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**Bile Acid Induced Diarrhoea
Pathophysiological and Clinical Aspects**

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ABSTRACT

Bile Acid Induced Diarrhoea

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A common cause for referral to gastroenterologists is chronic watery diarrhoea. Approximately 40% of these patients have idiopathic bile acid malabsorption (BAM) – a condition with unknown aetiology. The $^{75}\text{SeHCAT}$ test, which correlates inversely with faecal excretion and hepatic synthesis of bile acids, is used to diagnose BAM.

The aims of the thesis were to study different mechanisms behind BAM. We investigated the stability of the $^{75}\text{SeHCAT}$ test in diarrhoea patients having done the test twice, and in healthy controls. The $^{75}\text{SeHCAT}$ values were stable over time, suggesting that in clinical practice there is no indication for a second test. There was also a strong negative correlation between the $^{75}\text{SeHCAT}$ retention and the plasma marker for hepatic bile acid synthesis “C4” both in diarrhoea and in controls.

Impaired ileal absorption of bile acids may be secondary to a defective ileal reabsorption system. We assessed bile acid uptake in ileal biopsies from diarrhoea patients - both with normal and abnormal $^{75}\text{SeHCAT}$ test- and compared with the bile acid uptake in ileal biopsies from patients with normal bowel habits. Our data suggest that BAM is not caused by impaired bile acid uptake in the ileum.

We also tested whether BAM is associated with increased active small intestinal chloride secretion as estimated from small intestinal potential difference (PD) measurements. We recorded PD during manometry in patients with abnormal $^{75}\text{SeHCAT}$ test and compared the values with PD recording values in healthy controls. There was a higher PD in the fasting state in the BAM group and there was also a negative correlation between the $^{75}\text{SeHCAT}$ test values and the estimated chloride secretion.

It is known that budesonide has effect on symptoms of diarrhoea both in Crohn’s disease and in collagenous colitis. We investigated whether the improvement in symptoms in collagenous colitis is associated with an enhancement of bile acid uptake and/or changes in bile acid synthesis. After 8 weeks of budesonide treatment the $^{75}\text{SeHCAT}$ values increased significantly, synthesis rate decreased and the diarrhoea symptoms improved.

Conclusions: The $^{75}\text{SeHCAT}$ test is stable over a long period of time. C4, the plasma marker for bile acid synthesis, may be used in clinical practice instead of the $^{75}\text{SeHCAT}$ test. BAM does not seem to be caused by impaired absorption of bile acids in the ileum. A possible mechanism is increased small intestinal fluid secretion and motility, which in turn overrides the absorptive capacity of the colonic mucosa and leads to diarrhoea. The positive symptomatic effects of budesonide in collagenous colitis may in part be mediated by increased ileal absorption and lower colonic concentrations of bile acids.

Keywords: Diarrhoea, bile acid transport, bile acid synthesis, $^{75}\text{SeHCAT}$ reproducibility, C4, budesonide, collagenous colitis, in vitro, malabsorption, ASBT, intestinal secretion, potential difference, manometry.

LIST OF PAPERS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

- I. The bile acid turnover rate assessed with the ⁷⁵SeHCAT test is stable in chronic diarrhoea although it is slightly decreased in healthy subjects after a long period of time**
A Bajor, A Kilander, H Sjövall, M Rudling, K-A Ung
Submitted for publication
- II. Normal or increased bile acid uptake in isolated mucosa from patients with bile acid malabsorption**
A Bajor, A Kilander, A Fae, C Gälman, O Jonsson, L Öhman, M Rudling, H Sjövall, P-O Stotzer, K-A Ung
European Journal of Gastroenterology & Hepatology
- III. Enhanced motility-activated jejunal secretion is quantitatively related to reduced bile acid uptake in patients with bile acid malabsorption**
A Bajor, K-A Ung, L Öhman, M Simren, H Sjövall
In Manuscript
- IV. Budesonide treatment is associated with increased bile acid absorption in collagenous colitis**
A Bajor, A Kilander, C Gälman, M Rudling, K-A Ung
Alimentary Pharmacology & Therapeutics

ABBREVIATIONS

3 α -HSD	3 α -hydroxysteroid dehydrogenase
ATP	adenosine triphosphate
ASBT	apical sodium dependent bile acid transporter
BSEP	Bile Salt Export Pump
BMI	body mass index
C4	7 α -hydroxy-4-cholesten-3 one
CCK	cholecystokinin
CFTR	Cystic fibrosis transmembrane conductance regulator
CPF	CYP7A1 promoter binding factor
FGF	fibroblast growth factor
FTF	α -fetoprotein transcription factor
FXR	Farnesoid X receptor
HDL	High density lipoprotein
HNF-4	Hepatocyte nuclear factor 4
IBAM	idiopathic bile acid malabsorption
IBS	irritable bowel syndrome
IBABP	ileal bile acid binding protein
ILBP	ileal lipid binding protein
L-FABP	liver fatty acid binding protein
LRH-1	liver receptor homolog-1
MMC	migrating motor complex
MRP2	Multidrug Resistance Protein
NTCP	Na ⁺ taurocholate cotransporting polypeptide
OATP	organic anion transporter
PD	Potential difference
PM	after noon
PPAR α	Peroxisome proliferator-activated receptor α
⁷⁵ SeHCAT	⁷⁵ Se labelled homocholic acid-taurine
SHP	short heterodimer partner
TNF- α	Tumor necrosis factor α

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1. INTRODUCTION

Chronic diarrhoea is disabling and it is a frequent cause for referral to gastroenterologists. The diagnostic workup in this condition, as recommended by the American Gastroenterological Association, is aimed to exclude organic, infectious or metabolic disorders. It consists of careful history, physical examination, hematology, chemistry and stool tests, gastrointestinal endoscopy with biopsies and in some cases ultrasonography and radiologic tests [1]. However, this algorithm does not include any objective investigation to diagnose idiopathic bile acid malabsorption, a frequent cause of chronic diarrhoea [2-4]. This illustrates the controversy about the existence of this condition, - some authors recognize it as a disease entity, other authors consider it as part of the irritable bowel syndrome or functional diarrhoea [5].

1.1 Historical aspects - three types of bile acid malabsorption

There was experimental evidence already in the sixties that the absorption of bile acids occurs against a concentration gradient in the ileum but not in the jejunum. These animal studies postulated the presence of an active transport mechanism [6], and led to the conclusion that the diarrhoea following ileal resection is secondary to bile acid malabsorption [7].

Further evidence arrived when patients with ileal resection and diarrhoea were treated with cholestyramine, a bile acid binder, and those with a resection < 100 cm improved. However, patients with resections > 100 cm and a significant steatorrhoea did not respond to resins, probably because the bile acid pool was diminished and decreased further by cholestyramine treatment. The bile acid malabsorption was demonstrated by intravenous injection of sodium- taurocholate¹⁴C: more than 50% of the activity was excreted in the faeces in 24 hours– normally < 20% should be excreted [8].

Furthermore, patients with non-operated Crohn's disease with involvement of the terminal ileum also frequently have bile acid malabsorption, demonstrated by increased bile acid turnover rate as compared to "normals"[9]. Some other injuries to the terminal ileum may also cause bile acid malabsorption such as radiation therapy for gynaecological cancer [10].

In conclusion, ileal resection and/or ileal inflammation or radiation injury leads to bile acid malabsorption. The above mentioned disease entities with pathologically or anatomically defined ileopathy are generally described as **bile acid malabsorption type I** [11 12].

With the introduction of the $^{75}\text{SeHCAT}$ test it became possible to assess the bile acid turnover rate in different diarrhoea conditions [13].

Patients with bile acid malabsorption type 1 had accelerated bile acid turnover rate, i.e. < 10% of the orally administered bile acid analogue - $^{75}\text{SeHCAT}$ - is left after seven days in the enterohepatic circulation [14].

In the clinical situation we often investigate patients with morphologically intact digestive system, where the only abnormality is a reduced $^{75}\text{SeHCAT}$ retention. This condition is classified as **bile acid malabsorption type II** or **idiopathic bile acid malabsorption (IBAM)** and it was believed to be a rare condition previously. However, several studies demonstrated that bile acid malabsorption is present in at least 30% of diarrhoea cases with otherwise unexplained aetiology [2-4].

When **bile acid malabsorption** is associated with other diseases it is classified as **type III**, e.g. post-cholecystectomy [15], familial amyloidosis with polyneuropathy [16], collagenous colitis [17], after surgery for peptic ulcer [18], cystic fibrosis with pancreatic insufficiency [19-21] and myotonic dystrophy [22].

1.2 General description of bile acid synthesis and turnover

The exact aetiology of IBAM is unclear, but before discussing different explanatory models, we will briefly summarize current knowledge about the very complex systems for bile acid synthesis and reuptake. This system consists of six compartments: 1-liver, 2-biliary tree, 3-duodenum-small bowel, 4-terminal ileum 5-vena portae and 6-colon (Figure 1). For the sake of clarity, each system will first be described separately.

Bile acids are synthesized and conjugated in the liver (compartment 1), excreted through the biliary tree (compartment 2), into the small intestine (compartment 3), where bile acids participate in solubilization and absorption of dietary lipids.

After their mission in digestion is accomplished, bile acids are reclaimed in the terminal ileum (compartment 4) by an active transport mechanism. From the terminal ileum bile acids are transported via the portal vein (compartment 5) back to the liver, where they enter the hepatocytes finishing the circle of **enterohepatic circulation** [23], see Fig. 1 on next page.

There is only a minimal spill-over of bile acids from the small intestine into the colon (compartment 6). By measuring the faecal bile acid output it is estimated that almost 95% of the bile acids are reabsorbed per day in the terminal ileum. Bile acid concentrations in various tissues illustrate the enormous concentration differences between different compartments, see Table1 [23].

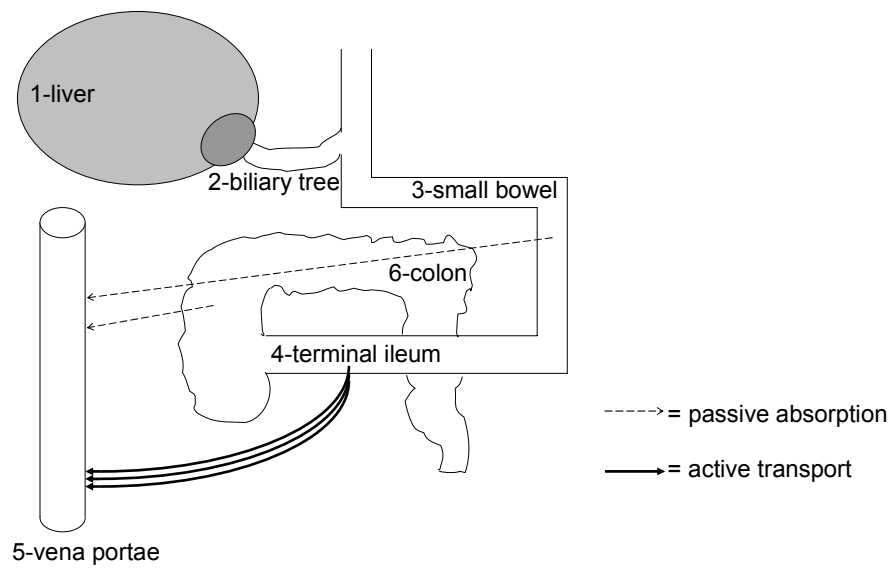


Figure 1. Overview of different compartments of the enterohepatic circulation.

Table 1. Concentrations of bile acids in various tissues.

Portal blood	20-50 $\mu\text{mol/L}$
Plasma fasting	< 5 $\mu\text{mol/L}$
Plasma postprandial	Because first pass extraction is constant, concentration rises several folds
Canaliculus and biliary ductulus	20-50 mmol/L
Gallbladder	300 mmol/L in some species
Small intestine	10 mmol/L
Caecum	1 mmol/L

1.2.1 Bile acid pool

The bile acid pool is the total amount of bile acids present in the enterohepatic circulation. Duane et al determined the bile acid pool with two different methods in 26 volunteers with “no evidence of hepatobiliary or other abnormality”, age between 9-34 years. The median value of the pool size was 2.7 g (25th to 75th percentile 2.5 to 3.1 g) [24].

