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Influence of Age, Sex, and Insulin on Osteoblast Function: Osteoblast Dysfunction in Diabetes Mellitus*

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ABSTRACT

The osteoblast function was evaluated in normal and diabetic children and adults by measurements of the serum concentration of the carboxy-terminal extension peptide of procollagen (PICP), total and skeletal alkaline phosphatase (ALP), and osteocalcin. Moreover, the osteoblast-stimulating growth factor, insulin-like growth factor I (IGF-I), was measured in the same samples. In normal children (n = 420; age, 5–20 yr), a marked pubertal increase of serum IGF-I (peak values at age 14–16 yr in both sexes), osteocalcin, and total and skeletal ALP (peak values earlier in girls than in boys) and a small increase in PICP were observed. All osteoblast markers and IGF-I were markedly lower in normal adults (n = 229; age, 21–69 yr) than in children. All osteoblast parameters showed a high degree of correlation ($P < 0.001$) with each other. In adolescents (n = 104) treated for insulin-dependent diabetes mellitus (IDDM), serum IGF-I (−19%), osteocalcin (−28%), and skeletal ALP (−28%) were markedly decreased, whereas total ALP was significantly increased (29%), and serum PICP remained normal. In adult IDDM (n = 125), both serum IGF-I (−41%) and osteocalcin (−24%) were decreased, but skeletal ALP and PICP remained normal. A similar abnormality in serum IGF-I and osteocalcin was observed in white (n = 61) and Pima Indian (n = 16) non-IDDM patients. The concentration of skeletal ALP was highly significantly correlated ($r \geq 0.9$) with total ALP in both normal and diabetic subjects, but the slope of the regression was significantly different, indicating the presence of other, probably intestinal, ALP in all types of diabetes.

In conclusion, the osteoblast function is significantly decreased in diabetic patients, which can best be characterized as a maturation defect, since the early osteoblast marker, PICP, remained normal in all types of diabetes, whereas a later marker, skeletal ALP, is frankly abnormal only in diabetic children. The most mature osteoblast marker, osteocalcin, is decreased in all types of diabetes irrespective of age. (*J Clin Endocrinol Metab* **80:** 1194–1202, 1995)

BONE turnover is decreased in experimental and autoimmune types of animal diabetes, as demonstrated by dynamic bone histomorphometry and biochemical evaluation (serum osteocalcin) of osteoblast function (1–5). Similar but less detailed observations have been made in human diabetes (6–10). Bone histomorphometry in diabetics with chronic renal failure also suggests a decreased bone turnover (11, 12) as well as a lower degree of secondary hyperparathyroidism than in nondiabetic patients (13). This decreased bone turnover may result in mild osteopenia in both animal and human diabetes (for review see Ref. 1).

Serum concentrations of insulin-like growth factor I (IGF-I) were also decreased in diabetic BB rats and correlated well with serum osteocalcin concentration and bone mineral apposition rate (5). This was not unexpected, as IGF-I and -II are potent bone-stimulating growth factors *in vitro* (14, 15) and *in vivo* (16, 17). In human diabetes, widely variable results on serum IGF-I have been reported, including high (18), normal (19, 20), or low (21–25) concentrations, but interference by IGF-binding proteins, known to be abnormal in diabetes, could be responsible for these discrepancies (26–28).

As IGF-I and osteocalcin were the best and simpliest hallmarks of diabetic bone disorders in diabetic BB rats (4, 5), we evaluated these and other biochemical osteoblast parameters in the serum of a large group of children and adults with insulin-dependent diabetes mellitus (IDDM). In addition, similar studies were performed in non-IDDM (NIDDM) white patients and in Pima Indians, known to have a particularly high genetic background for this disease. We confirm that both IGF-I and osteocalcin are decreased in all types of diabetes. The combined observations suggest an osteoblast defect caused by insulin deficiency or resistance.

Materials and Methods

Subjects

Serum samples were obtained from 229 healthy blood donors (75 men and 154 women; age range, 21–69 yr) and also from 420 healthy children and adolescents (205 girls and 215 boys; age range, 5–20 yr) after informed consent of the parents was given. Serum from diabetic children (44 girls and 60 boys) and adults (76 men and 49 women) with typical IDDM were taken at the time of their control visits at the pediatric or adult endocrinology clinic of the University Hospital Gasthuisberg. The children were treated with insulin for a mean period of 5.8 yr (range, 0.1–18 yr). IDDM in adults was confirmed by low C-peptide levels obtained 6 min after iv glucagon injection (peak levels <0.3 nM). The adult patients were treated with insulin for a period of 17 yr (range, 1–43 yr). Serum was also collected from 61 white patients (age range, 35–55

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yr) with NIDDM. This diagnosis was confirmed by clinical presentation and residual C-peptide concentration. Serum from Pima Indian females was selected among subjects who were participating in ongoing studies of the pathophysiology of diabetes in the Pima Indian community. Subjects were tested for diabetes by a 75-g oral glucose tolerance test after at least 3 days on a weight-maintenance diet. Thirty-seven subjects were studied: 16 with diabetes, 6 with impaired glucose tolerance, and 15 with normal glucose tolerance.

