Special Article

NUTRITION REVIEWS

Ethanol metabolism and its effects on the intestinal epithelial barrier

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Ethanol is widely consumed and is associated with an increasing global health burden. Several reviews have addressed the effects of ethanol and its oxidative metabolite, acetaldehyde, on the gastrointestinal (GI) tract, focusing on carcinogenic effects or alcoholic liver disease. However, both the oxidative and the nonoxidative metabolites of ethanol can affect the epithelial barrier of the small and large intestines, thereby contributing to GI and liver diseases. This review outlines the possible mechanisms of ethanol metabolism as well as the effects of ethanol and its metabolites on the intestinal barrier. Limited studies in humans and supporting in vitro data have indicated that ethanol as well as mainly acetaldehyde can increase small intestinal permeability. Limited evidence also points to increased colon permeability following exposure to ethanol or acetaldehyde. In vitro studies have provided several mechanisms for disruption of the epithelial barrier, including activation of different cell-signaling pathways, oxidative stress, and remodeling of the cytoskeleton. Modulation via intestinal microbiota, however, should also be considered. In conclusion, ethanol and its metabolites may act additively or even synergistically in vivo. Therefore, in vivo studies investigating the effects of ethanol and its byproducts on permeability of the small and large intestines are warranted.

INTRODUCTION

Beverages containing ethanol (i.e., ethyl alcohol) are widely consumed in many parts of the world, creating an increasing global health burden. It has been estimated that around two billion people drink ethanol on a regular basis.[1] Ethanol consumption has long been recognized as a major cause of liver disease,[2–4] but it can also affect the gastrointestinal (GI) tract and is associated with the development of oral, esophageal, and colorectal cancers.[5] Ethanol as well as its metabolites can cause damage that includes decreased intestinal motility and cytotoxic and mutagenic effects.[5–7] Another important effect is the ethanol-induced disruption of the epithelial barrier of the GI tract.[8] Studies performed in humans,[9,10] but mostly in animals,[11–13] have shown that both short- and long-term ethanol administration can result in increased intestinal permeability, which will ultimately enhance the translocation of luminal antigens (e.g., bacteria and endotoxins) into the portal circulation.[14,15] This can activate Kupffer's cells, subsequently leading to cytokine release, which results in hepatocellular injury and, consequently, alcoholic liver disease (ALD).[16,17] Data from human and animal studies indicate that increased intestinal permeability is also involved in inflammatory intestinal disorders such as inflammatory bowel disease[18] and irritable

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Key words: acetaldehyde, colon, epithelial barrier, ethanol, fatty acid ethyl esters

doi:10.1111/nure.12027

bowel syndrome.[19] Finally, decreased epithelial barrier function can result in increased susceptibility to carcinogens and may thereby contribute to the increased risk of alcohol-related cancers of the GI tract.[20–23]

Studies on the effects of ethanol intake on the intestinal epithelial barrier often focus on the small intestine, but ethanol and its metabolites can also reach the large intestine, depending on dosage, absorption, and metabolism.[24] Ethanol can be metabolized oxidatively and nonoxidatively, resulting in acetaldehyde, fatty acid ethyl esters (FAEEs), and phosphatidylethanol (PEth).[25] Many previous reviews have addressed the effects of ethanol and its main metabolite, acetaldehyde, on the GI tract, focusing on either carcinogenic effects or ALD. However, reviews on the effects of ethanol and all its (oxidative and nonoxidative) metabolites on small and large intestinal barrier function, as well as possible mechanisms of action, are limited.

This review aims to provide a broader understanding of the effects of ethanol and its oxidative and nonoxidative metabolites on intestinal barrier function. First, ethanol absorption and metabolism will be discussed, with a special focus on the GI tract. Subsequently, the effects of methanol and its metabolites on small and large intestinal barrier function, along with possible mechanisms of action, will be reviewed.

ABSORPTION AND METABOLISM OF ETHANOL

Pharmacokinetic studies have shown large individual variations in the absorption, distribution, and elimination of ethanol.[26,27] After oral ingestion, ethanol is absorbed from the GI tract by simple diffusion due to its small molecular size, moderate lipid solubility, and excellent water solubility.[28] Minimal absorption occurs in the mouth and esophagus, and about 20% and 70% is absorbed through the stomach and the proximal small intestine, respectively, indicating that the majority of ingested ethanol is absorbed before it reaches the colon.[29,30] A small proportion of ethanol is excreted unchanged: 1–5% via the lungs, 0.1–0.5% in sweat, and 0.5–2% in urine.[30,31] The rate of ethanol absorption in the GI tract depends on several factors, including rate of gastric emptying, sex body mass index, presence of food in the stomach, and ethanol dosage and concentration.[32,33] After being absorbed, ethanol reaches the circulation and is rapidly distributed throughout the body fluids, with the rate of distribution related mainly to the water content of various tissues and organs.[32] Therefore, in the terminal ileum and colon, ethanol concentrations approximate those in blood.[24,34,35] In general, the body water content is lower in females, which contributes to higher blood ethanol concentrations in women than in men after ingestion of similar doses per kilogram of body weight.[36,37]

Oxidative metabolism of ethanol

Although the majority of absorbed ethanol (i.e., 90–98%) is metabolized in the liver, metabolism also occurs in the tissues of the GI tract, including the oral cavity, the esophagus, the stomach, and the small and large intestines.[30,38–41] Ethanol is metabolized oxidatively into acetaldehyde by alcohol dehydrogenase (ADH), which is located in the cytosol of hepatocytes, by the microsomal ethanol oxidizing system (MEOS) cytochrome P450 2E1 (CYP2E1) in the microsomes, and by catalase in the peroxisomes (Figure 1).[25,30] Of these, ADH is the main enzyme involved, and 10 isoenzymes (grouped into 5 classes) with varying kinetic properties, substrates specificities, and tissue distributions have been reported (Table 1).[25,30,42–44] Class I ADH enzymes (with a low Km) are highly expressed in the liver, but ADH expression has also been reported in intestinal epithelial cells, being higher in the villous tip than in the crypt region.[45] The mucosa of the oral cavity, esophagus, and stomach is characterized by a high expression of class IV ADH.[46–48] The esophagus has the highest ADH activity in the GI tract, similar to that of the liver and approximately four times that of the stomach.[49] In small and large intestinal mucosa, class I ADH is predominant, with a Km of 1–2 mM for ethanol.[39] Interestingly, the activity of rectal ADH was found to be comparable to the activity of gastric ADH, suggesting that ethanol can be effectively metabolized to acetaldehyde in the rectal mucosa.[40] Evidence from both human and animal studies indicates that ethanol undergoes a first-pass metabolism in the stomach and liver, resulting in a significant decrease in the ethanol concentration reaching the blood.[50] An important role of the stomach is indicated by a clear decrease in first-pass metabolism after gastrectomy, after direct intraduodenal ethanol administration,[51] and in subjects with accelerated gastric emptying. [33] Gastric first-pass metabolism occurs predominantly by mucosal class IV ADH isoenzymes, especially σ-ADH.[39,52–54] The σ-ADH activity is lower in women than in men and, together with the lower body water content of women, contributes to the higher susceptibility of females to the injurious effects of ethanol.[55–57]

