Large intestine permeability is increased in patients with compensated liver cirrhosis

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Submitted 27 September 2013; accepted in final form 16 November 2013

Pijls KE, Koek GH, Elamin EE, de Vries H, Mascllee AA, Jonkers DM. Large intestine permeability is increased in patients with compensated liver cirrhosis. Am J Physiol Gastrointest Liver Physiol 306: G147–G153, 2014. First published November 21, 2013; doi:10.1152/ajpgi.00330.2013.—Intestinal barrier dysfunction, facilitating translocation of bacteria and bacterial products, plays an important role in the pathophysiology of liver cirrhosis and its complications. Increased intestinal permeability has been found in patients with liver cirrhosis, but data on small and large intestine permeability and tight junctions (TJs) in patients with compensated cirrhosis are scarce. We aimed to investigate both small and large intestine permeability in patients with stable compensated cirrhosis compared with healthy controls and evaluated the expression of TJ proteins in mucosal biopsies at duodenal and sigmoid level. Intestinal permeability was assessed in 26 patients with compensated cirrhosis and 27 matched controls using a multisugar test. Duodenal and sigmoid biopsies were available from a subgroup for analyses of gene transcription and expression of key TJ proteins by qRT-PCR and ELISA, respectively. Median 0–5-h urinary sucrose excretion and lactulose/rhamnose ratio were comparable between patients with compensated cirrhosis and controls, whereas 5–24-h urinary sucralose/erythritol ratio was increased in these patients. Downregulation of gene transcription was found for claudin-3 in duodenal biopsies and claudin-4 in sigmoid biopsies, and at the protein level occludin expression was significantly increased in both duodenal and sigmoid biopsies. This study shows that gastroduodenal and small intestine permeability are not altered, whereas large intestine permeability is increased in patients with stable compensated cirrhosis. Only limited alterations were found regarding the expression of TJ proteins in both the small and large intestine.

Liver cirrhosis; complications; intestinal epithelial barrier; permeability; tight junctions

Translocation of bacteria or bacterial products from the intestinal lumen into the systemic circulation is considered to play an important role in the development of liver cirrhosis and its complications (29, 40). Factors facilitating bacterial translocation are alterations in the composition of intestinal microbiota, the immune system, and disturbances of the intestinal epithelial barrier (5, 41).

Epithelial integrity is essential to prevent the entry of potentially harmful luminal substances, e.g., bacteria and their products, into the systemic circulation by means of transcellular and paracellular pathways. Although apart from an important role for transcellular bacterial translocation and involvement of a well-functioning chemical barrier (e.g., mucus and defensin secretion) and immune system, an increased paracellular permeability will enhance bacterial translocation. The paracellular pathway is regulated by the tight junctions (TJs), which are components of the apical junctional complex that seal the intercellular spaces (36). The TJs are composed of transmembrane proteins, such as occludin and members of the claudin family, and cytoplasmic plaque proteins, including the zonula occludens proteins (i.e., ZO-1, ZO-2, and ZO-3), that link the transmembrane proteins to the actin cytoskeleton (36, 37).

Previous studies have pointed to an increased small and even whole intestine permeability in patients with compensated and/or decompensated cirrhosis compared with healthy controls (6, 9, 11, 16), and it seems to be most pronounced in decompensated cirrhosis (27, 33). It is not completely clear whether the intestinal permeability is also increased in patients with compensated cirrhosis and thereby might be a risk factor for the progression toward decompensated cirrhosis. Studies on intestinal permeability have primarily focused on functional tests by measuring urinary excretion of orally administered test markers. Only two studies with contrasting results have assessed the expression of TJ proteins in duodenal biopsies of patients with cirrhosis (1, 13). Although recent evidence points toward a role of the microbiota and the damaging effects of alcohol in the large intestine (2, 14) in the pathophysiology of liver cirrhosis, studies investigating the epithelial integrity of the large intestine are scarce. One study, using a single test marker, found an increased large intestine permeability in patients with cirrhosis (25).

The aim of the present study was therefore to investigate intestinal permeability, not only of the small intestine, but also of the large intestine in patients with compensated cirrhosis and compare data with those obtained in healthy controls. Permeability was studied by using a multisugar test as well as by the expression of TJ proteins in mucosal biopsies of duodenum and sigmoid.

