Nutrition Related Hormonal Changes in Obese Children

TORU YASUNAGA***, SUSUMU FURUKAWA*, NORIYUKI KATSUMATA**,
REIKO HORIKAWA**, TOSHIAKI TANAKA**, AYAKO TANAE*** AND ITSURO HIBI***

*Department of Pediatrics, Yamaguchi University School of Medicine, Ube 755,

** Endocrine Research Laboratory, National Children's Medical Research Center, Tokyo 154,

***Division of Endocrinology and Metabolism, National Children's Hospital, Tokyo 154, Japan

Abstract. Children with simple obesity (SO) show increased linear growth with normal or high serum insulin-like growth factor-I (IGF-I) levels during prepubertal period, despite low GH secretion. We measured IGF-I, IGFBP-1, GHBP and other factors to clarify the hormonal relation between the nutrition and the linear growth in SO and compared these factors with children with normal short stature (NS). Subjects were 23 SO and 19 NS children, and their height standard deviation (SD) scores were 0.7 ± 0.2 SD and -3.4 ± 0.3 SD (mean $\pm$ SEM) ($P < 0.01$), respectively. Oral glucose tolerance test (OGTT) was performed in all the subjects and GH-releasing factor (GRF) test was also performed in 13 of SO and 17 of NS. The peak levels of GH in the GRF test were significantly lower in SO than in NS (12.8 ± 1.7 vs. 39.8 ± 6.9 ng/ml) and showed a significantly positive correlation with Σ IGFBP-1 ($r = 0.63$, $P < 0.01$). Serum GHBP level and IGF-I level were significantly higher in SO than in NS on pubertal stage matching. There was a positive correlation between GHBP and Σ insulin during OGTT ($r = 0.75$, $P < 0.01$). When the sum of the values during OGTT was expressed as Σ , Σ insulin, Σ C-peptide and Σ glucose were significantly higher in SO than in NS on pubertal stage matching. Basal and Σ IGFBP-1 were significantly lower in SO than in NS, but IGFBP-3 levels showed no significant difference between the two groups either in prepuberty or midpuberty. In conclusion, it can be hypothesized that the overnutrition causes hyperinsulinemia which increases GH receptor and IGF-I secretion despite low GH secretion. Hyperinsulinemia also may increase free IGF-I by lowering IGFBP-1. These two mechanism are supposed to be the nutrition related hormonal changes in SO and can explain the growth of SO. In addition, the increased free IGF-I may contribute the decreased GH secretion due to negative feedback in SO.

Key words: Obesity, GH, Insulin, Insulin-like growth factor-binding proteins, Short stature

(Endocrine Journal 45: 221–227, 1998)

IT HAS been reported that children with simple obesity (SO) show increased linear growth with normal or high serum insulin-like growth factor-I (IGF-I) levels during prepubertal period, despite low GH secretion [1, 2]. On the other hand, insulin secretion is increased in these children [3]. The hormonal mechanism of linear growth in obese children is not well understood. The importance of insulin on fetal growth is suggested in the small

fetus with pancreatic agenesis [4] and the large fetus with persistent hyperinsulinemic hypoglycemia [5], but the role of insulin in postnatal growth still remains unclear. It is speculated that insulin produces IGF-I directly or indirectly and contributes to the promotion of linear growth [6].

To date, six IGF-binding proteins (IGFBPs) have been found. IGFBPs are believed to modify IGF-I and -II action [7]. IGF-I and IGFBP-3 are known to be GH-dependent [8, 9] and also to be nutrition-dependent, since IGF-I and IGFBP-3 are increased by GH injection and decreased with dietary restriction in man [10, 11]. IGFBP-1 is one of the IGFBPs which is mainly synthesized in the liver

Received: February 25, 1997

Accepted: January 14, 1998

Correspondence to: Dr. Toru YASUNAGA, Department of Pediatrics, Yamaguchi University School of Medicine, 1144 Oaza-kogushi, Ube, Yamaguchi 755, Japan

and is decreased by insulin [12]. Since IGFBP-1 can cross the capillary endothelium and compete with IGF-I receptor, it is likely that IGFBP-1 contributes to the delivery of IGF-I to tissue and acts in an inhibitory manner [7]. We measured IGF-I, IGFBP-1, GHBP and other factors to clarify the hormonal relation between the nutrition and the linear growth in SO and compared these factors with children with normal short stature (NS).

