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Intestinal function in cholestasis and essential fatty acid deficiency

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CHAPTER 3

Intestinal capacity to digest and absorb carbohydrates is maintained in a rat model of cholestasis

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ABSTRACT

Cholestasis is associated with systemic accumulation of bile salts and by deficiency of bile in the intestinal lumen. During the past years bile salts have been identified as signaling molecules that regulate lipid, glucose and energy metabolism. Bile salts have also been shown to activate signaling routes leading to proliferation, apoptosis or differentiation. It is unclear, however, whether cholestasis affects the constitution and absorptive capacity of the intestinal epithelium *in vivo*. We studied small intestinal morphology, proliferation, apoptosis, expression of intestine-specific genes and carbohydrate absorption in cholestatic (1 wk bile duct ligation), bile-deficient (1 wk bile diversion) and control (sham) rats. Absorptive capacity was assessed by determination of plasma ^{2}H- and ^{13}C-glucose concentrations after intraduodenal administration of ^{2}H-glucose and naturally-enriched ^{13}C-sucrose, respectively. Small intestinal morphology, proliferation, apoptosis and gene expression of intestinal transcription factors (mRNA levels of *Cdx-2*, *Gata-4* and *Hnf-1α*, and Cdx-2 protein levels) were similar in cholestatic, bile-deficient and control rats. The (unlabeled) blood glucose response after intraduodenal administration was delayed in cholestatic animals, but the absorption over 180 minutes, was quantitatively similar between the groups. Plasma concentrations of ^{2}H-glucose and ^{13}C-glucose peaked to similar extents in all groups within 7.5 min and 30 min, respectively. Absorption of ^{2}H-glucose and ^{13}C-glucose in plasma was similar in all groups. The present data indicate that neither accumulation of bile salts in the body, nor their intestinal deficiency, two characteristic features of cholestasis, affect rat small intestinal proliferation, differentiation, apoptosis, or its capacity to digest and absorb carbohydrates.

INTRODUCTION

Cholestasis is associated with accumulation of bile salts in the body and by deficiency of bile salts in the intestinal lumen [1]. Bile salts facilitate dietary lipid absorption in the intestinal lumen and contribute to cholesterol homeostasis [2]. More recently, bile salts have been identified as signaling molecules. Through activation of the farnesoid X receptor (FXR), bile salts regulate various aspects of glucose and lipid metabolism as well as intestinal barrier function [2,3]. Watanabe *et al.* described a role for bile salts in the regulation of energy metabolism via the G protein-coupled bile acid receptor (GPBAR1) [4]. Finally, bile salts can activate MAPK pathways, leading to proliferation or apoptosis [2].

In children, cholestatic liver disease negatively affects nutritional status, growth and development, which cannot be explained by solely the inability to absorb lipids and lipid-soluble vitamins [5-7]. Sofar, it has remained unclear to what extent cholestasis, i.e. systemic accumulation and intestinal deficiency of bile salts, affects small intestinal epithelial proliferation, differentiation or apoptosis and, consequently, absorptive capacity *in vivo*.

In the small intestinal lumen sucrose is hydrolyzed into glucose and fructose by the brush border membrane enzyme sucrase. Glucose is actively transported across the apical membranes of enterocytes by the sodium-dependent glucose co-transporter SGLT-1. The majority of glucose is passively transported from the enterocyte into the circulation by the facilitated glucose transporter GLUT-2 [8]. Intestinal sucrase-isomaltase (SI) gene transcription is regulated by the intestine-specific transcription factors Gata binding protein 4 (GATA-4), hepatic nuclear factor 1α (HNF-1α) and caudal type homeobox transcription factor 2 (CDX-2) [9].

In the present study, we investigated whether cholestasis affects small intestinal constitution and the absorptive capacity for carbohydrates in rats.

MATERIALS AND METHODS

Rats and housing

Male Wistar rats, weighing 270-300 g at the beginning of the study, were obtained from Harlan (Horst, The Netherlands). They were individually housed in Plexiglas cages (25 x 25 x 30 cm) on a layer of wooden shavings under controlled temperature, humidity and on a 12:12-h light-dark cycle. Water and chow diet (Hope Farms, Woerden, The Netherlands) were available ad libitum. All experiments were approved by the Animal Experiments Ethical Committee of the University of Groningen.

Materials

6,6–^{2}H-glucose, 98% ^{2}H was obtained from Isotec Inc (Miamisburg, OH). Isotopic purity was confirmed by GC-MS. Cane sugar (Caribbean Gold, Amstelveen, The Netherlands) was used as naturally-enriched ^{13}C-sucrose.

