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Bile acids
fat digestion
growth hormone

Improved Micellar Dispersal of Dietary Lipid by Bile Acids during Replacement Therapy in Growth Hormone-deficient Children

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Summary

To date, no information is available on whether human growth hormone (hGH) exerts an influence on digestive processes in man. To test this, we studied the composition of the aqueous (micellar) phase during the digestion of two consecutive meals in growth hormone-deficient individuals and in control subjects, before and after replacement therapy with hGH.

Before treatment, the average micellar bile acid concentration was 5.0 ± 0.6 mM (normal adults and controls subjects: 6–15 mM) during the first meal, and 3.5 ± 0.9 mM (normal adults and control subjects 5–10 mM) during the second meal. After 1 year of treatment, the mean micellar bile acid concentration increased to 9.7 ± 1.0 mM ($P < 0.02$) during the first, and to 7.1 ± 0.3 mM ($P < 0.01$) during the second meal. Concomitantly with an increased micellar bile acid concentration, micellar lipid increased as well (effect of treatment: (*1*) fatty acid, first meal 6.3 ± 0.6 mM to 13.0 ± 0.7 mM, $P < 0.001$; second meal 4.5 ± 1.5 mM to 8.5 ± 0.3 mM, $P < 0.05$; (*2*) monoglyceride, as percentage of total fatty acids: first meal 25 ± 10 to 53 ± 7, $P < 0.02$; second meal 31 ± 7 to 62 ± 6, $P < 0.02$).

Short term treatment (10 days) did not have an effect on the concentration of micellar bile acids and lipids in the control subjects. There was no significant difference in the physical state of bile acids and lipids between patients and control subjects before and after treatment.

Speculation

The digestion of dietary lipid involves a sequence of important steps: after emulsification, lipolysis generates fatty acids and monoglyceride, which require dispersal by bile acids (above their critical micellar concentration) for transport through the aqueous milieu to the membrane of the enterocyte. The "physiologic micellar concentration" of bile acids in the intestine during a meal for the efficient micellar dispersal of lipid is between 3 and 4 mM.

The increase of the intestinal micellar bile acid concentration from borderline to normal adult levels after replacement therapy with growth hormone could be explained as follows. The increased concentrations of bile acids in the intestine after treatment was the result of an increased hepatobiliary secretion of bile acids. This could have been effected by a growth hormone-mediated increase of hepatic bile acid synthesis, or through increased storage capacity of the gallbladder. The latter is probably best explained by a growth hormone-stimulated secretin effect, rather than an effect of gallbladder emptying. Further, increased availability of bile acids for lipid digestion could also result from their improved intestinal conservation (absorption).

The clinical implications of the results reported in this study are indirect, but may point towards an interrelationship between hormonal stimulation and certain aspects of hepatobiliary function, which influence digestive processes.

In recent years, evidence has accumulated suggesting that hormones may influence digestive and/or absorptive processes in man and experimental animals. McBrien *et al.* (16) observed steatorrhea in individuals with Addison's disease, which was corrected by replacement therapy. Steatorrhea was also demonstrated in adrenalectomized rats (27). The fat malabsorption documented in hypoparathyroid patients reported by Miettinen and Perheentupa (18) was thought to be related to a decreased availability of bile acids in the intestinal lumen. Malabsorption of dietary lipid was observed in hypophysectomized dogs without structural abnormality of the jejunal mucosa (14). By contrast, no fat malabsorption was seen in rats after hypophysectomy on two different dietary regimens (25).

Occasionally, it was observed (4) that growth hormone-deficient individuals may have increased loss of fecal fat, the etiology of which has remained obscure. Since intestinal villous atrophy has never been observed in such patients, any increased fecal fat could be the result of fat maldigestion and/or malabsorption. In analogy to hypoparathyroid patients (18), we considered the possibility that a decreased amount of bile acids during digestion could result in insufficient solubilization of lipolytic products. Hence, we decided to investigate intraluminal aspects of fat digestion, *i.e.*, the efficiency of bile acid-mediated micellar dispersion of dietary lipid in growth hormone-deficient patients. To test this we studied the composition of the aqueous (micellar) phase during the digestion of two consecutive test meals in growth hormone-deficient individuals and in control subjects before and after replacement therapy with human growth hormone (29).

