Twenty-four-Hour Osteocalcin, Carboxyterminal Propeptide of Type I Procollagen, and Aminoterminal Propeptide of Type III Procollagen Rhythms in Normal and Growth-Retarded Children

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ABSTRACT. The relationships between spontaneous variations in serum 24-h osteocalcin (OC), carboxyterminal propeptide of type I procollagen (PICP), and aminoterminal propeptide of type III procollagen (PIIINP) concentrations and GH secretion, measured as GH response to provocative pharmacologic stimuli and spontaneous GH secretion during 24 h, were evaluated in prepubertal normal children and in GH-deficient and GH-secreting short normal children (SNC). All the subjects showed a circadian rhythm in smoothed 24-h OC and PICP mean data with higher nocturnal values in comparison with diurnal values. Conversely, serum PIIINP concentrations did not vary throughout the day. In children with classic GH deficiency and nonclassic GH deficiency, mean 24-h serum levels and smoothed 24-h mean data for OC, PICP, and PIIINP were significantly reduced ($p < 0.001$) with respect to age-matched controls. SNC showed mean 24-h OC concentrations similar ($p = \text{NS}$) to those we found in age-matched controls, but they had significantly lower ($p < 0.001$) diurnal 12-h mean data in comparison with controls. SNC also showed both 24-h PICP and PIIINP mean data and smoothed 24-h PICP and PIIINP mean data significantly lower (from $p < 0.02$ to $p < 0.001$) at all the time points of measurement in comparison with controls. Twenty-four-hour PICP and PIIINP mean data were positively related to spontaneous 24-h GH concentrations ($r = 0.77$, $p < 0.005$ and $r = 0.69$, $p < 0.005$, respectively) and growth velocity ($r = 0.85$, $p < 0.005$, and $r = 0.70$, $p < 0.005$, respectively), whereas 24-h OC mean data were not. Our study suggests that circadian serum PICP and PIIINP concentrations show GH dependency in children with classic GH deficiency and those with nonclassic GH deficiency, but this was less evident in SNC. Serum PICP and PIIINP concentrations may reflect somatic growth in children with short stature that is or is not related to GH deficiency. (*Pediatr Res* 35: 409–415, 1994)

Abbreviations

PIIINP, aminoterminal propeptide of type III procollagen
PICP, carboxyterminal propeptide of type I procollagen
GH, growth hormone
CGHD, classic GH deficiency
MGHC, mean GH concentration
NCGHD, nonclassic GH deficiency

OC, osteocalcin
SNC, short normal children

Some biochemical markers to evaluate bone metabolism and growth-related changes have been proposed. Of these, OC and type I and type III procollagen propeptides seem to be promising for application in clinical practice.

OC, or bone γ -carboxyglutamic acid (Gla) protein, is a major noncollagenous protein of the bone matrix specifically secreted by osteoblasts; a small fraction, less than 1%, is released into circulation, where it is measurable by RIA (1). Although physiologic functions of OC are still unknown, circulatory levels of this bone protein may represent a marker of bone formation, OC values being higher at ages when the bone mineralization rate is increased (2, 3).

Interstitial collagens are the major structural proteins of the extracellular matrix. Type I collagen is the most abundant body collagen and is a major product of osteoblasts, accounting for more than 90% of the organic bone matrix (4, 5). The same gene product also occurs in soft connective tissues, where type I collagen is present with a mixture of other collagens (5). Type III collagen is mainly located in soft connective tissues and is a major component of interstitial fibrils (6). Type I and type III collagens are derived from specific precursor molecules called procollagens, which are synthesized intracellularly. During the synthesis, large, soluble propeptide domains are released into the circulation from the precursor molecules (6); sensitive, reproducible, and specific RIA for PICP (7) and PIIINP (8) are now commercially available to measure circulatory levels of both propeptides. Serum PICP concentration seems to reflect osteoblast activity and could serve as an index of bone and linear growth (4, 9–11), whereas serum PIIINP concentration seems to reflect only somatic growth (8, 9, 11).

