CLINICAL AND PHYSIOLOGICAL ASPECTS OF ILEAL POUCH-ANAL ANASTOMOSIS

Effects on bile acid metabolism and gallbladder motility

Jan M.J.I. Salemans

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Een wetenschappelijke proeve
op het gebied van de Medische Wetenschappen

Proefschrift ter verkrijging van de graad van doctor aan
de Katholieke Universiteit Nijmegen, volgens besluit van
het College van Decanen in het openbaar te verdedigen
op vrijdag 1 december 1995 des namiddags om 1.30 uur precies

door

JOHANNES MARIA JOSEPHUS IGNATIUS SALEMANS
geboren op 16 september 1957 te Maastricht

Promotor:	Prof. Dr. J.B.M.J. Jansen
Co-Promotores:	Dr. F.M. Nagengast
	Dr. J.H. Kuijpers

Aan Viola en Max

The research presented in this thesis was performed at the Division of Gastroenterology and Hepatology (Head: Prof. Dr. J.B.M.J. Jansen), Department of Internal Medicine (Head: Prof. Dr. R.A.P. Koene), University Hospital Nijmegen, The Netherlands.

The investigations were supported by a grant from the Nederlandse Lever Darm Stichting.

The publication of this thesis was financially supported by: Astra BV, Byk BV, Glaxo BV, Janssen-Cilag BV, Pharmacia BV, SmithKline Beecham Farma, Tramedico BV, Yamanouchi Pharma BV, Zambon BV.

CIP-DATA KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Salemans, Johannes Maria Josephus Ignatius

Clinical and physiological aspects of ileal pouch-anal anastomosis : effects on bile acid metabolism and gallbladder motility / Johannes Maria Josephus Ignatius Salemans. - [S.l. : s.n.]
Thesis Katholieke Universiteit Nijmegen. - with ref. - With summary in Dutch.
ISBN 90-9008854-7
NUGI 742
Subject headings: ileal pouch-anal anastomosis / bile acids / gallbladder motility.

Omslagontwerp en boekverzorging:
Hans van Vugt [OptimaForma, Nijmegen]
Druk: Drukkerij SSN, Nijmegen
ISBN 90 9008854 7

Contents

GENERAL INTRODUCTION AND OUTLINE OF THE STUDIES

Backgrounds

Over the past decade proctocolectomy with ileal pouch-anal anastomosis has become the surgical treatment of choice for patients with ulcerative colitis or familial adenomatous polyposis. It enables the excision of the entire diseased colon with preservation of continence and avoidance of a permanent ileostomy. However, surgical techniques and understanding of physiology of ileal pouch surgery are continuing to evolve.

Ileal pouch surgery for ulcerative colitis and familial adenomatous polyposis started in 1983 in the Department of Surgery of the University Hospital Nijmegen. In a review published in 1992 (chapter 3) we were struck by the high incidence of pouchitis. In the literature several explanations for pouchitis are presented but none of these are entirely satisfactory. Among the explanations suggested, bile acid deconjugation as a result of bacterial overgrowth in the ileal pouch is often quoted. In view of the the combined expertise of the Departments of Gastroenterlogy and Surgery, we selected to study several aspects of bile acid metabolism in ileal pouch-anal anastomosis patients.

Introduction to bile acid metabolism

In man, the primary bile acids cholic and chenodeoxycholic acid are synthesized in the liver as the major end products of cholesterol metabolism, and secreted into bile as glycine or taurine conjugates. Conjugated bile acids play an imprtant role in solubilizing biliary cholesterol and digestion of dietary fat. Bile acids undergo effective enterohepatic ciculation: under normal conditions more than 95% is reabsorbed in the ileum per cycle. Only small amounts spill over into the colon and are deconjugated by the colonic bacterial flora. After deconjugation the primary bile acids cholic and chenodeoxycholic acid undergo bacterial 7α-dehydroxylation and are converted into the secondary bile acids deoxycholic and lithocholic acid respectively. Further bacterial and hepatic transformation results in the production of tertiary bile acids (Figure 1.1). Deoxycholic acid is passively reabsorbed by the colonic mucosa and takes part in the enterohepatic circulation. Lithocholic acid is insoluble at body temperature and is largely excreted via the faeces.

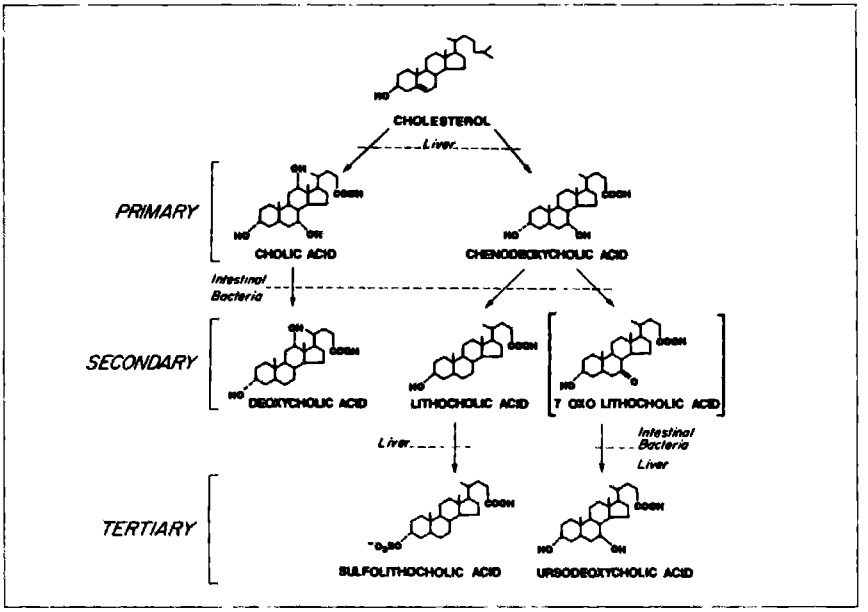

Figure 1.1 **Synthesis and transformation of the major bile acids.**

Outline of the studies

In Chapter 2 a review of the literature on the surgical and physiological aspects of proctocolectomy with ileal pouch-anal anastomosis is presented.

In Chapter 3 the immediate postoperative and long-term functional results of ileal pouch-anal anastomosis for ulcerative colitis and familial adenomatous polyposis are compared in all patients who underwent this procedure between 1983 and 1990 at the University Hospital Nijmegen.

In Chapter 4 the influence of ageing on fasting and postprandial conjugated and unconjugated serum bile acid levels is studied. In previous studies it has been suggested that reabsorption of conjugated bile acids is less effective in elderly subjects. Spill-over of conjugated bile acids into the colon may lead to an increased formation of secondary bile acids. Since secondary bile acids are co-mutagenic and enhance colonic mucosal proliferation, a decreased ileal bile acid reabsorption may contribute to an enhanced risk of colonic cancer in elderly subjects. In this study postprandial conjugated serum bile acid levels appeared to be decreased in elderly subjects. Therefore, subjects should be age-matched in studies comparing postprandial serum bile acid levels. An

impaired reabsorption of bile acids may theoretically lead to an increased stool frequency in elderly subjects who have undergone ileal pouch-anal anastomosis.

In Chapter 5 the usefulness of fasting individual unconjugated serum bile acid levels in predicting small intestinal bacterial overgrowth is evaluated. The results were compared to those obtained in healthy controls, and in patients with other malabsorptive states. Patients who had undergone ileal pouch-anal anastomosis or ileal resection were also included as control groups since these conditions may theoretically predispose to elevated unconjugated serum bile acid levels as a result of stasis in the ileal pouch and spill-over into the colon respectively.

In Chapter 6 the influence of proctocolectomy with ileal pouch-anal anastomosis on conjugated and unconjugated serum bile acid levels is evaluated. This study provides evidence that reabsorption of conjugated bile acids and biotransformation of primary into secondary bile acids is impaired after proctocolectomy with ileal pouch-anal anastomosis.

In animal studies it has been shown that colectomy may lead to elevated plasma cholecystokinin levels. This suggests that the colon contains a factor that inhibits the release of cholecystokinin from the proximal small intestine. In Chapter 7 plasma cholecystokinin levels and gallbladder volumes after proctocolectomy with ileal pouch-anal anastomosis are investigated in humans. This study showed that fasting and postprandial cholecystokinin levels are elevated after ileal pouch-anal anastomosis, and that fasting gallbladder volumes are decreased in these patients.

In order to examine whether ileal resection leads to alterations in bile acid absorption, deconjugation, 7α-dehydroxylation, and formation of ursodeoxycholic acid, we studied fasting and postprandial conjugated and unconjugated serum bile acid levels in patients who had undergone ileal resection for Crohn's disease. In addition, the effect of ileal resection on plasma cholecystokinin and gallbladder volume was evaluated since intraduodenal bile acids are known to modulate cholecystokinin release. This study, which is described in Chapter 8, provides further evidence that ileal resection leads to interruption of the enterohepatic circulation and that the formation of deoxycholic acid is greatly reduced, whereas the formation of ursodeoxycholic acid is increased in these patients. Ileal resection does not alter cholecystokinin release and gallbladder motility.

CLINICAL AND PHYSIOLOGICAL ASPECTS OF ILEAL POUCH-ANAL ANASTOMOSIS

Jan M.J.I. Salemans and Fokko M. Nagengast.
From the Division of Gastroenterology, Department of Medicine, University Hospital Nijmegen, The Netherlands.

Scand J Gastroenterol 1995; 30 Suppl 212: 3-12

2.1 Introduction

Proctocolectomy with ileal pouch-anal anastomosis (IPAA) is the surgical treatment of choice for severe chronic ulcerative colitis and familial adenomatous polyposis because the entire colonic mucosa is removed while anal function can be preserved and the necessity for permanent ileostomy is eliminated. Long-term functional results are generally gratifying since defecation frequency and degree of incontinence are acceptable in most patients. Pouchitis however, a non-specific inflammation of the ileal reservoir, is a major long-term complication occurring in a considerable number of patients. The etiology of pouchitis is unknown. Since pouchitis occurs more frequently, or even exclusively in ulcerative colitis patients it is supposed that pouchitis is a novel manifestation of inflammatory bowel disease. In this review the postoperative and long-term functional results, recent surgical developments, histological aspects, and physiological changes after pouch surgery are discussed.

2.2 Postoperative complications after ileal pouch-anal anastomosis

Although the mortality of proctocolectomy with IPAA is lower than 0.5% in the largest series (1-5), there is a substantial postoperative morbidity. The commonest major postoperative complications include anastomotic stricture, pelvic sepsis, fistula, and small bowel obstruction.

Anastomotic stricture — The reported incidence of anal strictures following IPAA varies between 8% and 15% (2,6,7). Most of the strictures respond favourably to a single dilatation. Repeated dilatations for recurrent strictures however, may result in incontinence. Recurrent strictures may increase the risk of pouchitis (8).

Pelvic sepsis — The incidence of pelvic sepsis following IPAA varies between 5% and 20% (1,3,7,9-11). Pelvic sepsis may result from anastomotic dehiscence or disruption of the suture line. Cuff abscess was the most common cause of pelvic sepsis at the time surgeons used to leave a rectal cuff through which the pouch was pulled down. Since the introduction of the stapled IPAA, which does not involve a rectal cuff, this complication has been

eliminated. Pelvic sepsis is the most common cause of pouch failure and results in pouch excision in approximately 5-10% of patients (7,11-13).

Pouch-vaginal and pouch-perianal fistula — The reported incidence of pouch-vaginal fistula varies between 7 and 11% (2,14,15). In a North-American multicentre study (14), 21 (7%) out of 304 women who underwent IPAA developed pouch-vaginal fistulas. Patients who had previously undergone subtotal colectomy for fulminant ulcerative colitis, or underwent IPAA on an urgent basis, as well as those who ultimately appeared to have Crohn's disease, and those who were operated without a temporary diverting loop ileostomy were at an increased risk of this complication. Twenty percent of these patients required pouch excision. In a study from St. Mark's Hospital in London, 17 (11%) out of 161 women developed a pouch-vaginal fistula(15). Postoperative pelvic sepsis and previous subtotal colectomy were associated with an increased risk. Eight patients (47%) required pouch excision. A variety of surgical procedures may be used to treat pouch-vaginal fistulas. Temporary ileal diversion alone is usually unsuccessful and should be combined with some sort of local treatment including transanal or transvaginal closure, sphincteroplasty, endoanal advancement flaps, or gracilis muscle interposition (14-16). However, the treatment of choice remains to be established. Fistulas from the ileal reservoir to the perianal area occur in approximately 5% of patients (2,17).

Small bowel obstruction — Small bowel obstruction is the most common early complication occurring in approximately 15-20% of patients undergoing IPAA (1,5,9,13,18,19). Approximately half of these patients require surgical intervention (20). The majority of episodes of small bowel obstruction occur after ileostomy closure.

2.3 Long-term results after ileal pouch-anal anastomosis

Functional results of IPAA tend to improve during the first year of follow-up, probably as a result of an adaptive process. The mean daily stool frequency declines from approximately 10 after one month to 7 after one year (4,5,13,21-23). Between 1 and 10 years after ileostomy closure stool frequency and rate of fecal incontinence remain unchanged (24).

The largest series with the longest follow-up to date on the quality of life

after IPAA has been reported by Kohler et al. (25) from the Mayo Clinic. They randomly selected 240 patients from 971 patients with chronic ulcerative colitis who had undergone IPAA between 1982 and 1989 (30 patients each year) for an assessment of their long-term functional results and quality of life. Hundred-sixty patients undergoing cholecystectomy in the same period (20 patients per year) served as controls. IPAA patients had more frequent stools and more fecal spotting than cholecystectomy patients. In spite of the altered bowel habits, 90% of IPAA patients had an excellent overall quality of life. Results were similar to patients who had undergone cholecystectomy. Moreover, quality of life and bowel habits remained steady in both groups of patients during the 8-year follow-up.

The presenting disease has a major impact on the incidence of postoperative complications and functional outcome. Several studies have compared the postoperative and long-term functional results of IPAA in patients with ulcerative colitis and familial adenomatous polyposis (5,8,13,26,27). The results of these studies are summarized in Table 2.1. Colitis patients tend to have a higher overall complication rate and more pouch-related septic complications. In most studies the long-term functional results are better in polyposis patients. Tjandra et al. (27) however, found very similar functional outcomes after IPAA in polyposis patients and ulcerative colitis patients. They studied 39 pairs of patients, individually matched for surgeon, technique of IPAA, duration of follow-up after pouch construction, age, and gender. The major difference between both groups was that pouchitis occurred more frequently in colitis patients than in polyposis patients.

2.4 Influence of pouch design on functional outcome

In an attempt to improve functional results after restorative proctocolectomy several pouch designs have been developed: the duplicated J-pouch, the rarely used lateral isoperistaltic pouch, the triplicated S-pouch, and the quadruplicated W-pouch. Nicholls and Pezim (28) compared functional outcome in 88 patients who had undergone IPAA with three different pouch designs (12 J-, 58 S-, and 18 W-pouches). Stool frequency was significantly higher in patients with J-pouches compared to those with W-pouches or S-pouches. Overall, there was an inverse relationship between reservoir volume and defecation frequency. J-pouches were significantly smaller than the other two designs. All patients with J- or W-pouches defecated spontaneously whereas

Table 2.1 **Comparison of postoperative and long-term functional results of IPAA for ulcerative colitis (UC) and familial adenomatous polyposis (FAP).**

study (reference)	*number of patients FAP vs UC*	*postoperative complications (%) FAP vs UC*	*stool frequency (per 24 hours) FAP vs UC*[3]	*nighttime incontinence (%) FAP vs UC*	*pouchitis (%) FAP vs UC*	*Conclusions, comments*
Becker and Raymond (5)	22 vs 78	overall: 13	4.4 vs 5.8[2]	?	0 vs 30[2]	Long-term results are better in FAP patients.
Dozois et al. (8)	94 vs 758	26 vs 29[1]	4.5 vs 5.8[2]	26 vs 40[2]	7 vs 22[2]	Long-term results are better in FAP patients, sepsis requiring reoperation more common in UC patients.
Salemans et al. (13)	21 vs 51	10 vs 25[1]	5 vs 7[2]	32 vs 43[1]	0 vs 44[2]	Long-term results are better in FAP patients.
Dayton et al. (26)	38 vs 239	similar in FAP and UC patients	4.2 vs 6.0[2]	5 vs 25[2]	0 vs 19[2]	Long-term results are better in FAP patients.
Tjandra et al. (27)	39 vs 39	21 vs 28[1]	6 vs 6[1]	51 vs 51[1]	10 vs 33[2]	FAP and UC patients have similar functional outcomes, but pouchitis is more common in UC patients.

1 = not significant
2 = $p<0.05$
3 = 1 year after ileostomy closure

only 41 percent of those with S-pouches did so. Sagar et al. (29) compared functional outcome between 20 patients with S- and 20 with W-pouches and found that the mean stool frequency in patients with W-pouches (3.5 per day) was significantly lower compared to those with S-pouches (6.0 per day). Patients with W-pouches had a greater efficiency of evacuation and their pouches were more capacious compared to those with S-pouches. In a study by Nasmyth et al. (30) defecation was less frequent in patients with S-pouches compared to patients with J-pouches. However, de Silva et al. (31) found no significant differences in stool frequency, degree of continence or urgency between 23 patients with J-pouches, 15 with S-pouches, and 23 with W-pouches, although patients with J-pouches required antidiarrhoeal medication more frequently compared to those with S- or W- pouches. Only 7 out of 15 patients with S-pouches could defecate spontaneously compared to 22 with W-pouches and all patients with J-pouches. Keighley et al. (32) compared 33 consecutive patients who were randomly allocated to reconstruction using a stapled J-pouch or a sutured W-pouch. The operation time was significantly shorter for J-pouches compared to W-pouches. The median daily stool frequency was similar in both groups. There was no incontinence, urgency or soiling in both groups.

In conclusion, the results of studies comparing the functional results of the different pouch designs are conflicting. However, stool frequency after IPAA seems to be inversely related to pouch capacity and tends to be lower with S- and W-pouches. On the other hand, operation time is longer and operative technique more complicated with the sutured S- and W-pouches compared to the stapled J-pouch. Spontaneous evacuation is impaired in a considerable percentage of patients with S-pouches. For these reasons the J-pouch is the most widely applied design.

2.5 Subtotal colectomy prior to ileal pouch-anal anastomosis

IPAA is usually carried out in two stages: the first operation consists of proctocolectomy, construction of the pouch, and a temporary diverting loop ileostomy. In the second operation, after a few months, the ileostomy is closed. However, IPAA is sometimes performed as a delayed procedure after subtotal colectomy with either ileorectal anastomosis (in polyposis patients), Hartmann's procedure and ileostomy, or ileostomy and sigmoidostomy (in ulcerative colitis patients), prior to proctectomy and construction of the pouch.

Cases of Crohn's disease presenting as 'indeterminate' colitis, can be recognized and excluded from pouch surgery. There has been debate as to whether delayed IPAA results in a lower postoperative complication rate and superior functional results, especially in malnourished patients with active ulcerative colitis receiving a high dose corticosteroids. A three stage procedure however, carries a number of disadvantages, including an increased number of operations, a longer hospital stay, and a higher cost.

Recent studies comparing the outcome of two-stage and three-stage IPAA are summarized in Table 2.2 (9,33-35). Galandiuk et al. (33) found that previous subtotal colectomy does not result in a decrease in postoperative complications overall, and that there is no functional advantage compared with the two-stage procedure. Therefore, they concluded that, in experienced hands, two-stage IPAA is the preferred approach in most patients with ulcerative colitis. However, this subject remains controversial, since Penna et al. (35) found lower rates of postoperative complications with the three-stage procedure. The functional outcome was similar in both groups. Since subtotal colectomy prior to IPAA did not comprise the results of this procedure, they argued that the three-stage procedure is valuable in a subset of patients, including those with steroid-resistant colitis.

In our experience (13), patients who had undergone subtotal colectomy prior to the ileal pouch procedure were at high risk for pouch failure. When all patients who had undergone subtotal colectomy prior to IPAA (n=27) were compared to the patients with a two-stage IPAA (n=45) the number of excised pouches was 11 (41%) and 1 (2%) respectively ($p<0.001$).

In conclusion, the results of these studies are somewhat contradictory, but they suggest that there is no advantage of subtotal colectomy prior to IPAA in patients who can be operated electively and when Crohn's disease can be excluded. Functional results are similar after two- and three-stage IPAA.

Table 2.2 **Studies comparing the outcome of IPAA for ulcerative colitis, carried out as a two-stage (without subtotal colectomy) or three-stage procedure (with prior subtotal colectomy).**

study (reference)	*type of study*	*number of patients two- vs three-stage*	*complications (%) requiring reoperation two- vs three-stage*	*conclusions, comments*
Nicholls et al.[1] (9)	prospective, non-randomized	57 vs 95	NS[2]; overall complication rate: 49% vs 51%[3]	Rates of pelvic sepsis and bowel obstruction similar in both groups; similar functional long-term results in both groups.
Galandiuk et al. (33)	retrospective, non-randomized	776 vs 95	no difference in reoperation rate	Rates of pelvic sepsis higher and bowel obstruction lower with the three-stage procedure; similar functional results in both groups.
Zenilman et al. (34)	retrospective, non-randomized	22[4] vs 25	5% vs 24%[3]	Previous colectomy associated with a greater incidence of postoperative small bowel obstruction; similar functional results.
Penna et al. (35)	prospective, non-randomized	78 vs 55	11.5% vs 1.8%[5]	Rates of postoperative complications lower with the three-stage procedure, similar functional long-term results in both groups.

1 = this study included both ulcerative colitis and familial adenomatous polyposis patients
2 = not stated
3 = not significant
4 = these patients had a history of previous abdominal surgery for unrelated disease and underwent a classical two-stage procedure
5 = $p<0.05$

Handsewn versus stapled ileal pouch-anal anastomosis — The classical pelvic pouch procedure (36,37) included transanal mucosectomy, a long rectal muscular cuff and a handsewn ileoanal anastomosis. In order to improve functional outcome and to reduce the incidence of postoperative anastomotic complications the stapled IPAA was introduced (38). This procedure avoids the time consuming mucosal proctectomy and leaves 1-2 cm of rectal mucosa (transitional zone) proximal to the dentate line to facilitate the ileoanal anastomosis.

Several groups have compared the clinical outcome of handsewn IPAA with mucosectomy versus stapled IPAA without mucosectomy (10,12,39). Wettergren et al. (12) studied 144 consecutive patients who underwent either handsewn (n=96) or stapled (n=48) J-pouch ileoanal anastomosis. The percentage of anastomotic leaks was similar in both groups (13% versus 15%) as was the percentage of pouches that were removed because of postoperative complications (5% versus 6%) but the incidence of anastomotic stenosis was significantly higher in patients with a handsewn anastomosis (23%) compared to those with a stapled anastomosis (6%). Two prospective, randomized studies (10,39) also showed a similar complication rate and similar functional results after either handsewn IPAA with mucosectomy or stapled IPAA without mucosectomy. The authors of these studies concluded that double-stapled IPAA does not offer any functional or technical advantage over hand-sutured anastomosis and they preferred full mucosectomy since the complete diseased colonic mucosa is removed.

The risk of cancer in the small segment of remaining rectal (transitional) mucosa after stapled IPAA does not seem to be very high, but follow-up is too short to draw firm conclusions. Persisting disease activity, dysplasia, and cancer in the remaining rectal mucosa have been documented in a few patients (40,41). Schmitt et al. (42) studied the incidence of inflammation and dysplasia in retained rectal mucosa after double-stapled IPAA for ulcerative colitis. They evaluated 56 patients who had undergone double-stapled IPAA with a mean of 1 cm of rectal epithelium left above the dentate line. No evidence of dysplasia was found in any of the biopsy specimens just above the dentate line, and the distal resection line revealed active ulcerative colitis in 19 (35%) patients. Only one of these patients experienced symptoms referable to active colitis.

Regeneration of rectal mucosa after rectal mucosectomy and IPAA could

not be demonstrated in pathologic specimens obtained from patients who had required pouch excision (43).

Temporary diverting ileostomy — Until recently a temporary defunctioning loop ileostomy has been employed routinely in patients undergoing IPAA in order to reduce the risk of anastomotic leakage and pelvic sepsis. However, the ileostomy itself and its closure may be a source of significant complications (13,44-50). Moreover, ileostomy closure prolongs total hospital stay (44,45).

Table 2.3 summarizes a number of studies comparing clinical outcome of IPAA with and without temporary diverting ileostomy. Cohen et al. (46) compared the surgical complication rate and outcome of 483 consecutive patients who had undergone IPAA performed with or without loop ileostomy. Three-hundred-twenty-five patients had a handsewn anastomosis with ileostomy, 87 had a stapled IPAA with ileostomy, and 71 patients had a stapled anastomosis without ileostomy. The rate of anastomotic leakage was significantly reduced in patients with a stapled IPAA with ileostomy compared with those with a handsewn anastomosis and ileostomy. The omission of the ileostomy was associated with a higher incidence of anastomotic leaks, but spontaneous healing occurred in almost all patients. Patients on steroids and patients who had undergone a true one-stage procedure, had a greater risk of anastomotic leakage. Functional results were excellent in all groups, even in the patients who had had an anastomotic leak.

In a prospective randomized study Grobler et al. (44) assessed the role of temporary ileostomy in patients receiving a stapled IPAA. Patients using steroids were excluded. The incidence of anastomotic leakage, pelvic sepsis, bowel obstruction and pouchitis were similar in patients with or without temporary ileostomy. Approximately half of the patients in the ileostomy group developed ileostomy-related complications and total hospital stay was longer with ileostomy.

In a non-randomized way Sagar et al. (45) similarly compared the clinical outcome after stapled IPAA with or without temporary ileostomy. In this study the use of high dose corticosteroids was no contraindication to ileostomy omission. The decision for or against temporary ileostomy was made during the procedure based on urgency, toxaemia, anastomotic tension, and integrity of the anastomoses. Avoidance of the ileostomy did not lead to an increased incidence of pelvic sepsis. The total hospital stay (including the hospitalization for ileostomy closure) was significantly reduced in the group of patients without an ileostomy.

In a study by Tjandra et al. (51) anastomotic leakage, pelvic abscess, and septic complications requiring relaparotomy were more common after IPAA without ileostomy, compared to a carefully matched control group of patients with ileostomy. Patients using high dose corticosteroids had an increased risk of complications. Functional results were similar in both groups. Sugerman and Newsome (52) however, found fewer acute complications and better stool control in patients with a stapled IPAA without ileostomy compared to a control group of patients with mucosectomy, handsewn IPAA, and temporary ileostomy.

In conclusion, omitting a temporary loop ileostomy does probably not lead to an increased number of pouch failures, at least in a group of well selected patients. In general, functional results are similar in patients who are operated without ileostomy compared to those without ileostomy. Whether the use of corticosteroids at the time of surgery increases the risk of anastomotic leaks is still matter of debate since data are scarce and conflicting. A temporary loop ileostomy itself is associated with a high incidence of complications and prolonged hospital stay.

2.7 Ileal pouch-anal anastomosis in 'indeterminate colitis' and Crohn's colitis

IPAA is generally considered contraindicated in patients with Crohn's disease because of the high risk of pelvic sepsis, fistulas, and pouchitis. However, preoperative distinction between ulcerative colitis and Crohn's colitis may be difficult, if not impossible. Occasionally, macroscopic and microscopic features of both ulcerative colitis and Crohn's colitis occur in the same patient. In such cases of 'indeterminate colitis', it is difficult to decide whether IPAA should be performed.

In a retrospective analysis of 514 colitis patients who had undergone IPAA, Pezim et al. (54) compared the outcome of 25 (5%) patients who had features of indeterminate colitis with that of 489 ulcerative colitis patients. No significant differences in complication rate, pouch function, incidence of pouchitis, or pouch excision rate were found between the two groups.

