Impaired Somatomedin Generation Test in Children with Insulin-dependent Diabetes Mellitus

ROBERTO LANES, BRIDGET RECKER, PAVEL FORT, AND FIMA LIFSHITZ

SUMMARY

Recent studies have suggested a partial block in somatomedin (SM) production or growth hormone (GH) action in IDDM. Twelve well-nourished diabetic children (9 males and 3 females with a mean age of 11.2 ± 3.3 yr), six with an HbA_{1c} of 7.9–11.2% (group A) and six with an HbA_{1c} of 12.5–15.6% (group B), were studied as follows: the GH response after 100 μg of oral clonidine and the SM generation capacity after i.m. administration of 0.2 U/kg/dose of human growth hormone (hGH) for 4 days. Group B diabetic subjects had a significantly higher mean ± SD GH increase after clonidine than did group A patients (Δ of 17.4 ± 4.9 versus 5.7 ± 6.0 ng/ml, $P < 0.01$); the basal GH of both groups were similar (1.6 ± 0.7 versus 2.3 ± 1.4 ng/ml). In contrast, the SM response to hGH was significantly decreased in group B children as compared with those in group A (Δ of 0.3 ± 0.3 versus 1.2 ± 0.4 U/ml, $P < 0.01$). The basal SM levels of both groups were normal for age. GH and SM correlated with HbA_{1c} levels ($r = +0.80$, $P < 0.01$; $r = -0.79$, $P < 0.01$, respectively); there was no correlation with plasma and urine glucose or serum cholesterol, cortisol, and transferrin. Our data indicate a blunted SM response to hGH in group B diabetic subjects; this defect in SM generation is apparently not present in group A subjects. A biologically inactive GH molecule and poor nutrition seem unlikely, but circulating inhibitory factors not picked up by our radioimmunoassay, the degree of diabetes control, or a still unclear metabolic derangement may be contributing to this defect. DIABETES 1985; 34:156–60.

Growth failure is a recognized complication of insulin-dependent diabetes mellitus (IDDM), particularly when metabolic control is inadequate,[1-3] but the mechanisms for growth retardation are not well understood. Both elevated[4] and normal[5] baseline growth hormone (GH) levels, and a hypersecretion of GH to different stimuli and during 24-h monitoring have been reported[6] in diabetic children. Baseline levels of somatomedin (SM) have been low,[7] normal,[8] or elevated.[9] To explain the discrepancy between GH and SM in IDDM, Nash[7] suggested that hypersecretion of GH in IDDM may compensate for a partial block in SM production.

To better understand the mechanisms controlling these hormonal derangements, we have administered human growth hormone (hGH) to 12 IDDM children in different degrees of diabetes control and evaluated their SM-generating capacity. This test has not to our knowledge been used before in diabetic subjects, except in one case reported by Winter et al.,[1] who gave 2 U of hGH for 5 days to a child with Mauriac syndrome, failing to alter his somatomedin activity. Diabetic subjects with the higher HbA_{1c} levels in our study had a blunted SM response in comparison with those with lower HbA_{1c} concentrations. This difference does not seem to be related to poor nutrition or biologic activity of GH.

MATERIALS AND METHODS

Patient population. The study group consisted of 12 IDDM children (9 males and 3 females) with a mean chronologic age of 11.2 ± 3.3 yr (ranging from 5.9 to 14.6 yr) (Table 1) who were diagnosed and followed thereafter at the pediatric diabetes center at North Shore University Hospital. All subjects were prepubertal or in very early puberty (Tanner 2, Table 1), and were growing above the fifth percentile in height and weight, maintaining a mean growth velocity of 6 ± 1.4 cm yearly, with an appropriate body weight for height. None had a medical problem other than diabetes, as determined by regular visits to our center every 3 mo, and by complete laboratory studies performed every 6 mo and just before the beginning of our study to rule out diabetic

This study was presented in part at the Annual Meeting of the Society for Pediatric Research, San Francisco, California, May 1984.

From the Department of Pediatrics, North Shore University Hospital, Manhasset, New York; and the Department of Pediatrics, Cornell University Medical College, New York, New York.