During childhood, serum levels of OC, PICP, and PIIINP change in relation to chronologic age with patterns resembling growth velocity curves (2, 8, 12, 13). In GH-deficient children, low baseline serum OC (14–18), PICP (11, 17, 19), and PIIINP (8, 11, 20) concentrations have been reported, and a positive correlation between serum OC (15, 16, 18), PICP (11, 17), and PIIINP (8, 11, 20, 21) concentrations and growth velocity during GH treatment has been found. In SNC, normal (22) or reduced (23) baseline serum OC levels have been observed. Trivedi *et al.* (11) found that SNC tended to have somewhat higher baseline serum PICP values than healthy children, whereas they had reduced baseline serum PIIINP concentrations (8, 11, 20).

In healthy young adults and prepubertal children, variations

in serum OC levels during a 24-h period have been observed (24–27); serum OC concentrations show considerable time point variability and nocturnal values are 20–50% higher than diurnal values (24–26). In GH-deficient children (28) as well as in SNC (29, 30), an abnormal pattern in serum OC values has been reported. Although some investigators did not find circadian PICP variation in healthy subjects ranging in age from 30 to 68 y (31), others (32) showed a significant PICP circadian rhythm with about 20% higher values at night than in the afternoon in premenopausal women. To our knowledge, no data are available for children. No apparent PIIINP circadian rhythm was discerned in children with GH deficiency (8) or idiopathic short stature (33).

In this study, we evaluated 24-h OC, PICP, and PIIINP variations in healthy, prepubertal, normal-statured children; in GH-deficient children; and in SNC to assess 1) whether 24-h spontaneous GH secretion affects the circadian concentrations of these biochemical markers and 2) the relationships among 24-h variations in serum OC, PICP, and PIIINP concentrations and growth.

MATERIALS AND METHODS

Patients. Forty prepubertal children (22 males, 18 females) aged 4.1–9.5 y were examined. All children had normal weight and length at birth, had normal renal, liver, and gastrointestinal function, and did not take drugs known to interfere with bone or mineral metabolism or collagen synthesis. Vitamin D status was normal in all the subjects (25-hydroxyvitamin D serum levels more than 45 nmol/L). Karyotype, examined in all females, was 46, XX. By using diagnostic criteria previously described (34), we subdivided 34 untreated patients with short stature into three groups: group A consisted of nine children with isolated CGHD, group B consisted of 13 children with isolated NCGHD, and group C consisted of 12 SNC. Diagnosis was made on the basis of the auxologic findings (Table 1) and biochemical data. Patients in group A had GH peaks $< 10 \mu\text{g/L}$ after two provocative pharmacologic stimuli (levodopa: 500 mg/1.73 m² and insulin tolerance test: 0.1 IU/kg of regular insulin i.v.) and reduced spontaneous MGHC for 24 h ($< 3 \mu\text{g/L}$) (34) (Fig. 1). Patients in group B had similar auxologic findings but normal GH response (peaks $> 10 \mu\text{g/L}$) to at least one provocative pharmacologic stimulus (levodopa or insulin tolerance test) and reduced MGHC ($< 3 \mu\text{g/L}$) (Fig. 1). Patients in group C were healthy, slowly growing short children: all were less than the 3rd percentile in height and growing at less than the 25th percentile for chronologic age (Table 1). They showed normal GH response (peaks $> 10 \mu\text{g/L}$) to at least one provocative pharmacologic stimulus (levodopa or insulin tolerance test) and normal MGHC ($> 3 \mu\text{g/L}$) (Fig. 1). The control group consisted of six prepubertal, normal-statured, healthy children, siblings of the patients in group A ($n = 2$, 1 male and 1 female), group B ($n = 3$, 1 male

and 2 females), and group C ($n = 1$, male) (Table 1). All these children had normal GH response (peaks $> 10 \mu\text{g/L}$) after two provocative pharmacologic stimuli (levodopa and insulin tolerance test) and normal MGHC ($> 3 \mu\text{g/L}$) (Fig. 1). There were no differences in baseline serum concentrations of ionized calcium, phosphate, magnesium, intact PTH, and 1,25-dihydroxyvitamin D, or in circadian rhythm in serum cortisol and thyroid hormones, between patients and controls (data not shown).

