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CHANGES IN SERUM INSULIN, BLOOD SUGAR, AND FREE FATTY ACID LEVELS FOUR HOURS AFTER ADMINISTRATION OF HUMAN GROWTH HORMONE TO FASTING CHILDREN WITH SHORT STATURE¶

Previous studies on normal and hypopituitary adults have demonstrated that administration of human growth hormone (HGH) is associated with changes in blood sugar (BS),[1,2] free fatty acids (FFA),[3,4] insulin like activity (ILA),[5] and plasma immunoreactive insulin (IRI).[6] The following investigation was carried out to study the acute effects of HGH on serum IRI, BS, and plasma FFA concentrations in normal and hypopituitary children.

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MATERIALS AND METHODS

A total of 17 children were studied. The clinical characteristics are summarized in Table 1. Seven (Group I, ages 9-15 years—heights from the 25th to the 75th percentile) were normal; five (Group II, ages 7-14 years—heights from the 1st to the 3rd percentile) were "normal" but considered genetically short because of a positive family history of short stature, a normal growth rate over the previous years and a lack of demonstrable endocrinopathies; and five (Group III, ages 9-11 years—heights significantly below the 1st percentile) had slow growth rates and deficiencies of one or more anterior pituitary hormones. Each subject had an evaluation of his CNS-pituitary function that included a protein-bound iodine, cholesterol, thyroidal I^{131}

TABLE 1. CLINICAL CHARACTERISTICS OF NORMAL AND SHORT STATURED SUBJECTS

Characteristics	*Groups*		
	I	*II*	*III*
	Normal	*"Genetic" short stature*	*Idiopathic hypopit.*
Number—total (male-female)	7 (5-2)	5 (4-1)	5 (4-1)
Age (yr.-mo.) mean	12-4	9-8	9-11
range	9-10 to 15-0	7-1 to 13-9	8-11 to 11-1
Stature (percentiles)	25-90	1-3	<1
Growth rates for age	Normal	Normal	Abnormal
Endocrine abnormalities*	None	None	Thyroid-4; adrenal-3

* See MATERIALS and METHODS for an explanation of endocrine evaluation.

uptake, wrist X-rays for determination of skeletal maturation, response of urinary 17-hydroxycorticoids (17-OH) and 17 ketosteroids (17 KS) to ACTH and metyrapone, water load test, measure of response to insulin-induced hypoglycemia and urinary gonadotropins. Those children whose heights were not below the 1st percentile showed no evidence of endocrine dysfunction. In contrast, all five patients with heights below the 1st percentile had one or more abnormalities: three had PBI concentrations < 3.5 μg%, four had 24 hour RAI uptakes of 15% or less, two had skeletal maturations significantly more delayed than linear growth, three had abnormal response to metyrapone, two had a poor urinary 17 OH steroid response to ACTH, and two had an abnormal water load test corrected by cortisol. A slow linear growth rate was present in all five. Four showed an abnormal response of either BS or FFA to insulin-induced hypoglycemia. None showed any evidence of sexual maturation but all were less than 11 1/12 years of age. All five had normal skull films.

Responsiveness to HGH was tested as follows: normally active and well subjects eating a normal diet with a 9:00 p.m. snack for 3 days were studied on the 4th day, at which time blood samples were obtained at 5:00 and 9:00 a.m. (i.e., after 8 and 12 hours of fasting) for serum IRI, BS, and FFA. Four to seven days later, the

same procedure was repeated, altered only by an IM injection of 2 mg. HGH immediately after the 8 hour fasting blood sample had been obtained.

Blood sugar was determined using the Somogyi-Nelson method[7] and FFA measured by the method of Dole.[8] Serum insulin concentration was measured using a double antibody radio-immunoassay.[9] Statistical analysis was carried out using the "Student's T" test.