Address reprint requests to Fima Lifshitz, M.D., Department of Pediatrics, North Shore University Hospital, 300 Community Drive, Manhasset, New York 11030.

Received for publication 19 January 1984 and in revised form 23 July 1984.

TABLE 1
Individual data of all patients

Patient no.	Age (yr)	Sex	Puberty (Tanner)	HbA_{1c} (%)	Clonidine stimulation GH (ng/ml)					Somatomedin generation SM (U/ml)			
					Basal	60 min	75 min	90 min	Δ	Basal*	Day 2	Day 4	Δ
Group A													
1	12	F	I	7.9	1.5	5.5	6.8	5.1	5.3	0.37	0.67	1.6	1.23
2	14	M	II	10	1.5	6.3	—	10.1	8.6	2.4	3.5	4.2	1.8
3	12.8	M	II	8.1	3.5	1.0	1.0	1.0	−2.5	0.3	0.78	1.9	1.6
4	11.2	M	I	10.6	4.6	1.5	8.4	16.2	11.6	0.7	0.9	1.8	1.1
5	11	F	I	11.2	1.5	1.8	1.5	1.5	0	0.72	1.9	1.6	1.18
6	12.5	M	II	11	1.2	3.5	9.7	12.8	11.6	0.64	0.81	1.1	0.46
Mean ± SD	12.5 ± 1.1			9.9 ± 1.5	2.3 ± 1.4	3.2 ± 2.2	5.4 ± 4.0	7.7 ± 6.2	5.7 ± 6.0	0.8 ± 0.7	1.4 ± 1.2	2.0 ± 1.1	1.2 ± 0.4
Group B													
7	14.5	M	II	13	1.2	1.2	1.6	10.8	9.6	0.95	1.3	1.3	0.35
8	13.9	M	II	15.6	2.9	15	19.2	9.4	16.3	0.76	0.9	1.0	0.24
9	7.9	M	I	14	2.1	22	23.3	23.6	21.5	0.63	1.2	0.87	0.57
10	5.9	F	I	15.8	1.4	6.4	21.2	22.7	21.3	0.43	0.7	0.73	0.3
11	12.3	M	II	13.5	1.6	—	6.4	23.3	21.7	3.0	2.4	2.8	−0.2
12	6.8	M	I	12.5	0.8	15.1	13.8	8.8	14.3	0.17	0.87	0.72	0.7
Mean ± SD	10.2 ± 3.8			14.0 ± 1.3	1.6 ± 0.7	11.9 ± 8.1	14.2 ± 8.6	16.4 ± 7.4	17.4 ± 4.9	0.9 ± 1.0	1.2 ± 0.6	1.2 ± 0.7	0.3 ± 0.3

*Normal ranges for somatomedin-C from Nichols Institute (U/ml): 6–11 yr (males, 0.22–2.8; females, 0.41–4.5); 11–13 yr (males, 0.28–3.7; females, 0.99–6.8); and 13–15 yr (males, 0.9–5.6; females, 1.2–5.9).

complications or other acute or chronic disease. Patients were on no medications other than insulin.

The children were divided into two groups based on their glycosylated hemoglobin (HbA_{1c}) levels being above or below 11.5%. Six children (patients 1–6) had a mean HbA_{1c} of 9.9 ± 1.5% (ranging from 7.9 to 11.2%) (group A), while six children (patients 7–12) had a mean HbA_{1c} of 14 ± 1.3% (ranging from 12.5 to 15.6%) (group B) (Table 1). Group A subjects (4 males and 2 females), with a mean chronologic age of 12.5 ± 1.1 yr had been diagnosed as having IDDM for 6 mo to 8 yr (mean 3.3 yr) before the study and were receiving a mean of 0.68 U of insulin/kg in 2 injections/day. Group B patients (5 males and 1 female), with a mean chronologic age of 10.2 ± 3.8 yr, were known to be diabetic for 5 mo to 8 yr (mean 3.7 yr) before this study, and were receiving a mean insulin dose of 0.98 U/kg in two daily injections. All patients had been on the above-mentioned dose of insulin for a minimum period of 6 mo and had shown only minimal fluctuation in their HbA_{1c} levels during this period (±10% of reported value) before this study. No apparent major differences in the degree of physical activity, dietary intake, and emotional stability (based on school performance, peer interrelationships, and family interactions) were determined during routine clinic interviews with an endocrinologist, nutritionist, and psychiatrist. Both groups had similar baseline levels of urinary glucose (20.7 ± 25.3 and 29.6 ± 42 g/24 h for groups A and B, respectively), fasting plasma glucose (181 ± 88.2 and 264 ± 82.1 mg/dl for groups A and B, respectively), cholesterol, triglyceride, cortisol, and transferrin (248.4 ± 50 and 273 ± 27 mg/dl for groups A and B, respectively).