Surgery

All rats were equipped with permanent catheters in the jugular vein and duodenum, as described by Kuipers *et al.* [10]. Bile duct ligated (cholestatic; $n = 5$) and bile-diverted (bile-deficient; n=7) rats were compared to sham-operated rats (control; $n = 6$). The experimental model allows for physiological studies in unanaesthetized rats with bile duct ligation and diversion without the interference of stress or restraint. These models have been proven

useful to analyze intestinal absorption capacity [11-13]. After surgery, the rats were allowed to recover for 1 wk.

Experimental procedures
Feces were collected for 24 h, between day 5 and 6 after surgery. Cholestatic, bile-deficient and control rats were subjected to a combined ^{2}H-glucose /^{13}C-sucrose absorption test at 1 week after surgery. On the day of the experiment, the rats received an intraduodenal bolus of 1 mg ^{2}H-glucose and 0.25 g ^{13}C-sucrose in 1 ml water, after an overnight fast. At t = 0, 7.5, 15, 30, 45, 60, 90, 120 and 180 minutes, blood samples were taken for determination of blood glucose concentrations and plasma ^{2}H- and ^{13}C-glucose enrichments. At the end of the experiment, the rats were sacrificed and the small intestine was collected for analysis. The small intestine was flushed with ice-cold PBS and was divided into the duodenum, the jejunum and the ileum. Material was harvested for histology and gene expression. Small intestinal mucosa was scraped for the determination of enzyme activity.

Analytical methods
Plasma bile salts were determined as described previously [14]. Fat ingestion, fecal fat excretion and net fat absorption were measured and calculated as described previously [11].

Stable isotope test
Blood glucose concentrations were measured with a Lifescan EuroFlash glucose meter (Lifescan Benelux, Beerse, Belgium). The sample preparation procedure of plasma ^{2}H-glucose enrichment and plasma ^{13}C-glucose enrichment was as described by Vonk *et al.* [15]. The ^{2}H enrichment was measured by GC-MS (Trace MS, Interscience, Breda, The Netherlands) [16]. The ^{13}C/^{12}C isotope ratio measurement of the glucose penta-acetate derivative was determined by Gas Chromatography/Combustion/Isotope Ratio Mass Spectrometry (GC-C-IRMS) using a Delta Plus instrument (Thermofinnigan, Bremen, Germany) [15]. Concentrations were calculated as described by Vonk *et al.* [16].

Disaccharidase activity
Enzyme activity levels of sucrase were measured in freshly scraped intestinal mucosa as described by Dahlqvist [17]. Activity levels were normalized to protein levels, measured by the BCA method as described by the manufacturer (Pierce, Rockford, IL).

RNA isolation and measurement of mRNA levels by real-time PCR (Taqman)
mRNA expression levels in duodenum, jejunum and ileum were measured by real-time PCR, as described previously [18]. PCR results were normalized to β-actin mRNA levels. The sequences of the primers and probes are listed in Table 1.

Histology
Morphology of jejunal sections of the small intestine was assessed by haematoxylin and eosin staining of formaline-fixated material. Proliferating cells were detected by staining of nuclear Ki-67 antigen. Apoptosis was assessed by staining DNA strand breaks with TUNEL staining according to the manufacturer's instructions (In Situ Cell Death Detection Kit, Roche, Mannheim, Germany). Crypt and villous length were determined by morphometric procedures.

Table 1. Primer and probe sequences.