Other investigators have estimated the bile acid pool size to approximately 3 grams in healthy humans. In celiac disease there is an enlarged pool of 9 grams and in patients after cholecystectomy the pool size may be somewhat reduced, but a study after 5 and 8 years in 12 female patients did not find any alteration of the pool size [25-27].

The composition of the bile acid pool is 50 % cholic acid, 30 % chenodeoxycholic acid, 20% deoxycholic acid and trace amounts of other bile acids [27]. In secondary bile acid malabsorption, after ileal resection the bile acid pool is reduced only if the liver cannot compensate for the faecal losses. This occurs if the length of the resected ileum is greater than 1 m [23].

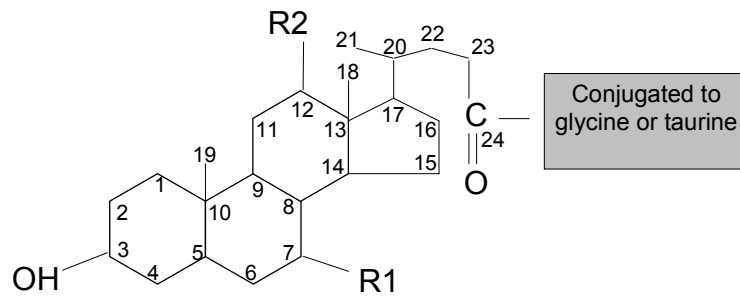
1.2.2 Bile acid chemistry

Bile acids are poorly metabolized, stable and indigestible molecules, perfectly “designed” to fulfil the role of solubilization and absorption of lipids in the gut. However, their function is considerably more sophisticated than only being “soaps”- owing to their chemical stability bile acids may also act as signalling molecules.

They have a very stable steroid ring core and a side chain. The steroid core or nucleus may have hydroxyl groups in different positions, which alter the solubility and biochemical properties of the compound. According to the number of hydroxyl groups there are mono-, di- and trihydroxy bile acids.

If an amino acid is attached to the side chain the bile acid is conjugated. Before this attachment occurs bile acids are called unconjugated. In humans most of the bile acids are conjugated to glycine and a smaller amount to taurine [23].

The general structure of the core, the numbering of the carbon atoms and the hydroxyl groups in different position are shown in Fig. 2.



Symbol	R1	R2	Hydrophilic property	Sort
CDCA	α -OH	H	+++	primary
CA	α -OH	α -OH	+++++	primary
DCA	H	α -OH	++	secondary
LCA	H	H	+	secondary
UDCA	β -OH	H	++++	secondary

Figure 2. Bile acid chemistry: CDCA = chenodeoxycholic acid, CA = cholic acid, DCA = deoxycholic acid, LCA = lithocholic acid and UDCA = ursodeoxycholic acid.

One of the tasks of the liver is to transform cholesterol, a hydrophobic, insoluble compound into hydrophilic bile acid molecules by hydroxylation and conjugation. The end products of the liver reactions are the primary bile acids, namely chenodeoxycholic and cholic acid. In the intestine, bacteria counteract the attempts of the liver to keep bile acids in water solution, by modifying the side chain through deconjugation and altering the nucleus through dehydroxylation. By these reactions the secondary bile acids are formed, namely deoxycholic-, lithocholic- and ursodeoxycholic acid (the last one only in trace amounts).

The bile acids are planar molecules with two “faces”. The hydroxyl groups are present only at one face of the molecule, making it hydrophilic. The other face lacks hydroxyl groups and therefore is hydrophobic. When several bile acid molecules are present the hydrophobic faces tend to aggregate to each other and the hydrophilic faces orientate toward the water molecules of a solution. If the aqueous concentration of bile acids reaches a critical value they form so called micelles which are small polymolecular aggregates which play a key role in lipid absorption [23].

1.3 Liver and the systems for bile acid synthesis

Compartment 1

1.3.1 Bile acid synthesis

There are at least two biosynthetic pathways for bile acid synthesis [28] involving more than 12 enzymatic reactions. The classic or neutral pathway is much more important in healthy humans, since it accounts for at least 80% of bile acid synthesis, and it is also the one involved in feedback regulation [29]. The end product of the classical pathway is cholic and chenodeoxycholic acid, in roughly equal amounts. The end product of the alternative pathway is mainly chenodeoxycholic acid in humans [30].

The classical pathway begins with conversion of cholesterol to 7 α -hydroxycholesterol by CYP7A1, cholesterol 7 α -hydroxylase, (gene symbol CYP7A1), which is the rate limiting **enzyme** [31]. The second particularly interesting step is the synthesis of the intermediate 7 α -hydroxy-4-cholesten-3 one (C4) which can be measured in peripheral blood and has been shown to mirror the bile acid synthesis.

These studies were performed in patients undergoing cholecystectomy for gallstone disease with liver biopsies taken during surgery. Some of the patients were pre-treated with cholestyramine, a bile acid sequestrant, and others were treated with bile acids to alter the feed-back inhibition of the enzyme. There was a strong correlation between the plasma concentration of C4 and the activity of the enzyme cholesterol 7 α -hydroxylase ($r=0.9$, $p<0.0001$) [32,33].

In a previous study with similar design, the activity of CYP7A1 was compared between patients treated with cholestyramine, chenodeoxycholic acid and untreated controls. In the liver biopsies, the enzyme activity was increased 5-fold in the cholestyramine group compared to untreated patients. On the other hand, the chenodeoxycholic acid treated group had an almost 6-fold lower enzyme activity compared to the untreated controls, suggesting that bile acids returning to the hepatocyte might have a strong regulatory influence on their own synthesis [34].

In a study by Bertolotti et al there was also a rather good correlation between hepatic **m-RNA** levels for gene expression of CYP7A1 and serum concentrations of 7 α -hydroxy-4-cholesten-3-one in 21 patients undergoing liver biopsy $r=0.5$, $p<0.05$ [35]. These patients were not treated with drugs interfering with bile acid metabolism.

The bile acid synthesis has been elucidated in different rodent models but there are substantial species differences. Humans have two peaks of bile acid synthesis during the day, one at 1 PM and the other at 10 PM. In rodents, there is only one peak during the night. This diurnal variation is independent of food intake [36].

The regulation of CYP7A1 is very complex and closely related to lipid metabolism. Peroxisome proliferator-activated receptor α (PPAR α), down-regulates the transcription of CYP7A1. The ligands – activators- for PPAR α are fatty acids, eicosanoids and drugs like fibrates [30]. It has been shown that bezafibrate, a drug used to treat hypertriglyceridemia reduces cholesterol 7 α -hydroxylase activity in patients with gallstone disease [37].

Hepatocyte nuclear factor 4 (HNF-4), a nuclear receptor, exerts an important stimulation of CYP7A1 transcription, by interaction with the transcriptional complex at the promoter level, in part independently of the FXR and SHP- cascade (see in section 1.3.3).

1.3.2 Feed-back regulation of hepatic bile acid synthesis

When the enterohepatic circulation is disrupted, either surgically, as in biliary diversion and ileal resection, or pharmacologically, as in treatment with bile acid sequestrants, the hepatic bile acid synthesis is increased [38]. Most of these studies on biliary diversion or ileal resection were done during 1960-1970.

Partial ileal bypass surgery was performed in the POSCH trial (Program on the Surgical Control of the Hyperlipidemias), when between 1975 and 1983, 838 patients were randomized: 417 to the diet control group and 421 to the diet plus partial ileal bypass intervention group. The operation involved bypass of either the distal 200 cm or the distal one third of the small intestine, whichever length is greater, with restoration of bowel continuity by an end-to-side ileocecostomy [39]. In this study the lipid lowering effect is partially due to bile acid malabsorption and a compensatory increase of bile acid synthesis.

In a similar way the enterohepatic circulation is disrupted when negatively charged bile acids bind to resins in the intestinal lumen and are not reabsorbed. In this situation, bile acids are lost in stools instead of returning back to the hepatocyte. The disruption of the feed-back loop leads to enhanced bile acid synthesis [40].

1.3.3 The Farnesoid X receptor and bile acid synthesis

The highly regulated bile acid homeostasis requires intracellular bile acid sensors. The most well characterized sensor is the Farnesoid X receptor – FXR or NR1H4, which has the typical structure of nuclear receptors, with a DNA binding domain and a ligand-binding domain.

It was first shown in 1999 that the natural and most active ligand of FXR is the primary bile acid chenodeoxycholic acid but also secondary bile acids, such as lithocholic acid and deoxycholic acid have some affinity to the receptor while ursodeoxycholic acid and cholic acid have not [41 42]. The affinity of FXR binding to bile acids is in the micromolar range, i.e. approximately 1000 times less than that of the binding of steroid hormones to steroid receptors (which is in the nanomolar range) [43].

FXR is present in liver, small intestine, colon, kidney and adrenal cortex and it is important not only for bile acid synthesis, but also for lipid and glucose metabolism [44]. When bile acids return to the liver via the enterohepatic circulation, they activate a heterodimeric nuclear receptor RXR- FXR by binding to it. This activation then induces the transcription of the nuclear receptor factor short heterodimer partner (SHP) which in turn indirectly blocks the transcription of CYP7A1 leading to reduced bile acid synthesis [45]. In other words, the interaction of hydrophobic bile acids with farnesoid X receptor (FXR), identified as the bile acid receptor, triggers overexpression of the co-repressor short heterodimer partner (SHP) [35].

The mechanism of this indirect blockade is through the involvement of the liver receptor homolog-1, LRH-1 or FTF, also called CYP7A1 promoter binding factor (CPF). SHP interacts with CPF, prevents its binding to CYP7A1 promoter and consequently the transcription is not initiated [45].

There is an indirect pathway of CYP7A1 blockade, by the interaction of bile acids with Kupffer cells resulting in synthesis of cytokines like TNF- α and interleukin-1 β . These cytokines activate protein kinase C, which in turn activates c-Jun N-terminal kinase leading to decreased transcription of CYP7A1 [45].

FXR also protects the hepatocyte from high intracellular bile acid concentrations by suppressing the expression of NTCP gene (Na⁺ taurocholate cotransporting polypeptide). NTCP is responsible for the bile acid influx from the portal venous system to the hepatocyte [46]. (For more details see section 1.8, page 13.

1.4 Bile acid transport through the biliary tree Compartment 2

From the basolateral surface of the hepatocyte, bile acids are transported by intracellular trafficking through the cytoplasm to the canalicular membrane. There are several putative transport proteins like liver fatty acid binding protein (L-FABP), glutathione S-transferase and 3 α -hydroxysteroid dehydrogenase (3 α -HSD), the latter being the most important one in the rat. These proteins are under the control of FXR. In humans these intracellular bile acid binding proteins are not well characterized [46].

Bile acids are excreted via the canalicular membrane into the bile. This process is energy consuming because the bile acid concentration gradient between the portal blood and bile canaliculus may be as high as 1000-fold [47]. To secure the energy supply of canalicular bile acid excretion, the transporter function is dependent on ATP hydrolysis. The two most important transporters are the Bile Salt Export Pump (BSEP) and the Multidrug Resistance Protein (MRP2). BSEP is regulated in a positive feed-forward fashion by bile acids through FXR [46].

1.5 Bile acid uptake in the proximal small intestine Compartment 3

Bile acid uptake in the proximal small intestine is mainly passive, but there is also an active bile acid uptake, which involves a sodium independent transporter, organic anion transporter subtype 3 (oatp-3). The function of this transporter is not well understood in humans, but the gene encoding this protein has been mapped both in humans and in rat [48].

