

Maturation of primary and permanent teeth in preterm infants

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Abstract

Aims—To elucidate the development of primary and permanent teeth and to interpret the effect of different calcium, phosphorus, and vitamin D supplementation in the neonatal period on dental maturation in preterm children.

Methods—Preterm infants were randomised to four groups to receive a vitamin D dose of 500 or 1000 IU/day and calcium and phosphorus supplemented or unsupplemented breast milk. The maturity of the primary and permanent teeth was recorded in 30 preterm children. Sixty children aged 2 years and 60 children aged 9–11 years served as controls. Bone mineral content/density was assessed in the preterm infants.

Results—The median (range) corrected teething age was 7 (2–16) months in preterm infants and 6 (2–12) months in controls ($p = 0.43$). The median (range) number of erupted teeth at 2 years of age was 16 (11–19) in preterm infants and 16 (12–20) in controls ($p = 0.16$). Maturation of the permanent teeth in the preterm infants was not delayed compared with the controls (mean Demirjian SDS 0.16 *v* 0.49, $p = 0.14$). Early dietary intake of either mineral or vitamin D did not affect maturation of the primary dentition in preterm children. Children receiving the higher vitamin D dose in the neonatal period had more mature permanent dentition than those receiving the lower dose, but mineral intake did not affect maturation of the permanent teeth. Dental maturation did not correlate with bone mineral status.

Conclusions—This is the first longitudinal study to follow primary and permanent tooth maturation in the same preterm children. Premature birth has no appreciable late sequelae in tooth maturation.

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Keywords: dental maturation; preterm; calcium; vitamin D; teeth

Tooth maturation can be delayed in systemic disorders such as hypothyroidism.¹ There are limited data for preterm children on the development of primary^{2–4} and permanent teeth.⁵ The effects of early dietary mineral and vitamin D intake on bone mineral status in preterm infants have been extensively explored.^{6–11} It is well known that breast milk contains too little calcium and phosphorus to enable intrauterine mineral accretion in preterm infants. Thus the

main cause of metabolic bone disease of prematurity is an inadequate supply of calcium and phosphorus, not vitamin D deficiency, as long as a dose of 160–1000 IU/day is used as a supplement.^{6–11} However, the effect of calcium, phosphorus, and vitamin D supplementation on dental maturation in preterm infants has not been reported. The aim of this study was to define the development of primary and permanent teeth in the same preterm infants and to investigate the effect of different calcium, phosphorus, and vitamin D supplementations in the neonatal period on dental maturation in children born preterm. A further objective was to investigate whether tooth development is associated with bone mineral status in preterm children.

Methods

Between August 1985 and May 1987, a cohort of preterm infants with a birth weight less than 2000 g were investigated to evaluate the effect of early dietary intake of mineral and vitamin D on bone mineral accretion and dental development. The study was conducted at the neonatal intensive care unit in Tampere University Hospital. The inclusion criterion was a gestational age of less than 37 weeks at birth. The exclusion criterion was any major congenital abnormality. The withdrawal criterion was failure of vitamin D administration according to the protocol (see below); in fact, there were no withdrawals. During the study period, the clinical practice in our hospital was to give all preterm infants unsupplemented breast milk and vitamin D 1000 IU/day. According to the study protocol, all preterm infants enrolled in the study were stratified according to birth weight and then randomly assigned to one of four groups to receive vitamin D supplementation either 500 or 1000 IU/day from the time of tolerance of full enteral nutrition until 6 months of chronological age, and to receive either unsupplemented breast milk (CaP–) or breast milk supplemented with calcium 108 mg/kg/day and phosphorus 53 mg/kg/day (CaP+) from the time of tolerance of full enteral nutrition until they reached a body weight of 2000 g. Primary dentition was examined at age 1 and 2 during routine visits in 30 children (16 girls and 14 boys). Permanent dentition was examined in the same preterm children at age 9–11. The controls were 60 healthy 2 year old children and 60 healthy children aged 9–11, all of whom had been born at full term; both groups of controls were matched for age and sex with the study children. Bone mineral content (BMC) was

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assessed by single photon absorptiometry at 3 months of age in eight of the preterm infants. At 9–11 years, bone mineral density (BMD) was measured by dual energy x ray absorptiometry in 29 of the preterm children. Plasma 25-hydroxyvitamin D (25(OH)D) and 1,25-dihydroxyvitamin D(1,25(OH)₂D) concentrations were measured at 3 and 6 months of chronological age in the preterm infants. The median birth weight of the preterm infants was 1505 (range 690–1930) g and the median gestational age 31.5 (range 23.7–35.0) weeks. Two of these children suffered from mild spastic diplegia, and three from bronchial asthma. The remaining 25 did not have any chronic illnesses. The group allocation was concealed from the dentist and those measuring plasma vitamin D metabolite concentrations and bone density.