Materials and Methods

The study has been approved by the Medical Ethics Committee of Maastricht University Medical Center (MUMC), conducted according to the revised version of the Declaration of Helsinki (October 2008, Seoul) and registered at the US National Library of Medicine (http://clinicaltrials.gov, NCT01081236). All subjects (patients and healthy volunteers) gave their written, informed consent before participation.

Patients. Patients with stable compensated liver cirrhosis aged between 18 and 75 yr were recruited from the outpatient clinic of our department. A diagnosis of cirrhosis was based on liver histology and/or clinical, laboratory, radiological, and/or endoscopic findings. Compensated cirrhosis was defined by the absence of clinically evident complications, i.e., ascites, variceal hemorrhage, hepatic en-
cerebral oedema, and/or jaundice. Exclusion criteria were malignancy, other chronic gastrointestinal diseases, renal failure, diabetes mellitus requiring insulin therapy, and major abdominal surgery interfering with gastrointestinal function. In the included patients, severity of cirrhosis was assessed according to the Child–Pugh classification and the Model for End-Stage Liver Disease score. Alcohol consumption was assessed by questionnaire and interview. Portal hypertension was based on the previous or current presence of esophageal, gastric, and/or anorectal varices during endoscopy and/or on the previous or current presence of splenomegaly, collaterals, and/or abnormalities of portal blood flow during abdominal ultrasound with Doppler.

Healthy volunteers were recruited by local advertisement and considered eligible for inclusion in the control group when they had a normal medical history, physical examination, and liver tests (i.e., alanine transaminase (ALT) <45 U/l and γ-glutamyl transpeptidase (GGT) <55 U/l for men, and ALT <35 U/l and GGT <40 U/l for women) and were matched for age, sex, and body mass index (BMI). Exclusion criteria included a history of gastrointestinal and/or liver diseases, major abdominal surgery interfering with gastrointestinal function, a history of alcohol abuse or excessive alcohol consumption (i.e., >2 alcoholic beverages per day or >14 per wk), use of medication that could affect intestinal permeability (e.g., nonsteroidal anti-inflammatory drugs (NSAIDs) and proton pump inhibitors (PPIs)), or intestinal microbiota in the 2 mo before the study.

Study design. This prospective case-control study consisted of two separate test days within a 4-wk period. All subjects were requested to abstain from alcohol consumption on the day before their test day. After an overnight fast, subjects received an oral multisugar drink to assess intestinal permeability, consisting of 1 g sucrose (Van Gils, Dinteloord, the Netherlands), 1 g lactulose (Centrafarm, Etten-Leur, the Netherlands), 0.5 g L-rhamnose (Danisco Sweeteners, Copenhagen, Denmark), 1 g erythritol (Danisco), and 1 g sucralose (Brenntag, Sittard, the Netherlands) dissolved in 150 ml of tap water. Urine was collected in plastic containers for 24 h in two separate fractions: 0–5 h and 5–24 h. No eating or drinking (except tap water) was allowed during the first 5-h urine collection. Before and after collection, weight of the containers was recorded, and aliquots of each fraction were stored at −80°C until further analysis.

On the other test day, a gastroduodenoscopy and/or sigmoidoscopy was performed after an overnight fast but without prior bowel cleansing. Mucosal biopsies were obtained from standardized locations: the second segment of the duodenum and the sigmoid (20 cm from the anal sphincter, respectively). Biopsies for gene transcription and protein expression were snap-frozen in liquid nitrogen and stored at −80°C until further analyses. Biopsies for histological evaluation of hematoxylin and eosin-stained sections by one experienced pathologist were fixed in 4% formaldehyde and embedded separately in paraffin.