FXR is involved also in the cross talk between the small intestine and the liver. When bile acids enter small bowel enterocytes, FXR stimulates the transcription of the signalling molecule fibroblast growth factor (FGF) 15 in mice, or its human orthologue FGF19 in man. FGF 19 returns to the liver by the portal vein and here suppresses bile acid synthesis [49].

The proximal small intestine will harbour relatively large amounts of bile acids involved in the fat absorption process. We will now discuss briefly how intestinal bile acid handling is linked to intestinal motility and secretion.

1.5.1 The motor and secretory activity of the small intestine in the fasting state

With the introduction of small bowel manometry, it was shown that in virtually all mammals as well as in humans, there is a cyclic motor activity during the fasting state [50]. This cycle, called the migrating motor complex (MMC) is divided into three phases by three different motility patterns. Phase I has no motor activity, in phase II there is an irregular and low frequency activity and in phase III the contractions are regular, with different frequencies in different parts of the intestine, i.e. three cycles/minute in the antrum, 10-12 in the duodenum and 7-9 in the distal ileum [51].

Not only the frequencies but the propagation velocities differ in different parts of the intestine, the speed in the duodenum being approximately 10 cm/minute, in the jejunum 7-8 cm/minute and in the ileum 1 cm/minute [52]. Not all phase III-s propagate throughout the small intestine; in the referred study of Kellow et al the majority of the complexes “died out” and only approximately 10% reached the ileocecal valve [52].

The duration of an MMC cycle is measured as the time between the end of two consecutive phase III periods and is approximately 100 min. In a study of Björnsson et al, the median value was 108.5 minutes [53]. There is a huge inter- and intra-individual variation between the duration of the MMC cycles [54].

The function of the MMC cycle is probably to clean the small intestine from its debris and to prevent bacterial overgrowth. Bacterial overgrowth is associated with motility disturbances like the absence of the MMC complexes [50]. Posserud et al found that 86%

of IBS patients with small intestinal bacterial overgrowth diagnosed with culture of jejunal aspirates had signs of enteric dysmotility in contrast to 39% of the IBS patients with negative jejunal cultures [55].

During phase III, the small intestine becomes a net secretory organ and the MMC can be seen as the motor component of a complex secretomotor rinsing programme [56]. There is a net chloride secretion during phase III which occurs against an electrochemical gradient, resulting in a potential difference between the gut lumen and the extracellular compartment with more negative lumen [57].

The use of phase III activated PD as a marker for electrogenic chloride secretion is based on the finding that phase III motor activity is not accompanied by any change in PD in patients with cystic fibrosis, a congenital disorder in which CFTR, the chloride secreting channel, does not open when activated [58].

1.5.2 Handling of bile acids in biliary system during the fed state

Bile acids secreted by the hepatocytes are excreted through the biliary system. Bile is concentrated and stored in the gall bladder and is released into the duodenum mainly in association with meals. The most important control mechanism for bile release into the duodenum is CCK release from chemosensitive enteroendocrine cells in the duodenal epithelium. CCK is released in response to duodenal lipids. CCK contracts the gall bladder and relaxes the sphincter of Oddi [59 60].

Smaller amounts of bile are also released intermittently in the fasting state, as part of the MMC rhythm. In late phase II, the gallbladder empties partially, and the sphincter of Oddi relaxes. Bile release occurs during a period with antegrade peristalsis in the duodenum (late phase II and early phase III). In late phase III, sphincter of Oddi closes, and when retroperistalsis occurs in late phase III, there is normally no bile present in the duodenum [56]. The physiological role of this very complex system is unknown.

1.5.3 The effect of bile acids on motility

There is a more rapid small bowel transit in idiopathic bile acid malabsorption compared with healthy controls, when transit time is measured with radio-opaque markers [61]. In contrast, when glycochenodeoxycholic acid was infused in the last 40 cm-s of the ileum, the motility of the jejunum and ileum was inhibited resulting in delayed transit [62].

In patients with “irritable colon”, when the terminal ileum was infused with bile acids, there was an exaggerated PD reaction [63]. The effects of physiological amounts of bile acids on small intestinal motility are not well characterized.

1.6 Bile acid uptake in the terminal ileum Compartment 4

1.6.1 Bile acid uptake

Daily bile acid losses are approximately 0,5 g through the faeces in humans with normal bowel habits [64]. The release of bile acids from the liver into the intestine varies between 15 to 25 g/day [65]. This means that bile acids are highly absorbed in the intestine and indicates the existence of active transport systems.

The ileal bile acid absorption efficiency during a single enterohepatic cycle was investigated in six healthy subjects by Galatola et al. They used an occluding balloon distal to ampulla of Vater, infused both ⁷⁵SeHCAT and ¹⁴C labelled taurocholate, and measured the faecal excretion of these markers after intestinal washout. In a single cycle, there was very little variation of bile acid uptake between individuals, the mean value of absorption being 96%, (range 95%-97%). There was however a substantial variability after 24 hours, suggesting that the number of enterohepatic cycling per day may differ widely [66].

The quantitatively most important transport protein is the apical sodium dependent bile acid transporter **ASBT** which is abundant in the distal 100 cm of the ileum [67]. The importance of this protein is emphasized by the fact that it exists in all vertebrates studied [65]. Interestingly, ASBT knockout mice are physically indistinguishable from wild type mice [68]. The gene SLC10A2 coding for ASBT is cloned in humans and a rare mutation of this gene leading to interruption of the enterohepatic bile acid circulation has been described [69].

The regulation of ASBT is species dependent. In human Caco-2 cell cultures, ASBT is positively regulated by retinoic acid and bile acids induce a negative feedback regulation of ASBT via an FXR-mediated, SHP-dependent effect [70]. Inflammatory cytokines inhibit ASBT expression in Caco-2 cell cultures by the so called c-Jun N-terminal kinase (JNK) dependent pathway [71].

ASBT is also up-regulated by peroxisome proliferator-activated receptor α (PPAR α), which is a regulator of fatty acid catabolism and also of hepatic bile acid synthesis [72]. PPAR α is activated by fatty acids, eicosanoids and drugs like fibrates.

Cholesterol feeding down-regulates ASBT in mice and the presence of cholesterol in media of human Caco-2 cell cultures has similar inhibitory effect. The mechanism is not fully understood, but a new pathway involving a partnership between SREBP-2 and HNF-1 α may be responsible [73].

Liver receptor homolog-1 (LRH-1; also called FTF, α -fetoprotein transcription factor in other species) is involved in the down-regulation of ASBT in some species e.g. the rabbit. The group of Guorang Xu identified a FTF functional binding element in the rabbit ASBT

promoter. They concluded that “a functional FTF binding site as well as functioning FXR are required for the negative feedback regulation of rabbit ASBT by bile acids. Only FXR-activating ligands can down-regulate rabbit ASBT expression via the regulatory cascade FXR-SHP-FTF” [74].

However, there are only a few in vivo studies about ASBT regulation in humans with one study showing down-regulation of ASBT in ileal Crohn’s disease and up-regulation during glucocorticoid treatment [75].

1.6.2 Intracellular bile acid transport in the small intestine

High concentration of bile acids can cause disruption of membranes due to their detergent properties. There are therefore mechanisms to monitor intracellular bile acid concentrations like with FXR, and also proteins having the task to bind bile acids and shuttle them through the cytoplasm.

The intestinal or **ileal bile acid binding protein** (IBABP), also known as **ileal lipid binding protein** (ILBP), with the gene symbol FABP6, is such a shuttle transporting bile acids from the apical to the basolateral surface of the enterocyte. IBABP also binds to cholesterol and fatty acids. It is up-regulated by bile acids through FXR, i.e. bile acids entering the ileal enterocyte increase the amount of IBABP [76]. The function and regulation of IBABP is not entirely known, because FXR knock-out mice have very low IBABP expression but increased intestinal bile acid absorption [77].

From the basolateral membrane bile acids are secreted to the portal venous circulation probably by a heterodimeric transporter composed of two subunits Ost α / Ost β [78 79].

1.7 Bile acid transport from the intestine to the liver Compartment 5

From the enterocyte, bile acids are transported tightly bound to albumin in the portal circulation. In plasma, besides albumin binding bile acids are also bound to lipoproteins, mainly HDL [47]. From the portal vein, bile acid are very efficiently extracted by the liver, between 70% and 95% are thus cleared by the first passage. The affinity is higher for trihydroxy than for dihydroxy bile acids, and the uptake system is seemingly unsaturable under physiological conditions.

To illustrate the efficiency of the system, Angelin et al found that the maximum postprandial portal venous bile acid concentration averaged 43.04 \pm 6.12 μ mol/liter, and the corresponding concentration in peripheral serum was 5.22 \pm 0.74 μ mol/liter [80].

1.8 Bile acid transport into the hepatocyte – back to Compartment 1

In the hepatocyte the main transport protein for bile acid uptake is **Na⁺ taurocholate cotransporting polypeptide** (NTCP, SLC10A1). It has high affinity to taurocholate, ($K_m \sim 6 \mu\text{mol/liter}$) but also transports unconjugated bile acids. Structurally it is very similar to the intestinal ASBT protein and is localized at the basolateral hepatocyte membrane.

The expression of NTCP is altered in pregnancy, cholestatic alcoholic hepatitis and advanced primary biliary cirrhosis where the NTCP gene expression is suppressed by elevated concentration of bile acids in order to prevent their entry to the hepatocyte. This suppression is also mediated through FXR [46].

Inflammation suppresses NTCP expression by the JNK dependent pathway in analogue fashion to the down-regulation of ASBT [46]. Like its intestinal counterpart (ASBT), NTCP is up-regulated by glucocorticoid treatment [81].

There is also a Na^+ independent uptake system in the hepatocyte, namely the **organic anion transporting polypeptides** with 4 different proteins, the most important being OATP1B1/SLC01B1 (formerly called OATP-C) with K_m for taurocholate between 14-34 $\mu\text{mol/liter}$. Of less importance are OATP1A2/SLC01A2 (OATP-A) and OATP1B3/SLC01B3 (OATP8) [47]. These transporters are responsible for most of the uptake of the unconjugated bile acids [46].

1.9 Bile acid transport through the colon - Compartment 6

1.9.1 Faecal losses

Even if bile acids have not been absorbed by the active transport system in the distal ileum, they can re-enter the enterohepatic circulation by passive diffusion. This passive absorption is strongly dependent on colonic pH and also whether bile acids are deconjugated and/or dehydroxylated by the colonic flora. Perfusion studies have shown that the rate of absorption is highest for chenodeoxycholic -, intermediary for deoxycholic - and lowest for cholic acid [82].

There are only few studies in the literature about bile acid malabsorption and quantification of faecal bile acids and even fewer studies about direct measurement of faecal bile acids and their relation to the ⁷⁵SeHCAT test [83-85]. The most accurate method for the assessment of faecal bile acid output is gas chromatography-mass spectrometry. In the clinical situation, less accurate enzymatic methods are frequently used [64].

Porter et al has determined faecal bile acid output in 10 healthy subjects with normal bowel habits and in 16 patients with diarrhoea. The median value in normal subjects was 0.315 g/day (0.2-0.73) and in diarrhoea 1.4 g/day (0.7-3.5). However, these patients had severe bile acid malabsorption secondary to systemic amyloidosis (3 patients), ileal surgery or radiation enteritis (5 patients). There was also a significant correlation between faecal weight and bile acid output [64].

1.9.2 Mechanisms of bile acid induced diarrhoea in the colon

The aqueous concentration of total bile acids in human caecum is approximately 0.6 mM (mM = μmol bile acid/ ml fluid) [86].

Colonic water secretion starts when bile acids reach the micellization concentration of 1-2 mM. However, there are differences between dihydroxy and trihydroxy bile acids, because cholic acid does not induce secretion, but chenodeoxycholic- and deoxycholic acid does. Deoxycholic acid induces net water secretion at 3 mM concentration and chenodeoxycholic acid at 5 mM [87].

