Utilization and Reference Values of Bone Turnover Markers: Osteocalcin and Procollagen Type 1 N-Propeptide

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Contents

Abstract

All body bones undergo continual remodeling. This process consists of bone resorption and bone formation which are closely coupled actions. Changes in bone tissue are accompanied by changes of biochemical markers. Plasma (and/or urine) concentration of these markers depends on bone resorption/formation activity, i.e., bone turnover rate. Osteocalcin is important noncollagenous protein

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in bone matrix, synthetized by osteoblasts and osteocytes. It is a good marker of bone turnover. Its undercarboxylated form has a role in regulation of energy metabolism. Osteocalcin is also involved in biosynthesis of testosterone or neurotransmitter production. Procollagen type 1 N-propeptide is a aminoterminal part of procollagen precursor cleaved by proteases during bone formation process. Its serum concentration is significantly related to newly formed collagen. Procollagen type 1 N-propeptide is supposed to be the most reliable biochemical marker of bone formation at present time. Reference values of both laboratory markers for central European population of children and adolescents are presented, and some data about reference intervals in adults from different ethnic groups are mentioned.

Keywords

Bone markers • Osteocalcin • Procollagen type 1 N-propeptide • Reference values • Children

List of Abbreviations

AMP	5′-adenosinmonophosphate
Atf4	Activating transcription factor 4
BMI	Body mass index
EDTA	Ethylenediaminetetraacetic acid
FoxO1	Forkhead box protein O1
mRNA	Messenger ribonucleic acid
OC	Osteocalcin
P1NP	Procollagen type 1 N-propeptide

Key Facts of Bone Turnover Markers

- For the prevention of osteoporosis in the future, it may be important to achieve a high amount and quality of bone tissue during childhood and adolescence.
- Bone turnover rate depends on activity of bone resorption and bone formation which are lifelong continual processes running in the whole skeleton.
- Bone turnover is minimally affected by some factors – for example, seasonality, daily meals, premenopausal menstrual cycles – while other individual lifestyle factors including lack of physical activity, smoking, and excessive alcohol intake could significantly damage bone health.
- Biochemical bone turnover markers are substances, measurable in blood and/or urine, whose concentrations are dynamically changed with bone turnover rate.
- Interpretation of results requires reliable age- and gender-specific reference databases. Detailed evaluation should be performed with regard to individual pubertal stage.

- Biochemical bone turnover markers could help to monitor changes of bone turnover very early, before significant changes of bone mineral density are measurable.
- They are very useful, but there is impossible to make a diagnosis of osteoporosis only according their values.

Definition of Words and Terms

Bone remodeling	Microscopic parts of bone tissue undergo bone resorption and followed bone formation. Thereby, the old or damaged bone tissue is replaced. This process is lifelong, genetically determined but influenceable by a lot of intrinsic and extrinsic factors.
Bone turnover markers	Bone-derived compounds, measurable in blood and/or urine, reflecting bone remodeling activity.
Bone turnover	In healthy skeleton, bone resorption and bone formation activities are closely coupled. Bone turnover rate depends on actual growth velocity, nutritional status, hormonal balance, and physical loading. Bone turnover is physiologically high during childhood with ascendant bone formation. Increased bone turnover with enhanced bone resorption is typical for postmenopausal bone loss.
Osteoblasts	Bone cells of mesenchymal origin. They are producing substances of bone matrix. They are responsible for bone formation. Osteoblasts embedded into bone tissue are called osteocytes.
Osteoclasts	Bone cells originated from monocyte-macrophage lineage. They are multinucleated and their main role is bone tissue degradation, that is, bone resorption.
Pubertal growth spurt	Significant increase of growth in height and weight during puberty. Pubertal growth spurt is managed by sex steroids and growth hormone and lasts for 2–3 years. There is very important role of estrogens which promote growth but also have maturational effect on long bones leading to epiphyseal fusion and final growth cessation in both genders.
Tanner stages	The most manifested somatic pubertal changes are growth in stature and development of sexual characteristics (it means development of external genitalia in boys, breast development in girls, and pubic hair development in boys and girls). They appear in typical sequences. It allows determine the staging system. Marshall and Tanner published the most frequently utilized system, commonly called "Tanner stages."

