Alterations in the Concentration of Cholesterol in Bile after Administration of Human Growth Hormone

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Extract

To determine the influence of human growth hormone (hGH) on gall bladder bile composition, bile (concentration and molar percentage of cholesterol, bile acids, and phospholipids) was examined in three growth hormone-deficient patients and in one control subject with constitutional short stature, but with adequate secretion of growth hormone. Investigations were made before and after 0.3, 4, and 12 months of therapy with exogenous hGH, 3 U/week. Two patients responded to hGH with accelerated growth (increase in height velocity from 1.3 and 0.2 cm/year to 10.5 and 3.0 cm/year, respectively), but, during the same time, the cholesterol concentration in bile increased progressively, raising the "lithogenic index" from 1.00 and 0.93 to 1.66 and 1.10, respectively. Normally, the "lithogenic index" is 1.00 or less. The third patient also showed some growth acceleration, but his bile did not become oversaturated with cholesterol. The bile of the control subject was excessively saturated with cholesterol after 0.3 months of treatment with hGH, raising the "lithogenic index" from 0.10 to 2.94, the highest yet reported in man. However, this abnormal response to hGH was not sustained, and bile composition returned to normal later on. The data obtained in this study suggest, but do not prove, that hGH might influence cholesterol secretion into bile.

Speculation

Normally, biliary cholesterol is kept in micellar solution by bile acids and phospholipids. An increase in the concentration of cholesterol in bile, without a concomitant increase of bile acids and/or phospholipids, causes oversaturation of bile with cholesterol, as expressed in changes of the lithogenic index. The lithogenic index is a quantitative expression of the cholesterol saturation in bile by a single figure which represents all three major biliary lipids. Oversaturation of bile with cholesterol is now recognized as a probable prerequisite for the formation of cholesterol gall stones. Further studies should now define the influence of hGH on biliary cholesterol secretion rates.

In health, plasma, hepatic, and biliary cholesterol are in rapid isotopic equilibrium and constitute the major portion of the rapidly exchanging cholesterol pool (35). Biliary cholesterol secretion is probably the result of an increased cholesterol synthesis in the liver (3, 27). Pharmacologic agents and hormones also influence hepatobiliary cholesterol dynamics. For instance, Clofibrate (23, 33), which lowers serum

cholesterol, was shown to increase the concentration of cholesterol in bile. Increases in the concentrations of biliary cholesterol were also observed after the administration of oral contraceptives (24). Various hormones, such as sex hormones, thyroxine (17, 23), and cortisone (17), influence hepatic cholesterol biosynthesis in rats.

Little information is available regarding the interrelations between growth hormone and hepatic and serum cholesterol in man. The administration of growth hormone to dogs (7) and rats (10) stimulated cholesterol synthesis in the liver, and a conspicuous rise in the concentration of biliary cholesterol was observed in the growth hormone-treated dogs. In the rat, the presence of growth hormone is prerequisite for the normal control of the serum cholesterol level (5). Untreated, growth hormone-deficient individuals tend to have hypercholesterolemia (19) and hGH (36) has been used effectively to lower serum cholesterol in hypercholesterolemic patients (6).

To obtain information about whether or not hGH might influence gall bladder bile composition, in particular with regard to the concentration of cholesterol, we have studied three growth hormone-deficient patients and one control subject before and after treatment with hGH.

PATIENTS AND METHODS

All individuals were admitted to the Clinical Research Center. Before the study of gall bladder bile composition, all subjects were tested for their ability to secrete growth hormone after insulin-induced hypoglycemia, arginine infusion, and by direct determination of serum growth hormone during nocturnal sleep. Other pituitary tropic hormones were also studied, and with the exception of the gonadotropins, no deficiency could be demonstrated.

The patients with isolated growth hormone deficiency were two Caucasian boys, aged 16 (RN) and 17 (WJ) years, and a Caucasian girl (DP), aged 15 years. The control subject, a Caucasian boy (WB) 14 years of age, had constitutional short stature, but adequate growth hormone secretion.