Biochemical procedures

Serum osteocalcin was measured by a homologous human osteocalcin RIA. The sensitivity of the assay in 0.05-mL samples was 2 ng/mL, and the between-assay variation coefficient was 7% (29). Serum IGF-I was measured by RIA after acid ethanol extraction using a polyclonal guinea pig antibiosynthetic human IGF-I antiserum (sensitivity, 0.2 ng/mL; between-assay coefficient of variation, 7.6%; ref. 5). Serum C-peptide was measured by RIA using a polyclonal guinea pig antiserum (code M1221, Novo Nordisk, Bagsvaerd, Denmark). Procollagen type 1 carboxy-terminal extension peptide was measured by a commercial RIA with a sensitivity of 1.2 ng/mL and a between-assay coefficient of variation of 5.2% (30). Total serum alkaline phosphatase (ALP) was measured by an optimized ALP assay (BM/Hitachi 747, Boehringer, Mannheim, Germany), and serum bone-specific ALP by the ostease kit (kindly provided by B. R. Regele, Hybritech, Liège, Belgium) as described by the manufacturer and evaluated recently (31). This solid-phase immunoradiometric assay is based on two monoclonal antibodies directed against different antigenic sites of human skeletal ALP. The sensitivity of the assays was 2 μg/L, and the within- and between-coefficients of variation were 6.7% and 8.1%, respectively.

Statistical analysis

All calculations were done with the SAS statistical package (release 6.04, SAS, Cary, NC). Groups were compared with an analysis of variance for normally distributed data and with the Wilcoxon test otherwise. In view of its high efficiency, the Spearman rank correlation was always taken, irrespective of the distribution of the data. Regression analyses were performed on the original scale (for interpretation purposes) and on a transformed scale (usually log-transformed) to satisfy statistical assumptions. For the calculations of the Z scores, a pooled estimate of the age- and sex-specific SD of the normal group was calculated using an analysis of variance model. The age- and sex-specific Z score of a diabetic individual was then calculated from the age and sex normal mean value.

Results

Type 1 diabetic children and adults

Serum IGF-I increased during puberty in normal and diabetic children, reaching the highest concentrations between 14 and 16 yr in both sexes (Fig. 1). In diabetic (pre)adolescent children, however, serum IGF-I was significantly lower ($P < 0.001$) than in normal children of the same age group, the mean decrease being about 22% in boys (Z score, −0.8) and 13% (Z score, −0.6) in girls. A similarly significant decrease was observed when normal and diabetic girls and boys were classified according to their pubertal instead of their chronological ages (data not shown). In normal and IDDM adults, serum IGF-I slowly decreased with age, but, as in children, consistently lower concentrations were found in diabetics (Z scores for men and women, −2.0 and −1.4, respectively; Fig. 1). Serum IGF-I was significantly higher in girls than in boys, whereas the opposite sex difference was observed in adults (Table 1).

Serum osteocalcin also fluctuated with age, but the peak levels were reached earlier in normal girls (age 10–12 yr) than in normal boys (age 14–16 yr), although the peak values were of similar magnitude (Fig. 2). Serum osteocalcin concentrations were significantly lower ($P < 0.001$) in diabetic children when compared with normal children, the mean decrease throughout puberty being 29% in boys (Z score, −0.6) and 27% in girls (Z score, −0.7) (Fig. 2). A similar decrease in serum osteocalcin was observed in diabetic children when classified according to Tanner staging (data not shown). Serum osteocalcin concentrations remained relatively stable in normal and type 1 diabetic adults, but at each age the concentrations were lower in diabetics than in normal subjects

