Daily Production and Metabolic Clearance of Growth Hormone in Juvenile Diabetes Mellitus

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Summary. Daily production (PR) of human growth hormone (HGH) was calculated in patients with juvenile diabetes and control subjects by determining metabolic clearance rate (MCR) of ^{131}I HGH, at equilibrium, and mean endogenous HGH levels throughout a 24 h day. Half hourly sampling or a constant withdrawal pump were used to obtain an integrated mean endogenous HGH level. MCR (liters/day) was significantly reduced in all diabetic subjects both in absolute terms (96 ± 15 vs 274 ± 37) and relative to surface area (62 ± 8 vs 171 ± 21) ($p < 0.01$). Mean HGH levels were 8.4 ng/ml in the diabetics

and 5.5 ng/ml in age matched controls. Daily HGH PR in the diabetic subjects (339 to 1365 $\mu\text{g/day}$) did not exceed values in the control subjects (1005–1426 $\mu\text{g/day}$). The results indicate that the elevated plasma HGH levels and increased HGH response to stimuli observed in diabetes, reflect reduced metabolic clearance, rather than increased pituitary secretion.

Key words: Growth Hormone, juvenile diabetes, metabolic clearance, integrated mean levels, daily production.

There is now convincing evidence from a number of studies that human growth hormone (HGH) can be diabetogenic in man [1]. However, whether excessive endogenous HGH secretion occurs or plays a role in the pathogenesis and complications of juvenile diabetes remains controversial. In support of such a concept, studies in patients with insulin dependent juvenile diabetes have documented higher plasma levels of HGH during the course of a normal 24 h day [2], or in response to stimulation with arginine [3], glucagon [3] or exercise [4] when compared to normal subjects. Furthermore, elevated levels of HGH following tolbutamide administration have been reported in "pre-diabetic" subjects [5]. It has been commonly implied that these elevated plasma levels indicate that pituitary release and/or secretion of HGH is increased in the diabetic state. However, elevated hormone levels could be present as a result of increased secretion or decreased metabolic blood clearance (MCR). The present study, designed to resolve this question, provides for the first time data on the integrated production rate of growth hormone in patients with insulin dependent juvenile diabetes of recent onset. In addition, the results confirm the recently reported reduction of HGH MCR in patients with diabetes [6, 7].

Materials and Methods

A. Subjects. Seven patients with juvenile onset, insulin dependent diabetes were studied; they ranged in age from 12 to 33 years. The duration of disease was 3–5 years except for patient 1, whose disease had been present for 12 years. This patient had undergone hypophysectomy 3 years previously in an attempt to control diabetic retinopathy. The degree of glycosuria and daily insulin dosage varied markedly in these pa-

tients. However, there was no clinical or laboratory evidence of liver disease and proteinuria was present only in case 1.

The control group consisted of eight subjects, four without a family history of diabetes, and four healthy siblings of patients with juvenile onset diabetes. None of these siblings had any significant disturbance in glucose tolerance. Studies were performed in a clinical study center after an overnight fast. The subjects were at rest, fasting throughout the study of metabolic clearance, and had received Lugol's iodine prior to infusion of radioactive growth hormone. Informed consent was obtained in each case. In the diabetic subjects the morning dose of insulin was omitted and given at the conclusion of the period of study of metabolic clearance.

B. Metabolic Clearance Rate of ^{131}I HGH. The MCR of ^{131}I labelled HGH (HGH*) was determined by the constant infusion method of Tait [8] as previously applied to growth hormone by Taylor *et al.*, [7] Mac Gillivray *et al.*, [9] and Kowarski *et al.* [10].

Growth hormone (supplied by the National Pituitary Agency) was iodinated to a specific activity of 50–150 $\mu\text{Ci}/\mu\text{g}$ by the chloramine-T method of Greenwood *et al.*, [11] using a Sephadex G-50 column previously washed with 2% human serum albumin. Column eluate fractions of 0.5 ml were collected into tubes containing 0.1 ml of 2% human serum albumin; samples from the protein peak were tested for immunoreactivity by a double antibody radioimmunoassay (RIA) procedure using excess rabbit anti-HGH serum. The fraction with the greatest immunoreactivity (80% or greater) was sterilized by Millipore filtration, and cultured (aerobically and anaerobically) prior to infusion. The interval between iodination and infusion was usually 3 days and did not exceed 5 days.

