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Increased Epithelial Cell Proliferation in the Colon of Patients with Acromegaly[1]

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ABSTRACT

To gain insight into the possible physiological mechanisms responsible for the increased incidence of colonic neoplasms in patients with acromegaly, a prospective cohort study was carried out in 30 patients with acromegaly. Seven patients had a newly diagnosed acromegaly and 23 were studied during follow-up. Serum growth hormone and insulin-like growth factor-1 (IGF-1) were determined on two separate occasions. During diagnostic endoscopy, mucosal biopsies were obtained for immunohistochemical determination of sigmoidal epithelial cell proliferation, expressed as labeling index (LI). Duodenal and fecal bile acid analyses were performed using gas-liquid chromatography. Results were compared with normal ranges of the laboratory.

An increased overall LI was found in 54% of the patients. Increased LI of the luminal, middle, and basal crypt compartments was found in 11, 64, and 28%, respectively. Similarly, comparisons of the mean ± SEM of the overall LI and the LI of the middle and basal compartments between acromegalic patients and a control group showed overall LI 10.0 ± 0.8% *versus* 5.7 ± 0.6% ($P < 0.001$), middle LI 12.1 ± 1.2% *versus* 5.0 ± 0.6% ($P < 0.001$), and basal LI 17.1 ± 1.3% *versus* 10.8 ± 1.3% ($P < 0.01$). Duodenal and fecal bile acid proportions were within the normal ranges of the laboratory. There was a positive correlation between growth hormone and overall LI ($r = 0.60$, $P < 0.001$) and between IGF-1 and total LI ($r = 0.55$, $P < 0.01$) by least square regression analysis. There was no correlation between duodenal bile acid composition and hormone levels. The proportion of secondary bile acids in feces correlated with growth hormone ($r = 0.55$, $P < 0.05$) as well as with IGF-1 ($r = 0.59$, $P < 0.05$). With multiple regression analyses, only a relation between overall LI and IGF-1 ($P = 0.007$) remained to hold true. Increased epithelial cell proliferation, most probably due to a direct stimulatory effect of especially IGF-1, contributes to the increased risk of colonic neoplasms in acromegaly.

INTRODUCTION

Acromegaly is a clinical syndrome characterized by growth of bone and soft tissue of the extremities as well as enlargement of the visceral organs due to excessive secretion of GH.[3] Several recent prospective studies have reported an increased risk of developing adenomas and adenocarcinomas in the colon of patients with acromegaly (1–4). Pathophysiological reasons for an increased risk of development of colonic neoplasms in this patient group may be 2-fold. On the one hand, the increased production of GH or of IGF-1, the main mediator of the physiological effects of GH, or both may have a direct stimulatory effect on colonic epithelial cell proliferation and thus promote neoplastic development. GH can stimulate mitogenesis directly by a rapid induction of c-myc expression (5). However, many growth-promoting properties of GH are mediated by IGF-1, which is produced in the liver, chondrocytes, kidneys, muscles, pituitary, and gastrointestinal tract (6, 7). This polypeptide is known to be involved in the growth stimulation of epithelial cells in either an endocrine, paracrine, or autocrine fashion (7). IGF-1 receptors are present in the normal colonic epithelial cell membrane of humans (8, 9). Also in human colon carcinoma cell lines and in freshly resected human colon cancers, a widespread existence of high-affinity binding sites for IGF-1 has been observed (10). Furthermore, in a number of such cell lines, incubation with IGF-1 enhanced cellular proliferation (10). On the other hand, GH excess may lead to a change in intestinal bile acid metabolism, resulting in increased intracolonic concentrations of secondary bile acids. These acids supposedly promote colonic tumor formation because of their cytotoxic and hence compensatory proliferation-inducing properties (11, 12). Patients with GH deficiency were reported to have a contracted bile acid pool size (13) and reduced bile acid concentrations in the small intestine (14). Exogenous human GH replacement therapy increased hepatic bile acid synthesis and bile acid pool size (13) and hepatobiliary secretion of bile acids (14) in GH-deficient children. Extrapolating these data to patients with excessive GH secretion, one might expect an increase of large intestinal bile acid concentrations in acromegalic patients.

In many studies, increased epithelial cell proliferation has been found in subjects at increased risk for colorectal cancer (15–19). Colonic epithelial cell proliferation is, therefore, considered to be a biomarker for the risk of development of colon cancer. To gain insight into the mechanisms responsible for the reported increased incidence of colonic neoplasms in patients with acromegaly (1–4), we evaluated the frequency of colonic neoplasia and the colonic epithelial cell proliferation in these patients in relation to their duodenal and fecal bile acid content and serum GH and plasma IGF-1 levels.

