Serum Levels of Type I Procollagen C-Terminal Propeptide, Insulin-Like Growth Factor-I (IGF-I), and IGF Binding Protein-3 in Obese Children and Adolescents: Relationship to Gender, Pubertal Development, Growth, Insulin, and Nutritional Status

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We measured fasting serum levels of type I procollagen C-terminal propeptide (PICP), insulin-like growth factor-I (IGF-I), and IGF binding protein-3 (IGFBP-3) in obese children and adolescents (obese subjects [OS]) to evaluate their relationship to growth, gender, pubertal stage, and weight excess (WE). The influence of insulin, growth hormone (GH), and weight loss was also studied. The study population consisted of 244 OS and 236 normal-weight subjects (NWS) matched for age, gender, and pubertal stage. At stage I, OS had a higher standard deviation score (SDS) for height than NWS of both genders. During the prepubertal phase, growth velocity (GV) was greater in OS than in NWS of both genders, but it was lower in female OS at stage II and male OS at stage III. PICP increased in puberty, with a more rapid decrease later in female OS and NWS; prepubertal values were higher in OS but were reduced at pubertal stage IV to V in comparison to NWS. Stepwise multiple regression analysis demonstrated that GV was the only anthropological variable correlating with PICP. IGF-I serum values increased significantly in puberty and were higher in OS than in NWS at stage I for both genders. IGFBP-3 values of OS exceeded those of NWS at stages I to III in males and I to II in females. No difference was observed for males versus females in each group, nor was any difference observed for the IGF-I/IGFBP-3 molar ratio between the two groups. Using stepwise analysis, a positive correlation between IGF-I and IGFBP-3 was observed in prepubertal but not in pubertal NWS. Fasting insulin values correlated with IGFBP-3 in OS, accounting for 24.8% of the variation in prepubertal subjects and 17.1% in pubertal subjects. No such correlation was observed in NWS. In prepubertal NWS, PICP and SDS of body mass index (BMI) correlated with IGF-I, accounting for 12.9% of the variation, and SDS of BMI correlated with IGFBP-3, explaining 27.8% of the variation. In prepubertal OS, no such correlations could be observed, but PICP and SDS of BMI accounted for 14.3% of the variation in the IGF-I/IGFBP-3 molar ratio. A significant reduction of IGFBP-3 and an increase of the IGF-I/IGFBP-3 molar ratio were detected after weight loss in 40 OS. In conclusion, we demonstrated that IGF-I and IGFBP-3 are influenced by age, gender, sexual development, and nutritional status. Also, an influence of insulin on IGFBP-3 serum levels was observed in OS. The relations of IGF-I to PICP in NWS and of the IGF-I/IGFBP-3 molar ratio to PICP in OS support the concept of IGF-I influence on skeletal growth. The increased IGFBP-3 serum values in OS suggest a possible role in controlling the growth stimulus induced by nutritional status.

NUMEROUS STUDIES have shown that children affected by simple obesity may be of greater height and may undergo faster bone maturation than normal-weight subjects (NWS).[1-9] However, in these obese prepubertal children, the accelerated growth and bone maturation will not result in excess height in adult life, because of a reduced spurt during pubertal development.[3,4,7-10]

There is general agreement that body fat excess may exert a growth-promoting effect,[3,4,10] but the mechanisms of this effect are not fully understood. In obese children and adults, secretion of growth hormone (GH) is reduced when measured either as 24-hour spontaneous secretion or after stimulation.[7,11-19] Conversely, the serum level of the major GH mediator, insulin-like growth factor-I (IGF-I), is either increased[12,13,15,17,20] or unchanged[7,11,15,21] in obese children and adolescents, but is reduced in obese adults relative to NWS in most[20-24] but not in all[25] studies.

Obesity-related hyperinsulinism has an important growth effect, and insulin could upregulate IGF-I production.[26] IGF activity is strongly modulated by specific binding proteins (IGFBPs). Only a few studies have focused on IGFBP-3 serum levels in adult individuals and experimental animals, and no data are presently available on IGFBP-3 serum levels in obese children and adolescents. This protein is the most prominent circulating IGFBP,[27] its levels being dependent on GH status.[28,29] In addition, IGFBP-3 is involved in regulation of the growth effect of IGF-I (for review, see Jones and Clemmons[30]).

Collection of several height measurements over a long period is required for a thorough evaluation of longitudinal growth. A number of biochemical markers have been proposed for evaluating the short-term dynamics of skeletal growth and metabolism (for review, see Robins[31]). Type I procollagen, an osteoblast product, has been indicated as a possible marker for investigating bone formation under physiologic conditions and in metabolic diseases.[32-35] Sensitive radioimmunoassays (RIAs) for the carboxy-terminal propeptide of type I procollagen (PICP) have been developed, and demonstrate effectiveness in growth evaluation.[31,33,35-37] However, no information in the literature is available on the serum levels of these growth markers in obese children compared with NWS.

The aim of our research was to test the hypothesis that PICP, IGF-I, and IGFBP-3 serum levels are influenced by gender, sexual development phase, and nutritional status in obese children and adolescents. Accordingly, we analyzed fasting serum levels of PICP, IGF-I, and IGFBP-3 in a large number of obese children and adolescents from a population-based survey and evaluated their relationship to gender, pubertal stage,

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growth, growth velocity (GV), insulin, GH, nutritional status, and weight reduction after food restriction.