PATIENTS AND METHODS

PATIENTS

All individuals who participated in this study were hospitalized in the Clinical Research Center, after informed consent had been obtained. The investigations on patients and controls were approved after review of the study protocols by the Committeee on Human Experimentation of the University of Oklahoma Health Sciences Center, Oklahoma City, Ok.

There were three male and two female patients aged 14–18 years with isolated growth hormone deficiency, and three control subjects, aged between 11 and 15 years. Two of the latter were girls with Turner's syndrome and the third, a boy, had constitutional short stature. The ability to secrete growth hormone was tested in all individuals by the following methods: measurement of the serum growth hormone levels after insulin-induced hypoglycemia, after arginine infusion, and at night, 2

hr after they had attained deep sleep. Although there was adequate secretion of growth hormone in the three control subjects, a totally inadequate secretion of growth hormone was observed in the growth hormone-deficient patients. The latter showed no deficiency of other pituitary trophic hormones, with the possible exception of gonodotropins and, hence, they received no other form of therapy. hGH was given in the following dosage: 2 units three times a week for 10 days (patients and control subjects), thereafter 3 units/week for 12 months (patients only).

METHODS

Intubation and Sampling of Jejunal Contents. After an overnight fast, a single lumen polyvinyl tube (OD 2.5 mm, ID 2.1 mm) with a metal collecting olive at the tip was placed under fluoroscopic control to a point about 20 cm distal to the ligament of Treitz. Thereafter, 300 ml of a well homogenized, liquid, corn oil-containing test meal of known composition (9), and containing polyethyleneglycol (PEG) was ingested within 3–7 min. There was never a problem with retching or vomiting. The individuals were then placed in a semireclining position.

Jejunal contents were obtained by siphonage and were collected for a period of 90 min after each test meal, at a rate of approximately 0.5–1.0 ml/min. The contents were heated immediately *ex vivo* to 70°c and kept at this temperature for 10 min to inactivate pancreatic lipase. Thereafter, the jejunal contents were pooled in three 30-min aliquots and kept at 37° until ultracentrifugation, which was done immediately after the end of the 90-min sampling period. Between meals, the individuals pursued recreational activity during which time nothing was ingested except 200 ml water. The second test meal was given in the early afternoon, 3–4 hr after sampling from the first meal had finished. Amount and composition of the meal and sampling techniques were identical.

Determination of pH. The pH was determined in all 30-min pooled samples with a pH electrode (Radiometer Copenhagen).

Isolation of Aqueous (Micellar) Phase. The pooled jejunal contents were well homogenized and centrifuged for 4 hr at 104,000 × *g*, at 37 –38 (Beckman-Spinco L2 65B preparative ultracentrifuge). After centrifugation, the aqueous phase was sampled by piercing the tube with a needle and about 70% of the aqueous phase was drawn into a calibrated syringe. A rather large sample of the micellar phase was aspirated in order to avoid sedimentation artifacts, which have been observed after prolonged (12 hr) centrifugation (21). Occasionally, slight turbidity of the micellar phase was observed, which was thought to be due to mesophasic monoglyceride (7). The bile acids and lipids were extracted from the aqueous phase with minor modifications as described (10).

Determination of Micellar Bile Acid and Lipid. Bile acids: The ethanolic phase was evaporated to dryness, and the residue dissolved in a measured amount of spec. grade methanol. One aliquot was used for bile acid determination by the 3-hydroxysteroid dehydrogenase method (13). The ratio of glycine to taurine conjugates, as well as the ratio of dihydroxy to trihydroxy bile acids was determined by the enzymatic method after separation of bile acids by thin layer chromatography (6). The coefficient of variation between replicate analyses was consistently below 3%.

Fatty acids and monoglyceride: Thin layer chromatograms of the aqueous phase lipid extracts disclosed mainly fatty acids, monoglyceride, and cholesterol, with negligible amounts of di- and triglyceride. The lipid extract was dissolved in acetone and one aliquot was titrated directly as nonglycerol-bound fatty acid. Another aliquot was subjected to mild alkaline hydrolysis, with subsequent extraction and titration as glycerol-bound fatty acid. The difference between the values of fatty acid determination before and after hydrolysis was considered chiefly monoglyceride fatty acid. The coefficient of variation between duplicate determinations was less than 4%.

Phospholipids: Phospholipids were extracted from the micellar phase with chloroform-methanol (2:1), and determined by a micromethod (8).

Physical State of Lipids. To determine the physical state of bile acids and fatty acids present in the aqueous phase, bile acids and total fatty acids were determined in the unspun homogenized intestinal contents and in the aqueous phase to arrive at the percentage of bile acid and fatty acid in solution.