Gene	GenBank	Forward Primer	Reversed Primer	TaqMan® probe
β-actin	NM_031144	AGC CAT GTA CGT AGC CAT CCA	TCT CCG GAG TCC ATC ACA ATG	TGT CCC TGT ATG CCT CTG GTC GTA CCA C
Asbt	NM_017222	ACC ACT TGC TCC ACA CTG CTT	CGT TCC TGA GTC AAC CCA CAT	CTT GGA ATG ATG CCC CTT TGC CTC T
Ibabp	NM_017098.1	CCC CAA CTA TCA CCA GAC TTC G	ACA TCC CCG ATG GTG GAG AT	TCC ACC AAC TTG TCA CCC ACG ACC T
Shp	NM_057133	ACC TGC AAC AGG AGG CTC ACT	TGG AAG CCA TGA GGA GGA TTC	TCC TGG AGC CCT GGT ACC CAG CTA GC
Gpbar1	NM_177936.1	ACT GGT CCT GCC TCC TTC TC	GCT GCA ACA CTG CCA TGT AG	TCC CTG CTT GCC AAT CTG CTG CT
Hnf-1α	NM_012669	CTC CAG CAG CCT GGT GTT GT	GAG GCC ATC TGG GTG GAG AT	CAC AGC CAC CTG CTG CCA TCC AAC
Gata-4	NM_144730.1	GAG ATG CGC CCC ATC AAG	GAC ACA GTA CTG AAT GTC TGG GAC AT	CTG TCA TCT CAC TAT GGG CAC AGC AGC TC
Cdx-2	NM_023963	GTC CCT AGG AAG CCA AGT GAA A	CTC CTG ATG GTG ATG TAT CGA CTA	CCT TCT CCA GCT CCA GCC GCT G
Sglt-1	NM_013033	GCT GGA GTC TAC GTA ACA GCA CA	GGG CTT CTG CAT CTA TTT CAA TG	TCC TCC TCT CCT GCA TCC AGG TCG
Glut-2	NM_012879	GCA TCA GCC AGC CTG TGT ATG	GCA GCA CAG AGA CAG CTG TGA	CCA TCG GCG TTG GTG CCA TCA AC
Si	NM_013061	TGT TTG GGT GAA TGA GTC AGA TG	CCC ACC ACT CGA TGG TTT G	ACT GTT AAT CCT GGC CAT ACC TCT CCA ATA A

Statistical analysis

Values represent means ± SE for the indicated number of rats per group. Using SPSS version 12.0.2 statistical software (Chicago, IL, USA), we calculated significance of differences. Significance of differences was calculated with regard to the treatments as well as the different intestinal segments. Body weight and food intake were normally distributed and tested with the One-Way ANOVA. When the One-Way ANOVA resulted in a significant difference among the groups ($P < 0.05$) the two-tailed T-Test was used to calculate differences among the treatments or intestinal segments separately. All other parameters were not normally distributed and therefore tested with Kruskal-Wallis H and subsequently with the Mann-Whitney U for differences among the treatment groups or intestinal segments when a significant difference ($P < 0.05$) was observed.

RESULTS

Characterization of the model

Plasma bile salt concentration was significantly higher in cholestatic rats compared to control and bile-deficient rats (187 ± 20 vs. 10 ± 4 and 1.0 ± 0.5 μmol/L, respectively), and significantly lower in bile-deficient rats compared to control rats (all, $P < 0.01$).

In accordance with the localization of intestinal bile salt reabsorption, expression of the apical sodium-dependent bile acid transporter (*Asbt*) and the ileal bile acid binding protein (*Ibabp*) was restricted to the ileum [19,20]. *Asbt* expression was not quantitatively changed in cholestatic or bile-deficient rats, while *Ibabp* expression was reduced in cholestatic rats (by ~35%) and significantly reduced in bile-deficient rats compared to control rats (by ~60%; $P < 0.05$, Fig 1). Expression of the short heterodimer partner (*Shp*) was similar in all three segments and significantly reduced in cholestatic and bile-deficient rats in the duodenum by ~60% and ~45% (both $P < 0.05$), in the jejunum by ~75% and ~70% (both $P < 0.01$) and in the ileum both by ~95% (both $P < 0.01$, Fig 1). Expression of *Gpbar1* was similar in duodenum, jejunum and ileum and was not affected by cholestasis or bile diversion (Fig 1).

After surgery the body weight decreased in all groups, but to a greater extent in cholestatic and bile-deficient rats compared to the sham-operated controls (93.7 ± 0.6 and 92.7 ± 0.7 vs. 97.5 ± 0.6% of initial weight at day 2 after surgery; both $P < 0.01$). Body weight of control rats remained stable over the experimental period, while body weight of bile-deficient rats

increased over time to the level of control rats at day 7. Body weight of the cholestatic rats remained stable over the experimental period and was significantly lower than body weight of control rats over the entire experimental period (approximately -4%, $P < 0.05$). Average food intake during the experimental period was similar in control and cholestatic rats (4.5 ± 0.2 and 4.1 ± 0.1 % of body weight). Average food intake of bile-deficient rats was significantly higher than that of cholestatic rats (4.9 ± 0.1 vs. 4.1 ± 0.1 % of body weight, $P < 0.01$) [10].