In a rat model, colonic secretion of Cl^- occurred between the taurodeoxycholate concentrations of 0.5-2 mM. With further increase of TDCA concentration, irreversible cytotoxic effects were seen [88].

Because the solubility of bile acids is pH dependent, high colonic pH perpetuates their secretory effects [89]. Increased colonic permeability, mediated by enteric neurons and increased colon motility are also diarrhoea-promoting effects of the high luminal concentrations of bile acids [90].

The chemical reactions of deconjugation, dehydroxylation and modification of the side chain are carried out by bacteria [91]. The anaerobic bacterial flora in the colon can generate 15–20 different bile acid metabolites [92] but degradation of the steroid ring to CO_2 probably does not occur in the colon. There are also bacteria which do not occur in living organisms that are able to completely metabolize cholesterol and also bile acids to CO_2 [92,93].

By using colonic epithelial cell cultures it has been shown that the secretory effect of bile acids is determined by the steroid ring structure and the length of the side chain. When cholic acid is converted to deoxycholic acid by bacterial dehydroxylation, a non-secretory bile acid is converted into a secretory one. When chenodeoxycholic acid, a secretory bile acid, is dehydroxylated, the result is lithocholic acid, which lacks secretory properties [87]. To sum up, the colonic bacterial flora might determine the chemistry of the bile acid pool in the large intestine and hence the secretory properties – from constipation to diarrhoea. The fact, that bile acid chemistry highly regulates the secretory response in the colon, suggests the existence of a bile acid “sensor” or “receptor”.

There are also important inter-individual variations between the caecal bile acid composition with at least 90% in deconjugated form. The bile acid pool in the caecum is composed of approximately 15% cholic -, 30 % deoxycholic -, 20% chenodeoxycholic -, 20% lithocholic- and 2% ursodeoxycholic acid. It is also important that up to 25% of bile

acids are present in 3 β -hydroxy isoform. These 3 β -hydroxy bile acids are promptly absorbed and are reesterified after returning to the liver to α -hydroxy isoform thus contributing to the enterohepatic circulation [⁸⁶].

The exact mechanism by which bile acids induce diarrhoea is not known. Studies performed from the sixties to early eighties have shown that the colon is a net absorbing organ even in the presence of severe inflammation. The only inflammatory bowel disease where net fluid secretion is reported is collagenous colitis [⁹⁴]. In this disease the colonic crypts are not affected as in contrast to ulcerative colitis or Crohn's disease. High concentrations of bile acids enough to activate fluid secretion are unlikely to occur in the physiologic state [⁸⁶].

However bile acids might increase rectal sensitivity. In an experiment with 11 healthy subjects, rectal infusion of deoxycholic acid at 1 and 3 mmol/l concentrations increased the sensitivity of the rectum to distension, and promoted urgency to defecate. Seven subjects could not tolerate infusion with 3 mmol/l deoxycholic acid [⁹⁵].

In the small intestine, bile acids (e.g. deoxycholic acid) at mM concentrations do induce fluid secretion, and this response is at least partially due to activation of enteric neurons [⁹⁶].

1.9.3 The composition of the bile acid pool in different conditions of bile acid malabsorption

The data on bile acid pool composition are generally based on small numbers of experiments. In the study of Fracchia et al, 13 patients with idiopathic bile acid malabsorption have been compared to 23 controls. They found a significant decrease of cholic acid expressed as percentage of the bile acid pool, but no alteration of the ratio between dihydroxy-to trihydroxy bile acids. They concluded that “the mechanism of diarrhoea does not seem to depend on an enrichment of the bile acid pool with dihydroxy bile acids” [97].

In secondary bile acid malabsorption as in Crohns disease, Lapidus et al found a significant decrease in the deoxycholic acid fraction and a prominent increase in the ursodeoxycholic acid fraction [98].

1.10 Aetiology of bile acid malabsorption type II (idiopathic bile acid malabsorption)

Rare cases of **idiopathic bile acid malabsorption** were described as secondary to a mutation in the ileal sodium-dependent bile acid transporter gene (SLC10A2) [69]. This is a severe condition, with diarrhoea and steatorrhoea present already at birth, weight deficiency and impaired lipid absorption. There is also a knockout mouse model of bile acid malabsorption with targeted deletion of the *slc10a2* gene. Interestingly, these animals do not have diarrhoea or weight loss despite a 10 fold increase in faecal bile acid output [68].

In adult onset bile acid malabsorption there was no dysfunctional mutation of the (SLC10A2) gene in 13 patients [99] and alternative explanations were suggested as a cause of the disease.

Van Tilburg et al have previously shown an increased uptake of taurocholic acid in idiopathic bile acid malabsorption [100]. They constructed brush border membrane vesicles from ileal tissue obtained from diarrhoea patients, including those with bile acid malabsorption. Furthermore, in a subsequent study they found an expanded bile acid pool in these patients.

Their findings indicate that high concentrations of bile acids in the small bowel may overload the saturable active uptake, causing the increased spill over of bile acids to the colon [101]. This idea is in line with a study in our clinic by Sadik et al who demonstrated an accelerated small bowel and distal colonic transit as well higher body mass index (BMI) in patients with adult onset bile acid malabsorption [61].

1.11 Treatment of bile acid malabsorption

Bile acid binding resins: There are only a few placebo-controlled trials for treatment of bile acid malabsorption. One study investigated the effect of enterocoated cholestyramine on symptoms of diarrhoea in patients with ileal resection and Crohn's disease and found significant effects [¹⁰²]. Another study investigated the effect of cholestipol on symptoms in critically ill patients starting enteral feeding after prolonged fasting. The authors postulated that these patients had a relative luminal excess of bile acids leading to choleric diarrhoea and found beneficial effect of bile acid binders [¹⁰³].

There are numerous uncontrolled, open label studies in bile acid malabsorption showing beneficial effects of resins [^{3 8 104}]. One potential drawback is the very high discontinuation rate of medication. This has been systematically assessed in 363 patients when resins were used for cholesterol lowering indication and it was found that only 17% was still taking the drug after 4 years [¹⁰⁵].

Cholesevelam is a new bile acid binder with at least four times higher affinity to bile acids compared to the older cholestyramine and cholestipol [^{106 107}]. This new drug was tested in five patients with bile acid malabsorption and intolerance to cholestyramine showing excellent effect on diarrhoea symptoms [¹⁰⁸].

There are anecdotic reports about the effect of unspecific chelators such as **aluminium hydroxide** on bile acid malabsorption [^{109 110}].

Loperamide: Although it is widely used in diarrhoea conditions, there is only one placebo-controlled trial, namely in radiation enteritis showing good effect on symptoms [¹¹¹].

Corticosteroids and budesonide: In Crohn's disease and in collagenous colitis there is clinical evidence for induction of remission both with conventional corticosteroids and with budesonide [^{112 113}]. Budesonide is a corticosteroid designed to achieve a topical effect in the terminal ileum and proximal colon [¹¹⁴].

The mechanism behind the often dramatic improvement of the symptoms by budesonide treatment is still unknown. In animal models, corticosteroids up-regulate the expression of the Apical Sodium Dependent Bile Acid Transporter (ASBT) [¹¹⁵].

In a study in healthy humans Jung et al have shown that ASBT protein expression increased after budesonide treatment. They suggested that induction of ASBT by budesonide leading to a reduced bile acid load on the colon could be an important mechanism behind the symptomatic improvement in patients with Crohns disease [⁷⁵]. There are no clinical trials with corticosteroids or budesonide in bile acid malabsorption type II.

Diet and lifestyle: Amelioration of diarrhoea may be achieved with low fat diet, both in patients with bile acid malabsorption secondary to radiation enteritis [¹¹⁶] or Crohn's disease [¹¹⁷]. This diet has not been tested systematically in bile acid malabsorption type II.

Bile acid replacement therapy: Patients with extensive ileal resection (>100 cm) did not respond to cholestyramine treatment in the study of Hofmann et al [⁸]. It was postulated that these patients had diminished bile acid pool and cholestyramine further reduced it, accentuating the initial steatorrhea. Replacement therapy with the synthetic bile acid cholylsarcosine improved fat absorption and resulted in weight gain in an open label study in patients with short bowel syndrome [¹¹⁸] or in a case report [¹¹⁹].

FXR agonists: Bile acids bind to FXR and indirectly suppress the rate limiting enzyme of bile acid synthesis, cholesterol 7 α -hydroxylase [⁴⁵]. There are FXR agonists, which bind more potently to FXR than natural bile acids, like 6 α -Ethyl-Chenodeoxycholic acid [¹²⁰] and nonsteroid ligands such as GW4064 [¹²¹].

Theoretically, these compounds reduce bile acid synthesis and consequently could be tested in bile acid malabsorption. However, these compounds are primarily designed for cholestatic liver diseases, as they also promote bile acid excretion from the hepatocyte.

2. AIMS OF THE PRESENT STUDY

As mentioned previously, relatively little is known about the causes of idiopathic bile acid malabsorption. The treatment of this condition is symptomatic by using bile acid binding resins.

The present thesis had the following aims:

To evaluate the diagnostic accuracy and reproducibility of the $^{75}\text{SeHCAT}$ test and whether it declines with aging (**paper I**).

To test the hypothesis that active bile acid uptake in the distal small intestine is reduced in bile acid malabsorption (**paper II**).

To test the hypothesis that patients with bile acid malabsorption have an increased fluid secretion in the small intestine - which may result in bypass of normally functioning distal absorption systems - (**paper III**).

To evaluate the role of colonic inflammation (collagenous colitis) in bile acid malabsorption and to test whether the improvement in symptoms in collagenous colitis during budesonide treatment is associated with an enhancement of bile acid uptake and/or changes in bile acid synthesis (**paper IV**).

3. SUBJECTS AND METHODS

3.1 Subjects and inclusion criteria

Informed consent was obtained from all participants and the local Ethics Committees and the Radiation Protection Committee at the Göteborg University approved the study.

Healthy controls (Paper I, III and IV)

Twenty nine healthy subjects with normal bowel habits underwent a ^{75}SeH CAT test in 1989 [2]. These historical data were used as reference values for the ^{75}SeH CAT test in **paper IV**. Between 2004 and 2006, 19 of the subjects were located and 16 reported that they were still healthy with normal bowel habits and they agreed to undergo a second test. On this occasion the hepatic synthesis of bile acids was estimated by measure of the plasma marker (C4) in 14 subjects. Body mass index (BMI) was registered at both occasions (**paper I**).

The small intestinal manometry and PD measurement were performed in 18 healthy volunteers (**paper III**).

Control patients with normal bowel habits (Paper II)

In the experiment concerning bile acid uptake capacity in ileal biopsies seventeen patients with normal bowel habits served as controls. They were recruited during colonoscopy and if the macroscopic appearance of the bowel was abnormal, suggesting inflammation or malignancy than the patients were not included in the study.

To investigate the correlation between ileal ASBT concentrations and bile acid uptake, multiple biopsies were taken during surgery from five patients operated for continent urinary diversion with an ileal reservoir. These patients had normal bowel habit.

Diarrhoea patients (Paper I, II and III)

In **paper I**, the patients were recruited retrospectively. All ^{75}SeH CAT tests performed between 1986 and 2001 at the Department of Nuclear Medicine in Skövde Hospital were reviewed and patients who had undergone two ^{75}SeH CAT tests were identified. The values from the first and second ^{75}SeH CAT tests among the patients with unchanged conditions were compared with regard to factors known to interfere with the absorption of bile acids. The age at the first investigation, gender, clinical diagnosis and time between the two ^{75}SeH CAT tests were registered.

Patients with chronic diarrhoea who were referred to our gastroenterology unit were included prospectively in the study in **paper II**. The symptom duration was at least three

months. Those with abnormal macroscopic appearance at colonoscopy, suggesting inflammatory bowel disease or malignancy, were excluded. All in all 53 patients with macroscopically normal ileum were included and none of them had previous bowel surgery.

