Nutritional supplementation and the development of linear enamel hypoplasias in children from Tezonteopan, Mexico[1-3]

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ABSTRACT The purpose of this study was to compare the effect of nutritional intake during tooth-crown formation on the subsequent development of linear enamel hypoplasias (LEHs) in Mexican nonsupplemented (control) adolescents (n = 42) and adolescents who had received daily nutritional supplements since birth (n = 42). The proportion of individuals with LEHs was nearly two-fold greater (74.4%; 95% CI 64.7–84.1%) in the control than in the supplemented group (39.5%; 95% CI 28.6–50.4%; χ^2 = 9.44; P = 0.001). Although the estimated peak age at formation, ~2–2.5 y, is similar in both groups, the proportion of early (before 1.5 y) and late (after 3.0 y) LEHs was greater in the control group. LEH was also more common in females and was associated with an increase in illness days and a decrease in growth velocity. Results of this study suggest that mild to moderate undernutrition during enamel formation is causally linked to the formation of LEHs. *Am J Clin Nutr* 1991;53:773–81.

KEY WORDS Nutritional status, chronic malnutrition, dental development, dental enamel hypoplasia, Mexico, developing countries

Introduction

Enamel is an epithelial tissue with a variety of unique developmental characteristics. It is highly susceptible to a wide variety of physiological perturbations (stressors) during amelogenesis (1–3). Because enamel is unable to remodel, the record of past physiological perturbations is semipermanently recorded in the pattern of developmental defects of enamel (2–5). Finally, because of the regular and ring-like nature of enamel apposition, the locations of these developmental defects on tooth crowns provides an estimate of individuals' ages at the time of physiological disruption (3, 4, 6). Because of the unique characteristics of enamel, it can yield a relatively permanent and chronologic record of physiological stress, potentially useful in epidemiological studies. However, for this potential to be more fully realized, the relationship between enamel development and common stressors affecting infants and children during tooth-crown development needs to be better understood.

Experimental studies have shown that severe deficiencies of micronutrients, along with a wide variety of other systemic physiological stressors, can lead to an enamel hypoplasia (7–9), a developmental defect caused by a disruption in enamel-matrix apposition (3–5). For example, M Mellanby (10) demonstrated that vitamin D deficiency could lead to an enamel hypoplasia in dogs, with the severity of the dental defect roughly associated with the intensity of deficiency. Similarly, H Mellanby (11) demonstrated that the degree of developmental disruption in enamel in offspring of vitamin A–deficient rats was associated with the duration of maternal, pregestational vitamin A deficiency. Experimental studies have shown that protein and energy deficiencies can lead to delayed dental development and reduced size and weights of teeth (12–14). Work by Navia and colleagues (15, 16) demonstrated the separate effects of protein and energy restriction on tooth size and eruption during key periods of development.

A variety of human studies suggests a relationship between enamel hypoplasias and protein-energy malnutrition (PEM). These defects have been frequently noted in prehistoric and historic populations believed to be suffering from mild to moderate degrees of undernutrition. Swardstedt (17) showed that the prevalence of these defects was inversely related to socioeconomic status in a medieval Swedish population from Westerhus, and Goodman et al (18, 19) found an increased prevalence of defects that paralleled a temporal increase in other indicators of early undernutrition, such as a decreased growth velocity, at Dickson Mounds, IL (~950–1300 AD). Analyses of the skeletons of former 17th–19th-century slaves from Barbados (20) and inhabitants of a 19th-century upper New York state poorhouse (21) have shown high prevalence of enamel hypoplasias. Nearly all (92.3%) permanent dentitions of African Americans (many of whom were former slaves) who were buried in the First African Baptist Church cemetery in Philadelphia (1823–1843) displayed one or more enamel hypoplasias (22).

More direct evidence for the relationship between undernutrition and enamel hypoplasias has been noted in studies of contemporary populations. Sweeney et al (23) found an increased prevalence of enamel hypoplasia on deciduous incisors of Guatemalan children with third- vs second-degree malnutrition, whereas Sawyer and Nwoku (24) found that children hospitalized in Nigeria for undernutrition displayed an increased prevalence

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of severe enamel hypoplasia compared with well-nourished control subjects. Enwonwu (25), in a study of Yoruba children from Western Nigeria, did not find any gross enamel hypoplasias of deciduous teeth in "well-fed" children, whereas he noted an increased prevalence of defects in village children with poorer diets and lower socioeconomic status. He concluded that enamel development was susceptible to disruptions from stressors associated with low socioeconomic status, with severe malnutrition being prominent among these factors (25).

Studies based mainly on the more severe forms of malnutrition and on deciduous teeth were extended to milder forms of malnutrition and permanent teeth in a study in Mexico by Goodman et al (26). These authors found a high prevalence (46.7%) of enamel hypoplasias on permanent teeth of children from the Solis Valley and determined that on the basis of their position on the anterior teeth, most enamel hypoplasias developed in the second to third year. Also noted were statistical associations between the presence of an enamel hypoplasia and low height-for-age and socioeconomic status (27).