SUBJECTS AND METHODS

Recruitment of Obese Subjects and NWS

The obese subjects (OS) selected for this study, 124 males and 120 females aged 6 to 16 years, were part of an extensive research project on growth and ponderal development of school children in three counties of central Italy (Perugia, Terni, and Rieti). Informed consent was obtained from the parents of children included in this study. The study involved weight, height, and triceps skinfold measurements of the schoolchildren recorded from 1992 to 1995. For statistical analysis, the following parameters were used: height, standard deviation score (SDS) of height for age, weight, and pubertal stage according to Tanner and Whitehouse[38]; Quetelet or body mass index ([BMI] weight in kilograms divided by the square of the height in meters) computed using the tables of Rolland-Cachera et al[39]; triceps skinfold evaluated according to the tables of Tanner and Whitehouse[40]; body weight excess (WE) for the stature age calculated from the ratio of actual body weight (ABW) to ideal body weight (IBW) for height and age according to Tanner and Whitehouse[38] (WE = ABW/IBW × 100); and excess of BMI ($BMI_{50}E$) calculated from the ratio of the actual BMI (Q) and the 50th percentile of BMI according to age and gender (M) ($BMI_{50}E = Q/M \times 100$) according to Cole.[41] Overweight subjects were selected according to the following criteria: WE more than 120%, BMI more than 90th percentile, and triceps skinfold more than 85th percentile. They were invited to attend programed visits during which anamnestic, auxological, and clinical data, as well as information regarding life-style and nutritional behavior, were recorded. The OS group was composed of subjects who had consented to the program of diagnostic evaluation and therapy. Analogous recruitment of NWS could not be used because such recruitment was not part of the original research program. Furthermore, there were no substantial reasons to include normal subjects in diagnostic or treatment protocols. Thus, the control group consisted of patients attending our Pediatric Clinic who were age-, gender-, and pubertal development–matched NWS (116 males and 120 females). NWS were selected according to the following criteria: WE between 85% and 115% and BMI and triceps skinfold between 25th and 75th percentile. NWS attended our Pediatric Clinic for minor diseases or for previous diagnoses not confirmed. In selecting NWS and OS, the following conditions resulted in exclusion from the study: acute pathologies (febrile diseases, traumas, seizures, etc.), dysmorphic pathologies, drug therapy, lesions of the central nervous system, short stature, or clinical conditions that could affect the endocrine-metabolic state, ponderal or statural growth, or pubertal development. There were no ethnic differences or differences in socioeconomic status or environmental exposure between OS and NWS. The height and BMI of the two groups of subjects subdivided according to age (2-year intervals) and gender are reported in Table 1.

To calculate GV (centimeters per year), height measurements were recorded at intervals of 6 to 8 months in subjects younger than 9 years and at 4 to 5 months in subjects older than 9 years. GV evaluation in OS was recorded before nutritional or therapeutic intervention was started. Age and auxological parameters are those found at the time of serum sample collection and stimulation tests. GV was grouped according to the mean age at the time of two measurements. There was a difference of 2 to 4 months between the GV age and the age for the other variables.

The study was performed in collaboration with the three county Directors of Education, and was approved by the Ethics Committee of the region of Umbria.

Serum Sample Collection and Stimulation Tests

Blood samples were collected during routine analysis after overnight fasting. Sera were separated and stored at −30°C until determination of PICP, IGF-I, and IGFBP-3 in both OS and NWS and serum insulin in NWS.

An oral glucose tolerance test ([OGTT] 1.75 g glucose/kg body weight; maximum, 75 g) was performed on OS, and blood samples were collected at 0, 30, 60, 90, and 120 minutes to assay blood glucose and serum insulin. All OS had normal glucose tolerance by OGTT. The L-Arginine test (0.25 g/kg body weight infused intravenously over 30 minutes) was used in 49 prepubertal OS (27 males and 22 females) to evaluate the serum GH response. The total insulin area under the curve of the OGTT and total GH area under the curve of the L-arginine test were computed by trapezoidal integration of the values at 0, 30, 60, 90, and 120 minutes.

Evaluation of Weight Reduction Induced by a Calorie-Deficient Diet

To evaluate the influence of weight reduction on PICP, IGF-I, and IGFBP-3, 40 OS aged 8 to 12.5 years were included in weight-control programs in our Clinic. A protein-sparing modified-fast diet (800 kcal/d; protein intake, 2.2 to 2.5 g/kg ideal body weight per day) was administered to eight male and six female OS for 8 to 10 weeks. The other OS (15 males and 11 females) followed a balanced calorie-deficient diet (1,200 kcal/d; protein intake, 1.5 to 2.0 g/kg ideal body weight per day) for 10 to 12 weeks. Vitamin and mineral integration was used for all OS regardless of the type of diet. During weight reduction, the children were seen at 1 to 2-week intervals and adjustments of dietary intake were made when necessary. Overweight indices (actual weight, BMI, WE, and $BMI_{50}E$) were recorded and fasting PICP, IGF-I, IGFBP-3, and insulin serum values were determined both before weight loss and after. All OS had a reduction of at least 10% of ideal body weight for height.

