Biochemical Measurements of Bone Metabolism in Childhood and Adolescence

Biochemische Messungen des Knochenstoffwechsels in Kindheit und Adoleszenz

E. Schoenau, F. Rauch

Summary: This article gives an overview of the physiology of biochemical indices of bone metabolism, their origin, the problems of interpreting their activities and the most important clincal applications in childhood and adolescence. Markers of bone formation are osteoblast products which enter the circulation like alkaline phosphatase (bone isoform), osteocalcin and type I procollagen peptides. Both traditional and new markers for bone resorption, detected in urine analyses, are matrix (collagen) degradation products deriving from osteoclast activity. They include urinary hydroxyproline, hydroxylysine glycosides, total or free pyridinoline crosslinks and cross-linked N- or C-telopeptides. Many studies have shown that both the old and new markers of bone metabolism are useful tools for basic bone biology research, for defining physiological phenomena in clinical studies, or in drug trials as growth modifying therapies (growth hormone) for following-up individual patients. But for the exact interpretation of bone marker analyses it is absolutely necessary to define the factors which may have an influence on measurement of the markers, such as age, sex, puberty, height velocity, circadian rhythms, diet, liver function, and kidney clearance rates.

Keywords: bone markers; collagen markers; bone turnover; growth; bone disorders; osteogenesis imperfecta.

Zusammenfassung: Diese Übersicht beschäftigt sich mit den biochemischen Parametern des Knochenstoffwechsels und beschreibt nicht nur die Mechanismen ihrer Entstehung, sondern auch die Anwendung dieser Kenngrößen in der klinischen Diagnostik bei Kindern und Jugendlichen und die Interpretation der Meßergebnisse. Die Marker des Knochenaufbaus werden von Osteoblasten gebildet wie die Knochen-Isoform der Alkalischen Phosphatase, das Osteocalcin und die Typ I Prokollagenpeptide. Die klassischen wie die neu eingeführten Marker des Knochenabbaus, die im Urin nachgewiesen werden, sind hingegen Endprodukte des osteoklastären Knochenabbaus wie das Hydroxyprolin, Hydroxylysin-Glykoside, freie und gebundene Pyridinolin-Crosslinks und vernetzte N-oder C-Telopeptide. In vielen Studien konnte gezeigt werden, daß die Marker des Knochenaufbau wie -abbaus wichtige Werkzeuge für die Erforschung der Knochenbiologie, für die Untersuchung physiologischer Prozesse im Rahmen klinischer Studien und für die Therapiekontrolle von Patienten, die mit wachstumsmodulierende Medikamente behandelt werden, darstellen. Für die Interpretation der Meßergebnisse müssen eine Reihe von Einflußgrößen wie Lebensalter, Geschlecht, Pubertät, Wachstumsgeschwindigkeit, zirkadiane Rhythmen, Ernährung sowie Leber- und Nierenfunktion berücksichtigt werden, um zu einer zuverlässigen Aussage zu gelangen.

Schlüsselwörter: Marker für Knochenaufbau; Kollagenmarker; Knochenstoffwechsel; Wachstum; Knochenstoffwechselstörungen; Osteogenesis imperfecta.

A variety of new assays for parameters of bone and collagen metabolism have been developed in recent years. These assays have generated much enthusiasm among researchers in the field of adult metabolic bone disease and are now increasingly used by pediatricians. Due to the process of skeletal growth, however, bone metabolism differs considerably between children and adults. Thus, there is a specific pediatric perspective of bone metabolism and of the biochemical parameters used in its evaluation.

The molecular basis and clinical use of markers of bone metabolism in adult medicine has been extensively reviewed [1–4]. Their application in childhood is reflected by their increasing use by pediatricians [5, 6]. Here we present a brief overview of the physiology of biochemical indices of bone metabolism, their origin, the problems of interpretation of their activities and the most important clinical applications in childhood and adolescence.

Endocrine and Metabolic Center, Children's Hospital, University of Cologne, Germany.
Correspondence: Prof. Dr. med. Eckhard Schoenau, Endocrine and Metabolic Center, Children's Hospital, University of Cologne, Joseph-Stelzmann-Str. 9, 50924 Cologne, Germany.
Fax: +49 (221) 4 78 46 35
E-mail:eckhard.schoenau@medizin.uni-koeln.de