The CYP2E1-dependent MEOS, a pathway for ethanol metabolism that is present in several different cells, including hepatocytes, accounts for less than 10% of ethanol metabolism under normal conditions.[58] The MEOS becomes active only when high concentrations of ethanol (Km 7–10 mM) are present, and its activity is increased during chronic alcohol consumption.[59] It plays a key role in the pathogenesis of ethanol-related diseases

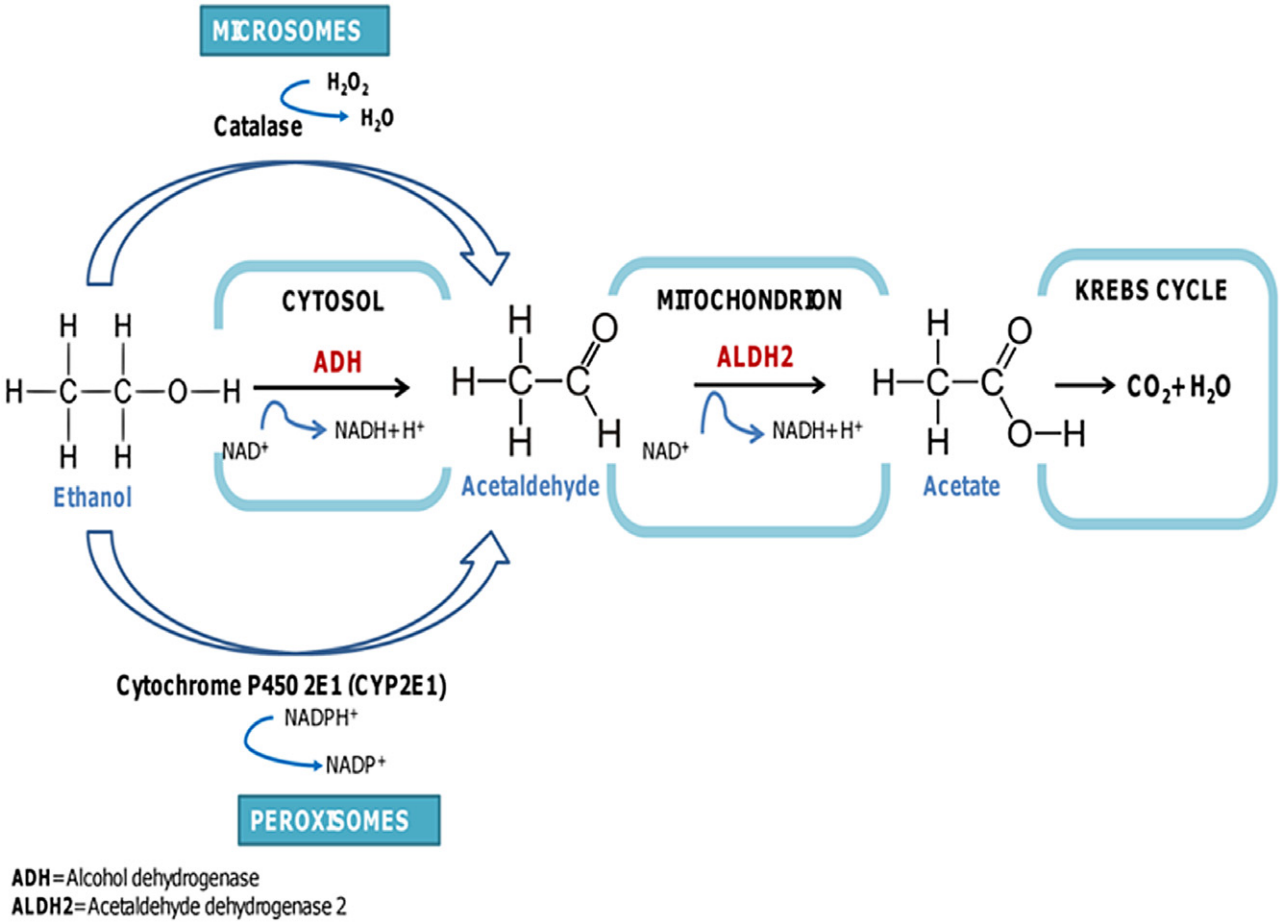

Figure 1 **Oxidative ethanol metabolism in the liver cells (hepatocytes). Adapted from Zakhari.**[25]

and carcinogenesis, as it potentiates the generation of free radicals and activates several xenobiotics, including carbon tetrachloride, to form potentially carcinogenic products.[58,60] Catalase is also able to oxidize ethanol, generating acetaldehyde and water in the presence of hydrogen peroxide (H_2O_2).[25] Although catalase activity has been observed in human gastric and intestinal mucosae, no data from human studies in vivo are available on its role in ethanol metabolism.[61]

Acetaldehyde is rapidly metabolized in the liver and, to a lesser extent, in the oral cavity, esophagus, stomach, intestine, and pancreas via oxidation by aldehyde dehydrogenase (ALDH) into acetate (Figure 1).[46,47] Acetate is conjugated to form acetyl coenzyme A and is oxidized, mainly in the skeletal muscles, into CO_2 and H_2O_2.[62] Thus far, 10 ALDH isoenzymes have been characterized (Table 2).[63,64] In addition to being expressed in the liver, class I ADH isoenzymes are also expressed in gastric

Table 1 **Kinetic properties and tissue distribution of ADH isoenzymes.**

Class	Gene	Protein	Km (mM)	V_{max} (min^{-1})	Tissue distribution
1	*ADH1A*	α	4.0	30	Liver, small and large intestines
	*ADH1B*1*	ß1	0.05	4.0	Liver, lung, kidney
	*ADH1B*2*	ß2	0.9	350	Liver, lung, kidney
	*ADH1B*3*	ß3	40.0	300	Liver, lung, kidney
	*ADH1C*1*	γ1	1.0	90	Liver, stomach
	*ADH1C*2*	γ2	0.6	40	Liver, stomach
II	*ADH4*	π	30.0	20	Liver
III	*ADH5*	χ	>1,000	100	Gingiva, tongue
IV	*ADH7*	σ (μ)	30.0	800	Liver, esophagus, stomach
V	*ADH6*		Unknown	Unknown	Liver, stomach

Table 2 **Distribution of ALDH isoenzymes among different body tissues and organs.**