The outcome of IPAA in patients who were operated upon for presumed ulcerative colitis but ultimately were found to have Crohn's disease has been described by several groups. Hyman et al. (55) reviewed the records of 362 ileal pouch patients with a preoperative diagnosis of ulcerative colitis and

Table 2.3.1 **Studies comparing clinical outcome of IPAA with and without temporary diverting ileostomy.**

study (reference) year of publication	*type of study*	*number of patients with vs without diverting ileostomy*	*type of operation with vs without diverting ileostomy*	*use of steroids with vs without diverting ileostomy*	*incidence of pouchitis with vs without ileostomy*	*anastomotic leaks and pelvic sepsis with vs without ileostomy*
Jarvinen and Luukkonen (53) 1991	retrospective, non-randomized	15 vs 16	HSA/HSA	?	20% vs 5%[1]	0% vs 6%[1]
Grobler et al. (44) 1992	prospective, randomized	23 vs 22	SA/SA	patients on steroids were excluded	9% vs 23%[1]	4% vs 5%[1]
Sagar et al. (45) 1992	retrospective, non-randomized	28 vs 30	SA/SA	29% vs 33%	?	10% vs 11%[1]
Cohen et al. (46) 1992	retrospective, non-randomized	71 vs 87	SA/SA	?	?	7% vs 18% [2]
Tjandra et al. (51) 1993	non-randomized	50 vs 50	SA/SA	40% vs 40%	18% vs 22%[1]	4% vs 14%[2]
Sugerman and Newsome (52) 1994	retrospective, non-randomized	63 vs 68	HSA/SA	? vs 63%	28% vs 31%[1]	33% vs 15%[2]

HSA = handsewn IPAA with mucosectomy, SA = stapled IPAA without mucosectomy, ? = not stated,
1 = not significant, 2 = $p<0.05$

Table 2.3.2 **Conclusions, comments.**

Jarvinen and Luukkonen (53) 1991	Incidence of complications and functional results similar in both groups, hospital stay shorter without ileostomy.
Grobler et al. (44) 1992	Incidence of complications and functional results similar in both groups, hospital stay shorter without ileostomy.
Sagar et al. (45) 1992	Incidence of complications similar in both groups, hospital stay shorter in patients without ileostomy.
Cohen et al (46) 1992	Higher incidence of anastomotic leaks in patients without ileostomy and in those taking steroids.
Tjandra et al. (51) 1993	Higher incidence of anastomotic leaks in patients taking steroids, functional results similar in both groups.
Sugerman and Newsome (52) 1994	Incidence of complications and functional results similar in both groups, hospital stay shorter without ileostomy.

analyzed the outcome of 25 (7%) patients who were postoperatively proven to have Crohn's disease. Sixteen patients had a functioning pouch, seven had required pouch excision, one was diverted, and one had died. In a subgroup of nine patients in which there was a clinical feature suspicious for Crohn's disease preoperatively, eight patients had their pouch removed ($p<0.01$). Deutsch et al. (56) reported on 9 (3%) out of 272 patients who appeared to have Crohn's disease. Four patients had eventually their pouches removed, five patients had functioning pouches: three with no complications and two with persistent perianal disease. Grobler et al. (57) found a marginally higher complication rate in 20 patients with pathological features of Crohn's disease. Pouch excision or a persistent stoma was necessary in 30% of patients with Crohn's disease compared to 15% in patients with definite ulcerative colitis. However, functional results were acceptable if the pouch could be retained. These data demonstrate that patients with Crohn's colitis have an increased risk of complications and pouch failure. Therefore, IPAA should not knowingly be performed in these patients.

2.8 Pouchitis

Ileal pouch inflammation or pouchitis is one of the major long-term complications of IPAA. Clinically, pouchitis is characterized by increased stool frequency, bleeding, abdominal pain, and systemic symptoms such as fever,
26 arthralgia, fatigue and weight loss. There is much confusion surrounding pouchitis because the syndrome has not been tightly defined. The reported incidence of pouchitis varies widely between 7% and 42% (1,4,5,8,13,58-65). This variation can partly be explained by the use of different criteria (endoscopic and histologic confirmation) to establish the diagnosis (Table 2.4). Therefore, it has been proposed that the definition of pouchitis should include endoscopic (increased vascularity, bleeding, ulceration) and histopathological (acute inflammation, ulceration, chronic changes) criteria (66-68). The incidence of pouchitis tends to increase with prolonged follow-up, as is shown in successive reports from the Mayo Clinic (1,58,65) (Table 2.5).

Table 2.4 **Incidence of pouchitis.**

study	*number of patients*	*incidence of pouchitis*	***diagnosis*** *clinical*	*endoscopy*	*biopsy*
Becker and Raymond (5)	100	18 (18%)	+	+	-
Salemans et al. (13)	72	19 (30%)	+	+	+
Dayton et al. (26)	277	45 (16%)	+	-	-
Tjandra et al. (27)	78	17 (22%)	+	-	-
de Silva et al. (31)	61	13 (21%)	+	+	+
Lohmuller et al. (58)	734	212 (29%)	+	-	-
Rauh et al. (69)	215	30 (14%)	+	+	-
Mc Mullen et al. (70)	73	11 (15%)	+	-	-
Schoetz et al. (71)	104	7 (7%)	+	-	-
Fonkalsrud et al. (72)	145	34 (23%)	+	-	-
Penna et al. (73)	41	0 (0%)[1]			

[1] = this study included only familial adenomatous polyposis patients

The etiology of pouchitis is unknown. It has been suggested that pouchitis is the result of bacterial overgrowth, particularly of anaerobic bacteria, secondary to stasis in the ileal pouch (74-77). The generally satisfactory response to treatment with metronidazole supports this hypothesis. However, bacterial

overgrowth alone is probably not sufficient to explain pouchitis, since bacterial overgrowth is present in virtually all pouches. Moreover, quantitative cultures of pouch effluents did not show higher bacterial counts in patients with pouchitis compared to those without pouchitis (74,78,79).

Ruseler-van Embden et al. (80) recently investigated the composition of ileal reservoir microflora in patients with and without pouchitis. An increased number of aerobes and a decreased ratio of anaerobes to aerobes in patients with pouchitis was found compared to those without pouchitis. Since anaerobes are largely responsible for the production of short-chain fatty acids, this microbial imbalance may explain the markedly decreased amounts of short-chain fatty acids in output from patients with pouchitis (81). Short-chain fatty acids, especially butyrate, are considered to be the major source of energy for colonic epithelium. Since the pouch epithelium undergoes colonic metaplasia (64,78), lack of short-chain fatty acids might result in mucosal damage. Irrigation of the pouch with short-chain fatty acids has shown to be beneficial in patients with pouchitis (82).

Several authors have suggested that pouchitis is a novel manifestation of inflammatory bowel disease persisting after total colectomy with IPAA (58,64,66,83-85). In most large series (8,58) pouchitis is more common in patients with ulcerative colitis than in those with familial adenomatous polyposis, and indeed may well be restricted to colitis patients (5,13) (Table 2.6). Only a few cases of pouchitis in familial polyposis patients have been reported by the Mayo Clinic (8,58) but these cases were poorly documented since endoscopy and histological confirmation was not performed in these patients. The presence of backwash ileitis in ulcerative colitis patients does not seem to predispose to later development of pouchitis, and an inflamed terminal ileum is not considered a contraindication for IPAA in ulcerative colitis patients (86).

Table 2.5 **Increasing incidence of pouchitis with prolonged follow-up. The Mayo Clinic experience.**

study	*year of publication*	*number of patients*	*incidence of pouchitis*
Metcalf et al. (65)	1985	188	15 (8%)
Pemberton et al. (1)	1987	390	55 (14%)
Lohmuller et al. (58)	1990	734	212 (29%)

Pouchitis is associated with the development of conditions such as arthritis, iridocyclitis, erythema nodosum, and pyoderma gangrenosum, which are characteristic extracolonic manifestations of ulcerative colitis (58,83,87). These findings suggest that immunological mechanisms play a role in the pathogenesis of pouchitis. Lohmuller et al. (58) found that patients with extraintestinal manifestations of inflammatory bowel disease are at higher risk of pouchitis than patients who never had extraintestinal manifestations. This may have implications for the selection of colitis patients for IPAA.

Transient mucosal ischemia may cause oxygen-derived free radical production by xanthine oxidase, precipitating pouchitis after IPAA. Therefore, Levin et al. (88) studied the effect of allopurinol, a xanthine oxidase inhibitor, in patients with acute and chronic pouchitis. Acute pouchitis resolved promptly in four of eight patients. Seven of the 14 patients with chronic pouchitis responded completely with no recurrence of symptoms during treatment. Thus, allopurinol either terminated an episode of acute pouchitis or prevented pouchitis from recurring in 50 percent of patients. These data support a role for mucosal ischemia and oxygen free radical production in the etiology of pouchitis.

Pouchitis usually responds favourably to treatment with metronidazole orally or rectally. However, recurrence rates are as high as 60% (31,58). Successful treatment of pouchitis with sulfasalazine (69), topical 5-aminosalisylic acid (89), topical or systemic steroids (31,69), and short-chain fatty acids irrigation (82) has been reported in patients who do not respond to metronidazole. However, until now, no controlled trials comparing medical treatments have been reported.

In conclusion, pouchitis is a major long-term complication after IPAA. Since pouchitis is probably confined to patients operated upon for ulcerative colitis, it is likely that pouchitis is a novel manifestation of inflammatory bowel disease. Luminal and microbiological factors may play a supplementary role in its pathogenesis.

2.9 Histopathological alterations in ileal pouches

Shepherd et al. (64) studied mucosal biopsy specimens from the ileal reservoirs of 92 patients who had undergone restorative proctocolectomy. Chronic inflammation (infiltration with lymphocytes and eosinophils) was found in

almost all, as was villous atrophy of varying severity. Acute inflammatory changes (infiltration with polymorphs) and ulceration were associated with pouchitis. The severity of acute inflammation was increased in ulcerative colitis patients compared to those with familial polyposis and pouchitis was present only in patients who had had ulcerative colitis. Although the mucosa of some ileal pouches acquire certain colonic characteristics, complete colonic metaplasia does not occur (90). The proportions of epitheloid cells and tingible body macrophages have been found to be increased in pouches with pouchitis compared to pouches without pouchitis or normal ileum (91). Since an increase of these macrophage subpopulations are characteristic of inflammatory bowel disease, their presence in pouchitis suggests that ulcerative colitis and pouchitis have similar pathogenetic mechanisms.

2.10 Sexual function and pregnancy

The most common sexual complication in males after IPAA is retrograde ejaculation, which occurs in 1-10% of men, whereas impotence has been reported in up to 1.5% (5,61,92,93).

Sexual function in women after restorative proctocolectomy has been studied by Metcalf et al. (94). Fifty women with a continent ileostomy (Kock's pouch) and 50 women with IPAA were interviewed regarding their preoperative and postoperative sexual function. Frequency of intercourse increased and the incidence of dyspareunia decreased after operation in both groups. Only one patient in each group reported a postoperative disturbance in ability to achieve orgasm. Overall, the majority of women in this study experienced enhanced sexual function after operation, which they attributed mainly to improved health.

Nelson et al. (95) described 20 women who underwent IPAA and subsequently had at least one successful pregnancy and delivery. Eleven deliveries were vaginal with episiotomy, and nine were cesarean sections. No maternal deaths occurred. The frequency of nocturnal stooling increased in the IPAA patients during pregnancy, and the increase persisted for three months after delivery. In contrast, the frequency of daytime stools and the incidence of incontinence were not greatly altered by pregnancy or delivery. Moreover, postpartum pouch function was not influenced by the type of delivery. Therefore, normal pregnancy appears to be possible after IPAA. However, the route of delivery should be individualized in these patients.

Bile acid metabolism — The fecal bile acid output is increased in ileal pouch patients compared to healthy, non-colectomized volunteers (96,97). Retention of ^{75}Se-taurohomocholate (SeHCAT) is decreased in these patients compared to nonoperated colitis patients (98) or healthy controls (99,100). Postprandial conjugated bile acid levels increase to a lower extent in ileal pouch patients compared to healthy subjects (101). Therefore, reabsorption of bile acids is impaired after IPAA. Bile acid malabsorption might be expected in these patients for several reasons. Firstly, the mucosa of ileal pouches show histologic signs of inflammation in the vast majority of patients (66). Secondly, reabsorption of bile acids may be impaired since the relative mucosal surface of the terminal ileum is smaller after construction of a reservoir. Finally, stasis in the ileal pouch may lead to deconjugation of bile acids as a result of bacterial overgrowth. Bile acid malabsorption may lead to alterations in bile composition and saturation index. Therefore, the risk of gallstone formation may be increased in ileal pouch patients. To assess the influence of colectomy on bile composition and saturation index Harvey et al. (102) collected bile samples at the time of abdominal surgery in patients with ulcerative colitis before or after colectomy. The precolectomy group consisted of 17 patients who were sampled at the time of colectomy. The postcolectomy group consisted of 11 patients who had undergone (sub)total colectomy previously (and were operated upon for conversion from conventional ileostomy to a pelvic pouch or other reasons). The bile composition in the precolectomy group was similar to control patients without gallstones, and few had crystals in their bile. In the postcolectomy group, cholesterol concentrations were very high, all biles were supersaturated, and almost all patients had cholesterol crystals in their bile. However, to date there is still no evidence that patients with an ileal pouch are at greater risk for cholelithiasis.

Water and electrolyte balance — Changes in water and sodium balance after IPAA are similar to those after conventional ileostomy. Santavirta et al. (103) studied water and electrolyte balance in 30 patients with IPAA, 10 patients with conventional ileostomy, and 9 nonoperated patients with quiescent ulcerative colitis. Daily urinary excretion of sodium in nonoperated patients was significantly higher than in patients with an ileal pouch or conventional ileostomy. Daily fecal weight, urinary volume, and urinary excretion of sodium were similar in patients with IPAA and conventional ileostomy. Using tri-

tiated water and a bromide dilution technique, Christie et al. (104) showed that the body content of water and extracellular fluid are normal in patients with IPAA.

Malabsorption — Hylander et al. (105) found moderate steatorrhoea in approximately 30% of patients three months after ileostomy closure, but fecal fat excretion normalized with time. The absorption of carbohydrates, amino acids, and bile acids by the ileal pouch mucosa after proctocolectomy with IPAA has been found to be markedly decreased compared to normal ileum (106). Impaired intestinal absorption of D-xylose and low serum iron levels have been reported in ileal pouch patients. Nevertheless, clinical signs of malnutrition or malabsorption rarely occur in these patients (100). Low vitamin B_{12} levels and decreased Schilling tests have been found in IPAA patients (78,97,105). Bacterial overgrowth in the pouch may contribute to vitamin B_{12} malabsorption, since some microbial species utilize dietary vitamin B_{12} from the host.

Motility of the small intestine — Soper et al. (107) found that small bowel transit is markedly slowed in most patients after proctocolectomy with IPAA compared to conventional ileostomy or healthy non-colectomized subjects. However, gastric emptying of liquids is not altered in these patients (107). Installation of oleic acid in the ileal pouch slows gastrointestinal transit and increases plasma levels of peptide-YY, neurotensin and enteroglucagon (108). These hormones are believed to play a role in the adaptive response after large bowel resection. Basal and postprandial plasma levels of peptide-YY have been reported to be increased after IPAA compared to healthy controls (109). Infusion of peptide-YY induces a dose related inhibition of mouth to caecum intestinal transit time and of the rate of gastric emptying (110). These data suggest that peptide-YY may play a major role in the adaptive response of the intestine to proctocolectomy with pouch construction.

Fasting and postprandial plasma cholecystokinin levels are elevated and fasting gallbladder volumes are decreased after proctocolectomy with IPAA in humans (111). These findings suggest that the colon contains a factor that inhibits the release of cholecystokinin.

2.12 General conclusions

Proctocolectomy with IPAA has become the treatment of choice for severe ulcerative colitis and familial adenomatous polyposis. The procedure should not knowingly be performed in patients with Crohn's colitis. The procedure

carries a low mortality but a considerable morbidity. The surgical procedure has become less complicated and less time consuming since the introduction of stapling devices. However, the complication rate has not declined apparently. Without mucosectomy some transitional and rectal mucosa is left behind and uncertainty remains as to whether these patients are at risk of dysplasia and cancer. Using the stapling techniques, omission of the temporary loop ileostomy probably does not increase the number of pouch failures, at least in a group of selected patients. Pouchitis, occurring in approximately 30% of ulcerative colitis patients, is the most frequent late complication and may lead to pouch excision.

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POSTOPERATIVE AND LONG-TERM RESULTS OF ILEAL POUCH-ANAL ANASTOMOSIS FOR ULCERATIVE COLITIS AND FAMILIAL ADENOMATOUS POLYPOSIS

Jan M.J.I. Salemans, Fokko M. Nagengast, Evert-Jan C. Lubbers*, Han J.H. Kuijpers*.
From the Division of Gastroenterology, Department of Medicine, and the *Department of Surgery, University Hospital Nijmegen, The Netherlands.

Dig Dis Sci 1992;37:1882-1889

3.1 Abstract

The immediate postoperative and long-term functional results of 51 ulcerative colitis patients and 21 familial adenomatous polyposis patients who underwent ileal J-pouch anal-anastomosis (IPAA) were compared in this study. The incidence of postoperative complications requiring reoperation was not statistically different in both groups. The mean daily stool frequency was significantly higher in colitis patients. Pouchitis occurred in 44% of colitis patients but not in polyposis patients ($p < 0.005$). Symptoms of pouchitis included bloody diarrhea, urgency, abdominal pain, weight loss, fever and arthritis. Six colitis patients required pouch excision because of intractable pouchitis. The overall pouch excision rate was 22% in ulcerative colitis patients and 5% in familial adenomatous polyposis patients ($p = 0.16$). Patient satisfaction was good in 46% of ulcerative colitis patients and 76% of polyposis patients ($p < 0.05$). Our data demonstrate that the long-term outcome of IPAA is more favourable in polyposis patients than in colitis patients. Pouchitis is a major long-term complication occurring exclusively in colitis patients.

3.2 Introduction

Total colectomy and ileal pouch-anal anastomosis (IPAA) is an attractive surgical alternative for colectomy and permanent ileostomy in patients with chronic ulcerative colitis and familial adenomatous polyposis because the entire colonic mucosa is removed while anal function can be preserved and the necessity for permanent ileostomy is eliminated (1,2). Long-term functional results are generally gratifying as defecation frequency and degree of incontinence is acceptable in most patients. Pouchitis however, a nonspecific inflammation of the ileal reservoir, is a major long-term complication occurring in 8-44% of patients (3-15). Symptoms of pouchitis include bloody diarrhea, urgency of defecation associated with abdominal cramps, malaise, and occasionally fever and arthritis. Little is known about the pathogenesis of pouchitis. It has been suggested that pouchitis is the result of bacterial overgrowth in the ileal pouch (4-6). The generally satisfactory response to treatment with metronidazole supports this hypothesis. Bacterial overgrowth however, is probably not the sole etiological factor because pouchitis occurs less frequently in familial adenomatous polyposis patients than in ulcerative

colitis patients (4-6). Therefore several authors have suggested that pouchitis is a novel manifestation of inflammatory bowel disease persisting after total colectomy with IPAA (5,16-21).

It is still a matter of debate whether to perform subtotal colectomy with ileorectal anastomosis or total colectomy with IPAA in patients with familial adenomatous polyposis. The risk of cancer in the rectal stump after ileorectal anastomosis is approximately 10 percent and makes indefinite proctoscopic screening necessary (22). Still many surgeons prefer ileorectal anastomosis in familial adenomatous polyposis arguing that it is less prone to complications and provides better long term results. These arguments are usually based on results achieved in series of IPAA performed in ulcerative colitis patients. However, results of the procedure are probably better in polyposis than in colitis because polyposis patients are usually younger and in a better physical condition at the time of proctocolectomy than colitis patients. Moreover, polyposis patients are less prone to pouchitis. The aim of this study was to compare the immediate postoperative and long term results of IPAA in patients with ulcerative colitis and familial adenomatous polyposis and to evaluate the occurrence, symptoms, and outcome of pouchitis.

3.3 Patients and methods

3.3.1 PATIENTS

Between July 1983 and May 1990, 72 patients underwent IPAA for either ulcerative colitis (51 patients) or familial adenomatous polyposis (21 patients) at the Nijmegen University Hospital. Forty-four of the patients were male; 28 were female. The mean age (SD) at the time of the ileal pouch procedure was 34 (13) years (range 10-61 years) in the ulcerative colitis patients and 27 (11) years (range 10-55 years) in the polyposis patients ($p < 0.05$). Twenty-seven (38%) patients had undergone subtotal colectomy with ileorectal anastomosis (4 colitis and 4 polyposis patients) or ileostomy (18 colitis patients and one polyposis patient) prior to the construction of the ileal pouch. In the remaining 45 patients the initial operation included abdominal colectomy, mucosal proctectomy, and endorectal IPAA. All ulcerative colitis patients except those who had undergone colectomy with ileostomy (33 patients) but none of the polyposis patients used corticosteroids at the time of the ileal pouch procedure.

3.3.2 SURGICAL PROCEDURE

In all patients a 'J' reservoir was constructed. The pouch was created by folding the terminal ileum back on to itself and anastomosing the limbs side to side. The rectal mucosa was removed from the rectal stump down to the dentate line via a transanal approach. The ileal pouch was extended into the pelvis endorectally and its apex opened and sutured circumferentially to the dentate line. In all patients a temporary loop ileostomy was established. At a second operation the temporary ileostomy was closed and ileal continuity reestablished. The mean interval (SD) between construction of the IPAA and ileostomy closure was 6.1 ± 4.4 months in colitis patients and 4.4 ± 3.9 months in polyposis patients ($p < 0.01$).

3.3.3 ASSESSMENT OF RESULTS

Immediate postoperative data included mortality and morbidity requiring reoperation within 30 days after IPAA and ileostomy closure. Follow-up data included stool frequency, degree of incontinence, use of loperamide, occurrence of pouchitis, social functioning, and patient satisfaction. The records of all patients were studied retrospectively in June 1991. Patients with incomplete follow-up data were contacted by telephone for answers to a follow-up questionnaire. Stool frequency per 24 hours and per night was an estimate by the patients of the average number of bowel movements and was recorded 1, 3, 6, 12 and 24 months after ileostomy closure. Incontinence was defined as involuntary loss of mucus or stool requiring a perineal pad. The presence of incontinence during the day and night and use of loperamide were recorded 12 months after ileostomy closure. Pouchitis was defined as episodes with abdominal cramping, bloody diarrhea, increased stool frequency, urgency, malaise, and/or fever associated with endoscopic and histologic signs of acute inflammation. Endoscopic signs of inflammation included mucosal hyperaemia with loss of vascular pattern with or without ulceration. Histological signs of acute inflammation were significant neutrophil infiltration and ulceration (18). Patients whose ileostomies were closed were asked whether they were able to work full-time or not and whether they preferred the pouch to the ileostomy.

3.3.4 STATISTICAL ANALYSIS

Proportions were analyzed by chi-square tests with Yates's modification when appropriate. Comparisons of continuous variables were made using Student's t test or, when appropriate, the rank-sum test. The risk of pouchitis

in the colitis and polyposis groups was estimated using the Kaplan-Meier life table analysis. The two actuarial curves were compared using the log-rank test. A p-value < 0.05 was considered statistically significant.

3.4 Results

3.4.1 IMMEDIATE POSTOPERATIVE RESULTS

One polyposis patient died of sepsis after the ileal pouch procedure and one colitis patient died of a cardiac arrhythmia after ileostomy closure. After the initial operation 13 (25%) ulcerative colitis patients and 2 (10%) polyposis patients required one or more laparotomies because of postoperative complications. After ileostomy closure 13 (30%) colitis patients and four (20%) polyposis patients needed reoperation (Table 3.1). These differences were not statistically significant. Small bowel obstruction was the most frequent complication requiring relaparotomy after both the ileal pouch procedure and ileostomy closure.

Table 3.1 **Postoperative complications requiring reoperation in ulcerative colitis (UC) and familial adenomatous polyposis (FAP) patients after the ileal pouch procedure (IPAA) and after ileostomy closure.**

	IPAA			Ileostomy closure	
	UC (n=51)	FAP (n=21)		UC (n=43)	FAP (n=20)
small bowel obstruction	5 (10%)	2 (10%)	small bowel obstruction	4 (9%)	2 (10%)
anastomotic dehiscence	6 (12%)		anastomotic leakage	4 (9%)	
other	2 (4%)		rectovaginal fistula	3 (7%)	
			other	2 (5%)	2 (10%)
total	13 (25%)	2 (10%)		13 (30%)	4 (20%)

3.4.2 LONG TERM FUNCTIONAL RESULTS

Analysis of stoolfrequency, use of loperamide, occurrence of pouchitis and incontinence was performed in all patients whose ileostomies were taken down. In four colitis patients the pouch was removed before ileostomy closure and in three colitis patients the ileostomy was still not closed at the time of evaluation. Mean follow-up after ileostomy closure in the remaining 43 colitis and 20 polyposis patients was 34 months (range 2-92) and 63 months (range 6-90) respectively. Pouch excision rate was calculated in all colitis and polyposis patients.

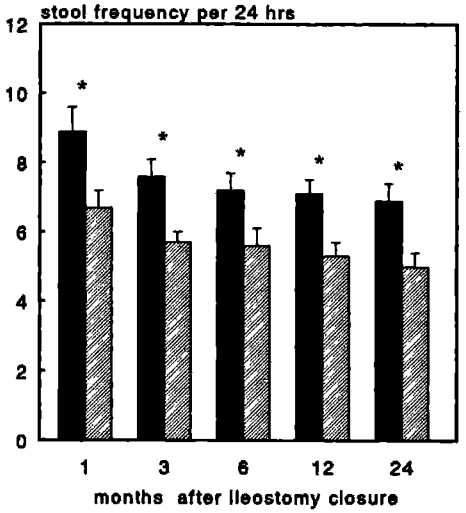
Stool frequency — The mean stool frequency per 24 hr after ileostomy closure decreased gradually in both patient groups but was significantly lower in polyposis patients any time after ileostomy closure (Figure 3.1a). Nocturnal stool frequency was significantly lower in polyposis patients one month after ileostomy closure and tended to be lower in these patients thereafter (Figure 3.1b).

At one year eight (26%) colitis patients and one (5%) polyposis patient had more than eight stools per 24 hr. At that time five (16%) colitis patients and one (5%) polyposis patient had more than two stools during the nighttime.

Pouchitis — The overall incidence of pouchitis during follow-up was 44% in ulcerative colitis patients (19 out of 43 patients) and 0% in polyposis patients ($p < 0.005$). The presence of pouchitis was confirmed by endoscopic and histological examination in all patients with symptoms compatible with pouchitis. Life table analysis of risk of pouchitis for both ulcerative colitis and familial adenomatous polyposis is shown in Figure 3.2. The probability of pouchitis occurring within five years after ileostomy closure in colitis patients was 57% versus 0% in polyposis patients ($p < 0.001$). The mean time to the first pouchitis episode was 14 months (range, 1-48 months).

The incidence of pouchitis was not affected by sex or age. The occurrence of pouchitis did not affect the incidence of incontinence. The mean number of stools per 24 hr during episodes of pouchitis increased significantly (7.7 vs 13.5 stools/day, $p < 0.001$). The stool frequency in patients with pouchitis before the first pouchitis episode was similar to that in ulcerative colitis patients who never developed pouchitis.

Pouchitis occurred in 8 (36%) of the 22 colitis patients who had undergone subtotal colectomy prior to the ileal pouch procedure and in 11 (38%) of 29 colitis patients in whom IPAA was the initial procedure (NS).



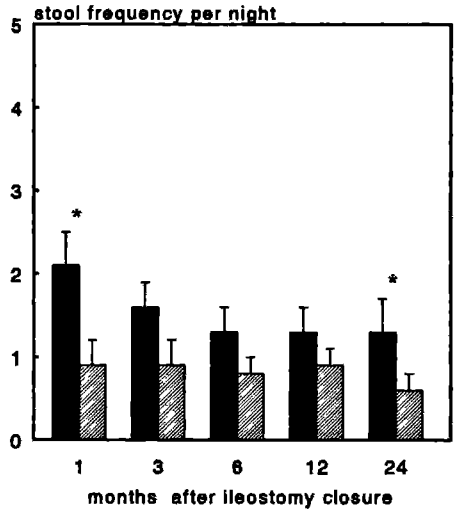


Figure 3.1 Twenty-four hr (a) and nocturnal (b) stool frequency (mean + SEM) 1, 3, 6, 12 and 24 months after ileostomy closure in ulcerative colitis patients (closed bars) and familial polyposis patients (hatched bars) (* $p < 0.05$).