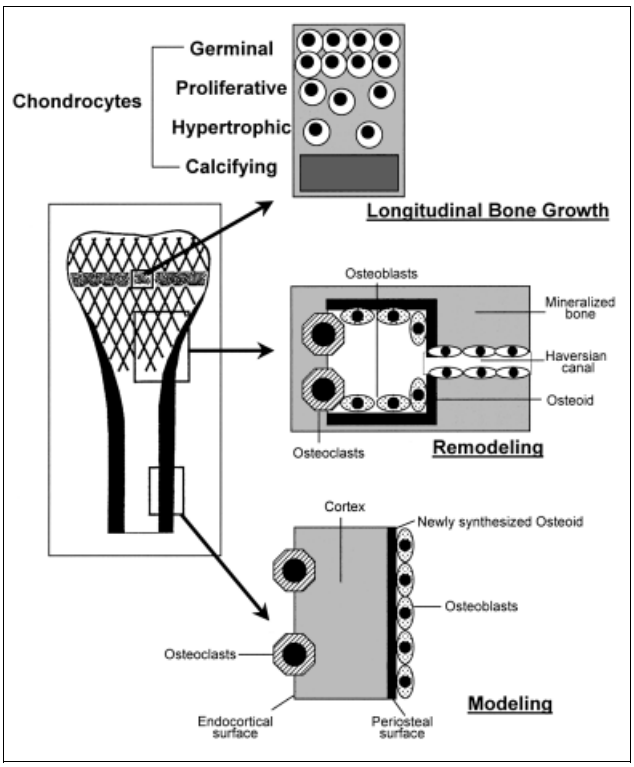

Figure 1 Description of the three most important skeletal-biologic principles during the process of bone development in childhood and adolescence

Basic skeletal-biologic principles

Figure 1 demonstrates the most important biologic processes which are responsible for the optimal development of the skeleton during childhood and adolescence. Knowledge of these processes is the basis for understanding and interpretation of bone marker analyses [6, 7].

Bone growth in length
Growth plates at the distal and proximal ends of long bones are designed for controlled bone lengthening as well as structural support and load bearing. Under the influence of various stimulation factors (e.g. growth hormone, GH, insulin-like growth factors, IGF), the chondrocytes of the resting zone divide rapidly and form well-oriented columns lying in the longitudinal axis of the bone. After proliferating, these cells secrete an extracellular matrix of collagen type I, II, V, X, XI; proteoglycans and other noncollagenous proteins. In the lower zones of the growth plate, the chondrocytes hypertrophy, mineralize the matrix, and secrete alkaline phosphatase. Eventually these hypertrophic chondrocytes die. Osteogenic cells differentiate into osteoblasts and congregate on the longitudinal tubes of calcified cartilage and deposit a thin layer of mineralizing osteoid called primary spongiosa. Osteoclasts move in to remove this primary spongiosa, followed by osteoblasts to lay down mature, secondary spongiosa or 'lamellar' bone [8]. Thus, the cartilagenous growth plate is replaced by bone.

Remodelling
Bone remodelling occurs throughout life and is a continuous process of coupled bone resorption and formation. This turnover is regulated by mechanical use, systemic hormones, and local factors. The end product of remodelling is the maintenance of a mineralized bone matrix which is well adapted to the change of physical bone loading. Bone remodelling occurs in small areas called bone multicellular units (BMUs), which occur on the surface of trabeculae and also within cortical bone. In an optimal situation, the resorbed bone is complete replaced by new bone, but in cases of underloading (e.g. during immobilization), "unused" bone is lost [9].

Modelling
Modelling is the process of shaping or sculpting the skeleton during growth. The process is also responsive to the mechanical forces that are placed on the skeleton while elongation is taking place at the growth plate. In modelling, osteoclasts and osteoblasts are not regulated by a direct coupling process. Bone formation follows bone resorption and the process usually results in a net increase of bone mass during childhood and adolescence [10].

Markers of bone metabolism
Biochemical markers of bone turnover are used clinically as non-invasive indices to evaluate the activity of the modelling and remodelling processes. Assays for these parameters all rely on the measurement, in serum or urine, of enzymes or matrix proteins synthesized by osteoblasts or osteoclasts that are released into body fluids, or of bone matrix degradation products released by the action of osteoclasts [11]. Biochemical markers of bone metabolism are usually classified as markers of bone formation and resorption (Fig. 2).

Markers of bone formation

Markers of bone formation are the products of osteoblasts in different stages of differentation [12]. Production of type I collagen is an early event, taking place during proliferation of osteoblasts precursor cells. The expression of alkaline phosphatase (AP) characteristically starts immediately after cessation of cell proliferation and declines as matrix mineralization commences. Among the genes expressed during matrix mineralization are those for the calcium-binding protein osteocalcin.

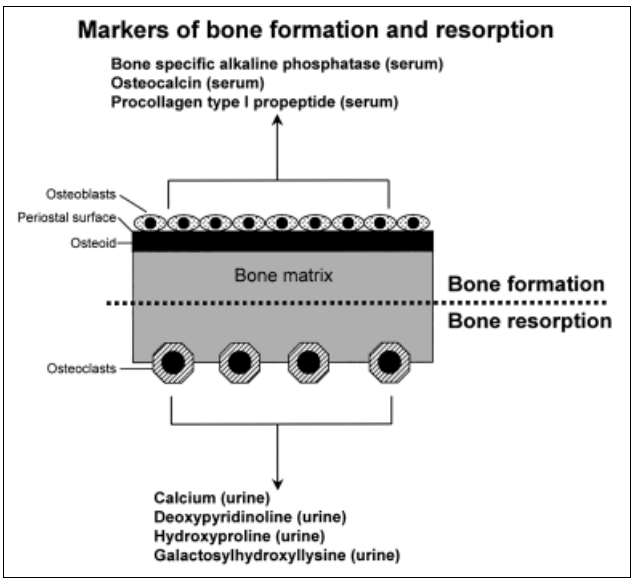

Figure 2 Markers of bone formation and resorption

Table 1 Specificity of bone markers

Parameter	Low	Medium	High
Formation:			
Bone AP			+
Osteocalcin			+
Procollagen PICP, PINP	+		
Resorption:			
Hydroxyproline (OHP)	+		
Galactosylhydroxylysine (GHLy)		+	
Pyridinoline (PYD)		+	
Deoxypyridinoline (DPD)			+
Crosslinked N-telopeptide			+
Crosslinked C-telopeptide		+	

