High-Turnover Osteopenia in Preterm Infants: Determination of Urinary Pyridinium Cross-Links of Collagen

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Osteopenia is a frequent condition in preterm infants, but its pathogenesis is uncertain. In the present study, we measured longitudinal changes in the excretion of pyridinium cross-links of collagen (specific markers of bone resorption) and evaluated the relationship between collagen cross-links and other indexes of bone and renal function in preterm infants. In these infants, urinary collagen cross-links were markedly increased on day 7 and day 30 of life and at estimated full-term gestation. The values were several times higher than those of older children and almost comparable to those of healthy full-term infants. Cross-link excretion did not correlate with β_2-microglobulin (B_2M) or *N*-acetyl-β-D-glucosaminidase (NAG) activity (markers of renal function), indicating that cross-link excretion is not influenced directly by infantile renal function. High serum osteocalcin and low bone mineral density (BMD) in the lumbar spine were also observed at estimated full-term gestation. There was no significant correlation between collagen cross-link excretion and either serum osteocalcin or spine BMD. We conclude that a state of high bone turnover underlies the development of osteopenia in preterm infants.

PRETERM INFANTS are at risk of osteopenia.[1,2] The clinical symptoms of osteopenia vary from mild demineralization to nontraumatic fractures. Previous studies have suggested that the osteopenia of prematurity is probably due to insufficient intake of minerals and/or vitamin D.[1,2] However, the exact mechanism by which insufficient mineral intake leads to osteopenia is unknown at present. Recently, quantitative measurements of the pyridinium compounds pyridinoline (Pyd) and deoxypyridinoline (D-Pyd) in the urine have become available to assess ongoing bone resorption in the pediatric population.[3-5]

The purpose of this study was to document postnatal changes in urinary Pyd and D-Pyd and to evaluate the relationship between collagen cross-links and other indexes of bone and renal function in preterm infants, in an attempt to understand the pathogenesis of the osteopenia of prematurity.

SUBJECTS AND METHODS

Subjects

A total of 24 preterm infants (16 boys and eight girls) who were hospitalized in our neonatal care unit in 1996 and had a normal urinary tract (confirmed by ultrasonography) were enrolled in the present study. The gestational age was 32 ± 2 weeks (range, 26 to 34) and the birthweight was appropriate for age (1,637 ± 407 g; range, 976 to 2,364). The Apgar score (1 minute) was 7 ± 2 (range, 1 to 9). Eighteen infants had respiratory failure and required supplemental oxygen during the first 8 ± 6 days of life (range, 1 to 24), and 15 were ventilated for a total of 6 ± 6 days (range, 1 to 22). Eight preterm infants received surfactant-replacement therapy just after birth.

All infants were fed a preterm formula (>70% of total feedings) in addition to their own mother's milk. The formula (Soft-Curd LW 94; Meiji, Tokyo, Japan) contained 70 kcal, 2.0 g protein, 65 mg Ca, 41 mg P, and 270 IU vitamin D per deciliter.[6] Feedings were increased as tolerated to a maximum total volume of 150 mL/kg/d in each infant. The infants started enteral feeding at the age of 4 ± 3 days (range, 1 to 9) and attained 100 and 150 mL/kg/d at 15 ± 9 days (range, 5 to 39) and 26 ± 20 days (range, 8 to 69), respectively. Seven of these preterm infants received parenteral supplementation of amino acids for a total of 6 ± 2 days (range, 3 to 9). Informed consent was obtained from the parents of all infants.

Study Design

Spot urine samples were collected between 10 AM and 1 PM from each infant on day 7 and day 30 of life and on the estimated day of full-term gestation. The urine sample was centrifuged, and the supernatant was stored at −30°C until analysis. Urinary Pyd and D-Pyd were assayed by high-performance liquid chromatography with fluorescence detection according to the method described previously.[5] The overall assay variance was ±3.5% and ±7.5%, respectively. Normal adult values for Pyd and D-Pyd using this technique were 17.7 to 41.9 and 2.2 to 6.1 nmol/mmol creatinine (Cr), respectively. To determine the possible contribution of alterations in renal function to cross-link excretion, β_2-microglobulin (B_2M) (B_2M kit II; Eiken, Tokyo, Japan)[7] and *N*-acetyl-β-D-glucosaminidase (NAG) (NAG test; Shionogi, Osaka, Japan)[8] concentrations were also measured using the same urine sample. All urinary values were corrected for Cr excretion (Creatinine HR-II Test; Wako, Osaka, Japan).