Fat balance was measured from day 5 to 6 after surgery (Fig 1). Fat ingestion was slightly, but not significantly higher in bile-deficient rats compared to control rats, as previously found [11]. Fecal fat excretion was significantly higher in cholestatic and bile-deficient rats compared to control rats (1.2 ± 0.1 and 1.2 ± 0.1 vs. 0.2 ± 0.0 mmol fatty acids/day, respectively, each $P < 0.01$). The resulting net fat absorption was significantly lower in cholestatic rats compared to control rats (2.9 ± 0.1 vs. 3.7 ± 0.2 mmol/day, respectively, $P < 0.05$), while net fat absorption in bile-deficient rats was not significantly different from the control rats. Cholestatic and bile-deficient rats had significantly lower coefficients of fat absorption than control rats (71.8 ± 0.5** and 73.1 ± 0.9** vs. 93.9 ± 0.2 % of ingested amount, respectively, each $P < 0.01$).

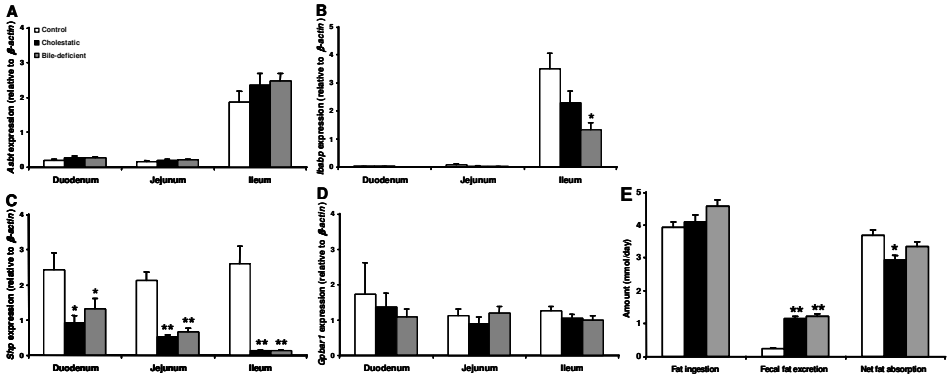

Figure 1. Gene expression of intestinal bile salt transporters/receptors and fat balance in control (white bars), cholestatic (black bars) and bile-deficient (grey bars) rats. (A) Duodenal, jejunal and ileal *Asbt* expression, (B) *Ibabp* expression, (C) *Shp* expression, and (D) *Gpbar1* expression, all normalized to *β-actin* levels. (E) Fat balance, measured from day 5 to 6 after surgery. Data represent means ± SE of 5-7 rats per group. *$P < 0.05$ and **$P < 0.01$ vs. control group.

Jejunal morphology, proliferation and apoptosis are not affected in cholestatic rats

Jejunal sections of control, cholestatic and bile-deficient rat intestines were stained with HE, Ki-67 and TUNEL to assess morphology, proliferation and apoptosis, respectively (Fig 2). Villous and crypt morphology, proliferation and apoptosis did not differ between the groups. Villous length was similar in control, cholestatic and bile-deficient rats (394 ± 36, 445 ± 26 and 510 ± 27 µm, respectively, Table 2), however, villous length in bile-deficient rats showed a trend towards being higher than villous length in control rats (Kruskal-Wallis H: $P = 0.064$ and Mann-Whitney U of bile-deficient rats vs. control rats: $P = 0.032$) [12]. Crypt/villous ratios were similar in control, cholestatic and bile-deficient rats (0.29 ± 0.02, 0.30 ± 0.02 and 0.28 ± 0.00, respectively, Table 2).

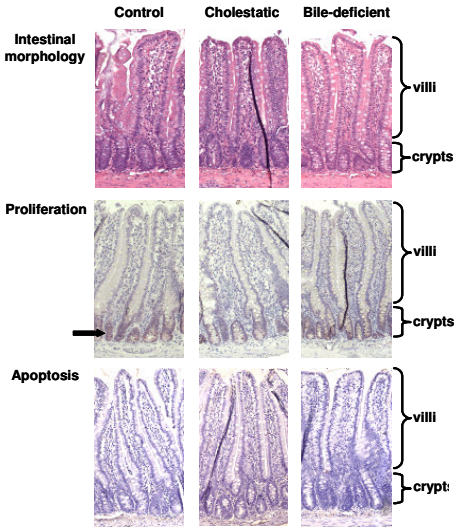

Table 2. Villus length and crypt-to-villus ratios of control, cholestatic and bile-deficient rats.