ANALYSES

PEG was determined turbidimetrically (12). All intraluminal indices were corrected for dilution. There was no significant difference of PEG concentration in spun and unspun samples. Peroral suction biopsies (Pediatric Crosby-Kugler capsule, College Park Instruments, College Park, Md.) were done in patients and control subjects to rule out morphologic changes of the villous architecture. Gas-liquid chromatography of bile acid methylester-acetates was done using 2.5% OV-17 on AW-DMCS Gas-chrom Q (Applied Science Laboratories, State College, Pa.). Statistical analyses were done using the rank sum test (28). Stool fat balance studies could not be performed, since prolonged hospitalization was unacceptable, as most individuals would have had to discontinue school for such studies.

RESULTS

AQUEOUS (MICELLAR) PHASE CONSTITUENTS DURING DIGESTION

Patients (Table 1). During treatment, all patients except one (JH) responded well to exogenous hGH with a good acceleration of height velocity (20).

Variation between meals: It has been estimated that during the collection of intestinal contents during the first meal, less than 5% of the circulating bile acid mass has been aspirated. This is unlikely to have had a significant effect on intraluminal concentrations of bile acids during the second meal.

In general, there was a decrease of aqueous phase bile acids and lipids from the first to the second meal. Only once was there a significant decrease of bile acid concentration from the first to the second meal in the four growing patients, 10 days after treatment. As in the control groups, before and after treatment with hGH, the concentration of aqueous phase fatty acid decreased during the digestion of the second meal, whereas the percentage of monoglyceride increased.

Influence of treatment: The concentrations of jejunal aqueous phase bile acids during the two test meals in patients, normal adults (19), and control subjects before and after treatment are presented in Figures 1 and 2. Treatment with hGH led to a consistent increase of bile acid and fatty acid in the aqueous phase. This increase was more conspicuous during the second meal. Before treatment, the concentration of bile acid and fatty acid in patients were lower than in control subjects ($P < 0.02$), but this difference was nearly equalized after 4 months of therapy with hGH. After 12 months of therapy, the concentration of bile acids and fatty acids, as well as the percentage of monoglyceride fatty acid, nearly doubled, and these changes were significant (Table 1).

Concomitant with improved aqueous dispersal of dietary lipid during treatment, the fraction of nonaqueous phase lipid decreased, whereas the amount of total lipid in the intestinal lumen remained fairly constant during the two meals with little variation from individual to individual. Although a statistically significant decrease of the means for phospholipids during treatment was obtained, an exact interpretation of these changes cannot be made, as biliary phospholipid secretory rates were not measured.

Patient JH (Table 2): Since this patient did not show any response to hGH as measured by an increase in height velocity, the values of the aqueous phase lipids are presented separately for comparison with the growing patients presented in Table 1.

Table 1. *Patients (n = 4): Bile acids and lipids in the aqueous phase during digestion of two consecutive test meals (mean ± SE)*[1]

Treatment with human growth hormone	First meal				Second meal			
	Bile acid, mM	Phospholipid, mM	Fatty acid, mM	% Monoglyceride fatty acid	Bile acid, mM	Phospholipid, mM	Fatty acid, mM	% Monoglyceride fatty acid
Before	5.0 ± 0.6[1]	1.95 ± 0.3[2]	6.3 ± 0.6[3]	25 ± 10[4]	3.5 ± 0.9[5]	0.75 ± 0.1[6]	4.5 ± 1.5[7]	31 ± 7[8]
After 10 days	7.0 ± 0.6[9]	2.55 ± 0.4[10]	9.5 ± 2.8[11]	39 ± 7[12]	4.5 ± 0.5[13]	1.23 ± 0.3[14]	6.5 ± 1.2[15]	50 ± 14[16]
After 4 mo	8.7 ± 1.6[17]	2.30 ± 0.1[18]	10.5 ± 1.8[19]	35 ± 9[20]	6.1 ± 1.3[21]	1.03 ± 0.04[22]	6.3 ± 1.3[23]	50 ± 3[24]
After 12 mo	9.7 ± 1.0[25]	1.76 ± 0.3[26]	13.0 ± 0.7[27]	53 ± 7[28]	7.1 ± 0.3[29]	1.27 ± 0.2[30]	8.5 ± 0.3[31]	62 ± 6[32]

[1] Superior numbers identify the micellar indices during the different treatment periods and the two meals, and are identical to italic numbers in the table, below.