Study design. During pharmacologic stimulation, blood samples for GH measurements were drawn every 20 min for 2 h in both tests. Hypoglycemia was achieved in all subjects (patients and controls) during the insulin tolerance test. To evaluate spontaneous GH secretion, we drew samples from each child every 20 min for 24 h (from 0800 to 0800 of the following day), and all 73 samples for each child were measured (34).

In all children, we also collected samples to evaluate spontaneous 24-h variations in serum OC, PICP, and PIIINP at the same time point at which GH concentrations were measured. All subjects were adapted to the study room for 12 h overnight. Blood was sampled through an indwelling venous catheter (Abbocath T-22G, Abbott, Campoverde, Latina, Italy) placed in the antecubital vein in each subject at least 8 h before the test. Samples for GH, OC, PICP, and PIIINP were stored at -20°C within 30 min of being drawn until analyzed. Meals were given at 0800, 1200, 1600, and 2000 h with calcium and phosphorus intake adequate for age and sex; physical activity was restricted and standardized during daytime within our department. All subjects were investigated from November to February. In three CGHD children, four NCGHD children, and two SNC, OC, GH, PICP, and PIIINP circadian variations were reevaluated during March and April.

Informed consent was obtained from the parents of each child, and the study was approved by the ethical committee for human investigation of our department.

Methods. To allow a comparison between different ages and genders, we expressed height as a z score with respect to height SD according to the method of Tanner *et al.* (35). Growth velocity was expressed as cm/y and as z score with respect to growth velocity SD by using Tanner tables (35). Standing height was measured with a wall-mounted stadiometer. Bone age was evaluated by using the Greulich and Pyle method (36).

All the examined biochemical parameters were detected by RIA using the following commercial kits: GH Spectria (Farnos Diagnostica, Turku, Finland); OC (Inctar Co., Stillwater, MN); and CPP-I and APP-III (Farnos Diagnostica). For GH, interassay and intraassay coefficients of variation and sensitivity were 7.8%, 5.1%, and 0.3 $\mu\text{g/L}$, respectively; for OC, 8.9%, 6.3%, and 0.2 $\mu\text{g/L}$; for PICP, 4.7%, 3.5%, and 1.2 $\mu\text{g/L}$; and for PIIINP, 6.5%, 5.2%, and 0.2 $\mu\text{g/L}$.

Statistical analysis. We considered the value of 3 $\mu\text{g/L}$ the cutoff limit between normal ($\geq 3 \mu\text{g/L}$) and reduced ($< 3 \mu\text{g/L}$) spontaneous 24-h MGHC (34). For each study day, the mean 24-h values of OC, PICP, and PIIINP were obtained by calculating the average of serum concentrations of these parameters of each subject for all the time points; results are expressed as mean $\pm$ SD and as z score with respect to control subjects' SD for OC, PICP, and PIIINP by using the following formula: measured individual value – mean value for age-matched controls/SD for age-matched controls. The mean patterns of each parameter were smoothed by the method of running means (37).

We also calculated mean 12-h values of GH, OC, PICP, and PIIINP for both nocturnal and diurnal periods by measuring the average of serum concentrations of these parameters of each subject for all daytime points (from 0800 h to 1900 h) and nighttime points (from 2000 to 0700 h). Data are expressed as mean $\pm$ SD.

Mean values were compared by analysis of variance. Multiple regression analysis was performed to determine correlation coefficients. A $p < 0.05$ was considered significant.