Assessment of intestinal permeability. Intestinal permeability was assessed using a validated multisugar test (38, 39). Urine sugar concentrations were determined by isotopic ion-exchange high-pressure liquid chromatography (Model PU-1980 pump; Jasco, Easton, MD) with mass spectrometry (Model LTQ XL; Thermo Fisher Scientific, Waltham, MA) as described by van Wijck et al. (38). Sucrose in 0–5 h urine, lactulose/rhamnose (L/R) ratio in 0–5 h urine, and sucralose/erythritol (S/E) ratio in 5–24 h urine were used as indicators for gastroduodenal, small and large intestine permeability, respectively.

qRT-PCR. Gene transcription of TJ and associated proteins, i.e., occludin, claudin-3, claudin-4, ZO-1, and myosin light chain kinase (MLCK), in mucosal biopsies was determined by qRT-PCR. Total RNA was isolated from the frozen biopsies using TRIzol reagent (Invitrogen, Carlsbad, CA). RNA was purified with the RNeasy Mini Kit (Qiagen, Venlo, the Netherlands). The concentration of the purified RNA was measured with the NanoDrop. Finally, 500 ng of total RNA was used as a template for the cDNA reaction, which was synthesized using the iScript cDNA Synthesis Kit (Bio-Rad, Veenendaal, the Netherlands). The cDNA was diluted to a concentration of 4 ng/μl. Each reaction contained 5 μl cDNA template solution, 12.5 μl iQ SYBR Green Supermix (Bio-Rad), 1 μl forward and reverse primers (10 μM), and 5.5 μl sterile water. Primer sequences have been listed in Table 1. Housekeeping genes included were 18S rRNA and GAPDH. Reactions were run on the My IQ Single Color RT-PCR Detection System (Bio-Rad). PCR conditions used were 3 min at 95°C; followed by 40 amplification cycles of 10 s at 95°C and 45 s at 60°C. Data were presented as normalized expression ratios.

ELISA. Protein levels of occludin and claudin-4 were determined in mucosal biopsies of duodenum and sigmoid. Frozen biopsies were ground with a mortar and pestle cooled in liquid nitrogen and resuspended in 100 μl of ice-cold PBS containing 10 μl/ml Protease Inhibitor Cocktail (Sigma-Aldrich, Zwijndrecht, the Netherlands). After centrifugation for 20 min (10,000 revolution/min, 4°C), the supernatant was stored at −80°C until further analysis. Total protein content in the supernatant was quantified using a BCA Protein Assay Kit (Pierce, Rockford, IL). Occludin and claudin-4 in supernatant were determined by ELISA. In short, 96-well plates were coated overnight (4°C) with 50 μl of tissue supernatant (1:10 dilution in PBS containing 10 μg/ml protein) or negative control (PBS). Thereafter, 50 μl of blocking agent (3% wt/vol BSA in PBS) was added for 1 h at 37°C. The plate was washed, and then the wells were incubated with primary antibodies, polyclonal rabbit anti-occludin, or monoclonal mouse anti-claudin-4 (1:100 dilution in PBS containing 3% BSA) (Invitrogen, Bleiswijk, the Netherlands) for 1 h at room temperature. The plates were washed, followed by the addition of secondary antibodies, horseradish peroxidase (HRP)-conjugated anti-rabbit IgG (RayBiotech, Heerhugowaard, the Netherlands), and HRP-conjugated anti-mouse IgG (Dako, Heverlee, Belgium) (1:1000 dilution in Assay Diluent, RayBiotech), and incubated for 2 h at room temperature. After being washed, TMB One-Step Substrate Reagent (RayBiotech) was added and incubated for 30 min in the dark to allow color development. The reaction was stopped by the addition of Stop Solution (RayBiotech), and absorbance of the color was measured at 450 nm by the Synergy HT Multi-Detection Microplate Reader (Bio-Tek, Abcoude, the Netherlands). Data were expressed as optical density (OD) read at 450 nm.

Power calculation and statistical analysis. The primary outcome parameter of the study was small intestine permeability. The number of subjects was determined using PS Power and Sample Size Calculations.
lations, based on previous studies in patients with compensated and decompensated liver cirrhosis (42) and in healthy subjects using indomethacin as a stressor to increase small intestine permeability (35). For the present study, 25 subjects were required per group to achieve an 80% power for detection of a statistically significant difference between both groups \((P < 0.05)\) with \(\alpha = 0.05, \delta = 0.017,\) and \(\sigma = 0.021.\)

Statistical analyses were performed using SPSS version 20.0. Data were tested for normality by using the Kolmogorov-Smirnoff test. Subsequently, continuous variables were presented as medians (range) and compared between groups using the Mann-Whitney \(U\)-test for nonparametric data. Dichotomous variables were compared with the \(\chi^2\) test. A \(P\) value <0.05 was considered statistically significant using a two-tailed test.