Class	Gene	Allele	Tissue distribution
I	*ALDH1*	*ALDH1*	Liver, stomach, brain (cytosol)
II	*ALDH2*	*ALDH2*1*	Liver (mitochondrion)
		*ALDH2*2*	Liver, stomach (mitochondrion)
III	*ALDH3*	*ALDH3*	Stomach, lung, liver (cytosol)
IV	*ALDH4*	*ALDH4*	Liver, kidney (mitochondrion)
V	*ALDH5*	*ALDH5*	Testes, liver, brain, stomach (mitochondrion)
VI	*ALDH6*	*ALDH6*	Salivary gland, stomach (cytosol)
VII	*ALDH7*	*ALDH7*	Kidney, lung (microsomes)
V1II	*ALDH8*	*ALDH8*	Parotid gland (microsomes)
XI	*ALDH9*	*ALDH9*	Liver, kidney, muscle (cytosol)
X	ALDH10	ALDH10	Liver, heart (mitochondrion)

epithelial cells and in the small and large intestines. They possess a low Km (0.6–4 mM) and a high maximal velocity. Therefore, they oxidize ethanol, even at low concentrations, at a constant rate.[25,65] Since mucosal ALDH activity is lower than ADH activity in the large intestine, accumulation of the reactive and toxic metabolite, acetaldehyde, is highly expected.[41,66]

Ethanol metabolism varies between different ethnic groups. For instance, the variant allele of ADH, *ADH1C*1*, is more frequent in Asians than in Caucasians or Africans, and Asians metabolize ethanol more readily into acetaldehyde, resulting in accumulation of the latter.[67,68] In addition, the variant allele *ALDH2*2*, which encodes an inactive subunit of the enzyme ALDH2, is dominant and highly prevalent (28–45%) in Asians but is rare in other ethnicities.[65] Homozygous carriers of the *ALDH2*2* allele lack ALDH2 activity and consequently experience strong facial flushing and physical discomfort due to high blood acetaldehyde levels following ethanol consumption.[69] These adverse effects are less severe in heterozygous carriers, who have 10–50% of the ALDH2 activity seen in subjects who do not carry the *ALDH2*2* allele, but heterozygous carriers are also at increased risk of developing ethanol-related GI cancers because they can metabolize only small amounts of acetaldehyde.[69–71]

Apart from those who consume large amounts of ethanol (>80 g daily) or engage in binge drinking (i.e., more than five drinks [>100 g] within 2 h), many people worldwide consume moderate amounts of alcohol (2 standard drinks) on a regular (i.e., at least weekly) basis.[32,72] Concentrations of ethanol after consuming two standard drinks (i.e., total of 28 g of ethanol) in luminal contents are found to be approximately 6.5–9.4 g/dL in the stomach, 6.5–9.4 g/dL in the jejunum, and 0.1–0.2 g/dL in the ileum as well as in the colon.[24]

Nonoxidative metabolism of ethanol

While most studies have focused on oxidative metabolism, ethanol can also be metabolized nonoxidatively via at least two pathways (Figure 2). First, ethanol may react with membrane phospholipids. Phospholipase D catalyzes transphosphatidylation, thereby generating PEth, an abnormal phospholipid.[25,73] Since the PEth is not a normal constituent of membranes, it is poorly metabolized and, upon intracellular accumulation, disrupts the cell signaling that normally restricts proliferation in different tissues, including intestinal epithelial cells.[74] With a half-life of 4 days, PEth can be detected in blood and is considered a sensitive biomarker for both long-term and heavy ethanol consumption (>50 g/d) as well as for moderate alcohol consumption (40 g/d).[75–79] Furthermore, PEth has also been detected in rat small intestine[80] and human colonic tissue.[74] One hour after intake of ethanol (50–140 g/dL), serum PEth concentration has been found in the range of 45–138 ng/mL, reaching maximum concentrations of 74–237 ng/mL after between 3 days and 6 days.[77]

Secondly, ethanol may react with free fatty acids in a reaction catalyzed by fatty acid ethyl ester (FAEE) synthase[25,73] and cholesterol esterase,[73,81] generating FAEEs.[73,82] FAEEs can also be generated by transesterification of ethanol and fatty acyl-coenzyme A in a reaction catalyzed by acyl-coenzyme A: ethanol *O*-acyltransferase.[73,83] FAEEs have been detected in hair, heart, leukocytes, brain, adipose tissue, and meconium.[84–90] In the GI tract, FAEEs have been found to accumulate in the pancreas and the liver.[91,92] Since fatty acids and ethanol are absorbed by intestinal mucosa, the intestine is considered to be another site where FAEE synthesis can occur.[83] FAEE synthase activity with subsequent FAEE synthesis has been demonstrated in duodenal mucosa.[83] However, little is known about the concentrations of FAEEs present in the intestine after ethanol intake and their local effects on intestinal cell physiology. FAEEs have a half-life ranging between 16 h and 99 h and can therefore also be used as biomarkers for prior ethanol ingestion.[93,94] Ex vivo, inhibition of oxidative ethanol metabolism in the liver and pancreatic homogenates of rats by 4-methyl pyrazole, an ADH