Methods

Serum PICP concentrations were measured by RIA[34] using a commercial kit (Pharmos Diagnostica, Turku, Finland). The sensitivity

Table 1. Anthropometric Characteristics in NWS and OS Subdivided According to Gender and Age

Characteristic	Age Group (yr)									
	6-8	8-10	10-12	12-14	14-16	6-8	8-10	10-12	12-14	14-16
NWS	Males (n = 116)					Females (n = 120)				
No.	21	24	23	24	24	20	25	26	25	24
Height (cm)*	121.3 ± 8.9	132.9 ± 5.7	143.8 ± 7.4	157.9 ± 9.3	169.9 ± 8.2	121.5 ± 5.9	134.4 ± 8.4	144.8 ± 8.9	155.9 ± 8.2	160.9 ± 4.2
SDS of BMI*	−0.13 ± 0.88	−0.28 ± 1.04	−0.11 ± 1	0.28 ± 0.98	0.16 ± 1.05	0.26 ± 1.08	−0.15 ± 0.97	0.18 ± 1.22	0.16 ± 1.24	0.25 ± 1.2
OS	Males(n = 124)					Females(n = 120)				
No.	24	27	25	25	23	22	23	26	27	22
Height (cm)*	125.8 ± 6.7	138.3 ± 6.3	151.7 ± 7.3	161.9 ± 8.7	170.8 ± 7.9	129.5 ± 6.7	137.2 ± 6.9	147.7 ± 6.5	157.9 ± 5.3	161.6 ± 4.3
SDS of BMI*	3.04 ± 1.14	3.13 ± 0.88	3.06 ± 0.96	2.98 ± 0.91	2.59 ± 0.87	3.29 ± 1.17	3.47 ± 0.95	3.26 ± 0.8	3.17 ± 0.91	3.03 ± 0.74

*Mean ± SD.

of the method was 1.2 μg/L. Interassay and intraassay coefficients of variation (CVs) were less than 6%. IGF-I was extracted by the acid-ethanol method as described by Daughaday et al,[42] modified by a cryoprecipitation step,[43] and serum concentrations were measured using a specific double-antibody RIA (Incstar, Stillwater, MN). The sensitivity of the assay was 20.6 μg/L. The cross-reactivity of the antiserum with insulin and IGF-II was less than 1%. Interassay and intraassay CVs were less than 7.5%. To validate the assay method,[44,45] both the extraction technique and G-75 Sephadex chromatography under acid conditions[46] were performed in 36 serum samples. Values obtained by the two methods were positively correlated with each other ($r = .71$). The mean IGF-I serum concentration determined after acid-ethanol extraction was 9.4% lower than after acid chromatography. Serum IGFBP-3 concentrations were measured by a double-antibody RIA[19] using a commercially available kit (Bioclone Australia, Marrickville, Australia). The sensitivity of the serum assay was 3.5 μg/mL. No cross-reactivity of IGFBP-3 antiserum was detected with GH or IGF-I at the standard serum concentrations. Interassay and intraassay CVs were less than 6%. The molar ratio of IGF-I and IGFBP-3 was calculated using the molar weight of 30.5 kd for IGFBP-3 as previously reported.[18] Insulin and GH serum concentrations were measured by RIA using commercial kits (Techno Genetics, Cassina de' Pecchi, Italy). In our laboratory, peak GH serum values during the L-arginine test were greater than 10 μg/L in more than 85% of normal subjects (mean ± SD, 14.8 ± 4.1 μg/L).

Statistical Analysis

ANOVA, paired and unpaired *t* tests (Bonferroni correction was used for the *t* test in which multiple comparisons were performed), Wilcoxon signed-rank test, Spearman rank correlation analysis, and multiple regression analysis (stepwise) were performed using Statgraphics software (Graphic Software System, Rockville, MD). The level of statistical significance was set at P less than .05. Statistical requirements for meeting normal (gaussian, with 0 mean and 1 SD) distribution were assessed by the Kolmogorov-Smirnov test, and a logarithmic or square-root transformation was used when necessary. The distribution for BMI at any particular age tends to be positively skewed. To express the SDS of BMI, when necessary, we used the Box-Cox power transformation according to the method proposed by Cole et al.[47,48] The equation to calculate an exact SDS is as follows:

$$SDS = \frac{[BMI/M]^{L} - 1}{L \times S}.$$

The smoothed L (Box-Cox power), M (median), and S (CV) values at 6-month intervals were kindly furnished by Dr Rolland-Cachera et al[39] for the BMI values detailed in their report.

RESULTS

Adiposity Indices, Insulin, and GH Values

Age, adiposity indices, insulin (basal and integrated under the OGTT curve), and GH (basal and integrated under the L-arginine curve) values in NWS and OS subdivided according to gender and pubertal stage are reported in Table 2. When subdivision according to pubertal stage was taken into account, age showed a gaussian curve of distribution and the mean ± SD was similar between OS and gender- and pubertal stage–matched NWS. BMI, expressed as SDS, was significantly higher ($P < .01$), as expected, in OS versus NWS of the same gender and pubertal stage. Moreover, ANOVA did not reveal differences between the two genders and the different pubertal stages within either group. Also, fasting insulin and integrated values under the OGTT curve were normally distributed. Using ANOVA, fasting insulin mean values varied significantly among the pubertal stages in NWS (males, $F = 6.05$, $P < .01$; females, $F = 10.5$, $P < .01$) and in OS (males, $F = 2.8$, $P < .05$; females, $F = 5.0$, $P < .01$). Mean values (males plus females) were greater in pubertal than in prepubertal subjects in both

Table 2. Age, Anthropometric Characteristics, and Insulin (basal and integrated OGTT) and GH (integrated L-arginine test) in NWS and OS Subdivided According to Gender and Pubertal Stage

