Collagen Cross-Links and Early Postnatal Growth in Newborns With Intrauterine Growth Retardation

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This study assessed growth and skeletal metabolism in full-term newborns with intrauterine growth retardation (IUGR) and determined the value of the urinary excretion of collagen cross-links in predicting postnatal catch-up growth. We studied 38 newborns (16 females) born at term with a birth weight less than the 10th centile of the reference and a ponderal index ([PI] 100 × weight in g/length in cm³) of 2.27 ± 0.19. The sample was divided into 23 children with proportionate ([P] PI ≥ 10th centile of the reference) and 15 with nonproportionate ([NP] PI < 10th centile of the reference) IUGR. The weight, head circumference, length, and knee-heel length of the newborns at days 7, 14, 30, 60, and 90 were measured. The height of 23 of the 38 children was also assessed at 27 ± 6 months of life. Urinary collagen cross-links were analyzed by high-performance liquid chromatography at day 14 and day 60. Most of the infants (68%) underwent catch-up growth, and the growth performance at 3 months was independent of the proportions at birth. Children who did not show catch-up growth in the first trimester of life failed to normalize in height in the following 2 years. The urinary excretion of pyridinoline (Pyd) was not related to the anthropometric measurements. In P children, urinary excretion of deoxypiridinoline (Dpd) at day 14 significantly correlated with the gain in length during the first 3 months, accounting for 25% of the variance. In NP children, these correlations between urinary Dpd and the gain in length were not significant. The evaluation of urinary Dpd excretion at 2 weeks of age might help to determine the therapeutic regimen in IUGR children.
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THE TERM INTRAUTERINE growth retardation (IUGR) is applied to full-term infants that are smaller than expected for gestational age. IUGR infants are a heterogeneous group. Rosso and Winick[1] proposed a classification of IUGR based on the clinical characteristics of newborns: the distinct types of neonates differ in pathophysiology and postnatal consequences. Both the timing and duration of the intrauterine insult determine the physical condition and body composition of the infant at birth. Infants with proportionate IUGR have low birth weight and retarded skeletal development. These children are more likely to remain shorter and lighter than normal infants. Several studies[2-4] showed that 30% of children born with proportionate IUGR stay below the 3rd centile of weight and height for age throughout life. In contrast, infants with nonproportionate IUGR have reduced fat deposition but not a reduction of skeletal size; these neonates are more likely to exhibit catch-up growth during the first few months of life. Adequate early nutritional management appears to be an important factor for optimal catch-up growth,[5] and the first months of extrauterine life seem to be crucial for the growth outcome of IUGR infants.[6]

The intrauterine insults that cause IUGR also affect bone development. In humans, IUGR results in a stunting of linear skeletal growth and a radiographic absence of distal femoral and proximal tibial epiphyses.[7] At birth, the bone mineral content (BMC) is significantly lower in IUGR infants versus term infants who are appropriate for gestational age.[8] On the other hand, little is known about the dynamics of bone formation and bone resorption in utero, particularly the normal changes that occur throughout gestation and in clinical situations that result in low bone mass at birth. Calcium-regulating hormones may mediate the effects of IUGR on BMC,[8-10] although there are conflicting data on the role of parathyroid hormone and vitamin D. Harrast and Kalkwarf[11] reported that the carboxy-terminal propeptide of type I collagen, another marker of bone formation, in amniotic fluid is inversely associated with fetal growth and is decreased in IUGR infants. The same study showed that the cross-linked carboxy-terminal telopeptide of type I collagen, a marker of bone resorption, was normally represented in IUGR newborns. Thus, bone metabolic changes in IUGR children resemble those observed in malnutrition, with reduced bone formation and normal or increased bone resorption leading to reduced BMC.

Since the first months of life are a critical period, the early detection of metabolic defects leading to poor performance appears essential to identify and plan suitable corrective measures. We have shown that urinary excretion of the collagen cross-links pyridinoline (Pyd) and deoxypiridinoline (Dpd) can predict growth velocity in malnourished children.[12] In the present study, we have investigated whether collagen cross-links may be predictive of catch-up failure in IUGR children.

The aim of this study was to evaluate growth and skeletal metabolism in full-term newborns with IUGR. In particular, we studied whether the early assessment of the urinary excretion of

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collagen cross-links is related to growth velocity during the first 3 months of extrauterine life in children with IUGR.