Regarding the eleven patients from **paper III**, bile acid malabsorption was defined as a combination of diarrhoeal symptoms during at least three months and a subnormal value for the $^{75}\text{SeHCAT}$ test (<10% retention at day seven). Celiac disease was excluded by serological tests and/or duodenal biopsies, and infectious causes were excluded by routine faecal cultures and microscopy. All but one patient had their gall bladder in situ and another patient has performed a gastric banding operation previously. One patient had ulcerative colitis with endoscopically normal terminal ileum, otherwise none of them had inflammatory bowel disease or microscopic colitis.

Patients with collagenous colitis (Paper IV)

Patients investigated for chronic diarrhoea who were diagnosed with collagenous colitis after a standard clinical work-up or had a previously known collagenous colitis with a relapse were included in the study. Conventional criteria for collagenous colitis were used -microscopic inflammation including an increased number of intraepithelial lymphocytes, inflammatory cells in the lamina propria with mainly mononuclear cells, epithelial damage such as flattening and detachment and a subepithelial collagen layer of at least 10 μm [¹²²⁻¹²⁶]. For comparison, previously published $^{75}\text{SeHCAT}$ retention data from 29 healthy controls were provided as described in the healthy control section.

Methodological comments: The definition of diarrhoea is poorly standardised. When we included our patients, one criterion was the reported duration of diarrhoeal symptoms of at least 3 months. Not all patients registered their bowel habits, but those referred to the $^{75}\text{SeHCAT}$ test routinely underwent a symptom registration during the test week.

3.2 Symptom recording

At the start of the $^{75}\text{SeHCAT}$ test the patients received a questionnaire to record the number of bowel movements, stool consistency, the symptoms of abdominal pain, distension and flatulence. The scores used were 0, absent; 1, mild; 2, moderate; 3, severe. The consistency of faeces was estimated as 1, watery; 2, loose; 3 firm. The arithmetic mean of the scores was calculated for each symptom as well as for consistency. See questionnaire Fig. 3.

VAR VÄNLIG REGISTRERA DINA MAGBESVÄR!

Namn:

Personnummer:

Period:

BESVÄRSTYP	Må	Ti	Ons	Tors	Fre	Lö	Sön
Buksmärta (0-3)							
Bulsvullnad (0-3)							
Riklig gasavgång (0-3)							

0 = Inga besvär

1 = Lätta besvär

2 = Medelsvåra (stör, men förhindrar ej arbete)

3 = Svåra besvär (t.ex. arbete avbryts eller sänkläge intas)

AVFÖRINGAR	Må	Ti	Ons	Tors	Fre	Lö	Sön
Antal per dygn							
Konsistens							
Nattliga avföringar (X = Ja)							

1 = Vattentunn

2 = Lös (välling, gröt)

3 = (Formad)

Egna kommentarer (t ex andra magbesvär): _____

Aktuella mediciner: _____

Ev. utsatta mediciner och datum:

3.3 The ⁷⁵SeHCAT test (Paper I-IV)

The test was introduced by Thaysen and has been described in previous articles [13]. A capsule containing 0.3 MBq ⁷⁵SeHCAT was swallowed in the morning after one night fasting. The geometric mean of frontal and posterior measurements with an uncollimated gamma camera was calculated. The initial value, representing 100%, was measured 3 hours after ingestion of the capsule. A repeated measurement was performed after 7 days. Retention values less than 10% on day 7 were considered abnormal.

This method was used in **paper II, III and IV**. In **paper III** it was used a simplified method where measurements were performed using an uncollimated gamma camera (Starcam System) with the patient in a supine position and the gamma camera positioned at a distance of 60 cm. The basal value (100%) was obtained with a measurement over the capsule. A new measurement was taken over the abdomen after seven days. The abdominal retention was calculated as a fraction of the basal value. A retention value of > 10% on day 7 was considered as normal [2].

All medication with a potential effect on diarrhoea was excluded during the study week, i.e. bile acid binding resins, loperamid, codein, etc.

Methodological comments: Gamma ray attenuation could be more pronounced in patients with considerable overweight. It has been performed both kind of measurements in the Department of Nuclear Medicine at Sahlgrenska University Hospital using so called phantom models. These measurements didn't show substantial differences between the results of two methods (personal communication, L. Jacobsson). During a time period between 1990 and 2005 it has only been used the simplified ⁷⁵SeHCAT measurement as described in the method section.

Theoretically, the cut off value for abnormal ⁷⁵SeHCAT test should be the (Mean value of 29 healthy controls – 1.96 x SD) = (39.1% -1.96 x 17.7) = 4.4%. In our clinics the cut off value is considered 10% retention at day seven like in other centres [127].

However, these values are somewhat arbitrary, as Fernandez-Banares et al has used <11% and Wildt et al <15% as abnormal retention [4 128]. The rationale behind the 10% cut off value is based on the study by Williams et al where eight patients with ⁷⁵SeHCAT retention > 10% but < 15% did not respond to cholestyramine treatment, in contrast to the 23 patients with ⁷⁵SeHCAT retention <5% responding to resin therapy [129]. It should be emphasized, that in the study of Wildt et al, the best response to treatment was also in the patients with idiopathic bile acid malabsorption and/or a retention value of < 5% [128].

3.4 Assay of 7 α -hydroxy-4-cholesten-3-one (C4) (Paper I, II and IV)

For the estimation of hepatic bile acid synthesis, one ml of blood serum was assayed for C4 by high performance liquid chromatography. The blood samples were taken in the morning under fasting conditions and were frozen immediately to -80° Celsius. The samples were then analyzed at the laboratory of the Department of Endocrinology, Karolinska University Hospital, Huddinge as described previously [130].

Methodological comments: As plasma C4 relative to total plasma cholesterol is a better marker for hepatic bile acid synthesis than the absolute C4 concentration, the ratio of C4/chol was also calculated and evaluated, but for simplicity the plasma C4 concentration was applied [131]. There were no differences between the results when the two different markers for hepatic bile acid synthesis were used.

3.5 Assay of bile acid uptake (Paper II)

Technical procedure

Twelve biopsy specimens were taken from the ileum approximately 10 cm-s proximal from the ileocecal valve for the bile acid uptake assessment. Patients were excluded if biopsy specimens could not be obtained from the ileum.

The biopsies were incubated for 45 minutes in Krebs solutions at 37° C containing three different concentrations of 14 C labelled taurocholate, a β particle emitting compound. The taurocholate concentrations were 100, 200 and 500 μ moles and four biopsies were used for each concentration.

After the incubation the biopsy specimens were freeze-dried, weighed and then dissolved in Soluene[®]-350 in order to mix with a scintillation cocktail. The absorbed radioactivity was measured using a liquid scintillation β -counter.

Estimation of taurocholate uptake

For each series the measured radioactivity (the mean value of the four measurements) was plotted on the Y axis and the taurocholate concentrations in the incubation media were plotted on the X axis (100, 200 and 500 μ moles on the X axis). The taurocholate uptake follows a straight line at concentrations between 100 and 500 μ moles in the medium [67]. This indicates that the active uptake is saturated above the concentration of 100 μ moles.

The three points of the diagram were connected to a line which was extended to 0 μ moles concentration – a point where the extended line meets the y axis. The maximal ability for

active bile acid uptake of the mucosal specimens was calculated from the intercept of this extended straight line with the y axis, at which point the concentration of taurocholate is zero and there is no passive absorption.

Methodological comments: This method was validated previously, when different tissues were tested [67]. According to methodological study, the estimated ¹⁴C taurocholate uptake was high in the terminal ileum and approximately 75% lower in the ileum 100 cm proximally from the ileocaecal valve, very low in the duodenum and in the right colon. The uptake capacity was measured using devitalized tissue from the terminal ileum and also using an inhibitor of the transporter protein (ASBT) showing very low uptake.

The incubation time of 45 min is based on the study of Hosie et al who has reported a linear uptake within the interval of 5 to 45 minutes [132].

3.6 Western blot analysis to quantify the ASBT protein

In five patients who were operated for continent urinary diversion with an ileal reservoir, multiple biopsies were taken during surgery, both for the bile acid uptake assay and for the western blot analysis. The method is described in detail in **paper II**.

Methodological comments: The depth of the biopsy may affect the amount of the ASBT protein since it includes non epithelial components in a variable manner. To circumvent this potential artefact the ASBT concentration was corrected to villin concentration of the very same biopsies. Villin is a marker for epithelial cells displaying a brush border and it is present in epithelial cells of the gastrointestinal, urinary and respiratory tract. Its synthesis increases during the maturation of the enterocyte, which takes place when the enterocytes migrate from crypts to the tip of the villus [133].

Since the Western-blot analysis is only semi-quantitative, ranks have been used instead of absolute values.

3.7 Small intestinal manometry and mucosal potential difference

The experimental setup is described in more detail in **paper III** and in previous articles [56, 134]. Briefly, in the morning after an overnight fast, the subjects were intubated transnasally with a multilumen polyvinyl tube containing eight separate channels, six of which were used in the experiment. The tip of the tube was placed in the proximal jejunum, under fluoroscopic guidance.

The pressure was recorded in the proximal jejunum channel (1), duodenojejunal junction (2), at three channels with 1.5 cm distance in between in the mid duodenum in the papilla region (3-5), and one channel in the antrum (6). Each pressure-recording channel (except those used for recording of transmural PD) was perfused with water at a rate of 30 ml/hr.

The low-compliance water perfused system is considered the standard device for intraluminal pressure recordings [¹³⁵ ¹³⁶].

We also recorded simultaneously the transmural potential difference (PD) through the jejunal recording point at the end of the tube (1) and the middle recording point in the descending duodenum (4), using an infusion of isotonic saline (instead of water) as a flowing electrode. PD was measured between calomel half-cells and a common reference electrode connected to a subcutaneous infusion of saline.

This mode of recording enabled us to measure PD and pressure at the same time and location. Intestinal motor activity and PD were recorded for three hours in the interdigestive state. The subjects were then given a standardized test meal (for details see **paper III**) and recordings were continued for another hour.

Data processing

The raw data were stored as ASCII files and were processed by specially designed software in Matlab code. A phase III of the MMC complex was identified by the computer software defined as 10-12 contractions/minute, duration of minimum 120 seconds and distal propagation. Phase II was defined as the time window of 15 minutes before phase III and phase I as the interval of 15 minutes after the phase III complex. The data were also checked manually by a person unaware of the automatically generated results.

The main recorded motor parameters were the number of phase III –s and the time duration of these complexes. The software had registered all contractions and consequently the contraction frequency was calculated both in fasting and fed state. The closely spaced side holes allowed us to recognize the propagation direction of the pressure waves [53].

Recorded secretory parameters: were those concerning the PD curve. Mean PD during fasting and fed conditions in phase I, II and III were calculated by the software. The maximum PD during phase III was also manually checked.

The **propagation velocity** of the phase III complex was calculated by dividing the distance between the duodenal and jejunal recording points (in fact a constant) with the time interval needed for the end of the phase III to reach from the duodenal to the jejunal recording point.

The length of the small intestinal segment with ongoing phase III activity the “**motility length**” was calculated as the product of [phase III propagation velocity] and [time duration of phase III], (cm/min * min = cm), between the mid duodenal (4) and proximal jejunal (1) recording channel.

The amount of **chloride secretion** during phase III was estimated as the following product: [mean phase III PD] *[phase III duration]*[phase III propagation velocity]* [number of phase III:s /3 hour recording period], (mV * min *cm/min *a digit = mV).

Methodological comment: First, it was shown by Geall et al that PD between the peripheral venous blood and the gastrointestinal mucosa is the same as between the serosa and mucosa [¹³⁷]. Afterwards it was concluded by Wingate et al that the PD between the mucosal and serosal surface is the same as between the mucosa and a subcutaneous compartment [¹³⁸]. Accordingly, an intravenous or a subcutaneous electrode shows the same electric potential as the serosal one. To sum up, the PD between the mucosal and serosal surface of the intestine can be measured indirectly by measuring the PD between the mucosa and a subcutaneous compartment in the forearm.

