Growth Hormone Bioactivity, Insulin-Like Growth Factors (IGFs), and IGF Binding Proteins in Obese Children

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In obese children, both spontaneous and stimulated growth hormone (GH) secretion are impaired but a normal or increased height velocity is usually observed. This study was undertaken to explain the discrepancy between impaired GH secretion and normal height velocity. We evaluated the GH bioactivity (GH-BIO), GH serum level by immunofluorimetric assay (GH-IFMA), insulin-like growth factor-I (IGF-I), IGF-II, and IGF binding protein-1 (IGFBP-1), IGFBP-2, and IGFBP-3 in 21 prepubertal obese children (13 boys and eight girls) aged 5.7 to 9.4 years affected by simple obesity and in 32 (22 boys and 10 girls) age- and sex-matched normal-weight controls. The results were as follows (obese versus [*v*] controls): GH-IFMA, 4.84 ± 3.54 *v* 23.7 ± 2.04 μg/L ($P < .001$); GH-BIO, 0.60 ± 0.45 *v* 1.84 ± 0.15 U/mL ($P < .001$); IGF-I, 173.8 ± 57.2 *v* 188.6 ± 132.6 ng/mL (nonsignificant); IGF-II, 596.1 ± 139.7 *v* 439.3 ± 127.4 ng/mL ($P < .001$); IGFBP-1, 23.25 ± 14.25 *v* 107 ± 165.7 ng/mL ($P < .05$); IGFBP-2, 44.37 ± 62.18 *v* 385.93 ± 227.81 ng/mL ($P < .001$); IGFBP-3, 3.31 ± 0.82 *v* 2.6 ± 0.94 μg/mL ($P < .05$); and IGFs/IGFBPs, 1.32 ± 0.32 *v* 1.07 ± 0.34 ($P < .05$). In conclusion, in prepubertal obese children, not only immunoreactive but also bioactive GH concentrations were low. In these subjects, therefore, nutritional factors and insulin may contribute to sustain normal growth also by modulating several components of the IGF-IGFBP system.

IN OBESITY, both spontaneous and stimulated growth hormone (GH) secretion are impaired.[1-4] However, despite the low GH levels, normal or moderately increased insulin-like growth factor-I (IGF-I) serum levels are observed in children,[5] although low levels have also been reported.[6] Furthermore, growth velocity is commonly normal or increased.[7] Body weight reduction is followed by an improvement in GH secretion together with a decline in IGF-I[2] and a reduction in linear growth velocity.[7] Altogether, body weight seems to be the main determining factor for these findings. The GH receptor could be the potential link between overweight and low GH and normal/high IGF-I serum levels.[8] GH binding protein, which probably reflects the liver GH receptor status, has been found to be elevated in obese children and to correlate with the body mass index (BMI) and IGF-I.[8] Thus, an increased binding capacity of liver GH receptors would explain the normal/high IGF-I serum levels in obese children, although an upregulation of GH receptors due to the hyposomatotropism cannot be excluded. On the other hand, an enhanced GH bioactivity (GH-BIO) could also play a role, but this hypothesis has not been tested. Moreover, little is known about whether IGF binding proteins (IGFBPs) play a role in the growth process in obese children.

The aim of our study was to evaluate in a group of prepubertal obese children (1) the GH-BIO using the Nb2 cell bioassay, (2) the serum values for IGFs and IGFBPs and their correlation with growth velocity, and (3) the effects of fat on GH, IGFs, and IGFBPs.