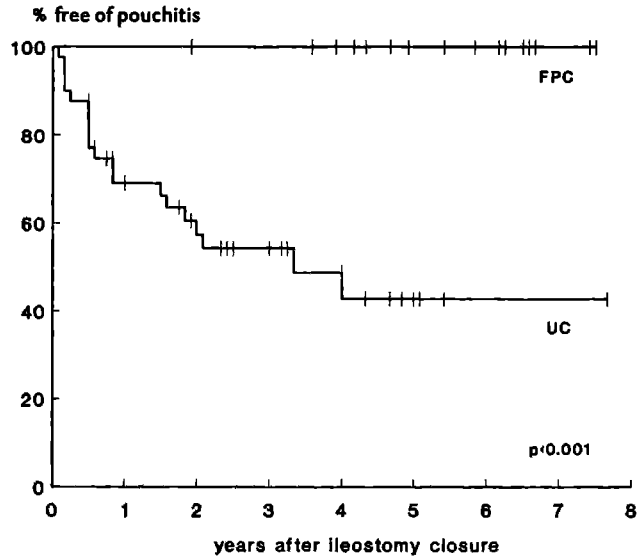


Figure 3.2 Life table analysis of risk of pouchitis after ileostomy closure in ulcerative colitis patients and familial polyposis patients. Pouchitis did not occur in polyposis patients. The risk of pouchitis was significantly higher in ulcerative colitis patients.

Initial episodes of pouchitis were treated with metronidazole in all patients. When this treatment was unsuccessful, local or oral corticosteroids were prescribed (13 patients). Recurrent episodes were treated similary. Twelve (63%) of the 19 patients with pouchitis responded favourably to medical treatment. Pouchitis occurred only once in two patients (11%). Ten (53%) patients had recurrent pouchitis. The remaining 7 (37%) patients developed chronic pouchitis that responded poorly to medical treatment. In 6 (32%) patients from the latter group the pouch was removed because of intractable pouchitis. In the seventh patient the pouch was still not removed at the time of evaluation. Histological examination of the removed pouches showed no signs of Crohn's disease.

Symptoms of pouchitis included bloody diarrhea, urgency, increased stool frequency, abdominal cramps, fatigue, fever, and weight loss (Table 3.2). Five patients with pouchitis (26%) developed arthritis. Arthritis occurred concomitantly with the onset of pouchitis episodes and affected knees, ankles, elbows, and wrists. Three of these patients required permanent ileostomy because of intractable pouchitis. In all arthritis disappeared rapidly after pouch excision. Arthritis did not occur in patients without pouchitis. The remaining two patients who developed arthritis responded favourably to oral corticosteroids.

Endoscopy showed inflammation (hyperaemia, oedema, loss of vascular pattern) of the ileal pouch mucosa in all pouchitis patients. Ulceration was seen in 16 (84%) patients. Mucosal biopsies from the ileal reservoir showed acute (infiltration of polymorphonuclear cells) and chronic inflammation (total or partial of villous atrophy and infiltration of lymphocytes, plasma cells, and eosinophils) in all patients with pouchitis.

Table 3.2 **Symptoms of pouchitis (n=19)**

symptom	number of patients (%)
increased stool frequency	13 (68)
abdominal pain	14 (74)
bloody stools	15 (79)
fever	8 (42)
weight loss	8 (42)
fatigue	15 (79)
arthritis	5 (26)

Incontinence — One year after ileostomy closure one ulcerative colitis patient and one polyposis patient were incontinent during daytime. During nighttime 16 (43%) colitis patients and 6 (32%) polyposis patients required a pad because of incontinence (NS).

Use of loperamide — One year after ileostomy closure 19 (51%) ulcerative colitis patients and 12 (63%) polyposis patients used loperamide in order to decrease stool frequency (NS).

Pouch excision — There was a tendency towards a lower rate of pouch excision in polyposis patients but this failed to achieve statistical significance. In 11 (22%) of the 51 ulcerative colitis patients and in one (5%) of the 21 polyposis patients, the pouch was removed and a permanent ileostomy constructed (p=0.16). Reasons for pouch excision were anastomotic dehiscence in three ulcerative colitis patients, pelvic abscesses in one colitis patient, intractable pouchitis in six colitis patients, incontinence in one colitis patient, and unacceptable high stool frequency in one polyposis patient. The probability of pouch excision (Figure 3.3) occurring within five years after the ileal pouch procedure was 26% in ulcerative colitis patients versus 6% in polyposis patients (p=0.07).

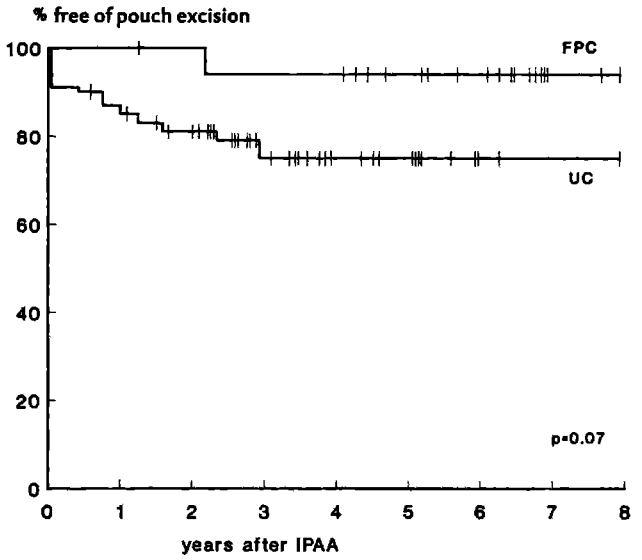


Figure 3.3 Life table analysis of risk of pouch excision in ulcerative colitis patients and familial polyposis patients after ileal pouch-anal anastomosis. There was a tendency towards a lower rate of pouch excision in polyposis patients but this failed to achieve statistical significance.

Ten (45%) out of 22 ulcerative colitis patients and one (20%) out of 5 polyposis patients who had undergone subtotal colectomy prior to the ileal pouch procedure required pouch excision. This difference was not statistically significant. When all patients with prior subtotal colectomy (n=27) were considered as a group and compared to the patients in which proctocolectomy with IPAA was the initial procedure (n=45) the number of excised pouches was 11 (41%) and 1 (2%) respectively ($p < 0.001$).

Final outcome — In three ulcerative colitis patients the ileostomy was still not closed at the time of evaluation. These patients were not included in the evaluation of final outcome. In the ulcerative colitis group the operation was unsuccessful in 12 patients (one postoperative death, 11 pouch excisions). The remaining 36 patients all preferred their pouch to the ileostomy. However, 14 of these patients (including 7 patients with pouchitis) felt unable to work full-time because of fatigue. Therefore, at the long-term the procedure was entirely successful in only 22 ulcerative colitis patients (46%). In the polyposis group the procedure failed in two patients (one postoperative death, one pouch excision). The remaining 19 patients all preferred the pouch to the ileostomy. Two of these patients were unable to work full-time

because of fatigue. Finally, 16 (76%) polyposis patients felt very satisfied with their pouch. The difference between the two groups was statistically significant ($p < 0.05$).

3.5 Discussion

In this study the immediate postoperative and long-term functional results of IPAA in 51 ulcerative colitis patients and 21 familial adenomatous polyposis patients were compared. The interval between the ileal pouch procedure and ileostomy closure was longer in colitis patients than in polyposis patients. Ileostomy closure was carried out at the time that the patients were recovered from the ileal pouch procedure. Precise data demonstrating that polyposis patients are in better health at the time of the ileal pouch procedure than colitis patients are lacking in this retrospective study. Nevertheless, polyposis patients are usually operated electively whereas colitis patients undergo (procto)colectomy when severe and disabling disease is not responding to medical treatment. The majority of colitis patients but none of the polyposis patients used corticosteroids at that time. Postoperative complications requiring relaparotomy after both the ileal pouch procedure and ileostomy closure occurred more frequently in ulcerative colitis patients, but these differences did not reach statistical significance.

The major long-term complication after IPAA was pouchitis, which occurred in 44% of colitis patients but not in polyposis patients. The estimated risk of pouchitis five years after ileostomy closure in colitis patients was 57%. As duration of follow-up in ileal pouch patients increases, the incidence of pouchitis also tends to increase (5). Even after three years of follow-up some new cases of pouchitis arose in our series.

The incidence of pouchitis was higher compared to many other reports (3-15), where the incidence ranges from 8% to 44%. This wide range probably reflects the lack of a uniform diagnostic standard. Moskowitz et al. (18) suggested that for an unequivocal diagnosis symptoms of pouchitis should be accompanied by endoscopic and histological features of acute inflammation. In our study all patients with clinical pouchitis fulfilled these criteria.

In some reports (9,10,15) colitis patients and polyposis patients were considered as one group. When colitis patients would have been considered as a separate group the rate of pouchitis in these patients would probably have been higher. Nevertheless, the rate of pouchitis in our colitis patients is hig-

her than in the colitis patients in the Mayo Clinic series (31%) (5). It is difficult to explain this discrepancy. In our series pouchitis was carefully looked for by endoscopy and histology, whereas in the Mayo Clinic series pouchitis was merely a clinical diagnosis.

The cause of pouchitis remains unclear. Bacterial overgrowth due to fecal stasis in the ileal pouch has been suggested as a possible pathogenic factor. In a study by Go et al. (23) ileum effluents of Kock's continent ileostomy patients showed significantly higher counts of anaerobic microorganisms (eg, *Bacteroides*) than in ileum effluents of patients with a conventional ileostomy. Nasmyth et al. (17) also found higher numbers of *Bacteroides* in ileoanal pouch effluents compared with conventional ileostomy effluents. However, quantitative cultures of pouch effluents from patients with pouchitis did not reveal higher numbers of anaerobes compared with controls without evidence of pouchitis (24). These findings were confirmed by Luukkonen et al. (25) who found significantly higher anaerobic counts in pouch patients compared to conventional ileostomy patients, but no specific changes in fecal bacteriology were found in patients with pouchitis. However, since many patients respond to metronidazole, anaerobic bacterial overgrowth may contribute to the pathogenesis of pouchitis.

Several authors have suggested that pouchitis is a novel manifestation of inflammatory bowel disease after proctocolectomy (5,16-21). In most studies pouchitis occurred exclusively in ulcerative colitis patients. Only a few cases of pouchitis in polyposis patients have been reported by workers from the Mayo Clinic (4,5). These cases were poorly documented because endoscopy was not performed in these patients. In the Mayo Clinic population ulcerative colitis patients were at much higher risk of pouchitis compared to polyposis patients. Moreover, ulcerative colitis patients with extraintestinal manifestations of inflammatory bowel disease before proctocolectomy were at higher risk of pouchitis compared to those without extraintestinal manifestations. In agreement with our experience, in some patients from the Mayo Clinic a temporal relationship between flares of extraintestinal manifestations and pouchitis was observed (5). These findings support the hypothesis that pouchitis is a novel manifestation of inflammatory bowel disease.

It has been suggested that the pouch mucosa, in an adaptive response to its new luminal environment, undergoes colonic metaplasia and thus may become vulnerable to immune damage in predisposed people (16,19,26-29). In a study on mucosal characteristics of pelvic ileal pouches using routine histology, mucin histochemistry, and monoclonal antibodies directed towards colo-

nic and small bowel specific proteins (29), villous atrophy and colonic type sulphomucin was found in all pouchitis patients. However, sucrase-isomaltase, a small bowel specific disaccharidase, was present in all pouches irrespective of the presence of villous atrophy or pouchitis. It was concluded that although some ileal pouches, especially those with signs of acute inflammation, acquire certain colonic characteristics, complete colonic metaplasia does not occur.

Another factor that has been suggested to play a pathogenic role in pouchitis is bacterial deconjugation and dehydroxylation of primary bile acids (16,30). Under normal circumstances the greatest part of the conjugated bile acid pool is transported actively by the ileal mucosa into the portal venous system. Less than ten percent of the bile acid pool passes the cecal valve and is deconjugated by the colonic bacterial flora. After deconjugation the unconjugated primary bile acids are dehydroxylated by the colonic bacteria to secondary bile acids. Loss of hydroxyl groups makes bile acids more lipophilic than the corresponding primary bile acids (31). Under experimental conditions secondary bile acids like deoxycholic acid cause an increase of water and salt permeability in colonic mucosal cells followed by cell death (32). Stasis and bacterial overgrowth in the ileal pouch may lead to deconjugation and dehydroxylation of bile acids. These secondary bile acids may exert a toxic effect on the ileal pouch mucosa. This mechanism plays at best a supplementary role in the pathogenesis of pouchitis because it does not explain why pouchitis does not occur in familial adenomatous polyposis.

Patients who had undergone subtotal colectomy prior to the ileal pouch procedure were at high risk of pouch excision. The incidence of pouchitis was similar in the colitis patients who had undergone subtotal colectomy prior to the ileal pouch procedure compared to those in whom the pouch procedure was the initial operation. This suggests that subtotal colectomy prior to IPAA is a risk factor for pouch excision. In three patients the pouch was removed because of complete anastomotic dehiscence and in one patient because of pelvic abscesses, probably because of incomplete dehiscence. Traction on the pouch-anal anastomosis is probably higher in patients who have undergone previous intestinal surgery due to adhesions and mesenterial retraction.

In conclusion, the rate of complications requiring relaparotomy after both IPAA and ileostomy closure is considerable both in ulcerative colitis and familial polyposis patients. Long-term functional results are better in familial adenomatous polyposis compared to ulcerative colitis since pouchitis does

not occur in polyposis patients. Moreover, the stool frequency is lower in polyposis patients and patient satisfaction is better in these patients. Pouchitis is the major long-term complication of IPAA occurring in approximately 40% of ulcerative colitis patients but not in polyposis patients. Pouchitis may lead to pouch excision in a considerable number of patients. Many pouchitis patients show a favourable response to metronidazole, although this response is usually temporary. Patients who have undergone subtotal colectomy prior to the ileal pouch procedure are at higher risk of pouch excision. These results have important implications for the information that should be given to patients who are candidates for IPAA.

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EFFECT OF AGEING ON POSTPRANDIAL CONJUGATED AND UNCONJUGATED SERUM BILE ACID LEVELS IN HEALTHY SUBJECTS

Jan M.J.I. Salemans, Fokko M. Nagengast, Albert Tangerman, Annie van Schaik, Wim P.M. Hopman, Anton F.J. de Haan*, Jan B.M.J. Jansen.
From the Division of Gastroenterology, Department of Medicine, and *Department of Medical Statistics, Nijmegen University Hospital, The Netherlands.

Eur J of Clin Invest 1993, 23:192-198

4.1 Abstract

A decreased ileal absorption of bile acids in elderly subjects may lead to an increased exposure of the colonic mucosa to secondary bile acids. This may contribute to an enhanced risk of colorectal cancer. In this study fasting and postprandial conjugated and unconjugated serum levels of cholic, chenodeoxycholic, and deoxycholic acid in 12 elderly and 12 younger subjects were investigated. Intestinal transit time, gallbladder emptying and jejunal bacterial flora were also studied in both age groups. Fasting levels of conjugated and unconjugated serum bile acids were similar in both age groups. Postprandial levels of all individual conjugated bile acids increased to a significantly higher extent in the younger subjects. Postprandial unconjugated serum bile acid levels did not differ significantly between both age groups, although unconjugated deoxycholic levels tended to increase to higher levels in the elderly. Results of jejunal bacterial counts, gallbladder emptying and intestinal transit time were similar in both groups. These data suggest that conjugated bile acids are reabsorbed less effectively in elderly subjects.

4.2 Introduction

Colorectal cancer is a disease of elderly subjects. It has been suggested that a decreased ileal absorption of bile acids in elderly subjects may lead to an increased exposure of the colonic mucosa to secondary bile acids (1). This may contribute to an enhanced risk of colorectal cancer since secondary bile acids are thought to have co-carcinogenic effects on the colonic mucosa (2-4). The primary bile acids cholic (CA) and chenodeoxycholic acid (CDCA), that escape to the enterohepatic circulation are deconjugated and converted in the large bowel into their secondary bile acids deoxycholic (DCA) and lithocholic acid respectively. It has been demonstrated that the conversion of CA into DCA is increased in elderly subjects compared to younger subjects (1).

In order to find further evidence that ageing influences bile acid reabsorption in the small intestine we studied fasting and postprandial conjugated and unconjugated serum levels of CA, CDCA, and DCA in twelve young adults and twelve elderly subjects. To our knowledge this is the first time that postprandial serum levels of individual bile acids have been studied in different age groups.

To investigate whether impaired intestinal motility and small intestinal

bacterial overgrowth in the elderly may be responsible for age dependent differences in bile acid metabolism, intestinal transit time, gallbladder emptying, and jejunal bacterial flora were also studied in both age groups. Since elevated unconjugated serum bile acid levels are a useful marker for small intestinal bacterial overgrowth (5-9) both conjugated and unconjugated serum bile acid levels were studied in both age groups.

4.3 Subjects and methods

Subjects — Twenty-four healthy subjects were studied; 12 were younger than 60 years (mean age 37 years, range 22-59 years; 7 female, 5 male) and 12 were older than 60 years (mean age 67 years, range 60-82 years; 7 female, 5 male). None of the subjects had abdominal symptoms, a history of gastrointestinal disease or abdominal surgery. Routine liver function tests were normal in all subjects. Informed consent was obtained from each subject and the protocol had been approved by the Ethics Committee of the University Hospital Nijmegen.

Experimental protocol— The subjects were studied after an overnight fast. Venous blood samples for analysis of serum bile acid levels were obtained before and at 30, 60, 90, 120, 150, 180, 210, and 240 minutes after consumption of a standardized breakfast. The breakfast consisted of one slice of bread, 5 g of margarine, 20 g of cheese, one boiled egg, 150 ml of yogurt and one cup of tea containing 5 g of sugar (21 g of protein, 21.5 g of fat, 34 g of carbohydrate, 413 kcal) and was ingested within 15 minutes. At time 0 minutes 12 g of lactulose (Legendal^{R,} Zambon, The Netherlands) in 100 ml water was ingested.

Analysis of serum bile acids — Venous blood samples were allowed to clot. After centrifugation the separated serum was frozen at -20°C for subsequent analysis. Serum levels of the conjugated and unconjugated fractions of CA, CDCA and DCA were measured by capillary gas-liquid chromatography, a highly accurate and sensitive method (10). 7α,12α-dihydroxy-5-ßcholanoic acid (Steraloids, Wilton, NH, USA) was added as an internal standard. Bile acids were extracted from serum using C18-bonded silica cartridges (SepPak, Waters Associates, Milford, MA, USA) (11). Separation of conjugated and unconjugated bile acids was carried out by means of column chromatography

using the lipophilic anion exchanger diethylaminohydroxypropyl Sephadex LH-20 (Lipidex-DEAP, Packard Instruments, Groningen, The Netherlands) (11). The conjugated fractions were subjected to enzymatic hydrolysis by cholylglycine hydrolase (from *Clostridium perfringens*, Sigma, St. Louis, MO, USA). After enzymatic hydrolysis the deconjugated bile acids were extracted and
60 eluted on Lipidex-1000 columns (10). The bile acids were converted to methyl esters by 2,2-dimethoxypropane (Merck, Darmstadt, Germany). After methylation the trimethylsilyl ether derivates were prepared by addition of a solution of pyridine, hexamethyldisilazane and trimethylchlorosilane (3:2:1 by vol.). Immediately before application to the gas chromatograph the silylation reagent was dried under nitrogen and the samples were redissolved in 50 µL hexane. Two µL of this solution was used for injection. Separation and quantification of individual bile acids was performed on a Packard 430 gas-liquid chromatograph (Packard Instruments, Delft, The Netherlands) with a flame ionisation detector and equipped with a 25 m* 0.22 mm glass capillary column (CP-Sil-5 CB, Chrompack, Middelburg, The Netherlands). The chromatograph was equipped with an automatic solid injection system. Helium was used as carrier gas. The injection temperature was 285°C and the individual bile acids were separated by computer based stepwise increment of column temperature from 150-290°C. The flame ionisation temperature was 280°C. Bile acids were identified by comparing the retention times of the individual bile acids to those of reference bile acids.

Bacterial examination of jejunal fluid — Jejunal fluid was obtained under anaerobic conditions after an overnight fast by means of a radio-opaque polyvinylchloride tube. The samples were collected at least 15 cm beyond the duodenojejunal junction. The position of the tube was checked fluoroscopically. To prevent contamination with saliva the tip of the tube was sealed with an agar plug. Before sampling the agar plug was expelled by inflating nitrogen through the tube. Immediately after sampling 0.5 ml of jejunal fluid was added to 4.5 ml of a solution containing 1.0 g glucose, 4.0 g starch, 10.0 g tryptose, 5.0 g NaCl, 3.0 g K_2HPO_4, 0.5 g KH_2PO_4 0.5 g $MgSO_4$ 0.5 g cysteine-HCl and 3.0 g yeast extract dissolved in 1000 ml H_2O. The procedure was continued under anaerobic conditions with an oxygen tension of less than 5 p.p.m. (12). After homogenization, serial dilutions of the homogenate were spread on a non-selective medium containing 22.5 g agar with 7% blood obtained from sheep. Anaerobic cultures and aerobic cultures were continued for eight and three days respectively at 37°C. For aerobic culture,

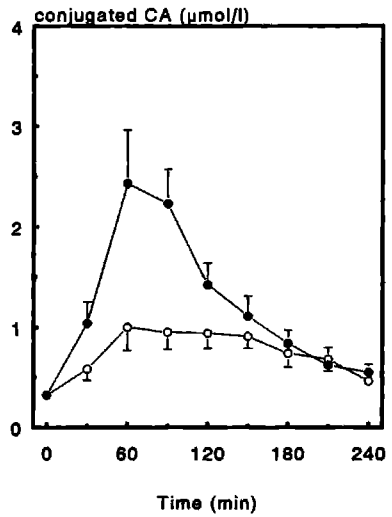
three selective media were used in addition to the above mentioned non-selective medium. Direct microscopic counting of bacteria was done using the method described by Holdeman (13).

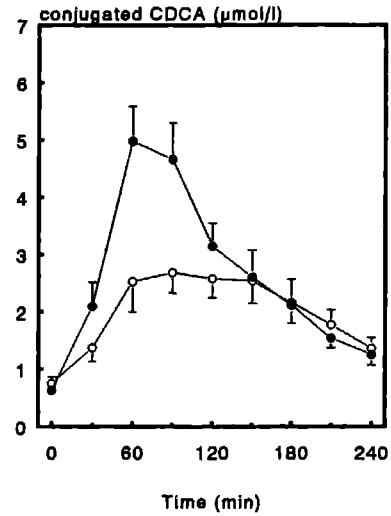
Gallbladder emptying — In a subgroup of five younger and five elderly subjects gallbladder volumes were measured by real-time ultrasonography. Gallbladder volumes were calculated by the sum of cylinders method using a computerized system (14,15). Two longitudinal and two transverse images of the gallbladder were obtained at 0, 15, 30, 45, 60, 75, 90, 105, 120, 150 and 180 minutes. The mean of two measurements was used for further analysis.

Small intestinal transit time — Small intestinal transit time was measured by the lactulose H_2 breath test. The hydrogen concentration in expired air was measured according to the method described by Dolmans et al. (16). End expiratory breath samples were collected under basal conditions and every 15 minutes thereafter. The time between ingestion of lactulose until a sustained rise in breath hydrogen concentration of at least 15 parts per million (ppm) was defined as small intestinal transit time.

Total intestinal transit time — Total intestinal transit time was measured by the single stool method using radio-opaque markers according to Cummings and Wiggins (17).

Statistical analysis — Results were expressed as the mean ± SEM unless stated otherwise. Bile acid curves were tested by standard repeated measurements analysis of variance. To eliminate skewness of the bile acid distribution a logarithmic transformation of the data was applied for statistical testing. Firstly, time effects were studied. Secondly, the hypothesis that time effects were equal for both groups was tested. When time effects were equal the mean bile acid curves were considered to be parallel. When the bile acid curves were non-parallel time effects were described separately for both groups. The correlation between age and the sum of individual integrated serum bile acid levels of the three major conjugated bile acids (CA + CDCA + DCA) was calculated using Pearson's correlation coefficient. Intestinal transit times and gallbladder volumes were compared with the one-tailed Student's t-test. Integrated bile acid values and jejunal bacterial counts were compared with the one-tailed Student's t-test after logarithmic transformation to eliminate skewness in the distribution. One-tailed tests were used since the hypothesis





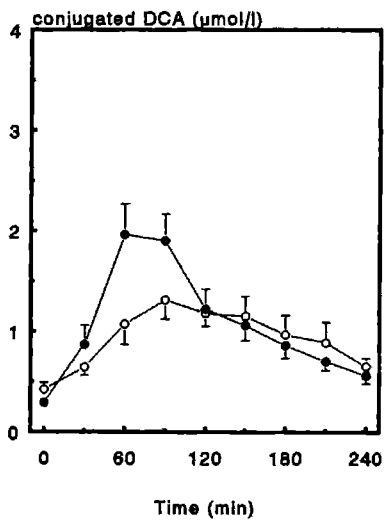


Figure 4.1 Fasting and postprandial conjugated serum levels (mean ± SEM) of cholic (CA), chenodeoxycholic (CDCA) and deoxycholic acid (DCA) in the younger subjects (closed markers) and the elderly subjects (open markers). All bile acid curves of both age groups were non-parallel (CA: $p<0.001$, CDCA: $p<0.01$; DCA $p<0.001$; repeated measurements analysis).

was aimed at one-directional changes: a slower transit time, slower gallbladder emptying and more bacterial overgrowth at older age. A p-value <0.05 was considered statistically significant.

4.4 Results

Conjugated bile acids — Fasting conjugated serum bile acid levels were not significantly different between both age groups. Postprandially, the conjugated bile acid levels increased rapidly in all subjects ($p<0.001$). The levels of all individual conjugated bile acids increased to a higher extent in the younger subjects compared to the elderly subjects. Repeated measurements analysis revealed that the curves of all conjugated bile acids were non-parallel in both age groups (Figure 4.1). The peak levels were reached within two hours and tended to occur slightly later in the elderly subjects but no significant differences between the two age groups were observed. After the peak, the conjugated bile acid levels decreased gradually and levelled off to approximately twice baseline values four hours after the meal. Postprandial levels of CDCA were approximately twice as high as CA and DCA levels in both groups.

The integrated conjugated bile acid values of both age groups are shown in Table 4.1. Integrated CA and CDCA levels were significantly higher in the younger subjects. The integrated DCA levels were similar in both age groups.

Figure 4.2 shows the correlation between age and the sum of the individual integrated conjugated bile acids levels (CA + CDCA + DCA). A significant negative correlation was found ($r=-0.56$; $p<0.01$) between both parameters.

Unconjugated bile acids — Fasting unconjugated serum bile acid levels were not significantly different in both age groups. Repeated measurements analysis revealed significant time trends of unconjugated CDCA ($p<0.01$) and DCA ($p<0.001$) but not CA. However, these trends were not significantly different between both age groups. Levels of CDCA and DCA reached a maximum at 90 and 120 minutes respectively. No differences between the time to peak concentration in the younger and elderly subjects were observed (Figure 4.3). After the peak a slow decrease was observed to approximately baseline levels, four hours after the meal.

The integrated unconjugated serum bile acid values are shown in Table 4.2. No significant differences between the two age groups were observed.

Table 4.1 Integrated conjugated serum bile acid levels (CA=cholic, CDCA=chenodeoxycholic DCA=deoxycholic acid in μM.min; mean ± SEM) in the younger and elderly subjects.

	young	*old*	*p-value*
CA	304 ± 41	185 ± 26	p<0.05
CDCA	662 ± 67	500 ± 64	p=0.05
DCA	269 ± 30	232 ± 31	NS

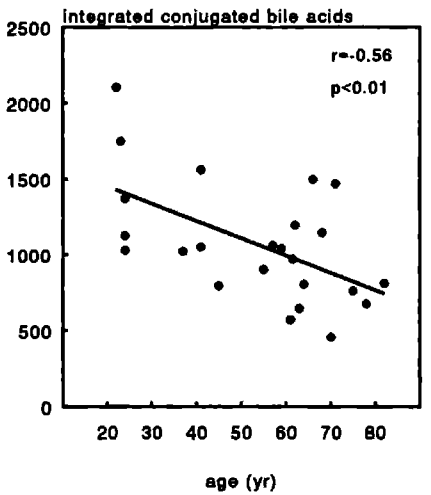


Figure 4.2 Correlation between age and the sum of the individual integrated values of conjugated CA, CDCA, and DCA.