Alkaline phosphatase

Total alkaline phosphatase (TAP) activity is still the most widely used marker in clinical work [13]. Alkaline phosphatases are a group of enzymes (molecular weight about 100 kD) that catalyze the hydrolysis of phosphate esters at an alkaline pH. In human serum, tissue-nonspecific (liver, bone, and kidney), intestinal, and placental AP have been identified, all encoded by different genes. The enzyme has a serum half-life of 24–48 h and is cleared mainly via the liver. Activity of TAP in serum is measured photometrically using p-nitrophenol as substrate, though different methods may yield considerably different results.

The two major circulation AP isoforms, bone and liver, are difficult to distinguish, because they have identical amino acid sequences and differ only in their pattern of posttranslational glycosylation. Many methods for the determination of AP have been described, using heat inactivation wheat-germ lectin precipitation, wheat-germ lectin affinity electrophoresis, and more recently, ELISA and radio-immunoassays [14–16]. Bone AP exhibits a marked age-dependency; in growing children over the age of 4 years, bone AP constitutes about 75–90 % of total TAP activity in serum. After puberty this proportion declines to 50 % (Table 1).

Osteocalcin

Osteocalcin is a 49-residue polypeptide (molecular mass 5800) produced by osteoblasts during the matrix mineralization phase under the control of 1,25-vitamin D_3. Osteocalcin is found exclusively in mineralizing tissues and is not expressed in growth plate chondrocytes. The precise physiological role of osteocalcin in the skeleton is still unknown, but it has recently been demonstrated that osteocalcin- deficient mice have increased bone formation [17]. Osteocalcin the only marker of bone metabolism which is exclusively found in mineralized tissue, and could give the closest reflection of bone formation.

In addition to intact osteocalcin, various molecular fragments circulate, which might result from bone matrix degradation. Not all assays can distinguish between these molecular forms. A number of radioimmunoassays have been developed to quantify osteocalcin serum levels, but even those which are specific for intact osteocalcin differ considerably in results [18].

Type I procollagen propeptides

Collagen is the predominant protein in bone and comprises about 90 % of the organic bone matrix, of which 97 % is type I collagen [10]. It is synthesized as a larger protein, type I procollagen. After secretion of procollagen in the extracellular space, the N- and C-terminal propeptides are cleaved by specific endopeptidases and are released in the circulation. Thus, one molecule of each propeptide is released for each collagen molecule excreted. Type I collagen is not, however, only found in bone, but also in soft tissues such as the skin and liver. Collagen type I propeptides are rapidly cleared from serum by liver uptake via different receptors, and due to their size, are not filtered by the renal glomerula. Therefore, levels of procollagen propeptides may be elevated in the liver disease, but are not influenced by renal function [19]. While the C-terminal propeptide (procollagen type I C-terminal propeptide, PICP) has been commercially available since 1990 and has been analysed in many pediatric studies, the various assays for the N-terminal propeptide (procollagen type I N-terminal propeptide, PINP) have not been studied in children in any detail.

Markers of bone resorption

Bone resorption includes dissolution of calcium salts and subsequent enzymatic breakdown of the organic matrix, which is mainly composed of type I collagen. Breakdown of collagen fibres results in a mixture of peptides and free amino acids. The parameters of bone resorption most widely used at present are products of collagen degradation.

Hydroxyproline
Hydroxyproline (OHP) arises during the post-translational modification of collagenous proteins in a vitamin C-dependent process. It is found in the triple-helical regions of all types of collagen and also in other proteins with collagen-like domains, such as the complement C1q. During collagen breakdown, OHP is released into the circulation in free and peptide-bound forms. About 90 % of all OHP is metabolized in the liver, the rest being excreted into the urine [1].

Urinary OHP can be measured by colorimetric or liquid-chromatographic methods, which are technically demanding [20, 21]. Despite these limitations, urinary OHP has been the most widley used marker of bone resorption for more than 30 years and, up to now, there are no studies in pediatric populations to prove that the newer markers of bone resorption are clinically more useful than urinary OHP.

Pyridinium cross-links
During the extracellular maturation of fibrillar collagens, intra- and intermolecular cross-links arise in all tissues. In bone, the predominant collagen cross-links are pyridinoline (PYD) and deoxypyridinoline (DPD). These connect the non-helical end (telopeptide) of one collagen molecule to the helical region of another molecule [22].