Subjects and Methods

Subjects

The subjects were twenty-three SO (one young woman included) aged 5–20 years (11.5 ± 0.6 , mean $\pm$ SEM, male/female: 16/7) without endocrinological abnormalities and nineteen NS aged 5–14 years (9.5 ± 0.6 , mean $\pm$ SEM, male/female: 11/8), who had no endocrinological abnormalities, bone malformation nor malformation syndromes. Height standard deviation (SD) scores calculated by the Japanese standard [13] were 0.7 ± 0.2 SD (mean $\pm$ SEM) in SO and -3.4 ± 0.3 SD in NS. Simple obesity is defined as more than 20% overweight. Ideal weight was calculated by Murata's method [14]. The mean overweight percentages were 64.1% in SO, and 0.9% in NS. The pubertal stage was graded according to Tanner's pubertal staging.

The number of subjects in each Tanner's pubertal stage was I: 9, II-III: 9, IV-V: 5 in SO and I: 13, II-III: 6 in NS. All subjects had normal liver function, and there were no significant differences between the two groups in HbA1c levels assayed by standard high-performance liquid chromatography ($5.6 \pm 0.1\%$ in SO and $5.4 \pm 0.1\%$ in NS).

Methods

Oral glucose tolerance test (OGTT, 1.75 g/kg glucose, p.o. maximum 75 g) was performed after overnight fasting. Blood samples were drawn at 0, 30, 60, 90, 120 and 180 min. In thirteen of the SO and seventeen of the NS subjects, the growth hormone-releasing factor (GRF) test (somatostatin acetate, Sumitomo, Japan, 1 μ g/kg, iv. max. 100 μ g) was performed after overnight fasting and the samples were taken at 0, 15, 30, 60, 90 and 120 min. The sum of the values during the test was

expressed as Σ .

Hormone measurements

Insulin (Eiken, Japan) and C-peptide (Daiichi Radioisotope, Japan) were measured by radioimmunoassay (RIA). Serum IGFBP-1 (Medix Biochemica, Finland) was measured by immunoenzymometric assay as described previously [15]. These were measured on the samples in the course of OGTT. The intra- and interassay coefficients of variations of each assay were 4.6–8.2%, 3.0–5.5% and 2.7–6.2%, respectively. The lower limits of sensitivity were 1.25 μ U/ml, 0.05 ng/ml and 0.4 μ g/l.

Serum IGFBP-3 (Eiken, Japan) was measured by immunoradiometric assay (IRMA), IGF-I (Fujisawa, Japan) by immunoenzymometric assay after acid-ethanol extraction, and serum growth hormone-binding protein (GHBP) by ligand-mediated immunofunctional assay as described previously [16] in the basal sample. The intra- and interassay coefficients of variations of each assay were 3.1–6.2%, 3.0–2.7%, 6.9–9.7%. The lower limits of sensitivity were 1.0 μ g/ml, 11.7 ng/ml and 0.38 ng/ml. IGFBP-3, IGF-I and GHBP were measured in serum samples drawn after overnight fasting.

Serum GH was measured by RIA (Eiken, Japan), and all samples were measured in the same assay during the course of the GRF test. The intra- and interassay coefficients of variations of the assay were 4.3–6.3% and the lower limit of sensitivity was 1 ng/ml.

Statistical analysis

Mann-Whitney U test was used to analyze the difference between the two groups; Wilcoxon signed-rank test was used to analyze paired data. Pearson's correlation coefficients were used for simple linear and logarithmic correlations. The results are expressed as the mean $\pm$ SEM. $P < 0.05$ is considered to be statistically significant.