Table 4.2 Integrated unconjugated serum bile acid levels (μM.min; mean ± SEM) in the younger and elderly subjects.

	young	*old*	*p-value*
CA	66 ± 9	64 ± 11	NS
CDCA	87 ± 12	93 ± 17	NS
DCA	98 ± 21	122 ± 20	NS

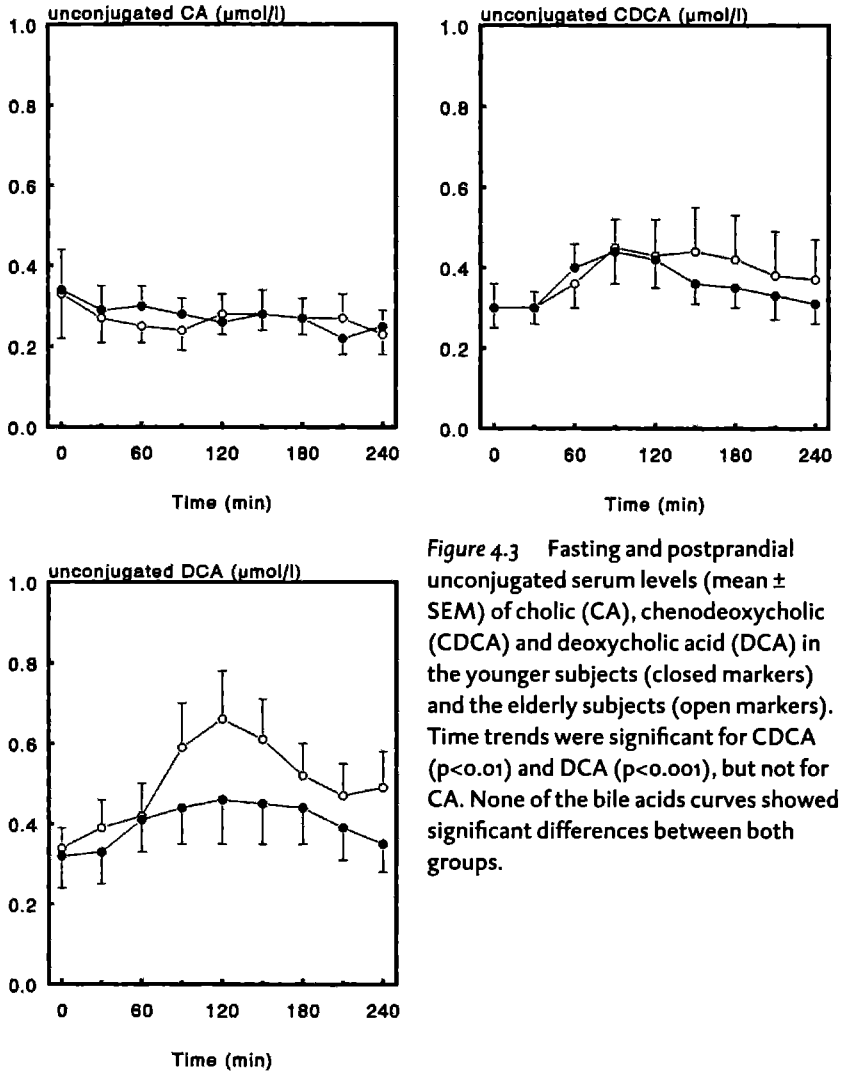


Figure 4.3 Fasting and postprandial unconjugated serum levels (mean ± SEM) of cholic (CA), chenodeoxycholic (CDCA) and deoxycholic acid (DCA) in the younger subjects (closed markers) and the elderly subjects (open markers). Time trends were significant for CDCA ($p<0.01$) and DCA ($p<0.001$), but not for CA. None of the bile acids curves showed significant differences between both groups.

Ratio of unconjugated and conjugated serum bile acids — The ratio of the integrated unconjugated and conjugated serum bile acid levels are shown in Table 4.3. The ratio of unconjugated and conjugated DCA levels tended to be higher in the elderly subjects ($p=0.07$). The ratios of the unconjugated and conjugated primary bile acids were not significantly different between both age groups.

Table 4.3 **Ratio of integrated unconjugated and conjugated serum bile acid levels (mean ± SEM) in the younger and elderly subjects.**

	young	*old*	*p-value*
CA	0.24 ± 0.04	0.45 ± 0.12	NS
CDCA	0.14 ± 0.02	0.24 ± 0.07	NS
DCA	0.34 ± 0.04	0.58 ± 0.10	p=0.07

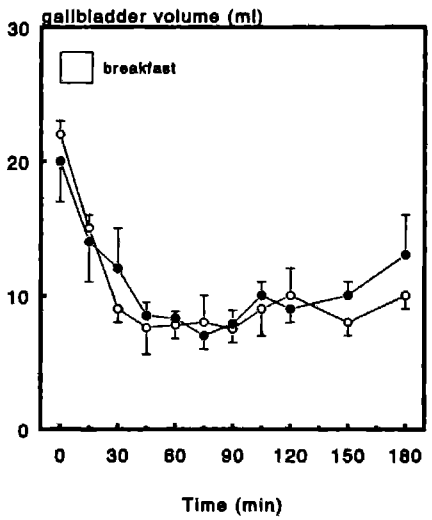


Figure 4.4 **Postprandial gallbladder emptying in five younger (closed markers) and five elderly subjects (open markers). No significant differences between both age groups were observed.**

Bacterial examination of jejunal fluid — The sum of jejunal aerobic and anaerobic bacteria in the elderly subjects (median and range: $10^{4.6}$, $10^{2.4}$-$10^{6.7}$ per ml) was not significantly different from that in the younger subjects ($10^{3.9}$, $10^{2.4}$-$10^{6.5}$ per ml).

Gallbladder emptying — No significant difference was found between fasting gallbladder volumes in the younger (22 ± 1 ml) and elderly subjects (20 ± 3 ml). The meal induced significant decreases in gallbladder volume in both age groups without statistically significant differences in the rate and extent of gallbladder emptying between the groups (Figure 4.4).

Small intestinal transit time — The mean small intestinal transit time as measured by the lactulose H_2 breath test was 142 ± 11 minutes in the younger subjects and 135 ± 16 minutes in the elderly subjects. The difference between the two age groups was not statistically significant.

Total intestinal transit time — The mean total intestinal transit time was 45 ± 6 hours in the younger subjects and 49 ± 4 hours in the elderly subjects. The difference between the two age groups was not statistically significant.

4.5 Discussion

Conjugated serum bile acid levels are well known to increase rapidly after meals in healthy subjects (18-22). However, the influence of age on postprandial bile acid levels has not been studied so far. In the present study fasting and postprandial conjugated and unconjugated serum levels of individual bile acids were compared in elderly and younger healthy subjects. All major conjugated bile acid levels increased to a higher extent postprandially in the younger subjects compared to the elderly subjects. We found a significant negative correlation between age and integrated postprandial conjugated serum bile acid levels. Since the fraction of bile acids in the portal blood cleared by the liver remains relatively constant and is independent of the load delivered to the liver (23,24) systemic bile acid levels are thought to be proportional to the load of bile acids absorbed from the intestine. Therefore, the observed differences in this study between both age groups suggest that the postprandial conjugated bile acid load in the portal blood is smaller in elderly subjects. Several mechanisms may account for a smaller conjugated bile acid load in portal blood in elderly subjects. Firstly, the active ileal reabsorption of conjugated bile acids may be less effective in elderly subjects. Secondly, the amount of bile acids delivered to the duodenum after gallbladder emptying may be smaller in elderly subjects as a result of impaired gallbladder emptying or a decreased secretion of bile acids by the liver. Finally, small intestinal bacterial overgrowth in elderly subjects may lead to deconjugation of bile acids in the small intestine. The observed differences in the present study between both age groups however, cannot be explained by delayed gallbladder emptying or impaired intestinal motility in elderly subjects since gallbladder emptying and small intestinal transit time were similar in both age groups. Moreover, we found no evidence for small intestinal bacterial over-

growth in the elderly age group since jejunal bacterial counts and unconjugated serum bile acid levels were similar in both age groups.

Using a double isotope dilution method van der Werf et al. (1) studied age dependent differences in bile acid metabolism and 7α-dehydroxylation. Eleven young adults and 11 elderly subjects were studied. They found a higher input rate of DCA from the large bowel into the enterohepatic circulation in elderly subjects compared to younger subjects. The poolsize and synthesis rate of CA were similar in both age groups, whereas the poolsize of DCA was higher in the elderly. Since CA is transformed into DCA by the colonic flora these findings suggest that the active ileal absorption of conjugated bile acids is decreased in elderly subjects. The lower postprandial conjugated serum bile acid levels in our study give support to this hypothesis. Moreover, postprandial unconjugated levels of DCA and the ratio of integrated unconjugated and conjugated DCA levels tended to be higher in the elderly subjects. This suggests that the load of CA exposed to the colonic flora is increased in this age group.

Previously, we have shown that the fecal secondary bile acid concentration is higher in elderly subjects compared to young adults (25). The concentration of fecal primary bile acids was similar in all age groups. Although it was suggested that the observed discrepancy was related to a lower dietary fibre intake in the elderly subjects, the higher fecal secondary bile acid concentration may well be explained by a decreased reabsorption of conjugated bile acids in the small intestine.

It must be stated however, that our findings do not prove that conjugated bile acids are reabsorbed less effectively in elderly subjects. A decreased excretion of bile acids by the liver in elderly subjects may be another explanation for the observed differences between in both age groups. Bile acid synthesis rate, duodenal excretion of bile acids, and bile acid pool sizes were not measured in the present study. Age dependent differences of bile acid synthesis have been studied by some other groups. Einarsson et al. (26) found a significant negative correlation between age and total bile acid synthesis in a group of 18 healthy subjects. However, no significant correlation was found between age and bile acid secretion into the duodenum. In the study by van der Werf et al. (1), no significant differences in pool size and synthesis rate of CA were observed. Similarly, Valdivieso et al. (27) found no significant reduction in CA pool size or synthesis in older women. Thus, former studies gave little evidence for a lower duodenal excretion of conjugated bile acids in the elderly.

By others it has been suggested that primary bile acids are displaced from the enterohepatic circulation by DCA in elderly subjects because slowing of colonic transit increases DCA absorption (1,28,29). In the present study however, total intestinal transit times were similar in both age groups.

Bacterial transformation of primary bile acids into secondary bile acids has been implicated in colonic carcinogenesis. In animal models it has been shown that secondary bile acids may have (co-)carcinogenic effects on colonic mucosa (2-4). Since colorectal cancer is a disease of elderly subjects several investigators have searched for age dependent alterations in bile acid metabolism. In the present study further evidence was found for a decreased active ileal absorption of conjugated bile acids with increased spill-over into the large bowel in elderly subjects. A higher exposure of secondary bile acids to the colonic mucosa may contribute to the increased risk of colorectal cancer in these subjects.

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UNCONJUGATED SERUM BILE ACID LEVELS IN PATIENTS WITH SMALL INTESTINAL BACTERIAL OVERGROWTH AND OTHER MALABSORPTIVE STATES

Jan M.J.I. Salemans, Albert Tangerman, Annie van Schaik, Egbert W. van der Hoek*, Jan B.M.J. Jansen, Fokko M. Nagengast.
From the Division of Gastroenterology, Department of Medicine, Nijmegen University Hospital, and *Department of Medicine, Carolus Hospital, Den Bosch, The Netherlands.

Submitted

5.1 Abstract

Unconjugated serum bile acid levels have been found to be elevated in patients with small intestinal bacterial overgrowth. In order to determine sensitivity and specificity of unconjugated serum bile acids as a marker for small intestinal bacterial overgrowth we studied individual and total unconjugated serum bile acid levels in 24 healthy subjects, 11 patients with culture proven bacterial overgrowth, 19 patients with other malabsorptive states, 10 patients with ileal resection, and in 11 patients who had undergone proctocolectomy with ileal pouch-anal anastomosis. Bile acids were measured using capillary gas-liquid chromatography. Individual as well as total unconjugated serum bile acid levels were significantly elevated in patients with bacterial overgrowth compared to controls and the other groups. Total unconjugated serum bile acid levels were outside the normal range in 9 out of 11 patients with bacterial overgrowth (sensitivity 82%), but within the normal range in all patients with other malabsorptive states (specificity 100%). The positive and negative predictive values of elevated total unconjugated serum bile acid levels as a marker for small intestinal bacterial overgrowth were 100% and 90% respectively. Individual unconjugated bile acids provided a poorer discrimination between patients with bacterial overgrowth and the other groups. It is concluded that determination of total unconjugated serum bile acid levels is of clinical value in the evaluation of patients with suspected small intestinal bacterial overgrowth. The sensitivity and specificity of total unconjugated serum bile acid levels are superior to those of individual unconjugated bile acid levels.

5.2 Introduction

Small intestinal bacterial overgrowth is characterized by the presence of diarrhoea, malabsorption, and weight loss (1). In patients with clinically significant bacterial overgrowth, the number of bacteria in the small intestinal fluid usually exceeds 10^7 ml^{-1}. Both anaerobic and aerobic bacteria may be found. The golden standard for the diagnosis of small intestinal bacterial overgrowth is a properly collected and appropriately cultured aspirate from the proximal small intestine. The specimen should be obtained under anaerobic conditions, serially diluted, and cultured on selective media under aerobic and anaerobic conditions. Intestinal culture requires intubation of

the small intestine and time-consuming microbiological analysis. Therefore, several non-invasive tests have been developed to demonstrate bacterial overgrowth, but none of them have proved entirely satisfactory. The glucose-H_2 breath test gives false negative results in the absence of H_2 generating bacteria (2,3). Moreover, the glucose-H_2 may not adequately distinguish patients with bacterial overgrowth from those with other malabsorptive states, including coeliac disease. The specificity of the ^{14}C-glycocholate breath test (4,5) is poor since this test does not differentiate bacterial overgrowth from ileal damage or resection resulting in excessive breath $^{14}CO_2$ production due to bacterial deconjugation within the colon of the unabsorbed bile salt. Although the sensitivity and specificity of the ^{14}C-xylose breath test (6) have been reported to be excellent (2,7), the use of a radioisotope is a major drawback.

Unconjugated serum bile acids levels have been found to be elevated in patients with small intestinal bacterial overgrowth (8-12). However, little is known about unconjugated serum bile acid levels in other malabsorptive or maldigestive states including coeliac disease and chronic pancreatitis with exocrine pancreatic insufficiency. In order to investigate whether unconjugated serum bile acids offer a good discrimination between these conditions, we measured fasting individual unconjugated serum bile acids in healthy controls, patients with small intestinal bacterial overgrowth, patients with untreated coeliac disease, and patients with chronic pancreatitis and exocrine insufficiency. Patients who had undergone ileal resection were included as a disease control group, since unconjugated serum bile acid levels may be elevated in these patients as a result of spill-over of unabsorbed bile acids into the colon. A group of patients who had undergone proctocolectomy with ileal pouch-anal anastomosis was also included since stasis in the ileal pouch may theoretically lead to bacterial overgrowth and deconjugation of bile acids. Serum bile acids were measured using capillary gas-liquid chromatography, a highly sensitive and accurate method for individual serum bile acid analysis.

5.3 Subjects and methods

5.3.1 SUBJECTS

Healthy subjects — Twenty-four healthy volunteers participated in this studied (mean age 52 years, range 22-82 years; 10 male, 14 female). None of these subjects had a history of abdominal surgery or gastrointestinal disease.

Stools were collected during 48 hours for analysis of fat excretion. None of the healthy subjects had steatorrhoea (fat excretion < 7 gram per day). In all healthy subjects results of a jejunal culture and a glucose H_2 breath test were normal ($<10^7$ microorganisms per ml and an increase in H_2-excretion <15 ppm in breath following oral administration of 100 g glucose, respectively).

Small intestinal bacterial overgrowth — Eleven patients (mean age 60 years, range 30-77 years; 8 male, 3 female) with culture proven small intestinal bacterial overgrowth participated in the study. The diagnosis of small intestinal bacterial overgrowth was established when a cultured jejunal aspirate demonstrated $>10^7$ microorganisms per ml in a patient with a gastrointestinal disorder known to predispose to small intestinal bacterial overgrowth with signs of malabsorption (weight loss, anaemia, diarrhoea, reduced urinary excretion of xylose, positive glucose H2 breath test, steatorrhoea). The causes of bacterial overgrowth, results of jejunal culture, and fecal analysis of fat excretion in the individual patients are shown in Table 5.1.

Coeliac sprue — Eight patients (mean age 48 years, range 30-67 years; 3 female, 5 male) with untreated coeliac disease were studied. The diagnosis of coeliac disease was established in patients with malabsorption, a flat jejunal mucosa, and clinical improvement after withdrawal of gluten from the diet.

Chronic pancreatitis — Eleven patients with chronic pancreatitis and steatorrhoea (mean age 46 years, range 27-64 years; 7 male and 4 female) participated in the study. Pancreatic enzyme supplements were stopped at least 4 days before stool collection and blood sampling for serum bile acid analysis.

Ileal resection — Ten subjects who had undergone ileal resection for Crohn's disease (7 male, 3 female; mean age: 42, range 24-57 yr) participated in the study. Ileal resection had been performed 1 to 14 yr (median: 9 yr) before the time of this study. The diagnosis of Crohn's disease had been confirmed by histological examination of the surgical specimen in all patients. The length of the resected terminal ileum ranged from 30 to 70 cm (mean ± SEM: 44 ± 8 cm). None of the patients had signs of active Crohn's disease or small bowel obstruction at the time of investigation.

Table 5.1 **Diagnosis and results of jejunal culture, stool fat excretion, and total unconjugated serum bile acid levels in patients with small intestinal bacterial overgrowth.**

patient	*Sex*	*age (yr)*	*diagnosis*	*bacteria log/ml*	*predominant microorganisms*	*fat excretion (g/d)*[1]	*total unconjugated bile acids (μM)*[2]
1	M	66	progressive systemic sclerosis	8.4	E. coli	11.2	2.57
2	M	77	truncal vagotomy, partial gastrectomy	8.3	Enterobacteria, E. coli	9.1	1.46
3	F	66	small bowel diverticula	8.9	Anaerobes, Enterobacteria	8.8	3.82
4	M	70	malignant jejunal stenosis	8.8	Enterobacteria	6.7	4.07
5	M	67	intestinal pseudo-obstruction	9.7	Anaerobes, Enterobacteria	12.1	2.01
6	M	56	posttraumatic jejunal stenosis	9.7	Anaerobes, E. coli	2.4	2.62
7	F	30	Crohn's disease, ileal stenosis	9.5	Anaerobes, E. coli	ND[3]	9.35
8	F	55	Crohn's disease, jejunal stenosis	10.8	Anaerobes, Strep. fecalis	50.0	7.90
9	M	70	intestinal pseudo-obstruction	9.6	Anaerobes, E. coli	11.2	2.57
10	M	47	progressive systemic sclerosis	8.6	Anaerobes, Enterobacteria	18.3	3.86
11	M	52	progressive systemic sclerosis	9.0	E. coli	15.2	4.44

1 Normal range stool fat excretion <7g/24h.

2 Normal range total unconjugated serum bile acid levels <2.5 μM.

3 ND = not determined

Ileal pouch-anal anastomosis — Eleven patients who had undergone proctocolectomy with ileal pouch-anal anastomosis (8 patients for ulcerative colitis and 3 for familial adenomatous polyposis coli; 7 male, 4 female; mean age: 41, range 28-61 year) were studied. The patients had been operated on 2 to 7 years (median: 6 years) before this study.

Informed consent was obtained from each subject and the protocol had been approved by the Ethical Committee of the Nijmegen University Hospital. None of the subjects had undergone cholecystectomy. Routine liver function tests were normal in all subjects and none of the subjects had been using antibiotics at least 3 weeks before the study.

5.3.2 METHODS

Analysis of serum bile acids — Venous blood samples were collected after an overnight fast and were allowed to clot. After centrifugation serum was frozen at -20°C for subsequent analysis. Serum levels of the conjugated and unconjugated fractions of cholic (CA), chenodeoxycholic (CDCA) and deoxycholic (DCA) acid were measured by capillary gas-liquid chromatography. An internal standard, 7α,12α-dihydroxy-5-ßcholanoic acid, was added to the serum samples. Bile acids were extracted from serum using C18-bonded silica cartridges (SepPak, Waters Associates, Milford, MA, USA) (13). Separation of conjugated and unconjugated bile acids was carried out by means of column chromatography using the anion exchanger diethylaminohydroxypropyl Sephadex LH-20 (Lipidex-DEAP) (13). The conjugated fractions were subjected to enzymatic hydrolysis by cholylglycine hydrolase (from *Clostridium perfringens*). The deconjugated bile acids were extracted and eluted on Lipidex-1000 columns (14). The bile acids were converted to methyl esters by 2,2-dimethoxypropane (Merck, Darmstadt, Germany). After methylation the trimethylsilyl ether derivates were prepared by addition of a solution of pyridine, hexamethyldisilazane and trimethylchlorosilane (3:2:1 by vol.). Immediately before application to the gas chromatograph the silylation reagent was dried under nitrogen and the samples were redissolved in 50 µL hexane. Two µL of this solution was used for injection. Separation and quantification of individual bile acids was performed on a Packard 430 gas-liquid chromatograph (Packard Instruments, Delft, The Netherlands) with a flame ionisation detector and equipped with a 25 m * 0.22 mm glass capillary column (CP-Sil-5 CB, Chrompack, Middelburg, The Netherlands). The chromatograph was equipped with an automatic solid injection system. Helium

was used as carrier gas. The injection temperature was 285°C and the individual bile acids were separated by computer based stepwise increment of column temperature from 150-290°C. The flame ionisation temperature was 280°C. Bile acids were identified by the comparing retention times of the individual bile acids to those of reference bile acids.

Bacterial examination of jejunal fluid — Jejunal fluid was obtained by means of a gas-sterilized radio-opaque polyvinylchloride tube after an overnight fast. Samples of jejunal fluid were collected 15 cm beyond the duodeno-jejunal junction. The position of the tube was checked fluoroscopically. To prevent contamination with saliva the tip of the tube was sealed with an agar plug. Before sampling the agar plug was expelled by inflating nitrogen through the tube. Immediately after sampling 0.5 ml of jejunal fluid was added to 4.5 ml of Wensinck medium containing 1.0 g glucose, 4.0 g starch, 10.0 g tryptose, 5.0 g NaCl, 3.0 g K_2HPO_4, 0.5 g KH_2PO_4, 0.5 g $MgSO_4$, 0.5 g cysteine-HCl, and 3.0 g yeast extract dissolved in 1000 ml H_2O. The procedure was continued in an anaerobic chamber with an oxygen tension of less than 5 ppm. (15). After homogenization, serial dilutions of the homogenate were spread on a non-selective agar plate with 7% blood obtained from sheep. Aerobic and anaerobic cultures were continued at 37°C for two and seven days respectively. For aerobic cultures, four additional selective media (Mannitol salt agar, Kanamycin esculin azide agar, Sabouraud agar, Oxoid, Pharmachemie, Haarlem, The Netherlands and Levine EMB, Boom, Meppel, The Netherlands) were used. Counting of bacteria was done using the method described by Holdeman (16).

Statistical analysis — Bile acid levels were expressed as mean, median and range. In order to test the hypothesis that unconjugated serum bile acids (CA, CDCA, DCA, total, and percentage unconjugated serum bile acids) were different in at least one of the patient groups the Kruskal-Wallis one-way AOV was used. If so, groups were compared pairwise using the Mann-Whitney U-test to identify which of the groups were different from patients with bacterial overgrowth and from controls. A p-value <0.01 was considered statistically significant because of multiple comparisons. The mean of controls plus at least two standard deviations was chosen as cut-off level between normal and elevated serum bile acid levels, unless stated otherwise.

5.4 Results

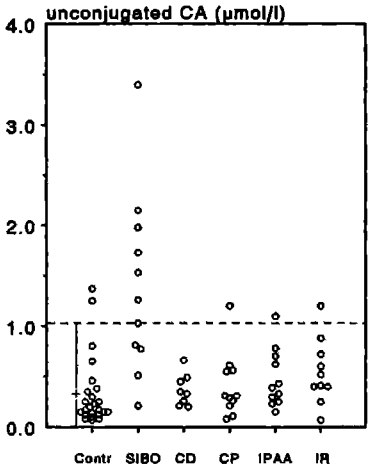
5.4.1 BACTERIAL EXAMINATION OF JEJUNAL FLUID

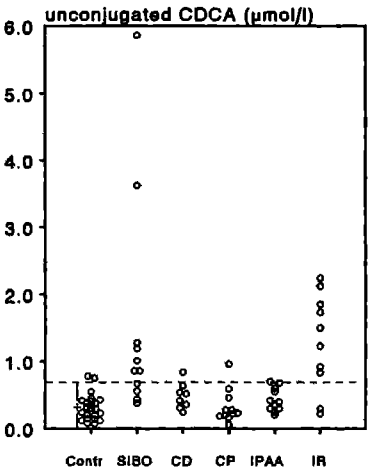
In all healthy subjects the sum of aerobic and anaerobic bacteria found in jejunal fluid was less than $10^{6.5}$ per ml. In all patients with suspected small intestinal bacterial overgrowth the number of bacteria exceeded 10^8 per ml (Table 5.1).

5.4.2 INDIVIDUAL UNCONJUGATED SERUM BILE ACIDS

Cholic acid — Unconjugated CA levels (Figure 5.1) were significantly higher in patients with small intestinal bacterial overgrowth (mean: 1.40, median: 1.26, range 0.21-3.40 μM) compared to healthy controls (mean: 0.35, median: 0.20, range: 0.07 -1.37 μM, p<0.0001), patients with coeliac disease (mean: 0.37, median: 0.34, range: 0.20-0.66 μM, p=0.003), patients with chronic pancreatitis (mean: 0.41, median: 0.31, range: 0.08-1.20 μM, p=0.004), and patients with ileal pouch-anal anastomosis (mean: 0.48, median: 0.39, range: 0.15-1.10 μM, p=0.006). However, there was a considerable overlap between the groups. Unconjugated CA levels in patients with bacterial overgrowth were also higher compared to patients with ileal resection (mean: 0.54, median: 0.47, range: 0.07-1.20 μM) but the difference did not reach statistical significance (p=0.012). No significant differences were observed between controls and patients with coeliac disease, chronic pancreatitis, ileal pouch-anal anastomosis, and patients with ileal resection.

Chenodeoxycholic acid — Unconjugated CDCA levels (Figure 5.1) were significantly higher in patients with small intestinal bacterial overgrowth (mean: 1.52, median: 0.86, range 0.38-5.86 μM) compared to the healthy controls (mean: 0.32, median: 0.30, range: 0.07-0.78 μM, p<0.0001), patients with coeliac disease (mean: 0.49, median: 0.47, range: 0.25-0.84 μM, p=0.009), patients with chronic pancreatitis (mean: 0.33, median: 0.23, range: 0.05-0.96 μM, p=0.001), and ileal pouch patients (mean: 0.43, median: 0.40, range: 0.20-0.70 μM, p=0.003). However, there was a considerable overlap between these groups. Unconjugated CDCA levels in patients with ileal resection (mean: 1.29, median: 1.37, range 0.22-2.24 μM) were significantly higher compared to controls (p=0.0005). No significant difference was observed between patients with bacterial overgrowth and patients with ileal resection (p=0.59). No significant differences were observed between controls and patients with coeliac disease, chronic pancreatitis, and ileal pouch-anal anastomosis.





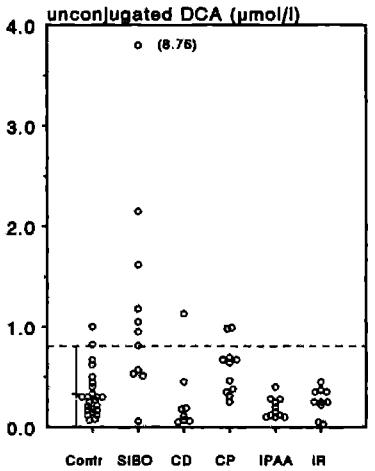


Figure 5.1. Unconjugated cholic acid (CA), chenodeoxycholic acid (CDCA), and deoxycholic acid (DCA) levels in controls (Contr), patients with small intestinal bacterial overgrowth (SIBO), coeliac disease (CD), chronic pancreatitis (CP), ileal pouch-anal anastomosis (IPAA), and in patients with ileal resection (IR). Error bars indicate mean ± 2sd.