Group	Villus length (μm)	Crypt-to-villus length ratio
Control	394 ± 36	0.29 ± 0.02
Cholestatic	445 ± 26	0.30 ± 0.02
Bile-deficient	510 ± 27	0.28 ± 0.00

Figure 2. Jejunal morphology, proliferation and apoptosis in control, cholestatic and bile-deficient rats. Intestinal morphology was assessed by HE staining, proliferation by Ki-67 staining and apoptosis by TUNEL staining. Crypts and villi are indicated in the pictures. Cells positively stained with Ki-67 are indicated by the arrow.

Expression of intestinal transcription factors is maintained in cholestatic rats

The expression of intestine-specific transcription factors was determined to assess effects of the experimental manipulations on enterocyte differentiation. CDX-2, HNF-1α and GATA-4 are known to cooperatively regulate sucrase-isomaltase gene transcription [9]. *Hnf-1α* expression was similar in duodenum, jejunum and ileum, while *Gata-4* expression was significantly lower in the ileum compared to the duodenum and jejunum (both $P < 0.01$; Fig 3) [21] No significant differences in *Hnf-1α* or *Gata-4* expression were found among the groups. *Cdx-2* expression increased slightly from the proximal to the distal part of the intestine of control rats (NS; Fig 3). No significant differences were found in duodenum, jejunum and ileum among all groups. Cdx-2 protein expression was similar in all groups (Fig 3).

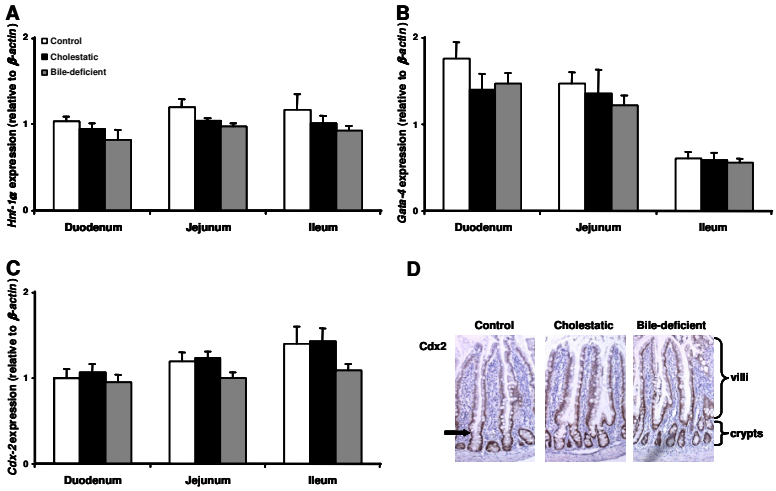

Figure 3. Expression of intestine-specific transcription factors in control (white bars), cholestatic (black bars) and bile-deficient (grey bars) rats. (A) Duodenal, jejunal and ileal expression of *Hnf-1α*, (B) *Gata-4*, and (C) *Cdx-2*, all normalized to *β-actin* levels. (D) Jejunal Cdx-2 protein expression. Cells positively stained with α-Cdx-2 are indicated by the arrow. Data represent means ± SE of 5-7 rats per group. *Gata-4* expression was higher in the duodenum and jejunum compared to the ileum ($P < 0.01$).

Blood glucose response is delayed in cholestatic rats

The blood glucose concentration in response to the intraduodenal bolus of labeled glucose and sucrose was delayed in cholestatic rats compared to bile-deficient and control rats and peaked at 15 min rather than at 7.5 min, respectively. Figure 4 shows significantly higher glucose concentrations in cholestatic rats compared to control and bile-deficient rats at 30 and 45 min after bolus administration. The area under the curve was not significantly changed in cholestatic rats compared to control and bile-deficient rats (1108 ± 51 vs. 1050 ± 40 and 1024 ± 28 mmol/L*min, respectively, NS).

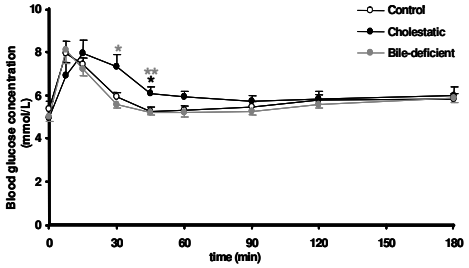

Figure 4. Blood glucose concentration in response to a 1 ml intraduodenal bolus containing ^{13}C-sucrose (0.25 g) and ^{2}H-glucose (1 mg) in control (white circles), cholestatic (black circles) and bile-deficient (grey circles) rats. Data represent means ± SE of 5-7 rats per group. *P < 0.05 vs. control group. $^{*}P$ < 0.05 and $^{**}P$ < 0.01 cholestatic vs. bile-deficient group.