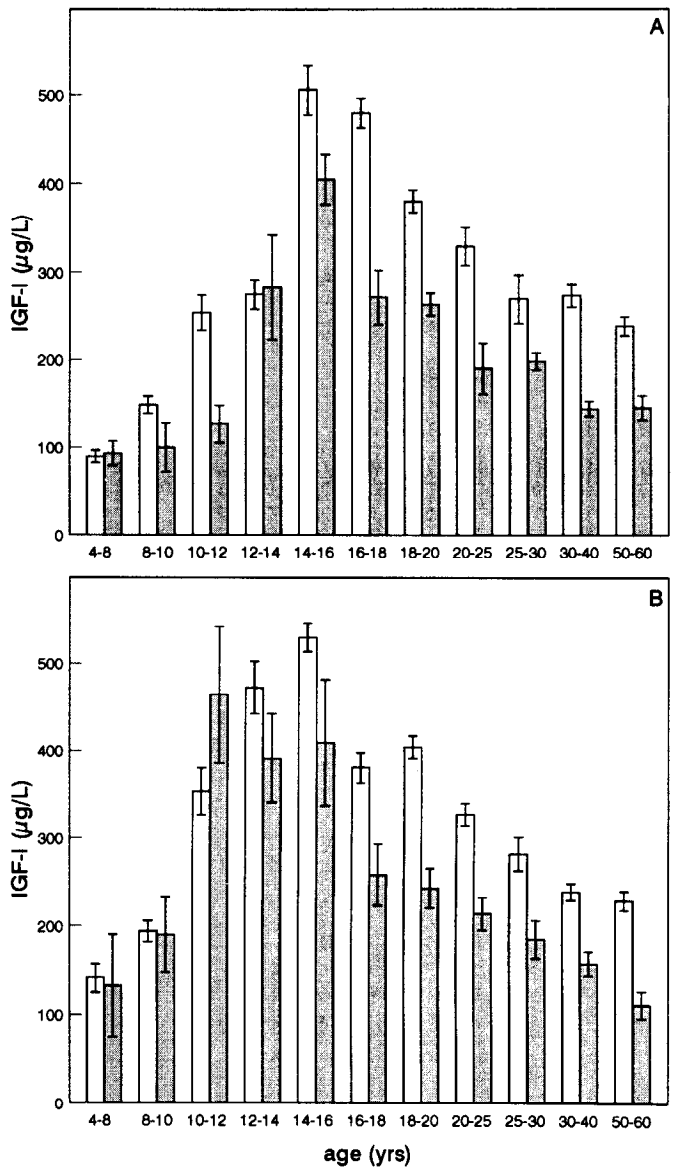

FIG. 1. Serum IGF-I concentrations in normal and type 1 diabetic children, adolescents, and adults. A, Serum IGF-I in normal and diabetic boys and men. B, Serum IGF-I in normal and diabetic girls and women. □, Nondiabetic subjects; ■, type 1 diabetic patients. All data are expressed as mean ± SEM. The data were analyzed with a multiple regression model with IGF-I on the original or log-transformed scale. For both scales, a significant positive age effect was found below the age of 16 yr and a negative effect above that age ($P < 0.0001$). A significant decreasing effect of IDDM was observed ($P < 0.0001$) in both sexes in children and adults.

TABLE 1. General and biochemical data on normal subjects and type 1 diabetic children and adults

Subjects	n	Age (yr)	IGF-I (μg/L)	Osteocalcin (μg/L)	Total ALP (U/L)
Children (<20 yr)					
Normal					
Female	205	13.4 (5–20)	368 (11–951)	60 (16–159)	157 (32–494)
Male	215	13.1 (5–20)	301 (76–833)[a]	74 (205–191)[a]	199 (71–451)[a]
Type 1 DM					
Female	44	13.5 (5–20)	321 (83–930)	44 (13–106)[b]	210 (63–527)[c]
Male	60	13.5 (5–20)	234 (56–688)[b,d]	52 (16–126)[c]	246 (98–489)[b]
Adults (>20 yr)					
Normal					
Female	154	41 (21–69)	235 (101–448)	24 (13–40)	48 (23–89)
Male	75	39 (21–69)	266 (159–415)[a]	26 (16–40)[e]	66 (34–114)[a]
Type 1 DM					
Female	49	40 (21–69)	152 (68–256)[c]	18 (9–32)[c]	79 (36–149)[c]
Male	76	43 (21–69)	141 (51–320)[c]	19 (11–30)[c]	86 (44–149)[b]

All data are represented by their arythmetric mean and 95% confidence limits. If necessary, a log transformation was applied to of the differences indicated in this table were calculated by a Student's t test when tested in a multiple regression analysis for skeletal ALP.

[a] $P < 0.001$ M/F.
[b] $P < 0.01$.
[c] $P < 0.001$ N/DM.
[d] $P < 0.01$.
[e] $P < 0.05$.

TABLE 2. Correlation matrix (calculated according to Spearman rank correlation test) between serum concentrations of markers of osteoblast function in normal subjects (children and adults) and in patients with type 1 diabetes (children and adults)

	IGF-I	PICP	Total ALP	Skeletal ALP	Osteocalcin
Normal					
PICP	−0.12[a]				
Total ALP	−0.21[b]	0.62[b]			
Skeletal ALP	−0.19[c]	0.66[b]	0.87[b]		
Osteocalcin	0.02	0.45[b]	0.47[b]	0.45[b]	
Type 1 diabetes					
PICP	0.30[b]				
Total ALP	0.38[b]	0.75[b]			
Skeletal ALP	0.49[b]	0.71[b]	0.91[b]		
Osteocalcin	0.25[b]	0.49[b]	0.70[b]	0.70[b]	
HbA_{1c}	0.12[a]	0.02	0.14[a]	0.08	0.07

[a] $P < 0.05$.
[b] $P < 0.001$.
[c] $P < 0.01$.

TABLE 3. General and biochemical data on white type 2 diabetes and their age-matched controls

	Controls		Type 2 diabetes	
	35–44 yr	45–55 yr	35–44 yr	45–55 yr
n	67	28	17	44
Age (yr)	40	49	40	51
Osteocalcin (μg/L)	22 (12–38)	20 (11–34)	15 (9–23)[a]	20 (9–36)
IGF-I (μg/L)	252 (157–388)	215 (145–305)	200 (72–437)[b]	186 (83–356)[c]
PICP (μg/L)	130 (67–216)	106 (55–186)	112 (55–202)	102 (38–231)
Total ALP (U/L)	58 (26–100)	67 (35–114)	78 (46–137)[a]	91 (41–172)[a]
Skeletal ALP (μg/L)	8 (4–16)	10 (2–43)	10 (4–23)	12 (4–33)
Diabetes duration (yr)			9 (0–25)	9 (0–25)
HbA_{1c} (%)[d]			6.8 (4.4–9.9)	7.3 (4.6–11.2)

All data are represented by their arythmitic mean and 95% confidence limits. If necessary, a log transformation was applied to achieve normality and confidence limits were then back transformed to the original scale.