SUBJECTS AND METHODS

Subjects and Experimental Design

Thirty-eight full-term (gestational age, 37 to 42 weeks) newborns (16 females) with a birth weight less than the 10th centile for gestational age according to the standards of Lubchenco et al[13] were selected. Infants with malformations or congenital diseases and twins were excluded. None of the mothers had signs of preeclampsia. Twelve of 38 newborns included in the study were born by cesarean section. The IUGR children underwent measurements of weight and supine length at birth and weight, supine length, and knee-heel length at 7, 14, 30, 60, and 90 days of life. To evaluate long-term growth, height was measured in a subgroup of the study subjects (23 children) at 27 ± 7 months of life. The ponderal index ([PI] 100 × weight in g/length in cm^3) was used to discriminate between children with proportionate (P) and nonproportionate (NP) IUGR. Children with a PI greater than or equal to the 10th centile of the reference values[14] were defined as P (23 babies), whereas those with a PI less than the 10th centile were defined as NP (15 babies). Catch-up growth was defined as a length and weight gain greater than the 50th centile of the distribution of normal infants.[15] Urine samples were collected at 14 and 60 days of life and were stored at −25°C until analysis. Anthropometric measurements and biologic samples were collected between 10 AM and noon. Infants were breast-fed and/or formula-fed according to the advice of the family pediatrician. The mothers were asked to keep a feeding diary.

The investigation was approved by the Ethical Committee of Tor Vergata University Medical School, and written informed consent was obtained from the parents of all of the children.

Anthropometry

Anthropometric measurements were made according to the method of Lohman et al.[16] Two observers were trained over a period of 2 months. The length was measured with a portable infantometer (Rollametre; Raven Equipment, Great Dunmow, Essex, UK). A pilot study on 66 term newborns showed that the within-observer technical error was 4 mm and between-observer error 6 mm. Knee-heel length was measured with a portable knemometer (Force Institute, Copenhagen, Denmark). The instrument was described by Michaelsen et al[17] and consists of a fixed graduated rod fitted with a knee cap and a sliding rod fitted with a heel cap and connected to a digital caliper with an accuracy of 0.01 mm. A series of 5 measurements were taken, each time removing and repositioning the knemometer. If the SD exceeded 0.8 mm, the entire series of 5 assessments were repeated. A pilot study on 97 term newborns showed that the within-observer technical error was 1 mm and between-observer error 1.6 mm. Weight-for-age and length-for-age *z* scores were calculated.[18]

Biochemical Analyses

Collagen cross-links and creatinine levels were measured in the urine samples. Urinary creatinine was determined by the Jaffé method with deproteinization. Creatinine was not affected by bilirubin values, as none of the infants had jaundice at the time of urine collection. The interassay variation was 4%. Collagen cross-links in the urine samples were measured by a high-performance liquid chromatographic method.[19] The interassay variations were 11% for Pyd and 13% for Dpd. Urinary Pyd and Dpd were corrected for urinary creatinine excretion.

Statistical Analysis

Statistical analyses were performed with the program STATISTICA for Windows 4.5 (StatSoft, Tulsa, OK, 1995). The results are reported as the mean ± SD. Student's *t* test was used for continuous variables and the chi-square test was used for categorical data. Statistical significance was defined as a *P* value of .05 or less.

RESULTS

Anthropometry

Table 1 shows the anthropometric data collected at birth. P and NP infants did not differ in gestational age, weight, length, and knee-heel length. Figure 1 shows the changes in weight, length, head circumference, and knee-heel length for age *z* score during the 3 months of study. At birth, the NP children were significantly shorter and lighter than the P group. The ratio of knee-heel length to crown-heel length was significantly greater in NP versus P children. During the 3 months of observation, both P and NP infants grew in weight, length, head circumference, and knee-heel length at an accelerated rate (Table 2). Repeated-measures ANOVA showed significant changes in anthropometric variables for age *z* scores since day 14. Weight and length growth rates in P and NP infants were not significantly different. Only the knee-heel length growth velocity was significantly higher in the P group versus the NP group. At 3 months, the differences between the two groups disappeared.