Neither cross-link is specific for collagen type I, but both PYD and DPD are absent from skin. During collagen breakdown, PYD and DPD are released into the circulation in free form and as part of variously sized peptides. Although this has not been studied in detail, it is assumed that, due to their unusual chemical structure, these molecules are not metabolized. They are excreted into the urine, where about 40–50 % of both types of cross-links are present in free form, the remainder being part of peptides. Urinary concentrations of total PYD and DPD can be measured by HPLC after acid hydrolysis of peptides and proteins. To allow a broader use of this marker, industry and academic institutions have joined efforts to quickly develop and commercialize a number of immunoassays for the determination of pyridinium cross-link-related peptides. Total PYD and DPD excretion have been shown to reflect bone turnover and bone resorption measured histomorphometrically [1, 11].

Cross-linked telopeptides
Two other 'cross-link-related' immunoassays for urine samples do not target the cross-link itself but recognize the N-telopeptide and a linear sequence of eight amino acids within the C-telopeptide of collagen type I [23, 24]. The fact that results obtained with these assays decrease after biphosphonate therapy in adults with Paget's disease has been cited as proof for their specificity for bone resorption.

Finally, a commercial radio-immunoassay has been developed which measures the cross-link containing C-telopeptide domain of type I collagen in serum samples. The assay has been shown to produce results which correlate with histomorphometrically determined bone resorption, but the clinical results in various metabolic bone diseases of adults have shed doupt on its specificity for bone resorption [1].

Galactosyl-hydroxylysine
Galactosyl-hydroxylysine (GHLy) is the predominant product of the post-translational glycosylation of hydroxylysine residues in skeletal collagen. Although it is also found in soft tissue collagen, the amount of GHLy per collagen molecule in these tissues is much lower than in bone, as most of it is processed to glycosyl-galactosyl-hydroxylysine (GGHLy). Like other components of the collagen molecule, GHLy is released during collagen breakdown and is excreted in the urine. Unlike urinary OHP, however, the urinary concentrations of GHLy seem not to be influenced by dietary collagen intake, and apparently, there is no significant metabolism of the compound after release from collagen [25].

Until now, this marker has not found widespread use, and data in children are limited [26]. The development of an immunoassay for its determination may make this parameter more widely available.

Tartrate-resistant acid phosphatase
Tartrate-resistant acid phosphatase (TRAP) is an enzyme of osteoclasts that is involved in bone matrix degradation. The enzyme is released into the circulation during the resorption process itself or after detachment of the osteoclasts from the bone surface. Although various catalytic and immunological assays for the determination of TRAP in serum samples have been developed, there still are major methodological problems, which have limited the popularity of this marker and few studies have used TRAP in pediatric populations [1].

Clinical applications in pediatrics

Rickets
Vitamin D deficiency rickets is the 'classical' metabolic bone disease in childhood, which today has become rare in Western countries. Elevated total AP activity is a characteristic finding and is very useful in monitoring the effect of treatment [27]. The value of other markers

is less certain: As the contribution of the liver isoform to total AP activity is negligible in children with increased bone turnover who do not simultaneously suffer from cholestatic liver disease [28], the determination of bone-specific AP does not offer any advantage in rickets [29[. Osteocalcin levels do not show a consistent pattern in children with vitamin D or calcium deficiency rickets [30–33]. Levels of PICP and ICTP are elevated [34, 32], but the clinical utility of these findings appears questionable.

In X-linked hypophosphatemic rickets, levels of plasma total alkaline phosphatase are also markedly elevated and often remain so despite current standard treatment with oral phosphate and calcitriol [35]. Few data are available on other bone markers in this disorder, but we have found preliminary evidence that parameters of bone resorption are inappropriately low compared to indices of bone formation, suggesting an imbalance between bone formation and resorption [36].

Other metabolic bone diseases are rare in childhood, and little information is available on markers of bone turnover in these conditions. Total AP, osteocalcin, and hydroxyproline were found to be decreased in hypoparathyroidism and were increased in hyperparathyroidism [37]. In idiopathic juvenile osteoporosis, PICP levels were not helpful in establishing the diagnosis [34].

Osteogenesis imperfecta

Osteogenesis imperfecta is an autosomal dominant disorder with increased bone fragility. Clinically, six types can be distinguished [38]. The disease is caused by mutations in one of the two genes for type I procollagen chains, which lead either to structural defects or decreased synthesis of collagen type I [38]. Accordingly, very low PICP serum levels are found in patients with osteogenesis imperfecta type I and somewhat higher values in types III and IV [39]. Although the sensitivity and specificity of PICP for osteogenesis imperfecta remain to be established, this parameter might be used to distinguish type I disease from child abuse and to screen for carriers in large pedigrees [39].

Bone histomorphometry in children with osteogenesis imperfecta revealed both enhanced bone formation and resorption [40] on the tissue level. However, results for biochemical markers of bone metabolism are discordant: Total AP levels are mostly in the normal range [41, 42], while osteocalcin has been reported to be decreased [42] or increased [41, 43]. Treatment with growth hormone increases levels of osteocalcin, but not total AP and PICP [42]. Some studies have found enhanced excretion of collagen degradation products in the urine of patients with osteogenesis imperfecta [44, 43], but it is still under debate, whether bone resorption is really increased in osteogenesis imperfecta or whether collagen markers simply reflect the degradation of mutated collagen molecules before they are incorporated into bone matrix [44].