PATIENTS AND METHODS

Patients. Thirty Caucasian patients (16 males and 14 females) with a median age of 56 (range, 30–71) years who visited the outpatient clinic of the Department of Endocrinology between June 1991 and July 1993 for acromegaly participated in this study. A GH-secreting pituitary adenoma had been diagnosed in 29, while in 1, no tumor could be detected. Seven of them had a newly diagnosed acromegaly, and 23 were studied during follow-up. The median duration of follow-up in the latter group was 10 (range, 1–39) years. Four of these patients had been treated by surgery, 5 by radiotherapy, and 13 had received both treatments. Twelve patients were treated with bromocriptine during the investigations. Because somatostatin is known to influence gastrointestinal function (20–22), only patients who never received octreotide, a somatostatin analogue, were included in the study. If necessary, the patients received supplementation therapy with thyroxine, cortisone acetate, or sex steroids. None of the patients studied had undergone previous colonoscopy or was known to have concomitant gastrointestinal disease. None of the patients had a family history of colorectal cancer. Screening colonoscopy was performed in all patients between 10:00 am and 2:00 pm after whole-gut lavage with saline polyethylene glycol solution. During colonoscopy, three biopsies for epithelial cell proliferation measurement were taken from macroscopically normal-appearing mucosa in the midsigmoid. In 14 patients, studies on intestinal bile acid composition were performed within a period of 3 weeks around the colonoscopy. Informed consent was obtained from all patients, and the study was approved by the Medical Ethical Committee of the University Hospital of Groningen.

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[3] The abbreviations used are: GH, growth hormone; IGF-1, insulin-like growth factor-1; LI, labeling index; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid.

Methods. Fasting morning blood samples for determination of GH and IGF-1 levels were collected on two separate occasions with a mean ± SD interval of 5 ± 5 weeks in the period around the colonoscopy. Serum GH (normal value, <5 μg/liter; Ref. 7) was measured by RIA (Farmos Diagnostica, Turku, Finland). Plasma IGF-1 (reference range for adults, 0.34–2.20 kU/liter) was determined by RIA with a kit purchased from Nichols Institute (San Juan Capistrano, CA). The individual results were expressed as the mean value of the two determinations.

Sigmoidal epithelial cell proliferation was determined after *in vitro* incubation of the endoscopically obtained mucosal biopsies with 5-bromo-2′-deoxyuridine (Serva, Heidelberg, Germany) and subsequent visualization of nuclear 5-bromo-2′-deoxyuridine incorporation using immunohistochemistry as described previously (18, 23). Epithelial cell proliferation was expressed as the LI, which is defined as the percentage of labeled nuclei of the total number of nuclei in longitudinally sectioned colonic crypts. After dividing crypts into three longitudinal compartments of equal length, the LIs of the luminal, middle, and basal compartments were also determined. The technician performing the scoring was unaware of the patients' characteristics. The normal ranges of the overall LI and the luminal, middle, and basal compartments in healthy individuals in our laboratory are 2.1–9.3%, 0–3.4%, 1.3–8.8%, and 2.3–19.3%, with the respective means ± SEM being 5.7 ± 0.6%, 1.0 ± 0.4%, 5.0 ± 0.6%, and 10.8 ± 1.3%. These normal values were calculated from the results of a group of healthy individuals, described previously (18), and were found to be unrelated to the age of the individuals.

Duodenal bile was collected for bile acid analysis using the Entero-Test (HDC Corp., Mountain View, CA) as described previously (23, 24). In brief, the duodenal bile acids adsorbed to the Entero-Test were eluted with 0.5 mM phosphate buffer (pH 7.0) and extracted from the buffer with Sep-Pak C_{18} cartridges (Waters Inc., Milford, MO). After hydrolysis and derivatization according to the method of Setchell and Matsui (25), bile acids were analyzed by gas-liquid chromatography, and the molar percentage of the major species, *i.e.*, CA, CDCA, DCA, and LCA, was calculated. The normal ranges in healthy individuals in our laboratory are 14–70% for CA, 13–57% for CDCA, 0–44% for DCA, 0–5% for LCA, and 0–3.88% for the di-/trihydroxy bile acid ratio.