	Tanner Stage							
Variable	I	II	III	IV-V	I	II	III	IV-V
NWS	Males (n = 116)				Females (n = 120)			
No.	49	23	22	22	50	24	22	24
Age (yr)*	8.9 ± 1.8	12.1 ± 0.9	13.5 ± 1.2	14.2 ± 14.2	8.5 ± 1.4	10.5 ± 1.2	11.6 ± 0.9	13.4 ± 1.1
SDS of BMI*	−0.21 ± 0.96	0.09 ± 1.02	0.15 ± 1.11	0.29 ± 0.96	−0.01 ± 1.04	0.25 ± 1.32	0.04 ± 1.16	0.41 ± 0.99
WE (%)								
Median	99.3	104.5	97.8	104.3	96.6	103.1	99.5	104.4
Range	82.2-119.9	85.6-117.4	87.8-119.5	84.8-119.9	80.7-114.5	82.1-118.9	80.6-119.9	83.9-115.4
$BMI_{50}E$ (%)								
Median	96.9	99.9	98.5	95.9	95.7	100.2	97.6	104.7
Range	85-117.8	80.8-119.9	79.5-115.2	80.9-119.9	84.8-119.3	82.4-119.5	85.4-119.5	86.5-119.7
Insulin (pmol/L)*	75.4 ± 26.8	75.7 ± 23.5	104.8 ± 41.5	93 ± 30.1	72.3 ± 30.6	95 ± 24.4	107.1 ± 29.8	105.4 ± 3.37
OS	Males (n = 124)				Females (n = 120)			
No.	54	24	24	22	51	24	22	23
Age (yr)*	9.3 ± 1.7	12.2 ± 1.1	13.1 ± 1.2	14 ± 1.2	8.8 ± 1.5	10.5 ± 0.9	1.3 ± 1	13.4 ± 1.5
SDS of BMI*	3.12 ± 1.17	2.98 ± 0.94	2.95 ± 0.7	2.61 ± 0.76	3.31 ± 1	3.4 ± 0.9	3.21 ± 0.85	2.99 ± 0.81
WE (%)								
Median	147.8	145.3	141.8	138	139	142.4	146.7	146.6
Range	130-220.9	131-183.7	130.5-169.3	132-181.8	130-185.5	132-186.6	139.5-183.5	131.4-181.8
$BMI_{50}E$ (%)								
Median	147.1	150.1	153.1	148.3	139.5	145.6	147.1	149
Range	120.1-260	135.4-220.2	140.1-230.5	143-202.1	125.5-241	130.1-215	136.7-235.6	141-221.1
Insulin (pmol/L)*	83.2 ± 29.7	98.8 ± 35.8	105.2 ± 36.1	91.8 ± 37.9	94.4 ± 49.5	137 ± 78.9	142.5 ± 59.5	118.9 ± 44.2
TIA (pmol/L)*	33,889 ± 15.234	37,044 ± 21,234	42,147 ± 15,359	52,175 ± 19,902	44,019 ± 28,108	59,272 ± 37,315	59,544 ± 40,506	50,586 ± 24,248
TGHA (ng/L)*	591 ± 276	—	—	—	582 ± 265	—	—	—
Males (n = 27); Females (n = 22)								

Abbreviations: TIA, total insulin area; TGHA, total GH area.
*Mean ± SD.

groups (prepubertal NWS 73.8 ± 28.7 *v* pubertal NWS 96.7 ± 30.4 pmol/L, $P < .01$; prepubertal OS 88.6 ± 39.3 *v* pubertal OS 115.6 ± 48.7 pmol/L, $P < .01$). Similarly, integrated values under the OGTT curves varied significantly in male OS ($F = 6.1$, $P < .01$) but not in females, and significantly increased during puberty when males and females were combined (prepubertal OS 39,130 ± 22,018 *v* pubertal OS 50,327 ± 26,525 pmol/L, $P < .01$). When comparing OS and NWS, fasting insulin mean values were higher in both prepubertal and pubertal OS ($P < .01$). Mean maximal serum levels of GH were subnormal after L-arginine stimulus in prepubertal OS (6.3 ± 2.9 μg/L). There was no significant correlation between insulin (basal and integrated area under OGTT) and GH (basal and integrated of L-arginine test) serum levels.

Height and GV

The SDS of height and GV (square root) for NWS and OS subdivided into pubertal stages is shown in Fig 1. The SDS for height was normally distributed in both groups regardless of gender or pubertal stage. OS had a significantly higher SDS for height than NWS at stage I to III in males and at stage I to II in females. When analyzed in relation to gender, the SDS for height in male NWS was significantly greater than in female NWS at stage IV to V, and was significantly lower in male OS than in female OS at stage I and significantly higher at stage III. The distribution of GV data tended to be skewed, with the right tail longer than the left. Therefore, for statistical analysis and graphic representation, square-root–transformed GV (SRGV) data were used to obtain the best approximation to a standard normal distribution of the data. SRGV was greater in male OS than in male NWS at stage I and lower at stage III. Similarly, SRGV was higher in female OS than in female NWS at stage I, but lower at stage II. When analyzed in relation to gender, male NWS had a greater SRGV than female NWS at stage III.