Results

Figure 1 shows GRF tests in SO and NS. The mean peak GH level for SO was 12.8 ± 1.7 ng/ml, and that for NS was 39.8 ± 6.9 ng/ml ($P < 0.01$), and showed a significantly positive correlation with Σ

IGFBP-I ($r=0.63$, $P<0.01$).

Figure 2 shows serum glucose, insulin, C-peptide levels during OGTT. The mean glucose levels were significantly higher at 60, 90, 120 and 180 min in SO than those in NS ($P<0.01$). The mean insulin and C-peptide levels in SO were significantly higher during OGTT than those in NS ($P<0.01$). On the other hand, the mean IGFBP-1 level was significantly lower in SO than that in NS at any time ($P<0.01$), although IGFBP-1 levels in both groups decreased gradually during OGTT. Σ glucose, Σ insulin and Σ C-peptide showed a significant negative correlation with Σ IGFBP-1 ($r=-0.57$, $r=-0.76$ and $r=-0.80$, respectively; $P<0.01$).

Table 1 shows the mean $\pm$ SEM data for both groups classified by Tanner stage. Σ glucose, Σ insulin and Σ C-peptide during OGTT were also significantly higher in SO than in NS on pubertal stage matching. IGF-I and Σ IGFBP-1 levels showed

Fig. 1. GH levels on GRF provocative test performed in 13 SO (●) and 17 NS (○). Peak GH levels in SO were significantly lower than in NS (*: $P<0.01$). The bars show the mean $\pm$ SEM.

Fig. 2. Glucose, insulin, C-peptide and IGFBP-1 concentrations during OGTT in SO (●) and NS (○). The bars show the mean $\pm$ SEM. *: $P<0.01$ and †: $P<0.05$ show significant difference at time matching.

Table 1. Clinical characteristics and laboratory findings in SO and NS classified by Tanner stage

	Tanner I			Tanner II-III			Tanner IV-V
	SO	NS		SO	NS		SO
Height SDS	1.19 ± 0.36	-3.71 ± 0.31	<i>P</i> <0.01	0.73 ± 0.15	-2.79 ± 0.37	<i>P</i> <0.01	-0.05 ± 0.49
% overweight	61.53 ± 6.16	1.15 ± 2.76	<i>P</i> <0.01	68.27 ± 8.25	0.18 ± 3.83	<i>P</i> <0.01	61.40 ± 7.83
Σinsulin (μIU/ml)	286.78 ± 44.80	119.96 ± 11.39	<i>P</i> <0.01	509.10 ± 68.81 [†]	180.35 ± 27.21 [†]	<i>P</i> <0.01	419.38 ± 41.31
ΣC-peptide (ng/ml)	45.78 ± 3.83	25.32 ± 1.80	<i>P</i> <0.01	64.51 ± 5.93 [†]	33.85 ± 4.45 [†]	<i>P</i> <0.01	67.82 ± 5.59*
Σglucose (mg/dl)	742.78 ± 25.90	691.62 ± 21.11	<i>P</i> <0.01	816.44 ± 27.33	720.83 ± 44.89	<i>P</i> <0.01	915.60 ± 47.01*
IGF-I (ng/ml)	109.06 ± 13.82	52.58 ± 9.03	<i>P</i> <0.01	182.51 ± 19.33*	99.91 ± 11.72*	<i>P</i> <0.01	307.15 ± 39.92*
ΣIGFBP-1 (μg/l)	15.39 ± 2.79	87.82 ± 9.37	<i>P</i> <0.01	7.53 ± 1.98 [†]	34.01 ± 4.63*	<i>P</i> <0.01	3.74 ± 1.53 [†]
IGFBP-3 (μg/ml)	2.77 ± 0.13	2.37 ± 0.18	n.s	3.27 ± 0.12 [†]	2.70 ± 0.28	n.s	4.32 ± 0.23*
GHP (ng/ml)	57.08 ± 9.01	14.19 ± 2.30	<i>P</i> <0.01	79.16 ± 14.37	14.20 ± 4.10	<i>P</i> <0.01	83.74 ± 14.28

*: *P*<0.01 and †: *P*<0.05 show significant difference from Tanner I.

a significant difference between SO and NS on pubertal stage matching (*P*<0.01), but IGFBP-3 levels showed no significant difference between them. There was a positive correlation between IGF-I and Σinsulin (*r*=0.67, *P*<0.01).