Feces were collected for 24 h. After dilution with distilled water (1:1, w/v) the individual stools were homogenized in a Waring blender (Waring Product Division, New Hartford, CT). Feces were subsequently freeze-dried and stored at room temperature until further processing. The procedures for extraction of fecal bile acids were basically those of Grundy *et al.* (26), with some modifications as described previously (23). Enzymatic determination of total 3α-hydroxy bile acids was performed in duplicate using a commercially available kit (Sterognost-3α Pho; Nycomed AS, Oslo, Norway; normal value, 600–1250 μmol/24 h). For determination of the molar composition of the major bile acids (CA, CDCA, DCA, and LCA) in the feces, gas-liquid chromatography was performed as described earlier (23). Normal ranges in healthy individuals in our laboratory are 38–70% for DCA, 20–44% for LCA, 0–10% for CA, and 0–26% for CDCA.

Statistical Analysis. The unpaired, two-tailed Student's *t* test was used to compare the results on epithelial cell proliferation between patients with acromegaly and the previously described control group. Possible correlations between serum GH or plasma IGF-1 levels on the one hand and the presence of colonic neoplasms, overall LI and LI of crypt compartments, duodenal bile acid composition, as well as total and proportional fecal secondary bile acid excretion, on the other hand, were analyzed by least square regression analysis. In addition, multiple regression analysis was performed to evaluate independent associations of overall LI and LI of crypt compartments as well as with serum GH and plasma IGF-1, duodenal bile acids, fecal bile acids, and clinical parameters (age, sex, duration of follow-up, previous treatment modalities, and bromocryptic therapy). The log analysis was also performed because this most probably reflects better the receptor occupancy. This may also be the case for the log of the LI. The level of statistical significance was set at $P < 0.05$.

RESULTS

Serum GH (normal value, <5 μg/liter) and plasma IGF-1 (reference range for adults, 0.34–2.20 kU/liter) levels were both elevated in nine patients. In eight patients, only GH and in one patient only IGF-1 was raised. In 12 patients, GH as well as IGF-1 levels were normal. Log GH and log IGF were analyzed and found to be related ($n = 28$; $r = 0.807$; $P < 0.001$). During colonoscopy, a solitary adenomatous polyp was found in seven acromegalic patients (23%). In five of these patients and in another six acromegalic patients (37%), hyperplastic polyps were observed. Additionally, in one patient (3%), an adenocarcinoma, as well as an adenomatous and a hyperplastic polyp, was found. After surgical resection, this adenocarcinoma proved to be restricted to the submucosa (Dukes A). Adenomatous lesions were distributed throughout the entire colorectum. There was no relationship between GH or IGF-1 levels and the presence of neoplastic lesions in the large bowel.

The required number of longitudinally sectioned crypts (18) to establish a reliable LI was achieved in 28 patients. Data on the LI of individual acromegalic patients and the normal range of the LI are depicted in Fig. 1. The overall LI was above the upper limit of the normal range in 15 (54%) of these acromegalic patients. Seven patients with normal GH as well as IGF-1 levels did have an elevated overall LI. In the crypt compartments, the same pattern was observed, particularly for the mid compartment, in which the LI was higher than the normal range in 18 patients (64%). Values above the upper limit of the normal ranges were found in only three patients (11%) for the luminal compartment and in eight patients (28%) for the basal compartment. The mean ± SEM of the overall LI in the patient group was higher than in previously studied controls, specifically 10.1 ± 0.8% *versus* 5.7 ± 0.6% ($P < 0.001$). The mean ± SEM of the middle and basal compartments in the patient group were also increased compared to controls: 12.1 ± 1.2% *versus* 5.0 ± 0.6% ($P < 0.001$) and 17.1 ± 1.3% *versus* 10.8 ± 1.3% ($P < 0.01$), respectively. No difference in the luminal compartment in the acromegalic patients *versus* the controls was observed: 1.1 ± 0.3% *versus* 1.0 ± 0.4%. The relative distribution of labeling within the crypts shows that proliferation predominantly occurs in the basal compartment. The labeling distribution (mean ± SEM) in the luminal, middle, and basal compartments are 2.7 ± 0.7%, 39.2 ± 1.9%, and 57.8 ± 2.4%, respectively. However, only the percentage of labeled nuclei in the middle