Whereas all these studies suggest a causal relationship between enamel hypoplasias and poor nutrition (and other stresses associated with low socioeconomic status), no studies have examined nutritional intake or status at the time of enamel development and related it in longitudinal fashion to the prevalence and pattern of enamel defects. This study is based on a follow-up of children from a long-term nutritional-supplementation study. It was designed to help answer the question of whether chronic mild to moderate undernutrition at the time of enamel development and of a degree frequently found in many developing areas of the world predisposes people to have an increased frequency of different types of enamel defects. The specific purposes of this study are *1*) to examine the risk of developing a linear enamel hypoplasia (LEH) in a group of control children characterized as having mild to moderate malnutrition, compared with a supplemented group of children residing in the same community but with improved nutritional status; *2*) to estimate and compare the age at development of LEHs in the nonsupplemented and supplemented children; *3*) to compare the pattern and prevalence of LEH to both past and current anthropometric measures; and *4*) to examine the role of respiratory and diarrheal disease as an additional variable that may further explain the relationship between nutritional status and the development of an enamel hypoplasia.

Subjects and methods

The nutritional-supplementation study

The sample was derived from people enrolled in Proyecto Puebla, a continuously operating nutritional supplementation program named after the state of Puebla and initiated by the Mexican National Institute of Nutrition in 1968 (28). Proyecto Puebla was designed to provide information on the effect of chronic undernutrition on functions such as disease resistance, growth, physical activity, and cognition.

Subjects in this study all reside in the rural Nahuatl (Aztec) Indian community of Tezonteopan, 180 km to the south of Mexico City and 25 km to the north of Atlixco in the central Mexican highlands, ~1600 m above sea level (28). Tezonteopan was originally selected for study because it was relatively isolated, with little migration, and typically poor, with inhabitants engaging in subsistence agriculture and eating a traditional diet based on maize and beans. About 30% of families own land, 40% belong to collectives (ejidos), 15% rent land, and 15% are landless day laborers. The nearest paved road is ~9 km from the town (28).

In 1968, the year in which the first study participants were born, 1495 individuals resided in Tezonteopan (43.8% aged < 15 y), with natality and mortality rates of 55.0/1000 and 14.5/1000, respectively, (28) and a mean yearly per capita income of $55 US (28). The majority of houses consisted of single rooms (84.8%) without electricity or plumbing (28). The town's water contained 0.5 mg fluoride/L (samples drawn in June 1988 and January 1990; N Tinnanoff, unpublished observations).

A 48-h weighted dietary survey that was completed at the start of the supplementation study indicated that 69% of energy was derived from tortillas, 11% from black beans, and 10% from sugar (28). The mean daily protein and energy intakes for all individuals in the community was 55.6 g [net protein utilization (NPU) 63%; NPU = ([nitrogen intake − nitrogen excretion]/nitrogen intake) × 100] and 8280 kJ, respectively. Preschool children consumed a similar diet with a daily average of 3030 kJ and 19.2 g protein, far below recommended levels (28). Chavez and Martinez (28) suggested that the population's mean energy and protein intakes are 10% and 37% below recommended levels and that the preschoolers are even further below the Mexican National Institute of Nutrition's recommended levels of 5225 kJ and 32 g protein.

The dietary data are supported by a cross-sectional growth study undertaken in 1977. Almost three-fourths (73.7%) of children between 1 and 4 y of age (n = 426) were found to be below 90% of the Mexican population's median reference values for weight-for-age (28; R Galvan, unpublished observations, 1972). The majority of these children was considered to have mild malnutrition [47.4%; Gomez classification (26) of 75–90% of weight-for-age] or moderate malnutrition (21.1%; 60–75% weight-for-age). A greater proportion of the females (31.5%) than males (21.2%) was classified as having second- or third-degree malnutrition (28).

The basic design of the supplementation study of Chavez and Martinez (28) involved the recruitment of two groups of mother-child dyads. The nonsupplemented group was recruited beginning in 1968. To be included in this group, mothers had to be free of obvious diseases, to have had between one and four pregnancies, to be between 18 and 36 y of age, and to be within ±1 Z score of the mean height (137–154 cm) and socioeconomic status for the community. Newborns needed to have a birth weight > 2.5 kg, to be free of disease at birth, and to receive an APGAR score (a measure of neonatal physiological status) of ≥ 8 (28).

Beginning the following year, the individuals in the supplemented group were recruited by use of the same criteria for inclusion as were used for the control subjects. Great effort was made to match as closely as possible the nonsupplemented individuals on a variety of criteria including family socioeconomic status, maternal weight and height, and infant's birth weight (28). This study was justified mainly because of a dearth of knowledge on the affect of moderate malnutrition on the growing child (28). Its protocol was approved by the Mexican National Institute of Nutrition's human subjects committee (28).