At a chronological age of 3 months, body weight and length were obtained from clinical charts. At 9–11 years of age, body weight and height were measured and pubertal status determined. Body weight was determined with a Secca Delta electronic scales model 707 and height with a Harpenden stadiometer.

DENTAL EXAMINATIONS

All dental examinations were performed by one investigator (LA) at the Oral Department in the Department of Otorhinolaryngology and Maxillofacial Surgery in the University Hospital of Tampere. The examinations were carried out using an artificial light in a dental chair.

Primary dentition

Maturity in the primary dentition was recorded in 30 preterm children at the ages of 1 and 2 during routine visits and in 60 control children at the age of 2. Maturity was assessed by counting the erupted teeth. A tooth was considered to be clinically erupted when the crown or part of it had penetrated the mucous membrane. Both chronological and corrected teething age (corrected teething age = chronological teething age in weeks – (40 – gestational age at birth in weeks)) were recorded. The age when the first primary tooth erupted was recorded from well baby clinic records in both preterm children and controls.

Permanent dentition

Maturity of the permanent dentition was recorded in the same 30 preterm children at the age of 9–11 years and in 60 control children. Dental maturity was assessed from panoramic radiographs using the method of Demirjian *et al*¹² and updated by Demirjian and Goldstein.¹³ In this method dental maturity is based on seven left mandibular teeth, the developmental stage of which is evaluated according to criteria given by Demirjian. The summed scores of all seven teeth give the dental maturity score, which can be converted into dental age by using the curves given by Demirjian and Goldstein.¹³

BONE DENSITOMETRY

At the age of 3 months, linear BMC (g/cm) was determined by single photon absorptiometry based on 28 keV γ radiation from an ¹²⁵I source

in eight of the infants.^{14 15} A piece of apparatus constructed in house consisting of source, collimated radiation detector, and plotter was used for measurements. Two repeated single photon absorptiometry scans were taken at the distal site of the left forearm, specifically at one third of the ulnar length measured proximally from the distal ulnar styloid. The left forearm was placed between two rubber gloves filled with water. The mean of two BMC measurements was used.

At the age of 9–11, BMD was measured by dual energy x ray absorptiometry (Norland XR-26; Norland Corp, Fort Atkinson, Wisconsin, USA) in the lumbar spine (L2–L4) of 29 participants. Measurement and analysis procedures are described in detail elsewhere.¹⁵ All scans and analyses were performed by the same experienced laboratory technician in a blinded fashion. The scanner was calibrated daily and its performance monitored by our quality assurance protocol.¹⁶ In addition to standard area BMD (g/cm²) measurements, a volumetric bone mineral apparent density (BMAD, g/cm³) in the lumbar spine was calculated by assuming the shape of vertebral bodies to be cylindrical.¹⁷ The in vivo precision (coefficient of variation) of the above measurements in our laboratory is about 1%.¹⁷

DETERMINATION OF PLASMA VITAMIN D METABOLITES

25(OH)D and 1,25(OH)₂D were measured at chronological age 3 and 6 months using 1 ml plasma samples to which [³H]25(OH)D₃ and [³H]1,25(OH)₂D₃ were added to monitor recovery throughout the assay. The samples were deproteinised and purified using the acetonitrile-C₁₈ Sep-Pak method.¹⁸ Thereafter the metabolites were further purified and separated by high performance liquid chromatography. A LiChrosorb Si 60 5(m) column (E Merck, Darmstadt, Germany) eluted with hexane/dichloromethane/methanol/propan-2-ol (76:16:5:3, by vol) was used. 25(OH)D was measured by a method based on binding to the competing protein,¹⁹ using serum from a pregnant woman diluted 1:20 000 in barbital acetate buffer, pH 8.6, and [³H]25(OH)D. Non-radioactive 25(OH)D served as the standard. 1,25(OH)₂D was measured by a radio-receptor method.²⁰ Interassay and intra-assay coefficients of variation for each of the metabolites ranged from 11.7 to 14.5%. The paediatric reference range for 25(OH)D in our laboratory is 30–130 nmol/l, and in the winter the lower limit is 17.5 nmol/l. The laboratory reference range for 1,25(OH)₂D is 48–175 pmol/l.