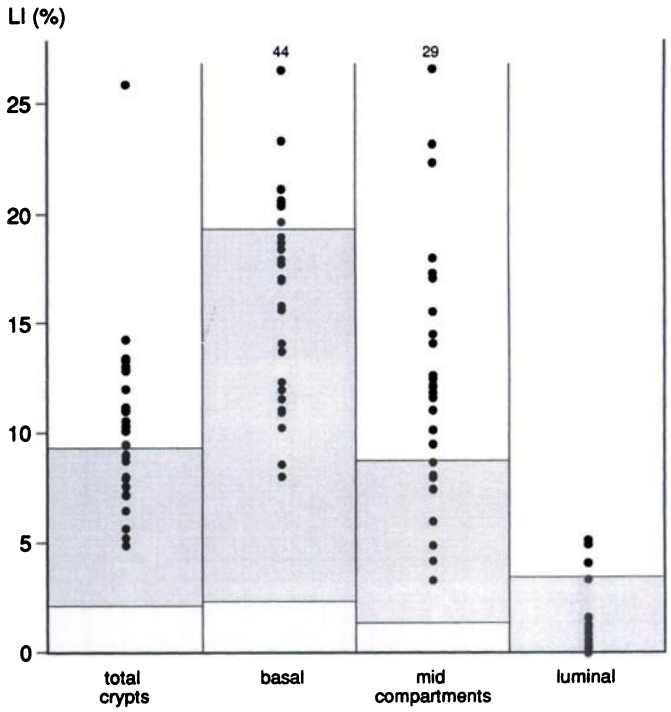

Fig. 1. The overall LI (%) and the LIs of luminal, middle, and basal crypt compartments in individual acromegaly patients (●), compared to normal values (*shaded areas*).

compartment was increased ($P < 0.02$) compared to that in the control group, with the latter being 30.5 ± 2.9%. The labeling distribution in the luminal and basal compartments is not different from that in the control group; 6.1 ± 2.5% and 63.7 ± 4.1%, respectively. If the extremely high GH value of 780 μg/liter in a newly diagnosed patient was deleted, there was a positive correlation between the serum GH level and the LI of whole crypts ($r = 0.62$; $P < 0.001$; $n = 27$). There was also a positive correlation between plasma IGF-1 level and the LI of whole crypts ($r = 0.55$; $P < 0.01$; $n = 28$; Fig. 2). Additionally, LIs of the three crypt compartments were correlated with both GH and IGF-1 levels, apart from a correlation between luminal LI and IGF-1 (Table 1). Multiple regression analysis showed only a relation between whole crypt LI and plasma IGF-1 ($P = 0.007$).

In 12 patients, the Entero-Test was performed successfully, and duodenal bile acid composition could be determined. In nine of these patients, elevated GH and/or IGF-1 levels were present. The relative percentages of the major duodenal bile acids, CA, CDCA, and DCA, and the ratio of di-/trihydroxy bile acids were within the normal range. Table 2 shows the 95% confidence interval of the mean of duodenal bile acid proportion in acromegalic patients with their corresponding normal ranges. There was neither a relation between the ratio of di-/trihydroxy bile acids and serum GH or plasma IGF-1 levels nor between this ratio and LI of whole crypts or crypt compartments.

In 12 of the 14 patients studied for fecal bile acid analyses, increased GH and/or IGF-1 levels were present. Total excretion of fecal bile acids was not different from normal, nor were the proportional concentrations of the individual bile acids DCA, LCA, CA, and CDCA. Table 3 shows the 95% confidence interval of the mean of total bile acids and bile acid proportions in feces in acromegalic patients, with their corresponding normal ranges. There were no correlations between serum GH, plasma IGF-1, or LI on the one hand and excretion of total or secondary bile acids on the other hand. However, the proportion of secondary bile acids in feces showed a significant correlation with GH ($r = 0.58$; $P < 0.05$) as well as with IGF-1 ($r = 0.59$; $P < 0.05$). Multiple regression analysis for duodenal and fecal bile acids with hormone levels and clinical parameters revealed no correlations.

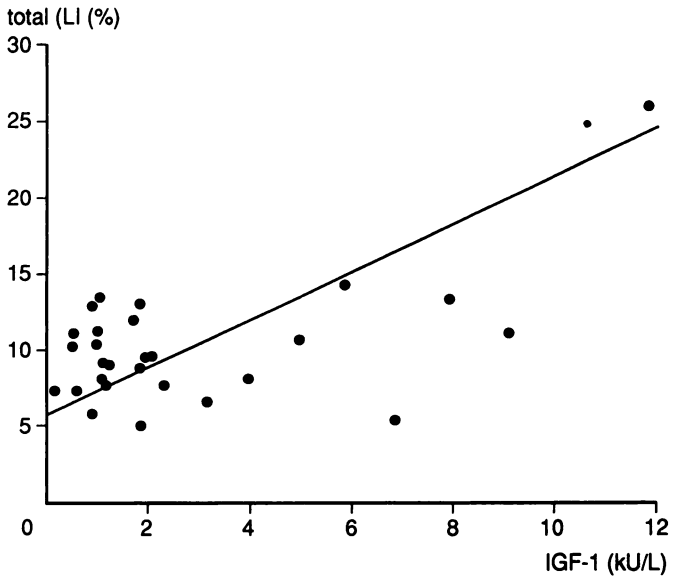

Fig. 2. Relation between plasma IGF-1 level and overall LI for each individual patient.