Serum GHBP levels in SO distributed above the 50th percentile for normal children, whereas most of those in NS were distributed in the lower normal range (Fig. 3). GHBP levels showed a significant difference between SO and NS on pubertal stage matching (*P*<0.01) (Table 1). There were positive correlations between GHBP and % overweight (*r*=0.73, *P*<0.01) and between GHBP and Σinsulin (*r*=0.75, *P*<0.01) (Fig. 4).

Figure 5 shows basal IGFBP-1 levels in SO and NS against the normal reference range. IGFBP-1 levels in NS were within the normal range, but almost all of those in SO were lower than the normal range.

Discussion

It is suggested that the growth of obese children is not GH dependent but nutrition dependent, since GH secretion by GRF is significantly smaller in SO than in NS as shown in this study and other studies [2, 17]. However, IGF-I level is significantly greater in obese children than in normal short children despite lower GH secretion. Therefore, it is demonstrated that IGF-I in obese children is not directly produced by GH. The mechanism to increase IGF-I level other than GH is assumed to be nutrition. However, the relationship between the nutrition and the hormonal regulation is not clear. GHBP is known to be influenced by the

Fig. 3. The distribution of GHBP levels of SO (●) and NS (○). The lines give the 5th, 50th and 95th percentiles of normal children from the top.

nutritional state [16]. GHBP levels are high in obese children [16] and low in undernourished patients [10]. Overnutrition in obese children causes hyperinsulinemia. Increased GHBP level in obese children is speculated to be caused by hyperinsulinism, since there was a strong positive correlation between GHBP and integrated insulin levels during OGTT. GHBP, which has the same structure as the extracellular domain of the GH receptor, is assumed to reflect the tissue GH-receptor concentration as a part of the GH axis [18, 19]. Increased level of GHBP in SO suggests increased GH sensitivity which relates to increased IGF-I synthesis. Relatively low IGF-I level in NS

Fig. 4. The correlation between Σ insulin and GHBP.

Fig. 5. Serum IGFBP-1 levels of SO (●) and NS (○) before glucose intake. The line gives the -2 SD, mean and $+2$ SD of normal children from the top, respectively.

can be explained by the relatively low level of GHBP, despite normal GH secretion to GRF, since many non-GHD short children have been shown to have empirically poor appetites. One of the growth mechanisms in SO is hypothesized as a nutrition-insulin-GHBP-IGF-I system (Fig. 6). A significant positive correlation between Σ insulin and IGF-I supports this hypothesis.

Another mechanism is assumed to be the system

Fig. 6. Hormonal mechanism of growth in obese children.

via IGFBP-1. Since IGFBP-1 shows diurnal variation and maximum level in the morning after overnight fasting [15], we have measured IGFBP-1 at OGTT performed after overnight fasting. There was a significant difference in basal IGFBP-1 level between SO and NS, which was caused by the difference in insulin secretion. Lee *et al.* reported that IGFBP-1 levels continued to increase under the pancreatic clamp despite glucose infusion and proved that IGFBP-1 is mainly regulated by insulin, not by glucose [20]. IGFBP-1 gradually decreased with time according to insulin secretion in both SO and NS. Low IGFBP-1 level in SO may activate IGF action by increasing free IGF-I levels, since there was no difference in IGFBP-3 between SO and NS. IGFBP-1 is believed to be an inhibitor of IGF-I. It is reported that IGFBP-1 prevents the binding of [125 I] (Thr⁵⁹) IGF-I to membrane receptors in human endometrium and choriocarcinoma JEG-3 cells which possesses IGF-I receptor [21, 22]. Furthermore, an increase in IGFBP-1 has been shown to inhibit the hypoglycemic actions of IGF-I in the fasting state [23]. It is conceivable that increased free IGF-I caused by lower IGFBP-1 potentiate the linear growth in SO. Increased free IGF-I may serve as an inhibitor of GH secretion in the GRF test in SO, since there was a significant positive correlation between Σ IGFBP-1 and peak GH levels on GRF test. Therefore, a nutrition-insulin-IGFBP-1-free IGF-I system is hypothesized