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SUBJECTS AND METHODS

Patients

After obtaining informed consent from the parents and before starting the dieting program, we evaluated 21 prepubertal obese children (13 boys and eight girls) aged 5.7 to 9.4 years attending the obesity outpatient clinic. The children were considered to be affected by simple obesity after exclusion of any syndromic, organic, and hormonal causes. None of the subjects were on any medication or showed evidence of systemic disease. For the purpose of homogeneity, the height[9] and BMI[10] were expressed as a standard deviation score (SDS). Bone age was evaluated by one of us (C.P.) according to the atlas of Greulich and Pyle.[11] The percentage of body fat was assessed from skinfold thickness using the equations of Slaughter et al.[12] Body fat mass was obtained by multiplying the percentage of body fat by body weight. Fat-free mass was calculated by subtracting body fat from body weight. To improve reproducibility, measurements of subscapular and triceps skinfolds were performed in triplicate to the nearest millimeter by the same observer (B.P.). As a control group, we evaluated 32 age- and sex-matched prepubertal children (22 boys and 10 girls) of normal height and weight-for-height. Clinical data and results of anthropometric measures are reported in Table 1.

Study Protocol

At 9 AM after an overnight fast, an indwelling venous catheter was inserted into an antecubital vein and maintained patent by a slow saline infusion. After a basal sample was obtained for assay of GH, IGF-I, IGF-II, IGFBP-1, IGFBP-2, IGFBP-3, C-peptide, and insulin, a solution of 10% arginine HCl (0.5 g/kg body weight) was infused over 30 minutes; after a further 90 minutes, an oral dose of L-dopa (250 mg for weight < 35 kg; 500 mg for weight > 35 kg) was administered. Arginine, which has an inhibiting effect on endogenous somatostatin,[13] was infused because of its potentiating effect on the GH response to L-dopa.[14] Blood samples for GH assay were taken every 30 minutes from the beginning of the test until 120 minutes after the L-dopa dose (total time, 240 minutes). Serum samples were stored at −20°C and assayed within 6 months.

Methods

Serum GH levels were measured using a time-resolved immunofluorimetric assay ([IFMA] Delfia; LKB-Wallac, Turku, Finland) based on the direct sandwiching technique in which two monoclonal antibodies are directed against two separate antigenic determinants on the hGH

Table 1. Clinical Data for the Obese Children and Controls

Parameter	Obese	Controls
Sex ratio (boys/girls)	13/8	22/10
Age (yr)	8.1 ± 1.3	7.9 ± 1.5
Height SDS	0.93 ± 1.35	0.42 ± 0.87
Height velocity SDS	1.75 ± 1.24*	0.37 ± 0.93
BMI SDS	7.06 ± 2.28*	−0.08 ± 1.09
Body fat (%)	37.58 ± 6.92*	19.55 ± 6.66
FM (kg)	17.15 ± 5.63*	5.46 ± 2.66
FFM (kg)	28.11 ± 6.14*	22.04 ± 5.26

Abbreviations: FM, fat mass; FFM, fat-free mass.
*$P < .001$ *v* controls.

molecule. The IFMA is highly specific (detection limit, 0.03 mIU/L) for the 22-kd form of hGH and has low cross-reactivity with other GH molecular variants or pituitary hormones. The intraassay and interassay coefficient of variation (CV) was 2.1% to 5.0% and 4.2% to 6.3%, respectively.

Serum GH values were also measured by the Nb2 cell bioassay performed according to the method of Tanaka et al[15] as modified by Walker et al,[16] with other minor changes as previously described.[17] The rat node lymphoma (Nb2) cell line (generously supplied by Dr Birgit Gellersen, Hamburg, Germany) was routinely cultured in 25-mL tissue culture flasks in Fisher's medium supplemented with 10% horse serum, 10% fetal calf serum (FCS), L-glutamine (2 mmol/L), gentamicin (50 μg/mL), and 2-mercaptoethanol (0.1 mmol/L) at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. Under these conditions, the cell population doubled in approximately 21 hours. Stationary cultures of Nb2 cells were obtained by transferring the cells from a medium supplemented with FCS to Fisher's FCS-free medium supplemented with 10% horse serum to reduce the rate of cell replication. After 24 hours, the cells were resuspended in microtiter plates at a density of 1×10^5 mL in 200 μL medium and incubated for 48 hours with increasing concentrations (0.05% to 1%) of patient sera and FCS, respectively. The cells were pulsed with 0.5 mCi/well ^{3}H-thymidine for 21 hours to determine the rate of DNA synthesis and then harvested using an automatic counter. A polyclonal antibody against human prolactin (hPRL; Hycor Biomedical, Portland, ME) was routinely added to each serum sample (final dilution, 1:4,000) to block the mitogenic effect of PRL. Each serum sample was assayed at least in duplicate. To calculate mitogenic activity, we used only the part of the growth response curve obtained from the sample dilutions that was parallel to the growth curve produced by standard lactogen dilutions. The mitogenic response of each serum sample was calculated by comparison to the mitogenic response of FCS (100% stimulation) arbitrarily considered to be 1 U/mL, using a slope ratio assay.[18] The intraassay and interassay CV was 6% and 7.6%, respectively, based on measurements of cell division in the presence of FCS.

