal values were calculated and used in further statistical analyses. Teeth deemed suitable for ablation were intact (i.e., not cracked or broken), and provided sufficient labial enamel (e.g., unworn or only slightly worn) for the analysis of prenatal and postnatal developmental periods within the enamel. The cleaning and embedding of each tooth and preparation of histological sections prior to elemental sampling were conducted following the standard protocols (Goodman and Rose, 1990). Once a longitudinal thin-section was removed from each tooth, the exposed surface of one embedded tooth half was prepared for LA-ICP-MS analysis. Each surface was cleaned using deionized-distilled water and polished using a new Buehler polishing cloth mounted on a high-speed wheel in order to remove saw marks.

Elemental analyses were conducted using a 266-nm Nd:YAG Cetac LSX-100 Laser Ablation System (Cetac Technologies, Omaha, NE), coupled with an ELAN 6000 ICP-MS (Perkin Elmer Instruments, Shelton, CT). Details regarding the instrument operating conditions, optimization procedures, and a photograph of an ablated tooth, have been published previously (Dolphin et al., 2005). The exposed surface of each tooth was oriented on the stage of a laser ablation cell so as to direct the trajectory of the laser within the preferred regions of enamel. Previous studies have shown that trace element values for prenatal and postnatal values differ significantly for this sample (see Goodman et al., 2003; Dolphin et al., 2005), representing that possible dietary or metabolic differences between, prenatal and postnatal regions of enamel were identified and sampled separately. Calibration curves comparing laser results (counts per second) with known concentrations of elements in calcium phosphate pressed pellets demonstrated good agreement (R^2 = 0.78–0.98), thus reducing concerns that differences in elemental ratios could be the result of fluctuations in laser parameters. These regions of the deciduous enamel were identified in relation to the location of the neonatal line as viewed via a video camera fitted with a zoom lens. Both the prenatal and the postnatal regions of enamel were discretely sampled for each tooth, with great care being taken to avoid surface enamel, micro-cracks in the enamel, any discolored areas, the dentine-enamel junction, and the neonatal line itself. This sampling method, discussed in greater detail elsewhere (Dolphin et al., 2005), involved ablating one line within the prenatal region of enamel and one line in the postnatal region.

Several elements were assessed simultaneously (^{25}Mg, ^{43}Ca, ^{66}Zn, ^{68}Zn, ^{88}Sr, ^{138}Ba, ^{208}Pb), however, only the results for ^{66}Zn and ^{68}Zn will be discussed here. Data were background-subtracted using the GEMOC GLITTER! 4.0 data reduction program (New Wave Research/ Merchantek Products, Freemont, CA) and adjusted for instrument drift using a National Institute of Standards and Technology (NIST612) glass standard. Adjustment for drift serves to further correct for fluctuations in laser parameters so that data are as representative of the actual enamel trace element contents as possible. Because calcium levels were uniform across regions of enamel and among individuals, and there was no calcium deficiency in this population, ^{43}Ca was used as an internal standard for the normalization of other elemental values. Some researchers choose to calibrate their intensity values (counts per second), however, there is considerable disagreement amongst LA-ICP-MS users regarding the question of what standard material is ideal for this purpose (Outridge and Evans, 1995; Craig et al., 2000; Neufeld and Roy, 2004; Bellis et al., 2006). Calcium is commonly used to normalize the counts per second values produced by laser ablation sampling, because this major element in tooth enamel has demonstrated high and relatively uniform levels throughout the enamel matrix (Cox et al., 1996; Lee et al., 1999). Thus, Zn/Ca ratios were used to facilitate intersample comparisons rather than concentrations. This approach is not recommended when making comparisons between samples analyzed with different instruments and/or operating parameters. Ratios derived from LA-ICP-MS data are appropriate and useful for relative comparisons between individuals and groups within this sample.