Deoxycholic acid — Unconjugated DCA levels (Figure 5.1) were significantly higher in patients with small intestinal bacterial overgrowth (mean: 1.65, median: 0.95, range 0.06-8.76 µM) compared to healthy controls (mean: 0.33, median: 0.29, range: 0.07-1.00 µM, p=0.001), patients with coeliac disease (mean: 0.28, median: 0.15, range: 0.05-1.13 µM, p=0.01), patients with ileal pouch-anal anastomosis (mean: 0.19, median: 0.15, range: 0.10-0.40

μM, p=0.001), and patients with ileal resection (mean: 0.25, median: 0.25, range: 0.00-0.45 μM, p=0.001). Unconjugated DCA levels in patients with chronic pancreatitis (mean: 0.58, median: 0.64, range 0.25-0.99 μM) did not differ significantly from those in patients with bacterial overgrowth (p=0.08). Again, there was a considerable overlap between patients with bacterial overgrowth and controls. No significant difference was observed between controls and patients with coeliac disease, ileal resection, or ileal pouch-anal anastomosis. Unconjugated DCA levels were significantly higher in patients with chronic pancreatitis compared to controls (p=0.005).

Total unconjugated serum bile acid levels — Total unconjugated serum bile acid levels (Figure 5.2) were significantly higher in patients with small intestinal bacterial overgrowth (mean: 4.57, median: 3.86, range 1.46-9.35 μM) compared to healthy controls (mean: 1.00, median: 0.93, range: 0.21-2.85 μM, p<0.0001), patients with coeliac disease (mean: 1.13, median: 1.01, range: 0.56-1.86 μM, p<0.001), patients with chronic pancreatitis (mean: 1.31, median: 1.07, range: 0.80-2.43 μM, p<0.001), patients with ileal pouch-anal anastomosis (mean: 1.10, median: 0.96, range: 0.47-1.90 μM, p<0.001), and patients with ileal resection (mean: 2.09, median: 2.24, range: 0.31-3.54

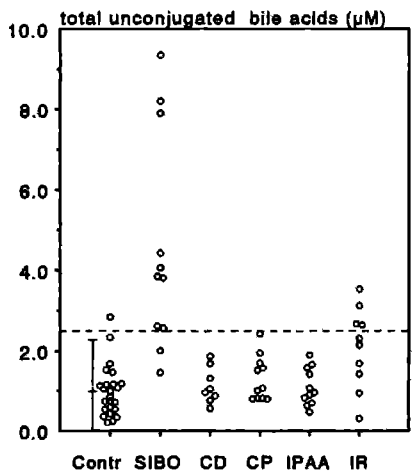


Figure 5.2. **Total unconjugated serum bile acid levels (CA+CDCA+DCA) in controls (Contr), patients with small intestinal bacterial overgrowth (SIBO), coeliac disease (CD), chronic pancreatitis (CP), ileal pouch-anal anastomosis (IPAA), and patients with ileal resection (IR). Error bar indicates mean ± 2sd. Cut-off level between normal and elevated total unconjugated bile acid levels was chosen at 2.5 μM.**

μM, p=0.018). Total unconjugated serum bile acid levels in patients with ileal resection were significantly higher compared to controls (p=0.006). No significant differences were observed between controls on the one hand and patients with coeliac disease, chronic pancreatitis, or ileal pouch-anal anastomosis on the other.

When 2.5 μM was chosen as cut-off level between normal and elevated total unconjugated serum bile acid levels, 9 out of 11 patients with bacterial overgrowth had levels outside the normal range (sensitivity = 82%). In all patients with other malabsorption syndromes (8/8 patients with coeliac disease and 11/11 with chronic pancreatitis) levels were within the normal range (specificity = 100%). The positive predictive value of elevated total unconjugated serum bile acids as a diagnostic criterium for small intestinal bacterial overgrowth in patients with malabsorption was 100% (9/9 patients). The negative predictive value was 90% (19/21 patients). The accuracy was 93% (28/30 patients).

Individual unconjugated bile acids provided a poorer discrimination between patients and controls compared to total unconjugated bile acids. Unconjugated CA, CDCA, and DCA levels were outside the normal range in 7 (64%) out of 11 patients with bacterial overgrowth (Figure 5.1).

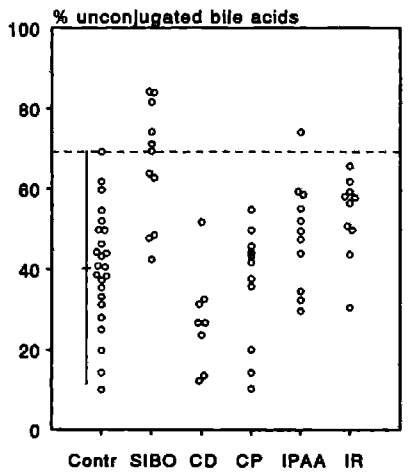


Figure 5.3. **Percentage unconjugated serum bile acid levels in controls (Contr), patients with small intestinal bacterial overgrowth (SIBO), coeliac disease (CD), chronic pancreatitis (CP), ileal pouch-anal anastomosis (IPAA), and patients with ileal resection (IR). Error bar indicates mean ± 2sd.**

Percentage unconjugated serum bile acid levels — Total unconjugated bile acids expressed as percentage of the sum of total conjugated and unconjugated bile acid levels (Figure 5.3) were significantly higher in patients with small intestinal bacterial overgrowth (mean: 66, median: 69, range: 42-84%) compared to the healthy controls (mean: 40, median: 41, range 10-69%, $p<0.001$), patients with coeliac disease (mean: 27, median: 27, range: 12-51%, $p<0.001$), chronic pancreatitis (mean: 36, median: 42, range: 10-55%, $p<0.001$). In patients with ileal pouch-anal anastomosis (mean: 49, median: 49, range: 30-74%, $p=0.018$) and ileal resection (mean: 53, median: 57, range 30-66%, $p=0.05$) the percentage unconjugated serum bile acid levels tended to be lower compared to the patients with bacterial overgrowth, but the difference did not reach statistical significance. Again, discrimination between the groups was poor because of a considerable overlap. No significant differences were observed between controls and patients with coeliac disease, chronic pancreatitis, ileal pouch-anal anastomosis, or ileal resection.

5.5 Discussion

In small intestinal bacterial overgrowth conjugated bile acids are deconjugated in the small intestine by bacterial enzymes. This results in malabsorption of fat and fat-soluble vitamins since unconjugated bile acids lack the capacity to form micelles. Unconjugated bile acids are rapidly absorbed in the small intestine and are less effectively extracted from the portal blood by the liver than conjugated bile acids (17).

Unconjugated bile acid levels in serum have been shown to be elevated in patients with small intestinal bacterial overgrowth (8-12,18). Lewis et al. (8) found elevated fasting total serum bile acid levels in patients with clinical signs of small intestinal bacterial overgrowth using thin layer chromatography. This increase was almost entirely due to the presence of high unconjugated bile acid levels. Conjugated bile acid levels were normal or slightly elevated. More recently, Setchell (9) found markedly elevated unconjugated bile acid levels throughout the day in a patient with small intestinal bacterial overgrowth using gas-liquid chromatography. In this patient a remarkable predominance of DCA and other secondary bile acids was found.

Bolt and co-workers (10) studied unconjugated serum bile acid levels using both gas-liquid chromatography-mass spectrometry and enzymatic fluoro-

metry in nine patients with a positive ^{14}C-glycocholate breath test and clinical signs compatible with small intestinal bacterial overgrowth. Unconjugated serum bile acid levels were significantly higher in patients with bacterial overgrowth but there was a considerable overlap between patients and controls. Six out of nine patients had clearly elevated total unconjugated serum bile acid levels. When individual unconjugated bile acids were measured by gas-liquid chromatography-mass spectrometry, CA provided the best discrimination: eight out of nine patients had levels out of the normal range. CDCA and DCA levels were elevated in 6 and 4 patients, respectively. Thus, in contrast to the patient described by Setchell (9), there was no predominance of secondary bile acids in the serum of these patients. However, like in the former studies bacterial overgrowth was not validated by jejunal culture but by a ^{14}C-glycocholate breath test. The ^{14}C-glycocholate breath test depends on bacterial cleavage of glycine from the conjugated bile acid, metabolism of the glycine moiety to $^{14}CO_2$ and measurement of ^{14}C in breath samples. This test has a low specificity (7) because false positive results are obtained in patients with idiopathic bile acid malabsorption and after ileal resection.

Masclee et al. (12) found significantly elevated total unconjugated serum bile acid levels using enzymatic fluorometry in patients with culture proven bacterial overgrowth compared to healthy subjects, patients with an accelerated intestinal transit and patients who had undergone an ileal resection. The sensitivity of the test as a marker for bacterial overgrowth was 90%.

In the present study, fasting total unconjugated serum bile acids were elevated in 9 out of 11 patients with culture proven bacterial overgrowth (sensitivity = 82%). Discrimination between patients and controls could not be improved by using individual unconjugated bile acid levels. Total unconjugated serum bile acids appeared to be within the normal range in all patients with other malabsorptive states (specificity = 100%), when 2.5 μM was chosen as cut-off level. In patients with malabsorption, the positive and negative predictive values were 100% and 90% respectively. When specificity and predictive values were calculated in all non-operated subjects regardless of having malabsorption 42 out of 43 subjects without bacterial overgrowth had levels within the normal range (specificity = 98%). In this population positive and negative predictive values were 90% (9/10) and 95% (42/44) respectively.

In contrast to Setchell (9) and in agreement with Bolt (12) we did not find a predominance of unconjugated secondary bile acids since unconjugated DCA levels were elevated in 7 out of 11 bacterial overgrowth patients, whereas both CA and CDCA levels were also elevated in 7 patients.

Probably as a result of spill-over into the colon, unconjugated CDCA levels were remarkably high in patients with ileal resection. However, CA and DCA levels were within the normal range. Total unconjugated serum bile acids were significantly higher in patients with ileal resection compared to controls.

In conclusion, this study demonstrates that determination of fasting total unconjugated serum bile acid levels is a sensitive and specific marker for small intestinal bacterial overgrowth, and that determination of individual unconjugated bile acids offers no advantage in terms of sensitivity or specificity over total unconjugated serum bile acid levels. Mass-spectrometry in addition to gas-liquid chromatography appears to be redundant in the identification of patients with bacterial overgrowth. This implies a major simplification of bile acid analysis. After ileal resection unconjugated CDCA levels are elevated, probably as a result of spill-over into the colon.

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POSTPRANDIAL CONJUGATED AND UNCONJUGATED SERUM BILE ACID LEVELS AFTER PROCTOCOLECTOMY WITH ILEAL POUCH-ANAL ANASTOMOSIS

Jan M.J.I. Salemans, Fokko M. Nagengast, Albert Tangerman, Annie van Schaik, Anton F.J. de Haan*, Jan B.M.J. Jansen.
From the Division of Gastroenterology, Department of Medicine, and *Department of Medical Statistics, University Hospital Nijmegen, The Netherlands.

Scand J Gastroenterol 1993;28:786-790

6.1 Abstract

In patients with ileal pouch anal anastomosis (IPAA) bile acid reabsorption may be impaired and stasis in the ileal pouch may lead to deconjugation and dehydroxylation of bile acids as a result of bacterial overgrowth. Therefore, we studied fasting and postprandial conjugated and unconjugated serum levels of cholic (CA), chenodeoxycholic (CDCA), and deoxycholic acid (DCA) in 11 patients who had undergone proctocolectomy with IPAA and in 11 healthy controls. Fasting levels of conjugated DCA but not CA and CDCA were significantly lower in IPAA patients. Postprandially, conjugated bile acid levels were significantly lower in IPAA patients. Postprandial unconjugated CA levels were significantly higher and CDCA levels tended to be higher in IPAA patients, whereas unconjugated DCA levels were lower in IPAA patients. These data suggest that reabsorption of conjugated bile acids is impaired after IPAA; that deconjugation of bile acids may result from bacterial overgrowth secondary to stasis in the pouch; and that dehydroxylation of bile acids is decreased after proctocolectomy with IPAA.

6.2 Introduction

Proctocolectomy with ileal pouch-anal anastomosis (IPAA) is an attractive and widely accepted surgical alternative to permanent ileostomy or continent ileostomy in patients with severe chronic ulcerative colitis and familial adenomatous polyposis since anal function is preserved and the necessity for permanent ileostomy is eliminated (1,2). Bile acid metabolism may be altered in these patients for several reasons. First, the terminal ileum, which is being used in the construction of the ileal reservoir, plays a key role in the reabsorption of conjugated bile acids. An increased fecal excretion of bile acids has been observed in patients with a Kock's continent ileostomy (3-5), and in IPAA patients (6,7). This suggests that ileal reabsorption of conjugated bile acids is impaired in these patients. Secondly, stasis of fecal contents in the ileal pouch may theoretically lead to bacterial overgrowth with abnormal deconjugation of bile acids. Finally, 7α-dehydroxylation of the primary bile acids cholic (CA) and chenodeoxycholic acid (CDCA) to the secondary bile acids deoxycholic (DCA) and lithocholic acid by colonic bacterial flora, is probably decreased after proctocolectomy.

The aim of this study was to investigate whether proctocolectomy with

IPAA leads to alterations in absorption, deconjugation, and 7α-dehydroxylation of bile acids. Since systemic bile acid levels are proportional to the load of bile acids absorbed from the intestine (8,9) and postprandial conjugated serum bile acid levels increase rapidly in healthy subjects (10-12) fasting and postprandial serum bile acid levels were compared in IPAA patients and in healthy non-colectomized subjects. Both conjugated and unconjugated serum bile acid levels were measured since unconjugated bile acids levels are usually elevated in patients with small intestinal bacterial overgrowth (13-17).

6.3 Subjects and methods

Subjects — Eleven subjects who underwent proctocolectomy with IPAA (8 ulcerative colitis and 3 familial adenomatous polyposis patients; 7 male, 4 female; mean age: 41, range 28-61 year) and eleven healthy sex and age matched controls (7 male, 4 female; mean age 41, range 27-61 year) were studied. Patients and controls were matched for age since postprandial serum levels of conjugated bile acids are influenced by ageing (18). The patients had been operated 2 to 7 years (median: 6 years) before this study. None of the IPAA patients had clinical signs of pouchitis at the time of investigation. None of the subjects had undergone cholecystectomy and none of the healthy subjects had a history of gastrointestinal disease. Routine liver function tests were normal in all subjects. Informed consent was obtained from each subject and the protocol had been approved by the Ethics Committee of the Nijmegen University Hospital.

Experimental protocol — All subjects fasted overnight and then ingested a standardized breakfast composed of one slice of bread, 5 g margarine, 20 g cheese, one boiled egg, 150 ml yogurt and one cup of tea with 5 g sugar (21 g protein, 21.5 g fat, 34 g carbohydrate, 413 kcal). Venous blood samples for analysis of serum bile acid levels were taken at 0, 30, 60, 90, 120, 150, 180, 210, and 240 minutes through an indwelling catheter placed in an antecubital vein. Blood samples were allowed to clot. After centrifugation the separated serum was frozen at -20°C for subsequent analysis.

Analysis of serum bile acids — Serum bile acid levels were measured by capillary gas-liquid chromatography, a highly accurate and sensitive method (19). 7α,12α-dihydroxy-5ßcholanoic acid was added as an internal standard. Bile

acids were extracted from serum using C18-bonded silica cartridges (SepPak, Waters Associates, Milford, MA, USA) (20). Separation of conjugated and unconjugated bile acids was carried out by means of column chromatography using the lipophilic anion exchanger diethylaminohydroxypropyl Sephadex LH-20 (Lipidex-DEAP, Packard Instruments, Groningen, The Netherlands) (20). The conjugated fractions were subjected to enzymatic hydrolysis by cholylglycine hydrolase (from *Clostridium perfringens*) (21). After enzymatic hydrolysis the deconjugated bile acids were extracted and eluted on Lipidex-1000 columns (Packard Instruments) (21). The bile acids were converted to methyl esters by 2,2-dimethoxypropane. After methylation trimethylsilyl ether derivates were prepared by addition of a solution of pyridine, hexamethyldisilazane and trimethylchlorosilane (3:2:1 by vol.). Separation and quantification of bile acids was performed on a Packard 430 gas-liquid chromatograph with a flame ionisation detector and equipped with a 25 m * 0.25 mm glass capillary column (CP-Sil-5 CB, Chrompack, Middelburg, The Netherlands).

Statistical analysis — Results were expressed as the mean ± SEM. Bile acid curves were tested by standard repeated measurements analysis of variance. To eliminate skewness of the bile acid distribution a logarithmic transformation of the data was applied. Firstly, time effects were studied. Secondly, the hypothesis that time effects were equal for both groups was tested. When time effects were equal the mean bile acid curves were considered to be parallel. When the bile acid curves were non-parallel time effects were described separately for both groups. Integrated bile acid values (area under the curve) were compared with the two-tailed Student's t-test after logarithmic transformation to eliminate skewness in the distribution. A p-value <0.05 was considered statistically significant.

6.4 Results

Conjugated bile acids — Fasting levels of the conjugated primary bile acids (CA and CDCA) were similar in IPAA patients and controls. Fasting levels of conjugated DCA however, were significantly lower in the IPAA group. After the test meal levels of all individual conjugated bile acids increased more rapidly and to a higher extent in the control subjects compared to the IPAA patients (Figure 6.1). In IPAA patients conjugated DCA levels were extremely low

Table 6.1 **Integrated conjugated serum bile acid levels in patients with ileal pouch-anal anastomosis (IPAA) and healthy controls.**

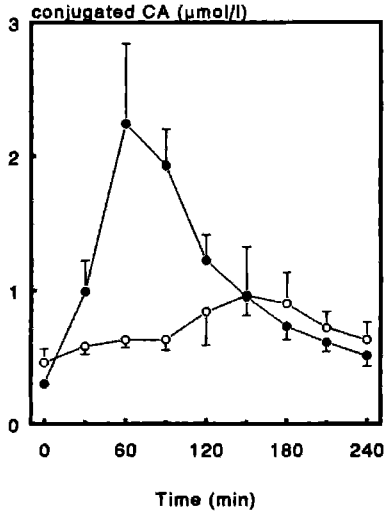
	controls (μM.min)	*IPAA (μM.min)*	*significance level*
CA	272 ± 40	175 ± 30	p<0.05
CDCA	601 ± 54	405 ± 40	p<0.05
DCA	270 ± 30	64 ± 12	p<0.001

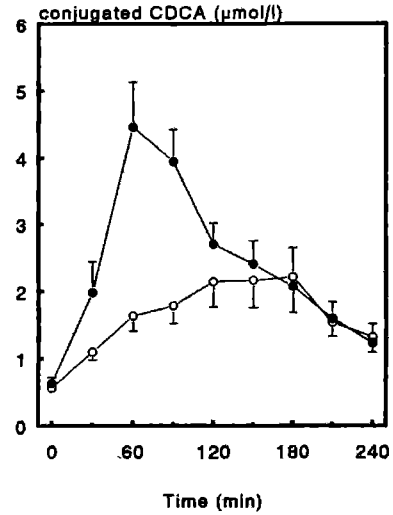
Table 6.2 **Integrated unconjugated serum bile acid levels in patients with ileal pouch-anal anastomosis (IPAA) and healthy controls.**

	controls (μM.min)	*IPAA (μM.min)*	*significance level*
CA	60 ± 10	110 ± 17	p<0.05
CDCA	89 ± 13	123 ± 15	NS
DCA	112 ± 23	48 ± 7	p<0.05

compared to the primary bile acid levels and showed no postprandial increase. Repeated measurement analysis revealed that the trends of all individual conjugated serum bile acid curves differed significantly between both groups (Figure 6.1). The integrated conjugated bile acid levels were significantly higher in the healthy controls compared to the IPAA patients (Table 6.1). The ratio of the integrated levels of conjugated DCA and the sum of the integrated levels of conjugated CA and CDCA levels was significantly lower in the ileal pouch patients (mean ± SEM: 0.11 ± 0.02) when compared to the controls (0.34 ± 0.06; p<0.005).

Unconjugated bile acids — Fasting unconjugated serum bile acids levels were not significantly different between both groups. Repeated measurements analysis revealed a significant postprandial increase of unconjugated DCA levels in the healthy subjects but not in IPAA patients whereas CA and CDCA levels showed no significant postprandial trend in both groups (Figure 6.2). The integrated unconjugated serum bile acid levels are shown in Table 6.2. Integrated CA levels were significantly higher in IPAA patients, whereas integrated DCA levels were significantly lower in the IPAA patients. Integrated CDCA levels tended to be higher in the IPAA patients but the difference did not reach statistical significance. The ratio of the integrated levels of





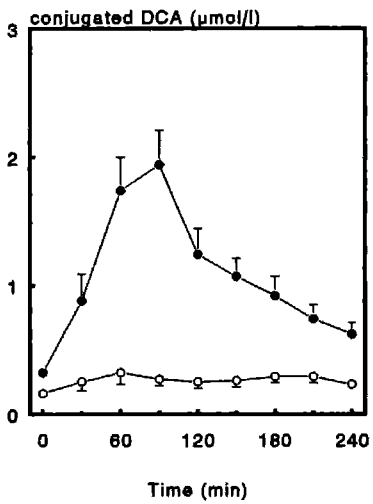


Figure 6.1 Fasting and postprandial conjugated serum levels (mean ± SEM) of cholic (CA), chenodeoxycholic (CDCA) and deoxycholic acid (DCA) in ileal pouch patients (open markers) and healthy controls (closed markers). All bile acid curves of both groups were non-parallel (CA: $p<0.001$, CDCA: $p<0.01$; DCA $p<0.001$; repeated measurements analysis).

unconjugated DCA and the sum of the integrated levels of unconjugated CA and CDCA levels was significantly lower in the IPAA patients (mean ± SEM: 0.23 ± 0.03) when compared to the controls (0.94 ± 0.29; $p<0.05$).

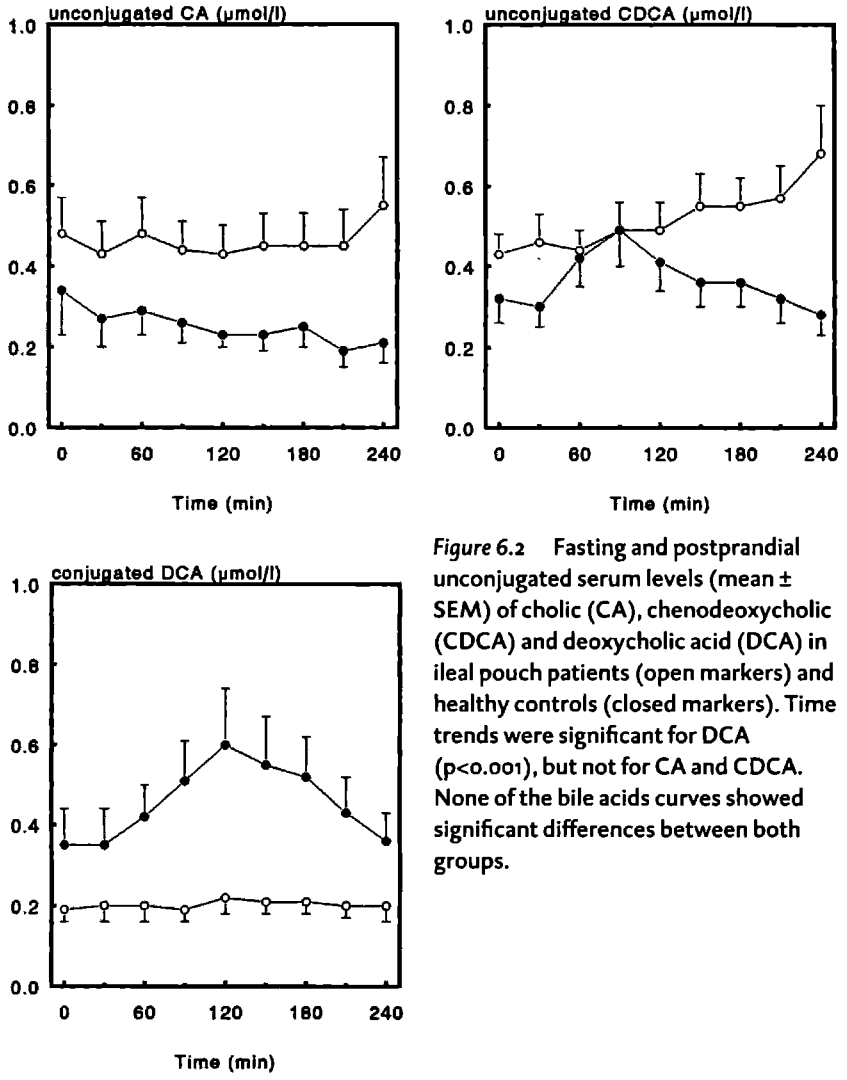


Figure 6.2 Fasting and postprandial unconjugated serum levels (mean ± SEM) of cholic (CA), chenodeoxycholic (CDCA) and deoxycholic acid (DCA) in ileal pouch patients (open markers) and healthy controls (closed markers). Time trends were significant for DCA ($p<0.001$), but not for CA and CDCA. None of the bile acids curves showed significant differences between both groups.

6.5 Discussion

In the present study fasting and postprandial serum levels of conjugated and unconjugated individual bile acids in patients who had undergone proctocolectomy with IPAA were compared with those obtained in healthy non-colectomized volunteers. Postprandial conjugated bile acid levels increased to a

lower extent in IPAA patients compared with the healthy subjects. This suggests that reabsorption of bile acids is impaired after proctocolectomy with IPAA since systemic bile acid levels are thought to be proportional to the load of bile acids absorbed from the intestine (8,9). Malabsorption of bile acids might be expected in these patients for several reasons. Firstly, the mucosa of ileal pouches show histologic signs of inflammation in the vast majority of patients (22). This may lead to an impaired reabsorption of conjugated bile acids with an increased loss of fecal bile acids. Secondly, reabsorption of conjugated bile acids may be impaired since the relative mucosal surface of the terminal ileum is smaller after construction of a reservoir. Finally, stasis in the ileal pouch may lead to bacterial overgrowth with abnormal deconjugation of bile acids.

Fecal bile acid excretion after colectomy with conventional ileostomy, continent Kock's ileostomy, and IPAA has been studied by various groups (3-7,23). Bile acid absorption, as determined by the fecal excretion of intravenously administered ^{14}C-cholic acid, was studied by Andersson et al. (3) in conventional ileostomy patients before and after conversion to a continent Kock's ileostomy. Fecal bile acid excretion increased more than two-fold after conversion to a Kock's pouch. Hylander et al. (4) found an increased fecal excretion of ^{14}C after oral ingestion of ^{14}C-glycocholic acid in both conventional ileostomy patients and Kock's ileostomy patients compared to non-colectomized healthy subjects. The fecal ^{14}C excretion was higher in the continent ileostomy patients compared to the conventional ileostomy patients. Pulmonary $^{14}CO_2$ excretion was increased in patients with a Kock's pouch, but not in those with a conventional ileostomy. This suggests that bile acid reabsorption is impaired after both colectomy with conventional ileostomy and Kock's ileostomy and that bile acids are deconjugated as a result of bacterial overgrowth in the ileal pouch. Go et al. (23) however, found no impaired reabsorption of bile acids in patients with a Kock's pouch using direct installation of 23-^{75}Se-homotaurocholic acid (SeHCAT) into the ileal pouch.

Pedersen et al. (6) found a significant higher excretion of bile acids in IPAA patients compared to healthy controls. IPAA patients excreted primary bile acids, whereas controls mainly excreted secondary bile acids. Natori et al. (7) found that the mean daily fecal bile acid output in IPAA and conventional ileostomy patients was 1.5 times that of healthy, non-colectomized volunteers. The output of secondary and unconjugated fecal bile acids was higher in IPAA patients compared to conventional ileostomy patients, suggesting that bacterial conversion is more prominent in IPAA patients.

The present study provides further evidence that reabsorption of conjugated bile acids is impaired in IPAA patients. Our data further suggest that conversion of primary to secondary bile acids (7 α-dehydroxylation) is decreased after proctocolectomy with IPAA since both conjugated and unconjugated DCA levels were extremely low in these patients. Integrated unconjugated CA levels were significantly higher in IPAA patients, suggesting that bacterial overgrowth in the ileal pouch leads to abnormal deconjugation of bile acids.