The nutritional supplement, in the form of a drink made by adding 64 g powdered milk with vitamin fortification and fla-

voring to 180 mL water, was given daily ad libitum after the first missed menstruation. Supplements were given to the mother during pregnancy and lactation and, thereafter, to the infant. The amount consumed was monitored and recorded (28). The milk was low fat (1.5%) during pregnancy and whole during lactation (LV Schlaepfer, unpublished observations, 1986). The supplement contributed a mean extra 915 kJ and 21.6 g protein/d during pregnancy and 1330 kJ and 17.7 g protein/d during lactation. The vitamin fortification contributed an extra daily dose of ~100 000 IU vitamin A, 20 mg thiamin, 10 mg riboflavin, 200 mg nicotinamide, 100 mg ascorbic acid, and 20 000 IU vitamin D (28; LV Schlaepfer, unpublished observations, 1986).

After ~4 mo the supplement was given directly to the child in the form of a bottle of reconstituted powdered whole milk (180 mL) and commercial semisolid baby foods. Later, sandwiches and glasses of low-fat milk were given to the children (28). The supplement given directly to the infants and children roughly contributed an extra 1254 kJ and an extra 20 g protein/d (28; LV Schlaepfer, unpublished observations, 1986). Supplementation has continued into adolescence; however, the amount consumed has decreased mainly because of the inconvenience of having to ingest the supplement at the Tezonteopan clinic.

Previous studies showed that the nutritional supplement had a profound effect on such things as growth, activity levels, and percent of days ill (28–31; LV Schlaepfer, unpublished observations, 1986). Physical activity was greatly increased in the supplemented group after the first year of life (28, 29). The incidence of parasitic and diarrheal diseases before 3 y of age was ~30% greater in the nonsupplemented group, and the nonsupplemented group suffered from nearly twice the number of days ill because of increased duration of illness (28, 30). Differences in growth between groups were profound. Height differed by nearly 10 cm at 2 y of age (28, 31; LV Schlaepfer, unpublished observations, 1986).

The dental study

The field recording of enamel defects was undertaken in June 1988. All available study participants who were aged ≥ 10 y ($\bar{x}$ age 15.5 y) came to the clinic for a dental cleaning and examination for developmental defects of enamel. A total of 84 individuals were examined, equally divided between the supplemented (n = 42; 21 male, 21 female) and nonsupplemented (n = 42; 21 male, 21 female) groups. On the basis of research in Solis, Mexico (26), we expected a LEH prevalence of ~60% in the control subjects and determined that a 20% decrease due to the supplement would be statistically detectible with a statistical-power measure of ~0.70.

Dental-enamel defects were recorded by type and location on the labial surfaces of all 12 anterior, permanent teeth. The recording of defect type followed the epidemiological standard for classification of developmental defects of dental enamel (DDE index) of the Federation Dentaire International (32). Previous studies in Mexico and elsewhere found this classification method high reliable (26, 33–35). The recorder was always blind to whether an individual received nutritional supplements. The protocol for this dental follow-up study was approved by the human subject committees of both the Mexican National Institute of Nutrition and Hampshire College.

Five types of defects were found: three types of dental opacities (demarcated opacities, diffuse opacities with fine lines, and diffuse opacities without lines) and two types of quantitative defects [pits-type hypoplasias and horizontal-line, or groove, hypoplasias (LEHs)]. LEHs were most often thin, horizontal bands of decreased enamel thickness, occasionally involving an area of totally missing enamel (combined FDI types 4 and 6). This paper only concerns LEH-type defects, which have been focused on the most in previous studies and are most likely to be due to systemic stressors such as undernutrition (2–4, 17–27).

After identification of defect types, all defects were drawn onto a tooth diagram to reflect their size and location (26). Missing teeth and areas of incomplete eruption or attrition were also noted on the tooth diagram. From these data, chronologies of defects were constructed by developmental ages at formation. By following the dental-development standard of Massler et al (36), incisor tooth crowns were divided into equal-width, half-year-long development zones and canine tooth crowns were divided into equal-width, year-long developmental zones. For example, the maxillary central incisor crown, which develops between birth and age 4.5 y, was divided into nine zones (birth–0.5 y, 0.5–1.0 y . . . 4.0–4.5 y) starting at the incisal edge and ending at the cemento-enamel line (26). The methodology for estimating the location of a defect on tooth crowns was shown to be highly repeatable (interobserver agreement 88.9%) in previous studies in Mexico by the same observer (AHG; 26).

Replicas of the upper (maxillary) teeth were made to provide a permanent record of the field observations. Two-stage impressions (casts), involving a base and wash layer, were made with vinyl polysiloxane. Dental replicas were made with super-hard resin, poured in four to six layers. Each pour was subject to a brief (3–5-min) vacuum to remove bubbles and allowed to harden over a 24-h period. Reliability of the field assessment of LEH was estimated by comparison with an assessment by the same observer (AHG) of 100 randomly selected anterior teeth (10% sample) from the completed dental casts.