[a] $P < 0.001$.
[b] $P < 0.01$.
[c] $P < 0.05$.
[d] Normal values, 3.4–6.4%.

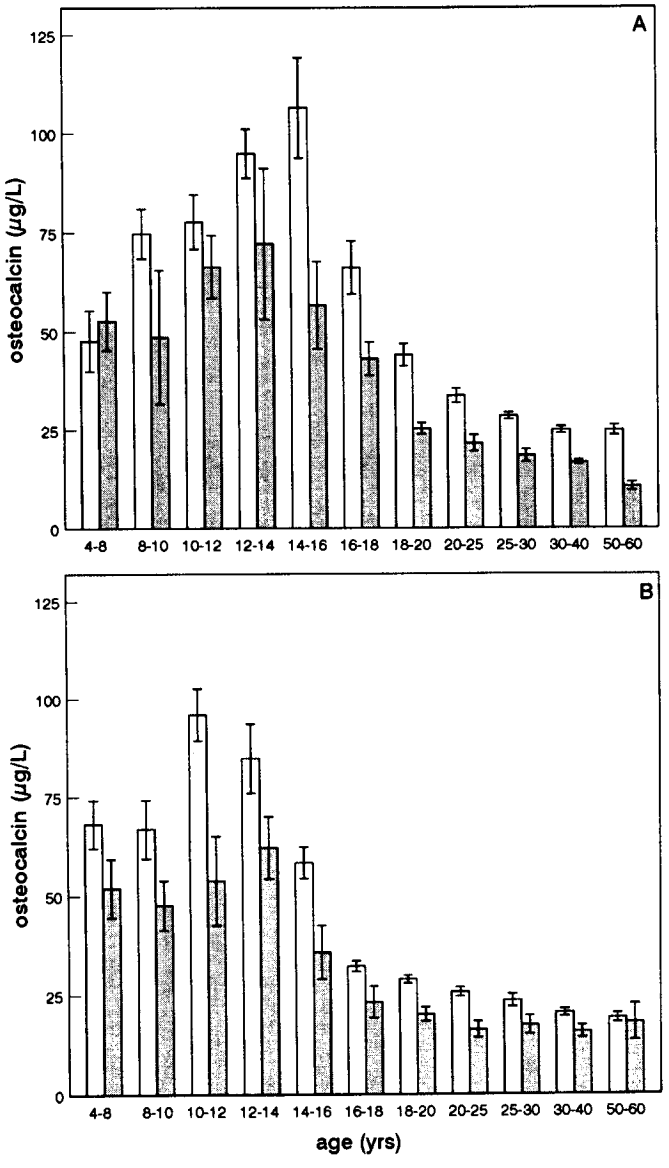

FIG. 2. Serum osteocalcin concentrations in normal and type 1 diabetic children, adolescents, and adults. A, Serum osteocalcin in normal and diabetic boys and men. B, Serum osteocalcin in normal and diabetic girls and women. Symbols are as in Fig. 1. On multiple regression analysis as described in Fig. 1, a positive age effect was observed below the ages of 16 and 12 in boys and girls, respectively, and a negative one above those ages ($P < 0.001$). A significant decreasing effect of diabetes was observed in both sexes ($P < 0.005$).

(Fig. 2) (Z scores for men and women, −1.9 and −1.1, respectively). Serum osteocalcin was higher in control postmenopausal compared with premenopausal women (37%), but in both age periods a significantly lower osteocalcin concentration was found in diabetic women.

Serum total ALP concentrations decreased after puberty in normal and diabetic boys and girls, but the decrease started earlier in girls than in boys (Fig. 3, A and B). In IDDM children and adults, total ALP concentrations were increased (Z scores: children, 0.9; adults, 1.6). A similar trend in age-related changes was observed for skeletal ALP concentration (Fig. 3, C and D). In contrast, the skeletal ALP concentration was lower in diabetic children than in normal children (Z score, −0.8). In adult male IDDM, no significant differences of skeletal ALP were observed, but in women a slight increase was found. The correlation between total and skeletal ALP in all normal and diabetic subjects was highly significant (r = 0.87 and 0.91 in normal and diabetic subjects, respectively) but the slope of the regression was significantly higher in diabetic (4.39) than in nondiabetic subjects (2.62; $P < 0.002$ by multiple regression analysis with or without taking age and sex into account; Fig. 4).

The serum concentration of the collagen type 1 carboxyterminal propeptide extension peptide (PICP) was higher in children and adolescents than in adults, but the decrease started earlier in girls (peak levels in age group 10–12 yr) than in boys (peak levels at 14–16 yr). No significant difference between girls and boys (except for the age at which the decrease started) or between men and women was observed. IDDM, in children or adults, had no significant influence on the serum concentration of PICP (Fig. 5).