Statistically significant differences of the means

	First meal vs. second meal	Influence of treatment
Bile acid	*9* vs. *13* ($P < 0.02$ ↓)	*1* vs. *25* ($P < 0.02$ ↑) *5* vs. *29* ($P < 0.01$ ↑)
Fatty acid	*27* vs. *31* ($P < 0.001$ ↓ ↓)	*3* vs. *27* ($P < 0.001$ ↑ ↑) *7* vs. *31* ($P < 0.05$ ↑)
% Monoglyceride fatty acid		*4* vs. *28* ($P < 0.02$ ↑) *8* vs. *32* ($P < 0.02$ ↑)
Phospholipid	*2* vs. *6* ($P < 0.02$ ↓) *10* vs. *14* ($P < 0.05$ ↓) *18* vs. *22* ($P < 0.001$ ↓)	*6* vs. *30* ($P < 0.05$ ↑)

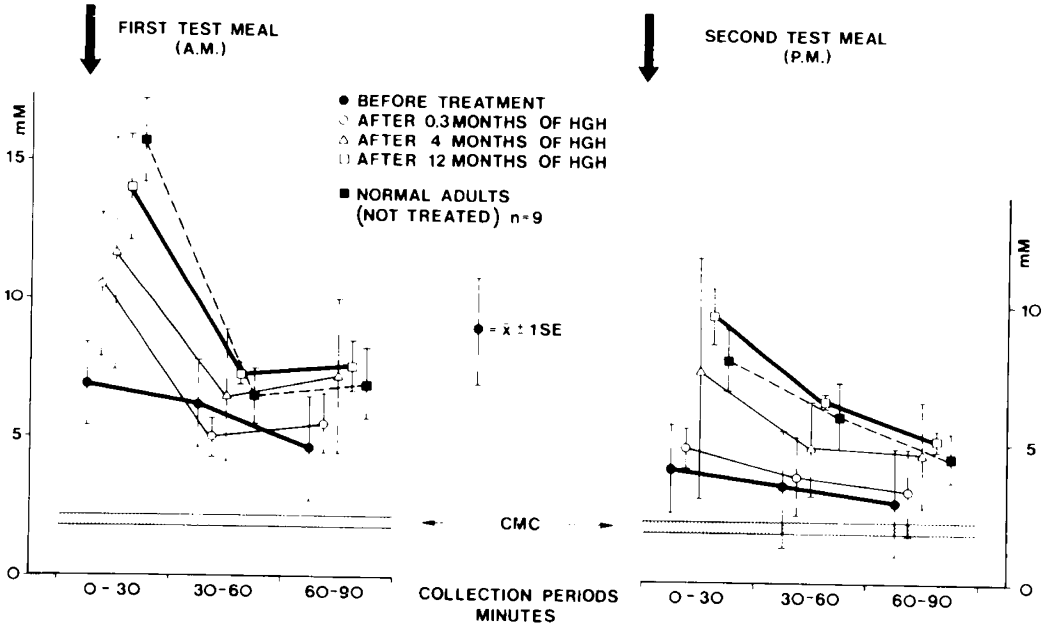

Fig. 1. Intraluminal concentration of aqueous phase bile acids after two sequential test meals in growth hormone-deficient individuals ($n = 4$) and in healthy adults. CMC: critical micellar concentration.

Only after 1 year of treatment was there a modest increase in the concentration of micellar bile acid and fatty acid. This increase has not been as great as compared with patients presented in Table 1, and was only noted after the first, but not during the second meal.

Control Subjects. There was essentially no change of bile acid, phospholipid, fatty acid concentration, and percentage of monoglyceride fatty acid between the first and second meal. Short term treatment with hGH had little influence on the composition of the aqueous phase lipids. It was of interest, however, to see that, although the concentration of fatty acid was less during the second meal, that of fatty acid derived from monoglyceride increased.

PHYSICAL STATE OF BILE ACIDS AND LIPIDS

During the digestion of both meals, most bile acids (84–98%, first meal, 78–96% second meal) and a considerable amount of fatty acids (39–60% first meal, 26–49% second meal) were in solution. There was no significant difference between patient and control groups.