Once each individual datum point (~200 per ablation) was background subtracted, calcium normalized, and adjusted for drift, outliers (beyond ±3 standard deviations) were removed. Mean adjusted intensity ratios were then calculated for each prenatal and postnatal enamel sample per individual participant. The calculation of means was preferred in order to get a general picture of trace element levels in these developmentally distinct regions of enamel, rather than trying to pinpoint the exact timing of elemental fluctuations—which would prove difficult to investigate given that elements are incorporated during enamel secretion *and* during mineralization, which are offset in their timing by several weeks to months (Balasse, 2002).

The total amount of zinc in each sample was estimated based upon the data collected for the two isotopes, ^{66}Zn and ^{68}Zn. Because the percent natural abundance for ^{66}Zn is 27.90% and for ^{68}Zn is 18.75% (Rosman and Taylor, 1998), each total intensity ratio represented 46.65% of the total amount of zinc in the enamel sample. Knowing this, mean total zinc intensity ratios were calculated for each sample from each individual. These values were entered into the Solis NCRSP dataset alongside the corresponding data for each mother–infant dyad.

Statistical analyses were carried out using SPSS 16.0. Although many other variables pertaining to socioeconomic status, maternal anthropometry, maternal morbidity, infant anthropometry, infant morbidity, and infant cognitive development were examined in relation to the zinc intensity ratios calculated for each infant's prenatal and postnatal enamel (Dolphin, 2006), the focus here will be strictly upon maternal dietary variables during pregnancy and lactation. Spearman's rho bivariate correlations were calculated between multiple dietary variables and the enamel zinc intensity ratios because they are resistant to outliers and distribution problems.

RESULTS

Individuals' prenatal and postnatal Zn/Ca ratios often differed, with postnatal values generally being higher (mean = 0.050) than those for the prenatal enamel (mean = 0.020). Prenatal enamel values for Zn/Ca are less variable (standard deviation = 0.086) than for the postnatal period (standard deviation = 0.852). It could be suggested that this prenatal lack of variability reflects a buffering of the fetus from environmental fluctuations faced by their mother, such as those associated with diet. Although there may very well be a higher degree of prenatal buffering than is possible via breastfeeding after birth, the idea that buffering is so strong as to make it impossible to see signs of nutritional variation or stress does not hold for this population. For example, a high frequency of prenatal striae of Retzius has been documented for the Solís individuals included in this study (Acosta et al., 2003). Thus, the buffering argument only goes so far, and certainly leaves room for other explanations such as those related to maternal dietary quantity and quality.

Although the mean postnatal Zn/Ca ratio is higher than the mean from prenatal enamel, the difference is not statistically significant. However, because previous data published (Dolphin et al., 2005) from a larger Solís Valley dental sample ($N = 36$) did demonstrate a significant difference between prenatal and postnatal Zn/Ca ratios, these regions of enamel were sampled separately.

Pregnancy diet and prenatal enamel

Nutrient intake. Table 2 presents descriptive statistics for several nutrients measured from the diets of Solís Valley mothers during pregnancy. With these nutrients, it was possible to consider the impact of dietary quantity (energy), meat consumption (zinc), a possible zinc absorption inhibitor (calcium), and likely zinc absorption enhancers (protein, vitamins A and C) on enamel zinc values.

Bivariate correlations between the amount of these nutrients consumed by mothers during pregnancy and the prenatal Zn/Ca ratios of their children's dental enamel is also shown in Table 2. There are several nutrients that are significantly correlated with prenatal zinc, but interestingly, zinc intake is not one of them ($r = -0.337$; $P = 0.058$). Energy and protein intake during pregnancy are both negatively correlated with the zinc content of prenatal enamel. None of the remaining nutrients known to enhance zinc absorption (vitamins A and C) appear to have any effect on the Zn/Ca ratios in prenatal enamel.