Gall bladder bile was obtained by duodenal intubation and siphonage after intraduodenal instillation of essential amino acids (11). The bile was kept at 37°, and aliquots were prepared for the analysis of total bile acids (16) (procedure modified by Admirand, Picott, and Small (30)), phospholipids (8), and cholesterol (31). Bile was also examined microscopically under polarized light for cholesterol crystals. All analyses were done before treatment with hGH, after 10 days (0.3 month) of 2 U/i.m./24 hr, and after 4 months of 3 U, i.m./week in all individuals. Repeat analyses of gall bladder bile

were done in all growth hormone-deficient patients after 12 months of treatment (3 U, i.m./week), and in *RN*, 6 months after the cessation of treatment. The hormone was always administered in the evening, since such timing would correspond most closely to physiologic growth hormone secretion in health.

RESULTS

Results of the determinations of biliary bile acids, phospholipids, and cholesterol were plotted on triangular coordinates (Figs. 1 and 2), where a single point represents the percentage moles of the three major organic anions in bile (1).

Biliary cholesterol saturation, as defined by the lithogenic index (20) utilizing the model previously mentioned (1), was within normal limits before administration of hGH in the three patients and in the control subject. However, the bile in *patient RN* became progressively more concentrated with cholesterol as treatment progressed (Table 1 and Fig. 1), and, after 12 months of treatment, cholesterol crystals were identified in the bile. During the time of growth hormone administration, there was also an excellent acceleration of height velocity (centimeters per year) which was sustained throughout the treatment period, as seen in Table 1. A repeat analysis of gall bladder bile 6 months after the cessation of treatment demonstrated undersaturation of bile with cholesterol, a normal lithogenic index, and no cholesterol crystals. Height velocity had dropped to near pretreatment levels.

Patient DP did not show any appreciable change in gall bladder bile composition or in height velocity during the early phase of treatment (Table 1). However, after 4 months, there was modest growth acceleration and the concentration of biliary cholesterol also had increased to some extent. Continued treatment with hGH did not result in maintenance

of height velocity, which indicated no further response to hGH. During the next 8 months, the cholesterol concentration in bile decreased, whereas that of bile acids and phospholipids had increased and bile composition remained normal.

Exogenous hGH had only a slight effect on the concentration of biliary cholesterol in *patient WJ* (Table 1). Acceleration of height velocity increased only during the first 4 months of treatment. Of interest was the marked increase in biliary cholesterol (crystals) in the control subject (*WB*, Table 1), after 10 days of growth hormone administration. Subsequently, the bile composition returned to pretreatment values. The growth spurt was probably not caused by administration of hGH, inasmuch as a similar height velocity was maintained after withdrawal of hGH, because the patient entered puberty.

Although the serum cholesterol levels were within the normal range in each individual, changes of serum cholesterol were observed during treatment with hGH (Table 1). It decreased in the two growth hormone-deficient patients, *RN* and *WJ*; it would thus appear that hGH had only an effect on the serum cholesterol, but not on biliary cholesterol in the latter. In *patient DP*, the serum cholesterol rose at the time when growth response and biliary cholesterol concentrations decreased. In the control subject *WB*, a rise in the serum cholesterol was observed when biliary cholesterol concentrations fell towards pretreatment levels.

The sera of all individuals were not tested for antibodies to exogenous hGH. It is unlikely, however, that *WJ* had developed high titer antibodies in hGH early, nullifying the effect of growth hormone. The development of antibodies to hGH in *patient DP* during the late phase of the study cannot be excluded.

DISCUSSION

The procedure utilized for the stimulation and sampling of gall bladder bile was well reproducible in each patient and

Fig. 1. Percentage of molar composition of gall bladder bile (bile acids (BA), phospholipids (PL), cholesterol (C)) represented on triangular coordinates in two growth hormone-deficient patients. *RN* sustained good growth response, *WJ*; no sustained growth response after treatment with human growth hormone (hGH). The enlarged triangle is a partial reproduction of the original triangular coordinate (2), depicted in the left upper corner.

Fig. 2. Percentage of molar composition of gall bladder bile (bile acids (BA), phospholipids (P), cholesterol (C)) represented on triangular coordinates in one growth hormone-deficient patient (DP) and in the control subject with constitutional short stature (WB). *hGH*: human growth hormone.