Ehlers-Danlos syndrome type VI

This is a rare disorder affecting connective tissue in many organs [45], which is caused by a deficiency in lysyl-hydroxylase, an enzyme involved in the post-translational modification of the procollagen molecule. As PYD is synthesized from three hydroxylysine residues, while DPD is made up of one lysine and two hydroxylysine residues [46], the lack of lysyl-hydroxylation leads to the preferential synthesis of DPD. This can be easily diagnosed in urine samples: The PYD/DPD ratio (about 4:1 in healthy subjects) is reversed to roughly 1:4 in individuals affected with Ehlers-Danlos syndrome type VI [47, 48].

Secondary bone disease

Osteopenia of prematurity

Total AP is a useful parameter to evaluate bone metabolism in preterm babies [49, 50]. Elevated levels during the first weeks of life indicate a mineralization deficit and are associated with growth delay at the age of 18 months [51]. Total AP levels are about 2–3 times above adult levels at birth and further rise during the first 3 to 6 weeks of life [49, 50]. It is important to know that the AP isoenzyme pattern in the neonatal period is different from later life [49, 52]: A so-called 'fetal intestinal' isoform, which is not detectable at birth, increases after the start of enteral feeding and peaks about 2 weeks afterwards. The maximum level of the fetal intestinal isoform is negatively correlated with gestational age and amounts to 30 % to 50 % of total AP levels in very premature babies [49]. Bone-specific AP levels might therefore reflect bone metabolism better than total AP in these babies, but it is uncertain whether immunoassays can distinguish between the unusual AP isoforms of this period of life [52]. No reports are available on the value of the newer markers of bone metabolism in osteopenia of prematurity.

Renal osteodystrophy

Chronic renal failure in children is often associated with slow growth and other skeletal disorders including secondary hyperparathyroidism, aluminium-related low turnover bone disease, osteomalacia, and adynamic osteopathy [53]. Although the distinction between these conditions may have important therapeutical implications and bone biopsies are too invasive to be repeated regularly, few studies have analysed the value of bone markers other than total AP in children with renal failure.

However, total AP may not be a good indicator of bone metabolism in these patients, because in conditions associated with slow growth, the relative input of the bone isoform to total AP decreases. In fact, total AP has been shown to be a poor predictor of bone histology in children undergoing peritoneal dialysis [54, 55]. Renal bone disease might be an indication to determine bone-specific AP, which has been shown to correlate

with histomorphometrically determined bone formation rate in adults [56]. Studies in children are not yet available.

The value of other bone markers in renal osteodystrophy remains to be elucidated. Urinary markers and serum assays of small molecules which are eliminated via the kidneys (such as osteocalcin and ICTP) are probably not useful when renal clearance is impaired. In contrast, serum levels of PICP are independent of renal function [57], and have been found to reflect dynamic parameters in bone histomorphometry in adults with predialytic renal failure [57].

Bone involvement in serious acute and chronic illnesses

Seriously ill children do not grow well and, not unexpectedly, levels of bone markers are low in such patients. This has been consistently demonstrated for osteocalcin, bone-specific AP, PICP, PYD, and DPD in disease states as diverse as malnutrition, phenylketonuria, acute lymphatic leukemia, active rheumatic diseases, and severe burns [58–65]. Bone markers also reflect the acceleration of growth during the successful therapy of these disorders [58, 59, 65].

Glucocorticoid treatment

Long-term glucocorticoid therapy in children leads to slow growth and inhibits bone formation and synthesis of type I collagen by mechanisms which have not been entirely elucidated [66]. These effects can be expected to lower the levels of markers of type I collagen. In fact, PICP and ICTP were found to decrease rapidly after the start of treatment with oral glucocorticoids in children with a variety of different diseases [62, 67]. This effect is dose dependent [68] and quickly reversible: in children with inflammatory bowel disease, PICP increased markedly within 2 months after glucocorticoid dosage was reduced, reflecting the rise in growth velocity [69]. Markers of collagen metabolism also indicate the systemic effects of inhaled corticosteroids in asthmatic children: One month of treament with 800 µg of inhaled budesonide resulted in significantly decreased levels of PICP, PINP, ICTP, PYD, DPD, and NTX [70]. Thus, markers of collagen metabolism appear to be very sensitive indicators of the systemic effects of glucocorticoid treatment.

Perspectives

Normal growth and growth disorders

Recently, much interest has focussed on the search for biochemical markers of longitudinal growth in children. The rationale for using biochemical markers of growth rather than 'simple' height measurements is that height measurements are operator- and instrument-dependent, they are insensitive over short time periods, and they are necessarily retrospective. Research in this field is driven by the hope to find an early predictor of the long-term success of a growth-promoting treatment in an individual child, so that therapy can be adjusted accordingly. As longitudinal growth is primarily a function of the growth plate cartilage, a marker which is specific for growth plate activity should give the best reflection of current longitudinal growth. Such markers are being developed, but no results are currently available. DPD and osteocalcin are not present in growth plate cartilage, but bone AP, GHLy, OHP, and PYD are. The levels of these markers might, therefore, also be influenced by growth plate cartilage metabolism, though the extent of this influence is unknown.