Introduction

The skeleton provides protection for organs, represents a storage system for minerals, and creates points of attachment for skeletal muscles. It is the largest organ in organism. Findings from recent years support theory that skeleton is a true endocrine organ (Oldknow et al. 2015). The whole skeleton undergoes permanent remodeling process. Bone resorption is coupled to bone formation. Bone-derived compounds reflecting bone remodeling activity represent two main categories – bone formation and bone resorption markers. In adults, approximately 20% of bone tissue is replaced annually varying by site and type (Carey et al. 2006). The remodeling of cortical bone is slower than trabecular one. To monitor these dynamic changes, laboratory bone turnover markers are measured in blood and urine. In postmenopausal women, they have shown association with bone loss and fracture risk (Garnero 2000). The bone turnover rate in healthy premenopausal women was considered to be ideal metabolic situation of skeletal tissue. Significant changes of biochemical markers could be found within several months, whereas changes in bone mineral density could be recorded after about 2 years at least. Therefore, biochemical markers are useful tool for treatment monitoring. But there is no possibility to make a diagnosis of osteoporosis only according their values. In children, rapid bone turnover and high growth of bone mass during childhood result in significantly elevated bone marker levels. Bone markers in children reach the circulation during bone growth, modeling, and remodeling. They are changed in time according to growth velocity. Measurement in urine brings about some problems in childhood: the child should be able to comply with the instructions for obtaining a second void fasting urine (Mora et al. 1998); there are significant circadian and intraindividual variation in urinary bone markers (Schönau and Rauch 1997); their concentration should be expressed in relation to creatinine which is subject to change with muscle mass accrual (Szulc et al. 2000). Thus, the measurement of bone markers in blood seems to be more convenient in children and adolescents and become the preferred. It is necessary to take in account that markers of bone turnover have different degree of intraindividual variation. Their level could be influenced by a lot of factors: age, gender, ethnicity, fasting, physical activity, calcium supplementation, and others. Long-term corticosteroid therapy results in suppression of bone formation (van Staa et al. 2002). Its role could play menstrual cycle, with high osteoblastic activity during the luteal phase (Nielsen et al. 1990) and higher bone resorption within the follicular period (Chiu et al. 1999). There are different opinions of seasonal variation from insignificant changes (Blumsohn et al. 2003) to significant increase during the winter (Woitge et al. 1998). Regarding interindividual variation, parameters of bone metabolism are highest in infants up to 3 years of age. Then they are stable (but higher than in adults) until pubertal growth spurt, in which they are influenced by pubertal stage rather than age (Mora et al. 1999). Somatic growth in childhood and adolescence comprises bone modeling, bone remodeling, epiphyseal bone growth, and soft tissue accrual. There is no marker in children specific for these processes. Thus, results should be corrected to growth velocity and pubertal development,

and large population of healthy children is needed to obtain sex- and age-specific pediatric reference intervals for bone markers.