Table 1. Auxologic findings of subjects*

	CGHD	NCGHD	SNC	Controls
<i>n</i>	9	13	12	6
Gender	5 M/4 F	7 M/6 F	7 M/5 F	3 M/3 F
Chronologic age (y)	6.8 $\pm$ 1.9	7.0 $\pm$ 2.2	6.0 $\pm$ 1.9	6.9 $\pm$ 1.7
Bone age (y)	4.2 $\pm$ 1.3	4.9 $\pm$ 1.6	5.2 $\pm$ 2.1	6.4 $\pm$ 1.8
Statural age (y)	4.0 $\pm$ 1.5	4.6 $\pm$ 1.7	4.3 $\pm$ 1.9	6.8 $\pm$ 1.6
Bone age/chronologic age ratio	0.62	0.65	0.82	0.93
Height z score	-3.2 $\pm$ 0.6	-3.1 $\pm$ 0.8	-2.3 $\pm$ 1.2	-0.2 $\pm$ 0.2
Growth velocity (cm/y)	2.8 $\pm$ 0.6	2.9 $\pm$ 0.7	4.4 $\pm$ 0.3	5.8 $\pm$ 0.6
Growth velocity z score	-3.4 $\pm$ 0.7	-3.2 $\pm$ 0.7	-1.8 $\pm$ 0.2	-0.3 $\pm$ 0.3

* Values are mean $\pm$ SD.

Fig. 1. *a*, GH secretion after provocative pharmacologic stimuli (*L*, levodopa; *I*, insulin tolerance test), and *b*, spontaneous 24-h MGHC of the subjects. Data are expressed as single value for males (*M*) and females (*F*). The area above the broken line indicates normal values.

RESULTS

Figure 1 summarizes the results of GH response to provocative pharmacologic stimuli and spontaneous 24-h MGHC in all subjects. All CGHD and NCGHD children had 24-h MGHC below the cutoff limit of 3 μg/L (Fig. 1), with a mean (1.6 ± 0.4 μg/L and 1.7 ± 0.5 μg/L, respectively) significantly reduced ($p < 0.001$) in comparison with that of SNC (4.8 ± 1.1 μg/L) and controls (7.0 ± 1.0 μg/L). In SNC, each individual value of 24-h MGHC was more than 3 μg/L (Fig. 1), but the mean of all MCGH was significantly lower ($p < 0.01$) than that of controls.

In both CGHD and NCGHD children, mean serum 24-h OC (5.2 ± 1.4 μg/L and 5.5 ± 1.3 μg/L, respectively), PICP (18.3 ± 2.0 μg/L and 18.8 ± 2.0 μg/L, respectively), and PIIINP (4.1 ± 0.9 μg/L and 4.1 ± 1.0 μg/L, respectively) levels were significantly reduced ($p < 0.001$) in comparison with those found in controls (OC: 14.5 ± 2.5 μg/L, PICP: 32.4 ± 4.6 μg/L, and PIIINP: 7.9 ± 1.0 μg/L). In SNC, mean 24-h serum OC concentration (12.2 ± 2.1 μg/L) was not significantly different ($p = \text{NS}$) compared with that of controls, but it was significantly higher ($p < 0.001$) than that found in CGHD and NCGHD children. In SNC, mean serum 24-h PICP (24.6 ± 3.1 μg/L) and PIIINP (6.6 ± 1.1 μg/L) concentrations were significantly higher ($p < 0.001$) than those in CGHD and NCGHD children and significantly reduced ($p < 0.001$ for PICP and $p < 0.02$ for PIIINP) in

comparison with the controls. Data expressed as *z* score in respect to control subjects' SD are reported in Figure 2: all CGHD and NCGHD children had mean serum 24-h OC, PICP, and PIIINP values below -2.0 *z* score, whereas SNC had mean serum 24-h OC, PICP, and PIIINP values either below -2.0 *z* score or within the normal limit for age. However, there was a clear overlap between both CGHD and NCGHD children and SNC, and between SNC and controls for OC, PICP, and PIIINP (Fig. 2).