In untreated GH-deficient patients, levels of osteocalcin, PICP, TAP, bone AP, ICTP, DPD, GHLy are lower than in healthy control groups [71–74]. There is considerable overlap with normal children, however, and these markers cannot be used to diagnose GH-deficiency in individual patients. Positive associations with growth velocity after 1 year of GH treatment have been reported for the increase of PICP, PYD, DPD, GHLy, osteocalcin, pyridinoline cross-linked telopeptide of type I collagen and bone alkaline phosphatase [7, 26, 71, 75].

Figure 3 shows the relationship between GHLy and DPD excretion after 4 weeks and the corresponding growth velocity after 1 year GH treatment in GH-deficient patients. It is unclear, if and how this information can be used to improve the care of children with growth disorders. A recently published study described that the combination of biochemical bone markers with early auxology and bone age determination allows after 3 months growth hormone therapy an accurate prediction of the first year growth velocity of the first year on GH-treatment [75]. The clinical advantage is the early detection of non-responders and/or the possibility of early individual growth hormone dosage adaption. More studies addressing this question are needed if markers of bone metabolism are to play a role in the monitoring of growth-modifying treatments.

Problemes associated with the interpretations of bone markers

Different biological processes

The fact that different biological processes occur in bone complicates the use of markers. Osteoblasts and osteoclasts are involved in all of these processes – bone growth, modelling, and remodelling. Bone and collagen markers are not specific to the different processes. Bone growth and modelling are responsible for higher levels of bone breakdown products during childhood compared to adulthood. Bone markers are the same in a child undergoing elevated bone remodelling with a low growth rate as they are in a child undergoing normal growth. Knowledge of growth activity is necessary for the exact interpretation of bone and collagen markers in childhood and adolescence.

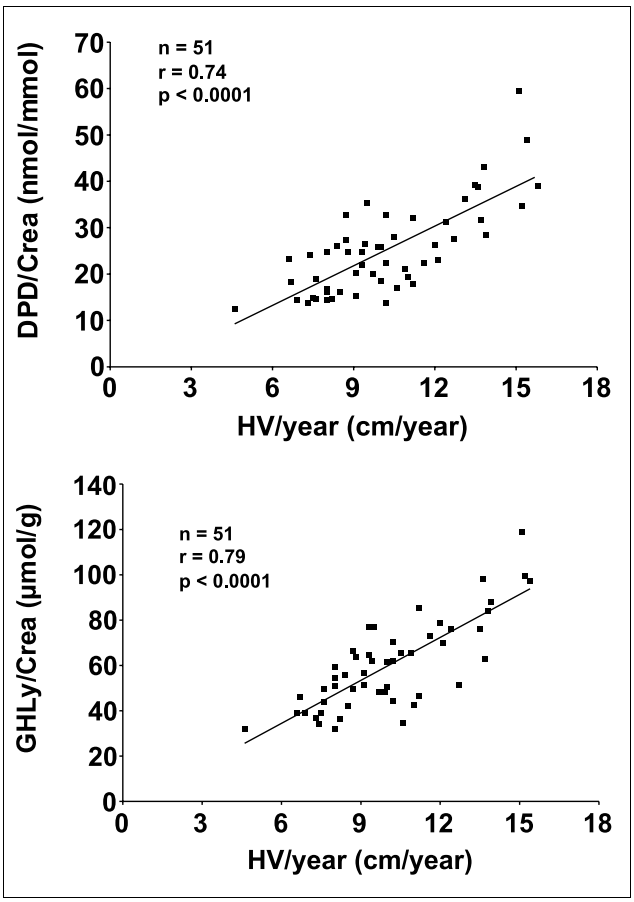

Figure 3 Relationship between the bone resorption marker DPD and GHLy 4 weeks after beginning a growth hormone therapy and the growth velocity of the first year on treatment

Example: Alkaline phosphatase activity is the sum of:

growth plate activity + remodelling + modelling

Specificity

Table 1 shows the specificities of the different bone markers for the description of bone formation and resorption activity. No marker has 100 % specificity. Even the so-called 'gold standard' for bone resorption – DPD – has been detected in many other joint tissues [1]. The low specificities of type I procollagen peptides and OHP do not exclude a high sensivitiy for the analysis of bone turnover. As any laboratory parameter, these measurements must be interpreted together with all the additional information available on the patient in question.

Day-to-day variation

In adults, for example, individuals studied had up to 62 % intra-individual variation, even in the widely used DPD marker [1]. Future studies have to show whether urine pooling over 3–5 days can resolve this problem.

Diurnal variation

Significant diurnal variation has been found for nearly all markers [71, 76]. The excretion of degradation products is more pronounced during the night and early morning (Fig. 4). Osteocalcin serum levels rise during the day. For this reason, serum and urine samples have to be drawn under standardized conditions.

Normalization to creatinine

When results are normalized to creatinine, spot urine can be used, making sampling easier. It must be remembered, however, that creatinine excretion reflects body muscle mass, which may be altered as a consequence of disease or therapeutic intervention or growth (Fig. 5). Furthermore, normalization to creatinine introduces additional analytical and biological variability. Therefore, whenever feasible, it is preferable to obtain timed urine collections and relate results to an index of body size.