After all dental observations were completed, data were obtained regarding which subjects were in the supplement and control groups, and growth and illness data on a subsample were collected. The growth data include height, weight, and head circumference at 6-mo intervals from birth to 2 y (n = 22), and height, weight, and head circumference at the time of dental study (n = 78). The illness data include the number of days ill per 3-mo period from upper-respiratory and gastrointestinal (diarrheal) illnesses from birth to 6 y (n = 22). Statistical analyses of the prevalence and chronological distribution of LEHs in the supplemented and control groups, as well as associations between LEH and illness and anthropometric measurements, were carried out by use of SPSS-X software (37).

Because teeth develop during different periods (36) and vary in their susceptibility to developmental disruption (1, 3, 26, 38), data are first presented on the prevalence of LEH by tooth between the supplemented and nonsupplemented groups. Second, a summarized prevalence of developmental disruption is presented by combining data from antimeres (contralateral teeth) and other sets of teeth. Third, the chronology of LEH in the supplemented and control group is presented and compared for the maxillary central incisors, the most hypoplastic teeth. Finally, we present results of analyses of the relationship between

LEH and past and present anthropometric status and past illnesses.

Results

Reliability

Of the 100 buccal tooth surfaces examined on casts, 34 cases of LEH were observed, compared with an original assessment of 31 cases of LEH in the field. Twenty-seven times a defect was recorded in both the field and on the casts, yielding a proportion of specific agreement of 0.83. A LEH was not found in either the field or on the casts in the remaining 62 cases, yielding an overall proportion of observed agreement of 0.89 (39). The Kappa statistic (K), a measure of agreement, yielded a value of 0.75 (SE of K = 0.12; $Z = 6.25$, $P < 0.001$). Landis and Koch (40) suggest that values > 0.75 represent excellent agreement.

Prevalence of linear enamel hypoplasias

The prevalence of LEHs was relatively similar for contralateral teeth but greatly varied by individual tooth types (**Table 1**). The lowest prevalence of defects in both the supplemented and control groups was found on the maxillary left canine (supplemented 2.6%; control 15.0%) and maxillary left incisor (supplemented 2.4%; control 16.7%). The greatest prevalence of defects was found on the maxillary central incisor (supplemented 38.1% for left and 40.5% for right; control 71.4% for left and 66.7% for right).

The prevalence of defects in the control group was greater than in the supplemented group for all 12 teeth examined. The differences in prevalence between groups approached significance (Mantel-Haenszel estimation of χ^2, one-tailed $P < 0.10$ and $P > 0.05$) for three teeth (mandibular right canine and left lateral and central incisors) and was significant ($P < 0.05$) for seven of nine remaining teeth (maxillary left canine, left lateral incisor, right and left central incisor, mandibular left canine, right lateral incisor, and right central incisor). The relative risk (RR) of a LEH in the control vs the supplemented groups ranged from 1.59 (mandibular left central incisor) to 6.96 (maxillary left lateral incisor).

The proportions of individuals with LEHs in the same zone (±1 zone to allow for developmental variation and measurement error) on contralateral teeth (such as left and right upper canines) are presented in **Table 2.** This method eliminates asymmetric defects that are less likely to be due to systemic stress (3, 5). Nearly three-fourths (72.2%) of defects were found on both antimeres [37/52 (71.2%) in the supplemented group and 72/99 (72.7%) in the nonsupplemented group]. The difference between the supplemented and control groups in proportions of contralateral teeth with matching defects was significant for all maxillary teeth and approached significance for the mandibular canine and central incisor (Table 2). The relative risk of a LEH in the control vs the supplemented group ranged from 1.5 to 5.0 for the six anterior tooth types.

The proportion of individuals with one or more matched LEHs on anterior maxillary and mandibular teeth, a combination of the maxillary central incisor and mandibular canine (the two most hypoplastic teeth), and on any of the anterior teeth studied was also computed (Table 2). Differences between groups were not significant for the mandibular tooth prevalence but were significantly different for the maxillary tooth prevalence ($P = 0.009$), the central incisor-canine combination ($P = 0.001$), and the total prevalence ($P = 0.001$). The proportion of individuals with LEHs is 39.5% (95% CI = 28.6–50.4%) in the supplemented group and 74.4% (95% CI = 64.7–84.1%) in the control group. The supplemented and control groups clearly differed in the overall prevalence of LEH.