PICP

PICP fasting serum values in NWS and OS are reported in Fig 2, subdivided according to gender and pubertal stage. A unimodal distribution was observed in both NWS and OS regardless of gender and pubertal stage. PICP mean values of

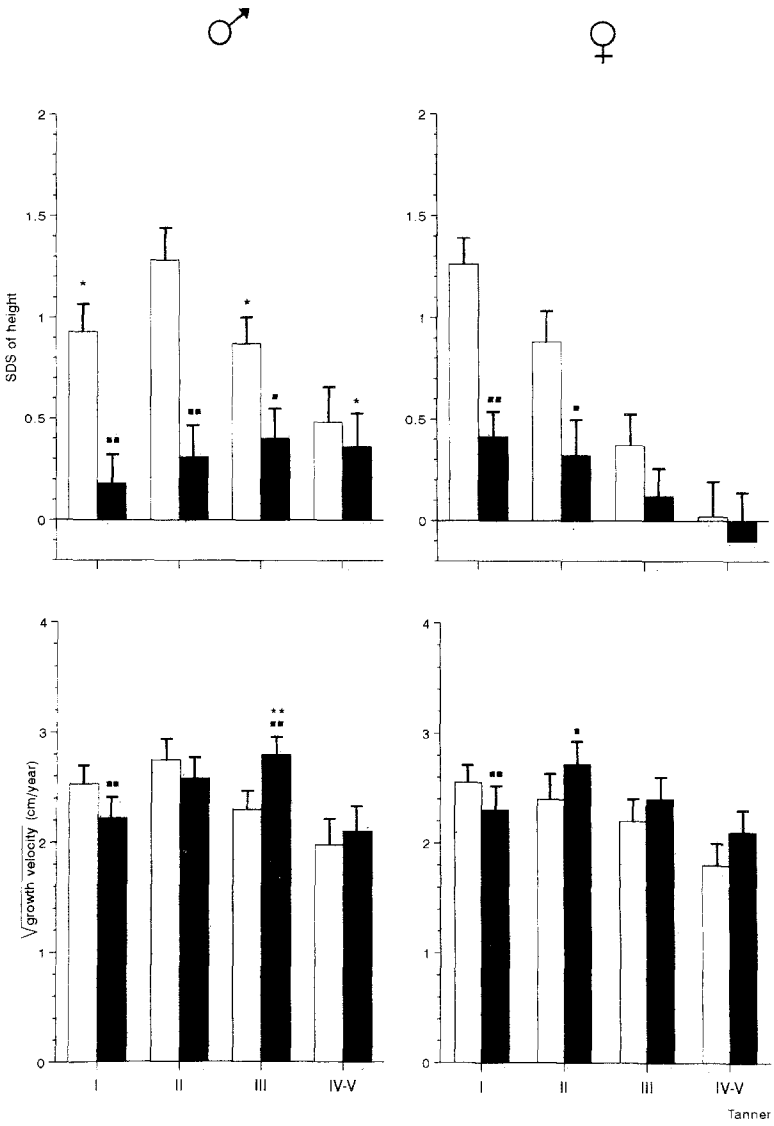

Fig 1. SDS of stature and SRGV (mean ± SE) in OS (□) and NWS (■) according to gender and pubertal stage. ■$P < .05$, ■■$P < .01$: NWS *v* OS. *$P < .05$, **$P < .01$: males *v* females.