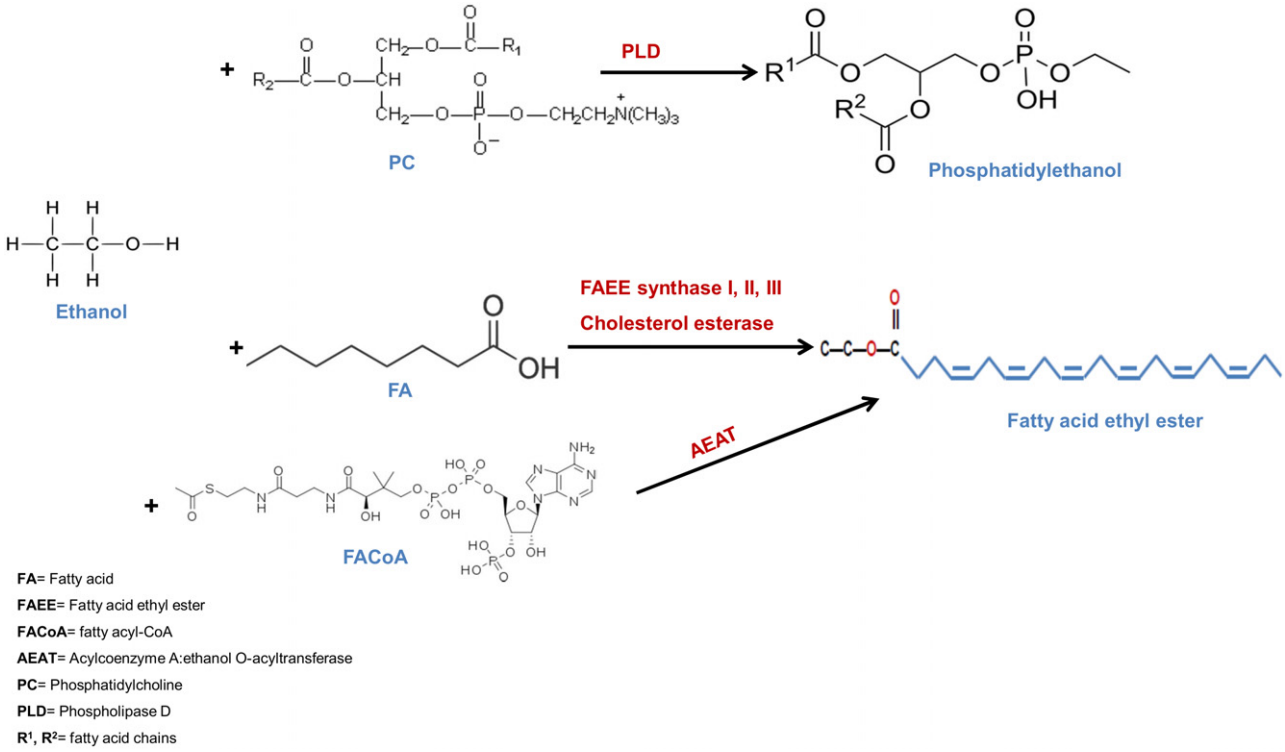

Figure 2 **Nonoxidative ethanol metabolism. Adapted from Zakhari[25] and Best and Laposata.[73]**

inhibitor, by diallyl sulfide, an MEOS inhibitor, and by aminotriazole, a catalase inhibitor, has been shown to result in a shift toward the nonoxidative pathways, resulting in the generation of FAEEs.[91] Thus, a low ADH activity in chronic ethanol abusers may lead to accumulation of FAEEs and, consequently, increased risk for the injurious effects of FAEEs.[95]

Role of microbiota in the endogenous production and oxidation of ethanol

In addition to being ingested orally, ethanol can be produced endogenously by bacterial fermentation of carbohydrates.[96] The endogenously produced ethanol is absorbed and transferred via the portal vein to the liver, where it is metabolized.[97] Although blood ethanol concentrations are usually very low in sober subjects (40–45 µg/dL), conditions associated with intestinal bacterial overgrowth, such as jejunoileal bypass surgery and tropical sprue, can lead to endogenous ethanol production of up to 1 mM (approximately 4.6 mg/dL) and of 2–31 mM (approximately 9.2–142.6 mg/dL) in blood and jejunal aspirates, respectively.[98,99] In addition, endogenously produced ethanol has also been found in the cecum of normally fed rats, with concentrations of 0.9 mM (4.14 mg/dL) reported.[97]

Several bacteria and yeasts can ferment sugars to ethanol,[100] including some that can be found in the GI tract. For example, gastric overgrowth of *Helicobacter pylori*[101] and small intestinal overgrowth of coliform bacteria such as *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Escherichia coli* can contribute to endogenous ethanol production.[98] Coliform bacteria and, for example, *Clostridium* spp., may contribute to ethanol production in the colon as well.[100]

Endogenous and exogenous ethanol can also be further metabolized by the GI microbiota.[102,103] For instance, oral bacteria and yeasts, including *Streptococcus viridans*[104] and *Candida* strains,[105] and gastric bacteria such as *H. pylori*[106] have been found to possess ADH activity. Intestinal bacteria belonging to the Enterobacteriaceae family, such as *E. coli*, have also been shown to oxidize ethanol aerobically into acetaldehyde by an ADH-dependent reaction.[107–109] Furthermore, Salaspuro et al.[108] have demonstrated that *E. coli* is also able to convert ethanol into acetaldehyde under microaerobic, microaerophilic, and anaerobic conditions.

Several bacteria, such as members of the Enterobacteriaceae family, are known to possess catalase activity in addition to ADH activity. Tillonen et al.[110] demonstrated that human colonic contents could indeed generate acetaldehyde via catalase-dependent pathways.

Jokelainen et al.[111] have shown that in vitro incubation of human colonic contents with ethanol concentrations found in vivo can result in dose-dependent acetaldehyde production. Data on colonic luminal

acetaldehyde levels in humans are scarce, most likely due to the volatility of acetaldehyde, but some data from animal studies are available. A marked increase in mucosal acetaldehyde was found in rats with blind loops and concomitant bacterial overgrowth[103] as well as in the cecum and rectum of rats and the colon of pigs (271 μM, approximately 1.2 mg/dL) after administration of 2.5–4.5 g/kg ethanol.[112–115] Furthermore, rectal mucosal acetaldehyde concentrations were found to be higher in conventional than in germ-free rats,[112] and cecal levels of acetaldehyde could be effectively reduced by pretreatment with ciprofloxacin,[115] pointing to a role of the intestinal microbiota in acetaldehyde production.[112]

Besides exhibiting ADH activity, facultatively anaerobic bacteria such as Enterobacteriaceae have also been found to exhibit ALDH activity.[116] However, the capacity of the intestinal microbiota to metabolize acetaldehyde by ALDH is rather low.[117] This, combined with the low levels of ALDH in colonic mucosa,[118] results in the accumulation of acetaldehyde in the large intestine.[66] Intracolonic production and accumulation of acetaldehyde is suggested to cause colorectal carcinogenesis[119] and to be an important determinant for blood acetaldehyde levels and subsequent hepatotoxicity.[66]

In summary, ethanol as well as its oxidative and nonoxidative metabolites can be found in the contents of both the small and the large intestines, either as a direct consequence of ethanol ingestion or via the systemic circulation, which involves the action of ethanol-metabolizing enzymes in the GI tract and microbiota.

Ethanol-induced changes in the intestinal microbiota

Besides demonstrating the role of bacteria in ethanol metabolism, mounting evidence has shown that ethanol can also result in quantitative and qualitative changes in the intestinal microbiota. Yan et al.[120] have demonstrated in a mouse model that intragastric feeding of ethanol (30.9 g/kg per day) for 3 weeks induced small intestinal bacterial overgrowth and cecal dysbiosis. In rats, intragastric administration of ethanol (8 g/kg/d) for 10 weeks was shown to induce ileal and colonic dysbiosis.[121]

In humans, long-term ethanol consumption was found to be associated not only with small intestinal bacterial overgrowth[122,123] but also with alterations in the composition of the mucosa-associated microbiota in sigmoid biopsies.[124] Mutlu et al.[124] found a lower abundance of Bacteroidetes and a higher abundance of Proteobacteria in alcoholics than in healthy controls. In a randomized crossover study performed in healthy volunteers who consumed red wine, dealcoholized red wine, or gin for 20 days, all interventions resulted in changes in the fecal microbiota, as demonstrated by quantitative PCR and denaturing gradient gel electrophoresis, with changes differing among groups.[125] In line with the above, a reduction in the proportion of Bacteroidetes and an increase in the Proteobacteria were also demonstrated by 454 pyrosequencing in a mixed group of patients with hepatitis-B or ethanol-related liver cirrhosis versus healthy individuals. Furthermore, the authors also reported changes on the family level, including, for example, increased numbers of Enterobacteriaceae and Streptococcaceae and reduced numbers of Lachnospiraceae.[126] Moreover, probiotic and synbiotic interventions have been demonstrated to attenuate liver injury in a rat model of alcoholic steatohepatitis[127] and liver dysfunction in cirrhotic patients,[128] respectively, supporting a role for the gut microbiota in ethanol-induced liver diseases.