At full-term gestation (38 ± 1 weeks; range, 37 to 41), when the infants were 46 ± 24 days old (range, 16 to 101) and weighed 2,381 ± 169 g (range, 2,094 to 2,694), the lumbar spine (L1-L4) bone mineral density (BMD) was measured with a dual-energy x-ray absorptiometry (DXA) unit (QDR-1000/W; Hologic, Waltham, MA) according to the standard lumbar spine protocol.[6] BMD values determined by this unit were 0.305 ± 0.019 g/cm^2 (range, 0.262 to 0.331) in 16 normal full-term newborns. On the same day, fasting blood samples were obtained for measurement of Ca, P, and intact osteocalcin by two-site immunoradiometric assay (BGP IRMA kit; Mitsubishi-Kagaku, Tokyo, Japan; normal adult range, 2.5 to 13 ng/mL),[6] as well as urine samples for measurement of Pyd, D-Pyd, B_2M, NAG, and Cr levels.

Statistical Analysis

Data are expressed as the mean ± SD and/or range. Statistical comparisons were examined using the paired or unpaired Student's *t* test where appropriate. Correlation coefficients were determined by linear regression analysis. The significance threshold was attained for *P* less than .05.

RESULTS

Urinary excretion of Pyd and D-Pyd varied widely in preterm infants at each age. The mean levels were 622 ± 188 and 89 ±

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29 nmol/mmol Cr at 7 days and 827 ± 232 and 111 ± 25 nmol/mmol Cr at 30 days, respectively (Fig 1). The mean levels of Pyd and D-Pyd at 30 days were significantly higher than at 7 days ($P < .005$ in both).

At estimated full-term gestation, the mean urinary excretion of Pyd and D-Pyd was 793 ± 170 and 110 ± 26 nmol/mmol Cr, respectively (Fig 2). At this age, lumbar BMD values were 0.150 ± 0.034 g/cm^2 (range, 0.090 to 0.213), which is approximately 50% of the value in full-term newborns ($P < .001$). Serum concentrations of intact osteocalcin, Ca, and P were 44 ± 30 ng/mL (range, 11 to 140), 9.7 ± 0.3 mg/dL (range, 9.0 to 10.4), and 6.1 ± 0.6 mg/dL (range, 5.1 to 7.3), respectively. No significant correlation was found between the urinary excretion of Pyd and D-Pyd and the spine BMD ($r = .09$ and $-.08$, respectively) or serum osteocalcin ($r = -.21$ and $-.25$, respectively) at estimated full-term gestation.

Urine Pyd and D-Pyd correlated significantly with each other ($r = .88, .70$, and $.81$ at 7 days, 30 days, and estimated full-term gestation, respectively, $P < .001$ for all). There was no significant correlation between the urinary collagen cross-links Pyd or D-Pyd and the renal markers B_2M or NAG at all ages (Table 1).

DISCUSSION

The pyridinium compounds Pyd and D-Pyd are maturation products of the lysyl oxidase–mediated cross-linking pathway of type I collagen in bone and cartilage.[9,10] Pyd and D-Pyd are more specific and sensitive for the degradation of mature type I collagen than hydroxyproline. Accumulating clinical evidence indicates that quantitative measurement of these compounds in urine by high-performance liquid chromatography provides a valid index of bone resorption.[3-5,9] Pyd and D-Pyd follow a characteristic pattern with age, with high levels during childhood, rapidly decreasing levels afterward, and stabilization at low levels during adulthood. However, little information is available on the levels in preterm infants. In this study, we measured longitudinal changes in the excretion of Pyd and D-Pyd in preterm infants in an attempt to identify the pathogenic mechanisms of the osteopenia of prematurity.