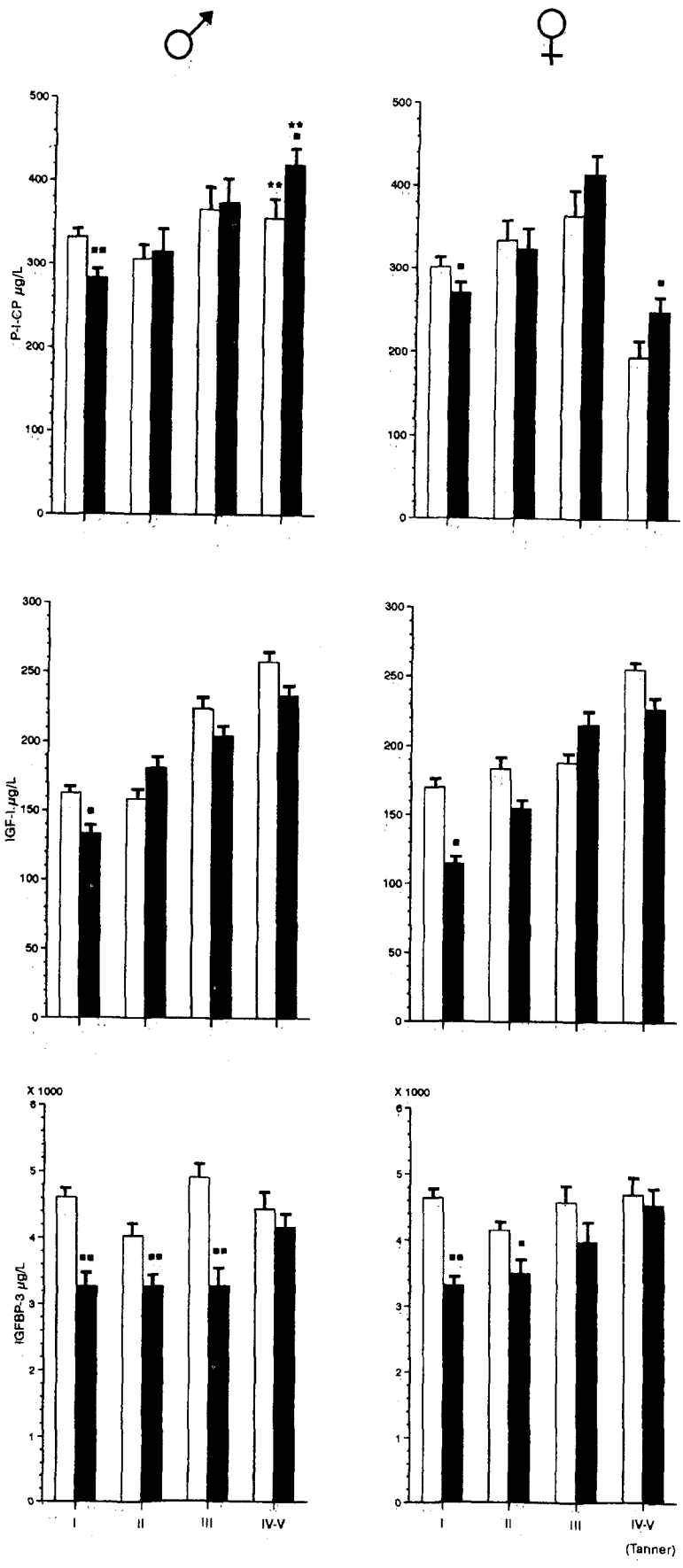

Fig 2. Fasting serum values (mean ± SE) for PICP, IGF-I, and IGFBP-3 in NWS and OS according to gender and pubertal stage. ■$P < .05$, ■■$P < .01$: NWS *v* OS. *$P < .05$, **$P < .01$: males *v* females.

NWS were increasing during pubertal development (males, F = 10.5, $P < .01$; females, F = 15.1, $P < .01$). However, in female NWS, a significant reduction was observed in late puberty, with mean PICP values significantly lower with respect to those in males at stage IV to V. The pubertal increase of PICP was evident also in male OS (F = 5.5, $P < .01$), but not in OS females, who showed a significant reduction of mean PICP values in late puberty (F = 12.0, $P < .01$). As a consequence, at stage IV to V, male OS had higher values than female OS. On comparing the two groups, mean PICP values were significantly higher in prepubertal OS of both genders, but the pubertal increase was less evident, and as a result, PICP values were significantly reduced in both males and females versus NWS at stage IV to V.

Stepwise multiple regression analysis was performed separately for all NWS and OS, using fasting PICP serum values as the dependent variable and age, gender, SRGV, SDS of BMI, WE, and $BMI_{50}E$ as independent variables. SRGV correlated significantly in both groups and accounted for 26.3% of the variation in PICP ($P < .01$) in NWS and 23.6% ($P < .01$) in OS. The significance of the correlation remained unchanged when the other variables were entered in the regression analysis. Values remained significant when PICP versus GV (unmodified) were examined in Spearman rank correlation analysis (prepubertal male OS, $r = .349$, $P < .01$; pubertal male OS, $r = .562$, $P < .01$; prepubertal female OS, $r = .421$, $P < .01$; pubertal female OS, $r = .733$, $P < .01$; prepubertal male NWS, $r = .295$, $P < .05$; pubertal male NWS, $r = .422$, $P < .01$; prepubertal female NWS, $r = .351$, $P < .05$; pubertal female NWS, $r = .410$, $P < .05$).

IGF-I and IGFBP-3

IGF-I and IGFBP-3 values had a unimodal distribution in both groups of subjects with respect to gender and sexual development. The mean ± SD in both groups of subjects subdivided by pubertal stage are reported in Fig 2. In both NWS and OS, IGF-I mean values varied significantly (male NWS, F = 12.3, $P < .01$; female NWS, F = 23.9, $P = .01$; male OS, F = 15.8, $P < .01$; female OS, F = 10.5, $P < .01$) with an increase in puberty. No difference was observed between the two genders in the two groups subdivided according to pubertal stage. When comparing OS and NWS, mean IGF-I values of OS were significantly higher than those of NWS in both prepubertal males and females (Fig 2).

IGFBP-3 mean values also tended to increase during puberty; differences detected by ANOVA were significant in NWS (males, F = 4.0, $P < .01$; females, F = 7.1, $P < .01$) and in male OS (F = 3.5, $P < .05$) but not in female OS. No significant difference was found on comparing both genders for each group. When comparing the two groups, mean values were significantly greater in OS than in NWS at pubertal stages I, II, and III in males and I and II in females (Fig 2). No significant difference was found in the mean IGF-I/IGFBP-3 molar ratio of the two groups regardless of gender or pubertal stage.

Two different stepwise multiple regression analyses were performed examining the two groups separately after subdivision into prepubertals and pubertals. Since IGF-I, IGFBP-3, and insulin are interrelated, type 1 stepwise multiple regression analysis was made by entering fasting insulin and IGF-I as independent variables and IGFBP-3 as the dependent variable. From the analysis, a correlation resulted between IGF-I and IGFBP-3 in prepubertal NWS, the former accounting for 38.2% of the variation in the values for the latter. This correlation was not evident in OS, in whom only fasting insulin correlated with IGFBP-3, accounting for 24.8% of its variation in prepubertal subjects and 17.1% in pubertal subjects (Table 3). In OS, no significant correlation resulted either from basal and integrated GH values during the L-arginine test versus IGF-I, IGFBP-3, or the IGF-I/IGFBP-3 molar ratio or from integrated insulin values during the OGTT versus IGF-I, IGFBP-3, and the IGF-I/IGFBP-3 molar ratio.

In type 2 stepwise multiple regression analysis, anthropometric parameters (age, gender, SDS for height, GV, SDS for BMI, WE, and $BMI_{50}E$) and PICP were considered in the analysis as independent variables and IGF-I, IGFBP-3, or the IGF-I/IGFBP-3 molar ratio were considered separately as dependent variables. In prepubertal NWS, PICP and SDS of BMI together accounted for 12.9% of the variation in IGF-I, and SDS of BMI alone accounted for 27.8% of the variation in IGFBP-3. In contrast, results differed in prepubertal OS, as the correlations were no longer significant, but PICP and SDS of BMI accounted for 14.3% of the variation in the IGF-I/IGFBP-3 molar ratio. There was no significant correlation in pubertal subjects of the two groups (Table 4).