In addition to possibly enhanced translocation of endotoxins and direct effects of bacteria on the epithelial barrier, alterations in the composition and activity of gut microbiota can also result in changes in the production or breakdown of ethanol and acetaldehyde. In an in vitro study, for example, Nosova et al.[129] demonstrated that *Bifidobacterium* spp. and, to a greater extent, *Lactobacillus GG*, are weak acetaldehyde generators but have a high acetaldehyde-metabolizing capacity, which correlates positively with bacterial concentrations. Nevertheless, additional data on the effects of different dosages and durations of ethanol intake on the intestinal microbiota and subsequent ethanol-related microbial metabolic activity in humans are warranted.

EPITHELIAL BARRIER DISRUPTION MEDIATED BY ETHANOL AND ITS METABOLITES

Intestinal epithelial barrier

The GI epithelium is composed of a continuous monolayer of intestinal epithelial cells, which facilitate a selective passive entry of luminal nutrients, ions, and water while restricting access of pathogenic substances and microorganisms by means of transcellular and paracellular pathways.[130] The transcellular pathway contains lipophobic and lipophilic pores located in the brush border membrane of enterocytes.[131] The paracellular pathway is regulated via apical intercellular junctional proteins known as tight junctions (TJs) and via associated proteins known as the adherens junctions (AJs).[131] The TJs are composed of transmembrane proteins (e.g., claudins), integral membrane proteins (e.g., occludin), junction adhesion molecules, and cytoplasmic zona occludens (ZO) proteins (e.g., ZO-1, ZO-2, and ZO-3), which connect the TJ complex intracellularly with the actin cytoskeleton.[132] The TJs are regulated by both intra- and

extracellular signaling molecules. Intracellular signaling molecules that regulate the assembly and disassembly of the TJs include, for example, myosin light chain kinase (MLCK),[133] Rho GTPases,[134] protein kinase C,[135] mitogen-activated protein kinases,[136] protein tyrosine kinase,[137] intracellular calcium,[138] and zonulin.[139,140] Extracellular modulators of the TJs include, for example, nutrients, xenobiotics such as nonsteroidal anti-inflammatory drugs, and cytokines (e.g., interferon-γ, tumor necrosis factor-α, and interleukin-1β).[141–145] Intestinal epithelial barrier function in vivo can be assessed noninvasively by measuring ingested test probes (sugars, polyethylene glycol, radioactively labelled chromium-EDTA), analyzing TJ structures, or measuring the sequelae of barrier dysfunction, such as bacterial translocation and production of serum/plasma endotoxins.[146–149]

Effects of ethanol on small and large intestinal barrier function

Ethanol and its oxidative and nonoxidative metabolites can be found throughout the GI tract, where they can interfere with several functions, including those of the intestinal barrier. It has been shown that, after oral administration of 0.8 g/kg ethanol as a 25% solution, luminal levels of ethanol can reach more than 400 mg/dL and can be maintained for 60 min in the stomach, in the proximal jejunum, and in the duodenum, whereas levels in the ileum were approximately 200 mg/dL, parallel to those in blood.[24] Ethanol concentrations found in the colon are comparable to those in blood.[24,34,35] Hence, the continuous presence of ethanol in the GI tract, which results from equilibration throughout the vascular space, may account for the ethanol-induced epithelial barrier dysfunction in both the upper and the lower GI tract. Few studies have investigated the effects of ethanol intake on GI tract barrier function in humans (Table 3). Most studies are performed in long-term ethanol abusers, defined as individuals with a consumption of more than four drinks (>80 g alcohol) per day,[32] the majority of whom are also diagnosed with ALD. Overall, an increase in small intestinal permeability was observed in long-term ethanol abusers (alcoholics)[9,10,14,150–153]; likewise, gastroduodenal permeability increased in nonalcoholics following administration of a single dose of ethanol.[151,154] Millan et al.[155] reported histological changes in the small intestine following administration of a single dose of ethanol in nonalcoholics, although barrier function was not assessed. Hirsch et al.[150] did not find changes in the permeability of the small intestine of long-term abusers after 3 days of abstinence. However, in a similar group of patients, the ethanol-induced increase in gastroduodenal permeability was found to persist for at least 7 days in the presence of aspirin.[152] In patients with ALD, an increase in small intestinal permeability has been found to be associated with high levels of endotoxins in blood.[9,14,156,157] Animal studies have demonstrated that both short- and long-term ethanol administration, at dosages ranging from 6 g/kg/day to 8 g/kg/day, can increase intestinal permeability and induce endotoxemia,[12,13,15,158] subsequently leading to liver injury,[12,13,15] intestinal inflammation,[159] and rectal carcinogenesis.[112,160,161] Moreover, ethanol, when present along with alterations in intestinal permeability and immune status, has been shown to lead to small intestinal bacterial overgrowth, contributing to an increase in endotoxin translocation and an exacerbation of intestinal tissue damage after burn injury in rats.[162,163] Data from studies in humans have confirmed that small intestinal bacterial overgrowth can also occur in patients with ALD, in whom increased numbers of aerobic and anaerobic bacteria were found in jejunal aspirates.[122,123] Data on the effects of ethanol on human colonic barrier function are lacking. In animals, oral administration of ethanol (3–4.5 g/kg) in rats as well as ex vivo exposure of rat colon to acetaldehyde (40–160 μM, approximately 176–704 μg/dL) resulted in increased colon permeability.[164] Antibiotics and doxantrazole, a mast cell membrane stabilizer, significantly inhibited these effects, pointing to a mechanistic role for the enteric microbiota and mast cell activation.[164] As for human data, it has recently been shown that moderate red wine consumption can increase small intestine and colon permeability in patients with inflammatory bowel disease.[154]

In vitro studies on the effects of ethanol and acetaldehyde on epithelial integrity

The majority of data on ethanol-induced barrier dysfunction results from in vitro studies, all using Caco-2 cell monolayers. It was found that ethanol in concentrations of 0.1% up to 10% (92–920 mg/dL) significantly decreased transepithelial electrical resistance and increased permeation markers (see Table 4). In two studies, ethanol up to a 5% (4.6 g/dL) concentration failed to increase paracellular permeability.[165,166] In contrast, ethanol at concentrations of 2.5% (2.3 g/dL) and above has been shown to increase paracellular permeability by compromising the cell viability.[167,168] Ethanol metabolism into acetaldehyde has been suggested to be required for ethanol-induced barrier disruption. Indeed, acetaldehyde at concentrations ranging from 25 μM to 760 μM (0.11–3.3 mg/dL) has also been demonstrated to increase permeability (see Table 5). The concentrations of ethanol tested in vitro were comparable to those found in the human upper GI tract,[24] whereas concentrations of acetaldehyde were comparable to those found in the rat colon (0.12–3 mM, approximately 0.53–13.2 mg/dL).[169]