Study protocol. At 8 a.m. and before their injection of insulin, fasting blood for HbA_{1c}, glucose, cholesterol, triglycerides, transferrin, cortisol, GH, and SM were obtained. Glucose was also measured in a 24-h urine sample collected the previous day.

All patients then received a single dose of 100 μg of oral clonidine and blood samples for the determination of growth hormone were drawn in a recumbent position at 0, 60, 75, and 90 min, while cortisol levels were measured at 0 and 90 min. Blood pressure was measured every half-hour. After completion of this test, all children returned to their usual

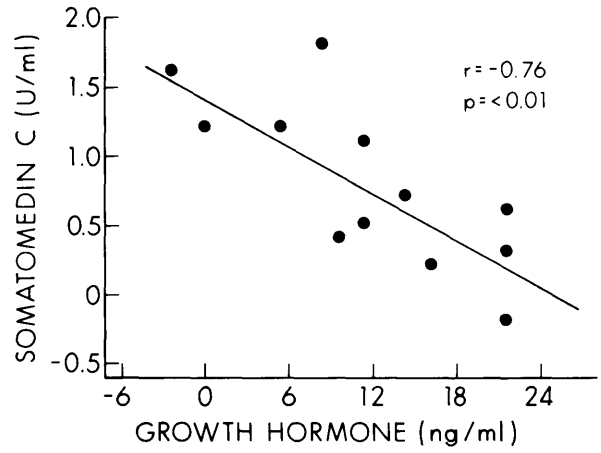

FIGURE 1. Relationship of Δ plasma somatomedin to Δ serum GH concentration in study patients.

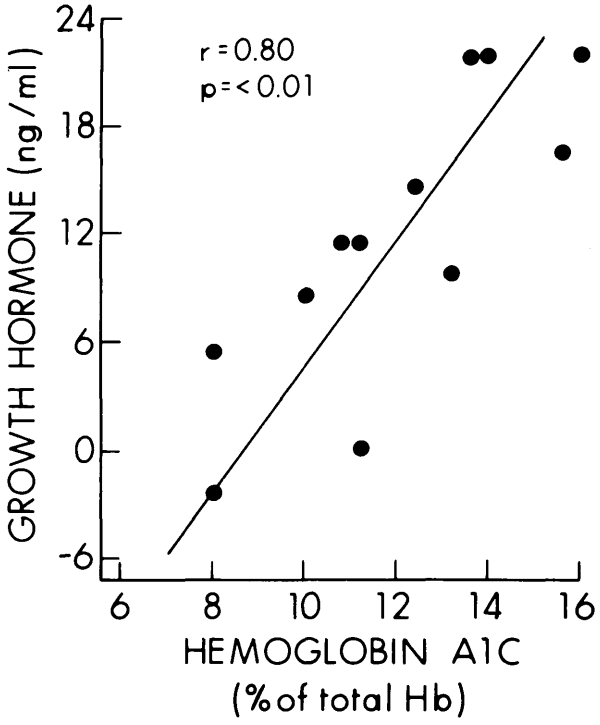

FIGURE 2. Relationship of HbA_{1c} levels to Δ serum GH concentrations in study patients.

insulin and dietary regimens as well as to their regular daily activities.