Table 1 *Mean ± SEM concentrations of serum GH (normal value, <5 μg/liter) and plasma IGF-1 (normal range, 0.34–2.20 kU/liter) versus compartmental LI and their interrelated correlation coefficient*

	GH (μg/liter; $n = 27$)	IGF-1 (kU/liter; $n = 28$)
Hormone levels	10.3 ± 2.6	2.78 ± 0.55
Luminal LI (%)	1.1 ± 0.3 ($r = 0.40^a$)	1.1 ± 0.3 ($r = 0.36$)
Middle LI (%)	12.1 ± 1.2 ($r = 0.51^b$)	12.1 ± 1.2 ($r = 0.45^a$)
Basal LI (%)	16.9 ± 1.4 ($r = 0.57^b$)	17.1 ± 1.3 ($r = 0.50^b$)

[a] $P < 0.05$.
[b] $P < 0.01$.

Table 2 *Duodenal bile acid composition (n = 12)*

	95% CI[a] (x)	Normal range
LCA (%)	2–6	0–5
DCA (%)	12–22	0–44
CDCA (%)	28–42	13–57
CA (%)	34–56	14–70

[a] CI, confidence interval.

Table 3 *Fecal bile acid excretion and composition (n = 14)*

	95% CI[a] (x)	Normal range
Total BA (μM/24 h)	614–1390	600–1250
LCA (%)	22–30	20–44
DCA (%)	48–60	38–70
CDCA (%)	9–17	0–26
CA (%)	3–9	0–10

[a] CI, confidence interval.

DISCUSSION

Acromegaly represents a unique model for the evaluation of growth-promoting effects of increased concentrations of circulating GH and IGF-1 on the human colonic epithelium *in vivo*. The prevalence of (pre)malignant adenomatous lesions in the acromegalic patients in this study is in accordance with those observed in studies described previously (1–4). The present study shows that in patients with acromegaly, there is a relation between colonic epithelial cell proliferative activity and circulating GH and IGF-1 levels. The results strongly suggest that this is due to a direct stimulatory action of GH or IGF-1 or both on the colonic epithelium. The other proposed mechanism, *i.e.*, a GH-induced increase of secondary bile acids in the colon, seems to be only of marginal importance. Although there was a significant correlation between both GH and IGF-1 levels and proportional fecal concentrations of secondary bile acids, total excretion of these bile acid species and of total fecal bile acids were normal, as was duodenal bile acid composition. In addition, there was no correlation between any of the bile acid parameters and LI.

The increase in epithelial cell proliferative activity observed in this study is in agreement with the finding that the colonic mucosal activity of the enzyme ornithine decarboxylase, which also reflects mucosal cell proliferation, is increased in acromegalic patients with elevated IGF-1 levels (27). In some patients without or with only minimally increased GH or IGF-1 concentrations, the colonic epithelial cell proliferation was also elevated. It may well be that, although fasting GH and IGF-1 were (near) normal in these patients, an abnormal rhythm of secretion did occur. Besides an increased epithelial cell proliferation in whole crypts in these acromegalic patients, cell proliferation in the middle compartment was found to be particularly increased. Hyperproliferation and a shift of the proliferative compartment toward the lumen are cytokinetic abnormalities that frequently occur in the macroscopically normal-appearing mucosa of patients with colorectal neoplasia (16, 17, 28–30). Persistent hyperproliferation has been suggested to be of substantial significance in

colorectal carcinogenesis, since neoplastic transformation is facilitated in tissues with high proliferation (31, 32).

An alteration in the distribution of DNA-synthesizing cells toward the crypt surface has been proposed as a premorphological marker of an intrinsic risk of neoplastic transformation (29). It is a proliferative pattern also noted in adenomatous polyps (33). Thus, the increased epithelial cell proliferation and the alteration of the proliferative pattern, in all likelihood due to stimulatory actions of GH and IGF-1, probably form the basis of the increased prevalence of colonic adenomas and carcinomas in patients with acromegaly.

This study underscores the relevance of colonoscopic follow-up screening of acromegalic patients, even if the disease is clinically quiescent. Furthermore, this study suggests that treatment with recombinant GH may not be as harmless as it seems to be. The pharmacokinetics of exogenously administered recombinant GH do not resemble endogenous GH concentrations and may result in high plasma IGF-1 levels (34, 35). It has thus to be realized that current trends to administer recombinant human GH to GH-deficient adults (34) and even to healthy persons to delay the process of normal aging (36, 37) may carry a risk for the development of colon carcinomas. Our data indicate that these aspects deserve further attention.

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