STATISTICAL ANALYSIS

The data were analysed using the statistical software SPSS Windows version 6.1 (SPSS Inc, Chicago, Illinois, USA). For variables with skewed distributions, median and range were given as descriptive statistics, and the differences between the groups were tested by the Mann-Whitney U test or Kruskal-Wallis non-parametric analysis of variance when appropriate. Means (SD) were given as descriptives for

Table 1 Development of deciduous teeth in children born preterm and at term

	Girls				p Value*	Boys				p Value*
	Preterm		Term			Preterm		Term		
	n	Median (range)	n	Median (range)		n	Median (range)	n	Median (range)	
Chronological age at eruption of first tooth (months)	15†	9 (5–17)	32	6 (3–12)	<0.01	15†	7 (6–15)	28	6 (2–10)	<0.01
Corrected age at eruption of first tooth (months)	15†	8 (3–16)	32	6 (3–12)	0.02	13‡	5 (2–12)	28	6 (2–10)	0.18
Number of teeth at age 2 years	16	16 (11–18)	32	16 (12–20)	0.22	14	16 (15–19)	28	16 (12–20)	0.52
Demirjian SDS	22	0.30 (–2.41–1.48)	109	0.42 (–0.46–1.74)		18	0.27 (–1.24–1.88)	95	0.33 (–0.66–2.62)	0.46

*Mann-Whitney U test.

†n = 15 + 13 = 28; information was missing on eruption of first tooth in two children.

Table 2 Development of deciduous teeth in children born preterm who received mineral supplemented (CaP+) or unsupplemented (CaP–) breastmilk and vitamin D 500 IU/day or 1000 IU/day in the neonatal period

	CaP+				CaP–				p Value*
	500 IU/day		1000 IU/day		500 IU/day		1000 IU/day		
	n	Median (range)	n	Median (range)	n	Median (range)	n	Median (range)	
Chronological age at eruption of first tooth (months)†	4	10 (6–15)	4	9 (7–10)	11	8 (6–17)	9	9 (5–11)	0.93
Corrected age at eruption of first tooth (months)†	4	7 (3–12)	4	6 (6–8)	11	6 (2–16)	9	7 (3–8)	0.91
Number of teeth at 1 year of age‡	4	4 (0–8)	4	4 (1–8)	11	5 (0–8)	10	6 (1–8)	0.75
Number of teeth at 2 year of age	4	16 (16–17)	5	16 (14–18)	11	16 (11–18)	10	16 (15–19)	0.99

*Kruskal-Wallis non-parametric analysis of variance.

†n = 4 + 4 + 11 + 9 = 28; information missing on eruption of first tooth in two children.

‡n = 4 + 4 + 11 + 10 = 29; data missing on number of teeth at 1 year of age in one child.

the variables with normal distribution, and they were tested by Student's *t* test or two way analysis of variance when appropriate. Spearman's correlation coefficient (*r*) was used to evaluate the strength of association between selected quantitative variables. The α level was set at 0.05.

ETHICAL CONSIDERATIONS

The study was approved by the ethical committee of Tampere University Hospital, and written informed consent was obtained from the parents.

Results

PRIMARY DENTITION

The median (range) chronological age when the first tooth erupted—that is, teething age—was 9 (5–17) months in the preterm infants. The corrected teething age was 7 (2–16) months, which corresponds well to that of the control children: 6 (2–12) months ($p = 0.43$). At a chronological age of 1 year, 5 (0–8) teeth (median (range)) had erupted in the preterm infants. At a chronological age of 2 years, the corresponding number was 16 (11–19) teeth in preterm children and 16 (12–20) in the controls ($p = 0.16$). Maturation of primary teeth was thus not significantly delayed in the whole study group of preterm children. However, when the preterm children were divided according to sex, the corrected age at the time of eruption of the first tooth in preterm girls was significantly later than in control girls, whereas the teething age was not delayed in preterm boys (table 1). Furthermore, eruption of the first tooth occurred significantly later in preterm girls than in preterm boys ($p = 0.01$), whereas no difference between boys and girls was seen in term children ($p = 0.64$). At 2 years of age, the median number of erupted teeth was 16 in both preterm and control boys