Beginning that same day, a somatomedin generation test was performed as follows: an intramuscular injection of 0.2 U of hGH/kg body wt was administered at home every evening for four consecutive days to each child, and fasting venous blood samples for measurements of SM were obtained 12 h after the second and fourth injections of hGH. To evaluate diabetes control during hGH administration, fasting blood glucose was measured on days 2 and 4 of this somatomedin generation test and urines were tested at home for glucose and ketones 4 times a day during this period. No changes in insulin dose were required, as these parameters remained similar to baseline.

The studies were performed with the approval of the North Shore University Hospital committee on research and publications and with informed parental consent.

Determinations and calculations. Plasma glucose was measured by the glucose-oxidase method using a Beckman Glucose Analyzer (Beckman Instruments, Fullerton, California).

Total glycosylated hemoglobins were measured using the Corning Electrophoresis Glytrac Glycosylated Hemoglobin Set by Corning Medical, Palo Alto, California.[11] Specific radioimmunoassay techniques were used for the determination of serum GH and cortisol using chemically prepared RIA kits (Quantitope ^{125}I-HGH by Kallestad Laboratories, Austin, Texas,[12] and ^{125}I-cortisol by Aria II System, Becton Dickinson Immunodiagnostics, Salt Lake City, Utah[13]). Intra- and interassay coefficients of variation were 5% and 4.2% and 5% and 7.7%, respectively, for growth hormone and cortisol. All samples were assayed in duplicate.

Blood for SM was collected in EDTA tubes and the plasma frozen and shipped to Nichols Institute in Los Angeles for somatomedin radioimmunoassay.[14] This is a direct RIA procedure in which the EDTA plasma is diluted and incubated with a high-affinity antibody. Intra- and interassay coefficients of variation for this assay were 6% and 9%, respectively. Glycosuria and ketonuria were determined using the Clinitest and Acetest tablets, Ames Laboratories (Elkhart, Indiana). Human growth hormone for the somatomedin generation test was supplied by the National Hormone and Pituitary program.

The groups of patients were compared using the Mann-Whitney rank test. In each group, linear regression analysis was used to correlate the clinical and laboratory data. Results are expressed as means ± SD. Basal SM levels were compared with the normal values for children given by Nichols Institute taking into account chronologic age and sex (Table 1).

RESULTS

RESPONSE TO STIMULATION TESTS

Clonidine. Oral clonidine induced a significantly higher GH response in group B than in group A subjects (Table 1). The mean Δ ± SD GH increase after oral clonidine was 17.4 ± 4.9 ng/ml in group B, which was significantly different from a Δ of 5.7 ± 6.0 ng/ml in group A children ($P < 0.01$). Two children in group A were unable to increase their GH concentrations above baseline after clonidine ingestion.

Baseline GH levels of both groups were similar and not elevated. The mean ± SD baseline GH value was 1.6 ± 0.7 ng/ml in group B, which was not significantly different from 2.3 ± 1.4 ng/ml in group A.

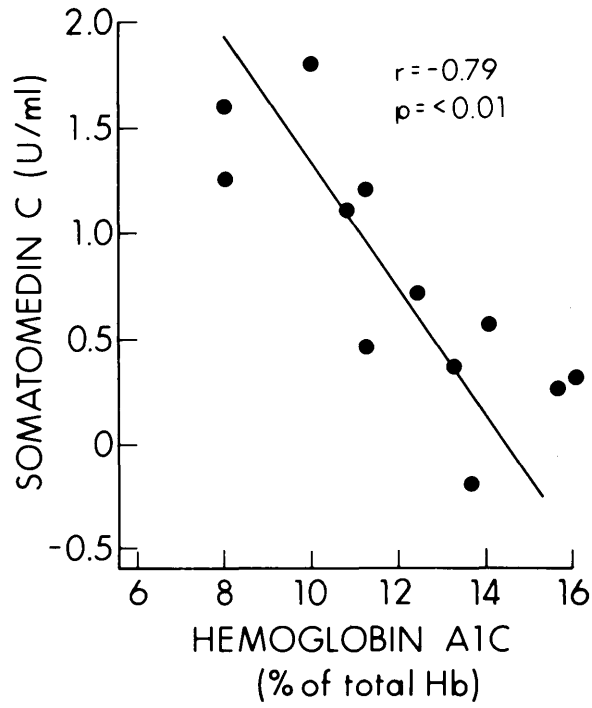

FIGURE 3. Relationship of HbA_{1c} levels to Δ plasma somatomedin concentration in study patients.