as another growth mechanism in SO (Fig. 6).

IGFBP-3 is almost unaffected by the over-nutritional state, since our data showed no difference between SO and NS in IGFBP-3. In the Zucker rat, plasma IGFBP-3 levels were similar in obese and lean animals [24]. In human prepubertal simple obese children, there is a tendency to find higher IGFBP-3 levels, but no significant difference is found [25]. It has no apparent diurnal variation [26]. It can be concluded that IGFBP-3 has no role in the nutrition related growth.

In conclusion, it can be hypothesized that the overnutrition causes hyperinsulinemia which increases GH receptor and IGF-I secretion despite

low GH secretion. Hyperinsulinemia also may increase free IGF-I by lowering IGFBP-1. These two mechanism are supposed to be the nutrition related hormonal changes in SO and can explain the growth of SO. In addition, the increased free IGF-I may contribute the decreased GH secretion due to negative feedback in SO.

Acknowledgements

Special thanks go to Dr. A. D. Fair for proof-reading this English paper.

References

1. Vinolo M, Naseli A, Di Battista E, Mostert M, Aicardi G (1988) Growth and development in simple obesity. *Eur J Pediatr* 147: 242–244.
2. Loche S, Cappa M, Borrelli P, Faedda A, Crino A, Cella SG, Corda R, Muller EE, Pintor C (1987) Reduced growth hormone response to growth hormone releasing-hormone in children with simple obesity: Evidence for somatomedin C mediated inhibition. *Clin Endocrinol* 27: 145–153.
3. Deschamps I, Giron BJ, Lestrade H (1977) Blood glucose, insulin, and free fatty acid levels during oral glucose tolerance tests in 158 obese children. *Diabetes* 26(2): 89–93.
4. Lemons JA, Ridenour R, Orsini EN (1979) Congenital absence of the pancreas and intrauterine growth retardation. *Pediatrics* 64: 255–257.
5. Soliman AT, Alsalmi I, Darwish A, Asfour MG (1996) Growth and endocrine function after near total pancreatectomy for hyperinsulinaemic hypoglycemia. *Arch Dis in Child* 74: 379–385.
6. Houston B, O'Neil IE (1991) Insulin and growth hormone act synergistically to stimulate insulin-like growth factor-I production by cultured chicken hepatocytes. *J Endocrinol* 128: 389–393.
7. Jones JL, Clemmons DR (1995) Insulin-like growth factors and their binding proteins: Biological actions. *Endocr Rev* 16: 3–34.
8. Blum WF, Albertsson-Wikland K, Rosberg S, Ranke MB (1993) Serum levels of insulin-like growth factor I (IGF-I) and IGF binding protein 3 reflect spontaneous growth hormone secretion. *J Clin Endocrinol Metab* 76: 1610–1616.
9. Blum WF, Ranke MB (1990) Use of insulin-like growth factor-binding protein 3 for the evaluation of growth disorders. *Horm Res* 33 (Suppl 4): 31–37.
10. Counts DR, Gwirtsman H, Carlsson LM, Lesem M, Cutler GB Jr (1992) The effect of anorexia nervosa and refeeding on growth hormone-binding protein, the insulin-like growth factors (IGFs), and the IGF-binding proteins. *J Clin Endocrinol Metab* 75: 762–767.
11. Young SC, Underwood LE, Celniker A, Clemmons DR (1992) Effects of recombinant insulin-like growth factor-I (IGF-I) and growth hormone on serum IGF-binding proteins in calorically restricted adults. *J Clin Endocrinol Metab* 75: 603–608.
12. Snyder DK, Clemmons DR (1990) Insulin-dependent regulation of insulin-like growth factor binding protein-1. *J Clin Endocrinol Metab* 71: 1632–1636.
13. Suwa S, Tachibana K, Maesaka H, Tanaka T, Yokoya S (1992) Longitudinal standards for height and height velocity for Japanese children from birth to maturity. *Clin Pediatr Endocrinol* 1: 5–13.
14. Murata M (1987) Underweight and obesity (definition and classification). *Pediatr Medicine* 20: 666.
15. Yasunaga T, Tanaka T, Katsumata N, Tanaka A, Hibi I (1996) Diurnal variation of serum IGFBP-1 in three boys and fasting IGFBP-1 levels in normal children. *Clin Pediatr Endocrinol* 5: 99–102.
16. Horikawa R, Tanaka T, Kokai Y, Katsumata N, Nagashima A, Satoh M, Tanaka A, Hibi I (1994) Growth hormone-binding protein measurements in normal children and children with short stature using ligand-mediated immunofunctional assay. *Clin Pediatr Endocrinol* 3: 35–43.
17. Van Vliet G, Bosson D, Rummens E, Robyn C, Wolter R (1986) Evidence against growth hormone-releasing factor deficiency in children with idiopathic obesity. *Acta Endocrinol Suppl* 279: 403–410.
18. Leung DW, Spencer SA, Cachianes G, Hammonds RG, Collins C, Henzel WJ, Barnard R, Waters MJ,