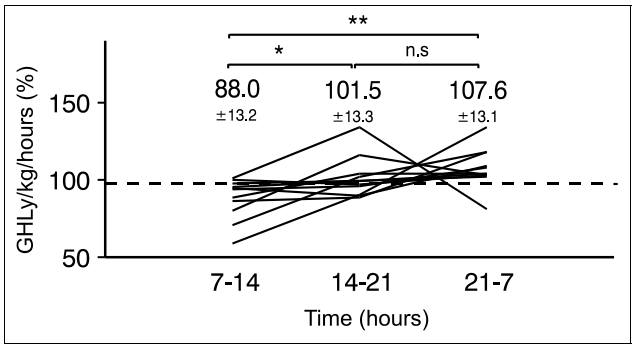

Figure 4 Diurnal rhythm of urines collagen degradition products (GHLy)

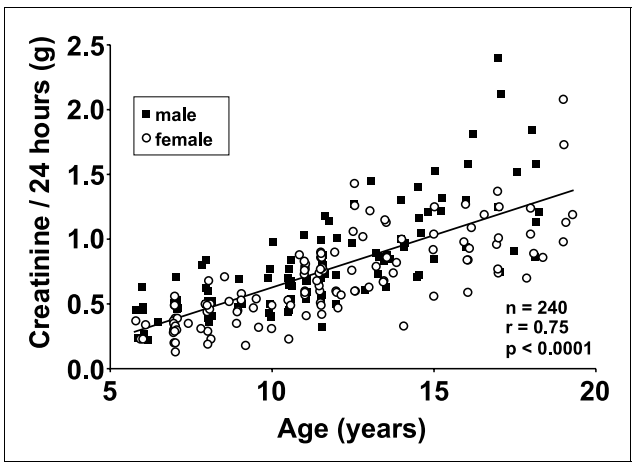

Figure 5 Age-dependent change of creatinine excretion

Conclusion

At the present time, the newer biochemical markers of bone turnover are interesting research tools for the analysis of the complex processes in the growing skeleton. They are useful in evaluating the side-effects of treatment with glucocorticoids and could possibly be of value in monitoring the effects of growth-modifying treatments. PICP may be helpful for diagnosing osteogenesis imperfecta type I. However, knowledge about the origin and metabolic pathways of these markers in health and disease is of critical importance for the interpretation of findings. As these issues are almost completely unresolved for the newer immunoassays for markers of bone metabolism, great caution is needed in the evaluation of results. Clearly, more research is needed, before any of these markers can be regarded a validated parameter for pediatric use.

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Appendix: Reference values (Tables 2 – 10)

When analysing markers of bone metabolism in children, it should be noted that a 'normal' result may be due to the combination of two contrasting pathologic effects, for example slow growth and enhanced bone turnover. Thus, from a theoretical point of view 'reference' data for markers of bone metabolism should not only be presented as a function of age, but also of height velocity. However, height velocity dependent reference values are not available for any bone marker.

Table 2 Reference ranges for alkaline phosphatase

Age (years)	TAP (U/L)		BAP-Elpho (U/L)		BAP-ELISA (U/L)		BAP-IRMA (µg/L)	
	girls	boys	girls	boys	girls	boys	girls	boys
5.0 – 9.9	297 ± 74	262 ± 81	259 ± 65	225 ± 55	95 ± 26	79 ± 33	66 ± 18	51 ± 22
10.0 – 14.9	304 ± 108	385 ± 102	252 ± 107	325 ± 112	87 ± 36	124 ± 38	52 ± 22	69 ± 18
15.0 – 19.9	124 ± 53	148 ± 56	102 ± 91	111 ± 67	23 ± 11	22 ± 56	15 ± 8	12 ± 3

Values are mean±SD.
Abbreviations: TAP, total alkaline phosphatase; BAP, bone-specific alkaline phosphatase; Elpho, electrophoresis; IRMA, immunoradiometric assay; ELISA, enzyme-linked immunosorbent assay.
Assays:TAP: Monotest®; Boehringer Mannheim Diagnostica; Mannheim, Germany. BAP-Elpho: Hydragel; ISO-PAL; Sebia; Issy-les-Moulineaux; France. BAP-IRMA: Tandem®-R Ostase™; Hybritech Inc., CA, USA. BAP-ELISA: Alkphase-B™; Metra Biosystems Inc., Mountain View, CA, USA. Source: [7].

Table 3 Reference ranges for osteocalcin

Age (years)	Both genders (ng/ml)
1–10	10–50
10–15	10–100
15–20	10–50
21–30	4–20

Assay: OSCAtest®, Henning Berlin GmbH, Berlin, Germany. Source: manufacturer.

Table 4 Reference ranges for procollagen type I C-terminal propeptide

	Age (years)	P1CP (µg/L)
		mean±SD
Both genders	0–0.25	2200±350
	0.26–0.5	1700±710
	0.51–0.75	1040±480
	0.76–1.0	870±370
	1.1–2.0	570±170
	2.1–3.0	390±160
	3.1–4.0	410±130
		95 % reference interval
Girls	4–12	225–676
	13–14	108–567
	15–18	82–285
Boys	4–16	193–716
	17–18	105–452

Assay: Radioimmunoassay; PICP, Orion Diagnostica, Turku, Finland. Sources: [3, 13].