**RESULTS**

**Patients.** Twenty-six patients with compensated cirrhosis and 27 controls were included. Characteristics of subjects are given in Table 2. No significant differences with regard to age, sex, or BMI were observed between patients with cirrhosis and controls. Serum ALT and GGT levels were significantly higher in patients with cirrhosis compared with controls \((P = 0.025\) and \(P < 0.001,\) respectively). Cause of liver cirrhosis was alcohol related in 11 patients, autoimmune related in 6 patients, cryptogenic in 5 patients, chronic viral infection in 1 patient, hereditary hemochromatosis in 1 patient, and multifactorial in 2 patients. The majority of patients with alcohol-related cirrhosis \((n = 9)\) was completely abstinent. Only two patients consumed alcohol on a regular basis but not on the day before the test day. Signs of portal hypertension were present in 24 of the 26 patients. Furthermore, 17 and 9 patients were classified as Child-Pugh class A and B, respectively.

Drug therapy as part of the standard medical care was given to all patients, including, among others, PPIs \((n = 15),\) \(\beta\)-blockers \((n = 12),\) ursodeoxycholic acid \((n = 11),\) diuretics \((n = 7),\) lactulose \((n = 6),\) glucocorticosteroids/immunosuppressives \((n = 4),\) antibiotic prophylaxis \((n = 1),\) and antiarrheal agents \((n = 1),\) and NSAIDs \((n = 1).\)

**Gastroduodenal, small and large intestine permeability.** Gastroduodenal permeability, reflected by the 0–5-h urinary sucrose excretion, was not different between patients with cirrhosis and controls \([5.979 (0.547–24.880)\) vs. \(6.265 (2.226–30.469)\) \(\mu\)mol; \(P = 0.881;\) Fig. 1A]. The 0–5-h urinary L/R ratio, indicating small intestine permeability, was also similar in both groups \([0.023 (0.010–0.115)\) vs. \(0.023 (0.006–0.069);\) \(P = 0.455;\) Fig. 1B]. However, large intestine permeability was significantly increased in patients with cirrhosis compared with controls, as reflected by the increased 5–24-h urinary S/E ratio \([0.019 (0.008–0.051)\) vs. \(0.015 (0.008–0.034);\) \(P = 0.006;\) Fig. 1C]. The 0–24-h urinary S/E ratio, indicating whole intestine permeability, was also significantly increased in patients with cirrhosis compared with controls \([0.020 (0.010–0.037)\) vs. \(0.016 (0.008–0.028);\) \(P = 0.003.\)

Subanalysis based on the etiology of cirrhosis revealed a significantly increased 0–5-h urinary L/R-ratio in patients with alcohol-related cirrhosis \((n = 11)\) vs. non-alcohol-related cirrhosis \((n = 15)\) \([0.050 (0.010–0.115)\) vs. \(0.020 (0.011–0.033);\) \(P = 0.016;\) Fig. 2], whereas the 0–5-h urinary sucrose excretion, 5–24-h, and 0–24-h urinary S/E ratio did not differ between these subgroups of patients (data not shown). The 0–5-h urinary L/R ratio was also significantly increased between the subgroup of patients with alcohol-related cirrhosis and the controls \([0.050 (0.010–0.115)\) vs. \(0.023 (0.006–0.069);\) \(P = 0.009;\) Fig. 2]. Use of PPIs and NSAIDs, which have been reported to be associated with a decreased barrier function \((8, 24),\) did not affect the urinary excretion of sucrose, the L/R ratio, or the S/E ratio in the patient group (data not shown).

**Gene transcription of TJ and associated proteins in duodenal and sigmoid biopsies.** Duodenal and sigmoid biopsies were obtained from 12 and 13 of the patients with cirrhosis, respectively, and from 22 controls. Gene transcription of claudin-3 was significantly downregulated in duodenal biopsies of patients with cirrhosis vs. controls \([0.608 (0.363–1.397)\) vs. \(1.400 (0.722–3.911);\) \(P < 0.001],\) whereas no significant differences were found for claudin-4, occludin, ZO-1, and MLCK between both groups (Table 3).

In sigmoid biopsies, gene transcription of claudin-4 was significantly downregulated in patients with cirrhosis compared with controls \([0.866 (0.550–1.910)\) vs. \(1.299 (0.192–5.310);\) \(P = 0.031].\) No significant differences in gene transcription were found for occludin, claudin-3, ZO-1, and MLCK (Table 3).