The major long-term complication after ileal pouch-anal anastomosis is pouchitis, an non-specific inflammation of the ileal reservoir, occurring in 11-44% of patients (24-28). The etiology of pouchitis is unclear but it has been suggested that stasis and bacterial overgrowth with deconjugation of bile acids in the reservoir may play a role in its pathogenesis (29,30). Patients with pouchitis usually respond favourably to treatment with metronidazole. Higher counts of anaerobic bacteria have been found in effluents of Kock's and ileoanal pouches compared to effluents from conventional ileostomies (31). However, culture of pouch effluents from patients with pouchitis did not reveal higher numbers of anaerobes compared to patients without pouchitis (32,33). Since pouchitis almost exclusively occurs in ulcerative colitis patients but not in familial adenomatous polyposis patients several authors have suggested that pouchitis is a novel manifestation of ulcerative colitis (22,27,28). In the present study no essential differences were found between serum bile acid profiles in ulcerative colitis and familial polyposis patients.

In conclusion, the postprandial rise of conjugated bile acid levels was significantly lower in patients with IPAA compared to that of the healthy controls. The postprandial serum levels of unconjugated CA and CDCA were higher in the IPAA patients compared to the controls although in the case of CDCA the difference did not reach statistical significance. The postprandial unconjugated DCA levels, however, were significantly lower in the IPAA patients. These data suggest that reabsorption of conjugated bile acids is impaired after IPAA and that bile acids are deconjugated by bacteria in the ileal pouch. Bacterial overgrowth in the ileal pouch, however, does not lead to 7 α-dehydroxylation, indicated by very low DCA levels in the IPAA patients.

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PLASMA CHOLECYSTOKININ LEVELS AND GALLBLADDER VOLUMES AFTER PROCTOCOLECTOMY WITH ILEAL POUCH-ANAL ANASTOMOSIS

Jan M.J.I. Salemans, Paul W.L. Thimister, Wim P.M. Hopman, Han J.H. Kuijpers[1], Gerd Rosenbusch[2], Fokko M. Nagengast and Jan B.M.J. Jansen.
From the Division of Gastroenterology, Department of Medicine, Surgery[1], and Radiology[2], Nijmegen University Hospital, The Netherlands.

Surgery 1995; 117:705-711

7.1 Abstract

The colon and rectum contain regulatory peptides in mucosal endocrine cells, which suggests a hormonal role. In animal studies colectomy leads to increased plasma levels of cholecystokinin. Little is known about the effects of proctocolectomy with ileal pouch-anal anastomosis (IPAA) on the release of cholecystokinin (CCK) in man. Therefore we studied the effects of this procedure on fasting, postprandial, and bombesin stimulated plasma CCK levels and gallbladder volumes. Ten patients who had undergone proctocolectomy with IPAA and 12 healthy volunteers participated in the study. Fasting and postprandial plasma CCK levels and gallbladder volumes were studied for 3 hours at 15 min intervals. In a second experiment plasma CCK levels were measured before and during intravenous administration of bombesin in six ileal pouch patients and five healthy volunteers. Fasting plasma CCK levels were higher ($p<0.05$) in IPAA patients (2.6 ± 0.3 pM) compared to controls (1.7 ± 0.2 pM). Integrated postprandial plasma CCK levels were also distinctly higher ($p<0.01$) in patients (978 ± 126 pM.180min) than in controls (588 ± 60 pM.180min). Mean fasting gallbladder volume was significantly ($p<0.01$) decreased in IPAA patients (18 ± 2 mL) compared to controls (28 ± 2 mL). Postprandial gallbladder emptying as measured by percentage change was similar in both groups. After infusion of bombesin, integrated plasma CCK responses were higher ($p<0.05$) in patients (161 ± 20 pM.20min) than in controls (90 ± 12 pM.20min). In conclusion: fasting, postprandial, and bombesin stimulated plasma CCK levels are elevated in IPAA patients compared to controls. Fasting gallbladder volume is decreased after IPAA. These findings suggest that the colon contains a factor that inhibits the release of CCK.

7.2 Introduction

Proctocolectomy with ileal pouch-anal anastomosis (IPAA) is an attractive and widely accepted alternative to permanent ileostomy in patients operated upon for severe ulcerative colitis or familial adenomatous coli, since the normal route of defecation is preserved (1,2). Long-term functional results are generally gratifying as defecation frequency and degree of incontinence is acceptable in the majority of patients (3-7).

The colon and rectum contain regulatory peptides in mucosal endocrine cells, which suggests a hormonal role (8). Harper et al. (9,10) demonstrated

that extracts from colonic and ileal mucosa of cats and pigs, markedly inhibited both pancreatic protein secretion and gallbladder contraction. Intraluminal perfusion of the colon with nutrients induces endocrine effects (11-16). Perfusion of the colon with oleic acid inhibits the release of cholecystokinin (CCK) and pancreatic enzyme secretion in dogs (15). Moreover, it has been shown that subtotal colectomy results in an increased postprandial CCK release in rats and dogs (17,18). These findings suggest that the colon contains a factor that suppresses the release of CCK. Little is known, however, about the effects of proctocolectomy on gastrointestinal physiology and circulating gut hormone responses in man.

The aim of this study was to examine the effect of proctocolectomy with IPAA on the release of CCK and gallbladder motility in man. Therefore, we have studied fasting and postprandial plasma CCK levels and gallbladder volumes (GBV) in IPAA patients. The results were compared with those obtained in healthy volunteers. In order to study the release of CCK independently of a meal, we further have studied CCK secretion during intravenous administration of bombesin, a neuropeptide that potently stimulates the release of CCK (19-21).

7.3 Subjects and methods

Subjects — Twelve healthy subjects (seven male and five female) and 10 patients who had undergone proctocolectomy with IPAA (three male and seven female; three cases of familial adenomatous coli and seven cases of ulcerative colitis) were studied. In all patients a J-pouch had been constructed 6 months to 7 years (median: 4 years) before this study was performed. The diverting loop ileostomy had been closed in all. None of the IPAA patients had experienced pouchitis. Mean age, weight, length, and body mass index (BMI) were similar in both groups (Table 7.1). None of the healthy subjects had a history of previous abdominal surgery or gastrointestinal disease and none of the IPAA patients had undergone cholecystectomy. Fasting plasma CCK levels were determined in an additional 5 IPAA patients and 3 healthy volunteers (both groups, total 15 subjects). To assess whether postprandial plasma CCK levels change with time of follow-up the correlation between the integrated postprandial plasma CCK levels and the length of time after operation was calculated. Informed consent was obtained from each subject and the protocol was approved by the Ethics Committee of the Nijmegen University Hospital.

Table 7.1 **Gender, mean age, weight, height and body mass index (BMI) of patients with ileal pouch-anal anastomosis (IPAA) and healthy volunteers (controls). No significant differences between both groups were observed.**

	Controls	*IPAA*	*p value*
sex	7M, 5F	7M, 3F	0.67
age (yr/range)	36.3 (22-61)	35.7 (22-49)	0.70
weight (kg)	77 ± 3	77 ± 3	0.99
height (m)	1.77 ± 0.03	1.75 ± 0.03	0.78
BMI (kg/m^2)	24.9 ± 0.9	25.2 ± 0.9	0.80

Experimental protocol — All subjects reported at 8:30 AM at the gastrointestinal research laboratory after an overnight fast. The subjects ingested a standardized breakfast (at t=0 min) composed of one slice of bread, 5 g of butter, 20 g of cheese, one boiled egg, 150 mL yoghurt, and one cup of tea with 5 g of sugar (21 g of protein, 21.5 g of fat, 34 g of carbohydrate). Venous blood samples for analysis of plasma CCK levels were drawn before (at t=-5 and 0 min) and at 15-minute intervals from 0 to 120 min and at 30-minute intervals from 120 to 180 minutes after the meal through an indwelling catheter placed in an antecubital vein. Blood was collected into glass tubes containing EDTA. After centrifugation the separated plasma was frozen at -20 °C until analysis. GBV was measured at the same time points.

In a second experiment, the response of plasma CCK to intravenous administration of bombesin (5 ng/kg/min) was measured in 6 IPAA patients (two male, four female; mean age, 34 years, range 23 to 41 years) and 5 healthy volunteers (three male, two female; mean age, 47 years, range 43 to 53 years) after an overnight fast. Three of the six IPAA patients had also participated in the first experiment. None of the IPAA patients had experienced episodes with pouchitis. None of the healthy volunteers in the bombesin experiment had participated in the first experiment. Bombesin was administered through an indwelling catheter in an antecubital vein. Blood samples for determination of plasma CCK levels were drawn from a catheter in an antecubital vein in the opposite arm before and at 5-min intervals for 20 minutes during bombesin infusion.

Gallbladder emptying — GBV was measured by means of real-time ultrasonography with the sum of cylinders method using a computerized method (22). In this method the longitudinal scan of the gallbladder is divided into a series of cylinders of equal height, with diameters perpendicular to the longitudinal axis of the gallbladder image. The uncorrected volume is the sum of volumes of these separate cylinders. To correct for the displacement of the longitudinal image of the gallbladder from the central axis, a correction factor is calculated from the longitudinal and transverse scans of the gallbladder. GBV is calculated by multiplication of the uncorrected volume with the square of the correction factor. Two longitudinal and two transverse images of the gallbladder were obtained at each time point. The mean of two measurements was used for further analysis. The variation of GBV measurements using this method ranges from 6.2% to 10.0%. Gallbladder emptying and percentage gallbladder emptying were calculated using the following formulas:

Maximum gallbladder emptying = GBV_0 - GBV_{min}
Percentage gallbladder emptying = 100%*(GBV_0 - GBV_{min})/GBV_0
were GBV_0 = mean fasting GBV (average of GBV at t=-5 and 0 min) and GBV_{min} = smallest postprandial gallbladder volume.

Plasma CCK-levels — CCK was measured in plasma by a sensitive and specific radioimmunoassay (23,24). The antibody employed (T204), was raised in rabbit after the fourth immunization with albumin-coupled crude porcine CCK, and used in a final dilution of 1:80,000. The antibody binds to all carboxy-terminal CCK-peptides containing the sulphated tyrosyl region. The antibody shows less than 2% cross-reactivity with sulphated gastrins and does not bind to unsulphated forms of gastrin or structurally unrelated peptides, like insulin, secretin, pancreatic polypeptide, bombesin and neurotensin. Synthetic $hCCK_{33}$ coupled to ^{125}I-hydroxyphenylpropionic acid succinimide ester (Bolton-Hunter reagent) was used as label and as standard preparation. The detection limit of this assay is 0.5 pM. The intra-assay variation ranges from 4.6 to 11.5% and the inter-assay variation from 11.3 to 26.1%.

Statistical analysis — Results were expressed as mean ± SEM. Fasting plasma CCK levels and fasting GBVs were calculated as the mean of two basal measurements (-5 and 0 min). Integrated plasma CCK secretion in response to the meal or to bombesin infusion was determined by calculating the area under the plasma concentration-versus-time curve using the trapezoidal rule. Sta-

tistical analysis was performed using Student's t-test for unpaired results or the rank sum test when appropriate. A two-tailed p value of less than 0.05 was considered statistically significant. The correlation between integrated postprandial plasma CCK levels and length of postoperative time of follow-up was calculated with the Pearson correlation coefficient.

7.4 Results

Fasting and postprandial plasma CCK levels — Fasting plasma CCK levels were significantly higher ($p<0.05$) in IPAA patients (2.6 ± 0.3 pM) compared to the healthy controls (1.7 ± 0.2 pM). Postprandially, plasma CCK levels increased rapidly and significantly in both groups. In healthy volunteers the mean peak plasma CCK level was 4.2 ± 0.5 pM and in IPAA patients 7.8 ± 1.1 pM ($p=0.003$). The mean time to peak plasma CCK levels was similar in both groups: 58 ± 12 min in healthy controls and 50 ± 11 min in IPAA patients (NS). Plasma CCK concentration versus time curves are shown in Figure 7.1. Integrated postprandial plasma CCK levels were significantly higher ($p<0.01$) in the IPAA patients (797 ± 77 pM.180min) compared to the healthy controls (516 ± 57 pM.180min). Individual integrated postprandial plasma CCK levels are shown in Figure 7.2. The Pearson correlation coefficient between integrated postprandial plasma CCK levels and time of postoperative follow-up was -0.63 ($p=0.052$).

Fasting gallbladder volumes and postprandial gallbladder emptying — Fasting GBVs were significantly smaller ($p<0.01$) in IPAA patients (18 ± 2 mL) when compared to healthy controls (27 ± 2 mL). Individual fasting GBVs are shown in Figure 7.3. Mean fasting and postprandial GBVs are depicted in Figure 7.4. Maximum gallbladder emptying was 23 ± 2 mL in controls and 15 ± 3 mL in IPAA patients ($p<0.01$). The smallest GBVs were reached at 95 ± 13 min in IPAA patients (3 ± 1 mL), and at 81 ± 7 min (NS) in controls (5 ± 1 mL, NS). The percentage gallbladder emptying was similar in both groups (controls: $82 \pm 3\%$; IPAA patients: $81 \pm 4\%$, NS). No correlation was observed between fasting gallbladder volume and time of postoperative follow-up.

Plasma CCK responses to bombesin-infusion — Basal plasma CCK levels were similar in both groups (controls: 1.8 ± 0.3 pM, IPAA patients 1.8 ± 0.4 pM). During infusion of bombesin the integrated plasma CCK response was

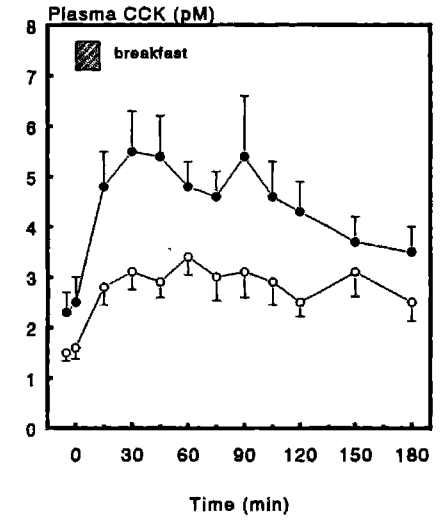

Figure 7.1 Fasting and postprandial plasma CCK versus time curves (mean ± SEM) in controls (open markers) and IPAA patients (closed markers).

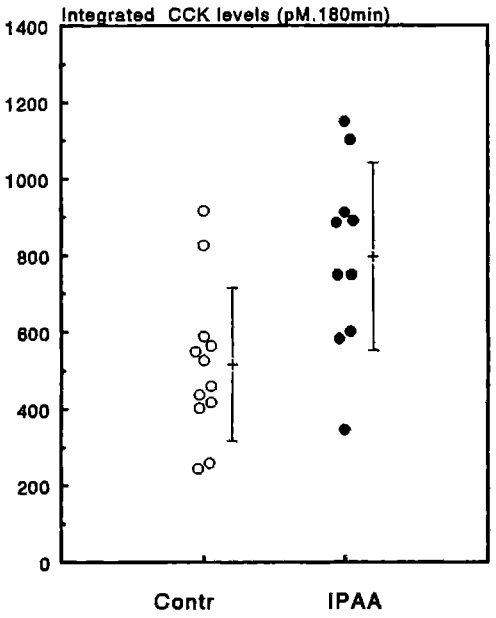

Figure 7.2 Individual integrated postprandial plasma CCK levels in healthy controls (open markers) and IPAA patients (closed markers). Integrated postprandial CCK levels were significantly higher in IPAA patients ($p<0.01$). Error bars indicate standard deviation.

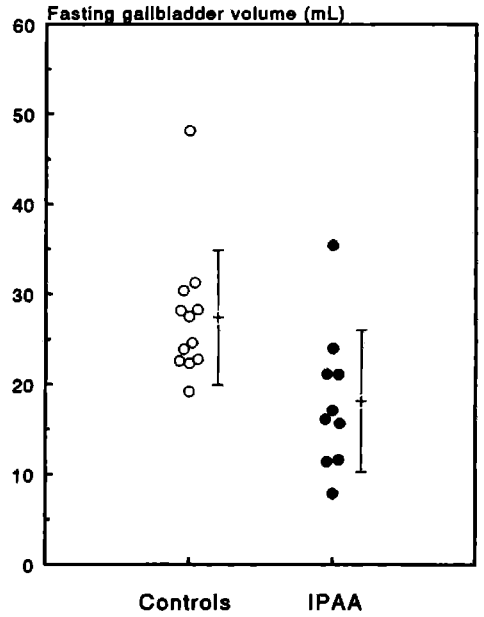

Figure 7.3 Individual fasting GBVs in healthy controls (open markers) and IPAA patients (closed markers). Error bars indicate standard deviation. Mean fasting GBV in the healthy controls (28 ± 2 mL) were significantly higher compared to IPAA patients (18 ± 2 mL, p<0.01).

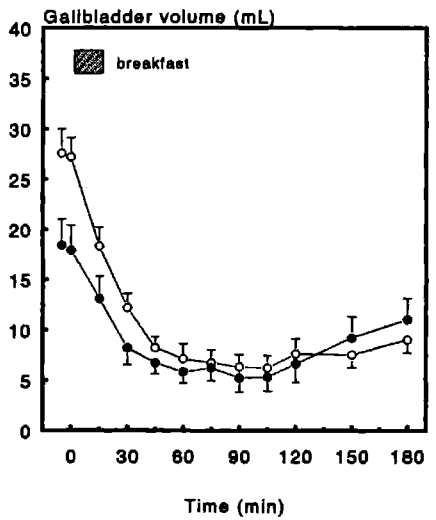

Figure 7.4 Mean (± SEM) fasting and postprandial GBVs in healthy controls (open markers) and IPAA patients (closed markers). Postprandial gallbladder emptying as measured by percentage change was similar in both groups.

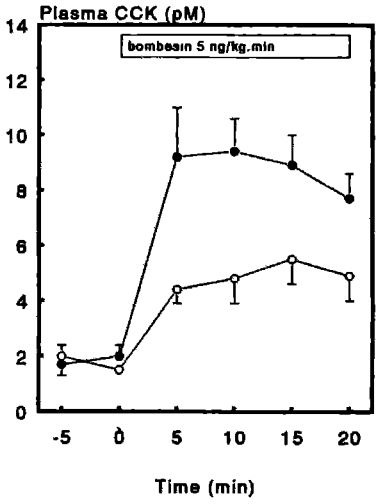

Figure 7.5 Plasma CCK response (mean ± SEM) to infusion of bombesin in 5 healthy controls (open markers) and 6 IPAA patients (closed markers). The integrated CCK response to bombesin infusion was significantly higher in IPAA patients ($p<0.05$)

significantly higher ($p<0.05$) in IPAA patients (161 ± 20 pM.20min) compared to controls (90 ± 12 pM.20min). Figure 7.5 shows the mean plasma CCK time curves in five healthy controls and six IPAA patients before and during infusion of bombesin.

7.5 Discussion

In the present study we have demonstrated that postprandial and bombesin-stimulated plasma CCK levels are increased after proctocolectomy with IPAA. To our knowledge this is the first time that the effect of proctocolectomy on plasma CCK levels and GBVs has been studied in human beings. Probably as a result of fasting hypercholecystokinaemia, fasting GBVs are decreased in IPAA patients. Basal plasma CCK levels were slightly, but significantly elevated after proctocolectomy compared to the healthy controls in our meal study. However, in the bombesin study plasma CCK levels were similar in both groups. This apparent discrepancy is likely to be due to the small number of subjects in the bombesin experiment compared to the meal study. Therefore, the power to detect a true difference in basal plasma CCK levels may

have been too low in the bombesin experiment (type II error).

Previously, it has been demonstrated that subtotal colectomy results in an increased postprandial plasma CCK release in rats and dogs (17,18). Pancreatic weight, digestive enzyme concentration, and secretion capacity increase following large bowel resection in rats (25). These effects are supposed to be induced by elevated plasma CCK levels.

Several mechanisms may account for the increased CCK levels after proctocolectomy. First, a negative feedback mechanism on the release of CCK exerted by an unknown inhibiting colonic factor may be eliminated after proctocolectomy. This hypothesis is supported by the finding that perfusion of the colon with oleic acid has been shown to inhibit pancreatic protein secretion in dogs (12), cats (13), and man (14,15). Inoue et al. (16) have demonstrated that this inhibition of pancreatic exocrine protein secretion in dogs is due, at least in part, to suppression of CCK release. Under normal conditions, however, nutrients like oleic acid do not enter the colon in concentrations used in these perfusion experiments. Therefore, it is unclear whether these nutrients play a functional role in a feedback control of proximal CCK release in healthy human beings.

Lluis et al. (26) have shown that a substance released from the colon inhibits pancreatic secretion by inhibiting CCK release in dogs. This substance, peptide YY (PYY), has been found primarily in mucosal endocrine cells in the ileum, colon, and rectum of several species (27-29) including man (30). Therefore, proctocolectomy may lead to lower circulating PYY levels and hence to elevated plasma CCK levels in human beings. Indeed, fasting PYY levels have been found to be decreased in patients who had undergone total colectomy (31) or colonic resection with ileostomy (32). This topic however, remains controversial since Armstrong et al. (33) have found increased fasting and postprandial plasma PYY levels as well as increased tissue levels of PYY one year after proctocolectomy with IPAA in dogs. It was suggested that an increased synthesis and release of PYY is an adaptive process that may contribute to a functional improvement by slowing small intestinal transit. This suggestion is supported by studies of Pietroletti et al. (34), who found elevated fasting and postprandial PYY levels after proctocolectomy with IPAA in humans. Although not of enough statistical power ($p=0.052$), we found a trend towards declining CCK levels with time postoperatively, probably as a result of adaptation. It could be speculated that during follow-up after proctocolectomy PYY synthesis in the terminal ileum and circulating PYY levels gradually increase as a result of adaptation. Assuming a suppressive effect of PYY on the

release of CCK, plasma CCK levels may initially increase after proctocolectomy and gradually decline during follow-up.

Previous studies have shown that ileal perfusion of fat emulsions markedly inhibits gastric emptying, small bowel transit, and jejunal motor activity (35-37). This 'ileal brake' correlates well to increased plasma levels of PYY (37). Intravenous infusion of PYY at physiological levels slows the mouth to caecum intestinal transit and the rate of gastric emptying (38,39). These data suggest that the ileal brake may be mediated by PYY. Small intestinal transit is also slowed in IPAA patients (40). It could be speculated that elevated PYY levels mediate this inhibition of small intestinal transit. Removal of the colon does not eliminate the ileal brake because it has been shown that oleic acid infusion into the ileal pouch slows gastric emptying and small intestinal transit and increases plasma levels of PYY (41,42). However, it is unclear to what extent stool frequency is influenced by the ileal brake mechanism following proctocolectomy with IPAA. As a result of inflammation of the ileal mucosa, the production of PYY might be decreased in patients with pouchitis. This might contribute to a rapid small intestinal transit and increased stool frequency in these patients.

Previously, we have reported that postprandial conjugated serum bile acid levels are decreased in IPAA patients compared to healthy subjects with an intact colon (43). An increased fecal excretion of bile acids has been observed in patients with a Kock's continent ileostomy (44-46) and in IPAA patients (47,48). Probably, bile acids are reabsorbed less effectively by the ileal-pouch mucosa in comparison to the mucosa of the normal terminal ileum. The bile acid pool and the bile acid output into the duodenum decrease if fecal losses are substantial. Because the release of CCK is inhibited by bile acids in the duodenal lumen (49,50), elevated plasma CCK concentrations after proctocolectomy may also be due to a decreased load of bile acids in the proximal part of the small intestine.

In conclusion, basal, postprandial, and bombesin stimulated plasma CCK levels are elevated after proctocolectomy with ileal pouch-anal anastomosis in humans. Probably as a result, fasting GBVs are decreased after proctocolectomy. The mechanisms responsible for hypercholecystokinaemia in IPAA patients are still incompletely understood, and need further investigation.

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BASAL AND MEAL STIMULATED SERUM BILE ACID LEVELS, PLASMA CHOLECYSTOKININ CONCENTRATIONS, AND GALLBLADDER VOLUMES AFTER ILEAL RESECTION

Jan M.J.I. Salemans, Fokko M. Nagengast, Albert Tangerman, Annie van Schaik, Wim P.M. Hopman, Jan B.M.J. Jansen.
From the Division of Gastroenterology, Department of Medicine, University Hospital Nijmegen, The Netherlands.

Submitted

8.1 Abstract

The aim of this study was to examine whether ileal resection leads to alterations in bile acid absorption, deconjugation, 7α-dehydroxylation, and formation of ursodeoxycholic acid (UDCA), and whether ileal resection affects plasma cholecystokinin (CCK) concentrations and gallbladder motility. Therefore, we have determined fasting and postprandial conjugated and unconjugated serum bile acid levels, plasma CCK levels, and gallbladder volumes in 8 patients who had undergone ileocaecal resection for Crohn's disease and in 12 healthy volunteers. After ileal resection, postprandial conjugated cholic (CA) and chenodeoxycholic acid (CDCA) levels were decreased and conjugated deoxycholic acid (DCA) levels were extremely low compared to controls. Unconjugated CA and CDCA levels were increased after ileal resection, whereas unconjugated DCA levels were similar in both groups. Unconjugated UDCA was present in the serum of 6 of the 8 patients with ileal resection but in none of the healthy volunteers ($p<0.01$). Fasting and postprandial CCK levels and gallbladder volumes were similar in both groups. These data demonstrate that ileal resection leads to malabsorption and increased deconjugation of conjugated bile acids, decreased 7α-dehydroxylation, and increased formation of UDCA. ileal resection does not alter the release of CCK and gallbladder motility.

8.2 Introduction

Interruption of the enterohepatic circulation by ileal resection leads to bile acid malabsorption and spill-over of bile acids into the colon. This is associated with increased fecal bile acid losses (1-5) and bile acid induced diarrhoea (1,6,7). Patients with Crohn's disease, particularly those with a history of distal ileitis or ileal resection, have an increased risk for cholelithiasis (8-13), possibly as a result of a decreased bile acid pool and a subsequent increase of biliary cholesterol saturation. Various data on bile lithogenicity in patients with Crohn's disease have been published. Most studies have reported a high frequency of cholesterol supersaturated bile (14-17), while others have found a decreased biliary cholesterol saturation in patients with Crohn's disease with ileal resection or ileal dysfunction (18,19).

Postprandial serum levels of conjugated cholic (CA) and chenodeoxycholic (CDCA) acid have been shown to be decreased after ileal resection (20-25).

However, little is known about the effect of ileal resection on deoxycholic acid (DCA), ursodeoxycholic acid (UDCA), and unconjugated serum bile acid levels. Lapidus and Einarsson have recently found a pronounced increase in the biliary UDCA fraction after ileal resection (19), which suggested that ileal resection should not lead to an increased risk of cholesterol gallstones.

The aim of the present study was to examine whether ileal resection leads to alterations in bile acid absorption, deconjugation, 7α-dehydroxylation, and formation of UDCA. Therefore, we have studied fasting and postprandial serum levels of conjugated and unconjugated individual bile acids in patients with Crohn's disease who had undergone partial ileal resection and right hemicolectomy. These data were compared to those obtained in healthy, non-operated volunteers. Serum bile acids were determined by capillary gas-liquid chromatography, a highly sensitive and accurate method for serum bile acid analysis (26).

Additionally, we studied fasting and postprandial plasma cholecystokinin (CCK) levels and gallbladder volumes in both groups. Intraduodenal bile acids are known to exert an inhibitory effect on the release of CCK (27-30). Since the output of bile acids into the duodenum has been shown to be decreased after ileal resection (15), we have tested the hypothesis that ileal resection may lead to increased cholecystokinin levels and decreased fasting gallbladder volumes.

8.3 Subjects and methods

Subjects — Eight subjects who had undergone partial resection of the terminal ileum in conjunction with partial right hemicolectomy for Crohn's disease (5 male, 3 female; mean age: 39, range 24-54 years) and 12 healthy controls (8 male, 4 female; mean age 43, range 22-70 years) participated in this study. Ileal resection had been performed 1 to 14 years (median: 9 years) before the time of this study. The diagnosis of Crohn's disease had been confirmed by histological examination of the surgical specimens in all patients. The length of the resected terminal ileum ranged from 30 to 70 cm (mean ± SEM: 44 ± 8 cm). None of the patients had signs of active Crohn's disease at the time of investigation. None of the subjects had undergone cholecystectomy and none of the healthy subjects had a history of gastrointestinal disease. Routine liver function tests (bilirubin, alkaline phosphatase, γ-glutamyltransferase) were normal in all subjects. None of the subjects had steatorrhoea (fecal fat excre-

tion in excess of 7 g/day as measured in a 48 hr stool sample). None of the subjects was using cholestyramine or other drugs at the time of investigation. Informed consent was obtained from each subject and the protocol had been approved by the Ethics Committee of the Nijmegen University Hospital.