The different parameters of bone formation (PICP, ALP, and osteocalcin) all showed high degrees of correlation in both normal and IIDM subjects (Table 2). The highest correlation was, as expected, observed between total and skeletal ALP (Fig. 4). The correlations between serum PICP or osteocalcin and total or skeletal ALP were also highly significant, but the correlation between PICP and ALP or ALP and osteocalcin was still better than the correlation between PICP and osteocalcin. The serum concentration of IGF-I was slightly but negatively correlated with PICP and ALP in normal subjects but was significantly and positively correlated with PICP, ALP, and osteocalcin in IDDM subjects (Table 2).

NIDDM in white subjects

In a group of well controlled white NIDDM subjects (age range, 35–55 yr; mean diabetes duration, 9 yr; range, 0–25 yr) treated with oral antidiabetics (n = 38) or insulin (n = 23), serum IGF-I was significantly decreased (Table 1), whereas serum osteocalcin was only decreased in the younger (35–44 yr) age group. Serum PICP was not significantly influenced by the presence of NIDDM, but serum total ALP activity was clearly increased (Table 3). The serum concentration of skeletal ALP activity, however, was not different in white NIDDM subjects compared with age-matched controls (Table 3). As in normal subjects and in IDDM, a significant correlation was observed between the three osteoblast markers (osteocalcin, PICP, and ALP; $r > 0.3$; $P < 0.01$). The correlation with serum IGF-I was not significant for any osteoblast marker. The HbA_{1c} concentration did not correlate with the serum concentrations of IGF-I, osteocalcin, or PICP but was highly correlated with total ALP activity (r = 0.48; $P < 0.001$).

NIDDM in Pima Indians

Three groups of Pima Indians were studied. In nondiabetic obese subjects, serum IGF-I, osteocalcin, and PICP were lower than in nonobese healthy white subjects, reminiscent of previous results on racial difference in (markers of) calcium homeostasis (32, 33). When white and Pima Indian