Foods and food groups. Table 3 shows descriptive statistics for the percentages of total energy provided by various foods and food groups during pregnancy. Because Zn concentrations and Zn/Ca ratios are used to estimate the relative meat consumption of past populations, several animal products were included. Not only are animal products (especially meat) high in zinc, some products, such as dairy and eggs, provide most of the vitamin A in this diet, possibly helping to enhance the absorption of zinc. Tortillas (high phytate + calcium) and legumes were investigated because they are known to strongly inhibit zinc absorption, while *pulque* (a traditional alcoholic beverage derived from the *maguey* plant) was the most important source of another zinc absorption enhancer, vitamin C, in the diet.

TABLE 2. Descriptive statistics for pregnancy (N = 26 individuals) and lactation (N = 24 individuals) nutrient intakes, and their Spearman's rho correlation statistics when compared to prenatal enamel Zn/Ca and postnatal enamel Zn/Ca ratios, respectively

	Pregnancy					Prenatal Zn/Ca		Lactation					Postnatal Zn/Ca	
Nutrient intake	Mean	S.D.	Min	Max	% RDA	*r*	*P*	Mean	S.D.	Min	Max	% RDA	*r*	*P*
Energy (kcal)	2447.6	559.8	1730.1	3809.0	94.1	−0.0532	0.005*	2762.6	639.7	1663.0	4769.6	92.6	0.154	0.473
Protein (g)	68.8	16.7	50.2	114.8	111.0	−0.402	0.042*	71.8	18.59	45.2	140.0	104.3	0.200	0.349
Calcium (mg)	1727.3	404.0	1138.0	2754.0	93.1	−0.242	0.233	1901.9	538.8	1146.3	3664.6	101.2	0.121	0.574
Zinc (mg)	12.2	2.6	8.4	19.7	83.3	−0.377	0.058	13.0	3.2	8.1	23.4	78.3	0.194	0.363
Vitamin A (mg)	382.6	334.7	50.0	1405.2	59.9	−0.331	0.098	349.4	573.7	56.8	3000.5	51.3	0.024	0.910
Vitamin C (mg)	33.2	17.3	10.5	77.0	–	−0.374	0.060	38.4	18.1	8.8	72.9	–	−0.380	0.067

*Significant difference ≤ 0.05.

TABLE 3. Descriptive statistics for percentages of energy contributed by various food groups during pregnancy (N = 26 individuals) and lactation (N = 24 individuals)

	Pregnancy				Prenatal Zn/Ca		Lactation				Postnatal Zn/Ca	
% of Total Energy	Mean	S.D.	Min	Max	*r*	*P*	Mean	S.D.	Min	Max	*r*	*P*
Animal Products												
Meat	2.66	2.19	0.00	8.70	0.194	0.343	2.24	1.10	0.01	4.23	−0.127	0.554
Dairy	1.30	1.78	0.00	5.63	−0.478	0.013*	1.37	2.38	0.00	8.28	−0.046	0.829
Eggs	1.45	1.82	0.00	4.17	−0.327	0.103	1.36	0.78	0.00	2.74	0.028	0.897
All	6.10	3.41	1.06	16.09	−0.211	0.301	5.44	3.05	1.28	15.66	−0.147	0.493
Plant Products												
Tortillas	63.90	8.22	51.25	76.23	0.510	0.008*	62.36	6.18	45.45	79.41	0.377	0.070
Legumes	7.28	5.13	1.95	21.98	0.082	0.692	4.40	2.26	0.00	9.76	0.163	0.448
Pulque	6.53	6.68	0.00	24.60	−0.155	0.451	7.64	8.40	0.00	28.55	−0.288	0.172
All	93.90	3.41	83.91	98.94	0.211	0.301	94.56	3.05	84.34	98.72	0.147	0.493

Their Spearman's rho correlation statistics when compared to prenatal enamel Zn/Ca and postnatal enamel Zn/Ca ratios, respectively, are also shown.
*Significant difference ≤ 0.05.

Trends for this subsample of Solís mother–child dyads correspond with those previously identified by Solís NCRSP researchers. For example, only 6.1% of all energy consumed comes from animal products, with the remaining overwhelming contribution to the diet coming from plant sources (93.9%). Some Solìs mothers ate no meat, dairy, or eggs during their pregnancy at all. For those who did consume some animal products, meat provided roughly twice as much energy as did the dairy items or eggs. Tortillas were the staple food in the Solìs Valley, with pregnant mothers deriving as much as 51.25%–76.23% of their diet from this one plant-based food.