Table 1. Gall bladder bile composition before and after treatment with human growth hormone (*hGH*)¹

Duration of treatment with hGH	BA + PL	Concentration, mM				Lithogenic index	Serum cholesterol, mg/100 ml	Height velocity, cm/year
	C	C	PL	BA				
<i>RN</i>								
Prior	6.7	10.1	15.8	52.0	1.00	207	1.3	
0.3 month	6.0	12.9	20.2	57.0	1.12			
4 months	4.9	14.5	19.0	52.0	1.32		10.5	
12 months	3.8	21.7	25.1	58.2	1.66	157	6.3	
6 months no hGH	8.2	12.7	24.4	79.5	0.86		1.4	
<i>WJ</i>								
Prior	9.3	10.6	22.6	76.5	0.75	167	2.0	
0.3 month	9.0	11.1	27.1	72.5	0.82			
4 months	7.1	13.5	26.9	68.5	0.94	147	8.3	
12 months	7.7	12.1	23.3	69.5	0.83		0.2	
<i>DP</i>								
Prior	7.2	10.3	20.9	60.4	0.93	157	0.2	
0.3 month	7.3	12.4	28.9	62.0	0.92			
4 months	5.8	19.7	28.7	86.1	1.10		3.0	
12 months	11.0	13.3	40.1	107.0	0.63	237	0.8	
<i>WB</i>								
Prior	73.8	1.6	28.0	90.0	0.10	146	2.8	
0.3 month	1.7	11.1	3.4	15.0	2.94			
4 months	17.5	5.0	22.9	64.0	0.40	187	8.0	

¹ Data on gall bladder bile composition before and after treatment with human growth hormone. *RN*, *DP*, and *WJ* are growth hormone-deficient individuals; *WB* is the control subject (constitutional short stature with normal secretion of growth hormone). The lithogenic index (20) is defined as the ratio between the actual amount of cholesterol present in bile and the maximal amount of cholesterol which can be dissolved in any bile sample (2). This relation defines the degree to which a given bile sample is saturated with cholesterol. For instance, samples with a lithogenic index greater than 1.0 would be oversaturated; samples with a lithogenic index smaller than 1.0 would be undersaturated with cholesterol. Height velocity is defined as growth in relation to time, expressed in centimeters per year. BA: bile acids; PL: phospholipids; C: cholesterol.

instance. Ample quantities of a dark brown or dark green, that bile composition of bile-rich duodenal fluid after clear and viscid bile were obtained. It has been shown (34) stimulation of gall bladder emptying was essentially identical

with gall bladder bile; however, admixture of gall bladder bile with hepatic bile could not be excluded in our samples; hepatic bile may show an even greater degree of cholesterol saturation than gall bladder bile (30). Because initial data (2) describing the limits of cholesterol solubilization in, and "lithogenicity" of human bile are undergoing revision (4, 13), and because the major attention in this study was focused on hGH-induced alterations of cholesterol concentrations in bile, the determinations of cholesterol, bile acids, and phospholipids were done in a nonisotropic system, *i.e.*, without prior filtration or ultracentrifugation of bile to eliminate nonmicellar cholesterol.

The increase in the concentration of biliary cholesterol in our studies suggests that growth hormone has an influence on the secretion of cholesterol into bile, but the exact mechanisms are unknown. The increase in the concentration of biliary cholesterol could have been a relative one, due to a decrease in hepatic bile acid synthesis and excretion, thus causing supersaturation of bile with cholesterol. However, biliary bile acid concentration data in our patients do not support this hypothesis. On the contrary, growth hormone may stimulate hepatic bile acid synthesis; in the rat, the activity of cholesterol 7 α -hydroxylase, the rate-limiting enzyme for hepatic bile acid synthesis (28), seems to be under pituitary control (9, 18). In man, no data have been published on the effect of exogenous hGH on bile acid synthesis. However, preliminary observations (25) indicate that a modest increase in bile acid synthesis and/or pool size of chenodeoxycholic acid occurs in man after short term administration of exogenous hGH.