Table 3 **Human studies exploring the effect of ethanol consumption on intestinal barrier function.**

Subjects	Dose of ethanol (route of administration)	Parameters assessed	Major findings	Reference
Nonalcoholics ($n = 20$)	45 g or 60 g (single intragastric dose)	Histological changes	Transient damage to the upper small intestine	Millan et al.[155]
Nonalcoholics ($n = 12$) Alcoholics ($n = 12$)	20 g (single oral dose) >80 g/day for >5 yrs	Intestinal permeability by PEG	Reversible altered permeability in alcoholics and nonalcoholics	Robinson et al.[10]
Nonalcoholics (15) Alcoholics ($n = 36$)	>80–150 g/day for >3 yrs >80–150 g/day for >3 yrs	Intestinal permeability by chromium-51-EDTA	Increased intestinal permeability in alcoholics > nonalcoholics	Bjarnason et al.[9]
Alcoholics with cirrhosis ($n = 88$) Alcoholic without cirrhosis ($n = 42$)	>80 g/day for ≥5 yrs >80 g/day for ≥5 yrs	Blood endotoxin levels	Increased endotoxin levels in patients with cirrhosis > patients without cirrhosis	Bode et al.[156]
Nonalcoholics ($n = 20$) Alcoholics ($n = 18$)	0.8 g/kg (oral, i.v.) 100 g/day for ≤5 yrs	Intestinal permeability by morphological changes	Increased gastroduodenal permeability in alcoholics. Reversible changes in villous integrity in alcoholics	Keshavarzian et al.[151]
Nonalcoholics ($n = 10$) Alcoholics ($n = 18$)	Nondrinkers 100 g/day for ≤5 yrs	Intestinal permeability by lactulose-mannitol test	Increased intestinal permeability in alcoholics with chronic liver disease	Keshavarzian et al.[157]
Nonalcoholics ($n = 18$) Alcoholics ($n = 19$)	Undefined Undefined	Intestinal permeability by lactulose-mannitol test on the third day of abstinence	No changes in intestinal permeability	Hirsch et al.[150]
Nonalcoholics ($n = 30$) Alcoholics with ($n = 19$) or without cirrhosis ($n = 35$)	Nondrinkers >60 g/day for >3 yrs >60 g/day for >3 yrs	Intestinal permeability by PEG and blood endotoxin levels	Increased intestinal permeability and endotoxin levels in ALD patients (with or without cirrhosis)	Parlesak et al.[14]
Nonalcoholics ($n = 26$) Alcoholics with cirrhosis ($n = 35$)	Undefined Undefined	Intestinal permeability by PEG 400 and PEG 3350	Increased intestinal permeability in patients with cirrhosis and ascites	Lee et al.[153]
Healthy subjects ($n = 7$) Patients with CD ($n = 6$) Patients with UC ($n = 8$)	0.4 g ethanol/kg (oral) 0.4 g ethanol/kg (oral) 0.4 g ethanol/kg (oral)	Intestinal permeability by lactulose-mannitol-sucralose test	Increased small intestinal and colon permeability in patients with CD and UC	Swanson et al.[154]

Abbreviations: ALD, alcoholic liver disease; CD, Crohn's disease; PEG, polyethylene glycol; UC, ulcerative colitis.

Table 4 **In vitro studies exploring the effects of ethanol on paracellular permeability using Caco-2 cell monolayers.**

Ethanol concentration[a]/ exposure time	Significant findings	Possible mechanisms	Reference
1%, 2.5%, 5%, 7.5%, 10%	Dose-related drop in TEER Increase in FSA permeability Disruption of TJ integrity Displacement of actin and myosin filaments	MLCK activation	Ma et al.[166]
0–15%	Increase in FSA permeability Disassembly of the microtubules	Oxidative stress	Banan et al.[167]
0–2.5%/24 h	Increase in FSA permeability Disruption of F-actin	NF-kappaB activation	Banana et al.[187]
5%/5 h	Drop in TEER Increase in FSA permeability Decrease in ZO-1, occludin, and claudin-1 protein levels	Zinc deficiency Oxidative stress	Zhong et al.[198]
5%/24 h	Drop in TEER Increase in FITC-D4 permeability Decrease in occludin protein levels	HNF-4α inactivation via oxidative stress and zinc deficiency	Zhong et al.[191]
0.1% and 1%/3 h	Drop in TEER Increase in FITC-D4 permeability Decrease in occludin protein levels	Induction of miR-212 expression with subsequent decrease in ZO-1 translation	Tang et al.[192]
0.2%/2 h	Drop in TEER Increase in FITC-D4 permeability Decrease in occludin protein levels	Stimulation of intestinal circadian clock gene expression	Swanson et al.[190]
0.2%/2 h	Drop in TEER Increase in FSA permeability Increase in p-Snail protein levels	iNOS- mediated ethanol-induced Snail activation	Forsyth et al.[193]
0.1–1%/3 h	Increase in FITC-D4 permeability Disruption of ZO-1 and occludin integrity Increase in acetylated microtubule protein levels	Hyperacetylation of microtubules	Elamin et al.[194]

[a] 1% equals 1 g/dL.
Abbreviations: FITC-D4, fluorescein isothiocyanate-labeled dextran 4 KD; FSA, fluorescein-5-(and-6)-sulfonic acid trisodium salt; HNF-4α, hepatocyte nuclear factor-4α; iNOS, inducible nitric oxide synthase; MLCK, myosin light chain kinase; PER2, period circadian protein homolog 2; TEER, trans-epithelial electrical resistance; ZO-1, zona occludens 1.

MECHANISMS OF INTESTINAL BARRIER DYSFUNCTION INDUCED BY ETHANOL AND ITS METABOLITES

Several mechanisms underlying the ethanol-induced barrier dysfunction have been proposed, including direct damage to epithelial cells, loss of integrity of TJs and/ or AJs, and changes in intestinal microbiota, each of which is discussed below.