INTRALUMINAL pH

Before treatment, jejunal pH during the first meal in patients was 6.3 ± 0.06, whereas in the control subjects it was 6.5 ± 0.04 ($P < 0.005$). During the second meal it was 6.3 ± 0.05 for patients and 6.5 ± 0.05 for control subjects ($P < 0.005$).

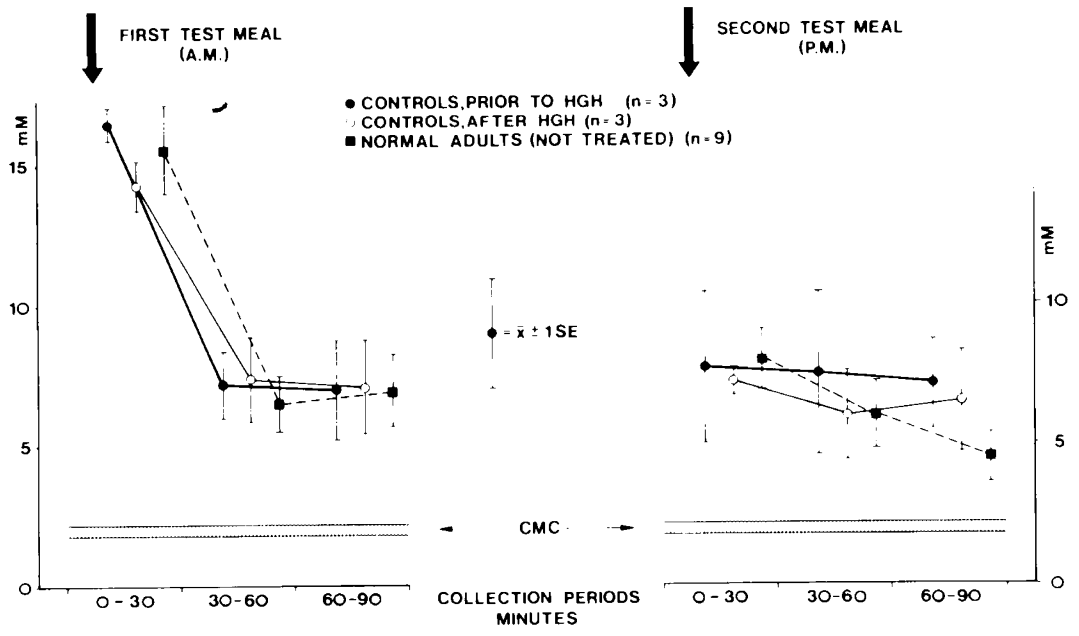

Fig. 2. Intraluminal concentration of aqueous phase bile acids after two sequential test meals in control subjects of short stature and in healthy adults. Influence of human growth hormone (2 units day × 10). CMC: critical micellar concentration.

Table 2. *Patient JH: Bile acids and lipids in aqueous phase during digestion of two consecutive test meals*

	First meal				Second meal			
Treatment with hGH[1]	Bile acid, mM	Phospholipid, mM	Fatty acid, mM	% Monoglyceride fatty acid	Bile acid, mM	Phospholipid, mM	Fatty acid, mM	% Monoglyceride fatty acid
Before	4.9	1.78	5.9	21	3.1	0.83	7.0	31
After 10 days	3.8	1.13	2.6		3.0	0.95	2.5	
After 4 mo	3.7	1.39	5.4	19	2.3	0.51	4.1	26
After 12 mo	7.9	1.85	7.9	20	4.3	0.95	5.6	6

[1] Human growth hormone.

Table 3. *Control subjects (n = 3): Bile acids and lipids in aqueous phase during digestion of two consecutive test meals (mean ± SE)*[1]

	First meal				Second meal			
Treatment with hGH[2]	Bile acid, mM	Phospholipid, mM	Fatty acid, mM	% Monoglyceride fatty acid	Bile acid, mM	Phospholipid, mM	Fatty acid, mM	% Monoglyceride fatty acid
Before	10.8 ± 1.3	2.48 ± 0.3	9.3 ± 0.9	18 ± 10	7.7 ± 2.5	1.10 ± 0.3	6.3 ± 0.7	34 ± 13
After 10 days	11.2 ± 1.2	2.05 ± 0.7	10 ± 3.7	22 ± 11	7.3 ± 1.3	1.14 ± 0.03	5.7 ± 0.9	42 ± 12

[1] No significant differences of the means of bile acids, etc., between first meal and second meal, without or with treatment.
[2] Human growth hormone.