and girls (table 1). There was no association between gestational age at birth ($r = 0.14$, $p = 0.31$) or birth weight ($r = -0.23$, $p = 0.08$) and the corrected age at eruption of the first tooth. However, the heavier the infant at 3 months chronological age, the sooner the first tooth erupted ($r = -0.68$, $p < 0.01$). The age when full enteral nutrition was tolerated ($r = -0.02$, $p = 0.88$) and the length of tracheal intubation ($r = 0.04$, $p = 0.77$) were not associated with the corrected teething age. Late teething correlated with fewer erupted teeth at 1 and 2 years of chronological age ($r = -0.57$, $p < 0.01$ and $r = -0.34$, $p = 0.03$) in the preterm children.

The median serum 25(OH)D concentration at 3 and 6 months chronological age did not differ between infants receiving vitamin D 500 IU/day or 1000 IU/day in the neonatal period (3 months: 143.5 *v* 174.5 nmol/l, $p = 0.31$; 6 months: 147.4 *v* 172.0 nmol/l, $p = 0.18$), neither was the median serum 1,25(OH)₂D concentration (3 months: 243.0 *v* 160.0 nmol/l, $p = 0.09$; 6 months: 282.0 *v* 195.0 nmol/l, $p = 0.09$).

Early dietary vitamin D supplementation did not affect maturation of the primary teeth (table 2). Furthermore, early dietary intake of vitamin D or mineral did not affect the chronological or corrected age at eruption of the first tooth or the number of erupted teeth at 1 and 2 years of chronological age (table 2). There was no association between BMC at 3 months of chronological age and maturation of primary dentition ($r = -0.30$ to 0.31, $p = 0.32$ –0.88). There was no difference in birth weight, gestational age, duration of tracheal intubation, age at tolerance of full enteral nutrition, or age when body weight reached 2000 g between the four feeding groups (data not shown).

Table 3 Group characteristics and development of permanent teeth at 9–11 years of age in children born preterm who received mineral supplemented (CaP+) or unsupplemented (CaP-) breastmilk and vitamin D 500 IU/day or 1000 IU/day in the neonatal period

	CaP+		CaP-		p Value*
	500 IU/day (n=4)	1000 IU/day (n=5)	500 IU/day (n=11)	1000 IU/day (n=10)	
Age (years)	10.7 (9.5–10.8)	10.5 (9.7–10.7)	10.7 (8.9–11.3)	10.9 (9.7–11.7)	0.24
Weight (kg)	28.6 (22.8–35.1)	31.5 (21.0–35.0)	32.2 (20.7–70.7)	39.0 (26.5–56.8)	0.22
Height (cm)	140.9 (129.0–144.7)	134.3 (129.5–150.3)	144.0 (126.3–162.2)	145.2 (132.5–151.7)	0.50
Demirjian SDS	-0.12 (-1.31–1.29)	1.0 (-1.67–1.48)	-0.20 (-2.42–1.18)	0.64 (-0.88–1.88)	0.14

Values are median (range).

*Kruskall-Wallis non-parametric analysis of variance.

PERMANENT DENTITION

Maturation of the permanent teeth in the whole group of children born preterm did not differ from that in the controls born at term (mean Demirjian SDS 0.16 *v* 0.49, *p* = 0.14). When the children were divided according to sex, neither the preterm girls (mean Demirjian SDS 0.08 *v* 0.58, *p* = 0.12) nor the preterm boys (mean Demirjian SDS 0.26 *v* 0.38, *p* = 0.74) differed from the term ones. The Demirjian SDS was more advanced, the taller the preterm children were at the time of evaluation of dental status (*r* = 0.42, *p* = 0.02). Maturation of the permanent teeth did not depend on body weight (*r* = 0.26, *p* = 0.15) or pubertal stage (*r* = 0.26, *p* = 0.16). If teething was late and maturation of the primary teeth at 1 year of chronological age was slow, maturation of the permanent dentition at 9–11 years of age was also slow. However, no association was found between the number of teeth at 2 years of chronological age and the Demirjian SDS at 9–11 years of age (*r* = 0.13, *p* = 0.36). Neither low birth weight (*r* = 0.17, *p* = 0.36) nor gestational age at birth (*r* = 0.08, *p* = 0.68) was associated with maturation of the permanent dentition.