Distribution of LEH by age at formation

To evaluate the pattern of LEH development by individuals' age at time of developmental disruption, the central incisor was

TABLE 1
Proportion of individuals with linear enamel hypoplasia (LEH)* in the supplemented and control groups

	Supplemented	Control	RR†	χ^2‡	One-tailed *P*
Maxilla					
Right canine	10.5 (4/38)	17.9 (7/39)	1.70	0.85	NS
Left canine	2.6 (1/39)	15.0 (6/40)	5.77	3.73	0.027
Right I2§	11.9 (5/42)	21.4 (9/42)	1.80	1.36	NS
Left I2	2.4 (1/42)	16.7 (7/42)	6.96	4.91	0.013
Right I1‖	38.1 (16/42)	71.4 (30/42)	1.87	9.30	0.001
Left I1	40.5 (17/42)	66.7 (28/42)	1.65	5.71	0.008
Mandible					
Right canine	20.0 (8/40)	34.1 (14/41)	1.71	2.02	0.077
Left canine	12.5 (5/40)	29.3 (12/41)	2.34	3.39	0.033
Right I2	16.7 (7/42)	33.3 (14/42)	2.00	3.07	0.040
Left I2	14.3 (6/42)	26.2 (11/42)	1.83	1.82	0.089
Right I1	16.7 (7/42)	33.3 (14/42)	2.00	3.07	0.040
Left I1	21.4 (9/42)	34.1 (14/41)	1.59	1.65	0.098

* Defined as a combination of Federation Dentaire International defect types 4 and 6 (32). Numbers in parentheses are frequencies of LEHs/sample size.
† Relative risk (control/supplemented).
‡ Computed by the Mantel-Haenszel method.
§ Lateral incisor.
‖ Central incisor.

TABLE 2
Proportion of LEHs matched on antimeres and combined for anterior maxillary and anterior mandibular teeth, upper central incisor and lower canine (CI combination), and all anterior teeth in the supplemented and control groups*

	Supplemented	Control	RR	χ^2	One-tailed *P*
Maxilla					
Canine	2.6 (1/38)	15.4 (6/39)	5.92	3.74	0.026
I2	2.4 (1/42)	11.9 (5/42)	4.96	2.84	0.046
I1	33.3 (14/42)	66.7 (28/42)	2.00	9.22	0.001
Total maxilla	36.8 (14/38)	64.1 (25.39)	1.74	5.65	0.009
Mandible					
Canine	12.5 (5/40)	26.8 (11/41)	2.14	2.59	0.054
I2	14.3 (6/42)	21.4 (9/42)	1.50	0.72	NS
I1	16.7 (7/42)	31.7 (13/41)	1.90	2.54	0.056
Total mandible	20.0 (8/40)	31.7 (13/41)	1.59	1.43	NS
CI combination†	40.0 (16/40)	73.2 (30/41)	1.83	8.97	0.001
Any combination	39.5 (15/38)	74.4 (29/39)	1.88	9.44	0.001

* Numbers in parentheses are frequencies of LEHs/sample size.
† Maxillary central incisors and mandibular canine.

divided into nine horizontal zones, roughly corresponding to successive, 6-mo periods (26). Overall, a total of 61 hypoplastic zones was found in the control group [1.45 ± 1.30 ($\bar{x}$ ± SD) per tooth], compared with 33 hypoplastic zones [0.76 ± 1.07 per tooth] in the supplemented group. The highest prevalence of defects was found for the zone corresponding to defects forming around 2.0–2.5 y for the supplemented group and 2.5–3.0 y for the control group (**Table 3**). The estimated mean age at development of a defect is 2.03 y in the supplemented group and 2.17 y in the control group. Frequency of defects was found to be significantly different between groups for three zones, those corresponding to defects developing around 0.5–1.0 y, 3.0–3.5 y, and 3.5–4.0 y.

Whereas the measures of central tendency suggest that there is little difference in the chronological distribution of LEH between groups, it is apparent that the main differences between groups occur before and after the peak time of disruption (**Fig 1**). When defects on the upper central incisor are divided into three developmental zones (birth–1.5 y, 1.5–3.0 y, 3.0–4.5 y) the most significant differences between supplemented and control groups were found for defects developing between birth and 1.5 y ($P = 0.006$) and between 3.0 and 4.5 y ($P = 0.002$) (Table 3). The relative risks of a LEH that develops before 1.5 y and after 3.0 y in the control vs supplemented group were 2.7 and 6.0, respectively. In comparison, a low relative risk (1.14) was found in comparing the frequency of LEHs that developed in the control and supplemented groups during their peak period of formation (1.5–3.0).

Gender, anthropometric status, and illness history

Because girls from Tezonteopan were found to have a higher prevalence of second- and third-degree malnutrition (weight-for-age) than were boys (28; R Galvan, unpublished observations, 1972), we compared the frequency of LEHs between males and females in our sample (**Table 4**). Confirming the anthropometric differences, there was a slight increase in prevalence of defects in females vs males. This difference was significant for the mandibular canine, where females had over three times the frequency

TABLE 3
Comparison of the chronological distribution of LEHs on maxillary central incisors in the control and supplemented groups*