120 *Experimental protocol* — All subjects fasted overnight and then ingested a standardized breakfast composed of one slice of bread, 5 g margarine, 20 g cheese, one boiled egg, 150 ml yogurt and one cup of tea with 5 g sugar (21 g protein, 21.5 g fat, 34 g carbohydrate, 413 kcal). Venous blood samples for analysis of serum bile acids and plasma CCK concentrations were taken at -5, 0, 15, 30, 45, 60, 75, 90, 105, 120, 150, and 180 minutes through an indwelling catheter placed in an antecubital vein. Blood samples were allowed to clot. After centrifugation the separated serum was frozen at 20°C for subsequent analysis.

Analysis of serum bile acids — Serum bile acid levels were measured by capillary gas-liquid chromatography (26). 7α, 12α-dihydroxy-5-ßcholanoic acid was added as an internal standard. Bile acids were extracted from serum using C18-bonded silica cartridges (SepPak, Waters Associates, Milford, MA, USA) (31). Separation of conjugated and unconjugated bile acids was carried out by means of column chromatography using the lipophilic anion exchanger diethylaminohydroxypropyl Sephadex LH-20 (Lipidex-DEAP, Packard Instruments, Groningen, The Netherlands) (31). The conjugated fractions were subjected to enzymatic hydrolysis by cholylglycine hydrolase (from *Clostridium perfringens*). After enzymatic hydrolysis the deconjugated bile acids were extracted and eluted on Lipidex-1000 columns (Packard Instruments, Groningen, The Netherlands). The bile acids were converted to methyl esters by 2,2-dimethoxypropane. After methylation trimethylsilyl ether derivates were prepared by addition of a solution of pyridine, hexamethyldisilazane and trimethylchlorosilane (3:2:1 by volume). Separation and quantification of bile acids was performed on a Packard 430 gas-liquid chromatograph with a flame ionisation detector and equipped with a 25 m * 0.25 mm glass capillary column (CP-Sil-5 CB, Chrompack, Middelburg, The Netherlands).

Plasma cholecystokinin levels — CCK was measured in plasma by a sensitive and specific radioimmunoassay (32,33). The antibody employed (T204), was raised in rabbit after the fourth immunization with albumin-coupled crude porcine CCK, and used in a final dilution of 1:80,000. The antibody binds to all

carboxy-terminal CCK-peptides containing the sulphated tyrosyl region. The antibody shows less than 2% cross-reactivity with sulphated gastrins and does not bind to unsulphated forms of gastrin or structurally unrelated peptides, like insulin, secretin, pancreatic polypeptide, bombesin and neurotensin. Synthetic human CCK_{33} was used as standard preparation and coupled to ^{125}I-hydroxyphenylpropionic acid succinimide ester (Bolton-Hunter reagent) also as label. The detection limit of the assay was between 0.5 an 1 pM in plasma. The intra-assay variation ranged from 4.6 to 11.5% and the inter-assay variation from 11.3 to 26.1%.

Gallbladder emptying — Gallbladder volumes were measured by real-time ultrasonography by the sum of cylinders method using a computerized method as previously described (34). The variation of volume measurements ranged from 4.6 to 11.5%. Two longitudinal and two transverse images of the gallbladder were obtained at each time point. The mean of two measurements was used for further analysis. Gallbladder emptying parameters were calculated using the following formulas:

Maximum gallbladder emptying = $GBV_0 - GBV_{min}$
Percentage gallbladder emptying = $100\%*(GBV_0 - GBV_{min})/GBV_0$
Where GBV_0 represents mean fasting gallbladder volume (mean of GBV at -5 and 0 min), and GBV_{min} represents the smallest postprandial gallbladder volume.

Statistical analysis — Results were expressed as mean ± SEM. Integrated bile acid values (area under the curve), fasting bile acid levels, fasting and postprandial plasma CCK levels, and gallbladder volumes were compared with the two-tailed Student's t-test for unpaired observations or the Mann-Whitney U test when appropriate. A p-value <0.05 was considered statistically significant.

8.4 Results

Conjugated serum bile acids — Fasting and postprandial conjugated serum bile acid levels are shown in Figure 8.1. Fasting levels of conjugated CA (controls: 0.37 ± 0.04 μM; ileal resection: 0.43 ± 0.07 μM, NS) and CDCA (0.70 ± 0.11 μM and 0.82 ± 0.14 μM respectively, NS) were similar in both groups. Fasting

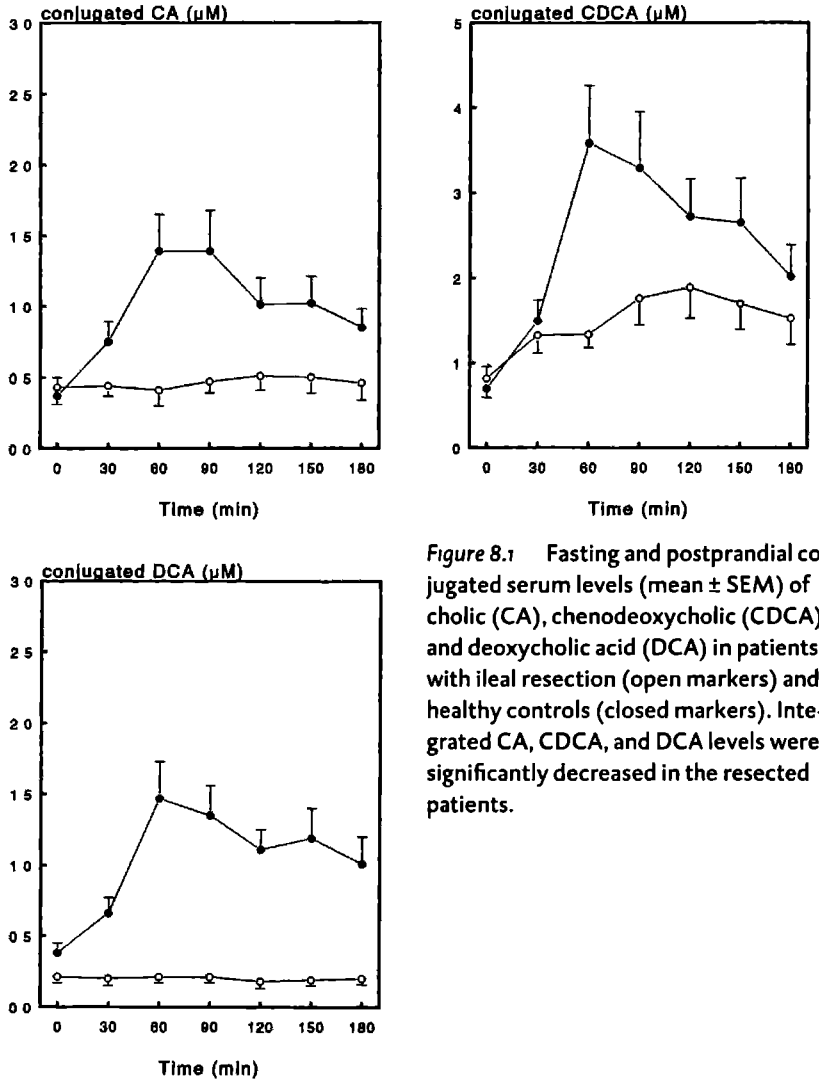

Figure 8.1 **Fasting and postprandial conjugated serum levels (mean ± SEM) of cholic (CA), chenodeoxycholic (CDCA) and deoxycholic acid (DCA) in patients with ileal resection (open markers) and healthy controls (closed markers). Integrated CA, CDCA, and DCA levels were significantly decreased in the resected patients.**

DCA levels however, were slightly decreased after ileal resection (0.21 ± 0.04 µM) when compared to the healthy subjects (0.38 ± 0.07 µM), but the difference did not reach statistical significance (p=0.09). Postprandially, all individual conjugated bile acid levels increased rapidly and significantly in the healthy volunteers. In the patients with ileal resection however, CA and DCA levels did not increase while CDCA levels increased to a lesser extent

compared to the healthy subjects. In the ileectomized patients postprandial DCA levels were extremely low compared to the healthy volunteers. The ratio of the integrated DCA to CA levels was significantly lower in the ileectomized patients (mean ± SEM: 0.47 ± 0.07) compared to the healthy subjects (1.17 ± 0.18; p<0.005). The integrated levels of all individual conjugated bile acids were significantly higher in the healthy subjects compared to the patients with ileal resection (Table 8.1). Conjugated UDCA was not detected or present in trace amounts in both patients and controls.

Table 8.1 **Integrated conjugated serum bile acid levels in healthy volunteers and patients with ileal resection (IR). Values are mean ± SEM. ND = not detected.**

	controls (μM.min)	*IR (μM.min)*	*significance level*
CA	185.4 ± 26.2	83.2 ± 31.9	p<0.005
CDCA	453.6 ± 67.3	275.7 ± 42.7	p<0.05
DCA	194.1 ± 24.8	35.8 ± 5.4	p<0.0001
UDCA	ND	ND	

Unconjugated serum bile acids — Fasting serum levels of unconjugated CA (controls: 0.31 ± 0.06 μM; ileal resection: 0.47 ± 0.09 μM, NS) and DCA (controls: 0.32 ± 0.06 μM, ileal resection: 0.28 ± 0.05 μM, NS) levels were similar in both groups but CDCA levels were higher in the ileal resection patients (controls: 0.32 ± 0.04 μM; ileal resection: 1.07 ± 0.22 μM, p=0.01). Fasting and postprandial unconjugated serum bile acid levels are shown in Figure 8.2. Integrated unconjugated bile acid levels are shown in Table 8.2. The Integrated CA and CDCA levels were significantly higher in the ileal resection patients. Integrated DCA levels were similar in both groups. The ratio of the integrated unconjugated DCA to CA levels was significantly lower in the operated patients (mean ± SEM: 0.68 ± 0.08) compared to controls (1.66 ± 0.25; p<0.005). In six of the operated patients unconjugated UDCA was detected in serum, but in none of the healthy subjects (p<0.01).

Fasting gallbladder volume and postprandial gallbladder emptying — None of the subjects had gallstones. Fasting gallbladder volume was similar in both groups (controls: 28 ± 3 mL; ileal resection: 27 ± 7 mL, NS). Mean fasting and postprandial gallbladder volumes are shown in Figure 8.3. Maximum gall-

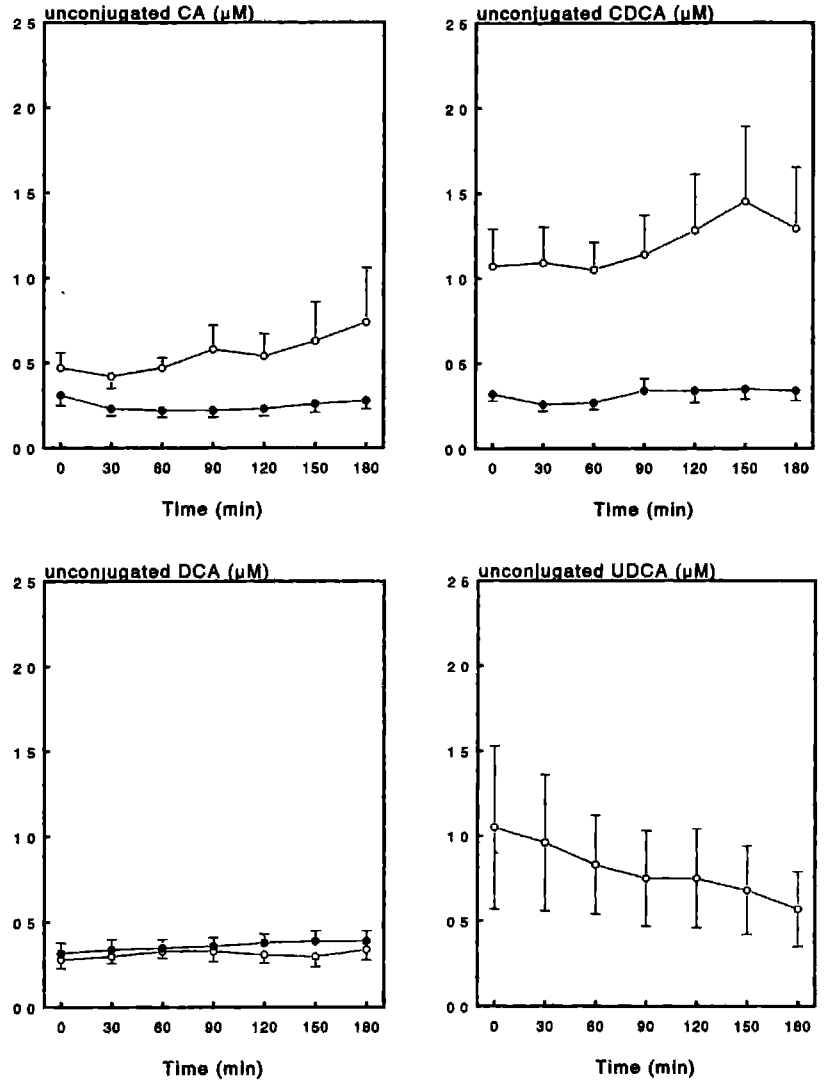

Figure 8.2 Fasting and postprandial unconjugated serum levels (mean ± SEM) of cholic (CA), chenodeoxycholic (CDCA), deoxycholic acid (DCA), and ursodeoxycholic acid (UDCA) in patients with ileal resection (open markers) and healthy controls (closed markers). Integrated unconjugated CA and CDCA levels were significantly higher in patients with ileal resection. Unconjugated DCA levels were similar in both groups. UDCA was not detected in controls.

Table 8.2 **Integrated unconjugated serum bile acid levels in healthy volunteers and patients with ileal resection (IR). Values are mean ± SEM. ND = not detected.**

	controls (μM.min)	*IR (μM.min)*	*significance level*
CA	43.7 ± 6.2	97.4 ± 22.7	p<0.05
CDCA	60.1 ± 7.3	232.9 ± 51.3	p<0.01
DCA	65.2 ± 8.9	56.1 ± 7.2	NS
UDCA	ND	143.6 ± 53.6	p<0.01

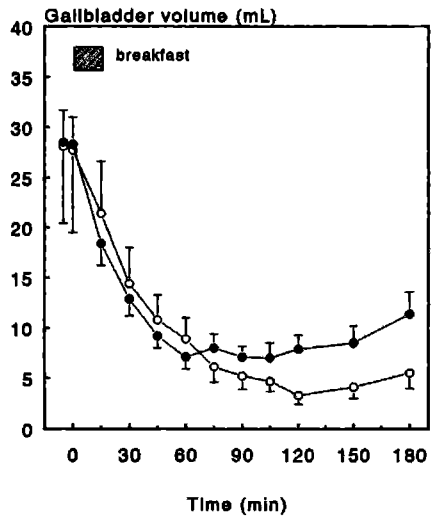

Figure 8.3. **Fasting and postprandial gallbladder volumes (mean ± SEM) in patients with ileal resection (open markers) and healthy controls (closed markers). Fasting gallbladder volume and postprandial gallbladder emptying were similar in both groups.**

bladder emptying was 22 ± 2 mL in controls and 23 ± 7 mL in the ileectomized patients (NS). The percentage gallbladder emptying was similar in both groups (controls: 78 ± 3%; ileal resection: 83 ± 5%, NS).

Fasting and postprandial plasma cholecystokinin levels — Fasting plasma CCK levels were similar in controls (3.4 ± 0.2 pM) and the patients with ileal resection (3.4 ± 0.2 pM). Postprandially, plasma CCK levels increased rapidly in both groups. The mean peak plasma CCK level was 6.7 ± 0.8 pM in the healthy subjects and 6.8 ± 0.7 pM in the ileectomized patients (NS). Plasma CCK concentration versus time curves are shown in Figure 8.4. Integrated

postprandial plasma CCK levels were similar in both groups (controls: 898 ± 73 pM.min; ileal resection: 961 ± 130 pM.min, NS).

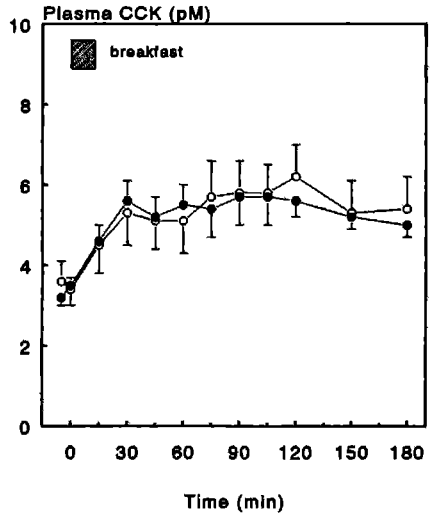

Figure 8.4. **Fasting and postprandial plasma cholecystokinin (CCK) (mean ± SEM) in patients with ileal resection (open markers) and healthy controls. Fasting and postprandial CCK levels were similar in both groups.**

8.5 Discussion

In accordance with previous studies, the present study demonstrates that postprandial conjugated bile acid levels are decreased in patients with Crohn's disease who have undergone ileal resection. These data provide further evidence that resection of the terminal ileum causes malabsorption of bile acids since systemic bile acid levels reflect portal venous bile acid levels (20,35).

Postprandial conjugated CA levels showed virtually no increase in patients with ileal resection. Conjugated CDCA levels however, increased significantly both in patients and healthy subjects, but the rise in the patients with ileal resection was significantly lower compared to the healthy subjects. This supports the hypothesis that CDCA is reabsorbed both passively throughout the small intestine and actively in the terminal ileum, whereas reabsorption of conjugated CA is fully dependent on an intact terminal ileum (35-37).

In former studies, postprandial serum bile acid levels in patients with ileal

resection have been measured using radioimmunoassays for the glycine conjugates of CA and CDCA (20-25). Radioimmunoassay for serum bile acid analysis is rather insensitive, and inaccurate because of cross-reactivity between CA and CDCA. Moreover, at present little information is available on serum levels of conjugated DCA and unconjugated bile acids in patients with ileal resections. Therefore, we used capillary gas-liquid chromatography combined with lipophilic anion exchanger column chromatography. This method is highly sensitive and offers the opportunity of measuring both the conjugated and unconjugated fractions of all major bile acids in serum (26,31).

Conjugated DCA levels were extremely low after ileal resection and the ratio of integrated levels of DCA to CA (both conjugated and unconjugated) was significantly decreased after ileal resection. Low DCA levels after ileal resection are probably due to a reduced exposure of CA to bacterial 7α-dehydroxylase as a result of rapid colonic transit. This is supported by studies on fecal bile acid patterns in patients with Crohn's disease showing a low excretion of DCA and an increased excretion of CA (2,38). Moreover, decreased molar percentages of DCA in bile have been found after ileal resection (15,19).

The increased risk for cholelithiasis in patients with ileal dysfunction or ileal resection has been attributed to a decreased bile acid pool and increased of biliary cholesterol saturation. However, Lapidus and Einarsson (19) found a low biliary cholesterol saturation and a more than tenfold increase in the relative concentration of ursodeoxycholic acid in bile after ileal resection. Since UDCA treatment of patients with gallstones may induce biliary cholesterol unsaturation and gallstone dissolution, they suggested that there should be no increased risk of cholesterol gallstone formation in patients with Crohn's disease and a history of ileal resection. Our findings give support to their data since UDCA was detected in sera of 6 of the 8 patients with ileal resections, but in none of the healthy subjects.

UDCA is the 7ß-hydroxyepimer of CDCA and is usually present only in small amounts in human bile. UDCA is formed from CDCA acid with 7-ketolithocholic acid as an intermediate. In the colon CDCA is oxidized to 7-ketolithocholic acid, which is subsequently reduced to UDCA by bacterial enzymes (39-41). Miwa et al. (39) demonstrated that the incorporation of deuterium into biliary UDCA in patients with Crohn's disease is increased after ingestion of deuterated CDCA. Therefore, malabsorption of CDCA in patients with ileal resection or ileal dysfunction may favour the formation of UDCA acid in the colon.

Integrated unconjugated CA and CDCA levels were significantly higher in patients with ileal resection, probably as a result of spill-over to the colon with deconjugation by the colonic flora. It is well known that unconjugated bile acids are reabsorbed by the colon (42,43). Although unconjugated serum bile acids levels are usually elevated in patients with small intestinal bacterial overgrowth (44-48) it is unlikely that this condition is responsible for the increased unconjugated CA and CDCA levels in our patients. None of our patients had either small bowel obstruction or steatorrhoea. The late increase of unconjugated CA and CDCA levels (Figure 8.2) also suggests deconjugation and subsequent absorption in the colon.

Intraduodenal bile acids exert a negative feedback on the release of CCK from the duodenum (27-30). Although the liver increases bile acid production after interruption of the enterohepatic circulation, the intraduodenal output of bile acids has been shown to be decreased (15). In the present study however, plasma CCK levels and gallbladder volume were similar in patients with ileal resection and controls.

In conclusion, postprandial conjugated bile acids are significantly decreased after ileal resection. This provides further evidence that ileal resection leads to malabsorption of conjugated bile acids. Formation of DCA is reduced after ileal resection, probably as a result of a decreased colonic transit time and a shortened exposure to bacterial 7α-dehydroxylase. Bile acid deconjugation however, is increased after ileal resection. Spill-over of conjugated bile acids into the colon leads to increased deconjugation and increased unconjugated serum bile acid levels. Probably as a result of CDCA malabsorption, the production of UDCA is increased in most patients with ileal resection. Bile acid malabsorption after ileal resection does not lead to alterations in CCK release, fasting gallbladder volume, and gallbladder emptying.

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Since the early 1980s proctocolectomy with ileal pouch-anal anastomosis is the surgical treatment of choice for severe chronic ulcerative colitis and familial adenomatous polyposis. It enables the excision of the entire diseased colorectal mucosa with preservation of continence and avoidance of a permanent ileostomy.

Chapter 2 — Chapter 2 presents a review of the literature concerning the ileal-pouch procedure. The most important postoperative complications and the long-term functional results are discussed. Comparative studies have shown that the results of the ileal pouch procedure are more favourable in polyposis patients compared to ulcerative colitis patients. Recent developments in pouch surgery, especially the use of new stapling devices, have made it possible to perform a one step procedure. Previously, a temporary diverting loop ileostomy was created to reduce the risk of anastomotic leakage and pelvic sepsis. A second operation was necessary to close the temporary ileostomy at the expense of a considerable complication rate.

The major long-term complication of ileal pouch-anal anastomosis is pouchitis. Pouchitis is an a non-specific inflammation of the ileal mucosa in the pouch with a high recurrence rate. Clinically, pouchitis is characterized by an increased stool frequency, bleeding, abdominal pain, fatigue, fever, erythema nodosum, and arthritis. The etiology of pouchitis is unknown. Bacterial overgrowth as a result of stasis in the pouch may contribute to the pathogenesis of pouchitis since many patients respond favourably to metronidazole. In most studies pouchitis occurred exclusively in ulcerative colitis patients. This suggests that pouchitis is a novel manifestation of inflammatory bowel disease.

Finally, some physiological aspects of proctocolectomy with ileal pouch-anal anastomosis, are discussed.

Chapter 3 — In chapter 3 we compared the immediate postoperative and long-term functional results of 51 ulcerative colitis patients and 21 familial adenomatous polyposis patients who underwent ileal J-pouch anal-anastomosis in the University Hospital Nijmegen between 1983 and 1990. Since familial polyposis patients are usually in a better physical condition and not using corticosteroids at the time of proctocolectomy, the incidence of postoperative

complications is likely to be lower in these patients than in ulcerative colitis patients. However, in this population the incidence of postoperative complications requiring relaparotomy was similar in both groups. Pouchitis was the major long-term complication. Pouchitis occurred in 44% of colitis patients but not in polyposis patients ($p<0.005$). Six colitis patients required pouch excision because of intractable pouchitis. The overall pouch excision rate was 22% in ulcerative colitis patients and 5% in familial polyposis patients ($p=0.16$). The mean daily stool frequency was significantly higher in colitis patients. Incontinence during daytime was rare. However, during nighttime 16 colitis patients (43%) and 6 (34%) polyposis patients required a pad because of soiling or incontinence (not significant). Patient satisfaction was good in 46% of ulcerative colitis patients and 76% of polyposis patients ($p<0.05$).

Chapter 4 — In this chapter the effects of ageing on serum bile acid levels are described. Previously, it has been shown that the formation of deoxycholic acid is increased in elderly subjects. It has been suggested that cholic acid is reabsorbed less effectively by the terminal ileum in elderly subjects compared to younger ones. As a result of increased spill-over into the colon bacterial dehydroxylation of cholic acid into deoxycholic acid may be increased. An increased exposure of deoxycholic acid to the colonic mucosa may contribute to an enhanced risk of colorectal cancer since secondary bile acids are thought to have co-carcinogenic effects on the colonic mucosa.

In order to find further evidence that ageing influences bile acid reabsorption in the small intestine, fasting and postprandial conjugated and unconjugated serum bile acid levels were measured in twelve young adults and twelve elderly subjects. Serum bile acid levels were measured by capillary gas-liquid chromatography, a highly accurate and sensitive method for individual bile acids (cholic, chenodeoxycholic, deoxycholic and ursodeoxycholic acid) analysis.

In the elderly subjects postprandial levels of conjugated bile acids increased to a lesser extent than in the younger subjects. This suggests that active ileal absorption of conjugated bile acids is less effective in elderly subjects. Unconjugated deoxycholic acid levels tended to be higher in elderly subjects, although the difference did not reach statistical significance. The observed differences between both age groups could not be explained by delayed gallbladder emptying, impaired intestinal motility, nor by small intestinal bacterial overgrowth, since gallbladder emptying, intestinal transit time, and small intestinal flora were similar in both groups.

Chapter 5 — Small intestinal bacterial overgrowth is associated with steatorrhoea, as a result of bacterial deconjugation of bile acids. Previous studies have shown that unconjugated serum bile acid levels are usually increased in patients with small intestinal bacterial overgrowth. However, it is unclear whether other causes of malabsorption, such as coeliac disease and exocrine pancreatic insufficiency, can be differentiated from small intestinal bacterial overgrowth on the basis of a characteristic serum bile acid profile.

In chapter 5 the results are reported of a study on unconjugated serum bile acid levels in patients with small intestinal bacterial overgrowth or other causes of malabsorption, such as untreated coeliac disease and exocrine pancreatic insufficiency. A group of healthy volunteers and a group of patients who had undergone ileal resection without evidence of malabsorption served as controls. The latter group was included since ileal resection may lead to elevated unconjugated serum bile acid levels as a result of spill-over and deconjugation in the colon. Moreover, a group of patients who had undergone proctocolectomy with ileal pouch-anal anastomosis was investigated since stasis in the ileal pouch may lead to bile acid deconjugation. It was shown that fasting unconjugated serum bile acid levels are significantly increased in patients with small intestinal bacterial overgrowth when compared to healthy volunteers, patients with other causes of malabsorption, and ileal pouch-anal anastomosis patients. Patients with ileal resection had elevated unconjugated primary bile acid levels and decreased unconjugated deoxycholic acid levels. The sensitivity and specificity of total unconjugated serum bile acid levels as a marker for small intestinal bacterial overgrowth in patients with malabsorption were 82% and 100% respectively.

Chapter 6 — This chapter describes the effects of proctocolectomy with ileal pouch anal anastomosis on serum bile acid levels. Eleven pouch patients and 11 sex and age matched healthy subjects participated in this study. Fasting levels of conjugated deoxycholic acid but not cholic and chenodeoxycholic acid were significantly lower in the pouch patients. Postprandially, conjugated bile acid levels were significantly lower in the pouch patients. Deoxycholic acid levels were decreased to a much lower extent compared to the primary bile acids, cholic and chenodeoxycholic acid. Postprandial unconjugated cholic acid levels were significantly higher and chenodeoxycholic acid levels tended to be higher, whereas unconjugated deoxycholic acid levels were significantly lower in the pouch patients. These data suggest that reabsorption of conjugated bile acids is impaired after proctocolectomy with ileal

pouch-anal anastomosis. Stasis in the pouch may lead to deconjugation of bile acids as a result of bacterial overgrowth. Dehydroxylation however, is decreased after proctocolectomy with ileal pouch-anal anastomosis, probably as a result of the absence of the colon.