A significant, although similar, decrease in cortisol levels was noted in both groups of patients after clonidine administration ($P < 0.01$); baseline cortisol levels were normal in both groups of diabetic children. A slight, but neither clinically nor statistically significant, drop in blood pressure was noted with clonidine ingestion in both groups of patients.

Somatomedin generation. Group B diabetic subjects had a blunted SM response to exogenous hGH as compared with group A subjects (Table 1). The mean $\Delta \pm$ SD somatomedin increase after exogenous hGH was 0.3 ± 0.3 U/ml in group B, which was significantly less than the Δ increase of 1.2 ± 0.4 U/ml noted in group A ($P < 0.01$).

Mean basal SM concentrations of both groups were in the low range of normal when compared with the mean nonstimulated SMC levels for age and sex given as a reference by Nichols Institute (Table 1). The mean $\pm$ SD baseline SM level of group B subjects was 0.9 ± 1 U/ml, which was not different from 0.8 ± 0.7 U/ml noted in group A (Table 1).

CORRELATION OF SERUM GH AND PLASMA SM WITH EACH OTHER AND WITH DIFFERENT PARAMETERS OF DIABETES CONTROL

When we compared the change in GH after clonidine with the change in SM levels after hGH administration, they were inversely correlated ($r = -0.76$; $P < 0.01$) (Figure 1). The Δ changes in GH and SM concentrations after stimulation correlated with metabolic control of the patients. Linear regression analysis of our data revealed a direct correlation between the GH change after oral clonidine and the HgA_{1c} concentrations of our patients ($r = 0.8$; $P < 0.01$) (Figure 2). On the other hand, an inverse correlation between the SM change after exogenous hGH and HbA_{1c} concentrations ($r = -0.79$; $P < 0.01$) was noted (Figure 3). A direct correlation between ΔGH increase and triglyceride levels was also noted ($r = 0.77$, $P < 0.05$), but this was not evident for ΔSM.

Neither GH nor SM change was related to age, height, height velocity in the preceding year, pubertal status, or dose of insulin of our patients.

DISCUSSION

Hypersecretion of GH and a blunted SM increase after stimulation was noted in the diabetic subjects with the higher HbA_{1c} levels in this study. This response was very different from that seen in the group of patients with lower HbA_{1c} concentrations, in spite of similar baseline GH and SM levels in both groups of patients. This difference seems not to be due to inadequate nutrition or to a biologically inactive growth hormone molecule in the poorly controlled subjects.

The normal baseline GH and SM levels of our patients may be a reflection of their appropriate nutrition. Patients with protein-calorie malnutrition have been reported to have elevated growth hormone and low SM levels; refeeding increases SM and lowers GH concentrations.[15,16] The discrepancy in baseline GH and SM concentrations noted between several previous studies in IDDM children and ours may be due to differences in the nutritional status of patients studied. Most other reports do not provide enough data to assess nutritional status, such as transferrin levels, which are believed to be one of the most sensitive indicators of the nutritional state available.[15]

Our group B patients had a blunted SM response to exogenous hGH when compared with the response noted in group A diabetic children. This blunted response tends to rule out a biologically inactive growth hormone molecule in these IDDM patients. In subjects who have been suspected of having a biologically inactive GH molecule, low baseline SM levels rise significantly after exogenous hGH administration.[17,18] Low plasma SM levels in IDDM could be due to impaired liver responsiveness secondary to a GH receptor defect. However, Maes et al.[19] have recently shown how, in streptozocin-induced diabetic rats, SM levels remain low after insulin therapy despite the restoration of the normal number of GH binding sites. Group A diabetic subjects more than doubled their basal SM levels after 4 days of hGH administration. This response is similar to that recently reported by Van Vliet et al.[20] and by Gertner et al.[21] in normal short children of similar ages who received hGH for 4 consecutive days, but clearly different from the response noted in our group B diabetic subjects who did not increase their SM concentrations above baseline.