	Supplemented	Control	χ^2	One-tailed *P*
Six-month developmental periods				
Birth–0.5 y	2.4 (1/41)	5.7 (2/35)	0.52	NS
0.5–1.0 y	7.1 (3/42)	22.5 (9/40)	3.82	0.026
1.0–1.5 y	11.9 (5/42)	14.3 (6/42)	0.45	NS
1.5–2.0 y	11.9 (5/42)	14.3 (6/42)	0.10	NS
2.0–2.5 y	23.8 (10/42)	26.2 (11/42)	0.06	NS
2.5–3.0 y	16.7 (7/42)	31.0 (13/42)	2.33	0.064
3.0–3.5 y	4.8 (2/42)	23.8 (10/42)	6.15	0.006
3.5–4.0 y	0.0 (0/42)	9.5 (4/42)	4.15	0.021
4.0–4.5 y	0.0 (0/42)	0.0 (0/42)	—	—
Eighteen-month developmental periods				
Birth–1.5 y	14.6 (6/41)	40.0 (14/35)	6.18	0.006
1.5–3.0 y	33.3 (14/42)	38.1 (16/42)	0.20	NS
3.0–4.5 y	4.8 (2/42)	28.6 (9/42)	8.47	0.002

* Numbers in parentheses are frequencies of LEHs/sample size.

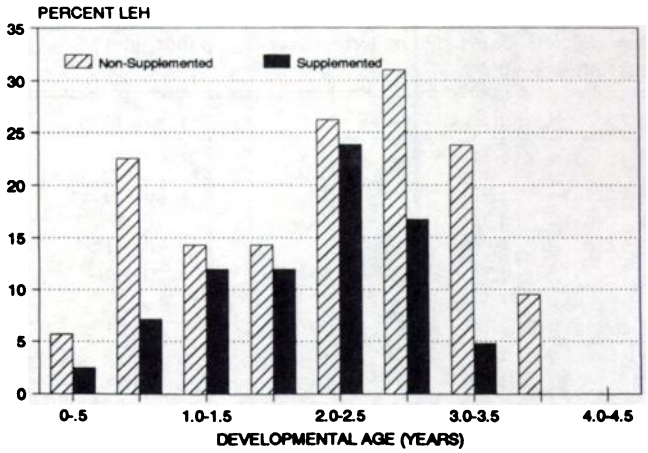

FIG 1. Comparison of the chronological distribution of linear enamel hypoplasias (LEHs) on the maxillary central incisors of individuals who received nutritional supplementation and those who did not. Data are based on dividing the crown into nine half-year developmental zones by following the developmental chronology of Massler et al (36).

of LEHs compared with males (30.0% vs 9.8%, $P = 0.011$). Overall, 60% (95% CI = 49.1–70.9%) of females displayed a symmetrical LEH on one or more pairs of anterior teeth, compared with 54.1% (95% CI = 43.0–65.2%) of the males. No evidence was found for an interaction between gender and supplementation, or for a gender difference in the chronological pattern of defects.

Head circumferences, heights, and weights were evaluated at the time of the dental evaluation. Whereas the mean percentile weight-for-age (based on NCHS reference values) (41) was significantly greater in the supplemented (37.8%) than the control group (22.3%), there was no difference in mean percentile weight-for-age for those with (30.5%) and without (32.5%) LEHs (**Table 5**). In fact, when the supplemented and control groups were studied separately, one finds a slight increase in mean weight-for-age with LEH (Table 5).

At the time of the dental observation the mean percentile height-for-age of the supplement group was 18.5%, compared with 5.9% for the control group ($t = 3.58$; $P = 0.001$). Differences in the current mean percentile height-for-age were also significantly different between individuals without and with LEHs (17.7% vs 8.2%; $t = 2.19$, $P = 0.034$; Table 5). To assess whether this difference was independent of supplementation, the mean percentiles were compared for those with and without LEHs by supplementation status. The mean percentiles of height-for-age of individuals in the control group with and without LEHs are nearly identical (6.0% and 5.7%, respectively). However, there is a slightly but not significantly greater mean height-for-age for individuals without LEHs (22.7%) vs those with LEHs (12.1%) in the supplemented group.

Mean head circumference was not found to be significantly different between the supplemented and control groups (53.43 ± 1.50 and 53.04 ± 1.18 cm, respectively). However, the mean head circumferences of individuals without LEH was significantly greater than those without LEH (Table 5). This mean difference is entirely a function of variation within the supplemented group, where the mean head circumference increased from 52.80 cm for those with LEHs to 54.05 cm for those without LEHs (Table 5).

The data on growth during the first 2 y of life on a subset of individuals (22 total; 15 in the control group and 7 in the supplemented group) suggests that the formation of LEHs was also associated with simultaneous decreases in growth velocity, especially in weight. For example, individuals with LEHs developing around 12–24 mo had nearly a 50% lower mean gain in weight (1.48 ± 0.33 kg) during this same time period when compared with the weight gain of those without LEHs (2.84 ± 1.59 kg; $t = 3.33$, $P = 0.003$). For the control group sample alone, the mean weight gains were 1.48 kg with concurrent LEH and 2.56 kg without concurrent LEH ($t = 1.78$; $P = 0.101$). Although sample sizes are small, these results suggest an association between growth faltering and formation of LEHs.