At the end of the period of observation, 7 (18%) babies (6 P) had not caught up in either weight or length, while 18 (47%) infants (11 P) showed catch-up growth in both weight and length. Considering weight and length separately, one third (36%) of the children showed a gain in weight below the 50th centile, while 39% did not catch up in length. The occurrence of catch-up growth was independent of the initial condition of P or NP (χ^2 not significant). The length at 2 years of life showed that children who did not recover at 3 months of life continued to have a negative *z* score; P and NP subjects did not show significant differences. The mother's height showed a positive and significant correlation with length growth velocity in P infants ($r = .66$ and $P = .001$), whereas no correlation was observed in NP children. The weight gain was also positively correlated with the mother's height in P infants ($r = .62$ and $P = .004$) but not NP infants. No correlation was observed between the father's height and length growth velocity and weight growth velocity of the infants during the 3 months of observation.

Table 1. Age and Anthropometry of the Newborns Studied at Birth

Parameter	P Group		NP Group		
	Mean ± SD	No.	Mean ± SD	No.	*P*
Sex ratio (male/female)	11/12		11/4		
Gestational age (wk)	38.6 ± 1.2	23	38.8 ± 1.1	15	NS
Weight (g)	2,333 ± 224	23	2,227 ± 204	15	NS
Length (cm)	46.1 ± 1.7	23	47.3 ± 1.6	14*	NS
Head circumference (cm)	32.0 ± 1.09	23	32.1 ± 0.65	15	NS
PI (g/cm^3)†	2.37 ± 0.15	23	2.09 ± 0.08	14*	<.01

Abbreviation: NS, nonsignificant.

*Birth length was not measured in 1 child.

†PI was defined as the ratio, 100 × weight (g)/length (cm^3).

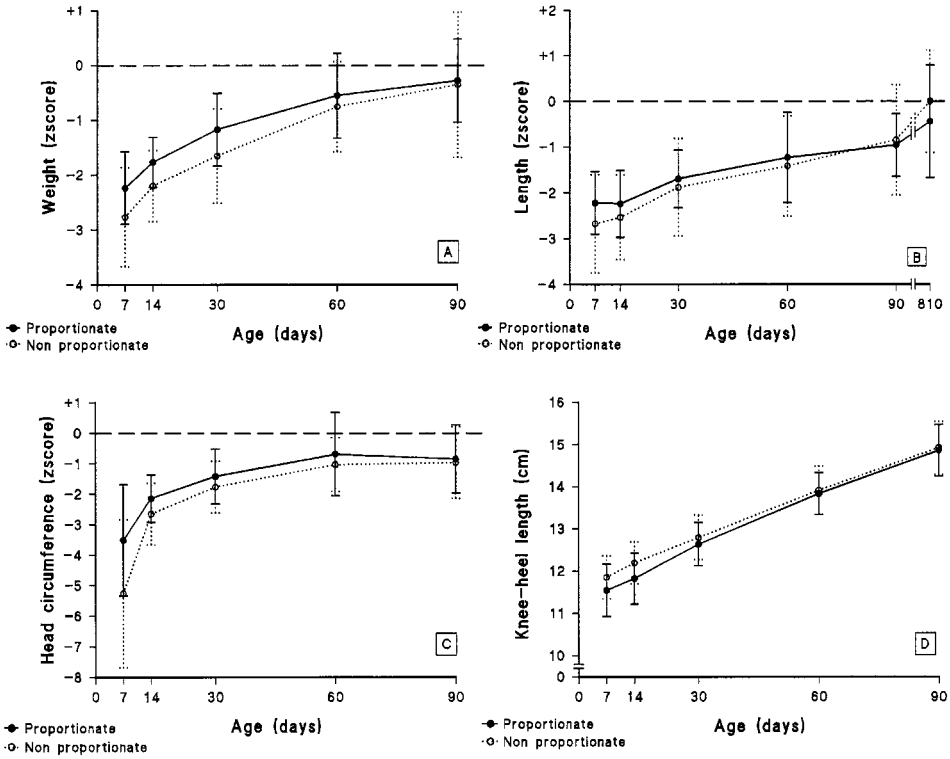

Fig 1. Pattern of growth in weight (A), length (B), head circumference (C), and knee-heel length (D) of the 2 newborn groups during the 3 months of study. The dotted line represents the value for non–growth-retarded children and the error bars indicate the SD.

Children's Diet

A feeding diary was kept for 27 neonates. Five P infants were exclusively breast-fed, 12 (4 P and 8 NP) were partially breast-fed, and 10 (5 P and 5 NP) were formula-fed. None of the children received a special formula for premature children. The different groups were compared by bivariate ANOVA. Breast-fed and formula-fed children did not show differences in the pattern of growth and urinary excretion of collagen cross-links.