Effects of Weight Reduction

Actual body weight, ideal body weight, BMI, WE, and $BMI_{50}E$ and fasting values for PICP, insulin, IGF-I, IGFBP-3, and the IGF-I/IGFBP-3 molar ratio in OS before weight loss and after are reported in Table 5. We observed a significant reduction in the ponderal indices and IGFBP-3 serum levels and a significant increase in IGF-I/IGFBP-3 molar ratio values after weight loss regardless of gender, age, duration, and type of diet. On the contrary, no significant variation was found in PICP and IGF-I serum values. No significant correlation was detected between the variations in IGFBP-3 and variations in several ponderal indices.

DISCUSSION

This study provides evidence that obese children are greater in stature compared with NWS, confirming previous observations of a growth-promoting effect resulting from obesity,[1-9] and the effect appeared to be most evident in prepubertal subjects. The SDS for height of our OS gradually decreased with age, such that differences between OS and NWS were no longer significant in late puberty. The GV of OS was also higher in prepuberty but tended to decrease later to less than that of NWS during puberty. Differences were observed between the two genders in relation to the pubertal development pattern. These data are in line with previous observations[3,4,7-10] that the

Table 3. Results of the First Stepwise Multiple Regression Analysis

Group	Independent Variable	Adjusted R^2	Dependent Variable	P
Prepubertal NWS	IGF-I	.382	IGFBP-3	<.01
Prepubertal OS	Insulin	.248	IGFBP-3	<.01
Pubertal OS	Insulin	.171	IGFBP-3	<.01

Table 4. Results of the Second Stepwise Multiple Regression Analysis

Group	Independent Variable	Adjusted R^2	Dependent Variable	P
Prepubertal NWS	SDS of BMI	.082	IGF-I	<.01
	PICP	.129		
	SDS of BMI	.278	IGFBP-3	<.01
Prepubertal OS	PICP	.115	IGF-I/IGFBP-3	<.01
	SDS of BMI	.143	molar ratio	

increased height of the obese child will not result in excess height in adult life. In evaluating these results, one must consider that the sampling procedure may have resulted in undue bias possibly affecting the comparison in height between the two groups of subjects. It is unlikely that our OS were associated with the recruitment of taller subjects. Yet the modalities of recruitment of OS could not be used for NWS. However, we did exclude NWS who presented conditions possibly affecting statural development, nutritional status, and pubertal development. In fact, the SDS for height of NWS (Fig 2) shows values close to 0 or even higher.

PICP serum values were studied, and upon fasting, variations were found in NWS of either gender, with an increase in puberty. This pattern was in part similar to the GV curves reported by others.[36,37] However, a gender-dependent effect was observed, as PICP serum values decreased at stage IV to V only in females. In prepubertal male and female OS, fasting mean values for PICP were significantly higher than in NWS, but the puberty-related increase was less evident, so that PICP serum levels were reduced in comparison to those in NWS in late puberty. The greater values for PICP in prepubertal OS might be explained by the fact that other tissues may contribute to the level of circulating PICP (~10%).[33] Nevertheless, the lack of correlation between PICP and adiposity indices using stepwise analysis and the fact that GV was the only influential factor in PICP of the two groups appear to be in contrast with the hypothesis that the high values observed in OS could be contributed by sources other than bone. The results of our study, conducted for the first time in OS, confirm the role of PICP as a growth marker, as reported in studies of normal subjects,[36,49] subjects with different pathologies, and subjects on GH therapy.[33,50-53] Our data suggest that PICP can be considered a sensitive and reliable marker of skeletal development even under conditions not genuinely affecting growth.

Table 5. Anthropometric Data and Fasting Serum Values for Insulin, PICP, IGF-I, and IGFBP-3 Before and After Weight Loss in 40 Subjects (23 males and 17 females) Undergoing a Hypocaloric Diet

Parameter	Before Weight Loss	After Weight Loss
Ideal body weight (kg)		
Median	41	42.4
Range	24-56.5	24.9-57
Actual body weight (kg)		
Median	62.7	57.7†
Range	34.6-105	31.2-98.1
BMI (kg/m²)		
Median	28.2	25.8†
Range	20.1-40.3	18.7-38.3
WE (%)		
Median	156.3	139.4†
Range	128.9-219	110.5-208
$BMI_{50}E$ (%)		
Median	151.6	136.7*
Range	122.8-204.7	114.6-194.5
Insulin (pmol/L)‡	124.9 ± 52.3	101.4 ± 51.4*
PICP (μg/L)‡	275.5 ± 103.2	302.7 ± 110.7
IGF-I (μg/L)‡	206.3 ± 99.1	209.4 ± 106.7
IGFBP-3 (μg/L)‡	3,934 ± 1,156	3,341 ± 967†
IGF-I/IGFBP-3 molar ratio‡	0.322 ± 0.19	0.395 ± 0.198*

*$P < .05$, †$P < .01$: before *v* after weight loss.
‡Mean ± SD.