Finally, an analysis of the association between illness and the formation of LEHs also suggests that individuals with LEHs had a slight (10–25%) but insignificantly greater frequency of days ill with gastrointestinal illnesses, upper-respiratory illness, and the total of upper-respiratory and gastrointestinal illnesses. For example, nonsupplemented individuals with LEH that developed

TABLE 4
Comparison of the prevalence of LEHs in males and females, with defects matched on antimeres and combined for anterior maxillary and anterior mandibular teeth, upper central incisor and lower canine, and all anterior teeth*

	Males	Females	RR	χ^2	Two-tailed P
Maxilla					
Canine	5.4 (2/37)	12.5 (5/40)	2.31	1.15	NS
I2	9.5 (4/42)	4.8 (2/42)	0.50	0.71	NS
I1	52.4 (22/42)	47.6 (20/42)	0.91	0.19	NS
Total maxilla	46.8 (18/47)	52.5 (21/40)	1.12	0.11	NS
Mandible					
Canine	9.8 (4/41)	30.0 (12/40)	3.06	5.17	0.022
I2	16.7 (7/42)	19.0 (8/42)	1.14	0.80	NS
I1	24.4 (10/41)	23.8 (10/42)	0.98	0.04	NS
Total mandible	19.5 (8/41)	32.5 (13/40)	1.67	1.76	NS
CI combination	53.7 (22/41)	60.0 (24/40)	1.12	0.33	NS
Any combination	54.1 (20/37)	60.0 (24/40)	1.11	0.27	NS

* Numbers in parentheses are frequencies of LEHs/sample size.

TABLE 5
Comparison of current anthropometric measurements for individuals with and without LEHs for the supplemented and control groups and the total sample*

	LEH	No LEH	*t* value	Two-tailed *P*
Percentile weight-for-age (%)†				
Total	30.5 ± 24.9 [40]	32.5 ± 23.6 [31]	0.35	NS
Control	25.4 ± 23.0 [25]	17.0 ± 18.4 [9]	−0.98	NS
Supplemented	38.9 ± 26.3 [15]	38.8 ± 22.9 [22]	−0.01	NS
Percentile height-for-age (%)†				
Total	8.2 ± 11.9 [41]	17.7 ± 21.9 [31]	2.19	0.034
Control	6.0 ± 9.5 [26]	5.7 ± 6.0 [9]	−0.11	NS
Supplemented	12.1 ± 14.8 [15]	22.7 ± 24.1 [22]	1.52	NS
Head circumference (cm)				
Total	53.12 ± 1.13 [41]	53.77 ± 1.23 [31]	2.33	0.023
Control	53.30 ± 0.88 [26]	53.10 ± 1.30 [9]	−0.43	NS
Supplemented	52.80 ± 1.46 [15]	54.05 ± 1.12 [22]	2.93	0.006

* $\bar{x}$ ± SD. *n* in brackets.
† On the basis of NCHS reference values (41).

around 30–36 mo averaged 214.5 illness d during this 6-mo period compared with 174.9 illness d for those without a LEH.

Discussion

Intraobserver reliability

Previous studies showed that LEHs could be reliably scored in clinical and field settings (26, 33–35). This study extends these results in demonstrating that field observations are highly consistent with observations from high-quality dental replicas. In fact, the slightly but not significantly greater frequency of defects observed on casts attests to their quality and potential in further studies of quantitative dental developmental defects.

Moderate to severe LEHs similar to the FDI standard (32) are easily discerned in the field. However, most defects are less severe than the standard types presented by FDI. These mild defects are the most difficult to score with high repeatability. Additionally, they may be neither symmetrical nor associated with histological irregularities (3), suggesting that they are not caused by systemic physiological disruption. Because some decreases in enamel thickness (a hypoplasia)—visible to the human eye—do not appear to be physiologically meaningful, research is needed to formulate minimum criteria for recognizing a linear depression as being a true enamel hypoplasia (and caused by systemic physiological perturbation). The method employed in this study of matching defects on contralateral teeth is one way to eliminate the less easily discerned defects.

Variation in prevalence of LEH by tooth type

Consistent with previous studies, substantial differences were observed in the frequency of LEH among the anterior teeth (1, 3, 38). Variation in frequency of defects among teeth is due to differences in time of crown formation as well as to variation in susceptibility to disruption (38). Teeth such as upper central incisors that are polar teeth in developmental fields are under the strongest genetic control of development compared with other nonpolar teeth (38); these polar teeth are least able to vary the timing of their development during physiologically stressful periods. Therefore, they are more likely to develop defective enamel (38).