Spearman's rho bivariate correlations between percentages of total energy consumed by pregnant mothers and the Zn/Ca ratios of their infants' prenatal enamel are presented in Table 3. Despite the premise in paleodietary research that zinc concentrations and Zn/Ca ratios can be used to reconstruct the meat or animal product component of the diet, there are no statistically significant correlations between mothers' meat intakes ($r = 0.194$; $P = 0.343$) or animal product intakes ($r = -0.211$; $P = 0.301$) and the zinc content of their children's prenatal enamel, with the exception of dairy products ($r = -0.478$; $P = 0.013$). Given that tortillas are known to inhibit zinc absorption because of their high phytate content, and that tortillas are the primary food staple in the Solís diet, is not surprising that the percentage of tortilla-derived energy is significantly correlated with prenatal enamel Zn/Ca ratios ($r = 0.510$; $P = 0.008$). It is important to note that the correlation between these variables is a positive one—with high tortilla intake (high phytate + calcium) among pregnant mothers being linked to high Zn/Ca ratios in their infants' prenatal enamel. Thus, despite the fact that tortillas reduce zinc bioavailability, they appear to be associated with an increased absorption of zinc in the enamel forming during gestation. No other plants, nor the plant product group as a whole, were correlated with the zinc content of prenatal enamel.

Lactation diet and early postnatal enamel

Nutrient intake. In Table 2, descriptive statistics for mothers' nutrient intakes during lactation are shown. The mean amount of each nutrient, except for protein and calcium, is below the RDA. The %RDA values are generally lower during lactation than pregnancy, despite the fact that the overall dietary pattern for mothers did not change (Allen et al., 1992).

Bivariate correlations presented in Table 2 illustrate the relationships among nutrients consumed by mothers during lactation and the Zn/Ca ratios of their infants' early postnatal enamel. There are no significant correlations between nutrient intakes and the zinc content of the postnatal tooth enamel. Even nutrients that were correlated with enamel zinc during pregnancy, such as energy (dietary quantity) and protein, do not approach significance.

Foods and food groups. Table 3 includes descriptive statistics for the percentages of total energy provided by various foods and food groups during lactation. As with pregnancy diets, lactation diets were heavily reliant upon plant products for the majority of energy consumed (94.56%). The primary food consumed was tortillas, with mothers taking from 45.46% to 79.41% of their energy from this one food item. The mean percentage of energy from tortillas did not change significantly from

TABLE 4. Spearman's rho correlations between mothers' pregnancy nutrient intake and the Zn/Ca intensity ratios of their infants' early postnatal enamel (N = 26)

	Postnatal total enamel zinc	
Pregnancy nutrients	*r*	*P*
Energy	−0.089	0.665
Protein	−0.047	0.820
Calcium	0.037	0.859
Zinc	−0.090	0.662
Vitamin A	−0.354	0.076
Vitamin C	−0.271	0.180

Significant difference ≤ 0.05.

TABLE 5. Spearman's rho correlations between percentages of total energy provided to mothers by various food groups consumed during pregnancy and the Zn/Ca intensity ratios of their infants' early postnatal enamel (N = 26)

	Postnatal total enamel zinc	
Pregnancy % of total energy	*r*	*P*
Animal Products		
Meat	−0.052	0.801
Dairy	−0.061	0.768
Eggs	−0.100	0.626
All	−0.117	0.571
Plant Products		
Tortillas	0.031	0.880
Legumes	0.234	0.250
Pulque	−0.303	0.133
All	0.117	0.571

Significant difference ≤ 0.05.

pregnancy to lactation, nor was there any major increase or decrease of other foods and food groups. The very low consumption of animal products (mean = 5.44%) during lactation is dominated by the intake of meat, dairy and eggs, as it was during pregnancy. These findings agree with those of the larger Solís NCRSP study in that the dietary pattern or quality remained consistent throughout pregnancy and lactation.