Osteocalcin

Osteocalcin – Characteristics

OC was isolated from bone tissue 40 years ago (Hauschka et al. 1975). It is a small protein (49 amino acids in human, 46–50 amino acid residues varying from different species) synthetized by osteoblasts and osteocytes. OC gene activity is regulated by 1-alpha, 25-hydroxyvitamin D3. Translation results in prepro-OC containing 98 amino acid residues. Following proteolysis will form the mature OC (Lee et al. 2000). Three vitamin K-dependent γ-carboxyglutamic acid residues are added. They are important for OC activity. OC is one of most important noncollagenous proteins in bone matrix. Carboxylated OC is able, in the presence of calcium, to bind to hydroxyapatite and regulate bone mineralization. Osteoblasts can secret OC to stimulate osteoblastic differentiation and osteocytic maturation (Shao et al. 2015). Small amount of OC circulates in blood in carboxylated or undercarboxylated form (Ferron et al. 2010). OC carboxylation status has an important role in energy metabolism management. Undercarboxylated OC is acting as a hormone with only little affinity to bone. Its effect is realized through several activating transcription factors, for example, FoxO1 and activating Atf4. They are involved in more processes including regulation of insulin sensitivity and glucose tolerance (Kode et al. 2012). According to recent research, undercarboxylated OC is able to stimulate beta cell proliferation (Klein 2014). OC is stored in bone tissue and during bone resorption released to circulation. Local pH is decreased during osteoclastic activity and allows OC decarboxylation (Shao et al. 2015). Thus, osteoclasts are able to regulate glucose metabolism indirectly by osteocalcin decarboxylation (Kanazawa 2015). Glucocorticoids suppress osteoblast activity and OC production (Ferron and Lacombe 2014); thyroid hormone stimulate OC synthesis in osteoblasts under regulation of AMP-activated protein kinase. Thus, energy metabolism has a direct link to bone tissue (Kondo et al. 2013). The evidence of expression of OC mRNA in adipose tissue (it means ability to product OC) contributes to the complex picture (Foresta et al. 2010). OC participates also in management of testosterone biosynthesis in males (Oury et al. 2011). It is insulin signaling in osteoblast which has positive influence to OC stimulated testosterone synthesis illustrating the existence of pancreas-bone-testis axis (Oury et al. 2013). Finally, osteocalcin is involved in regulation of neurotransmitter production and could play a role in brain function (Zoch et al. 2016). OC is stable as EDTA sample for up to 8 h at room temperature (Stokes et al. 2011), but has a short half-life (Blumsohn et al. 1995) with large interlab variation (Vasikaran et al. 2011b). If there is no possibility of immediate analysis, samples should be stored at −20 °C or lower. OC has a circadian variability with maximal levels in early morning and nadir between 11:00 and 15:00 h. Difference is about 20% (Wheater et al. 2013). The ratio of undercarboxylated and

carboxylated OC is not changed, but the total amount of undercarboxylated OC in circulation had the similar changes as the circadian rhythm of OC (Lee et al. 2000; Nishimura et al. 2007). It could be also influenced by vitamin K status and renal functions (Brown et al. 2009). On the other hand, OC shows negligible dietary influences – its analytical coefficient of variation is below 5% in both the fed and fasting states (Clowes et al. 2002).

Osteocalcin – Utilization

Serum concentration of OC has relation to osteoblast number and bone formation, and it was repeatedly documented to use it as laboratory marker of bone formation (Gundberg et al. 2012), but it is possible to say that OC reflects entire skeletal metabolic activity (Wheater et al. 2013). At present time, OC is supposed to be very good marker of bone turnover rather than bone formation. In healthy adults, OC levels correlate negatively with sclerostin (Amrein et al. 2012). Some authors studied the possibility to use OC plasma concentrations as a predictor of cardiovascular disease risk in seniors. In population older than 75 years, higher plasma OC concentration was related to higher risk of cardiovascular disease in women, while significant inverse relation was found in men (Holvik et al. 2014). In male adolescents, OC has inverse relation to leptin values and body adiposity (Jürimäe et al. 2015).

Osteocalcin – Reference Values

Serum concentration of OC is highest in the early morning and lowest in the afternoon. Rauchenzauner et al. gathered morning blood samples from 572 healthy children and adolescents to form reference curves for bone markers including serum osteocalcin. Values correlated to age and pubertal stage. Taller and heavier individuals for age had greater bone marker concentrations, which may be due to greater growth velocity (Rauchenzauner et al. 2007). In a group of Brazilian male adolescents, OC levels were related to bone age with maximal values between 13 and 15 years (da Silva et al. 2012). Reference values of OC in relation to age, sex, and pubertal stage were recently established in a group of 439 healthy children and adolescents in central Europe. OC missed postnatal peak, but its levels were higher than the adult reference interval throughout childhood (Bayer 2014). See Tables 1 and 2. OC peaked with the pubertal growth spurt at 2nd–3rd Tanner stage (Marshall and Tanner 1969; 1970) of breast development in girls (Fig. 1) and at 2nd–3rd Tanner stage of genital development in boys (Fig. 2). In group of 638 healthy premenopausal women, serum OC decreased with advancing age and independently and negatively correlated with BMI ($P<0.001$). The use of contraceptive pills in healthy premenopausal women was associated with 14–26% decrease of OC in comparison with non users ($P<0.005$) (Adami et al. 2008). Nabipour et al. found in group of 785 healthy adult Iranian individuals consecutive decrease in serum osteocalcin in women from second to third decade of life with following increase in women older than fifty. In men, the highest