For IGF assay, to avoid interference from binding proteins, samples were treated with acid ethanol. The IGF-I level was measured by double-antibody radioimmunoassay using immunochemicals and tracer provided by Medgenix (Fleurus, Belgium). The sensitivity of the assay was 150 pg/mL, and the intraassay and interassay CV was 6% and 7.5%, respectively. IGF-II levels were measured by double-antibody radioimmunoassay using a monoclonal antibody provided by Sera-Lab (Techno-genetics, Trezzano, Italy) and ^{125}I-IGF-II provided by Amersham (Aylesbury, Buckinghamshire, UK). The standard curve procedure was performed using recombinant IGF-II. The sensitivity of the assay was 90 pg/mL, and the intraassay and interassay CV was 6% and 9%, respectively. No cross-reactivity was found between IGF-I and IGF-II with the respective antibodies used in the assay to a maximum concentration of 500 ng/mL of both peptides.

The IGFBP-1 level was measured by immunoradiometric assay using reagents and tracers provided by Diagnostic Systems Laboratories (Webster, TX). The sensitivity of the assay was 125 pg/mL, and the intraassay and interassay CV was 2.5% and 4.6%, respectively.

IGFBP-2 levels were determined by double-antibody radioimmunoassay using a nonequilibrium technique as described by Clemmons et al.[19] Specific IGFBP-2 antiserum was purchased from Upstate Biotechnology (Lake Placid, NY), and the standard was a pure IGFBP-2 preparation obtained by DNA recombinant technology (ImmunoKontact, Frankfurt, Germany). Radioiodination of rIGFBP-2 was achieved by reacting 5 μg protein with 1 mCi ^{125}I-Na and 10 μg chloramine T in a final volume of 100 μL 0.5-mol/L sodium phosphate, pH 7.4. After terminating the reaction by addition of 50 μg sodium metabisulfite, unreacted iodide was removed by passage through a 0.7 × 50-cm column of Sephadex G-100 in 0.01 mol/L phosphate buffer plus 0.25% bovine serum albumin, pH 7.5. The specific activity was approximately 107 μCi/μg protein.

The IGFBP-3 level was measured by immunoassay using reagents and tracers provided by Bioclone Australia (Narrickville, New South Wales, Australia). The sensitivity of the assay was 3.5 ng/mL, and the intraassay and interassay CV was 4.25% and 6.6%, respectively.

The IGF/IGFBP molar ratio was calculated after converting the obtained values to nanomolars. The following coefficients were used for conversion from IGF-I, 0.131; IGF-II, 0.134; IGFBP-1, 0.04; IGFBP-2, 0.032; and IGFBP-3, 0.024.

Insulin was assessed by a radioimmunoassay (Liso-phase; Techno Genetics, Milan, Italy) with a sensitivity of 2 μU/mL = mU/L and an intraassay and interassay CV of 1.8% and 6.1%, respectively. C-peptide was assessed by a chemiluminescent enzyme immunoassay (Immulite; Diagnostic Products, Los Angeles, CA), which has a sensitivity of 0.3 ng/mL = μg/L and an intraassay and interassay CV of 8.2% and 5.9%, respectively.