- Wood WI (1987) Growth hormone receptor and serum binding protein: Purification, cloning and expression. *Nature* 330: 537–543.
19. Barnerd R, Waters MJ (1986) Serum and liver cytosolic growth hormone-binding proteins are antigenically identical with liver membrane 'receptor' types 1 and 2. *Biochem J* 237: 885–892.
20. Lee PDK, Jensen MD, Divertie GD, Heiling VJ, Katz HH, Conover CA (1993) Insulin-like growth factor-binding protein-1 response to insulin during suppression of endogenous insulin secretion. *Metabolism* 42: 409–414.
21. Rutanen EM, Pekonen F, Makinen T (1988) Soluble 34k binding protein inhibits the binding of insulin-like growth factor to its own cell receptors in human secretory phase endometrium: Evidence for autocrine/paracrine regulation of growth factor action. *J Clin Endocrinol Metab* 66: 173–180.
22. Ritvos O, Ranta T, Jalkanen J, Suikkari AM, Voutilainen R, Bohn H, Rutanen EM (1988) Insulin-like growth factor (IGF) binding protein from human decidua inhibits the binding and biochemical action of IGF-I in cultured choriocarcinoma cells. *Endocrinology* 122: 2150–2157.
23. Levitt MS, Denyer GS, Cooney GJ, Baxter RC (1991) Insulin-like growth factor-binding protein-1 modulates blood glucose levels. *Endocrinology* 129: 2254–2256.
24. Nguyen-Yamamoto L, Deal CL, Finkelstein JA, Van-Vliet G (1994) Hormonal control of growth in the genetically obese Zucker rat. I. Linear growth, plasma insulin-like growth factor-I (IGF-I) and IGF-binding proteins. *Endocrinology* 134: 1382–1388.
25. Prader A, Rappaport R (1994) Clinical Issues in Growth Disorders: Evaluation, Diagnosis and Therapy. London: Freund Publishing House Ltd. 105–118.
26. Jürgen JOL, Blum WF, Müller N, Ranke MB, Christiansen JS (1990) Circadian patterns of serum insulin-like growth factor (IGF)-I and IGF binding protein 3 in growth hormone deficient patients and age- and sex-matched normal subjects. *Acta Endocrinol (Copenh)* 123: 257–262.