Monomeric glucose absorption is maintained in cholestatic rats

Monomeric glucose absorption was assessed by determination of plasma appearance of ^{2}H-glucose after its intraduodenal administration. Plasma ^{2}H-glucose concentrations peaked at 7.5 min in control, cholestatic and bile-deficient rats (38 ± 4, 39 ± 6 and 41 ± 3 µmol/L, respectively; Fig 5). Areas under the curve were similar in cholestatic, control and bile-deficient rats (2220 ± 363, 1956 ± 315, and 1763 ± 222 µmol/L*min, respectively, NS).

In all groups, expression of the apical glucose transporter *Sglt-1* and that of the basolateral glucose transporter *Glut-2* were slightly lower in the ileum compared to the duodenum and jejunum (all $P < 0.05$; Fig 5). Jejunal *Sglt-1* expression was significantly increased in bile-deficient rats compared to control rats ($P < 0.01$). *Glut-2* expression was not significantly different between the three groups.

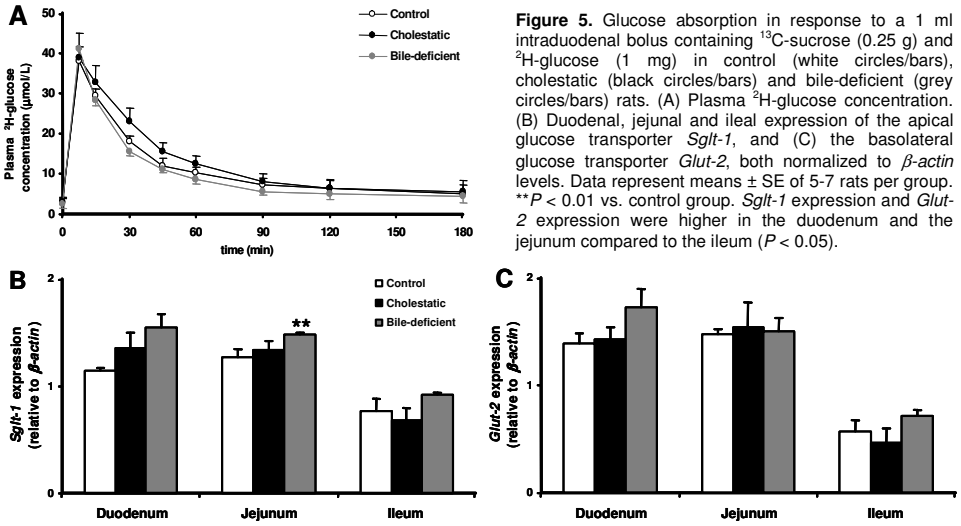

Figure 5. Glucose absorption in response to a 1 ml intraduodenal bolus containing ^{13}C-sucrose (0.25 g) and ^{2}H-glucose (1 mg) in control (white circles/bars), cholestatic (black circles/bars) and bile-deficient (grey circles/bars) rats. (A) Plasma ^{2}H-glucose concentration. (B) Duodenal, jejunal and ileal expression of the apical glucose transporter *Sglt-1*, and (C) the basolateral glucose transporter *Glut-2*, both normalized to *β-actin* levels. Data represent means ± SE of 5-7 rats per group. $^{**}P$ < 0.01 vs. control group. *Sglt-1* expression and *Glut-2* expression were higher in the duodenum and the jejunum compared to the ileum (P < 0.05).

Sucrose digestion is maintained in cholestatic rats

Sucrose digestion was assessed by appearance of plasma ^{13}C-glucose derived from ^{13}C-sucrose. Plasma ^{13}C-glucose concentrations peaked at 30 min in control, cholestatic and bile-deficient rats (3.4 ± 0.2, 4.1 ± 0.5 and 3.2 ± 0.3 mmol/L, respectively; Fig 6). Area under the curve was not significantly changed in cholestatic rats, when compared to control and bile-deficient rats (346 ± 42 vs. 312 ± 17 and 266 ± 21 mmol/L*min, respectively, NS). Sucrase enzyme activity was highest in the jejunum and lowest in the ileum in control rats (all $P < 0.01$; Fig 6). Duodenal sucrase enzyme activity was significantly lower in bile-deficient rats compared to control rats (6.9 ± 1.0 vs. 8.3 ± 0.8 μmol/mg protein.h, respectively, $P < 0.05$). The expression pattern of *sucrase-isomaltase (Si)* was less pronounced than that of sucrase enzyme activity, but jejunal expression was also significantly higher than ileal expression ($P < 0.05$). No differences were found among the groups.