**Protein levels of occludin and claudin-4 in duodenal and sigmoid biopsies.** Duodenal and sigmoid biopsies from 10 patients with cirrhosis and 22 controls were available for quantifying protein levels of occludin and claudin-4 by ELISA. Occludin levels were significantly increased in both duodenal and sigmoid biopsies of patients with cirrhosis vs. controls \([0.55 (0.31–0.67)\) vs. \(0.34 (0.20–0.46);\) \(P < 0.001;\) and \(0.69 (0.51–0.87)\) vs. \(0.46 (0.28–0.63);\) \(P < 0.001,\) respectively; Fig. 3, A and B]. No significant differences were found for claudin-4 levels (Fig. 3, C and D).

**Histology of mucosal biopsies.** Microscopically, only minor morphological changes were observed in duodenal biopsies of

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**Table 2. Characteristics of subjects**

<table>
<thead>
<tr>
<th></th>
<th>Patients with Cirrhosis ((n = 26))</th>
<th>Controls ((n = 27))</th>
<th>(P) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, yr</strong></td>
<td>61 (18–72)</td>
<td>60 (19–78)</td>
<td>0.539</td>
</tr>
<tr>
<td><strong>Sex, M/F</strong></td>
<td>17/9</td>
<td>17/10</td>
<td>0.854</td>
</tr>
<tr>
<td><strong>BMI, kg/m²</strong></td>
<td>25.8 (18.8–39.0)</td>
<td>26.0 (18.1–32.1)</td>
<td>0.689</td>
</tr>
<tr>
<td><strong>ALT, U/l</strong></td>
<td>28.0 (9.0–130.0)</td>
<td>22.0 (10.0–32.0)</td>
<td>0.025</td>
</tr>
<tr>
<td><strong>GGT, U/l</strong></td>
<td>51.0 (17.0–259.0)</td>
<td>22.0 (6.0–50.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Child Pugh class, A/B/C</strong></td>
<td>17/9/0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>Child-Pugh score</strong></td>
<td>5.0 (5.0–9.0)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>MELD-score</strong></td>
<td>8.0 (6.0–15.0)</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Continuous values are presented as medians (range). BMI, body mass index; ALT, alanine aminotransferase; GGT, \(\gamma\)-glutamyl transpeptidase; MELD, model for end-stage liver disease.

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**G149 INTESTINAL PERMEABILITY IN COMPENSATED CIRRHOSIS**

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patients with cirrhosis and controls that did not differ significantly between the groups, such as a (slightly) increased number of intraepithelial lymphocytes (\( \text{n}_1 / H11005 \text{2} \) vs. \( \text{n}_1 / H11005 \text{2} \), respectively), mild edema of the lamina propria (\( \text{n}_1 / H11005 \text{2} \) vs. \( \text{n}_1 / H11005 \text{3} \), respectively), and mild chronic inflammation (\( \text{n}_1 / H11005 \text{3} \) vs. \( \text{n}_1 / H11005 \text{2} \), respectively). None of the subjects had villous atrophy or signs of acute inflammation. In sigmoid biopsies, only mild edema of the lamina propria was observed in both groups (\( \text{n}_1 / H11005 \text{4} \) vs. \( \text{n}_1 / H11005 \text{4} \), respectively).

**DISCUSSION**

In patients with compensated cirrhosis, large intestine permeability but not gastroduodenal or small intestine permeability was significantly increased. In addition, altered gene transcription was found for claudin-3 in duodenal and claudin-4 in sigmoid biopsies. At the protein level, occludin expression was significantly increased in both duodenal and sigmoid biopsies.