In all subjects, a circadian rhythm in smoothed 24-h OC and PICP mean data was found, with higher nocturnal values in comparison with diurnal values (Fig. 3). Maximal peaks of OC were evident from 0000 to 0300 h in CGHD and NCGHD children and from 0100 to 0500 h in SNC and controls (Fig. 3); maximal peaks of PICP were present from 0200 to 0400 h in CGHD and NCGHD children, from 0300 to 0430 h in SNC, and from 0230 to 0430 h in controls (Fig. 3). Spontaneous maximal GH peaks greater than 5 μg/L were evident from 0000 to 0200 h in controls and SNC, whereas in CGHD and NCGHD children no GH peaks greater than 5 μg/L were observed throughout the 24-h period (data not shown). In all subjects, smoothed 24-h OC mean data showed greater variations during daytime than smoothed 24-h PICP mean data (Fig. 3). On the contrary, serum PIIINP levels did not vary throughout the day in any subjects (Fig. 3). When the smoothed 24-h OC, PICP, and PIIINP mean data of the CGHD and NCGHD children were

Fig. 2. Mean 24-h serum OC (a), PICP (b), and PIIINP (c) concentrations in the subjects. Data are expressed as z scores in respect to control SD for males (M) and females (F).

compared with those of the controls, significantly lower values for each parameter at all the time points of measurement were found (Fig. 3). In SNC, significantly lower smoothed 24-h OC mean data were demonstrated at all time points of measurement only during daytime, whereas nocturnal smoothed data were similar to those observed in controls (Fig. 3). In this group of patients, smoothed 24-h PICP and PIIINP mean data resulted in significantly lower values at all time points of measurement in comparison with mean data found in controls (Fig. 3).

In all subjects, diurnal 12-h MGHC was significantly lower than nocturnal 12-h MGHC; all three groups of patients had significantly reduced MGHC for both daytime and nighttime compared with controls (Table 2). Nocturnal 12-h OC and PICP mean data were significantly higher than diurnal 12-h mean data in either patients or controls, but to a lesser extent for PICP (Table 2). Nocturnal 12-h PIIINP mean data were similar to diurnal 12-h PIIINP mean data in either patients or controls

Fig. 3. Pattern of smoothed 24-h OC (a), PICP (b), and PIIINP (c) mean data of the subjects. CGHD: dotted line; NCGHD: broken line; SNC: solid fine line; controls: solid thick line. CGHD and NCGHD children vs controls: $p < 0.001$ at all the time points of measurement for OC, PICP, and PIIINP. SNC vs controls: for OC, $p < 0.01$ from 0600 to 1900 h and $p = NS$ from 2000 to 0500 h; for PICP, $p < 0.01$ from 0800 to 2300 h and $p < 0.001$ from 0000 to 0700 h; for PIIINP, $p < 0.001$ at all the time points of measurement.

(Table 2). In CGHD and NCGHD children, both nocturnal and diurnal 12-h mean data for OC, PICP, and PIIINP were significantly lower in respect to those of SNC and controls (Table 2). In SNC, only diurnal 12-h OC mean data were significantly lower in comparison with control data (Table 2), whereas 12-h PICP and PIIINP mean data were significantly lower than control data during both daytime and nighttime (Table 2).

Circadian pattern and daytime and nighttime mean data of GH, OC, PICP, and PIIINP did not vary with age, sex, and seasons (data not shown).