Underinsulinization of group B subjects may have contributed to their poor somatomedin response. Insulin has been shown to enhance somatomedin production in the isolated, perfused liver preparation,[22] and increased insulin secretion in children with growth hormone deficiency after pituitary surgery may explain their normal somatomedin levels.[23] Although group B children were receiving a larger dose of insulin than group A, we were unable to find a correlation between changes in GH and SM after stimulation and the dose of insulin administered. Whether intensive insulin therapy will normalize the hormonal response to stimulation of our patients as was recently shown by several groups,[8,24] with baseline somatomedins after the use of the insulin pump, remains to be seen.

The hypersecretion of growth hormone in group B children after stimulation with the new growth hormone-releasing agent, clonidine, is in agreement with previous studies reporting elevated GH levels after stimulation with various other stimuli. However, contrary to the reports of others,[4] all of our patients had normal basal GH concentrations. The theory of hypersecretion of GH compensating for a partial block in somatomedin production seems to be strengthened by the inverse correlation between the change in GH and SM after acute stimulation noted by us.

The relationship between diabetes control and the regulation of growth hormones is not well understood, and the correlation between somatomedin activity and diabetes control has varied among different studies.[8] Several previous studies have found a significant inverse correlation between baseline SM levels and the degree of diabetes control, as assessed by glycosylated hemoglobins.[25,26] This correlation seems to hold true even when SM secretion is maximally stimulated by hGH. The lack of correlation with plasma and urine glucose may be a reflection of the continuous variability of glycemia in these patients. The controversy in relation to the role of diabetes control as a determinant of growth and as a regulator of growth hormones remains unresolved. This regulation may be dependent on multiple factors, but the degree of diabetes control clearly seems to be an important one. Further studies on the effect of circulating growth-inhibiting factors, which have been reported to be detected

by bioassay but not by radioimmunoassay,[27] of receptor and postreceptor defects, and of the intracellular mechanisms involved in SM synthesis are needed.

ACKNOWLEDGMENTS

Human growth hormone was supplied by the National Hormone and Pituitary Program, University of Maryland School of Medicine, National Institute of Arthritis, Metabolism, and Digestive Diseases.

REFERENCES

[1] Guest, C. M.: The Mauriac syndrome: dwarfism, hepatomegaly, and obesity with juvenile diabetes mellitus. Diabetes 1953; 2:415–17.

[2] Birbeck, J. A.: Growth in juvenile diabetes mellitus. Diabetologia 1972; 8:221–24.

[3] Tattersall, R. B., and Pyke, D. A.: Growth in diabetic children: studies in identical twins. Lancet 1973; 2:1105–1109.

[4] Hansen, A. P.: Abnormal serum growth hormone response to exercise in juvenile diabetics. J. Clin. Invest. 1970; 49:1467–78.

[5] Molnar, G. C., Taylor, W. F., Langworthy, A., and Fatrurechi, V.: Diurnal growth hormone and glucose abnormalities in unstable diabetics. J. Clin. Endocrinol. Metab. 1972; 34:837–46.

[6] Hayford, J. T., Danney, M. M., Hendrix, J. A., and Thompson, R. G.: Integrated concentration of growth hormone in juvenile onset diabetes. Diabetes 1980; 29:391–98.

[7] Nash, H.: Growth failure, somatomedin and growth hormone levels in juvenile diabetes mellitus: a pilot study. Aust. NZ J. Med. 1979; 9:245–49.

[8] Tamborlane, W. V., Hintz, R. L., Bergman, M., Genel, M., Felig, P., and Sherwin, R. S.: Insulin-infusion pump treatment of diabetes: influence of improved metabolic control on plasma somatomedin levels. N. Engl. J. Med. 1981; 305:303–307.

[9] Cohen, M. P., Jasti, K., and Rye, D. L.: Somatomedin in insulin-dependent diabetes mellitus. J. Clin. Endocrinol. Metab. 1977; 45:236–39.

[10] Winter, R. J., Phillips, L. S., Green, O. C., and Traisman, H. S.: Somatomedin activity in the Mauriac syndrome. J. Pediatr. 1980; 97:598–600.