Table 1 Osteocalcin (µg/l) and P1NP (µg/l) serum concentration in healthy children 0–9 years (Bayer 2014) (With permission of Springer Science + Business Media)

Age	n	Osteocalcin		P1NP	
		Lower	Upper	Lower	Upper
0–1	18	20,8	144,3	227,2	4762,8
1.1–2	16	28,3	126,1	346,6	1088,0
2.1–3	16	30,7	85,4	178,3	645,7
3.1–4	15	23,9	98,4	135,2	746,1
4.1–5	17	22,8	129,3	85,7	901,7
5.1–6	18	42,1	128,2	111,5	768,4
6.1–7	17	30,9	122,2	187,6	887,3
7.1–8	15	12,5	232,5	49,9	1200,0
8.1–9	19	25,7	151,1	120,4	1021,0

Lower and upper limits correspond to 2.5 and 97.5 percentile

Table 2 Serum concentration of osteocalcin in healthy children 9.1–18 years (Bayer 2014) (With permission of Springer Science + Business Media)

Age	Osteocalcin µg/l						P	Test
	Boys			Girls				
	n	Lower	Upper	n	Lower	Upper		
9.1–10	16	12,2	110,6	15	18,4	251,7	0,847	KS
10.1–11	17	12,6	145,7	15	18,5	154,2	0,334	t
11.1–12	16	31,9	200,9	15	11,9	140,4	0,554	t
12.1–13	14	19,8	164,9	15	13,1	186,7	0,749	t
13.1–14	17	58,7	236,2	15	16,8	238,9	0,122	MW
14.1–15	16	25,7	241,0	16	15,4	88,8	0,000068	t
15.1–16	17	30,1	186,9	16	16,9	96,3	0,0169	KS
16.1–17	17	32,0	124,2	16	5,7	66,7	0,00004	t
17.1–18	19	13,5	160,1	16	20,7	45,6	0,003	KS

Lower and upper limits correspond to 2.5 and 97.5 percentile Statistical evaluation: *KS* .... Kolmogorov–Smirnov test, *t* . . ..two-sample *t*-test, *MW* . . .Mann–Whitney test, *P* . . .significance level (comparing boys and girls)

serum osteocalcin concentration was revealed in second decade, showing important differences according gender (Nabipour et al. 2008). Not only very young age is in relation to higher levels of OC but also low BMI could be another contributing factor in healthy adult women (Glover et al. 2008).

Procollagen Type 1 N-Propeptide

P1NP – Characteristics

Procollagen precursor is secreted from proliferating osteoblast to extracellular space. Then, the amino- and carboxy-terminals are split off by proteases and are released into the blood. Trimeric structure of P1NP is very quickly broken down by thermal