Urinary Excretion of Cross-Links

Figure 2 shows the urinary excretion of Pyd and Dpd at 14 and 60 days. At day 14, urinary excretion of collagen cross-links was not different between P and NP infants.

Relationships between anthropometric variables and urinary Pyd and Dpd were studied by linear regression analysis. The urinary excretion of Pyd and Dpd at days 14 and 60 did not correlate with birth weight and birth length or weight, length, and knee-heel length measured at 14 days. In P infants, the urinary excretion of Dpd at day 60 was significantly and positively related to length ($P < .05$; Fig 3) and weight ($P < .005$). Pyd was not correlated with length growth velocity in either of the groups. Dpd excretion at day 14 was positively correlated with the length gain in the following 3 months and explained 14% of the variance in the length growth velocity of children in the whole sample (Fig 4A). This increased to 24% in the P subjects (Fig 4B), whereas the correlation was not significant in NP infants (Fig 4C). The change in Pyd and Dpd excretion between day 60 and day 14 was weakly correlated ($P = .06$) with the length growth velocity. No relationship was

Table 2. Weight, Length, Head Circumference, and Knee-Heel Length Velocities Between 7 and 90 Days

Parameter	P Group		NP Group		
	Mean ± SD	No.	Mean ± SD	No.	*P*
Weight velocity					
g/d	32 ± 5	19*†	32 ± 5	15	NS
z score	0.61 ± 1.07	19*†	0.50 ± 1.13	15	NS
Length velocity					
mm/d	1.23 ± 0.25	20*	1.27 ± 0.14	13‡	NS
z score	1.87 ± 2.58	20*	2.04 ± 1.35	13‡	NS
Head circumference velocity (mm/d)	0.72 ± 0.09	20*	0.74 ± 0.09	15	NS
Knee-heel length velocity (mm/d)	0.40 ± 0.08	20*	0.35 ± 0.04	15	<.01

Abbreviation: NS, nonsignificant.

*Children were lost to follow-up study.

†Weight at day 90 was not measured in 1 child.

‡Length at day 7 was not measured in 2 children who were undergoing perfusion via a scalp vein.

