CLINICAL STUDY

The relationship between the GH/IGF-I axis and serum markers of bone turnover metabolism in healthy children

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Abstract

Context: There is evidence to suggest that IGF-I plays a role in regulating bone turnover.

Objective: To evaluate the relationships between serum concentrations of IGF-I and IGF-binding protein-3 (IGFBP-3), and bone metabolism markers in healthy children.

Design and setting: Prospective cross-sectional study.

Subjects and methods: A cohort of 579 boys and 540 girls, all healthy Caucasian, were included in this study. Serum IGF-I and IGFBP-3 concentrations, bone alkaline phosphatase (BAP) and CrossLaps (markers of bone formation and bone resorption respectively) levels were evaluated as a function of age, gender, pubertal stage and body mass index.

Results: Serum IGF-I SDS levels were positively correlated with BAP and CrossLaps SDS levels before and after puberty, and also with CrossLaps during puberty (weak correlation). Serum IGFBP-3 SDS levels were positively correlated with BAP and CrossLaps levels before, during (weak correlation) and after puberty (for BAP levels only).

Conclusions: This study demonstrated the independent association between serum IGF-I and IGFBP-3 concentrations with both serum bone formation and resorption markers in healthy children. Physiological differences before, during and after puberty in the association of serum IGF-I and IGFBP-3 levels with the serum bone metabolism markers were found. These differences may be related to differences in interactions between sex steroid hormones and the GH/IGF-I system, bone metabolism and growth during the pubertal transition. Improvements in our understanding of life course determinants of the IGF-I system and bone metabolism are required to shed further light on the role of the GH/IGF-I axis in bone remodelling.

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Introduction

Bone metabolism is affected by genetic, nutritional, lifestyle and hormonal factors. Serum levels of bone alkaline phosphatase (BAP) and CrossLaps are recognised markers of bone formation and resorption respectively. These markers have been demonstrated to reflect changes in growth of children (1–4). Insulin-like growth factor-I (IGF-I) is an important regulator of bone turnover at tissue level. It has been shown to enhance osteoblast proliferation, to stimulate type I collagen production and BAP activity and to modulate osteoblast–osteoclast interactions (5, 6). Circulating IGF-I levels also directly regulate bone growth and density, and epidemiological studies have suggested a causal relationship between serum levels of IGF-I and fracture risk and/or bone density or bone mass (5, 7–9). Serum

IGF-I binds mostly to IGF-binding protein-3 (IGFBP-3) which controls and modulates its tissue bioavailability, directly regulating growth (10). It has been suggested that the IGF-I to IGFBP-3 molar ratio could be used as a crude indicator of IGF-I bioavailability. Serum IGF-I and IGFBP-3 concentrations and circulating free IGF-I concentration are related to growth hormone (GH) secretion, although they are also influenced by other factors, including genetic and nutritional factors (11). GH increases the levels of markers of both bone formation and bone resorption (12), and the IGF system appears to be one of the most important factors affecting bone remodelling when administered exogenously to mice and rats or when overexpressed in bone (13). Bone remodelling markers may provide an early indication of the height velocity response to growth-promoting treatment. They may also be of considerable value for

assessing the effects of any underlying disorder and therapeutic interventions on bone turnover and growth (14–16). However, the interaction between the serum GH-IGF-I axis and biochemical markers of bone turnover is complex and not fully understood. Previous studies investigating correlations between biochemical markers of bone turnover, and serum IGF-I and IGFBP-3 concentrations in children have reported conflicting results (17–21).

In this study, we investigated whether serum levels of IGF-I and IGFBP-3, and IGF-I:IGFBP-3 ratio had independent associations on serum markers of bone turnover metabolism in healthy children.

Subjects and methods

In total, 579 boys and 540 girls participated in the study. All were recruited at a regional institute for health, at a median age (25th–75th percentiles) of 13.3 (10.7–16.0) years for boys and 13.4 (10.9–16.1) years for girls. Subjects who met the inclusion criteria and underwent routine medical check-ups provided by social security medical centres between May 2002 and December 2003 were asked to participate in the study. These patients lived in ten administrative areas (*départements*) in the western part of France. These medical centres are part of the French national health insurance system. They provide a biomedical examination, once every 5 years, to all individuals spontaneously attending or invited to attend the medical centre. This medical check-up includes a series of biological tests and a clinical examination. Subjects were asked for an additional blood sample for IGF-I, IGFBP-3, BAP and CrossLaps measurements during this spontaneous health evaluation. The inclusion criteria for this study were: age between 6 and 20 years, Caucasian. The exclusion criteria were a history of chronic disease or smoking and current treatment (including oral contraception) or disease (based on medical history and results of current health examination and biological records for liver, kidney, lipid and haematological function).