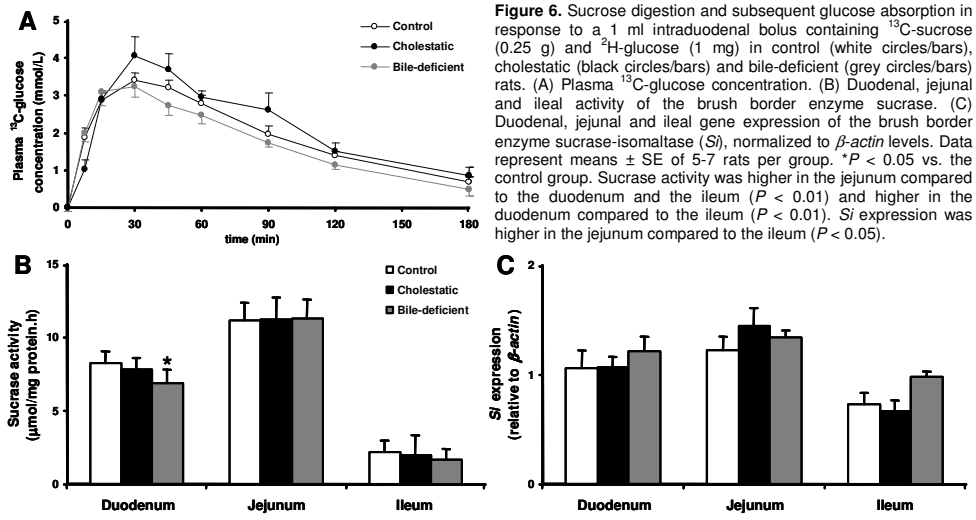

Figure 6. Sucrose digestion and subsequent glucose absorption in response to a 1 ml intraduodenal bolus containing ^{13}C-sucrose (0.25 g) and ^{2}H-glucose (1 mg) in control (white circles/bars), cholestatic (black circles/bars) and bile-deficient (grey circles/bars) rats. (A) Plasma ^{13}C-glucose concentration. (B) Duodenal, jejunal and ileal activity of the brush border enzyme sucrase. (C) Duodenal, jejunal and ileal gene expression of the brush border enzyme sucrase-isomaltase (*Si*), normalized to *β-actin* levels. Data represent means ± SE of 5-7 rats per group. *$P < 0.05$ vs. the control group. Sucrase activity was higher in the jejunum compared to the duodenum and the ileum ($P < 0.01$) and higher in the duodenum compared to the ileum ($P < 0.01$). *Si* expression was higher in the jejunum compared to the ileum ($P < 0.05$).

DISCUSSION

In this study, we investigated whether cholestasis; i.e. the combination of systemic accumulation of bile salts and the deficiency of bile salts in the intestinal lumen, affects the constitution and absorptive capacity of the rat small intestinal epithelium. We compared cholestatic rats with bile-deficient rats, without systemic accumulation, and with control rats. We found that short-term extrahepatic cholestasis in rats does not affect intestinal morphology, proliferation or apoptosis, nor the functional capacity of the intestine to digest sucrose and to absorb glucose.

Several studies describe the effect of the absence of bile components in the intestinal lumen on intestinal bile salt transporter expression in rats. They report that rat Asbt expression is not regulated by bile salts, while rat Ibabp expression is positively regulated by bile salts via Fxr activation [19,22-27]. In accordance with the literature, our data indicate that *Asbt* expression is not affected in cholestatic and bile-deficient rats. *Ibabp* expression was decreased in cholestatic rats and significantly decreased in bile-deficient rats. The increased expression of *Ibabp* in cholestatic rats compared to bile-deficient rats may be explained by the occurrence of retrograde transport of bile salts from the blood compartment into the

epithelial layer of the small intestinal lumen. However, expression of bile salt-sensitive *Shp* was markedly decreased in both cholestatic and bile-deficient rats. The strongest reduction was observed in the ileum, coinciding with the highest Fxr expression [28]. The bile duct-ligated rats had strongly elevated plasma bile salt levels and significantly reduced net fat absorption, in accordance with cholestasis. As previously described [10], cholestatic rats lost slightly more weight than bile-deficient and control rats, despite similar food intakes. The weight loss may be related to the decreased net fat absorption in cholestatic rats [13], compared with unchanged net fat absorption in bile-deficient rats fed a chow diet [11]. It is well-known that the composition of chow is variable between batches. Therefore we used only one batch to feed all rats before and during our experiments. Usage of a purified diet, such as AIN-93M, is not possible in this experimental setting, because the cornstarch in the diet is naturally enriched in ^{13}C as is the sucrose (cane sugar) we used to assess sucrose digestion. Pilot experiments showed that the enrichment of ^{13}C-glucose in the plasma is immeasurable, due to the high baseline enrichment in rats fed a diet containing cornstarch (unpublished observations).