In line with the results of others,[28,54] IGF-I serum levels of NWS and OS increased with pubertal stage in our study. Conflicting data have been reported on IGF-I serum levels in obese children, with evidence of either increased[12,13,15,17,20] or normal[7,11,15,21] levels. Such discrepancies have been attributed in part to methodological problems; however, it has recently become evident that studies on IGF-I in children and adolescents should consider age, gender, and pubertal development.[54] Accurate subdivision according to gender and pubertal stage allowed us to determine that prepubertal OS had higher IGF-I serum levels than NWS. Moreover, in our study, IGFBP-3 mean serum values were higher in OS than in NWS in stage I and early puberty. This pattern in obese children has not been previously described, and seems to be age- and growth-related. In fact, in advanced puberty, IGFBP-3 mean values for both genders of OS were similar to those of NWS in a phase with a more prominent spurt. Our finding in advanced puberty is in agreement with previous reports on adult OS.[18]

Approximately 80% of IGF-I in the circulation is bound to a 150-kd IGF-binding complex consisting of IGFBP-3 and an acid-labile protein. IGF-I levels are controlled by GH in subjects with a normal nutritional status. However, in agreement with previous studies,[11-13,55] our prepubertal OS had increased IGF-I serum levels in the presence of a blunted GH response to L-arginine. Another discrepancy emerging from our study concerns IGFBP-3. In fact, although GH is considered the most important regulator of IGFBP-3 production to the extent that IGFBP-3 is indicative of GH status for clinical purposes,[28,29,56] high serum levels of IGFBP-3 were found in OS. In this context, an important role might be played by receptor adaptation, which could increase peripheral responsiveness to GH, hyperinsulinism being considered as the most likely factor for increasing GH receptor function in obesity.[15,57-59]

Obesity is associated with hyperinsulinism, and insulin may stimulate IGFBP-3 production either directly or indirectly via effects on IGF-I, which is also considered one of the most important stimulating factors for IGFBP-3 production.[14,60] Our findings are, in part, in agreement with these reports, and show an important influence of insulin on IGFBP-3 production in obese children and adolescents, which was demonstrated by stepwise multiple regression analysis and was not observed in NWS.

The relationships among IGF-I, IGFBP-3, insulin, and GH are influenced by nutritional status. Chronic malnutrition, anorexia nervosa, and acute fasting are associated with elevated levels of GH and decreased levels of IGF-I[61,62] and IGFBP-3,[63] which increase with refeeding. Studies on obesity, a condition

of reduced GH production and increased insulin secretion, are limited to adults and animals. Nguyen-Yamamoto et al,[64] using Zucker rats, reported normal or increased IGF-I and IGFBP-3 serum levels in the presence of low GH levels. Conflicting data are reported for IGFBP-3 serum levels in obese adult humans. Reduced serum values for IGF-I, normal serum values for IGFBP-3, and a reduced molar ratio of IGF-I/IGFBP-3 have been reported by Rasmussen et al[19] in adult OS. In contrast, increased serum levels of both IGF-I and IGFBP-3 in adult obese women are reported by Frystik et al.[25] A positive correlation between IGF-I and IGFBP-3, previously reported by Blum et al,[56] was observed also in our NWS, in whom we demonstrated a relationship between IGF-I and skeletal growth and nutritional status, as indicated by the correlation with both PICP and SDS of BMI. The role of nutritional status in NWS was emphasized by the correlation between SDS of BMI and IGFBP-3. The relationship between IGF-I and both growth and nutritional status was still apparent in prepubertal OS because of significant correlations between the IGF-I/IGFBP-3 molar ratio and both PICP and SDS of BMI. No correlation between BMI and IGF-I was observed by Juul et al.[54] On the contrary, BMI together with age, gender, height, and pubertal maturation were all important factors in determining circulating levels of IGFBP-3 in a large number of healthy children.[65]

The influence of nutritional status on IGFBP-3 is confirmed in this investigation by the effect of dietetic restriction. Weight loss in OS was associated with a reduction of IGFBP-3 serum levels and insulin without any significant change in PICP values. In accordance with other studies,[11,14] IGF-I also remained unchanged in obese children after weight reduction. In animal studies, only a caloric restriction of up to 40% decreases IGF-I and IGFBP-3, while protease activity or the association of IGF-I with IGFBP-3 and the acid-labile subunit remained unaffected.[63] To the best of our knowledge, our study represents the first demonstration of a reduction in IGFBP-3 serum levels after weight loss in obese children. A slight reduction of IGFBP-3 after weight loss or a short fast has been previously reported in some[57] but not all[19,66] studies in adults. In our study, for technical reasons, evaluation of GH secretion during the L-arginine test was performed only in a limited number of prepubertal OS and was not repeated after weight loss. Therefore, we are not able to contribute to the definition of the role of GH in weight loss–related IGFBP-3 reduction. However, it should be noted that Rasmussen et al[19] failed to detect a correlation between IGF-I and IGFBP-3 and 24-hour GH secretion in adult OS during weight loss.

In conclusion, our study demonstrates that IGF-I and IGFBP-3 serum levels in obese children and adolescents are influenced by gender, sexual development phase, and nutritional status. Also, a possible influence of insulin on IGFBP-3 serum levels has been observed in OS. Both the control of IGFBP-3 release and its physiologic role remain to be clarified, as does the effect on IGF-I activity. The correlation between IGF-I and PICP, yet inconstant in NWS, is in line with the IGF-I impact on skeletal development. This influence is also emphasized in OS by the correlation between the IGF-I/IGFBP-3 molar ratio and PICP. The increased IGFBP-3 serum levels, reported in prepubertal and early pubertal phases in OS for the first time, suggest a possible role for IGFBP-3 in controlling the stimulus resulting from the nutritional status with effects on growth stature and bone maturation.

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