The study protocol was reviewed and approved by the faculty ethics committee. It was explained to all subjects and their parents who signed a written consent form for participation.

Height and weight were measured using a stadiometer and a calibrated scale respectively. They were expressed as a SDS for sex and chronological age (22). We also calculated body mass index (BMI; $\text{kg/m}^2 = \text{weight/height}^2$) in SDS for sex and chronological age (23). Pubertal development was assessed according to Tanner stage (24).

Blood samples were drawn from all patients between 0800 and 1000 h, after an overnight fast. Samples were stored at -20°C until analysis.

Serum IGF-1 concentrations were determined by fully automated two-site chemiluminescence immunoassays (Nichols Adavantage, Nichols Institute Diagnostics, Paris, France), with an interassay coefficient of variation (CV) of $<5.8\%$. Serum IGFBP-3 concentrations were measured by an immunoradiometric assay (IRMA) kit (ACTIVE IGFBP-3 IRMA) from Diagnostic Systems Laboratories (Cergy Pontoise, France), with an interassay CV of $<6.5\%$. Based on the molecular weight of IGF-I and IGFBP-3, the molar ratio of IGF-I/IGFBP-3 was calculated. Serum BAP concentrations were determined by IRMAs (Tandem-R Ostase, Beckman Coulter, Roissy, France), with an interassay CV of $<9.2\%$. The serum CrossLaps assay is an enzyme-linked immunoassay (serum CrossLaps ELISA, Nordic Bioscience Diagnostics A/S, Herlev, Denmark) specific for a β -aspartate form of the EKAHD-b-GGR epitope derived from the cross-linked degradation products of C-terminal telopeptides of type I collagen. The interassay CV was $<5\%$.

Statistical analysis

Results are expressed as medians (25th–75th percentiles) for quantitative variables and absolute numbers for qualitative variables. Comparisons between subjects of the same sex were performed with non-parametric tests (χ^2 or Fisher's exact test, as appropriate, for categorical variables and Wilcoxon's test for quantitative variables).

Relationships between IGF-I, IGFBP-3, IGF-I:IGFBP-3 molar ratio, CrossLaps and BAP levels and height in SDS were studied by multiple linear regression with adjustment for age, sex and pubertal stage. The same model was used to study the effect of BMI (SDS). Pearson's partial correlation coefficients were estimated for each pubertal stage and adjusted for BMI (SDS). The correlations assessed were those between IGF-I and IGFBP-3, BAP and CrossLaps levels and between serum IGF-I, IGFBP-3, IGF-I:IGFBP-3 ratio and bone turnover marker levels. The analysis was simplified by expressing serum concentrations as SDS. Age-specific reference intervals were estimated by the simplified parametric method (25). Basic data transformation and multiple regression techniques were combined to model the mean, s.d. and skewness. We checked that the assumptions of the model, including in particular the normal distribution of $\text{SDS} = (\text{measurement} - \text{mean})/\text{s.d.}$, applied at all steps. Goodness-of-fit plots and tests were used to assess the overall fit of the model. Bonferroni correction was used to compensate for multiple comparisons, with P values <0.01 considered significant.

Variables were log transformed where appropriate. We also checked for normality of the residuals and an absence of heteroscedasticity. All tests were two tailed. Statistical analyses were performed with the SAS 9.12 (SAS Inc., Cary, NC, USA) software package for PC.

Results

The characteristics of the study population are presented as a function of pubertal stage and sex in Table 1. Subjects were slightly taller than the French reference population, for both sexes, documenting the upward secular drift in height. For girls, the median age at menarche was 12.8 (11.9–13.6) years.

The age- and puberty-related changes in serum IGF-I, IGFBP-3, IGF-I:IGFBP-3 molar ratio, BAP and CrossLaps levels in normal subjects are shown in Figs 1 and 2 respectively. As expected, serum IGF-I, IGFBP-3 levels and IGF-I:IGFBP-3 molar ratio increased during childhood, with the highest values recorded during puberty. Serum IGF-I levels and IGF-I:IGFBP-3 molar ratio decreased slightly thereafter. Serum IGFBP-3 levels remained relatively constant in late puberty (Tanner stages 4–5) at a level higher than that earlier in puberty. Before puberty, levels were significantly higher in girls than in boys ($P < 0.01$). They peaked about 2 years earlier in girls than in boys and occurred during Tanner's pubertal stage 4.