RESULTS

Eight and 12 hour fasting IRI and BS concentrations were not significantly different in any of the groups so the data are combined for comparing

TABLE 2. EFFECT OF FASTING AND HGH ON SERUM IRI AND BS IN NORMAL AND SHORT STATURED CHILDREN

		Fasting (8° + 12°)		*Fasting* (12°) + *HGH**
*Groups***	*No. determinations*	*Mean ± S.E.M. (range)*	*No. determinations*	*Mean ± S.E.M. (range)*
A. IRI-microunits/ml.				
I	21	9.2±0.9 (2.5-15.0)	7	10.5±1.6 (2.5-14.0)
II	14	5.9±0.8 (3.0-12.0)	4	6.4±0.9 (5.0-9.0)
III	15	2.6±0.8 (0 -11.5)	5	2.1±0.8 (0 -4.5)
B. FBS-mg/100 ml.				
I	21	81±1.3 (73-97)	7	76±1.8 (73-86)
II	13	78±1.5 (71-88)	5	82±2.8 (74-88)
III	15	70±1.2 (63-79)	5	75±2.4 (65-80)

*2 mg. given IM immediately after obtaining 8 hr. fasting sample.
** Group I—Normal
Group II—"Genetic" short stature
Group III—Idiopathic hypopituitarism.

differences between groups and within groups before and after HGH (Table 2). Mean fasting IRI concentrations before and after HGH administration in the children with pituitary deficiencies (Group III) were significantly lower ($P < 0.01$) than those of the normal children (Group I) or children with genetic short stature (Group II). The mean fasting IRI concentration of Group II was significantly less ($P < 0.02$) than that of Group I without HGH. The same type of difference in serum IRI concentrations between Group I and II is apparent after HGH although the number of subjects and the variability of the IRI values were such that the differences observed were not significant. A single intramuscular

injection of 2 mg. of HGH appears to have no effect on serum IRI concentrations obtained four hours later in any of the groups.

The blood sugar concentrations of both Group I and Group II prior to HGH were significantly greater than the mean BS concentration of Group III ($P < 0.01$) but not significantly different from each other (Table 2). Following the single IM injection of HGH there were no significant differences among the three groups, since the mean BS concentration of the children in Group III had increased. The number of BS determinations on children in Group III after HGH, however, were not sufficient to establish a significant effect of HGH on BS concentrations in this group

TABLE 3. EFFECT OF FASTING AND HGH ON PLASMA FFA IN NORMAL AND SHORT STATURED CHILDREN

*Group**	*No. Ind.†*	*HGH Amt.***	*FFA—microeq./l—mean ± SE (range)* 8 hr. fast	12 hr. fast	12 hr.-8 hr. (Δ)
I	7	0	467±124 (266-1177)	502± 52 (301-650)	31±109 (—541-350)
	7	2 mg.	355± 43 (241-586)	780± 30 (447-1176)	424± 99 (158-882)
	14	0	411± 65 (266-1177)		
II	5	0	676± 74 (287-1137)	1074±205 (686-1811)	397±189 (—31-1097)
	5	2 mg.	444± 70 (241-661)	1170± 35 (866-1416)	725± 80 (474-892)
	10	0	560± 86 (241-1137)		
III	4	0	554± 81 (366-714)	1001±138 (610-1327)	398±144 (204-664)
	5	2 mg.	741± 93 (510-1058)	1555± 23 (1347-1784)	804±133 (289-1026)
	9	0	658± 65 (366-1058)		

* Group I—Normal
Group II—"Genetic" short stature
Group III—Idiopathic hypopituitarism.
** 2 mg. given IM immediately after obtaining 8 hr. fasting sample.
† No. Ind. = Number of Individuals.

The mean 8 hour fasting FFA concentration of the children in Group III (658 ± 65 μEq/L) was significantly greater ($P < 0.02$) than that of the normal children (411 ± 65 μEq/L) although the range of values overlaps in all groups (Table 3). Again, the children with genetic short stature have a mean 8 hour fasting value intermediated between, but not significantly different from Groups I and III (560 ± 86 μEq/L). The mean 12 hour fasting FFA concentrations of both the genetic and pituitary deficient short stature groups, however, are significantly different ($P < 0.01$) from the normal group with almost no overlap in the range of values. The large significant error of the mean (SEM) values for the 12 hour fasting concentration of FFA in both groups of short statured children is striking,

especially when compared to the values for the same group four hours after a single IM injection of 2 mg. of HGH. These findings suggest that both the genetic and pituitary-deficient short statured child are more susceptible than normal children to the stimulus of prolonged fasting, with respect to factors that mobilize FFA. All three groups of children showed a similar effect of HGH on mobilization of FFA by a greater than twofold increase with a marked decrease in standard errors of the mean. Thus the HGH administered appeared to be biologically active in all groups of subjects studied.