Approximately 15–20 μCi of HGH* was dissolved in 50 ml of 0.9% saline. After a priming dose of 3–5 μCi , constant infusion of a further 12–15 μCi was maintained for 150 min via a scalp needle in an antecubital vein, using a Harvard constant infusion pump. Samples of blood were obtained from a scalp needle (maintained patent with saline) in a contralateral antecubital vein prior to, and at 30, 60, 90, 120, 130 and 140 min after commencing the infusion. At the end of this period the infusion needle was withdrawn and the infusate continued at the same rate into 3 collecting tubes for an exact period of 5 or 10 min each.

To determine a mean endogenous HGH level, blood samples were collected at 30 min intervals for 24 h in 4 diabetic and 4 control subjects. During this period subjects were allowed to walk about and diabetic subjects received their appropriate diet and in-

However, the mean of the values at 120, 130 and 140 min and the mean of the 3 infusate samples were used for calculation.

The infusate was at least 80% precipitable on the day of study. Results are expressed in liters/day and liters/meters square/day by the appropriate corrections.

D. Determination of Endogenous HGH. Endogenous HGH levels were determined by a double antibody RIA, previously described from this laboratory [12] using ^{125}I HGH as the label. Samples were assayed at two dilutions. At least 6 weeks elapsed from infusion of HGH* to determination of endogenous HGH, to allow for decay of ^{131}I .

E. Production Rate. The production rate (PR) was calculated as: $\text{PR} (\mu\text{g}/24\text{h}) = \text{MCR} \times \text{mean endogenous HGH concentration (ng/ml)}$.

Table 1. Metabolic clearance of growth hormone in normal subjects and patients with diabetes mellitus

Diabetic						Normal					
Patient	Age	Sex	Surface area in meters ²	MCR liters/day	MCR liters/m ² /day	Subject	Age	Sex	Surface area in meters ²	MCR liters/day	MCR liters/m ² /day
1	33	F	1.8	132	73	1	35	M	1.81	456	252
2	16	F	1.6	148	93	2	33	F	1.54	425	276
3	20	F	1.65	90	55	3	33	M	1.80	239	133
4	12	F	1.16	55	47	4	27	M	1.75	221	126
5	13	M	1.55	125	81	*5	17	F	1.73	248	143
6	14	M	1.3	68	52	*6	15	F	1.65	219	133
7	16	F	1.7	55	32	*7	13	M	1.42	205	144
						*8	9	M	1.12	179	160
Mean $\pm$ SEM				96 $\pm$ 15	62 $\pm$ 8					274 $\pm$ 37	171 $\pm$ 21

MCR-liters/day $p < 0.01$.

MCR-liters/m²/day $p < 0.01$.

* Sibling of a diabetic.

sulin therapy. To obtain an even more accurate integrated mean endogenous HGH level, blood was withdrawn in 2 diabetic patients at a continuous rate of 1 ml per hour by a Sigmamotor constant withdrawal pump, as recently described by Kowarske *et al.*, [10].

C. Determination of MCR ^{131}I HGH. The measurement of immunoprecipitable HGH was begun within 3 h after ending the infusion in each subject. Duplicate 0.5–1.0 ml samples of plasma and infusate were incubated with excess rabbit anti-HGH serum for 18–24 h at room temperature and precipitated with goat anti-rabbit gammaglobulin serum. Precipitable radioactivity was measured in an automatic gamma spectrometer. Background counts and counts in a plasma sample without first antibody were subtracted. MCR was calculated from the formula of Tait:

$\text{MCR (ml/min)} =$

$$\frac{\text{Rate of infused precipitable } ^{131}\text{I HGH (cpm/min)}}{\text{Plasma precipitable } ^{131}\text{I HGH (cpm/ml)}}$$

This formula requires that equilibrium be established and this was considered to be present when the plasma precipitable ^{131}I labelled HGH reached stable levels. Equilibrium was achieved by 90 min in all cases.

Results

Table 1 compares the HGH* MCR in the diabetic and normal subjects. In the diabetic subjects the mean MCR was 96 ± 15 liters/day (Mean $\pm$ SEM) compared to 274 ± 37 liters/day for normal subjects. When correction was made for surface area, the MCR in diabetics was 62 ± 8.0 l/M²/day compared to 171 ± 21 l/M²/day for the normal subjects. Comparison by Students t test indicates that these differences are statistically significant ($p < 0.01$) both for the absolute values and after correction for surface area. The reduced MCR was also apparent in the subject who had undergone hypophysectomy and whose endogenous HGH levels were undetectable, except on 2 occasions in samples collected over a 24 h period (as shown in Fig. 1). Fig. 1 also shows the wide fluctuations in growth hormone concentration in 2 of the diabetic subjects. The horizontal lines indicate the mean growth hormone levels in the 2 subjects whose blood was obtained via the constant withdrawal pump. Despite the wide fluctuations, the mean HGH levels determined by the different sampling techniques were similar in the diabetic subjects. The mean HGH level in the entire

diabetic group (8.4 ± 0.58) was significantly higher ($p < 0.01$) than the mean of the normal group (5.6 ± 0.47).