Spearman's rho bivariate correlations between percentages of total energy provided to mothers by various foods and food groups consumed during lactation and the Zn/Ca intensity ratios of their infants' early postnatal enamel are presented in Table 3. There are no statistically significant relationships between any of the dietary variables and the zinc content of the tooth enamel. Despite the fact that it is often argued that the zinc content of hard tissues reflects dietary intake, there is no evidence for this when lactation diets and the early postnatal enamel of breastfed infants' are compared. This also means that none of the known inhibitors or enhancers of zinc absorption seem to have an effect on zinc uptake by enamel during the first months of life.

Pregnancy diet and early postnatal enamel

Nutrient intake. If lactation diets do not influence the zinc content of early postnatal enamel for the mother–infant pairs, it is worth considering whether the Zn/Ca ratios from this developmental period are instead influenced by nutrient intake and dietary patterns during pregnancy. Although the postnatal enamel clearly does not develop during pregnancy, infants do build stores of zinc in their livers during gestation (Krebs, 1999; Prentice, 2003), which are drawn upon to supplement the nutrients provided to them via breastmilk. This phenomenon may link the mothers' pregnancy diets with the availability of zinc to their infants shortly after birth.

Table 4 shows the results of spearman's rho bivariate correlations testing the relationship between mothers' intakes of nutrients during pregnancy and the Zn/Ca intensity ratios of their infants' early postnatal enamel. Neither variables associated with dietary quantity (energy) nor those that influence dietary quality (protein, calcium, Vitamin A and C) are significantly correlated with the early prenatal enamel zinc values. Zinc intake during pregnancy is also not linked to the zinc content of postnatal enamel.

Foods and food groups. Bivariate correlations between percentages of total energy provided to mothers by various food groups consumed during pregnancy and the Zn/Ca ratios of their infants' early postnatal enamel are presented in Table 5. There are no significant differences between any dietary variables and the enamel Zn/Ca ratios, thus it appears that those foods and food groups known to inhibit or enhance zinc bioavailability do not have any major effect on zinc uptake by developing postnatal enamel when they are consumed during pregnancy.

DISCUSSION

The data presented here demonstrate the complexity of the relationships among nutrient intakes, foods, and food groups and the zinc content of human dental enamel. Although maternal diets during pregnancy and lactation were virtually identical, the impact of these diets on the Zn/Ca ratios of tooth enamel formed by their infants during these periods was not. Prenatal Zn/Ca ratios were linked to the dietary intake of some nutrients (energy and protein) and some specific foods (tortillas and dairy), but not with the relative contribution of total animal products or total plant products. Postnatal enamel Zn/Ca ratios were not correlated with any dietary variables, whether they reflected consumption during the period of postnatal enamel formation (during breastfeeding) or not (during gestation). Based upon these results, it is possible to comment on what the limitations and possibilities are for the reconstruction of paleodiets from zinc concentrations or Zn/Ca ratios in hard tissues.

Zinc as a trophic level indicator

Zinc is still employed as a paleodietary indicator by some, despite mixed results and the cautions of several researchers, thus a goal of this article was to test whether zinc in tooth enamel truly reflects an individual's consumption of meat. If the assumption that zinc in hard tissues reflects meat or animal product consumption were to hold, it would be reasonable to expect that there would be a significant relationship between the relative intake of meat, or animal products as a general food group, and the zinc content of teeth formed while that diet was being consumed. The results presented here do little to support this assumption as mothers' meat consumption during pregnancy and lactation was not correlated with prenatal or postnatal enamel Zn/Ca ratios. Mothers' zinc intakes, derived from both animal and plant sources, also did not demonstrate any relationship to the zinc content of their infants' teeth.