This difference could have been due to a relative increase of H ions or a relative decrease of HCO_3 ions in patients. Throughout treatment, the pH remained essentially unchanged in patients and control subjects.

The pH had little influence on the physical state of bile acid in the range between 5.9 and 7.5. However, a moderate decrease of bile acid in solution was found in an occasional sample with pH of less than 5.7, whereas only 60–70% of total bile acids were in the aqueous phase.

RELATIONSHIPS BETWEEN AQUEOUS PHASE BILE ACIDS, PHOSPHOLIPIDS, AND FATTY ACIDS

Since conjugated bile acids above the critical micellar concentration for a mixed bile acid solution (≈2 mM) disperse polar and nonpolar lipids in an aqueous solution at physiologic pH during digestion, it was of interest to look at the relationship between micellar solvent (bile acid and phospholipid) and micellar solute (fatty acid). These relationships are presented in Figure 3, showing a good correlation ($r = 0.81$) between these indices. When only micellar bile acid was plotted against micellar lipid, the correlation was not as good ($r = 0.63$).

RATIOS OF GLYCINE TO TAURINE CONJUGATED BILE ACIDS (G/T RATIO) AND DIHYDROXY TO TRIHYDROXY BILE ACIDS

Treatment with growth hormone did not induce persistent changes of these indices, with the exception of patient RN, who showed a consistent decrease in the G/T ratio (1.4 to 1.0) and in the ratio of dihydroxy to trihydroxy bile acids (1.2 to 1.0) during treatment. These findings are probably accidental.

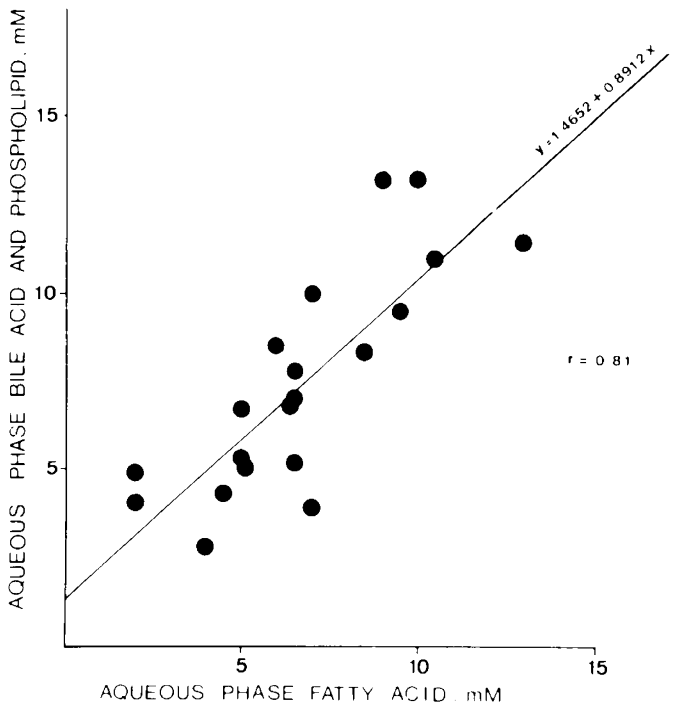

Fig. 3. Relationship between aqueous phase bile acid and phospholipid and fatty acid during digestion.

SECONDARY BILE ACIDS

The percentage of secondary (deoxycholate, lithocholate) and tertiary (ursodeoxycholate) bile acids remained essentiallly unchanged in three of the four patients who responded to growth hormone. However, in patient RN, there was a significant and persistent decrease of these bile acids from 19% to 7% during treatment with growth hormone. After cessation of treatment, the percentage of secondary bile acids increased again to 10%.

SMALL INTESTINAL BIOPSIES

No structural abnormalities or changes could be elicited by light microscopy in either patients or control subjects before and after replacement therapy.

DISCUSSION

Efficient absorption of dietary lipid is dependent on efficient dispersal of fatty acid and monoglyceride in bile acid micelles for transport to the intestinal mucosa (11). The physiologic micellar concentration of bile acids required for efficient lipid dispersal *in vivo* is probably between 3 and 4 mM (1, 15).

Data presented in this study demonstrated increases in the concentration of aqueous (micellar) phase bile acids and fatty acids in the jejunum during digestion of two sequential meals in four of five patients treated with hGH. Since hGH was the only variable, there is a strong suggestion that these increases were effected by hGH.