3.8 Statistical methods

Due to relatively low number of study participants and non-gaussian distribution of parameters we used nonparametric methods, Man-Whitney U test and Wilcoxon signed rank test. In order to evaluate the reproducibility of the ⁷⁵SeHCAT test, the repeatability coefficient was used in diarrhoea patients, as described by Bland and Altman [¹³⁹]. For further description see statistical methods **in paper I**. With a good concordance of this coefficient, 95% of the differences should to be less than two standard deviations (SD).

The relationships between the ⁷⁵SeHCAT values and other parameters were calculated using a logarithmic regression model based on the reasoning described by Sauter et al [¹⁴⁰].

The kinetics of bile acids follows a one-compartment model. In a one-compartment model the synthesis of the bile acids is increased exponentially with the decrease of its half-life (or with the decrease of the ⁷⁵SeHCAT retention values). This means that is appropriate to use exponential or logarithmic equation when relating the ⁷⁵SeHCAT retention values to the bile acid synthesis (C4) or to symptoms (Number of stools).

4. RESULTS

4.1 Diagnostic accuracy and reproducibility of the $^{75}\text{SeHCAT}$ test (Paper I)

In the retrospective study with chronic diarrhoea patients, 43 had performed the test twice. Four patients were excluded because of major intervention which could alter the outcome of the test, i.e. ileocaecal resection (2), cholecystectomy (1) and treatment for coeliac disease (1).

In the prospective study with healthy controls 16 had performed to tests with a time interval of sixteen years. The body mass index (BMI) was registered at both occasions and increased significantly by 12% (from $23 \pm 3.1 \text{ kg/m}^2$ to $25.7, \pm 4.6 \text{ kg/m}^2$, $p < 0.01$). The reproducibility index is illustrated in Fig.4.

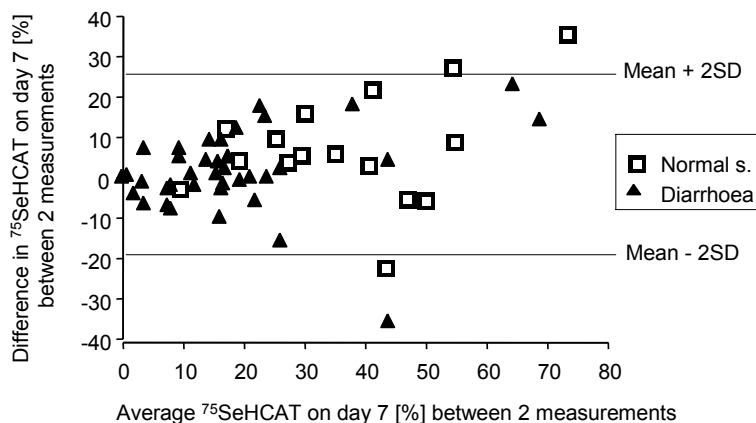
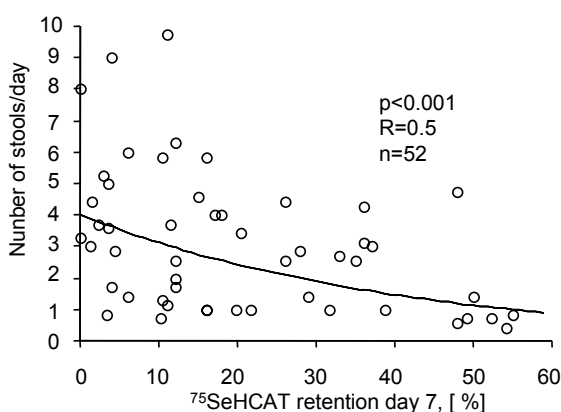


Figure 4. The reproducibility index of the $^{75}\text{SeHCAT}$ test.

Comments: The diagnostic consistency of the $^{75}\text{SeHCAT}$ test is excellent in diarrhoea patients, only 1 patient of 39 is outside the limit of $\text{Mean} \pm 2\text{SD}$. In healthy controls there is a greater difference, 3 of 16 are outside the limit of $\text{Mean} \pm 2\text{SD}$. However, in healthy controls there was a significant decrease between the two test values. This could be a true physiological phenomenon linked to the increase in body mass index BMI, by 13%, from a median of 23.2 kg/m^2 , (21 - 24.6) to 26.2 kg/m^2 , (22.5 - 27.8), $p < 0.01$) during the 16 years.

4.2 Correlation between symptoms, bile acid turnover rate ($^{75}\text{SeHCAT}$) and hepatic bile acid synthesis (C4) (Paper I, II, IV)

The average stool frequency per day during the week of the $^{75}\text{SeHCAT}$ measurement was registered in almost all participants: healthy subjects (**paper I**), diarrhoea patients (**paper II**) and patients with collagenous colitis (**paper IV**). When the data from the three studies is added together the following correlations were found and presented in Fig. 5, Fig. 6 and Fig. 7.



Correlation between the number of stools and $^{75}\text{SeHCAT}$ in all diarrhoea patients and healthy controls. (>10 stools/day are not presented).

Figure 5. $^{75}\text{SeHCAT}$ test and stool frequency $r = 0.5$, $p < 0.001$, $n = 52$. logarithmic regression.

In the individual studies, the correlations were the following:

- $^{75}\text{SeHCAT}$ test and stool frequency (**paper I**): $r = 0.321$, $p = 0.336$, $n = 11$ logarithmic regression.
- $^{75}\text{SeHCAT}$ test and stool frequency (**paper II**): $r = 0.492$, $p < 0.001$, $n = 41$ logarithmic regression in the mixed diarrhoea population.

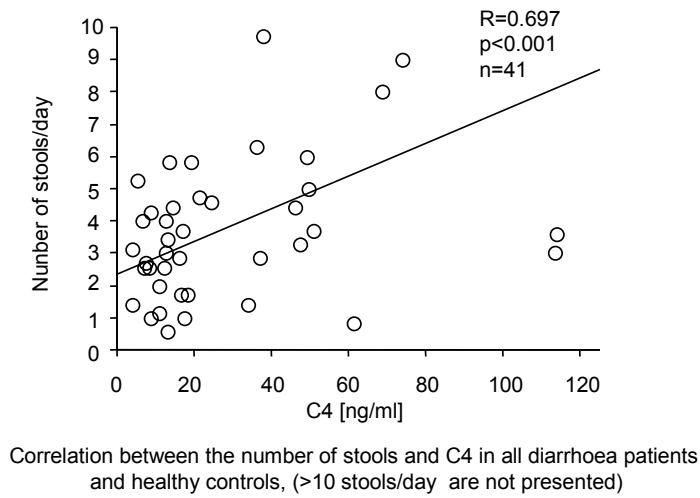


Figure 6. **C4 and stool frequency** $r = -0.697$, $p < 0.001$, $n = 41$ simple regression.

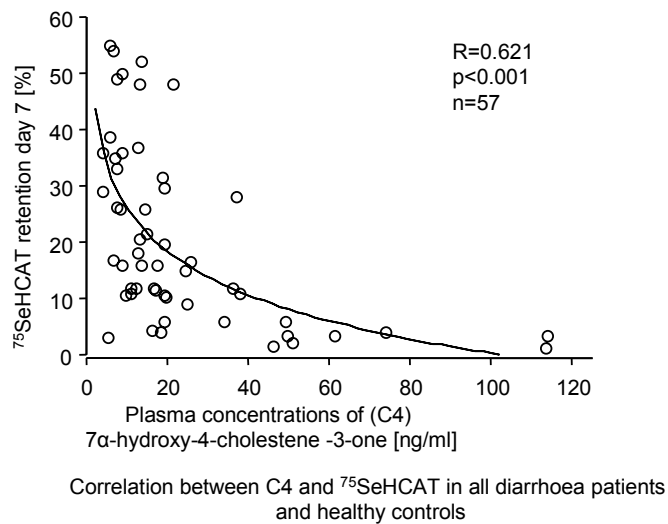


Figure 7. **⁷⁵SeHCAT test and C4** $r = 0.621$, $p < 0.001$, $n = 57$ logarithmic regression.

There was only minimal difference when the C4 values were corrected for plasma cholesterol. ⁷⁵SeHCAT test and C4/cholesterol $r = 0.624$, $p < 0.001$, $n = 53$.

In paper IV, in the collagenous colitis group we registered the stool consistencies and other symptoms like pain, abdominal gas and bloating both before and during budesonide treatment. The correlation matrix before treatment is presented in the table. The statistical significance was calculated by Fischer's test. A p value < 0.05 is marked with *, p<0.01 with** and p<0.001 with ***. Data from sixteen patients were used to compute the correlation matrix, see Table 2.

During budesonide treatment there were no significant correlations between the different parameters.

Table 2. The correlation matrix between symptoms and ⁷⁵SeHCAT test and C4.

	⁷⁵ SeHCAT	C4	Frequency	Consistency	Pain	Bloating	Gas
⁷⁵ SeHCAT	1	-.52*	-.57*	.36	-.67**	-0.145	0.61*
C4	-.52*	1	.73***	-.52*	.26	.14	-.22
Frequency	-.57*	.73***	1	-.51*	.41	.89	-.14
Consistency	.36	-.52*	-.51*	1	.064	.035	.11
Pain	-.67**	.26	.41	.064	1	.49	-.31
Bloating	-.145	0.14	.89	.035	.494	1	.340
Gas	.61*	-.21	-.14	.11	-.31	.34	1

Comments: The plasma C4 measurements and the ⁷⁵SeHCAT tests although strongly correlated they reflect different variables. In steady state condition C4 mirrors the hepatic synthesis which is an absolute value. The ⁷⁵SeHCAT tests mirrors the fractional turnover rate, i.e. a fraction or percentage. In some diseases it is possible that a high ⁷⁵SeHCAT tests corresponds to high C4 values – e.g. in celiac disease, where the bile acid pool is very large [²⁶]. In this disease the C4 test, which mirrors the absolute amount of bile acids reaching to colon, might be more accurate.

4.3 Bile acid uptake capacity in isolated biopsies from the ileum (Paper II)

The results are presented as median, (percentile 25 and 75). Patients with diarrhoea had a significantly higher uptake of taurocholate: 7.7 $\mu\text{mol}/\text{g}\cdot\text{min}$, (6.1 and 9.7), $n=53$, compared with the controls: 6.1 $\mu\text{mol}/\text{g}\cdot\text{min}$, (4.5 and 6.9), $n=17$ ($p<0.01$). See Fig. 8.

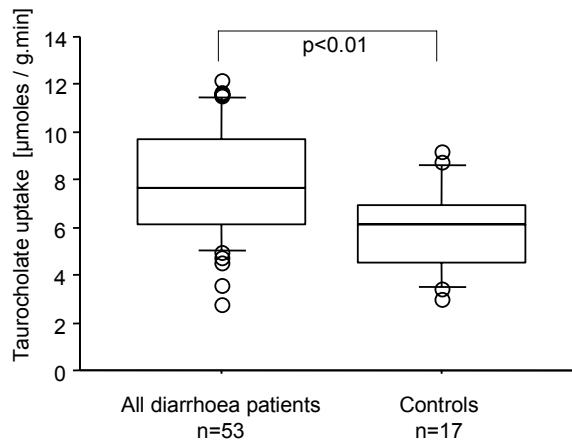


Figure 8. Bile acid uptake capacity in all diarrhoea patients compared to controls.

The uptake among patients with abnormal $^{75}\text{SeHCAT}$ values was significantly higher: 7.4 $\mu\text{mol}/\text{g}\cdot\text{min}$, (7 and 8.9), $n=18$, than in the controls ($p<0.01$), see Fig. 9.