Evidently, the simple trophic level model does not apply in this case. In fact, the only animal product variable that was significantly correlated with Zn/Ca ratios in teeth was dairy, which primarily reflects the intake of cow's milk. It is argued by some that dairy products enhance the absorption of zinc (Backstrand et al., 2002), in part due to its high concentration of vitamin A. Although the calcium in dairy products does not necessarily inhibit zinc absorption, when considering high phytate diets, the presence of calcium can contribute to the inhibitory effects of phytate (Lönnerdal, 2000). However, for this sample, calcium intakes were not correlated with the zinc content of prenatal enamel. It seems then that something about dairy products, other than their calcium component, was potentially affecting the uptake of zinc by infants' prenatal enamel. Knowing that cow's milk provided the majority of vitamin A in the Solís diet (Allen et al., 1992), this may explain the association between dairy intake and enamel zinc levels. Although dairy products certainly would have contributed to the calcium component of the diet, their effects would likely have been outweighed by addition of lime (calcium carbonate) to the ground corn used in making tortillas.

It could be argued, given that Zn/Ca ratios are being used to assess the elemental composition of the Solís deciduous tooth sample, that it is variation in the Ca component of mothers' diets that is responsible for the high levels of Zn in infants' prenatal enamel for those consuming the poorest quality diets. Calcium deficiency in rats has been shown to result in increased Zn utilization by bone (Weisstaub et al., 2003), so perhaps the Ca content of the diet is a better predictor of Zn uptake in enamel. In the Solís sample, however, mothers do not appear to have suffered from even moderate Ca deficiency as they reached their RDA for Ca intake during lactation (101.2% of RDA) and approached it during pregnancy (93.1%) (see Tables 2 and 3). As mentioned above, there are also no significant correlations between Ca intake and Zn levels in prenatal or postnatal enamel (see Tables 2 and 4). None of the data derived from this current study support the argument that Ca is the driving force in determining the Zn content of enamel formed while in utero, but it is a process that will be addressed in future studies with populations consuming more diverse diets with respect to Ca intake.

It appears then, that for the Solís mothers and infants, the total amount of zinc and meat consumption has less influence on the zinc content of prenatal or postnatal enamel than the enhancing effects of protein and vitamin A on the absorption of zinc. The lack of a clear connection between zinc and/or animal product intake and Zn levels in enamel suggests that one, or more likely several, of the other factors implicated in Zn absorption by hard tissues may be responsible for the variation in Zn/Ca ratios within this population.

Zinc as an indicator of bioavailability

Although the mechanisms governing zinc absorption are not fully understood, research has demonstrated that absorption is not determined by zinc intake alone. A combination of inhibitors and enhancers influence the bioavailability of zinc, or the amount of zinc that will actually be available for uptake by tissues. As such, it would stand to reason that the zinc content of hard tissues such as teeth would more likely reflect the *quality* of the mothers' overall diets than the *quantity* of zinc they consumed. The data presented here support this hypothesis when linkages between pregnancy diets and prenatal enamel are considered. For example, the protein intake and percentage contribution of dairy products, both known zinc absorption enhancers, were significantly correlated with Zn/Ca ratios in tooth enamel (vitamins A and C were not). Of the zinc inhibitors, legumes were not linked to the zinc content of prenatal enamel, but the percentage of tortilla consumption was. These associations, in light of the lack of correlations between zinc or meat intake, suggest that the Zn/Ca ratios of prenatal enamel provide a better retrospective measure of mothers' dietary zinc bioavailability and their overall nutritional status than of the degree of carnivory for this population.

One puzzling aspect of the data is that zinc absorption enhancers (dairy and protein) are negatively correlated with the zinc content of infants' teeth, while the major zinc absorption inhibitor (tortillas) is positively correlated with Zn/Ca ratios. The question then is: why are the poorest Solís mothers, who consume the largest proportions of tortillas, and, as a result, the highest amounts of zinc inhibiting phytates, giving birth to children who have absorbed the most zinc into their prenatal enamel? If these mothers have the poorest quality diets and severely impaired zinc bioavailability, and their children were ultimately at a greater risk for growth stunting in the months after birth (Allen et al., 1992), it seems entirely contradictory that their infants would have absorbed the most zinc during gestation. However, if the special circumstances concerning zinc absorption during pregnancy and lactation are considered, these results become more comprehensible.