Several reports have shown that intestinal permeability is increased in patients with liver cirrhosis (6, 9, 11, 16, 27, 33). Although, in most studies, mixed groups of patients with compensated and decompensated cirrhosis were included, we focused on patients with compensated cirrhosis and compared the results with age-, sex-, and BMI-matched controls. Gastroduodenal and small intestine permeability measured by the validated multisugar test were not affected in our patients. One should realize that several etiological factors leading to liver cirrhosis, such as alcohol and obesity (i.e., high-fat diet), have been associated with a disturbed intestinal barrier function (15, 23, 30, 34). Interestingly, in previous studies comparing subgroups of alcohol-related vs. nonalcohol-related cirrhosis, an increased small intestine permeability in alcohol-related cirrhosis was observed, confirming a disruptive effect of alcohol on the intestinal epithelial barrier (15). Therefore, this may be a risk factor for bacterial translocation and could contribute to the progression toward decompensated cirrhosis. Although higher serum total IgA levels were observed in the subgroup of alcohol-related vs. nonalcohol-related cirrhosis, which might indicate an increased mucosal exposure to bacteria (12), we could not find an association with small intestine permeability (data not shown). Both experimental and human studies have

**Table 3. Gene transcription of proteins associated with intestinal epithelial barrier function**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Patients with Cirrhosis</th>
<th>Controls</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Duodenal biopsies</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occludin, 11 vs. 22</td>
<td>0.824 (0.587–1.055)</td>
<td>1.050 (0.372–4.674)</td>
<td>0.158</td>
</tr>
<tr>
<td>Claudin-3, 11 vs. 21</td>
<td>0.608 (0.363–1.397)</td>
<td>1.400 (0.722–3.911)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Claudin-4, 11 vs. 22</td>
<td>0.835 (0.483–1.852)</td>
<td>1.229 (0.419–4.933)</td>
<td>0.061</td>
</tr>
<tr>
<td>ZO-1, 10 vs. 22</td>
<td>0.781 (0.418–1.888)</td>
<td>0.473 (0.266–2.461)</td>
<td>0.143</td>
</tr>
<tr>
<td>MLCK, 11 vs. 22</td>
<td>0.802 (0.200–1.087)</td>
<td>0.708 (0.353–2.896)</td>
<td>0.593</td>
</tr>
<tr>
<td><strong>Sigmoid biopsies</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occludin, 13 vs. 21</td>
<td>0.975 (0.540–1.910)</td>
<td>0.571 (0.133–2.744)</td>
<td>0.058</td>
</tr>
<tr>
<td>Claudin-3, 13 vs. 22</td>
<td>1.385 (0.682–2.118)</td>
<td>1.429 (0.258–5.854)</td>
<td>0.918</td>
</tr>
<tr>
<td>Claudin-4, 13 vs. 22</td>
<td>0.866 (0.550–1.910)</td>
<td>1.299 (0.192–5.310)</td>
<td>0.031</td>
</tr>
<tr>
<td>ZO-1, 13 vs. 21</td>
<td>0.822 (0.275–2.155)</td>
<td>0.703 (0.157–2.886)</td>
<td>0.280</td>
</tr>
<tr>
<td>MLCK, 13 vs. 22</td>
<td>1.305 (0.720–1.740)</td>
<td>1.189 (0.139–2.736)</td>
<td>0.838</td>
</tr>
</tbody>
</table>

All data are normalized expression ratios, presented as medians (range).
shown that acute (i.e., single, moderate or high intake) as well as chronic consumption of alcohol increases intestinal permeability (4, 19, 20, 22, 26, 32). As in the present study, only two patients with alcohol-related cirrhosis still consumed alcohol regularly and were requested to abstain from alcohol the day before the test day; an acute effect of alcohol on intestinal permeability is not expected. It should be noted that sizes of subgroups are rather small, and our study was not powered for subgroup analysis of patients with cirrhosis according to etiology.

Although we did not observe any change in the whole group of patients with compensated cirrhosis, we cannot exclude that gastroduodenal and small intestine permeability are affected in patients with decompensated cirrhosis. Indeed, some studies have shown that increased intestinal permeability in patients with cirrhosis is associated with the presence of ascites (18, 21, 42). These changes in intestinal permeability may result from characteristics of patients with cirrhosis, such as alterations in the intestinal microbiota and/or immune system, systemic and local inflammation or oxidative stress, and/or the presence of portal hypertension (28, 41). As in the present study, almost all patients had signs of previous or current portal hypertension; overall, we do not find an increased small intestine permeability, and we do not expect portal hypertension to be a major risk factor in our population. This may be in part related to the fact that the degree of portal hypertension in this population does not exceed a certain limit (16), such as in patients with decompensated cirrhosis in whom clinically evident complications resulting from portal hypertension (i.e., ascites, variceal hemorrhage, and/or hepatic encephalopathy) are present. Remarkably, we observed a significant increase in large intestine permeability in the patients with compensated cirrhosis measured by the S/E ratio in 5–24-h urine samples. Data on large intestine permeability are scarce. Our findings confirm those of a recent study reporting an increased 5- to 26-h urinary excretion of sucralose in patients with cirrhosis (25). By combining two test markers different in molecular size and transport mechanism, we were able to correct for potential confounders, such as gastrointestinal transit and renal function (7).