Chapter 7 — Previous studies have shown that the colon and rectum contain regulatory peptides in mucosal endocrine cells which suggests a hormonal role. It has been demonstrated that colonic perfusion with nutrients inhibits pancreatic enzyme secretion and the release of cholecystokinin. Moreover, it has been shown that subtotal colectomy results in an increased postprandial cholecystokinin release in rats and dogs. These findings suggest that the colon contains a factor that suppresses the release of cholecystokinin. Little is known however, about the effects of proctocolectomy on gastrointestinal physiology and circulating gut hormone responses in humans.

Chapter 7 describes the effects of proctocolectomy with ileal pouch-anal anastomosis on fasting, postprandial, and bombesin stimulated plasma cholecystokinin levels and gallbladder volumes. Twelve healthy volunteers and ten patients who had undergone proctocolectomy with ileal pouch-anal anastomosis participated in this study. Fasting and postprandial cholecystokinin levels were significantly higher in the pouch patients. Fasting gallbladder volumes were significantly decreased in the ileal pouch patients, probably as a result of fasting hypercholecystokininaemia. Postprandial gallbladder emptying was similar in both groups. In a second experiment it was demonstrated that intravenous administration of bombesin results in higher plasma cholecystokinin levels in ileal pouch patients compared to healthy controls. These findings suggest that the human colon contains a factor that inhibits the release of cholecystokinin. However, it could not be excluded that interruption of the enterohepatic circulation after proctocolectomy with ileal pouch-anal anastomosis plays a role in the increased release of cholecystokinin.

Chapter 8 — Interruption of the enterohepatic circulation by ileal resection leads to bile acid malabsorption and spill-over of bile acids into the colon. This is associated with increased faecal bile acid losses and a decreased duodenal bile acid output. Since intraduodenal bile acids exert an inhibitory effect on the release of cholecystokinin, ileal resection might increase cholecystokinin levels. To test this hypothesis we measured fasting and postprandial serum bile acids, plasma cholecystokinin, and gallbladder volumes in eight patients who had undergone ileal resection for Crohn's disease and in

twelve healthy volunteers. The results of this study are presented in chapter 8. Not surprisingly, postprandial conjugated serum bile acid levels were decreased in the patients with ileal resection. However, plasma cholecystokinin levels and gallbladder volumes were similar in both groups. These data suggest that elevated plasma cholecystokinin levels after proctocolectomy are not the result of interruption of the enterohepatic circulation.

Patients with Crohn's disease, particularly those with a history of distal ileitis or ileal resection, have an increased risk for cholelithiasis, possibly as a result of a decreased bile acid pool and a subsequent increase of biliary cholesterol saturation. However, various data on bile lithogenicity in patients with Crohn's disease have been published. Recently, Swedish investigators have found a low biliary cholesterol saturation and a more than tenfold increase in the relative concentration of biliary ursodeoxycholic acid after ileal resection.

In order to examine whether ileal resection leads to alterations in bile acid absorption, deconjugation, 7α-dehydroxylation, and formation of ursodeoxycholic acid, fasting and postprandial individual conjugated and unconjugated serum bile acid levels were measured in both groups. As expected, postprandial conjugated bile acid levels were decreased after ileal resection. Deoxycholic acid levels were extremely low after ileal resection, probably as a result of rapid colonic transit and shortened exposure to bacterial 7α-dehydroxylation. Unconjugated cholic and chenodeoxycholic acid levels were increased after ileal resection. Spill-over of conjugated bile acids into the colon leads to increased deconjugation and increased reabsorption of unconjugated bile acids. Ursodeoxycholic acid was present in the serum of six of the eight patients with ileal resection but in none of the healthy volunteers. This is probably due to malabsorption of chenodeoxycholic acid, which is in part converted into ursodeoxycholic acid in the colon.

Sinds het begin van de jaren tachtig wordt de ileo-anale pouch procedure steeds meer toegepast als alternatief voor colectomie met ileostoma bij patinten met colitis ulcerosa of familiaire adenomateuze polyposis. Bij deze operatie wordt, nadat het gehele colon is verwijderd, een reservoir –de pouch– geconstrueerd van het terminale ileum. Deze pouch wordt ingehecht op de anus met behoud van het sfincter-mechanisme. Het voordeel van deze operatie is dat de patiënt geen (definitief) stoma nodig heeft, terwijl toch het gehele colon, dat ernstig ziek is, kan worden verwijderd.

Hoofdstuk 2 — In Hoofdstuk 2 wordt een overzicht gegeven van de literatuur op het gebied van de ileo-anale pouch procedure. Allereerst worden de meest voorkomende postoperatieve complicaties zoals ileus, naadlekkage, naaddehiscentie en sepsis besproken. Vervolgens wordt een overzicht gegeven van de resultaten op de lange termijn, waarbij de nadruk ligt op functionele aspecten zoals de defecatie frequentie en de mate van continentie. Aan de hand van een aantal vergelijkende studies wordt aangetoond dat het resultaat van de ileo-anale pouch operatie meestal beter is bij polyposis patiënten dan bij colitis patiënten. Verder zal kort worden ingegaan op de verschillende reservoir typen (J-, W-, en S-pouch).

Recente chirurgische ontwikkelingen, zoals het gebruik van nieuwe hechttechnicken (stapler apparatuur), en daarmee samenhangend, het achterwege laten van het tijdelijk ontlastend dubbelloops ileostoma, hebben er de laatste jaren toe geleid tot dat de procedure in één stap kan worden uitgevoerd. Voorheen was een tweede operatie, en dus een tweede ziekenhuisopname, noodzakelijk om het dubbelloops ileostoma op te heffen. Deze ingreep ging vaak gepaard met ernstige complicaties zoals ileus.

De belangrijkste complicatie van de ileo-anale pouch procedure op lange termijn is pouchitis. Pouchitis is een aspecifieke ontsteking van het ileumslijmvlies in de pouch, met een sterke neiging tot recidiveren. Klinische verschijnselen van pouchitis zijn: toename van de defecatiefrequentie, bloedverlies bij de ontlasting, buikpijn, algehele malaise, en koorts. Daarnaast kunnen ook extraintestinale klinische verschijnselen optreden zoals artritis en erythema nodosum. De oorzaak van pouchitis is onbekend. Men veronderstelt dat stase van darminhoud in de pouch kan leiden tot bacteriële overgroei en daardoor tot ontsteking van het slijmvlies. Deze hypothese wordt

gesteund door het feit dat behandeling met metronidazol, een antibioticum dat gericht is tegen anaërobe bacteriën, vaak een gunstig effect heeft. Het is opvallend dat pouchitis alleen lijkt voor te komen bij colitis patiënten en niet bij polyposis patiënten. Dit suggereert dat colitis en pouchitis een gemeenschappelijke pathogenese hebben.

Ten slotte worden in dit hoofdstuk een aantal fysiologische aspecten van de ileo-anale pouch procedure besproken, zoals veranderingen in het galzuurmetabolisme, de water- en zouthuishouding en de motiliteit van maag en dunne darm.

Hoofdstuk 3 — In dit hoofdstuk wordt een overzicht gegeven van de postoperatieve complicaties en de functionele resultaten op lange termijn bij alle patiënten (51 colitis ulcerosa en 21 familiaire adenomateuze polyposis patiënten) die tussen 1983 en 1990 in het St. Radboud Ziekenhuis een ileo-anale pouch-operatie ondergingen. Aangezien colitis ulcerosa patiënten ten tijde van de operatie meestal corticosteroiden gebruiken en in een slechtere lichamelijke conditie verkeren dan polyposis patiënten werden de resultaten in beide groepen met elkaar vergeleken. Uit dit onderzoek bleek dat de incidentie van postoperatieve complicaties, waarvoor een relaparotomie noodzakelijk was, niet significant verschilde tussen beide groepen. Pouchitis was de belangrijkste complicatie op de lange termijn en kwam voor bij 44% van de colitis patiënten maar niet bij de polyposis patiënten ($p<0.005$). De pouch moest worden verwijderd bij 11 (22%) colitis patiënten en bij één (5%) polyposis patiënt ($p=0.16$). Bij zes colitis patiënten werd de pouch verwijderd in verband met pouchitis. De defecatiefrequentie na ileo-anale anastomose was significant hoger bij colitis patiënten dan bij polyposis patiënten. Incontinentie overdag kwam zelden voor. Gedurende de nacht waren 16 (43%) van de colitis patiënten en zes (32%) van de polyposis patiënten in meer of mindere mate incontinent (niet significant). Zestien polyposis patiënten (76%) en 22 colitis patiënten (46%) waren uiteindelijk zeer tevreden met hun pouch ($p<0.05$).

Hoofdstuk 4 — In hoofdstuk 4 wordt de invloed van leeftijd op de concentratie van galzuren in serum beschreven. Uit eerder onderzoek is gebleken dat de vorming van deoxycholzuur bij oudere mensen is toegenomen. Een verklaring hiervoor zou kunnen zijn dat cholzuur bij ouderen minder effectief wordt gereabsorbeerd in het terminale ileum dan bij jongeren. Door bacteriële enzymen wordt het cholzuur, dat niet in het terminale ileum is gereabsor-

beerd, in het colon gedeconjugeerd en vervolgens omgezet in deoxycholzuur (7α-dehydroxylatie). Er zijn aanwijzingen dat secondaire galzuren zoals deoxycholzuur een co-mutageen effect hebben op de colonmucosa. Het is derhalve mogelijk dat een afgenomen galzuurreabsorptie in het terminale ileum bij ouderen bijdraagt tot een toegenomen risico op coloncarcinoom.

In de studie beschreven in dit hoofdstuk werden nuchtere en postprandiale geconjugeerde en ongeconjugeerde galzuur concentraties in serum gemeten bij twaalf jongere en twaalf oudere gezonde vrijwilligers. De galzuren werden gemeten met behulp van capillaire gaschromatografie, een gevoelige methode waarmee het mogelijk is de lage serumconcentraties van de individuele galzuren (cholzuur, chenodeoxycholzuur, deoxycholzuur en ursodeoxycholzuur) nauwkeurig te meten. Het bleek dat de geconjugeerde galzuur concentratie in serum na een maaltijd bij ouderen significant lager was dan bij jongere personen. Dit suggereert dat het terminale ileum bij jongere mensen beter in staat is galzuren te reabsorberen dan bij oudere mensen. De verschillen tussen beide groepen konden niet worden verklaard door verschillen in darmpassagetijd, galblaasontlediging of dunne darmflora, aangezien deze parameters niet verschilden tussen beide groepen. Verder bleek dat de concentratie van ongeconjugeerd deoxycholzuur bij oudere mensen hoger was dan bij jongeren, ook al was dit verschil niet statistisch significant (p=0.09). Een afgenomen reabsorptie van cholzuur in het terminale ileum bij ouderen zou dit verschil, evenals de verhoogde vorming van deoxycholzuur bij ouderen, kunnen verklaren.

Hoofdstuk 5 — Bacteriële overgroei in de dunne darm gaat meestal gepaard met steatorrhoe. Deconjugatie van galzuren door bacteriële enzymen in de dunne darm speelt hierbij een belangrijke rol omdat ongeconjugeerde galzuren veel minder goed in staat zijn vet te emulgeren dan geconjugeerde galzuren. Uit eerder onderzoek is gebleken dat de concentratie van ongeconjugeerde galzuren in serum vaak verhoogd is bij patiënten met bacteriële overgroei in de dunne darm. Het is echter niet goed bekend of ook andere oorzaken van malabsorptie, zoals coeliakie en exocriene pancreasinsufficiëntie, gepaard gaan met verhoogde ongeconjugeerde serum galzuur concentraties. Ook is niet bekend of deze aandoeningen op grond van een kenmerkend profiel van individuele galzuren van elkaar te onderscheiden zijn.

In hoofdstuk 5 wordt beschreven wat de waarde is van het bepalen van ongeconjugeerde serum galzuur concentraties voor de diagnostiek van bacteriële overgroei in de dunne darm en voor de differentiële diagnostiek van

malabsorptie. Hiertoe werden nuchtere individuele serum galzuur concentraties gemeten bij gezonde vrijwilligers, patiënten met bacteriële overgroei, patiënten met onbehandelde coeliakie en patiënten met exocriene pancreasinsufficiëntie. Tevens werd een aantal patiënten onderzocht die een ileocaecaalresectie hadden ondergaan omdat spill-over van geconjugeerde galzuren

naar het colon kan leiden tot een toegenomen deconjugatie en verhoogde ongeconjugeerde serum galzuur concentraties. Ook werd een aantal patiënten met een ileo-anale pouch onderzocht omdat stase in de pouch kan leiden tot bacteriële overgroei en deconjugatie van galzuren.

In deze studie bleek dat nuchtere ongeconjugeerde serum galzuur concentraties significant verhoogd zijn bij patiënten met bacteriële overgroei in vergelijking met gezonde vrijwilligers, patiënten met coeliakie, patiënten met exocriene pancreasinsufficiëntie en patiënten met een ileo-anale anastomose. Patiënten die een ileocaecaalresectie hadden ondergaan hadden verhoogde primaire ongeconjugeerde galzuur concentraties in serum, terwijl de concentratie van deoxycholzuur juist verlaagd was.

De specificiteit en sensitiviteit van ongeconjugeerde totale serum galzuur concentraties als een marker voor bacteriële overgroei in de dunne darm bij patiënten met malabsorptie waren respectievelijk 82% en 100%.

Hoofdstuk 6 — In dit hoofdstuk worden de resultaten beschreven van een studie waarin het effect van proctocolectomie met ileo-anale anastomose op het galzuurmetabolisme werd onderzocht. Hiertoe werden nuchtere en postprandiale geconjugeerde en ongeconjugeerde serum galzuur concentraties gemeten bij elf patiënten met een ileo-anale anastomose en bij elf gezonde vrijwilligers. Beide groepen kwamen qua leeftijd en geslacht nauwkeurig met elkaar overeen. Na de maaltijd bleken de geconjugeerde galzuur concentraties significant lager te zijn bij de ileo-anale pouch patiënten. De deoxycholzuur concentratie was relatief veel sterker verlaagd dan die van de primaire galzuren. De concentratie van ongeconjugeerd cholzuur was significant hoger, terwijl de concentratie van ongeconjugeerd deoxycholzuur significant lager was bij de ileo-anale pouch patiënten. Deze gegevens suggereren dat de reabsorptie van geconjugeerde galzuren verminderd is na proctocolectomie met ileo-anale anastomose en dat stase in de pouch leidt tot deconjugatie van galzuren, waarschijnlijk ten gevolge lokale bacteriële overgroei. Door het ontbreken van het colon is de omzetting van cholzuur in deoxycholzuur sterk afgenomen bij deze patiënten.

Hoofdstuk 7 — Uit eerder onderzoek is gebleken dat in de mucosa van het colon endocriene cellen voorkomen, waarin bepaalde hormonen zoals peptide-YY en enteroglucagon, worden gevormd. De functie van deze peptiden is niet goed bekend. Wel is aangetoond dat perfusie van het colon met bepaalde nutriënten, een remmend effect heeft op de afgifte van cholecystokinine en de secretie van pancreasenzymen. Bij ratten en honden is bovendien aangetoond dat colectomie leidt tot verhoogde postprandiale cholecystokinine spiegels in het bloed. Deze bevindingen suggereren dat het colon een factor bevat, die een remmend effect heeft op de afgifte van cholecystokinine uit het proximale deel van de dunne darm, en dat deze factor geactiveerd kan worden door de mucosa van het colon bloot te stellen aan bepaalde stoffen. Het is echter onbekend wat het effect is van (procto)colectomie op de afgifte van cholecystokinine bij de mens.

In hoofdstuk 7 worden de resultaten beschreven van een studie waarin het effect werd onderzocht van proctocolectomie op de afgifte van cholecystokinine en de galblaasmotiliteit bij de mens. Dit onderzoek werd uitgevoerd bij twaalf gezonde vrijwilligers en tien patiënten die een proctocolectomie met ileo-anale anastomose hadden ondergaan. In deze studie bleek dat de nuchtere en postprandiale cholecystokinine spiegels significant hoger waren bij de ileo-anale pouch patiënten dan bij de gezonde vrijwilligers. Bovendien was het nuchtere galblaasvolume significant kleiner bij deze patiënten, waarschijnlijk als gevolg van de verhoogde basale cholecystokinine spiegels. De galblaas ontlediging was vergelijkbaar in beide groepen. In een tweede experiment werd aangetoond dat ook tijdens intraveneuze toediening van bombesine, een peptide dat de afgifte van cholecystokinine stimuleert, de cholecystokinine spiegels significant hoger waren bij de ileo-anale pouch patiënten. Deze gegevens suggereren dat ook het humane colon endocriene eigenschappen bezit, en dat de afgifte van cholecystokinine geremd wordt door een factor die afkomstig is uit het colon. Het is echter niet met zekerheid uit te sluiten dat onderbreking van de enterohepatische kringloop een rol speelt bij de verhoogde cholecystokinine spiegels na proctocolectomie met ileo-anale anastomose.

Hoofdstuk 8 — Resectie van het terminale ileum leidt tot onderbreking van de enterohepatische kringloop en gaat gepaard met een toegenomen fecaal galzuurverlies en een verminderde output van galzuren in het duodenum. Aangezien galzuren in het duodenum een remmende invloed hebben op de afgifte van cholecystokinine is het in theorie mogelijk dat cholecystokinine

spiegels verhoogd zijn na resectie van het terminale ileum. Om deze hypothese te onderzoeken werden bij acht patiënten, die in verband met de ziekte van Crohn een ileocaecaalresectie hadden ondergaan, en bij twaalf gezonde vrijwilligers nuchtere en postprandiale galzuur en cholecystokinine concentraties en galblaasvolumina gemeten. Zoals te verwachten waren de post-

prandiale geconjugeerde galzuur concentraties duidelijk verlaagd bij de patiënten die een ileum resectie hadden ondergaan. De plasma cholecystokinine spiegels en het galblaasvolume werden echter niet beïnvloed door resectie van het terminale ileum. Dit maakt het onwaarschijnlijk dat de verhoogde cholecystokinine spiegels na proctocolectomie veroorzaakt worden door onderbreking van de enterohepatische kringloop.

Uit verschillende studies is gebleken dat patiënten die een ileocaecaalresectie hebben ondergaan een verhoogd risico hebben op galstenen. Dit zou verklaard kunnen worden door een afname van de biliaire galzuur concentratie als gevolg van de onderbroken enterohepatische kringloop. Een afname van de galzuur concentratie in gal leidt tot een toename van de lithogene index. Studies naar de lithogeniciteit van gal na ileumresectie hebben echter tegenstrijdige gegevens opgeleverd. Recent vonden Zweedse onderzoekers dat na resectie van het terminale ileum de lithogene index juist verlaagd is - in tegenstelling tot eerdere studies van andere onderzoekers - en dat de relatieve biliaire concentratie van ursodeoxycholzuur, dat een gunstig effect heeft op de lithogene index, bij deze patiënten sterk verhoogd is.

Om de invloed van ileumresectie op deconjugatie, dehydroxylatie en de vorming van ursodeoxycholzuur te bestuderen werden nuchtere en postprandiale individuele geconjugeerde en ongeconjugeerde serum galzuur concentraties gemeten in beide groepen. De geconjugeerde serum galzuur concentraties waren duidelijk verlaagd na ileumresectie. De concentratie van deoxycholzuur was veel sterker verlaagd dan die van de primaire galzuren. Dit is waarschijnlijk het gevolg van een versnelde passage door het colon met een verkorte blootstelling aan bacterieel 7α-dehydroxylase. De ongeconjugeerde cholzuur en chenodeoxycholzuur concentraties waren verhoogd na ileumresectie. Spill-over van geconjugeerde galzuren naar het colon leidt tot een toegenomen deconjugatie en verhoogde resorptie van ongeconjugeerde galzuren. Ursodeoxycholzuur was aantoonbaar in het serum van zes van de acht patiënten die een ileumresectie hadden ondergaan, maar niet bij de gezonde vrijwilligers. Waarschijnlijk is dit het gevolg van malabsorptie van geconjugeerd chenodeoxycholzuur omdat chenodeoxycholzuur in het colon door bacteriële enzymen omgezet kan worden in ursodeoxycholzuur.

Dit proefschrift is tot stand gekomen dankzij het werk van velen. Het doen van promotie onderzoek is geen solistisch werk, maar kan alleen tot stand komen door creatieve samenwerking en intensief overleg. Zonder iemand tekort te willen doen, hecht ik er aan enkele personen in het bijzonder te bedanken.

In de eerste plaats ben ik dank verschuldigd aan de patiënten en gezonde vrijwilligers, die steeds bereid waren aan de soms vervelende experimenten deel te nemen. Zonder hun medewerking was dit onderzoek onmogelijk geweest.

Dr. F.M. Nagengast, beste Fokko, je was de initiator van dit onderzoek, en door de jaren heen de stuwende kracht. Jouw enthousiasme voor het wetenschappelijk onderzoek en de gastroenterologie werkte ronduit aanstekelijk. Jij hebt mij gevormd als onderzoeker, als gastroenteroloog in de stijl van Van Tongeren, en als endoscopist.

Professor dr. J.B.M.J. Jansen, beste Jan, ik wil je hartelijk danken voor je wetenschappelijke gedrevenheid en voor je niet aflatende stroom van ideeën, waarmee je mijn onderzoek na je terugkeer naar Nijmegen een extra impuls hebt gegeven. Daardoor, én door je uiterst vriendelijke manier van leiding geven was het werken aan dit proefschrift voor mij een waar genoegen.

Het bepalen van galzuurconcentraties in serum met behulp van de gaschromatograaf is een tijdrovende en bewerkelijke methode. Bij het opzetten en uitvoeren van de bepalingen hebben Annie van Schaik en dr. Albert Tangerman van het laboratorium Maag-, Darm-, en Leverziekten, een rol van onschatbare waarde gespeeld. Zij hebben de talloze monsters op nauwgezette wijze geanalyseerd. Beste Annie en Albert, de samenwerking met jullie heb ik als heel prettig ervaren en ik ben jullie daar dankbaar voor.

Dr. Wim Hopman en Willie Thimister hebben de cholecystokinine spiegels bepaald en een belangrijk aandeel gehad, samen met verschillende medewerkers van de afdeling Radiodiagnostiek, in het maken van de echo's van de galblaas. Ik ben hen zeer erkentelijk voor hun onmisbare hulp.

Voor het kweken van de monsters dunnedarmvocht onder aërobe en anaërobe condities ben ik dr. J.P. Koopman en zijn staf van het Centraal Dieren Laboratorium zeer erkentelijk.

Ton de Haan van de Medische Statistische Adviesafdeling ben ik veel dank verschuldigd voor het bewerken van de gegevens van verschillende studies, beschreven in dit proefschrift.

Dr. J. Kuijpers en dr. J.C. Lubbers van de afdeling Heelkunde wil ik bedanken voor het "aanleveren" van patiënten met een ileo-anale anastomose en voor de waardevolle opmerkingen bij het tot stand komen van het manuscript. Zij hebben de ileo-anale pouch procedure in het Radboud Ziekenhuis geïntroduceerd. Zonder hun initiatief had een belangrijk deel van dit onderzoek niet plaats kunnen vinden.

Mijn collega's en oud-collega's van de afdeling Maag-, Darm- en Leverziekten wil ik vooral bedanken voor hun vriendschap en voor de hilariteit tijdens de talloze lunchpauzes. Alle andere (ex-)medewerkers van de afdelingen Maag-, Darm-, en Leverziekten en Centrale Endoscopie bedank ik voor de geweldige samenwerking.

Lieve Reintje, Viola en Max, jullie wil ik niet alleen bedanken voor jullie geduld, dat ik nog al eens op de proef heb gesteld, maar ook voor de vaak succesvolle pogingen mij van het werk af te houden.

De auteur van dit proefschrift werd geboren op 16 september 1957 te Maastricht. In 1975 behaalde hij het diploma Atheneum-B aan het Stedelijk Lyceum te Maastricht. Vanaf 1975 studeerde hij geneeskunde aan de Katholieke Universiteit te Nijmegen. In 1980 legde hij het doctoraal examen af. Van mei tot oktober 1983 werkte hij als co-assistent in het St. Joseph Hospital te Kilgoris, Kenya. Het artsexamen werd afgelegd in 1984.

Van augustus 1984 tot maart 1985 was hij werkzaam als arts-assistent op de afdeling Intensieve Zorg van het Academisch Ziekenhuis St. Radboud te Nijmegen (hoofd dr. J.S.F. Gimbrère). Aansluitend volgde hij de opleiding Interne Geneeskunde in hetzelfde ziekenhuis (hoofd prof. dr. A. van 't Laar). Tijdens de opleiding tot internist verrichtte hij van april 1989 tot februari 1991 wetenschappelijk onderzoek op de afdeling Maag-, Darm- en Leverziekten (hoofd dr. J.H.M. van Tongeren) gesubsidieerd door de Nederlandse Lever Darm Stichting. In deze periode werd een begin gemaakt met het onderzoek, dat geresulteerd heeft in dit proefschrift. Na zijn registratie als internist volgde hij van juli 1991 tot juli 1994 de opleiding gastroenterologie op de afdeling Maag-, Darm- en Leverziekten van het Academisch Ziekenhuis St. Radboud te Nijmegen (opleiders prof. dr. J.B.M.J. Jansen en dr. F.M. Nagengast). Tot augustus 1995 bleef hij werkzaam op deze afdeling. In september 1995 werd hij geregistreerd als gastroenteroloog.

Sinds 1 augustus 1995 is hij werkzaam in het St. Joseph Ziekenhuis te Veldhoven waar hij samenwerkt met de internisten H. Chin A Fo, dr. W.M.M. Driessen, dr P.G.G. Gerlag, dr. J.J. Keuning, dr. G. Vreugdenhil, en dr. A.W.L. van den Wall Bake.

Hij is getrouwd met Reintje van der Leeuw. Zij hebben twee kinderen, Viola en Max.

CLINICAL AND PHYSIOLOGICAL ASPECTS OF ILEAL POUCH-ANAL ANASTOMOSIS

Stellingen

JAN M.J.I. SALEMANS, 1 december 1995

1

Pouchitis komt alléén voor bij colitis patiënten en niet bij polyposis patiënten. Dit suggereert dat pouchitis een nieuwe manifestatie is van colitis ulcerosa.

— DIT PROEFSCHRIFT —

2

Een afgenomen absorptie van geconjugeerde galzuren in het terminale ileum bij oudere mensen leidt tot een toegenomen expositie van secundaire galzuren aan het colonslijmvlies. Dit kan bijdragen aan een verhoogde kans op coloncarcinoom op hogere leeftijd.

— DIT PROEFSCHRIFT —

3

Verhoogde ongeconjugeerde serum galzuurspiegels zijn een zeer specifieke marker voor bacteriële overgroei in de dunne darm mits het terminale ileum intact is.

— DIT PROEFSCHRIFT —

4

Proctocolectomie met ileo-anale pouch anastomose leidt tot onderbreking van de enterohepatische kringloop van galzuren en tot een verminderde omzetting van primaire in secundaire galzuren.

— DIT PROEFSCHRIFT —

5

Cholecystokininespiegels in plasma zijn verhoogd na proctocolectomie. Dit wordt waarschijnlijk niet veroorzaakt door onderbreking van de enterohepatische kringloop, maar door het wegvallen van een factor, afkomstig uit het colon, die de afgifte van cholecystokinine remt.

— DIT PROEFSCHRIFT —

6

Captopril kan waarschijnlijk acute pancreatitis veroorzaken

— EIGEN WAARNEMING —

7

Het is zeer twijfelachtig of patiënten met een short bowel syndroom, die volledig afhankelijk zijn van totale parenterale voeding, met een dunne darmtransplantatie beter af zouden zijn.

8

Het feit dat de vooruitgang op medisch therapeutisch gebied geen gelijke tred houdt met de snelle ontwikkelingen op het gebied van de medische diagnostiek wordt in de media onvoldoende belicht.

9

Het spreekwoord: "Beter één vogel in de hand dan tien in de lucht" voldoet niet meer aan de huidige ecologische inzichten en zou thans moeten luiden: "Beter tien vogels in de lucht dan één in de hand".

10

Omdat improvisatie de essentie vormt van de jazz sterft de muziek van een jazzmusicus met zijn overlijden.

11

Zonder testosteron zou het een stuk rustiger zijn in de voetbalstadions, en waarschijnlijk ook daarbuiten.

12

De voorspellingen over de klimatologische veranderingen zijn net zo veranderlijk als het weer zelf.