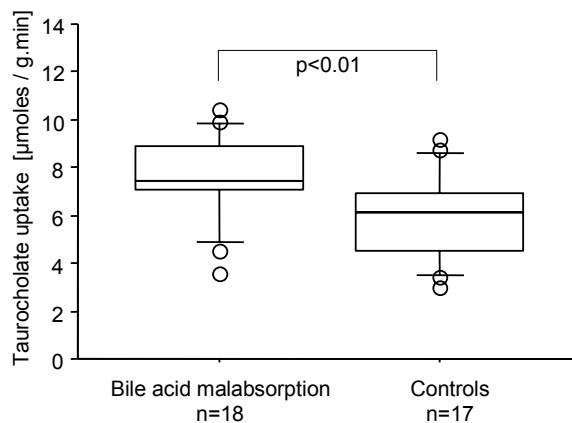


Figure 9. Bile acid uptake capacity in diarrhoea patients with bile acid malabsorption compared to controls.

There was no significant difference between patients with abnormal $^{75}\text{SeHCAT}$ test and those with diarrhoea and normal $^{75}\text{SeHCAT}$ values: $8.5 \mu\text{mol/ g}\cdot\text{min}$, (6.1 and 10), $n=23$ ($p=0.44$, ns)

The **correlation** between bile acid uptake capacity and ASBT/Villin ratio based on ranking showed identical ranking with the exception of 2 values, Fig 10.

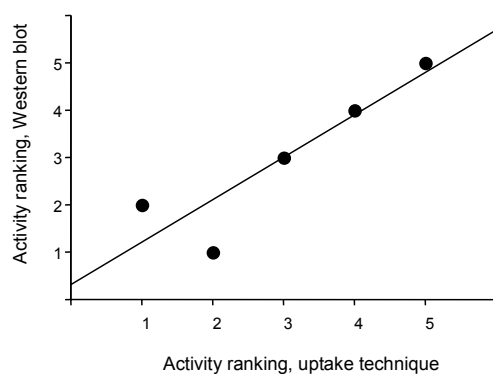


Figure 10. Correlation between the rankings of the two tests.

There was a **lack of correlation** between bile acid uptake capacity and the following parameters:

Gender: males $n=22$ and females $n=31$ ($p=0.8$) in the **diarrhoea group**, or in **controls**, males $n=8$, females $n=9$ $p=0.85$.

Age: $r=0.08$, $n=17$, ($p=0.77$) in controls and $r=0.15$, $n=53$, $p=0.3$ in diarrhoea patients.

$^{75}\text{SeHCAT}$ retention $r=0.141$, $n=41$, ($p=0.39$)

Hepatic synthesis of bile acids measured by the (C4) values $r=0.151$, $n=26$ ($p=0.47$).

4.4 Estimated chloride secretion in patients with bile acid malabsorption compared to healthy controls (Paper III)

In the fasting or interdigestive state the total amount of chloride secreted in the simultaneously contracting segment was calculated from the product of segment length and mean PD, and by multiplying this value with the number of phase III periods per 3 hour period (duration of experiment in fasting state). The estimated value for total chloride secretion (“interdigestive secretory index”) was higher in patients with bile acid malabsorption [$510.7 \text{ mV}\cdot\text{cm}$, (357.3-823.8), $n=9$] vs. controls [$307.8 \text{ mV}\cdot\text{cm}$, (180.2-374.9), $n=10$], ($p<0.01$), see Fig. 11.

In the fed state there were no significant differences in the magnitude of the motor or secretory response to feeding between controls and BAM patients. The time course and magnitude of the PD response to feeding was in fact almost identical in controls and BAM patients, and this was true in both duodenum and jejunum.

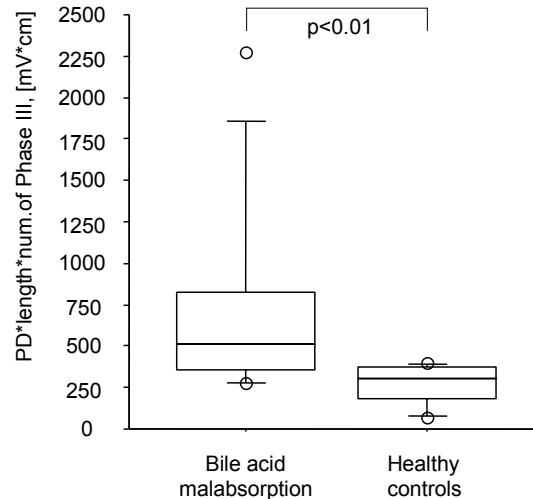


Figure 11. The estimated chloride secretion in patients with bile acid malabsorption compared to healthy subjects in the fasting state.

4.5 Correlation between estimated chloride secretion and the $^{75}\text{SEHCAT}$ test (Paper III)

The amount of **chloride secretion** during phase III was estimated as the following product: [mean phase III PD] *[phase III duration]*[phase III propagation velocity]* [number of phase III:s /3 hour recording period], (mV * min *cm/min *a digit = mV).

This estimation could be done in nine patients and the graph is presented in Fig.12 on the next page.

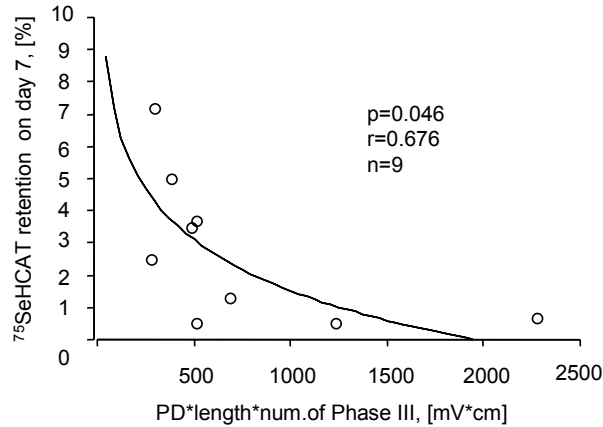


Figure 12. The correlation between the estimated chloride secretion and the ⁷⁵SeHCAT test in patients with bile acid malabsorption.

4.6 Budesonide treatment

4.6.1. The effect on bile acid turnover rate (⁷⁵SeHCAT test)

The ⁷⁵SeHCAT test was performed in 25 patients before treatment and after 8 weeks of budesonide therapy. Six patients (24%) had an abnormal value initially. The seventh day retention values increased from 18% (11% and 28%) to 35% (19% and 46%), ($p < 0.0001$). The 29 healthy controls ⁷⁵SeHCAT values were 38% (29% and 48%). Thus, the difference between the collagenous colitis group and healthy controls ($p < 0.0001$) disappeared during the treatment, ($p = 0.26$), see Fig. 13.

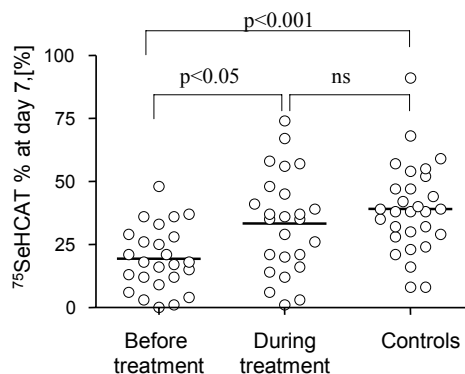


Figure 13. The ⁷⁵SeHCAT test in collagenous colitis patients before and during treatment compared to healthy control subjects.

4.6.2. The effect on bile acid synthesis (C4)

The plasma C4 concentrations were analyzed in 19 of the patients before and during budesonide treatment, see Fig .14.

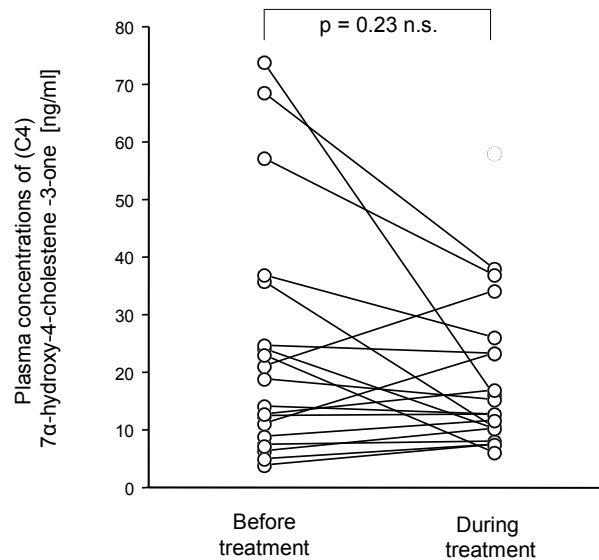


Figure 14. The estimated bile acid synthesis before and during budesonide treatment in patients with collagenous colitis.

The nine patients with initial plasma C4 concentrations over 20 ng/ml showed a significant drop of their C4 values during treatment, from 36 ng/ml (24 ng/ml and 60 ng/ml) to 23 ng/ml (10 ng/ml and 35 ng/ml)($p=0.04$). Their $^{75}\text{SeHCAAT}$ retention values increased from 12% (3% and 18%) to 21% (11% and 35%), ($p=0.03$).

In the whole group of investigated patients the C4 values decreased from 19 ng/ml (9 ng/ml and 33 ng/ml) to 13 ng/ml (10 ng/ml and 23 ng/ml), ($p = 0.23$ ns). Nine patients had higher and 10 had lower levels during the two measurements.

The C4 values decreased in 5 of the 6 patients with bile acid malabsorption but the small number didn't allow to reach statistical significance.

4.6.3. The effect on symptoms

The stool frequencies decreased from 4.15/day (3.5/day and 5.4/day) to 1.8/day (1.1/day and 2.3/day), ($p < 0.0001$), $n = 21$.

The scores for stool consistency improved from 1.7 (1.15 and 2) to 2.7 (2 and 2.9) ($p < 0.001$), $n = 20$.

The scores for pain improved from 1.3 (0.93 and 2) to 0.2 (0 and 0.6) ($p < 0.01$), $n = 18$.

The abdominal distension (bloating) score improved from 1 (0 and 1.5) to 0.15 (0 and 1) ($p < 0.01$), $n = 13$.

The scores for flatulence ameliorated from 1.3 (0.7 and 1.7) to 0.3 (0 and 1) ($p < 0.05$).

The improvement of the symptoms did not differ between the patients with initially high C4 values and those with $C4 < 20$ ng/ml. In the group with initially higher bile acid synthesis, with C4 values >20 ng/ml there was a tendency toward more frequent stools, 4.7/day (4.1/day and 7.5/day) compared to the group with C4 values <20 ng/ml, 4/day (3/day and 4.3/day), ($p = 0.06$) Otherwise, the symptom scores did not differ regarding consistency, pain, abdominal distension or flatulence.

5. GENERAL DISCUSSION – POSSIBLE CAUSES OF IDIOPATHIC BILE ACID MALABSORPTION

5.1 Short summary

The present thesis deals with intestinal absorption of bile acids with special reference to the mechanisms behind idiopathic bile acid malabsorption. In **paper I** we found that the turnover rate of bile acids is stable during a long period of time, both in diarrhoea and in health. Despite of the somewhat lower $^{75}\text{SeHCAT}$ retention values after sixteen years in healthy subjects, this decrease is of less magnitude to cause bile acid malabsorption. We also tested the relationship between age and the values of the $^{75}\text{SeHCAT}$ test in paper I, II and III and we can conclude that ageing is not correlated to this condition.

The BMI (body mass index) of the healthy subjects increased significantly during the study period at the same time when $^{75}\text{SeHCAT}$ values decreased. If the relationship between these two variables is real the accelerated turnover rate of bile acids reflected by the decreased $^{75}\text{SeHCAT}$ values may thus be a consequence of a westernized lifestyle.

The results of **paper II** suggest that this condition is not caused by an impaired bile acid uptake system at the level of the terminal ileum – the uptake capacity was increased in diarrhoea patients irrespectively of the $^{75}\text{SeHCAT}$ test values.