Table 2 lists the calculated production rate of HGH in 6 diabetic and 4 control subjects. No significant differences were present. In addition, among the diabetic group, none exceeded the production rates previously reported in normal subjects of comparable age [9, 10] when studied by similar techniques.

from the MCR and the endogenous concentrations. In the case of HGH this has presented some difficulty since the endogenous concentration fluctuates considerably, and particularly in diabetic subjects [2]. Indeed, in the only other report in which growth hormone production rates in patients with diabetes mellitus were estimated, the mean endogenous HGH during the equilibrium period of MCR was used [6]. This will clearly not take account of spontaneous daily

Fig. 1. Plasma growth hormone patterns in patients with juvenile diabetes. Patient 1 had undergone hypophysectomy. The horizontal lines represent the integrated mean endogenous growth hormone level determined on blood collected via a constant withdrawal pump

Table 2. Growth hormone production rates in patients with diabetes mellitus and control subjects

Diabetic				Control			
Patient	MCR liters/day	Mean HGH level ng/ml	Production rate $\mu\text{g}/24 \text{ h}$	Subject	MCR liters/day	Mean HGH level ng/ml	Production rate $\mu\text{g}/24 \text{ h}$
1	132	1.9	251	†5	248	5.75	1426
2	148	9.22	1365	†6	219	4.77	1045
4	55	6.16	339	†7	205	4.90	1005
5	125	9.27	1159	†8	179	6.79	1215
6	68	8.35	568				
7	55	9.0	495				
Mean	97	8.4*	785*		212	5.6	1173
$\pm \text{SEM}$	17	0.58	201		14	0.47	96

* Excluding patient 1 who had undergone hypophysectomy.

† Sibling of a diabetic.

Discussion

The mean MCR of HGH measured in the present normal subjects is similar to that previously reported [6, 7, 9]. The validity of the determination of metabolic clearance of endogenous HGH by means of constant infusion to equilibrium of HGH* has been confirmed by a number of investigators. These studies have shown that infused HGH* is cleared from the plasma at the same rate as infused unlabelled HGH or endogenous HGH, and that this clearance is independent of the plasma concentration of HGH [6, 7, 9, 13]. The daily production rate of a hormone can be determined

and particularly of nocturnal oscillations and, hence, may underestimate daily secretion rate. To circumvent this problem, frequent blood sampling over a 24 h period to calculate a mean endogenous HGH level has been used to determine HGH production rate [9]. A refinement to this approach has been the use of a constant withdrawal pump [10]. Excluding the patient who had undergone hypophysectomy the mean daily HGH level in our patients was 8.4 ng/ml, compared to a level of 7.3 ng/ml previously reported, in patients with diabetes, by Hansen and Johansen [2]. Moreover, these values were significantly higher than those in our normal subjects. Because of these high endogenous

HGH levels, as well as the augmented HGH responses to a variety of stimuli in diabetic and "prediabetic" subjects [3, 5], it has been implied that increased secretion of growth hormone may be a feature of the diabetic state and might play a role in the pathogenesis and/or complications of diabetes mellitus [14].

However, the present results demonstrate clearly that the daily production rate of HGH in patients with diabetes is not increased. Although the mean plasma HGH levels were elevated, the MCR was consistently reduced and the calculated production rate of HGH did not exceed values in the control group or the values of normal children and adults, as previously published [9, 10].

The mechanism for the reduced clearance of HGH in diabetic subjects is not defined, although it appears to be a consistent finding [6, 7]. In normal subjects the liver is the major site of clearance of plasma HGH [15]. Since most of our subjects had their disease for less than 5 years our results would support the concept that an early alteration of hepatic clearance of HGH is characteristic of diabetes mellitus [7]. Thus, it would seem likely that the elevated plasma levels of HGH in patients with diabetes mellitus reflect the reduced clearance, while daily production rate remains normal. Whether the "continuous" elevated plasma HGH levels contribute to the complications of diabetes cannot be ascertained from the present study.

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