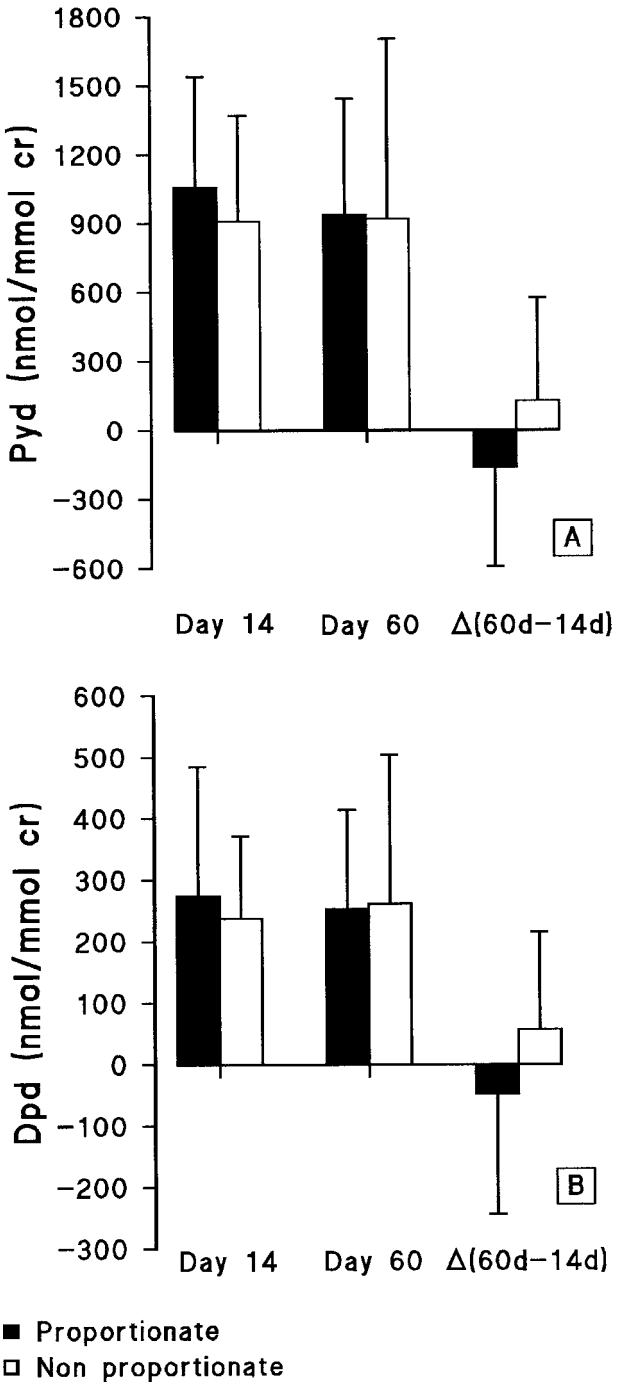

Fig 2. Urinary excretion of Pyd (A) and Dpd (B) at 14 and 60 days in the 2 groups of neonates. The error bars indicate the SD.

observed between the markers of skeletal metabolism at day 14 and day 60 and the knee-heel length gain.

DISCUSSION

Our results indicate that two thirds of the IUGR children underwent catch-up growth in the first 3 months of life. Catch-up growth was confirmed also by the knee-heel length gain. The knee-heel growth velocity in the IUGR children (2.66 ± 0.49 mm/wk) was higher than the rate (2.28 ± 0.45 mm/wk) found by Watson et al (personal communication, September 1993) in a sample of 20 normal healthy children measured at 6, 9, and 12 weeks. This growth performance can be explained by the endocrine pattern of these children. We have previously reported[20] that in most IUGR infants, the insulin-like growth factor–dependent growth-promoting machinery normalizes by 2 months of life, thus allowing early catch-up growth; no relationship was observed between endocrine variables and the urinary excretion of cross-links.

At 3 months, we did not observe clear growth differences between symmetric and asymmetric IUGR infants. However, we have observed that in the first 3 months of life, P children show a significantly higher knee-heel length velocity than NP children. A possible explanation for this different behavior is the worse nutritional condition of NP children, who would preferentially show normalized body composition and then accelerated skeletal growth. This finding is consistent with the results from Walker and Golden[21] in children recovering from protein-calorie malnutrition. Our results on the correlation between the maternal height and weight and the length gain in IUGR children are consistent with those reported by Markestad et al.[22]

Collagen cross-links are markers of bone resorption; bone formation markers are also available as growth markers.[23] Serum osteocalcin and procollagen type I C-propeptide have been shown to parallel growth velocity, and alkaline phosphatase has an age-related profile. Urinary collagen cross-links have been shown to correlate with length increments and have the additional advantage of being noninvasive markers suitable for studies in healthy infants. The assessment of markers of skeletal metabolism showed that at 2 weeks of life, there were no differences in bone resorption between P and NP groups, and in the following 2 months, there was no decrease in bone resorption in either group. We did not find a correlation between the urinary excretion of cross-links at day 14 and anthropometric measures at birth, while Watson et al (personal communication) have described a significant negative correlation between

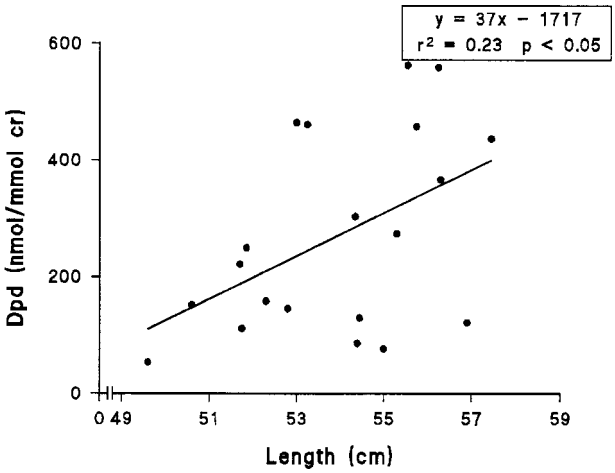

Fig 3. Correlation between length measured at day 60 and Dpd excretion at day 60 in P children.