[11] Nathan, D. M., Avezzano, E., and Palmer, J. L.: Rapid method for eliminating labile glycosylated hemoglobin from the assay for hemoglobin A1. Clin. Chem. 1982; 28:512–15.

[12] Schalch, D. S., and Parker, M. L.: A sensitive double antibody immunoassay for human growth hormone in plasma. Nature 1964; 203:1141.

[13] Vecseis, P.: Radioimmunological determination of plasma cortisol. Experientia 1972; 8:1104.

[14] Furlanetto, R. W., Underwood, J. E., Van Wyk, J. J., and D'Ercole, A. J.: Estimation of somatomedin-C levels in normals and patients with pituitary disease by radioimmunoassay. J. Clin. Invest. 1977; 60:648–57.

[15] Pimstone, B. L., Becker, D. J., and Hansen, J. D. L.: Human growth hormone and sulphation factor in protein-calorie malnutrition. *In* Endocrine Aspects of Malnutrition. Gardner, L. J., and Amacher, P., Eds. Santa Ynez, Kroc Foundation, 1973:73–90.

[16] Brasel, J. A.: Endocrine adaptation to malnutrition. Pediatr. Res. 1980; 14:1299–1303.

[17] Kowarski, A. A., Schneider, J., Ben-Galim, E., Weldon, V. V., and Daughaday, W. H.: Growth failure with normal serum RIA-GH and low somatomedin activity: somatomedin restoration and growth acceleration after exogenous GH. J. Clin. Endocrinol. Metab. 1978; 47:461–64.

[18] Plotnick, L. P., Van Meter, Q. L., and Kowarski, A. A.: Human growth hormone treatment of children with growth failure and normal growth hormone level by immunoassay: lack of correlation with somatomedin generation. Pediatrics 1983; 71:324–27.

[19] Maes, M., Ketelslegers, J. M., and Underwood, L.: Low plasma somatomedin-C in streptozotocin-induced diabetes mellitus: correlation with changes in somatogenic and lactogenic liver binding sites. Diabetes 1983; 32:1060–68.

[20] Van Vliet, G., Styne, D. M., Kaplan, S. L., and Grumbach, M. M.: Growth hormone treatment for short stature. N. Engl. J. Med. 1983; 309:1016–22.

[21] Gertner, J. M., Genel, M., Gianfredi, S. P., Hintz, R. L., Rosenfeld, R. G., Tamborlane, W. V., and Wilson, D. M.: Prospective clinical trial of human growth hormone in short children without growth hormone deficiency. J. Pediatr. 1984; 104:172–76.

[22] Daughaday, W. H., Phillips, L. S., and Mueller, M. D.: The effects of insulin and growth hormone on the release of somatomedin by the isolated rat liver. Endocrinology 1976; 98:1214–19.

[23] Costin, G., Kogut, M., Phillips, L. S., and Daughaday, W. H.: Craniopharyngioma: the role of insulin in promoting postoperative growth. J. Clin. Endocrinol. Metab. 1976; 42:370–79.

[24] Rudolf, M. C., Sherwin, R. S., Markowitz, R., Bates, S. E., Genel, M., Hochstadt, J., and Tamborlane, W. V.: Effect of intensive insulin treatment on linear growth in the young diabetic patient. J. Pediatr. 1982; 101:333–39.

[25] Winter, R. J., Phillips, L. S., Klein, M. N., Traisman, H. S., and Green, O. C.: Somatomedin activity and diabetic control in children with insulin-dependent diabetes. Diabetes 1979; 28:952–54.

[26] Blethen, S., Sargeant, D. T., Whitlow, M. E., and Santiago, J. V.: Effect of pubertal stage and recent blood glucose control on plasma somatomedin in children with insulin-dependent diabetes mellitus. Diabetes 1981; 30:868–72.

[27] Phillips, L. S., Vassilopoulou-Sellin, R., and Reichard, L. A.: Nutrition and somatomedin. VIII. The somatomedin inhibitor in diabetic rat serum is a general inhibitor of growing cartilage. Diabetes 1979; 28:919–24.