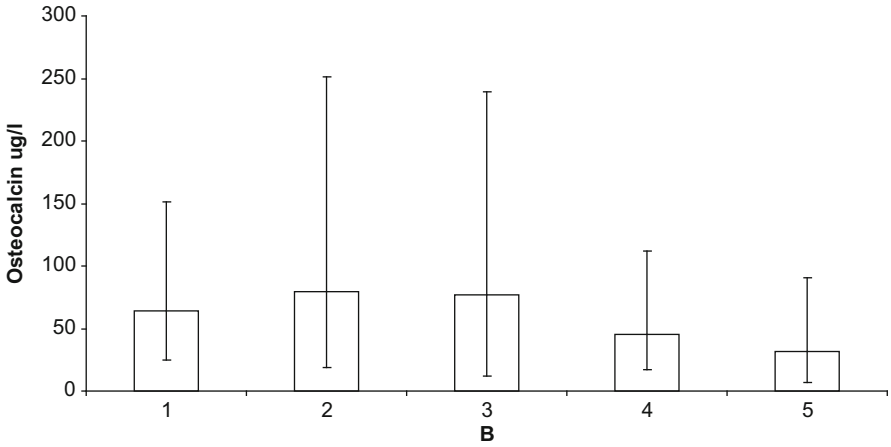

Fig. 1 Serum concentration of osteocalcin – relation to pubertal stage in girls (Bayer 2014) B. grade of breast development in girls (Tanner B) Lower and upper deviations correspond to 2.5 and 97.5 percentile (With permission of Springer Science + Business Media)

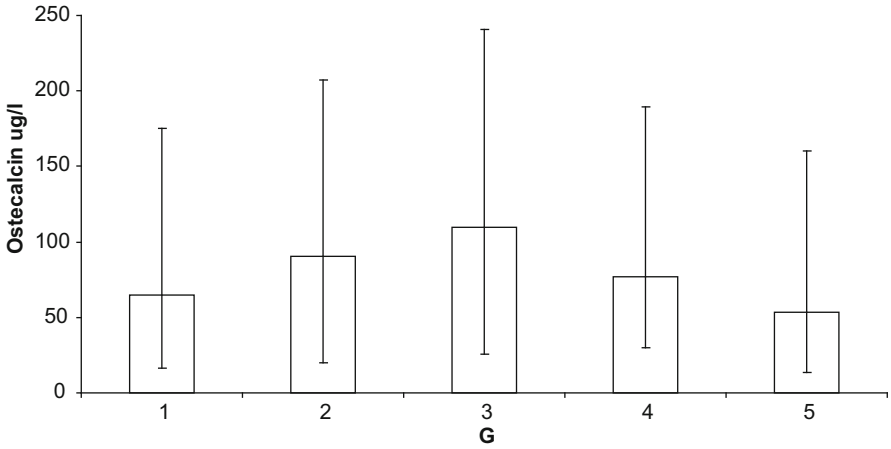

Fig. 2 Serum concentration of osteocalcin – relation to pubertal stage in boys (Bayer 2014) G grade of genital status in boys (Tanner G) Lower and upper deviations correspond to 2.5 and 97.5 percentile (With permission of Springer Science + Business Media)

degradation resulting in monomeric structure (Brandt et al. 1999). Trimeric form or both structure, so-called total P1NP, could be measured by current immunoassays (Wheater et al. 2013). The serum concentration of P1NP is directly related to the amount of newly formed collagen laid down in the bone – it is a very good bone formation marker. P1NP was confirmed as a more sensitive marker of type I collagen synthesis than P1CP (Crofton et al. 2004). P1NP has advantageous laboratory properties – it is stable in serum at room temperature (Stokes et al. 2011) – has

low interindividual variability and good assay precision (Vasikaran et al. 2011b). P1NP did not vary with season (Munday et al. 2006) and only small circadian rhythm was reported (Brown et al. 2009). But it is necessary to bear in mind that total P1NP concentration could be affected by delayed clearance of monomeric fraction, for example, in renal failure or metastatic bone disease (Marin et al. 2011). P1NP also shows imponderable dietary influences with coefficient of variation similar to OC (Clowes et al. 2002).

P1NP – Utilization

According Bone Marker Standards Working Group, P1NP represents a reliable laboratory marker of bone formation (Vasikaran et al. 2011a) due to its characteristics and good assay precision. During pubertal spurt, bone modeling increases with high P1NP levels corresponding to peak of growth hormone secretion but without significant association to systemic IGF-1 (Russell et al. 2011). Serum P1NP levels significantly correlated with total body bone mineral density changes in children after successful living-related liver transplantation and could be used as predictor of bone status in these patients (Kryskiewicz et al. 2012).