DISCUSSION

The present study has demonstrated significantly lower concentrations of serum IRI in fasting children with pituitary dysfunction and confirms the studies of others on hypopituitary adults.[10,11] Furthermore, these hypopituitary children show significantly lower 8 and 12 hour fasting BS concentrations and greater FFA concentrations than do normal children, including those with short stature. One of the explanations for these findings is that the hypopituitary children adapt more quickly to the fasted state than do normal children.[12] Luft, *et al.*[6] in studying three hypopituitary patients, demonstrated an apparent significant rise in FBS, serum IRI and plasma FFA afer HGH injections (10 mg. 2 times/day) for four days. In addition, long-term (5-10 days, 10 mg/d) HGH administration has been accompanied by deterioration in carbohydrate tolerance, and elevations in FBS and serum IRI. Similar observations of augmentation of growth hormone after insulin secretion in dogs have been reported.[13] However, Kipnis and Stein[11] found no significant change in either blood glucose or serum IRI during a five hour intravenous infusion of HGH to normal adults or to adults with hypopituitarism, although a significant rise of plasma FFA was seen in both groups. The responses of the children in this study given one intramuscular injection of 2 mg. of HGH were similar to those reported by the latter group.

Since changes in serum IRI concentrations have not been observed after single IM injections or five hour intravenous infusions, the effect of HGH on IRI secretion *in vivo* must not be directly on pancreatic islet cell tissue.

The children with genetic short stature who were studied come from families in which there were many other short individuals. Their heights were within two standard deviations of the average for age and they were growing at a normal rate. In addition, they had no clinical or laboratory evidence of endocrine dysfunction. For these reasons, the observed significant differences in fasting serum IRI and FFA concentrations between this

group and the children with normal stature were unexpected. These differences cannot be completely explained, since sufficient data on a normal group of children of the same age and degree of development are not available. However, previously reported studies[14] from our laboratories have shown that children from 3 to 9 years of age have fasting serum IRI concentrations significantly lower than infants up to 2 years, adolescents 10-16 years, and adults. Since two of the children in Group II were 7 years of age, it seems possible that the lowered mean IRI concentration in Group II may be attributed, in part, to the fact that the children were younger than those in Group I. The fact that the changes in IRI and FFA are in the same direction as those found in the children with hypopituitarism, although less marked, raises the possibility that the subjects included in Group II may have an alteration in HGH metabolism (synthesis, release, tissue response, etc.). The likelihood that there is a genetic determinant for this possible metabolic alteration warrants additional study.

SUMMARY

Serum immunoreactive insulin (IRI), fasting blood sugars (FBS), and free fatty acids (FFA) were obtained on two days after fast periods of 8 and 12 hours from seven normal children (Group I, ages 9-15 years—heights above the 25th percentile), five genetically short children (Group II, ages 7-14 years—heights between the 1st and 3rd percentile) and five children with idiopathic hypopituitarism (Group III, ages 9-11 years—heights significantly below the 1st percentile). On the second day, 4 to 7 days after the first study, two milligrams of human growth hormone (HGH) were administered intramuscularly after obtaining the 8 hour blood sample. Within each group mean serum IRI values after 8 and 12 hour periods of fast were not significantly different. Mean IRI values in the children in Group I were 9.2 microunits/ml., in Group II, 5.9 microunits/ml., and in Group III, 2.3 microunits/ml., the IRI concentrations in the hypopituitary children being significantly lower ($P < 0.01$) than in the other two groups. Four hours after HGH, mean IRI concentrations were not significantly increased in any of the groups. Mean FBS values at 8 hours in the hypopituitary group were lower than the other two groups but were not significantly increased in any group after HGH. Mean 8 and 12 hour fasting FFA in the hypopituitary subjects and mean 12 hour fasting FFA in the genetically short subjects were significantly greater than those in the normal subjects. All three groups showed the expected increases (two to threefold) in FFA after HGH.

These results show that children with hypopituitarism have lower fasting concentrations of IRI and blood sugar but higher and more variable

concentrations of FFA. A single IM injection of HGH, capable of increasing plasma FFA in normal as well as hypopituitary subjects, did not increase serum IRI or blood sugar concentrations significantly in any of the subjects. An unexpected finding was that mean fasting IRI levels were significantly lower in genetically short children as compared with controls.

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