In light of numerous *in vitro* data in the literature, we anticipated cholestasis to induce either proliferation or apoptosis in the small intestinal epithelium. Conjugated bile salts in concentrations found during cholestasis induce proliferation in the rat small intestinal cell line IEC-6, and in the human colon carcinoma cell line Caco-2, which gains small intestinal epithelial features upon differentiation [29-31]. In contrast to enterocytes, relatively low concentrations (50 µM) of conjugated bile salts induce apoptosis in human hepatoma cell lines and primary rat hepatocytes [32-34]. Bile salts in cholestatic concentrations can also be indirectly implicated in inhibition of differentiation and function of small intestinal epithelial cells. Suh and Traber demonstrated that the intestine-specific transcription factor Cdx-2 is an important regulator of differentiation in the small intestine [35]. Differentiation is regulated by phosphorylation and subsequent activation of Cdx-2 via the MAPK/ERK route. Phosphorylated Cdx2 inhibits transcription of the sucrase-isomaltase gene [36]. MAPK/ERK, in turn, appears to be activated by various bile salts [37]. However, our present results indicate that cholestatic concentrations of bile salts do not affect proliferation, apoptosis or differentiation in rat small intestinal epithelium *in vivo*. The discrepancy between the *in vitro* and *in vivo* data can possibly be explained by the fact that enterocytes might be more resistant in an *in vivo* setting, related to protective environmental factors. Besides, the absorption of nutrients and of bile salts takes place in different intestinal segments. In cholestatic rat models, enterocytes are exposed to bile salt concentrations only at their basolateral membrane, while intestinal cell lines are exposed to both sides or are not (completely) polarized. Bile salts do not need to enter the cells to activate cellular signaling routes. Kawamata *et al.* reported that membrane-type G protein coupled receptor Gpbar1 is expressed in the intestine [38]. In our rats, *Gpbar1* was expressed at similar amounts in duodenum, jejunum and ileum. It is unknown whether Gpbar1 is expressed at apical or basolateral membrane domains. Finally, several *in vitro* studies have addressed intestinal cell exposure to high concentrations of unconjugated bile salts in the context of colon cancer. Small intestinal cells *in vivo* are, however, normally not exposed to free bile salts in those concentrations and compositions.

Data addressing to what extent cholestasis or intestinal bile deficiency affect small intestinal function *in vivo* is scarce. In accordance with our current results several rat models of cholestasis or intestinal bile deficiency have been reported to have unchanged small intestinal function. Borges *et al.* reported that obstructive jaundice did not affect jejunal

absorption of glucose in rats [39]. Sucrase enzyme activity was shown to be unaffected in cholestyramine-fed and bile-diverted rats [40]. Corresponding with unaffected or minimally affected epithelial integrity, we have previously shown that fat absorption in 1 wk bile-diverted rats could be quantitatively reconstituted within hours using the infusion of model bile [41].

Vonk *et al.* developed a stable isotope test for the quantification of lactose digestion and glucose absorption in humans [15,16]. We adapted this test to quantify intestinal digestion and absorption of sucrose and glucose in rats. In the future, this test might be utilized or adapted to quantify the effect of other clinical conditions on intestinal absorption of carbohydrates or other macronutrients.

Malnutrition in children with cholestasis has a profound effect on mortality rate of children with end-stage cholestatic liver disease [5,7,42]. The association between cholestatic liver disease and fat malabsorption is well-known. Previous studies in our group have demonstrated that cholestatic rats and bile-deficient rats have severe fat malabsorption [11-13], as confirmed in the present study. Rings *et al.* showed that the absorption of free fatty acids but not fat digestion was rate-limiting for overall fat absorption in neonates that are known to have a mild 'physiological' cholestasis during the first months of life [43]. Our data indicate that intestinal deficiency of bile salts, with or without systemic accumulation of bile salts, does not impair intestinal carbohydrate digestion or absorption in rats. These observations suggest that increasing the dietary carbohydrate intake above conventional levels could be used in preventive or therapeutic fashion for a poor nutritional status induced by fat malabsorption in cholestatic children.

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