As in previous studies, the highest frequency of defects was found on the central incisors (1, 3, 19, 26, 38). Only two individuals without a symmetrical LEH on the upper central incisors had such symmetrical LEHs on another set of contralateral anterior teeth (Table 2). Stated differently, of the individuals without any missing teeth, 42 were recognized to have a LEH on the basis of analysis of upper central incisors alone, whereas 44 individuals were so assessed on the basis of an analysis of all anterior teeth. These data suggest that studies designed to gain a rapid assessment of nutritional status may be able to focus on the observation of upper central incisors. Conversely, data on the other teeth provide a means of confirming data from the central incisors and may have additional significance as less sensitive assays of physiological disruption. Regardless of which teeth are studied, these data further point toward the importance of reporting data by tooth type (1, 3, 38).

Effect of nutritional supplementation on LEH formation

The risk of developing a LEH was nearly twice as great in the nonsupplemented group as it was in the supplemented group. The decrease in prevalence of defects with supplementation suggests a clear relationship between dietary intake and the development of LEH. LEH appears to be sensitive to variations in nutrient intake on the order of 1200 kJ/d.

However, because supplemented individuals have greater intakes of a variety of nutrients, it is difficult to ascertain which nutrients might be most critical to the formation of a LEH. Although previous studies showed that there are associations between LEH and specific nutrient deficiencies (1, 7), these studies only suggest that a specific deficiency might be etiologically significant and cannot be used to determine if the specific deficiency is a sufficient cause or how common a cause it is in humans. A review of the literature combined with the results of this study further suggest that LEH is likely to be multifactorial in cause, perhaps similar to anthropometric measures of nutritional status, with both intake of a variety of nutrients and concurrent disease states potentially playing significant etiological roles. Further research with experimental animals and humans is clearly required to begin to tease out the relative importance of and interactions among these factors.

Developmental ages at LEH development

The estimated peak age at formation of a LEH on the upper central incisor is ~24–36 mo developmental age in both groups, with a slightly earlier mean age at formation (2.03–2.17 y) in the supplemented vs the control groups. This small mean difference in developmental age may be somewhat less than the actual difference in chronological age because developmental timing may be relatively delayed in the control group individuals with poorer nutritional status (42). Whereas poor nutritional status can significantly delay eruption times, less is known of its effect on the timing of matrix formation. It is, however, likely to be less significant, especially for a highly canalized tooth.

The peak age at formation of a LEH in both groups is similar to that found in another study in Mexico (26) and in a number of studies of prehistoric and historical populations (3). This peak also follows closely after the median age at completion of weaning (~20–24 mo) in this community (28) and is congruent with the age at which the incidence of illness is greatest (28, 30).

The greatest relative difference in frequency of LEH between supplemented and control groups occurs before 1.5 y and after 3.0 y, or before and after weaning and the time of greatest illness. It is as if all children are at great risk of LEH immediately after weaning, but the supplemented individuals are afforded greater protection before and after weaning. These data also support the hypothesis that common respiratory and gastrointestinal illnesses are an immediate cause of LEH, especially in individuals with compromised nutritional status.

Male-female differences, anthropometry, illness, and LEH

A variety of epidemiological studies has shown that LEH is more common in males than females and vice-versa (3). In this study, slightly more females than males had one or more LEHs (60.0–54.1%). These results are consistent with a previous study in the Mexican highlands (26) and the greater proportion of females with moderate to severe undernutrition in Tezonteopan (28).

The variation in association between LEH and both current and past anthropometric measures is explicable in terms of critical periods of development and catch-up potential of the anthropometric measures. The fact that there is no association between LEH and current weight-for-age is likely due to the ability of children to catch up in weight growth. Conversely, LEH developing earlier in life is associated with adolescent head circumference (Table 5). Because head circumference is rapidly increasing during the time of enamel formation, one might expect that a permanent decrease in mean head circumference is associated with LEH. Finally, a decrease in weight velocity between 2 and 3 y is associated with a concurrently developing LEH, although, as noted above, this relationship does not carry through to weight at the time of dental examination. This paradoxical relationship is likely due to the sensitivity of weight to physiological disruption. When a stressor is virulent enough to cause a LEH, it may also lead to a decrease in weight gain.

Both upper-respiratory and gastrointestinal illnesses were moderately associated with the development of a LEH. The potential importance of illness is indirectly suggested by the above-noted commonality in peak ages at incidence of illness and peak ages at formation of LEH. However, the role of illness in the etiology of LEH needs to be further explored.

In summary, the prevalence of LEH was shown to be reduced in a population after nutritional supplementation during the time of enamel development. This longitudinal study confirms results from previous studies that examined the relationship between current nutritional status and the previous formation of enamel defects. Associations between LEH and both growth velocity at the time of defect formation and current nutritional status further support the view that mild to moderate undernutrition is a main cause of LEH.

The peak age at formation of enamel defects, ~24–36 mo, is also the age at which weaning has been completed and illness reaches its greatest incidence. The web of potential interactions involving socioeconomic conditions, feeding patterns, dietary intake, and illness patterns suggests that all of these variables need to be examined in concert if one is to better understand the causal relationship between nutritional status and LEH. Because of enamel's ability to provide a chronological record of past physiological perturbations, LEH may prove to be a unique indicator of nutritional status.

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