In **paper III** we could show an inverse correlation between the $^{75}\text{SeHCAT}$ retention values and the estimated small bowel secretion, i.e. with increasing level of bile acid malabsorption there is a parallel increase in fluid secretion in the small bowel.

There was a significant inverse correlation between the $^{75}\text{SeHCAT}$ test and hepatic bile acid synthesis both in health and in diarrhoea (**paper I and II**). To our knowledge this is the first measurement in healthy subjects and also in diarrhoea where all the patients had macroscopically normal terminal ileum and none of them had bowel resection.

Concerning the mechanism of action of budesonide in collagenous colitis we found that the treatment significantly increased $^{75}\text{SeHCAT}$ values and improved the symptom scores (**paper IV**). It also decreased hepatic bile acid synthesis in patients with initially high synthesis rate, as determined from the serum marker C4. These results suggest that the effect of budesonide in collagenous colitis may partly be linked to a reduced bile acid load on the colon.

5.2 Impaired ileal bile acid uptake system

A key finding of the thesis is that bile acid malabsorption diagnosed by the $^{75}\text{SeHCAT}$ test is characterized by normal or high ileal uptake capacity. This is in line with the study by van Tilburg et al, which also showed an increased uptake using brush border membrane vesicles prepared from terminal ileal biopsy specimens from 10 patients who fulfilled the criteria of idiopathic bile acid diarrhoea [100]. Furthermore, in a subsequent study they demonstrated that these patients have an expanded bile acid pool [101].

In two studies of Montagnini et al no functional ASBT mutations were identified in 13 patients with bile acid malabsorption or in a family with bile acid malabsorption in three consecutive generations [99 141]. Furthermore, there were no mutations or polymorphisms in the genes for several of the nuclear receptors known to be important for ASBT expression: the farnesoid X receptor (FXR) and peroxisome proliferator activated receptor alpha (PPAR alpha).

Interestingly, in a recent study with a mouse model of postinfective gut dysfunction the ileal bile acid absorption was decreased, despite of an increased ASBT expression [142]. The authors postulated that the transport function of the protein may have been inhibited after infection. However, the results of **paper II** show an increased bile acid uptake capacity of the ileal biopsies and are contradicting the idea of diminished transport function. An alternative explanation may be that the excretion of bile acids through the basolateral membrane is altered.

We did not find any reduced bile acid uptake capacity of the few patients with collagenous colitis investigated in **paper II**. Interestingly, these patients had normal $^{75}\text{SeHCAT}$ values. However, in **paper IV**, the $^{75}\text{SeHCAT}$ values were abnormal in almost in a quarter of the patients with collagenous colitis initially and normalized during budesonide treatment.

5.3 Increased bile acid pool

The maintenance of the pool is mainly achieved by de novo synthesis in the hepatocyte and by active absorption in the ileum. The hepatic enzyme system is highly inducible and in extreme cases such as biliary fistulas the daily synthesis of bile acids can increase up to 20-fold [23]. We also found a wide range in the plasma concentrations of C4 pointing to great variation in the hepatic synthesis rate of bile acids.

The transport proteins of the ileal system, where the most studied is ASBT, are inducible too but probably with a lower magnitude i.e. only 1-2 fold. Jung et al found in healthy controls an increase of the ASBT protein expression 1,34 fold after 21 days of budesonide treatment [75]. The relationship between ASBT protein concentration in the gut and its effect on bile acid uptake it is not known today.

The bile acid pool size was determined in previous studies in healthy subjects with a median value of 2.7 g (25th to 75th percentile 2.5 to 3.1 g) [143]. The fecal bile acid losses in healthy subjects can vary between 0.08-0.84 g/day with average of 0.4 g/day [144]. If we assume that a healthy subject reabsorbs the amount of bile acids equal to the pool size minus daily losses, the reabsorbed amount is approximately $2.7\text{g} - 0.4\text{g} = 2.3\text{g}$ per day. The reabsorption rate is $2.3\text{g}/2.7\text{g} = 0.85$. In seven days this calculation should be repeated six more times, with each day leaving 0.85 of the amount of the previous days, thus it remains $(0.85)^6 = 0.377$ or 37.7% of the original pool. This estimate is in line with the median value of 38 % of the ⁷⁵SeHCAT at day 7 in a former investigation [17].

We found that bile acid malabsorption patients have an increased uptake capacity, approximately 1.2 times compared to patients with normal bowel habits. If we assume that the daily amount of bile acids they absorb is $2.3\text{g} * 1.2 = 2.76\text{ g/day}$ and at day 7 the median ⁷⁵SeHCAT is 3.5 % or (0.035), then their first day retention will be $\sqrt[6]{0.035} = 0.57$. This means that approximately 43 % of their bile acid pool is lost every day. If patients with bile acid malabsorption can absorb 2.76 g/day but lose each day 43% of the pool then their total bile acid pool should be $2.76\text{ g} * 100/57 = 4.84\text{ g}$, almost double sized compared to normal subjects. This assumption is in line with the finding of van Tilburg et al in 8 patients with BAM where the average pool size was 7 mmol, compared to 3.7 mmol in healthy subjects [101].

If the high uptake in ileal biopsies mirrors an increased total in vivo re-absorption of bile acids from the small intestine this should normally lead to a suppression of the hepatic bile acid synthesis. The increased synthesis in bile acid malabsorption therefore suggests that the feedback system may be hampered. The high synthesis will lead to higher concentrations of bile acids within the gut and subsequently may overload the saturable active uptake system.

In a study by Sadik et al patients with bile acid malabsorption had an increased body mass index [61]. High fat intake increases the synthesis rate of bile acids in animal models [145].

A low-fat diet was found to decrease the excretion of bile acids, both in ileostomy patients as well as in patients with bile acid diarrhoea caused by pelvic irradiation for malignant gynaecologic tumours [116 146]. Seemingly, there is a connection between high fat intake and bile acid excretion which in turn may cause with time an increased bile acid pool.

5.4 Shorter ileal segment with active bile acid uptake

The length of the segment with ASBT protein expression is not known in patients with bile acid malabsorption. If the segment with active absorption is shorter than normal the total absorption of bile acids may be decreased although the active uptake in the very distal part of the small bowel is up-regulated.

5.5 Faster motility

If the luminal bile acids “bypass” the reabsorbing epithelium due to reduced contact time (e.g. faster motility of the small intestine), this might explain a seemingly impaired absorption despite normal capacity. A study by Sadik et al shows that patients with bile acid malabsorption type 2 have a more rapid small bowel transit measured with radioopaque markers compared with healthy controls [61].

Since there is a hepatic feedback system that increases bile acid synthesis when bile acid reabsorption is reduced, the bile acid load on the intestine will increase further which will result in increased secretion and motor activity etc, i.e. a classical positive feedback circuit [30]. It is theoretically possible that the beneficial effect of cholestyramine in these patients is to break this positive feedback circuit. If the hypothesis is correct, it might also explain the beneficial effect of loperamide, since opiates have an inhibitory effect on enteric secretomotor neurons, and also reduce intestinal motor activity [147].

5.6 Increased small bowel secretion

In the duodenum, phase III is preceded by partial gall bladder emptying, i.e. the mucosa of the receiving segment contains quite high concentrations of bile acids which has been proposed to contribute to the maintenance of motor activity [148]. During the MMC cycle the motor activity is coupled to the secretory activity, e.g. net fluid secretion occurs mainly during the late part of the MMC cycle when there is high motor activity and a more lumen negative PD [149].

We found an increased jejunal PD in patients with bile acid malabsorption compared to healthy controls in **paper III**. Furthermore, there was an inverse correlation between the ⁷⁵SeHCAT retention values and the estimated chloride secretion in bile acid malabsorption, e.g. with increasing level of bile acid malabsorption there was a parallel increase in chloride secretion. This finding might be in line with the assumption of increased bile acid pool in patients with bile acid malabsorption type II.

The present data suggest that in patients with bile acid malabsorption as diagnosed by a pathological ⁷⁵SeHCAT test, the small intestine may generate an increased fluid load on the colon. Depending on contact time in the colon, this fluid will be partially reabsorbed.

Colonic transit time is reduced in bile acid malabsorption [61] and bile acids may per se increase colonic motility [150]. Even a modest increase in the fluid load from the small intestine may therefore be symptom generating in these patients. Interestingly, bile acids are also able to generate propulsive motility in the ileum, and administration of bile acids to patients with “irritable colon” was shown to increase ileal PD (i.e. probably generate secretion) [63].

The current findings may account for the puzzling observation of **paper II**, that despite normal or increased ileal bile acid reuptake capacity, patients with bile acid malabsorption lose bile acids via the stools. However, if there is an increased fluid secretion in the small intestine, then luminal bile acids may bypass the reabsorbing epithelium due to “dilution effect”. This dilution effect was tested previously in a study by Fellous et al, when the administration of an osmotic laxative resulted in lower ⁷⁵SeHCAT test values (22%) compared to untreated subjects, who had 44% retention after seven days [151].

Surprisingly, the PD response to feeding was almost identical in controls and BAM patients. This finding should be interpreted with some caution, since the test meal will change the chemical environment in the lumen which may per se induce electrochemical gradients that may mask small group differences.

The results of **paper III** suggest that patients with diarrhoea and reduced ability to retain bile acids produce elevated amounts of chloride in the proximal small intestine in association with phase III of the migrating motor complex. Increased ileal fluid load, possibly in combination with a reduced ileal transit time, might hydrodynamically reduce bile acid reuptake despite a normal active bile acid absorption capacity.

5.7 The effect of budesonide in collagenous colitis

Probably, the pathophysiology of bile acid malabsorption is different in idiopathic bile acid malabsorption (type II) compared to type III.

Patients with collagenous colitis often have bile acid malabsorption and they also respond to resin treatment [17]. When bile acid malabsorption is present, the condition can be classified as type III. In a recent study, TNF alpha could not be detected in the faecal samples of these patients [152], in contrast to patients with Crohn’s disease [153]. Inflammatory cytokines repress ASBT protein expression in Caco-2 cell cultures and in animal models [71] and ASBT is also down-regulated in Crohn’s disease [154]. However, in contrast to Crohn’s disease, in collagenous colitis there is no inflammation in the terminal ileum [17].

In collagenous colitis there is a colonic inflammation, and probably an altered reactivity of the colonic epithelium to bile acids.

In vitro experiments have shown that the predominant diarrhoea mechanism in collagenous colitis is a reduction of the net Na⁺ and Cl⁻ absorption accompanied by electrogenic chloride secretion. There is also a down-regulation of tight junctions which

contributes to the diarrhoea by leak flux mechanisms [155]. The effects of bile acids on the colonic mucosa resemble in part these mechanisms. Thus, there are data showing that bile acids induce diarrhoea by reduced absorption and by increased permeability which is suggested to be partly due to influence on the tight junctions [90].

Budesonide has well established anti-inflammatory features, most studied in Crohn's disease [114]. Direct anti-inflammatory influences on the colonic mucosa may also contribute to the benefit of budesonide in collagenous colitis.

Budesonide treatment significantly increased the $^{75}\text{SeHCAT}$ values and improved the symptom scores. It also decreased hepatic bile acid synthesis in patients with initially high synthesis rate, as determined from the serum marker C4. The results suggest that budesonide increases the intestinal active uptake of bile acids indicating that its clinical effect in collagenous colitis may partly be linked to a reduced bile acid load on the colon.

5.8 Conclusions- Bile acid induced diarrhoea

The findings of this thesis suggest that the term "idiopathic bile acid malabsorption" is not appropriate, hence there is no reduced absorption capacity in the terminal ileum of these patients. The values of an abnormal $^{75}\text{SeHCAT}$ test are inversely correlated to chloride secretion in the proximal small bowel indicating that bile acids play a role in the pathophysiology of diarrhoea. A more suitable term for this disease entity would be bile acid induced diarrhoea.

Further studies are needed to investigate the volume of the bile acid pool in bile acid induced diarrhoea and its connection to increased body mass index and westernized lifestyle.

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