Long term treatment with exogenous hGH at low physiologic doses (3 units/week) led to a consistent increase of the concentration of aqueous phase bile acids, and this was particularly noticeable during the second meal. After 1 year, the values obtained during the first as well as during the second meal were indistinguishable from those of healthy adults (19) or the three control subjects. Consonant with the increase of the bile acid concentration in the aqueous phase, there was a rise in the total concentration of fatty acid in the aqueous phase. This was obviously the result of an improved and more efficient micellar dispersion of fatty acids and monoglyceride by bile acids, which were available in the intestine.

To explain the increased concentration of bile acids in the intestine in patients after replacement therapy, several possibilities exist. (*1*) Increased hepatic synthesis of bile acids with consequent increased hepatobiliary secretion is possible. It is not known whether growth hormone has a direct or indirect (via the somatomedines) influence on cholesterol 7α-hydroxylase, the rate-limiting enzyme for hepatic bile acid biosynthesis (24). In the hypophysectomized rat (2, 3) bile acid synthesis rates decreased because of a decreased turnover of primary bile acids, *i.e.*, cholic and chenic acids, without changes in pool sizes. (*2*) hGH may have increased bile acid secretion irrespective of any influence on bile acid synthesis, either by more efficient gallbladder emptying or by an increased gallbladder storage capacity (secretin effect). (*3*) Concentration of bile acids during the second meal after treatment could be explained also by their more efficient intestinal conservation. However, no information exists about whether growth hormone exerts an influence on the absorption of bile acids in the intestine. The decrease of secondary bile acids in patient RN may indicate decreased exposure of bile acids to bacteria because of improved absorption.

Polar lipids such as fatty acids and monoglyceride are efficiently dispersed by bile acids above a certain concentration (critical micellar concentration). *In vitro*, the critical micellar concentration for a mixed bile acid solution is in the vicinity of 2 mM (11). *In vivo*, the bile acid concentration needed for adequate micellar dispersal of dietary lipid is higher, and 3 to 4 mM seem to be required in adults (1) and newborns (15). A bile acid concentration of 4 mM was not always reached by three of the four growing patients during the digestion of the second meal before and after short term replacement therapy. Concomitant with the lower concentration of aqueous phase bile acids, the amount of fatty acids in growth hormone-deficient patients was also lower when compared with the control subjects, healthy adults (19), or healthy young children (22, 23).

Studies in model systems have demonstrated that phospholipids, which are also amphiphiles, are capable of swelling the bile acid micelle (5), enhancing their solubilizing properties. The role of endogenous phospholipid during digestion of dietary lipid is still uncertain, but is probably of importance (17). In the present study, the correlation between combined bile acid and phospholipid and fatty acids in the aqueous phase was much better than between bile acids and fatty acids alone, suggesting that phospholipids do indeed contribute towards more efficient dispersal of dietary lipid.

It would have been preferable to have had digestive studies after three consecutive meals, as was done in patients with bile acid deficiency after ileal resection (26). This way, a "decompensated" state with regard to intraluminal digestive events in growth hormone-deficient patients would have become more apparent.

Although this study did not prove an influence of hGH on fat absorption, an improvement of intraluminal bile acid dispersal of dietary lipid was obvious. It was unfortunate not to have been able to compare results of the digestive studies with fecal fat balances, because, for reasons stated above, we were not able to hospitalize patients for the period of time required to do proper fecal fat balance studies.

The clinical implications of the results reported in this study are indirect and might be viewed in the interrelationship between hormonal stimulation and hepatobiliary function which influences digestive processes.

CONCLUSION

To test the influence of replacement therapy in growth hormone-deficient individuals on micellar dispersion of dietary lipid, analyses on the composition of the aqueous (micellar) phase were done in five growth hormone-deficient patients aged 14–18 years, and in three control subjects aged 11–15 years during digestion of two consecutive test meals. Studies

were done before treatment and after short term and long term treatment with hGH.

Before therapy with hGH, the concentration of jejunal aqueous phase bile acids was significantly lower in the patients as compared with the control subjects, particularly during the second meal. Consonant with low aqueous phase bile acid concentrations, there were also decreased amounts of lipid in solution.

Although hGH had no influence on the composition of the aqueous phase during digestion in the control subjects, significant increases of aqueous phase bile acids and lipids were observed in four of the five growth hormone-deficient patients after continued treatment with hGH. After 1 year of treatment, aqueous phase bile acids and fatty acids were equal to those of the control subjects and normal adults.

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