Complexity of zinc metabolism

Many physiological changes occur during pregnancy and lactation, including shifts in the metabolism of nutrients such as zinc. These shifts will vary depending upon the mother's lifestyle, health, and her prior nutritional status, as well as genetic determinants of fetal needs for growth (King, 2000a). Within a few weeks of conception, the placenta secretes hormones that alter nutrient metabolism in such a way that helps to maintain maternal homeostasis in light of increased zinc needs during pregnancy and in preparation for lactation (King, 2000b). More zinc must be made available for fetal and maternal tissue growth, fetal mineral accretion/storage, the production of amniotic fluid, and then for the production of breastmilk (Swanson and King, 1987; Krebs, 1999). During pregnancy the amount of zinc absorption is estimated to be 3.2 mg/day as opposed to 2.5 mg/day for nonpregnant women, with the need rising to 4.5 mg/day for women who are lactating (IZiNCG, 2004).

There are several adaptive biological strategies for meeting increased demands for zinc. Increased food consumption, improved intestinal absorption, decreased excretion, and mobilization of long-term zinc stores in bone (during pregnancy and lactation) all work together to help the mother achieve sufficient zinc absorption (Prentice, 2003). Women do not typically increase their zinc intake during pregnancy (King, 2000a), as was the case for the Solís Valley mothers, thus it has been argued that reduced excretion and enhanced zinc absorption are primarily responsible for meeting the zinc needs of the mother and child during pregnancy (Prentice, 2003; Donangelo et al., 2005).

Two studies in particular provide insight into the processes of shifting zinc absorption among pregnant and lactating women. In the first, Fung et al. (1997) used isotopic tracers to examine changes in fractional zinc absorption (FZA) from prepregnancy, into pregnancy, and during lactation for a group of Californian women who consumed a good quality diet. FZA was estimated from isotopic enrichment of urine, and the relationship to maternal plasma zinc status was assessed. The authors found that FZA was negatively correlated with plasma zinc during the third trimester, while the FZA during lactation was unrelated to zinc concentrations in breast milk. Thus, these findings indicated that those individuals with lower zinc levels in their blood during pregnancy were those who ultimately absorbed the most zinc when compared to their high plasma zinc peers. Also, the notion that mechanisms regulating zinc homeostasis differ between pregnancy and lactation was supported (Fung et al., 1997).

The second study also used FZA to assess plasma zinc concentrations throughout pregnancy/lactation (Donangelo et al., 2005). This work focused on a sample of Brazilian women who habitually consumed a marginal zinc diet with a high phytate:zinc molar ratio. FZA was found to increase significantly during pregnancy and lactation. The greatest increases in absorption were experienced by those women who consumed the poorest quality diets. Donangelo et al. suggest that this inverse relationship between FZA and plasma zinc concentrations acts to provide enough zinc to new tissues such that the fetus is buffered from impaired growth and poor quality breastmilk (2005).

High prenatal Zn/Ca ratios in the teeth of Solís Valley infants, whose mothers consumed the poorest quality diets, are less puzzling in light of what is currently known regarding shifting metabolic mechanisms related to reproduction. The argument that these mechanisms serve to protect the growing fetus from impaired growth and function is supported by the fact that almost all Solís infants were only very slightly below international standards regarding length, weight, and weight-for-length at birth (Allen et al., 1992)—even if their mothers had diets with the poorest zinc bioavailability.