Table 5 Reference ranges for hydroxyproline/creatinine

Age (years)	Girls (µg/mg)	Boys (µg/mg)
0.02–0.20	833 ± 259	833 ± 259
0.21–0.5	454 ± 147	454 ± 147
0.51–1.9	217 ± 59	217 ± 59
2.0 –5.9	150 ± 34	150 ± 34
6.0–13.9	110 ± 25	110±25
14.0–15.9	45 ± 10	105 ± 30
16.0–17.9	27 ± 6	38 ± 13

Values are mean±SD.
Values were determined in morning fasting urine specimen (1 µg/mg = 1.16 µmol/mmol). Assay: Hypronosticon®, Organon Teknika, Oberschleißheim, Germany. Source: [5].

Table 6 Reference ranges for total deoxypyridinoline, high performance liquid chromatography

Age (years)	tDPD (nmol/day)	tDPD/Cr (nmol/mmol)
4.0–10.0	167 ± 62.2	52.1 ± 16.5
10.1–12.0	240 ± 117	42.6 ± 10.1
12.1–14.0	343 ± 117	54.0 ± 14.5
14.1–16.0	280 ± 143	25.0 ± 11.5
16.1–18.0	102 ± 3.0	11.2 ± 0.7
young adults	78.0 ± 41.6	9.1 ± 5.3

Values are mean±SD.
Values were determined in 24-hour urine samples
Abbreviations: tDPD, total deoxypyridinoline; Cr, creatinine.
Assay: High-performance liquid chromatography [11]; Source: [9].

Table 7 Reference ranges for free deoxypyridinoline, enzyme-linked immunosorbent assay

Age (years)	Girls	Boys
4–12	1.4–8.2	1.4–8.2
13	0.8–6.3	3.9–10.0
14	0.2–5.7	2.8–8.9
15	0–5.2	1.8–7.9
16	0–4.7	0.7–6.8
17	0–4.2	0–5.8
18	0–3.7	0–4.7

The values are 2.5^{th} – 97.5^{th} percentiles (nmol/kg body weight/24 hours)
Values were determined in 24-hour urine samples.
Assay: ELISA; Pyrilinks-D™, Metra Biosystems Inc., Palo Alto, CA, USA. Source: [8].

Table 8 Reference ranges for free DPD, chemiluminescence assay

Age	Girls	Boys
4.0–10.0	19.5 ± 7.2	16.5 ± 5.0
10.1–12.0	15.1 ± 5.3	14.2 ± 5.3
12.1–14.0	11.6 ± 4.5	12.8 ± 6.0
14.1–16.0	6.6 ± 1.4	13.8 ± 7.2
16.1–20.0	7.1 ± 3.2	8.2 ± 4.8

Values are mean±SD (nmol/mmol creatinine).
Values were determined in 24-hour urine samples.
Assay: IMMULITE-Pyrilinks-D™ (DPC Biermann Inc., Bad Nauheim, Germany).
Source: Osteologic Laboratory Cologne, unpublished.

Table 9 Reference ranges for N-telopeptides of collagen type I (NTx)

Age	Girls	Boys
<1	2218 (1488–2896)	1988 (1387–2675)
1.0–1.9	1207 (816–1550)	1219 (859–1649)
2.0–2.9	953 (623–1387)	827 (663–1245)
3.0–3.9	760 (610–975)	721 (578–1018)
4.0–4.9	704 (537–910)	631 (494–940)
5.0–5.9	728 (646–923)	640 (481–777)
6.0–6.9	569 (404–760)	610 (477–760)
7.0–7.9	541 (464–666)	588 (498–799)
8.0–8.9	560 (447–824)	545 (386–713)
9.0–9.9	459 (382–606)	558 (442–674)
10.0–10.9	515 (374–664)	425 (322–593)
11.0–11.9	588 (399–1099)	532 (386–681)
12.0–12.9	543 (450–876)	659 (429–902)
13.0–13.9	298 (185–367)	462 (326–648)
14.0–14.9	223 (167–326)	399 (292–528)
15.0–15.9	217 (163–258)	376 (230–481)
16.0–16.9	148 (107–180)	292 (172–434)
17.0–17.9	163 (127–204)	163 (103–223)
18.0–18.9	116 (86–176)	292 (213–539)
19.0–19.9	112 (56–245)	125 (99–150)

The values are median (25^{th} percentile – 75^{th} percentile) and represent the median for age- and sex-group in the first line and the range from 25th to 75th percentile in the second line (pmol bone collagen equivalents/μmol creatinine).
Values were determined in random spot urines.
Assay: Osteomark™, Ostex International, Seattle, WA, USA. Source: [1].

Table 10 Reference ranges for Type I collagen C-terminal telopeptide (ICTP)

Sex	Age range	95 % reference interval (μg/litre)
Girls	4–8	5.7–14.9
	9–13	7.2–20.0
	14–15	4.1–13.3
	16–18	2.9–8.5
Boys	4–11	5.1–17.0
	12–16	7.5–22.8
	17–18	2.7–15.3

Assay: Radioimmunoassay; ICTP, Orion Diagnostica, Turku, Finland. Source: [3]