Our results showed that the urinary excretion of Pyd and D-Pyd in preterm infants was greater than 10 times the normal adult values and several times higher than the values found in

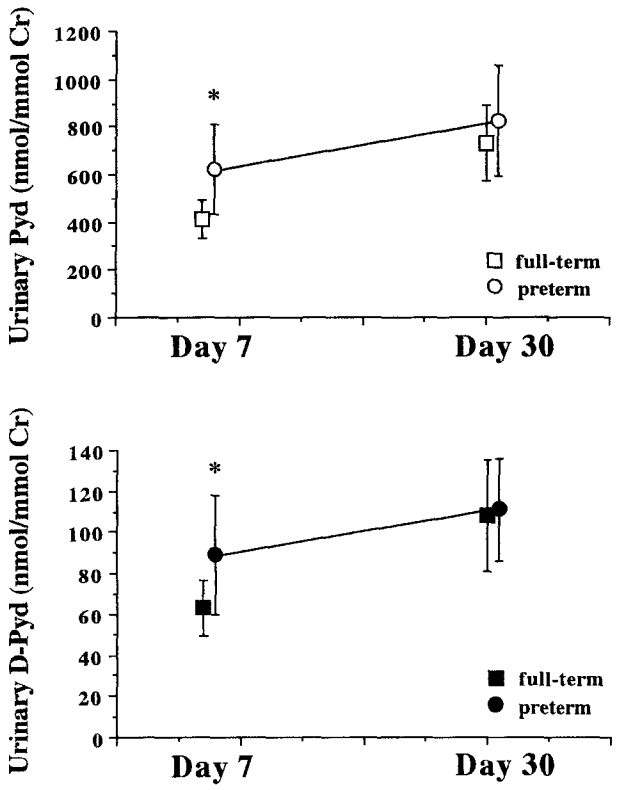

Fig 1. Urinary excretion of Pyd and D-Pyd on day 7 and day 30 of life. Data are expressed as the mean ± SD. Values for full-term infants of the same age are also shown.[5] *$P < .005$ *v* full-term infants.

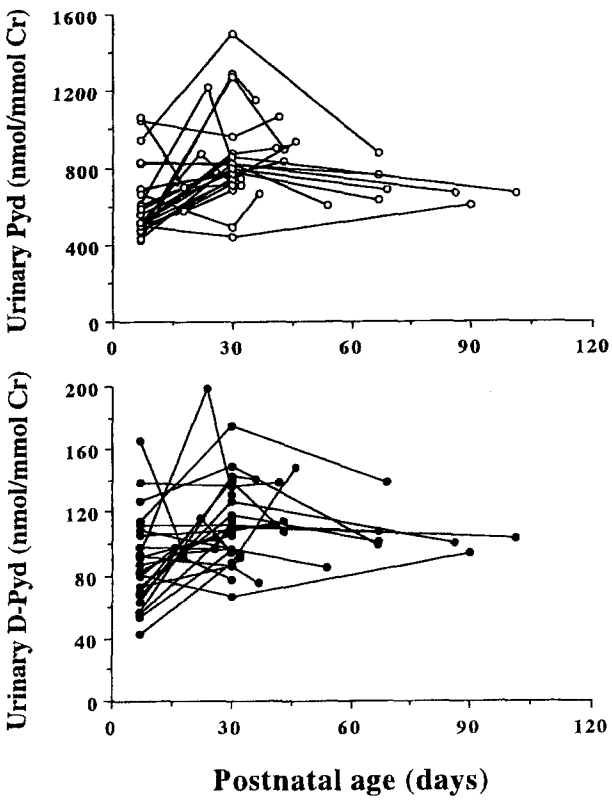

Fig 2. Serial changes in urinary Pyd and D-Pyd of preterm infants.