The work of Fung et al. (1997), Donangelo et al. (2005), Prentice (2003), and King (2000a,b) may also provide some perspective on why correlations were found between maternal pregnancy diets and infants' prenatal enamel Zn/Ca ratios, but not between lactation diets and early postnatal zinc values. Because infants draw from multiple sources of nutrients after birth, including breast milk, hepatic zinc stores, and zinc mobilized from their mothers bones, it may likely be even more difficult to link early postnatal Zn/Ca ratios in tooth enamel to mother's diet alone. This is not to say that bone remodeling during pregnancy would not contribute Zn from maternal reserves, but that this turnover is greater during lactation (Cross et al., 1995; Gulson et al., 1998). It should also be mentioned that, although most Solís Valley mothers were still breastfeeding at 6 months, some weanling foods had begun to be introduced by this point, and the canines included in this sample would have developed until 9 months (well into the weaning period). Thus, the inclusion of canines in the sample may have contributed to a slight clouding of associations during the lactation period.

Future directions

The data presented here do not support a link between dietary intake and the Zn content of hard tissues, yet they have stimulated some discussion of the possible impacts of Zn bioavailability and reproductive status in determining these values. Fortunately, it is possible to push further in testing the relationships between dietary quality and Zn absorption. For example, the authors are currently comparing the findings of this study with equivalent data derived from an equally well-controlled sample. A sister project to the Solís NCRSP was conducted at the same time in Kalama, Egypt, whereby longitudinal data for the same variables, using the same methods, and overseen by the same team of researchers were collected. Deciduous teeth were also collected from the children of those women who participated in the Kalama study, and have been sampled using the same LA-ICP-MS methodology outlined here for the Solís sample. Because Kalama individuals ate more meat than the individuals from Solís, and consumed foods with much lower phytate:zinc molar ratios, a comparison of data derived from these two populations should provide fuller insights into the link between meat consumption, Zn bioavailability, and Zn/Ca ratios in teeth. Other plans for these two samples include conducting analyses of strontium and barium concentrations in the deciduous enamel of these teeth in order to provide alternative lines of evidence regarding trophic level distinctions within and between them.

More work is needed to ascertain the full range of conditions under which Zn may and may not be useful as a dietary indicator. The results of this study speak to the association between meat consumption and Zn/Ca ratios in enamel formed during pregnancy and the first months of the postnatal period only. These results may not apply to all individuals at all stages of life, and we would recommend that similar research with samples of teeth representing later postnatal periods, such as the third molar, be pursued in order to address this issue more fully.

CONCLUSIONS

Here, we have presented findings from the first study in humans of the effect of dietary intakes and dietary quality on the Zn content of enamel. Longitudinal data collected from a contemporary group of Mexican mothers and their infants were used to test whether the zinc content of dental enamel reflects the proportion of meat consumed in the diet. Neither prenatal nor postnatal Zn/Ca ratios of tooth enamel reflected the relative contribution of meat, animal products, or zinc to the diet. Further, it was determined that Zn/Ca ratios of prenatal enamel were indicative of the overall zinc bioavailability (quality) of mother's diets during pregnancy. Distinct results for the prenatal and postnatal enamel correspond with literature regarding the different sources of zinc that are accessed during periods of fetal/infant development. As such, this work indicates that techniques for the microsampling of discrete developmental regions in teeth can reveal new information that is not possible via traditional bulk analyses. The results of this research also demonstrate that a consideration of the metabolic state of each individual is essential if one hopes to responsibly interpret chemical data retrieved from their hard tissues.

The relationships between diet, nutrition, metabolic status, and the zinc content of tooth enamel presented here are complex and do not support traditional trophic level arguments regarding the meaning of zinc concentrations and ratios in bones and teeth. Even with some general understanding of the kinds of foods consumed by past peoples, how they were prepared, and the relative contribution of particular food groups to the diet, along with information regarding the individuals' disease burden and metabolic status, it would be very difficult to assess the meaning of zinc values in hard tissues. In the majority of cases, this level of information is not available to researchers working with skeletal and dental remains, and this study demonstrates that understanding how and why Zn levels in teeth are determined is not easily done even with living populations. Future research with other populations having longitudinal dietary, health and growth data, as well as associated tooth banks, and groups that consume more varied diets, should enable a fuller exploration of the meaning, for bioarchaeologists, of trace element concentrations in human hard tissues.

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