Throughout the prepubertal period, from an age of 6 years, serum BAP and CrossLaps levels increased slightly in both sexes. During this period, similar values were obtained in girls and boys. These levels peaked during mid-puberty (Tanner stage 3) in both sexes, dramatically decreasing thereafter. They were higher in boys than in girls between the ages of 12 and 20 years, with the maximum difference between the sexes observed between the ages of 13.5 and 15.5 years (Tanner stage 5; $P < 0.0001$).

Influence of height

In both sexes, regardless of age and pubertal stage, height (SDS) was positively associated with serum log IGF-I ($\beta_{\text{coefficient}} = 0.09$ for boys and 0.07 for girls, $P < 0.0001$), log IGFBP-3 ($\beta_{\text{coefficient}} = 0.02$, $P = 0.001$ for boys; $\beta_{\text{coefficient}} = 0.03$, $P < 0.0001$ for girls), log IGF-I/IGFBP-3 molar ratio ($\beta_{\text{coefficient}} = 0.06$, $P < 0.0001$ for boys; $\beta_{\text{coefficient}} = 0.04$, $P = 0.0001$ for girls) values. No relationship was found between height and serum BAP or CrossLaps levels.

Influence of BMI

In both sexes, regardless of age and pubertal stage, BMI (SDS) was positively associated with serum log IGF-I values ($\beta_{\text{coefficient}} = 0.06$, $P < 0.0001$ for boys; $\beta_{\text{coefficient}} = 0.03$, $P = 0.0002$ for girls), log IGFBP-3 ($\beta_{\text{coefficient}} = 0.03$, $P < 0.0001$ for boys; $\beta_{\text{coefficient}} = 0.02$, $P < 0.001$ for girls), log IGF-I/IGFBP-3 ($\beta_{\text{coefficient}} = 0.04$, $P < 0.0001$ for boys; $\beta_{\text{coefficient}} = 0.03$, $P = 0.003$ for girls) and negatively associated with serum log CrossLaps levels ($\beta_{\text{coefficient}} = -0.03$, $P = 0.0002$ for boys; $\beta_{\text{coefficient}} = -0.06$, $P < 0.01$ for girls). No relationship was found between BMI and serum BAP levels.

Table 1 Clinical characteristics of the study population ($n = 1119$) as a function of pubertal stage and sex.

Pubertal stage (Tanner)	Boys ($n = 579$)				Girls ($n = 540$)			
	N	Age (years)	Height SDS	BMI SDS	N	Age (years)	Height SDS	BMI SDS
1	177	9.2 (7.9; 10.8)	0.90 (0.2; 1.69)	0.03 (−0.91; 1.13)	123	8.7 (7.4; 10.2)	0.95 (0.15; 1.68)	0.08 (−0.54; 1.10)
2	83	12.1 (11.3; 13.0)	1.01 (0.04; 1.84)	0.17 (−0.68; 1.41)	55	11.2 (10.6; 12.1)	0.78 (0.05; 1.84)	−0.19 (−1.00; 0.90)
3	54	13.8 (12.4; 14.9)	0.84 (−0.01; 1.75)	0.21 (−0.37; 0.97)	38	12.2 (11.4; 13.3)	0.96 (0.22; 1.77)	0.38 (−0.27; 1.17)
4	76	15.2 (14.3; 16.4)	0.77 (0.35; 1.36)	0.45 (−0.28; 1.30)	80	13.2 (12.4; 13.9)	1.09 (0.21; 2.03)	0.51 (−0.72; 1.40)
5	170	17.3 (15.4; 18.2)	0.70 (0.02; 1.43)	0.35 (−0.19; 1.11)	241	16.5 (15.3; 17.8)	0.52 (−0.18; 1.11)	0.19 (−0.43; 1.16)

Results are expressed as medians (25th–75th percentiles). Missing values for pubertal staging, $n = 19$ for boys and $n = 3$ for girls.

Figure 1 Cross-sectional measurements of serum IGF-I, IGFBP-3, IGF-I:IGFBP-3 ratio, bone alkaline phosphatase and CrossLaps levels in 1119 healthy subjects, as a function of chronological age and sex. Lines represent smoothed Loess fits.

Figure 2 Box and whisker plots of serum IGF-I, IGFBP-3, IGF-I:IGFBP-3 ratio, bone alkaline phosphatase and CrossLaps levels in 1119 healthy subjects as a function of pubertal stage and sex. The horizontal line represents the median; the box indicates the interquartile range.