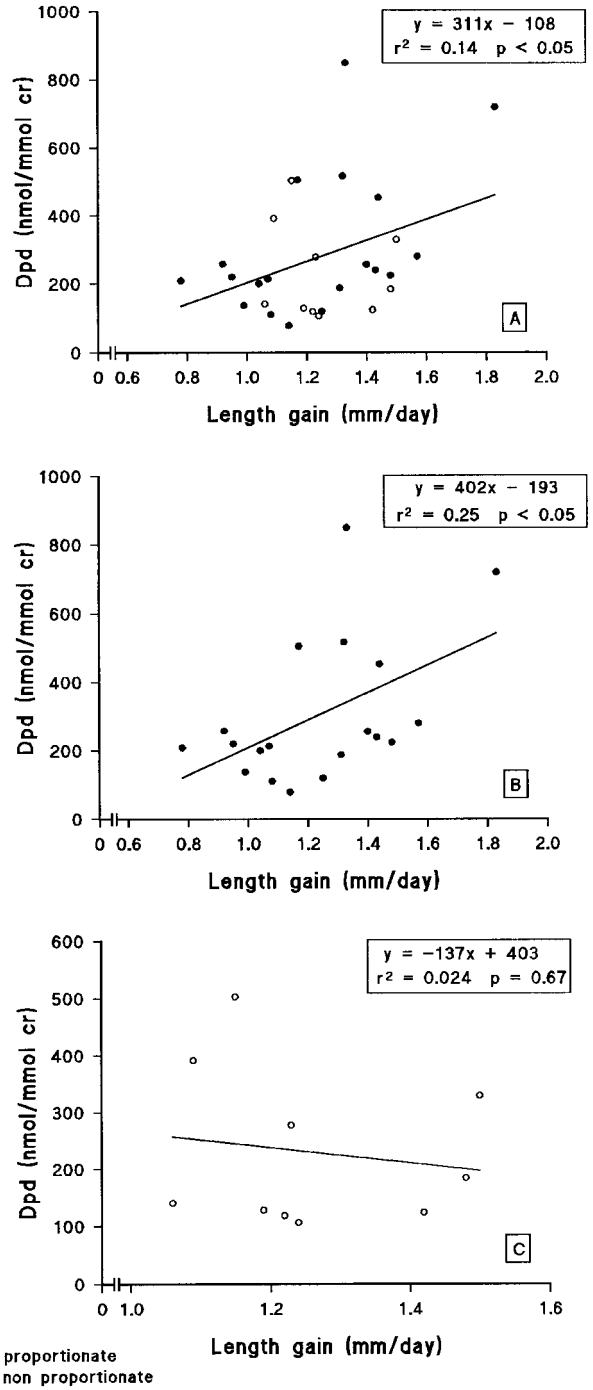

Fig 4. Correlation between urinary excretion of Dpd at day 14 and length gain in the total sample (A), P children (B), and NP (C).

birth weight and urinary Pyd excretion at 9 weeks of life in normal children. The correlation with anthropometry appeared at day 60 in P children only.

The main finding of this study is the positive correlation between the urinary excretion of Dpd at day 14 and the gain in length during the first 3 months of life. While Pyd urinary excretion reflects the metabolism of both bone and cartilage, Dpd is a specific marker of bone turnover.[23] It is possible that a larger proportion of cartilage in the skeleton of newborns, not related to longitudinal growth but still characterized by high turnover, might confound the relationship between Pyd and the length gain. However, Dpd was related to growth outcome in P infants only. The initial condition of skeletal growth retardation was worse in P children and, as expected, catch-up growth was faster in this group, as shown by the greater knee-heel length gain. The crown-heel length was not significantly higher either as a measurement sensitivity issue or, more probably, as a difference in segmental growth, with the legs growing faster in P children. Dpd excretion should thus parallel this growth pattern and should be higher in P than in NP children. We indeed observed higher cross-link excretion in P children, but the differences were not significant versus NP children.

The interpretation of these findings is confounded by the procedure used to express the urinary concentration of cross-links. Creatinine is currently used to normalize urinary dilution because it is excreted at a constant rate. However, creatinine is related to muscle mass[24] and is therefore excreted at a lower rate in children with reduced muscle mass such as NP children, accounting for the lack of correlation between Dpd at day 14 and growth velocity in NP children.

In conclusion, our findings suggest that catch-up growth in IUGR children is correlated with bone turnover in the first weeks of extrauterine life. The measurement of bone turnover at 14 days can be assessed by a simple and noninvasive method, ie, Dpd excretion. This might be helpful in deciding therapeutic interventions in IUGR children. To define suitable cutoff points, a larger population of infants should be studied. A further methodologic improvement of the test would be the implementation of timed (12 to 24 hours) urine collections and/or the collection of multiple urine samples to account for between-day variations in cross-link excretion.

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