No relationship was found between 24-h OC mean data and 24-h MGHC, and between each time point of measurement of

Table 2. Nocturnal 12-h and diurnal 12-h mean data of GH, OC, PICP and PIIINP in subjects*

	Nocturnal 12 h	Diurnal 12 h	Δ %†	p†
CGHD				
GH ($\mu\text{g/L}$)	$2.0 \pm 0.4\ddagger\§$	$1.3 \pm 0.5\ddagger\§$	34.2	<0.01
OC ($\mu\text{g/L}$)	$7.4 \pm 1.1\ddagger\§$	$3.5 \pm 1.7\ddagger\§$	52.4	<0.001
PICP ($\mu\text{g/L}$)	$19.6 \pm 2.4\ddagger\§$	$17.2 \pm 2.1\ddagger\§$	12.4	<0.02
PIIINP ($\mu\text{g/L}$)	$4.1 \pm 1.0\ddagger\§$	$4.1 \pm 0.9\ddagger\§$	0.7	NS
NCGHD				
GH ($\mu\text{g/L}$)	$1.9 \pm 0.4\ddagger$	$1.5 \pm 0.5\ddagger$	22.6	<0.02
OC ($\mu\text{g/L}$)	$7.9 \pm 1.1\ddagger$	$3.9 \pm 1.3\ddagger$	49.9	<0.001
PICP ($\mu\text{g/L}$)	$19.8 \pm 2.2\ddagger$	$17.8 \pm 2.3\ddagger$	10.2	<0.02
PIIINP ($\mu\text{g/L}$)	$4.1 \pm 1.0\ddagger$	$4.1 \pm 0.9\ddagger$	0.5	NS
SNC				
GH ($\mu\text{g/L}$)	$5.8 \pm 1.1\parallel\¶$	$3.8 \pm 1.2\parallel\¶$	34.5	<0.001
OC ($\mu\text{g/L}$)	$16.9 \pm 3.2\parallel\¶$	$6.9 \pm 1.3\ddagger\¶$	59.0	<0.001
PICP ($\mu\text{g/L}$)	$27.0 \pm 3.0\parallel\¶$	$22.7 \pm 2.1\ddagger\¶$	15.8	<0.001
PIIINP ($\mu\text{g/L}$)	$6.7 \pm 0.9\parallel\¶\ddagger\ddagger$	$6.45 \pm 1.0\parallel\ddagger\ddagger$	3.4	NS
Controls				
GH ($\mu\text{g/L}$)	8.0 ± 1.0	5.9 ± 0.9	25.9	<0.02
OC ($\mu\text{g/L}$)	19.4 ± 2.1	12.5 ± 1.7	35.7	<0.001
PICP ($\mu\text{g/L}$)	34.5 ± 4.6	28.7 ± 3.5	16.6	<0.001
PIIINP ($\mu\text{g/L}$)	7.9 ± 1.1	7.8 ± 1.0	1.6	NS

* Values are mean $\pm$ SD.

† Between diurnal and nocturnal mean data.

‡ $p < 0.001$ vs controls.§ $p = \text{NS}$ vs NCGHD.|| $p < 0.01$ vs controls.¶ $p < 0.001$ vs CGHD and NCGHD children.** $p = \text{NS}$ vs controls.†† $p < 0.02$ vs controls.

serum OC and GH concentrations during 24 h (data not shown). On the contrary, 24-h PICP and PIIINP mean data were positively related to 24-h MGHC (Fig. 4), as was 12-h PICP and PIIINP mean data to 12-h MGHC during both daytime and nighttime (daytime PICP versus daytime MGHC: $r = 0.63$, $p < 0.005$; nighttime PICP versus nighttime MGHC: $r = 0.75$, $p < 0.005$; daytime PIIINP versus daytime MGHC: $r = 0.57$, $p < 0.005$; and nighttime PIIINP versus nighttime MGHC: $r = 0.61$, $p < 0.005$). Moreover, 24-h PICP and 24-h PIIINP mean data were positively related to growth velocity (Fig. 5), whereas 24-h OC mean data were not (data not shown).