Table 1. Urinary Excretion of Markers of Renal Function and Relationship With Collagen Cross-Links

Parameter	Age (d) 7	Age (d) 30	Estimated Full-term (46 ± 24 d)
B_2M (mg/g Cr)	36.3 ± 26.8	19.7 ± 14.3*	23.3 ± 14.0
r v Pyd	.14	.02	−.27
r v D-Pyd	.03	.32	−.06
NAG (IU/g Cr)	46.3 ± 24.8	33.0 ± 16.0*	26.3 ± 9.6
r v Pyd	.20	.27	.27
r v D-Pyd	.12	.32	.27

NOTE. Data are the mean ± SD. There was no significant correlation between collagen cross-links and B_2M or NAG at all ages.

*$P < .05$ *v* 7 days.

older children.[4] Furthermore, the urinary levels significantly increased postnatally in the neonatal period; high urinary levels of Pyd and D-Pyd were also measured at estimated full-term gestation. The values for urinary cross-links in preterm infants were almost comparable to (or higher than) those in healthy full-term infants.[5] Although these findings may reflect immature transport systems in the renal tubules of preterm babies,[11] this seems unlikely, since the postnatal changes of urinary cross-links were different from those of B_2M and NAG (these usually decline postnatally[6,7]; Table 1) without showing any positive correlation with the indexes of renal function. Thus, our results indicate that osteoclastic bone degradation activity in preterm infants is much greater than in older children and adults and almost comparable to that in full-term infants. In addition, these data are consistent with previous results of urinary hydroxyproline measurement[12] and recent bone histomorphometric analysis in preterm infants.[13] The lack of a relationship with renal markers (B_2M and NAG) confirms the validity of analyzing Pyd and D-Pyd in preterm infants with immature and/or damaged kidney.[5]

At estimated full-term gestation, a significantly low bone mineral mass, expressed as BMD in the lumbar spine, was documented in the preterm infants. This observation supports previous findings that the postnatal increase in the bone mineral mass of preterm babies is less than that expected in utero.[1,2] Serum intact osteocalcin concentrations, a reflection of active osteoblastic bone formation,[10] were increased in our subjects, although the rate of increase relative to adult values appeared less pronounced than that of urinary Pyd and D-Pyd. There was no correlation between collagen cross-link excretion and either spine BMD or serum osteocalcin in the present study. These findings suggest that bone resorption and bone formation are uncoupled and that the increase of bone resorption exceeds that of bone formation in growing preterm infants, thus favoring the development of osteopenia in preterm infants. Normally, the rate of bone formation is higher than the rate of bone resorption in full-term infants.

Our results point to a state of "high-turnover" osteopenia in preterm babies. The cause(s) of increased bone turnover is probably complex. Our protocol of supplementation with minerals (using a preterm infant formula) may be suboptimal for the expected intrauterine bone mineralization.[1,2] However, neither hypercalcemia (>11.0 mg/dL) nor hypophosphatemia (<4.5 mg/dL) were documented in our subjects. Osteopenia due to phosphorus deficiency[14] was therefore not readily apparent. The enhanced bone resorption observed in preterm infants may be due to acidosis, immobilization, and/or functional derangement of local growth regulators produced by bone, cartilage, or marrow cells (eg, prostaglandins).[13] Otherwise, the increased bone turnover might represent a physiological phenomenon related to prematurity. In addition, the heterogeneity of our subjects could have contributed to the wide range of Pyd and D-Pyd levels found at each age. The mechanism(s) of increased bone turnover in preterm babies deserves further examination.

It is worthwhile to determine whether the osteopenia of prematurity has a long-term impact on skeletal development. Our recent study using DXA[6] shows that in preterm-born children, spontaneous resolution of lumbar spine osteopenia occurs during early childhood (by age 3 years).

In summary, the present study demonstrates that urinary excretion of the collagen cross-links Pyd and D-Pyd was markedly increased on day 7 and day 30 of life and at estimated full-term gestation in preterm infants, and that the urinary excretion is not influenced directly by infantile renal function, but probably reflects the state of high bone turnover. High serum osteocalcin and low BMD in the lumbar spine were also observed at estimated full-term gestation. We conclude that the state of high bone turnover underlies the development of osteopenia in preterm infants.

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