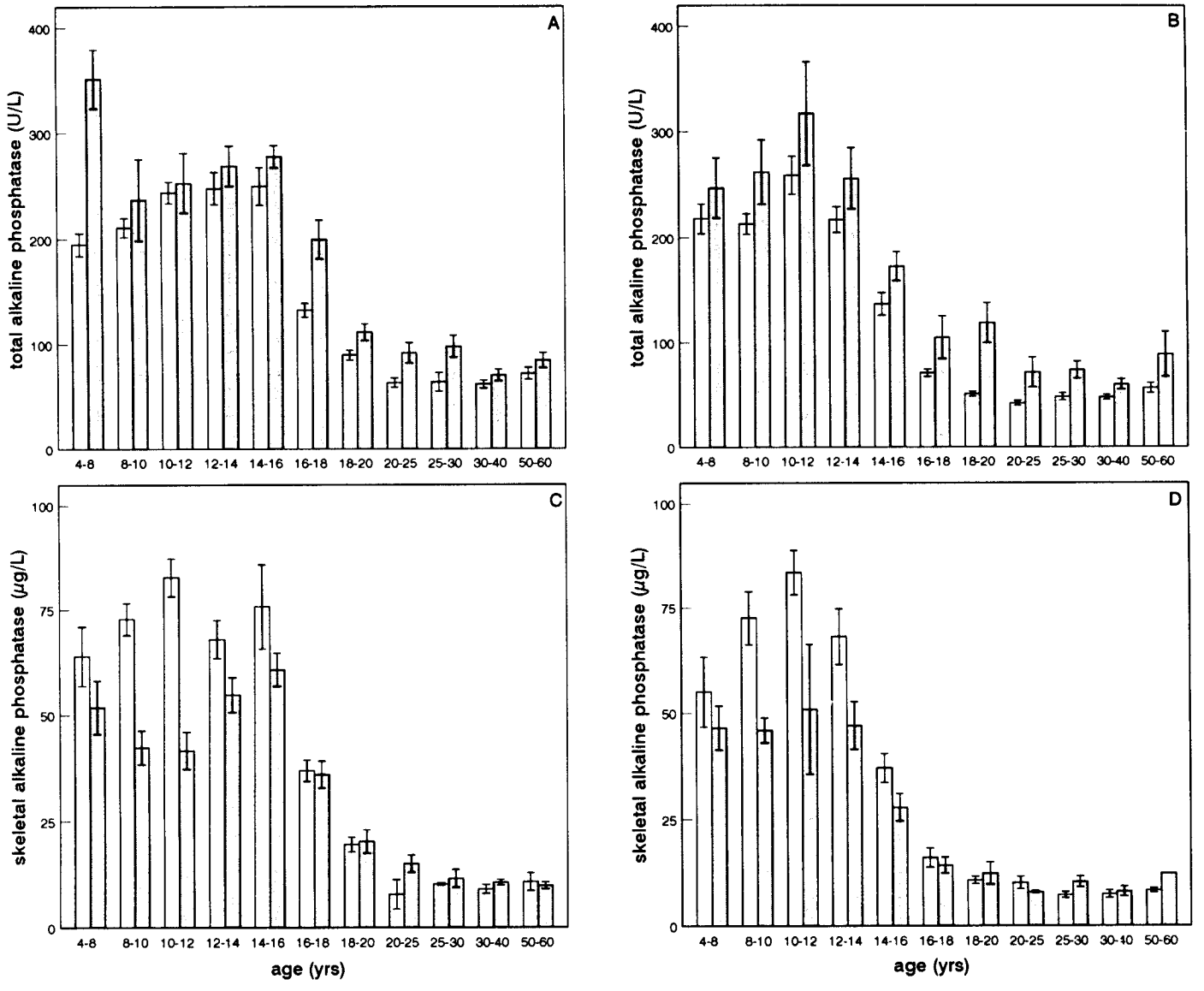

FIG. 3. Serum ALP concentration in normal and type 1 diabetic children, adolescents, and adults. A, Total ALP activity in normal and type 1 diabetic boys and men. B, Total ALP activity in normal and type 1 diabetic girls and women. C, Skeletal ALP activity in normal and type 1 diabetic boys and men. D, Skeletal ALP activity in normal and type 1 diabetic girls and women. Symbols are as in Fig. 1. On multiple regression analysis, diabetes significantly increased total ALP in both children and adults of both sexes ($P < 0.001$). For skeletal ALP, a significant decreasing diabetes effect was observed in children of both sexes ($P < 0.01$), whereas in adults a slightly increasing effect of diabetes was observed ($P = 0.03$).

NIDDM diabetic patients were compared, significantly lower serum osteocalcin (18 ± 7 *vs.* 14 ± 3 μg/L; $P < 0.001$) and IGF-I concentrations (194 ± 78 *vs.* 40 ± 28 μg/L; $P < 0.001$) were found in Pima Indian diabetics without significant differences in PICP concentrations. Serum IGF-I and osteocalcin decreased with increasing degrees of glucose intolerance ($P < 0.001$; Fig. 6), whereas serum PICP showed no consistant relation with fasting or 2 h after oral glucose glycemia (data not shown). The relation with plasma insulin was more complex, as serum IGF-I and osteocalcin showed a significant negative correlation with fasting but a positive correlation with insulin concentrations measured after iv glucose stimulation ($P < 0.01$). A positive correlation was again observed between serum concentrations of IGF-I and osteocalcin in sera from all Pima Indians combined ($r = 0.44$; $P < 0.01$).

Discussion

Osteoblasts secrete several proteins, but collagen type 1 is clearly their most abundantly secreted protein. During the formation of type 1 collagen, the carboxy-terminal propeptide (PICP; mol wt, ±100,000) splits off in a 1:1 molar ratio. This PICP is not incorporated into the bone matrix, so that serum PICP concentration is a marker of collagen synthesis by bone cells (30) and correlates reasonably well with bone formation (34–36). Osteoblasts also secrete a bone-specific

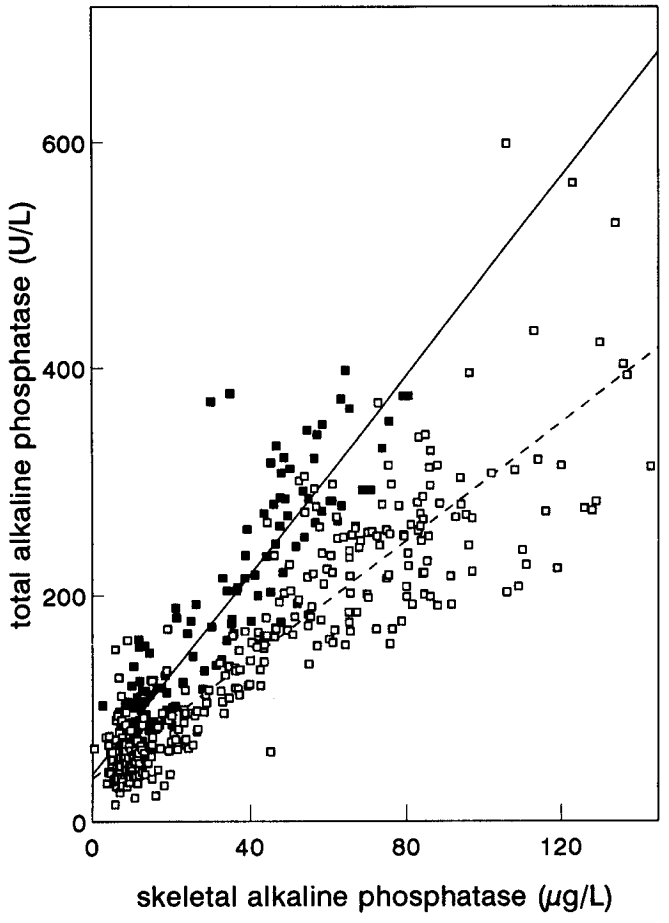

FIG. 4. Correlation between serum concentrations of total and skeletal ALP activity measured in normal (□) and type 1 diabetic subjects (■). For normal subjects (- - -): total ALP = 38 + 2.62 × skeletal ALP ($r = 0.90$; $P < 0.001$). For IDDM patients (—): total ALP = 42 + 4.39 × skeletal ALP ($r = 0.92$; $P < 0.001$). On multiple regression analysis a significant difference ($P < 0.002$) was observed in the slope of the regression between nondiabetic and diabetic subjects (with or without correction for age and sex).