Correlations between serum IGF-I and IGFBP-3 and between serum BAP and CrossLaps SDS values in normal children

After adjustment for BMI (SDS), serum IGF-I and IGFBP-3 SDS values were positively correlated at all pubertal stages (ρ between 0.37 and 0.59; $P < 0.0001$). Serum BAP and CrossLaps SDS values were also positively correlated during the prepubertal period ($\rho = 0.21$; $P < 0.001$), in late puberty and after puberty (Tanner stages 4 and 5) ($\rho = 0.47$ and 0.42 respectively; $P < 0.0001$), with no correlation found during early and mid-puberty (data not shown).

Correlations between serum IGF-I, IGFBP-3, IGF-I/IGFBP-3 and bone turnover marker (serum BAP and CrossLaps) SDS levels

Table 2 summarises the relationship between serum IGF-I, IGFBP-3, IGF-I:IGFBP-3 molar ratio and serum BAP and CrossLaps SDS values, as a function of pubertal stage and BMI (SDS). Serum IGF-I SDS levels were positively correlated with BAP and CrossLaps SDS levels before and after puberty, and also with CrossLaps during puberty (weak correlation). Serum IGFBP-3 SDS levels were positively correlated with BAP SDS and CrossLaps SDS levels before, during (weak correlation) and after puberty for BAP SDS levels only.

A significant positive correlation was found between serum IGF-I:IGFBP-3 ratio and CrossLaps SDS levels after puberty only.

Discussion

This large cross-sectional study does not simply provide reference values for serum IGF-I, IGFBP-3 and IGF-I/IGFBP-3 molar ratio levels and for serum BAP and CrossLaps levels, commonly used markers of bone formation and resorption respectively. We report here, for the first time, the simultaneous patterns of change in these parameters through childhood, as a function of both age and pubertal stage, in children of both sexes from a large cohort of carefully selected healthy Caucasian children. Our results show an interesting association between the GH/IGF-I axis and serum

markers of bone metabolism. Previous cross-sectional studies assessing the variation of IGF-I and IGFBP-3 throughout childhood have indicated that concentrations peak around mid-puberty and then gradually decline (26–28). We assigned the subjects to groups defined in terms of age, pubertal stage and sex, to follow the course of developmental changes. We confirmed previous findings suggesting that circulating levels of these biological parameters increase with age over the prepubertal period and during puberty. A significant difference between the sexes was found for serum IGF-I, IGFBP-3 levels and IGF-I:IGFBP-3 molar ratio before puberty. The values obtained were significantly higher in girls than in boys before puberty, with similar values between the sexes after puberty. These observations are consistent with previous studies (27–29). Puberty plays a key role in bone development. Age-related changes in bone remodelling markers have been documented in previous studies (3, 4, 16, 21, 30–39), but pubertal and sex-based differences in bone metabolism during childhood and all pubertal stages have been investigated only rarely and in a limited number of subjects (4, 21, 30, 35, 37, 39). We found differences in bone metabolism markers between the sexes, as a function of pubertal status. In the prepubertal period and during early puberty, serum BAP and CrossLaps levels increased slightly and were similar in both sexes. These levels increased during puberty, with significantly higher values recorded in boys than in girls in late puberty and after puberty, consistent with some of these previous studies of markers of bone formation and resorption as a function of pubertal stage and sex (4, 37). This increase in bone remodelling, which lasted longer in boys than in girls, may be related to the higher peak height velocity and peak bone mass at this time in boys than in girls (4, 21, 37). The dramatic decrease in levels of bone remodelling markers observed after puberty in both sexes probably reflects resorption inhibition by oestrogen. At this time, growth rate declines, but bone mineral mass continues to increase under the influence of sex steroids and IGF-I (40, 41).

The aim of this study was to determine whether serum markers of the GH/IGF-I axis may influence

Table 2 Pearson correlation coefficients for serum insulin-like growth factor-I (IGF-I), IGF-binding protein-3 (IGF BP-3), IGF-1:IGF BP-3 levels and serum bone alkaline phosphatase (BAP) and CrossLaps levels (SDS) in healthy children as a function of pubertal stage and adjusted for body mass index (SDS).