Surprisingly, the most mature permanent teeth were found in the preterm children who had received vitamin D 1000 IU/day, whereas mineral supplementation did not affect maturation (table 3). This impact was still seen after the effect of body weight had been eliminated by analysis of covariance. Maturation of the permanent teeth in the preterm children was not associated with lumbar bone mineral status at 9–11 years of age (*r* = 0.10, *p* = 0.72 for BMD and *r* = -0.05, *p* = 0.86 for BMAD). The prematurely born children receiving vitamin D 500 IU/day and those who had received 1000 IU/day had comparable BMD at 9–11 years of age (mean 0.615 g/cm² *v* 0.632 g/cm² (*p* = 0.61) for lumbar BMD and mean 0.104 g/cm³ *v* 0.108 g/cm³ (*p* = 0.47) for lumbar BMAD).

Discussion

No prospective studies such as the present one, monitoring dental maturation of both primary and permanent dentition within the same cohort of prematurely born children, have to our knowledge been previously reported. The preterm infants as a whole study group did not have a delayed corrected teething age. This is in accordance with the findings of Seow and coworkers² and Golden and coworkers,³ who showed that the chronological teething age in

children born preterm was delayed, but not the corrected teething age. It is noteworthy that the preterm girls in our study had their first tooth a median of two months later than girls born at term, whereas the corrected teething age of boys was not delayed. This is in agreement with a study in which male advancement was observed in the development of primary teeth during the first trimester,⁴ whereas, after the first postnatal year, tooth formation is more advanced in girls.^{18–21}

The results of the present study imply that different intakes of vitamin D and mineral in the neonatal period does not affect maturation of the deciduous teeth in children born preterm. It is well known that calcium and phosphorus supplementation in preterm infants in the neonatal period increases bone mineral accretion during the first few years.^{6–8} In the present study, BMC at 3 months of chronological age was not associated with maturation of the primary dentition. This is also in agreement with previous findings that eruption of the primary teeth was associated with somatic growth²¹ but not with skeletal maturity.²² There is moreover evidence that the chronology of the eruption of primary teeth is quite extensively genetically determined.^{23–24}

Seow and coworkers⁵ have reported that maturation of the permanent dentition in children born preterm is delayed at 6 years of age but that at 9 years of age it has caught up. This corresponds well to the results of our study, in which permanent teeth at 9–11 years of age were as mature in children born preterm as in controls. The mean Demirjian SDS of the control children in this study was 0.49, similar to that of children in other Finnish studies.^{25–26} In the present study, it is shown for the first time that a small number of primary teeth at 1 year of chronological age is associated with less mature permanent teeth at 9–11 years of age in children born preterm. Similarly, in children born at term, a weak significant correlation between maturation of the primary and permanent dentition has been observed.^{21–27}

This study indicates that maturation of permanent teeth is not affected by early dietary mineral intake. Interestingly, the preterm children receiving vitamin D 1000 IU/day had more mature permanent teeth at 9–11 years of age than those who had received vitamin D 500 IU/day. We suggest that the smaller vitamin D dose per se is not likely to have impeded the maturation of the permanent teeth, as there was no intergroup difference in the 25(OH)D concentrations at 3 or 6 months.

Even considerably smaller vitamin D doses than 500 IU/day have been reported to maintain the normal vitamin D status in the neonatal period.^{11 28} Moreover, giving a large vitamin D dose to preterm infants may have detrimental effects on bone mineralisation early in life.²⁹

In conclusion, bearing in mind the small number of subjects, early dietary mineral intake did not affect maturation of the primary or permanent dentition in children born preterm. Early dietary vitamin D intake had no impact on maturation of the primary dentition. Surprisingly, the children who had received the higher vitamin D dose of 1000 IU/day in the neonatal period had more mature permanent dentition than those who had received the smaller dose of 500 IU/day. This, however, needs further verification. Taking the group as a whole, maturation of both primary and permanent dentition in children born preterm does not differ appreciably from that in children born at term, indicating that premature birth has no appreciable late sequelae with respect to tooth maturation.

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