P1NP – Reference Values

Reference values of P1NP in relation to age, sex, and pubertal stage were established in a group of 439 healthy children and adolescents in central Europe. The highest levels of P1NP were observed during the first year of life. It slows down until 3 years of age and is then relatively stable up to the pubertal growth spurt (Bayer 2014). See Tables 1 and 3. P1NP peaks during 2nd–3rd Tanner stage of breast development in girls (Fig. 3) and during 2nd–4th Tanner stage of genital development in boys (Fig. 4). High bone turnover rate, reflecting bone growth, is decreased in young adult age with the end of puberty and seems to be stable until hormonal changes in advanced age. In adult Spanish men aged 65 ± 9 years, 95% P1NP ranges were 15–78 ug/l (Olmos et al. 2010). Similar age-related reference P1NP values were recently obtained in Australian population. In men aged 25–70 years, the interval was 15–80 ug/l. Values increased in older men, possibly due to changes in bone turnover (Jenkins et al. 2013). In the female Spain population aged 63 ± 9 years, 95% P1NP reference interval reached 19–100 ug/l (Martínez et al. 2009), and in Australian women with the corresponding age it was 15–75 ug/l. P1NP values in younger women were 25–90 ug/l (age less than 30 years), 15–80 ug/l (30–39 years), and 15-60ug/l (40–49 years) (Jenkins et al. 2013). Analogous results were found in a group of healthy Thai women – 95% confident interval was 40.79–48.35 ug/l in women with mean age 38.5 years (Bunyaratavej and Kittimanon 2005) as well as in premenopausal healthy Japanese women aged 30–44 years. Their P1NP plasma levels were 39.4 ± 15.4 ug/l (Nomura et al. 2013). Values generally increased in older women with probable high bone turnover.

Table 3 Serum concentration of P1NP in children 9.1–18 years (Bayer 2014) (With permission of Springer Science + Business Media)

	P1NP µg/l							
	Boys			Girls				
Age	n	Lower	Upper	n	Lower	Upper	P	Test
9.1–10	16	45,2	552,5	15	42,7	952,1	0,962	t
10.1–11	17	48,3	769,6	15	62,5	914,7	0,457	t
11.1–12	16	142,5	2501,7	15	65,3	855,8	0,938	KS
12.1–13	14	67,2	854,8	15	47,0	984,9	0,623	t
13.1–14	17	267,9	1514,6	15	37,1	1195,6	0,047	t
14.1–15	16	148,1	1200,0	16	58,5	451,4	0,0018	KS
15.1–16	17	81,8	961,4	16	45,6	600,2	0	KS
16.1–17	17	77,7	430,3	16	14,6	238,3	0,00021	t
17.1–18	19	38,7	494,5	16	36,3	143,9	0,0036	KS

Lower and upper limits correspond to 2.5 and 97.5 percentile Statistical evaluation: *KS* .... Kolmogorov–Smirnov test, *t* . . ..two-sample *t*-test, *MW* . . .Mann–Whitney test, *P* . . .significance level (comparing boys and girls)

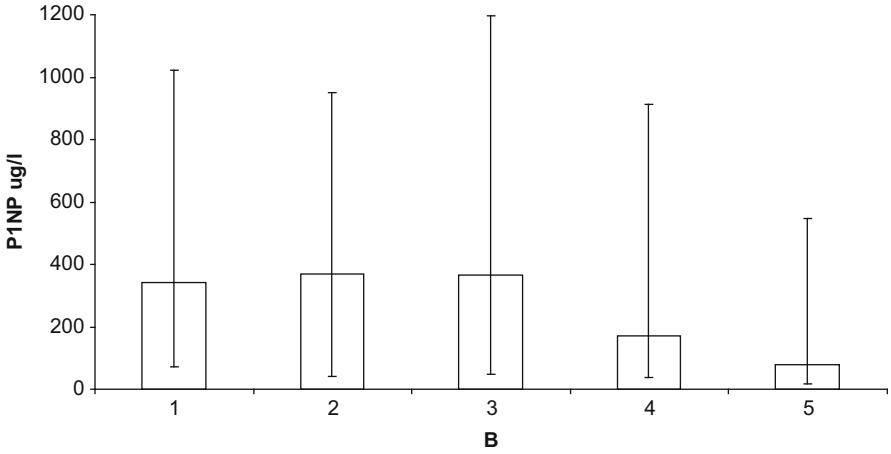

Fig. 3 Serum concentration of P1NP – relation to pubertal stage in girls (Bayer 2014) B grade of breast development in girls (Tanner B) Lower and upper deviations correspond to 2.5 and 97.5 percentile (With permission of Springer Science + Business Media)