Rh $\hat{o}$ (ρ)	IGF-I/BAP	IGF-I/CL	IGF BP-3/BAP	IGF BP-3/CL	(IGF-I:IGFBP-3)/BAP	(IGF-I:IGFBP-3)/CL
<i>Tanner stage</i>						
1	0.17*	0.30[‡]	0.12 ($P = 0.03$)	0.12 ($P = 0.04$)	0.11	0.11
2	0.12	0.32[‡]	0.17 ($P = 0.05$)	0.12	–0.03	0.07
3	0.16	0.24 ($P = 0.02$)	0.23 ($P = 0.03$)	0.05	–0.02	0.18
4	0.07	0.17 ($P = 0.03$)	0.10	0.06	–0.05	0.06
5	0.15*	0.13*	0.18[†]	0.07	0.09	0.12*

Statistically significant correlation coefficients (Bonferroni correction) indicated in bold. * $P \leq 0.01$; $^{\dagger}P \leq 0.001$; $^{\ddagger}P \leq 0.0001$.

serum BAP and CrossLaps levels in healthy individuals during childhood. All parameters displayed considerable variation for a given age and Tanner stage of puberty. The data were controlled for BMI and pubertal status, and a positive correlation was found between serum IGF-I, IGFBP-3 levels (weak correlations) and serum BAP and CrossLaps levels during the prepubertal period. After puberty, serum IGF-I remained positively correlated with both BAP and CrossLaps levels, and serum IGFBP-3 correlated with BAP levels only. However, no relationship was found during the prepubertal and pubertal periods when IGF-I:IGFBP-3 molar ratio was analysed. Our results are consistent with two studies performed in a limited number of subjects in whom serum IGF-I levels were not found to be important determinants of pubertal changes in bone turnover markers (18, 20), but contrast with two other studies reporting correlations in both sexes between serum IGF-I and bone metabolism marker levels during the pubertal period (17, 21). Our carefully selected study population was larger than the populations studied in these previous cross-sectional studies of children, making it possible to obtain more precise estimates of the relationships between serum markers of the GH/IGF-I axis and bone turnover metabolism at all pubertal stages. It is also interesting to note that in the few previous studies in which both bone turnover markers and bone mass were measured throughout puberty, bone remodelling markers were not found to be predictive of bone mineral accrual (42–44), with the exception of one study showing positive correlations in both sexes until mid-puberty (21). We investigated the relationship between serum markers of the GH/IGF-I axis and bone remodelling only in subjects under the age of 20 years. This relationship has also been investigated later in life but only in a cohort of elite athletes and in adults with acromegaly, in whom serum IGF-I levels were found to be an independent predictor of bone turnover and metabolism (45, 46).

IGF-I can stimulate bone remodelling when administered exogenously to mice, rats and humans or when overexpressed in bone (13, 47, 48). IGFBP-3 is the most abundant of the IGF-BPs in the human circulation and is thought to play an important role in mediating and modulating the biological actions of the IGF system. In animal models, IGFBP-3 overexpression has been shown to increase bone resorption and to have a negative effect on bone formation (49). However, systemic administration of the IGF-I/IGFBP-3 complex has also been shown to stimulate bone formation (50). We found only a weak relationship between serum IGFBP-3 and BAP levels during the prepubertal and pubertal periods, and between serum IGFBP-3 and CrossLaps levels during the prepubertal period. A positive relationship was found between serum IGFBP-3 and BAP levels after puberty.

Our study provides the first *in vivo* evidence in healthy children of a positive relationship between serum

concentrations of IGF-I and IGFBP-3 and bone remodelling levels before and after puberty. As sex steroid levels during puberty gradually increase to reach adult levels, the absence of a strong relationship during the pubertal period may be related to major changes in circulating oestrogen, testosterone and aromatised testosterone levels and in interactions between sex steroid hormones and the GH/IGF-I system, bone metabolism and growth (51). One of the weaknesses of this study was that we used cross-sectional rather than longitudinal data to examine whether serum IGF-I, IGFBP-3 and IGF-I/IGFBP-3 levels influenced serum BAP and CrossLaps levels in healthy children at various Tanner stages. In addition, although we studied associations with serum IGF-I:IGFBP-3 molar ratio, the validity of this parameter as a measure of free IGF-I bioavailability remains to be established.

In summary, this study sheds light on the complex physiological modulation of the circulating IGF-I system and bone metabolism parameters throughout childhood, and provides evidence of differences between pubertal stages in the relationships between serum IGF-I and IGFBP-3 levels and the components of bone metabolism. Both systems are complex, with multiple direct or indirect hormonal, genetic, nutritional and environmental determinants, which may vary during life (6, 40, 41, 51, 52). Further studies in adults are required to unravel changes in these biological parameters throughout an individual's life and to define more clearly the role of the GH/IGF-I axis in bone remodelling and the determinants of this process.

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