Statistical Analysis

Statistical analysis was performed by a Mann-Whitney nonparametric test. *P* values less than .05 were considered significant. Results are expressed as the mean ± SD. The statistical computer program Statgraphics Plus Version 1 for Windows (Manugistics, Rockville, MD) was used.

RESULTS

GH peaks during pharmacologic stimulation were significantly lower in obese children compared with controls when measured either by IFMA (4.84 ± 3.54 *v* 23.7 ± 2.04 μg/L, $P < .001$) or by Nb2 assay (0.60 ± 0.45 *v* 1.84 ± 0.15 U/mL, $P < .001$; Fig 1).

IGF-I was similar in obese and control children (173.8 ± 57.2

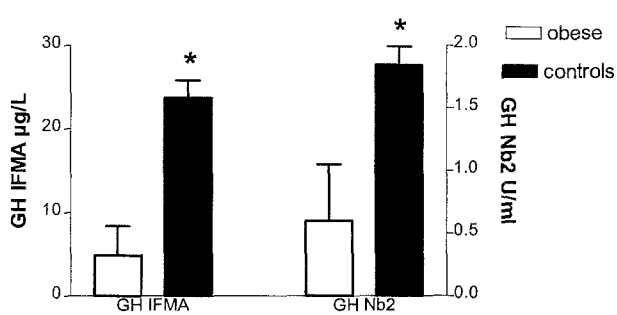

Fig 1. GH peak levels measured by IFMA and Nb2 cell assay in obese children and controls. *$P < .001$ *v* controls.

v 188.6 ± 132.6 ng/mL, nonsignificant), whereas IGF-II was significantly higher in obese children (596.1 ± 139.7 *v* 439.3 ± 127.4 ng/mL, $P < .001$; Fig 2).

Insulin (10.72 ± 4.40 *v* 2.02 ± 0.94 mU/L, $P < .001$) and C-peptide (1.94 ± 0.61 *v* 0.75 ± 0.48 μg/L, $P < .001$) were both higher in obese subjects versus controls.

IGFBP-1 (23.25 ± 14.25 *v* 107 ± 165.7 ng/mL, $P < .05$) and IGFBP-2 (44.37 ± 62.18 *v* 385.93 ± 227.81 ng/mL, $P < .001$) were significantly lower in obese subjects than in controls. IGFBP-3, on the contrary, was higher in obese subjects (3.31 ± 0.82 *v* 2.6 ± 0.94 μg/mL, $P < .05$).

The IGF/IGFBP molar ratio (Fig 2) was significantly higher in obese children than in controls (1.32 ± 0.32 *v* 1.07 ± 0.34, $P < .05$).

DISCUSSION

Our study clearly demonstrates that GH secretion is indeed suppressed and not artifactually low in obese children, since they show immunoreactive and bioactive GH levels similar to those observed in children affected by total GH deficiency.

However, despite hyposomatotropism, growth was maintained or even enhanced in these obese children, suggesting that growth factors were normally present. Indeed, we found similar IGF-I levels but higher IGF-II levels in obese children compared with controls. It is noteworthy that increased IGF-II levels have been found in newborn babies who are small for gestational age and show catch-up growth[20] and in some prepubertal children in whom overgrowth was associated with overexpression of this growth factor.[21] Nutritional factors are known to influence IGF peptide synthesis,[22] and normal or increased IGF-I levels are reported in overweight subjects,[5] declining after weight loss.[2] The liver GH receptors could be the potential link; in fact, it has been shown in rats that the number of hepatic GH receptors is regulated by nutrition[23] and the GH receptor concentration is correlated with IGF-I plasma levels.[23,24] Moreover, in humans, GH binding protein is also correlated with the BMI and with IGF-I.[8] Insulin, which modulates the rat liver GH receptor[25] and stimulates the accumulation of IGF-I RNA in the primary culture of hepatocytes, even in the absence of GH,[26,27] could play a major role in maintaining normal IGF levels in obese children. Moreover, we found clear qualitative changes in the IGFBP profile in obese children: IGFBP-1 and particularly IGFBP-2 were significantly lower in obese children compared with controls, whereas IGFBP-3 was slightly higher. Low IGFBP-1 levels have already been reported in obesity, presumably secondary to the high insulin levels present in this condition.[28] We believe the low IGFBP-2 could also be a consequence of the high insulin values present in these obese children, because insulin has been shown to be a negative regulator of hepatic IGFBP-2 levels in rats[29] and to negatively correlate with IGFBP-2 serum levels.[30] As extreme fasting increases IGFBP-2,[31] chronic overnutrition in our patients may have been responsible for the decreased IGFBP-2 levels, despite a low GH level, which is known to inhibit this IGFBP.[31] IGFBP-3, on the contrary, was higher in obese children, as already shown in adults,[32] and could be due to a direct effect of insulin and/or IGFs, which have been shown to increase IGFBP-3 in humans.[33]