The increase in large intestine permeability observed may be related to alterations in microbial composition by causing elevated endotoxin levels (1, 17) or by bacteria directly altering expression of TJ proteins (31). Changes in both fecal and sigmoid microbiota profiles have been reported in patients with cirrhosis compared with healthy controls (2, 3, 10). Furthermore, as high numbers and diversity of bacteria are present in the large intestine, an increased permeability may enhance the risk of bacterial translocation. However, reliable analyses of (site-specific) bacterial translocation in humans require invasive techniques for sampling.

To support the functional permeability changes found by the multisugar test, we evaluated the expression of TJ proteins in both duodenal and sigmoid biopsies. We did not observe significant alterations at the TJ gene level apart from a down-regulation of gene transcription of claudin-3 in the duodenal mucosa and claudin-4 in the sigmoid mucosa of patients with cirrhosis. Protein levels of occludin (but not of claudin-4) were significantly higher in duodenal and sigmoid mucosa of patients with cirrhosis compared with controls. After correction...
for multiple testing, gene transcription of claudin-4 was no longer significant.

The limited alterations on the gene and protein level are not clearly in line with the observed functional permeability changes. Inconsistent results are also reported by others showing altered and normal expression of TJ proteins in patients with cirrhosis with barrier dysfunction (1, 13), indicating that the relation between functional and structural changes of the intestinal epithelial barrier in these patients needs further clarification. In addition to gene transcription, it has to be taken into account that posttranslational protein breakdown and/or modifications can contribute to “functional” protein levels.

A strength of our study is that we investigated the epithelial barrier of both the small and large intestine and combined functional analyses using the multisugar test with TJ analyses in biopsies focusing on patients with compensated cirrhosis.

Some potential shortcomings should also be taken into account. The multisugar test provides accurate, site-specific information on intestinal permeability and uses low sugar doses (39), but the 24-h urine collection can be a practical limitation for routine use. Second, expression of TJ proteins could only be evaluated in a subgroup of patients with cirrhosis. Although we acknowledge that translocation of bacteria and their products can occur via both transcellular and paracellular pathways and may be facilitated by changes in immune defense (41), the observed increase in large intestine permeability may enable bacterial translocation. In the near future, it will also be interesting to examine biopsies from the ileum and proximal large intestine and to perform microbial analyses. Finally, it has to be noted that we included a heterogeneous group of patients with regard to etiology. Although we could demonstrate that patients with alcohol-related cirrhosis had an increased small intestine permeability, the small sizes of other subgroups precluded us from performing additional analyses to investigate whether these subgroups may also be more susceptible for an altered intestinal permeability. Furthermore, various factors, including malnutrition (25) and certain drugs (8, 24), may affect intestinal permeability. However, malnutrition was not observed in our study population, and use of PPIs and NSAIDs did not alter permeability results.

In conclusion, in patients with stable compensated cirrhosis, gastroduodenal and small intestine permeability were not altered, whereas the large intestine permeability was increased. Therefore, it would be interesting for future studies to further address the involvement of the large intestine regarding permeability and microbiota composition in bacterial translocation and in progression toward decompensated cirrhosis.

ACKNOWLEDGMENTS
We thank E. C. G. M. Schaepkens and H. J. H. M. Pieters for technical assistance.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
Author contributions: K.E.P., G.H.K., A.A.M., and D.M.J. conception and design of research; K.E.P. and H.d.V. performed experiments; K.E.P. analyzed data; K.E.P., G.H.K., E.E.E., A.A.M., and D.M.J. interpreted results of experiments; K.E.P. prepared figures; K.E.P. and D.M.J. drafted manuscript; K.E.P., G.H.K., E.E.E., A.A.M., and D.M.J. edited and revised manuscript; K.E.P., G.H.K., H.d.V., A.A.M., and D.M.J. approved final version of manuscript.

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