The administration of hGH to our patients may have resulted in an increase of hepatic cholesterol biosynthesis and a subsequent increase of hepatobiliary excretion of cholesterol. Experimental evidence in rats indicates that an increased biliary secretion of cholesterol is the result of an increased hepatic cholesterol synthesis (3, 27). Dogs and rats treated with growth hormone demonstrated marked rise in synthesis and concentration of hepatic cholesterol, which would indicate that growth hormone stimulates hepatic cholesterol synthesis. The growth hormone-induced stimulation of hepatic cholesterol synthesis should be mediated through 3-hydroxymethylglutaryl-CoA reductase, the rate-limiting enzyme for cholesterol biosynthesis (12). In experimental animals, the activity of this enzyme follows a diurnal rhythmicity (22, 29), suggestive of pituitary control. Pituitary secretion of growth hormone is greatest during nocturnal sleep (14, 15). If growth hormone does indeed influence hepatobiliary cholesterol dynamics, one would expect that bile collected in the morning is more lithogenic, *i.e.*, shows a greater cholesterol concentration than bile obtained later in the day, even in health. This has been demonstrated recently in man (21).

Although growth hormone has some influence on the insulin secretion in some growth hormone-deficient patients (26), we did not investigate this particular aspect because of the paucity of data on the interrelations between insulin and hepatic cholesterol synthesis.

The question of whether or not growth hormone has direct influence on the secretion of cholesterol into bile still remains to be answered. No experimental data are available to answer this question. We have not studied directly the secretion of cholesterol into bile before and after the administration of hGH in our patients. Such studies are necessary and would demonstrate clearly whether or not, how early, and at what rate cholesterol is secreted into bile after exogenous hGH.

The present investigation does not answer the question of whether or not patients receiving long term hGH are at risk for the development of bile oversaturated with cholesterol and, subsequently, gall stones. However, our study suggests, that growth hormone does influence hepatobiliary cholesterol dynamics in man.

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Brain	phenylalanine hydroxylase
fetus	tyrosine
liver	tyrosine hydroxylase
neonate	

Further Studies on the Nature of Phenylalanine Hydroxylation in Brain

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Extract

It has been reported by Wapnir *et al.* that fetal and neonatal rat brains are capable of converting phenylalanine to tyrosine at a rate that is about 10% that of livers from mature animals; this report led to the speculation that "the gestational role of phenylalanine hydroxylase is to supply tyrosine to the fetal brain."

As our present results show, however, the relatively high activity observed by Wapnir *et al.* in rat brain extracts is caused by a nonenzymatic hydroxylation of phenylalanine under their assay conditions. The critical features of their assay are the use of relatively high (2 mM) 2-amino-6,7-dimethyl-4-hydroxytetrahydropteridine (DMPH₄) concentrations and the reliance on a zero time control, i.e., a control to which trichloroacetic acid is added before incubation. Under these conditions, nonenzymatic hydroxylation of phenylalanine becomes significant. Although this nonenzymatic reaction is not troublesome when assaying tissues that have an active phenylalanine hydroxylase, such as liver, it can cause confusion when assaying a tissue with little or no hydroxylase, such as brain. Under the assay conditions used in our laboratory (0.2 rather than 2 mM DMPH₄), there is essentially no difference between a zero time control and a boiled enzyme control, i.e., there is no detectable nonenzymatic hydroxylation of phenylalanine under our conditions. It can

also be seen that there is no evidence for an enzyme-dependent hydroxylation under any of the assay conditions used.

With higher amounts of protein and longer times of incubation, some phenylalanine hydroxylation can be detected in a brain extract from newborn rats. The activity found is equal to 0.33 μ mol phenylalanine hydroxylated/hr/g protein. This value is less than 0.05% of the value for phenylalanine hydroxylation observed with liver extracts. That this low activity is caused by tyrosine hydroxylase is indicated strongly by the essentially complete inhibition of the reaction in the presence of 3-iodotyrosine.

Speculation

There is no detectable phenylalanine hydroxylation in brain other than the very small amount caused by tyrosine hydroxylase. This finding rules out the possibility that brain phenylalanine hydroxylase can have a role in brain development in the normal or phenylketonuric individual.

In 1971, Wapnir *et al.* (10) reported that fetal and neonatal (up to 11 days after birth) rat brains are capable of converting phenylalanine to tyrosine at a rate that is about 10% that of livers from mature animals. They concluded that the phenylalanine hydroxylase activity of brain was not caused by