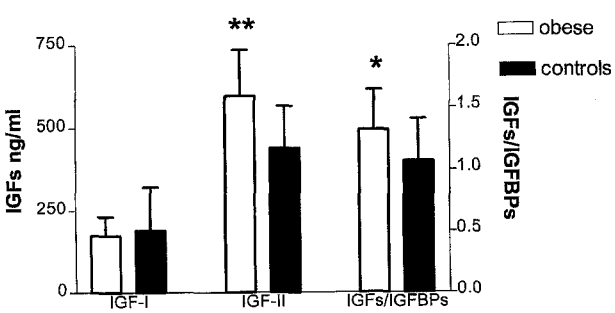

Fig 2. IGF-I, IGF-II, and IGF/IGFBP molar ratio in obese children and controls. IGFs, IGF-I + IGF-II; IGFBPs, IGFBP-1 + IGFBP-2 + IGFBP-3. *$P < .05$ and **$P < .001$ *v* controls. The following coefficients were used for conversion from ng/mL to nmol/L: IGF-I, 0.131; IGF-II, 0.134; IGFBP-1, 0.04; IGFBP-2, 0.032; and IGFBP-3, 0.024.

Considering the total amount of IGFs (I + II) versus their binding proteins (IGFBP-1 + IGFBP-2 + IGFBP-3), we observe that obese subjects show a significantly higher IGF/IGFBP molar ratio than controls, suggesting that in the former group there is an increase of the free form of IGF available for receptor interaction at the target tissue level.[32] To date, the IGF/IGFBP molar ratio appears to be the more accurate indicator of circulating IGFs delivered from the ternary and binary complexes. Indeed, the absolute concentration of free IGF measured by the assays currently available is inaccurate, because no method has satisfactorily eliminated the problem of the loss of equilibrium that occurs when the samples are diluted, so that the percentage of IGF bound to carrier proteins changes as a function of dilution of the sample.[34] The higher IGF/IGFBP molar ratio in obese children could therefore explain the sustained growth despite profound GH deficiency and also, at least in part, the suppression of GH secretion, which is known to be exerted by IGF peptides at the pituitary and hypothalamic level.

Moreover, high IGFBP-1 and IGFBP-2 values are reported in conditions of stunted growth such as intrauterine growth retardation,[35,36] in which the high serum levels are caused by accumulation of IGFBP-1 in blood due to reduced tissue transfer. Therefore, it is possible that low IGFBP-1 and IGFBP-2 in obese children may also be due to accelerated tissue transfer. Insulin may play a role because, in addition to directly inhibiting liver IGFBP-1 production,[37] it is also a potent enhancer of transcapillary transfer of IGFBP-1.[38]

In conclusion, we have found that in prepubertal obese children, both immunoreactive and bioactive GH concentrations are low. Therefore, in these subjects, normal growth seems to be sustained by nutritional factors and insulin, which also act in modulating several components of the IGF-IGFBP system.

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