Direct damage to epithelial cells

Ethanol and its metabolites can induce direct cell injury. Long-term ethanol ingestion has been reported to induce ultrastructural and histological changes in duodenal mucosa, including a decrease in the mean total mucosal surface area in chronic alcoholics.[170] Data on the effects of long-term moderate intake are not known. However, oral ingestion of a single dose of ethanol (1 g/kg, administered as a 35 g/dL solution) has been shown to result in histological changes in the duodenum, including subepithelial bleb formation, hemorrhagic erosions, and inflammatory cell infiltration.[171] In the rectal mucosa of individuals who consumed excessive amounts of ethanol, several ultrastructural changes were found, including inflammatory changes, a decreased number of mucin-secreting goblet cells, and alterations in cell organelles such as distorted mitochondria and dilated endoplasmic reticulum.[172]

Animal studies have demonstrated that acute intragastric administration of ethanol at 5–20% (4.6–18.4 g/dL) for 4 h can result in hemorrhagic erosions and epithelial cell loss in the proximal small intestine of rats.[173] Similar lesions have also been found in rats gavage fed for 14 days with 18.4 g/dL ethanol, including exfoliation and subepithelial edema of villous tips.[174] Administration of a single dose of ethanol (6 g/kg) in mice has also been demonstrated to induce severe injury in ileal

Table 5 **In vitro studies exploring the effects of acetaldehyde on paracellular permeability using Caco-2 cell monolayers.**

Exposure concentration/time	Significant findings	Possible mechanisms	Reference
100–760 μM/4 h	Reduction of TEER Increase in mannitol permeability	–	Rao[165,182]
650 μM/6 h	Decrease in TEER Increase in inulin permeability Tyrosine phosphorylation of ZO-1, E-cadherin, and β-catenin	Inhibition of protein tyrosine phosphatases	Atkinson & Rao[137]
100–600 μM/4 h	Decrease in TEER Increase in inulin and endotoxin permeability Dissociation of ZO-1, occludin, E-cadherin, and β-catenin	–	Seth et al.[183]
100–600 μM/4 h	Decrease in TEER Increase in inulin and endotoxin permeability Reorganization of occludin, ZO-1, E-cadherin, and β-catenin Reorganization of actin cytoskeleton	–	Sheth et al.[184]
100–600 μM/3–6 h	Redistribution of and reduction in ZO-1, occludin, E-cadherin, and β-catenin protein levels Protein tyrosine phosphorylation	Inhibition of protein tyrosine phosphatases	Basuroy et al.[186]
400 μM/0.5 h	Redistribution of E-cadherin and β-catenin Tyrosine phosphorylation of β-catenin Abolishment of interaction of β-catenin with E-cadherin	Tyrosine kinase activation	Sheth et al.[185]
100–760 μM/5 h	Decrease in TEER Increase in inulin permeability Redistribution of ZO-1, occludin, E-cadherin, and β-catenin. Reorganization of actin cytoskeleton Tyrosine phosphorylation of occludin, ZO-1, claudin-3, and E-cadherin	Tyrosine kinase activation	Samak et al.[136]
25–100 μM/3 h	Increase in FITC-D4 permeability. Disruption of ZO-1 and occludin integrity. Increase in acetylated microtubule protein levels	Hyperacetylation of microtubules	Elamin et al.[194]

Abbreviations: FITC-D4, fluorescein isothiocyanate-labeled dextran 4; TEER, trans-epithelial electrical resistance; ZO-1, zona occludens 1.

mucosa, including formation of submucosal blebbing and ulceration of microvilli.[15]

Long-term (16 weeks) ethanol administration induced various alterations in rat enterocytes, including enlargement, dilatation, and diminishment of the mitochondria, the smooth endoplasmic reticulum, and the rough endoplasmic reticulum, respectively.[175] Interestingly, these alterations were more prominent in the distal ileum than in the proximal jejunum, pointing to the bloodborne route of ethanol in inducing such effects rather than a first-pass effect on the ileal mucosa.[175]

Apart from the histological observations, most data on epithelial cell damage as well as further mechanistic insight come from in vitro experiments. In vitro studies using Caco-2 cell monolayers have shown that luminal (i.e., apical) exposure to high ethanol concentrations (i.e., 10–15%, approximately 9.2–13.8 g/dL) can decrease cell viability.[166,167] Ethanol at 13.8 g/dL has been found to induce cell apoptosis, an effect that was synergistically enhanced in the presence of estradiol, pointing to a possibly more severe effect of ethanol in females.[7,176] Low concentrations (i.e., <1%, <0.92 g/dL) have been shown to promote Caco-2 cell differentiation and to synergize with *E. coli* to induce cell apoptosis.[177,178] Studies investigating effects of acetaldehyde on intestinal cytotoxicity are scarce. However, at concentrations ≤1,000 μM (≤0.44 g/dL), acetaldehyde has not been found to compromise intestinal cell viability.[179] Further studies investigating wider ranges of concentrations are warranted.

Nonoxidative ethanol metabolites such as palmitoleic and palmitic acid ethyl esters (10–100 μM) have also been demonstrated to induce pancreatic acinar cell necrosis through mechanisms involving intracellular calcium release.[180] It has also been shown that FAEEs at concentrations (20–40 μM) reached in blood after moderate ethanol consumption can induce oxidative stress and decrease mitochondrial function in intestinal epithelial cells without compromising cell viability.[181] The

discrepancy between recent data and data from earlier studies can be explained by differences in cell type, dosage of ethanol, and duration of exposure to FAEEs. Data on the cell-damaging effects of PEth on intestinal epithelial cells are not available. Therefore, further study is required to determine the exact role of PEth and FAEEs in ethanol-induced intestinal epithelial damage.

Effects on tight junction integrity

Ethanol. The main mechanisms by which ethanol and its metabolites result in barrier dysfunction are through direct and indirect effects on the integrity of TJs (Table 4). Interactions between AJ proteins (E-cadherin and β-catenin), TJ proteins (ZO-1 and occludin), and cytoskeletal proteins are crucial for the organization of the TJ complex and for subsequent maintenance of the intestinal epithelial barrier.[137,182–186] Several mechanisms involving ethanol-induced disruption of epithelial TJs and AJs have been identified. Ma et al.[166] have shown that ethanol (1–10%, approximately 0.92–9.2 g/dL) in Caco-2 monolayers can reversibly disrupt the intestinal epithelial TJ integrity through MLCK activation and subsequent modulation of perijunctional actin and myosin filaments. Furthermore, incubation of Caco-2 cells with ethanol (2.5%, approximately 2.3 g/dL) for 24 h has been shown to induce nuclear factor-κB activation, thereby resulting in F-actin cytoskeleton instability and, consequently, intestinal barrier dysfunction.[187] Ethanol has also been found to affect intestinal cells by targeting a number of pre- and post-transcriptional regulators, including circadian clock genes and microRNA (miRNA; short ribonucleic acid molecules of an average of 22 nucleotides that bind to complementary sequences on target messenger RNA transcripts, resulting in translational repression), respectively.[188,189] Swanson et al. have demonstrated in the rat duodenum and proximal colon as well as in Caco-2 cells that ethanol-induced intestinal barrier dysfunction occurs through mechanisms involving upregulation of intestinal circadian clock gene expression.[190] In addition, in Caco-2 monolayers, ethanol downregulated the target gene ZO-1 of miRNA-212 and, consequently, decreased the mRNA and protein levels of ZO-1 through a mechanism involving hepatocyte nuclear factor-4α dysfunction.[191,192] In addition, ethanol has been found to induce activation of one of the transcription factors involved in an epithelial-mesenchymal transition program, known as Snail, resulting in upregulation of inducible nitric oxide synthase (iNOS) and, consequently, intestinal epithelial hyperpermeability.[193] Ethanol is also able to induce post-translational modifications in intestinal cell proteins. Very recently, it was demonstrated in a three-dimensional Caco-2 cell culture model that basal exposure to ethanol (40 mM, approximately 184 mg/dL) for 3 h can induce microtubule hyperacetylation, resulting in redistribution of ZO-1 and, consequently, loss of TJ integrity.[194] Such effects were independent of altered TJ-encoding gene expression.