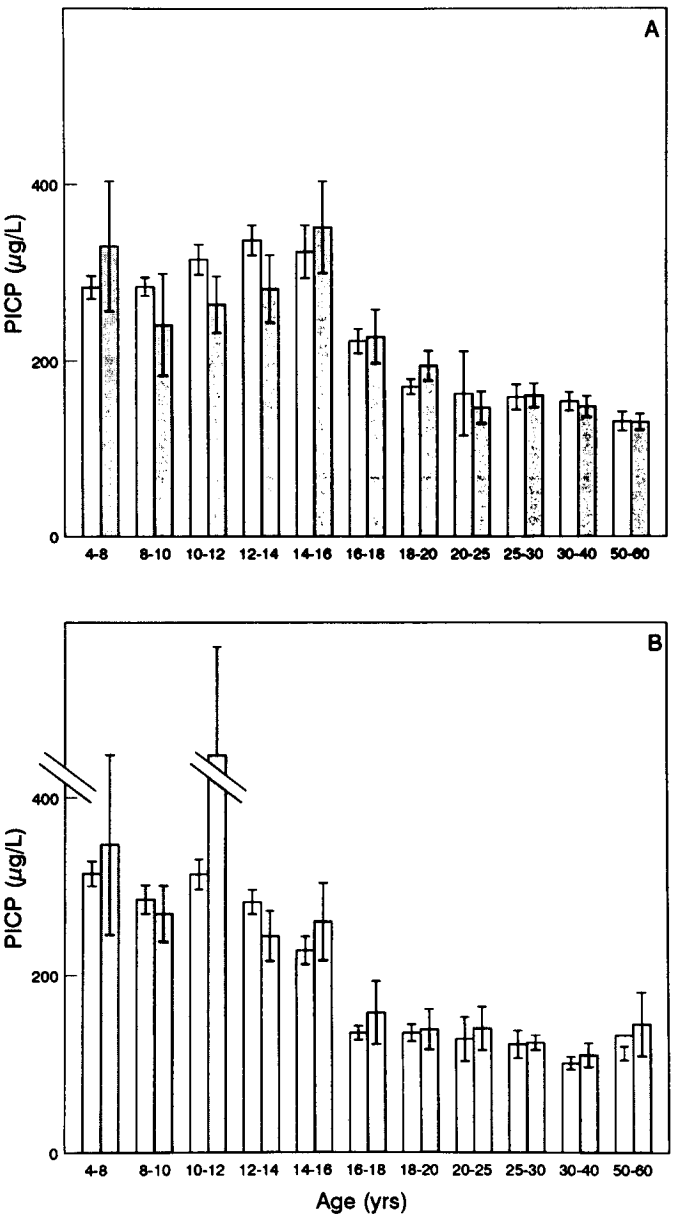

FIG. 5. Concentration of the type 1 PICP in sera from normal or type 1 diabetic subjects. A, Serum PICP concentrations in normal and type 1 diabetic boys and men. B, Serum PICP concentrations in normal and type 1 diabetic girls and women. Symbols are as in Fig. 1. On multiple regression analysis, no clear effect of diabetes on serum PICP was observed in children or adults.

ALP, but their most mature secretory products are osteocalcin and osteopontin (37). All these proteins are therefore also markers of bone formation (5, 38), although important dissociations between the two markers are well recognized (*e.g.* in Paget's disease). IGF-I and its binding proteins are also products secreted by osteoblasts, but their serum concentrations mainly reflect their synthesis and secretion by the liver. IGF-I, however, is an important factor responsible for the final maturation of osteoblasts (39, 40).

The concentration of all osteoblast markers was significantly higher before and during puberty than in adult patients. The most important peak phenomenon during the rapid growth phase was observed for IGF-I and with lower relative increase of osteocalcin and ALP and only a small pubertal increase of PICP. Similar data have previously been published in smaller groups of normal children or adults for osteocalcin (41, 42), ALP (34, 35), and PICP (34, 35). The markers of bone formation clearly correlated significantly with each other in normal and diabetic subjects. Since osteocalcin follows ALP expression, and both are preceded by collagen synthesis during the maturation of the osteoblast (37, 43), the highest correlation was observed between PICP and ALP on the one hand and ALP and osteocalcin on the other, with a somewhat lower, but still highly significant, correlation between the earliest and most mature osteoblast markers, PICP and osteocalcin.

In diabetic rats, marked abnormalities in bone metabolism have been described, characterized by decreased osteoblast number, decreased bone formation, and slow-turnover osteoporosis (1–10). The present study clearly confirms the existence of low IGF-I and osteocalcin concentrations in both children and adults with IDDM, even during reasonably good diabetes control.