DISCUSSION

During childhood, serum OC, PICP, and PIIINP concentrations vary with age, with patterns resembling growth velocity curves; higher values are observed during the first 2 y of life and at puberty, and the increase occurs earlier in girls than in boys (8, 11–13, 38, 39). Thus, OC, PICP, and PIIINP concentrations in serum may have clinical usefulness as biochemical markers of growth-related changes during childhood and adolescence. In addition, OC and PICP also reflect bone formation, being osteoblast products (4, 9–12, 15, 16), whereas PIIINP may give information about the activity of connective tissue during growth (8, 9, 11), because type III collagen accounts for 10–50% of total body collagen (6).

Our data confirm the presence of circadian OC rhythm in prepubertal, normal-statured, healthy children, as previously reported in young adults (24, 26, 27) and children (25), in GH-deficient patients (28), and in SNC (29, 30). In contrast to baseline serum OC concentrations, which showed an intraindividual coefficient of variation of 9–18% (27), circadian OC rhythm is remarkably constant in normal subjects (26, 27), as well as in CGHD and NCGHD children and in SNC tested. So, it may be more appropriate to assess osteoblast activity by measuring circadian OC rhythm than by measuring serum OC concentrations in single blood samples, even though this method

Fig. 4. Relationships between 24-h PICP mean data and 24-h MGHC (a), and between 24-h PIIINP mean data and 24-h MGHC (b). ■, CGHD; ▲, NCGHD; ●, SNC; ♦, controls.

is more invasive and difficult to perform in large series of patients. In agreement with the data of Hassager *et al.* (32) obtained in premenopausal women, we demonstrated a circadian PICP rhythm with higher values during the night also in prepubertal, normal-statured, healthy children, as well as in GH-deficient children and in SNC. We did not find any 24-h variation in serum PIIINP concentrations in all examined subjects, in agreement with previous data obtained in GH-deficient children (8) and in SNC (33).

In CGHD and NCGHD children, the reduction in 24-h mean data and in smoothed 24-h mean data of OC probably reflects reduced bone turnover and decreased bone formation, as is also supported by the findings of low baseline serum OC levels in these patients (14–18). In GH-deficient children, GH treatment at a dosage of 4 and 6 IU/d normalized the abnormal nighttime OC pattern, suggesting that circadian OC rhythm, which reflects circadian rhythmicity of osteoblast activity, could be related to circadian GH spontaneous secretion (28). Moreover, in GH-deficient children, baseline serum OC levels increased within 4 to 9 h after intramuscular GH administration, suggesting a direct effect of GH on OC levels independent of IGF-I increase (40). Our own previous data in CGHD and NCGHD children (17) did not confirm the rapid OC increase after GH administration, and the present study did not show any relationship between circadian OC rhythm and GH release evaluated by 24-h MGHC, diurnal MGHC, nocturnal MGHC, and number of pulses greater

Fig. 5. Relationships between 24-h PICP mean data and growth velocity (a), and between 24-h PIIINP mean data and growth velocity (b). ■, CGHD; ▲, NCGHD; ●, SNC; ◆, controls.

than 5 µg/L during 24 h, confirming other reports (27, 28, 41). Either 24-h mean data or smoothed 24-h mean data of OC may be used to assess some effects of GH deficiency and GH treatment on bone metabolism. Other factors, such as ionized calcium, PTH, and cortisol, may be involved in circadian OC fluctuations (42, 43). In CGHD and NCGHD children, reduced 24-h mean data and smoothed 24-h mean data of PICP and PIIINP revealed that poor growth is associated with decreased type I and type III collagen synthesis, confirming baseline measurement of serum PICP (11, 17, 19) and PIIINP (8, 11, 20). The relationships between 24-h PICP and PIIINP mean data and 24-h MGHC and growth velocity suggest that either spontaneous GH secretion or growth velocity may affect serum PICP and PIIINP concentrations. These data agree with the results of Trivedi *et al.* (8, 11), which showed a similar correlation between both baseline serum PICP and PIIINP levels and growth velocity, and between 24-h spontaneous GH secretion evaluated by area under the curve and serum PIIINP. So, the measurement of 24-h PICP and PIIINP mean data and circadian PICP and PIIINP rhythm may provide additional information to monitor growth rates in CGHD and NCGHD children and in SNC, even though an overlap in 24-h PICP and PIIINP mean data was found between CGHD and NCGHD children and SNC and between SNC and controls. In contrast to the data of Trivedi *et al.* (8, 11), no overlap in 24-h PICP and PIIINP mean data was observed between patients with GH deficiency and controls. The discrepancy between these data may be related to the major degree of

growth failure and reduced growth velocity of our own patients compared with those examined by Trivedi *et al.* (8, 11).