Potential Applications to Prognosis, Other Diseases, or Conditions

Referred laboratory markers (as well as other bone turnover markers) could provide good information about bone metabolism rate and on the efficacy of treatment in osteoporotic patients. However, their large biological variation limits their predictive value in individual patient. They would not be suitable to estimate bone loss as

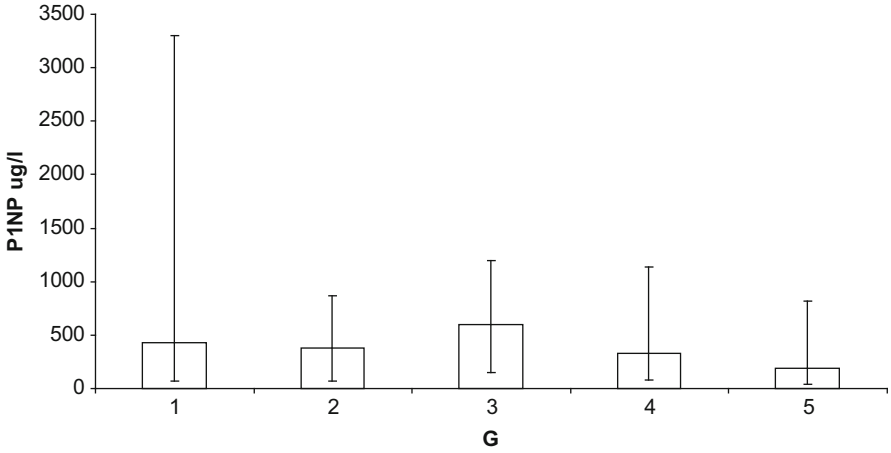

Fig. 4 Serum concentration of P1NP – relation to pubertal stage in boys (Bayer 2014) G grade of genital status in boys (Tanner G) Lower and upper deviations correspond to 2.5 and 97.5 percentile (With permission of Springer Science + Business Media)

alone, but they are very useful supplement to bone mineral density measurement. Laboratory markers can reflect treatment efficacy before bone mineral density changes reach significancy. Their early changes can be used to measure the clinical efficacy of an antiresorptive treatment and to reinforce patient compliance (Bergmann et al. 2009) and are broadly used as a surrogate for bone mineral density changes. During the treatment with anabolic agent such as parathormone, markers of bone formation increase very early after the initiation of therapy. Due to the coupling these changes are followed by increase in resorption markers (Finkelstein et al. 2010). Bone turnover markers can also monitor patients during treatment holidays (Wheater et al. 2013). According to recent research, OC plasma concentrations could serve as predictor of cardiovascular disease risk in women older than 75 years (Holvik et al. 2014).

Summary Points

- This chapter focuses on osteocalcin and procollagen type 1 N-propeptide, which are biochemical markers of bone metabolism.
- Osteocalcin is most important noncollagenous protein in bone matrix, synthetized by osteoblasts and osteocytes.
- Serum concentration of osteocalcin reflects activity of bone turnover.
- Undercarboxylated form of osteocalcin participates in regulation of energy metabolism.
- Osteocalcin is also involved in management of testosterone secretion and neurotransmitter production.

- Procollagen type 1 N-propeptide is a peptide split off in the process of collagen formation.
- Serum concentration of procollagen type 1 N-propeptide is relevant to new collagen amount.
- Procollagen type 1 N-propeptide is a reliable marker of bone formation.
- Reference values osteocalcin and procollagen type 1 N-propeptide for children, adolescents, and some data about adult population are presented.

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