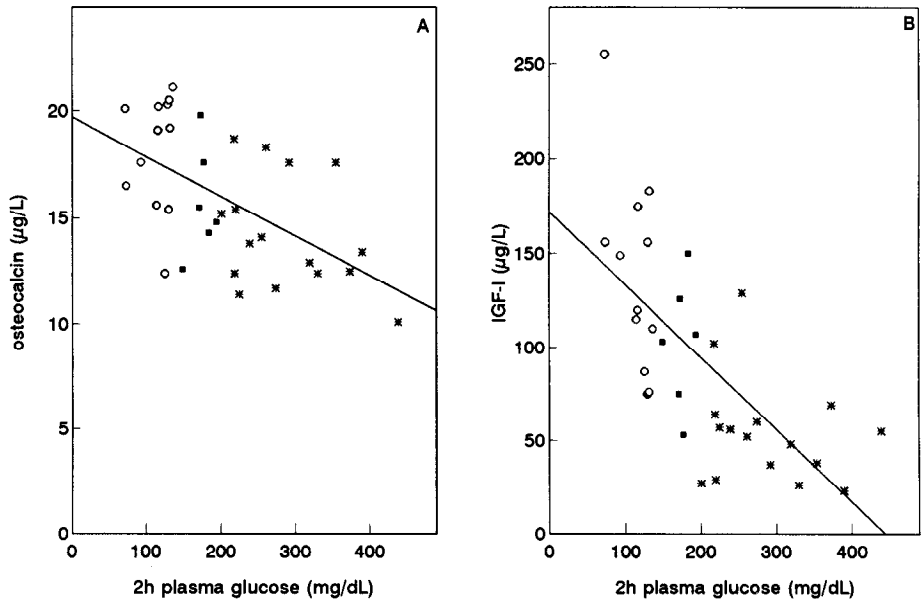

FIG. 6. Serum concentrations of osteocalcin and IGF-I in Pima Indians. Individual values from Pima Indians with normal (○), impaired glucose tolerance (■), or diabetic oral glucose tolerance (*) are plotted. For osteocalcin, r = −0.56; $P < 0.001$; for IGF-I, r = −0.68; $P < 0.001$.

In diabetic adolescents, the pubertal increase in serum IGF-I and osteocalcin was still present, but the values were lower than in control subjects of the same age. Previous studies also demonstrated low IGF-I concentrations in diabetic children before (21, 23) or during puberty (23, 26, 44) and in most adult IDDM patients (21–25). We now confirm this observation in a large group of diabetic adults with IDDM compared with a large age-matched control group. Moreover, similarly decreased IGF-I concentrations were observed in patients with NIDDM (of white or Pima Indian origin). Since IGF-I can influence the biological activity of the osteoblast, other biochemical parameters of osteoblast function were evaluated. There was, however, a poor correlation between serum IGF-I and either osteocalcin, total or skeletal ALP, and PICP in normal adolescents, probably due to a difference in timing (age) of the maximal secretion of IGF-I and the osteoblast markers. In normal adults (>20 yr), a significant correlation was observed between serum IGF-I and either PICP (r = 0.24; $P < 0.01$) or osteocalcin (r = 0.41; $P < 0.001$). A significant correlation was also found between IGF-I and these parameters in IDDM and NIDDM patients. This suggest that IGF-I, together with insulin or insulin action, plays at least a permissive role, especially for the final osteoblast proteins, skeletal ALP and, most importantly, osteocalcin. Serum osteocalcin was indeed lower in diabetic subjects when compared with the age- and sex-matched group. This difference was most marked in diabetic adolescents but persisted in adults with either type 1 or 2 diabetes. The relative decrease was smaller in white patients with NIDDM on insulin therapy, with normalization of HbA_{1c}, and was absent only in non-insulin-requiring NIDDM diabetic white patients older than 45 yr.

The effect of diabetes on serum ALP was complex, as its effect on total ALP differed from that on skeletal ALP. Previous studies already described increased concentrations of total ALP in diabetes (48, 49) and especially increased concentrations of the intestinal form of ALP (50–52). Our present data clearly confirm such increased total ALP concentration in diabetic patients. The concentration of skeletal ALP was, on the contrary, significantly decreased in diabetic adolescents, whereas the concentration of skeletal ALP was normal or slightly increased in adult diabetes. The possible (small) interference from liver ALP in this assay is unlikely to have influenced the results, as others markers of liver function (*e.g.* γ-glutamyltransferase) are normal in diabetics. The main secretory product of the osteoblast, type 1 collagen, as evaluated by serum PICP concentration, varied with age but not by sex or IDDM.

These data collectively demonstrate that the osteoblast function is abnormal in human diabetes and corresponds well with observations in insulin-deficient diabetic animals (2, 3). The best interpretation of the existing data points toward a defect of the osteoblast, as its primary secretion product, (pro)collagen, remained normal, with increasing abnormalities in the production of skeletal ALP and especially of the final maturation product, osteocalcin. Since osteocalcin is a good marker of the bone mineralization rate, it would be indicative of retarded bone formation or turnover in human diabetes as was observed in animal diabetes (5). The metabolic consequences of the osteoblast maturation defect in diabetes can correspond to the decreased growth velocity and final height observed in diabetic children as observed during longitudinal follow-up (50) or twin studies (51), the increased healing time of fractures (52), and the increased incidence of low bone mass and possibly increased prevalence of fracture (diabetic osteopenia) in human diabetes (1).

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