In SNC, mean 24-h serum OC concentrations were not different from those of controls, but OC values during diurnal hours were reduced compared with the diurnal values in the controls. These data might reflect reduced bone formation during daytime, suggesting an impairment in osteoblast cyclic activity to produce OC.

Recent reports showed that OC is released from osteoblast into bone matrix and blood after cleavage of a 26-residue propeptide known as pro-OC; this propeptide circulates in lower levels than does the intact OC molecule, because most of the propeptide is catabolized within the osteoblasts and a relatively small amount is released into circulation (16, 44). Serum pro-OC concentration seems to be more specific than intact OC as a marker of bone formation because it is not incorporated in bone matrix during bone formation (16). The lower serum OC level that occurs during diurnal hours in our SNC could result from a reduced osteoblast production of intact OC or from an increased osteoblast catabolism of pro-OC. We are not able to verify these suppositions because we measured serum OC concentrations by using rabbit antiovine OC antibody that recognizes a major epitope near the carboxyterminal portion of the molecule detecting both intact OC and carboxyterminal fragments of the molecule (45, 46); therefore, the method we used is unable to detect intact OC molecules alone or measure pro-OC peptide. Alternatively, the apparent normality of nighttime OC data in SNC could be related to increased bone resorption during nocturnal hours, because a fraction of immunoreactive OC fragments are released into blood during bone resorption by osteoclast, and the method to measure serum OC concentrations that we used also detects these fragments (16, 44–46). In contrast to our results, Markowitz *et al.* (30) in four children with idiopathic short stature demonstrated a reduced OC pattern during either daytime or nighttime using the same RIA we used. Differences in selection criteria of the patients may explain these conflicting data.

In contrast to OC, 24-h mean data and circadian pattern of PICP and PIIINP were significantly lower in SNC than in controls for both nighttime and daytime, suggesting that poor growth affects serum 24-h PICP and PIIINP concentrations, even if in the presence of normal GH secretion. The differences between the pattern of OC and that of PICP in SNC may be related to the possibility that these biochemical markers reflect different osteoblast functions, even though 24-h PICP mean data are likely influenced by soft connective tissue sources in addition to the organic bone matrix activity. Although 24-h PICP and PIIINP mean data were significantly higher in SNC in comparison with CGHD and NCGHD children, measurement of 24-h PICP and PIIINP mean data alone had no diagnostic value because we found a considerable overlap between these groups of patients and between SNC and controls. GH treatment was able to increase circadian PIIINP concentrations in children with SNC, but to a lesser extent than reported in GH-deficient children (33). So, 24-h PICP and PIIINP concentrations before and during GH treatment may be helpful in monitoring somatic growth in SNC.

In conclusion, our study demonstrates that 24-h PICP and PIIINP concentrations seem to be GH dependent in CGHD and NCGHD children, but this was less evident in SNC who showed normal GH secretion on the basis of the cutoff limits we used. The 24-h PICP and PIIINP levels were correlated to spontaneous GH secretion and growth velocity, whereas OC levels were not. Circadian variations in serum OC, PICP, and PIIINP concentrations in CGHD and NCGHD children and in SNC provide additional tools to assess bone formation (OC and PICP) and somatic growth (PICP and PIIINP) in respect to the measurement of baseline values. Future studies are necessary to know for certain the diagnostic and prognostic usefulness of the circadian evaluation of these biochemical markers in children with short stature related or not to GH deficiency.

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