A large number of studies have investigated the role of oxidative stress on the intestinal mucosa as a possible mechanism to explain barrier dysfunction. There is strong evidence to indicate the involvement of cellular oxidative stress in mediating ethanol-induced intestinal barrier dysfunction.[195] Evidence provided by Banan et al.[167,196] has shown that ethanol (2.5–15%, approximately 2.3–13.8 g/dL) can increase the paracellular permeability of Caco-2 monolayers via iNOS-mediated generation of reactive oxygen species (ROS), resulting in oxidation of the microtubule cytoskeleton and, consequently, disassembly of the TJs. The involvement of iNOS in ROS-mediated ethanol-induced intestinal hyperpermeability has been confirmed in rats gavage fed with ethanol (6 g/kg/day) for 10 weeks. In that study, inhibition of iNOS attenuated ethanol-induced gut leakiness and the associated endotoxemia.[197] Moreover, decreased intestinal antioxidant capacity has been found to play a crucial role in ethanol-induced intestinal disruption. Zhong et al.[198] demonstrated in mice that long-term ethanol (4.8%, approximately 4.4 g/dL) gavage for 4 months can induce ileal oxidative stress mediated by zinc deficiency, thereby sensitizing epithelial cells to ethanol, resulting in loss of TJ integrity.

Acetaldehyde. Evidence from in vitro and an ex vivo studies revealed that acetaldehyde has a higher potency than ethanol to induce intestinal barrier dysfunction,[164,165,182] thereby highlighting the relevance of this oxidative metabolite. Mechanistic studies have demonstrated in Caco-2 monolayers that acetaldehyde can induce redistribution of occludin and ZO-1 from the intercellular junctions into the intracellular compartments[137,183–185] leading to dissociation of these proteins from the actin cytoskeleton, resulting in paracellular hyperpermeability.[186,199] Acetaldehyde not only disrupts the TJs but also induces redistribution of E-cadherin and β-catenin, thereby disrupting the integrity of the AJs.[137,185] The role of several cell-signaling pathways that regulate barrier function, including protein tyrosine kinases[200–204] and protein tyrosine phosphatases,[203] in acetaldehyde-induced loss of TJ integrity has been explored. Basuroy et al.[186] have demonstrated that acetaldehyde induces tyrosine phosphorylation and disrupts the integrity of TJs and AJs in human colonic mucosa. Sheth et al.[184] have shown that acetaldehyde can induce protein tyrosine phosphorylation of E-cadherin and β-catenin, resulting in loss of interaction between these proteins and, consequently, barrier dysfunction. In

addition, acetaldehyde-induced inhibition of protein tyrosine phosphatase was found to disrupt the interactions between the AJ proteins and protein tyrosine phosphatase 1B and, consequently, to induce paracellular barrier dysfunction in vitro[137] and in human colon ex vivo.[186] Not only protein phosphorylation but also hyperacetylation can interfere with barrier dysfunction. Recently, in a three-dimensional Caco-2 cell culture model, it was demonstrated that exposure to acetaldehyde (25 μM, approximately 110 μg/dL) for 3 h can result in increased paracellular permeability through mechanisms involving hyperacetylation of microtubular protein (α-tubulin).[194]

Nonoxidative metabolites. Data on the effects of the nonoxidative metabolites, i.e., PEth and FAEEs, on the intestinal epithelial barrier function are limited. In one study, incubation of Caco-2 cells for 48 h with ethanol (0.05%, 46 mg/dL) was found to increase intracellular accumulation of PEth, resulting in claudin-1 endocytosis, disruption of claudin-1/ZO-1 TJs, and activation of ZO-1-associated nucleic acid binding proteins (ZONABs), which, consequently, promoted cell proliferation.[74] Similarly, high levels of accumulated PEth in colonic polyp sections obtained from long-term ethanol abusers were associated with remarkable ZO-1 mislocalization.[74] These data have been confirmed in vivo and ex vivo; for example, incorporation of PEth in membranes and disruption of ZO-1 and ZONAB localization was found to be correlated with increased cell proliferation in the colonic epithelium of mice (consuming 9.2 g/dL for 4 months) and in the adenomas of long-term ethanol consumers (30 g/d), respectively.[74] Although FAEEs have been found to possess cytotoxic activity in pancreatic cells[205,206] and liver cells,[207] research on their role in ethanol-induced barrier dysfunction has received little attention. However, it was recently shown in a Caco-2 three-dimensional cell culture model that exposure to ethyl ester oleate and ethyl ester palmitate at 20 μM or 40 μM concentrations can dose dependently induce ZO-1 and occludin redistribution and, consequently, a decrease in the paracellular barrier function.[181] Interestingly, these changes were partially attenuated by preincubation with the nutritional antioxidant resveratrol, pointing to the involvement of ROS generation in FAEE-induced intestinal barrier dysfunction.[181]

Modulation of tight junctions by microbiota

In addition to converting ethanol into acetaldehyde metabolically,[66] the intestinal microbiota can also modulate the TJs directly or indirectly via increased levels of lipopolysaccharide (LPS), an endotoxin derived from gram-negative bacteria.[208] As discussed previously, ethanol has been shown to alter the composition of the intestinal microbiota, which can result in increased levels of endotoxin.[124] Bode et al.[122] showed that ethanol can induce overgrowth of gram-negative anaerobic bacteria in the jejunum of alcoholics, predisposing them to increased levels of luminal LPS. Furthermore, short-term ethanol administration has been shown to increase plasma LPS levels approximately fivefold within 30–90 min in rats[209] and in mice,[210] whereas 4 weeks of ethanol feeding increases the plasma LPS levels 15-fold in rats.[211] In humans, long- and short-term ethanol consumption has been found to induce excessive and transient increases in blood LPS levels, respectively.[122,212,213] LPS has been shown to induce epithelial hyperpermeability via a poly (ADP-ribose) synthetase (PARS)-dependent mechanism[214] and MLCK activation.[215] Since increased levels of circulating LPS can potentiate an increase in intestinal permeability,[208] ethanol and LPS in combination may represent a two-hit insult on intestinal epithelial barrier integrity.

Intestinal bacteria can also modulate intestinal barrier integrity directly via mechanisms involving changes in TJ protein expression and distribution. The effects differ between bacterial strains. For example, enteropathogenic *E. coli* has been demonstrated to disrupt TJs in vitro[216] and to increase intestinal epithelial permeability in vivo.[217] In contrast, several probiotic bacteria, including *E. coli* strain Nissle 1917,[218,219] *Bifidobacterium infantis*,[220] and *Lactobacillus plantarum* MB452,[221] have been shown to promote intestinal barrier integrity in vitro by increasing expression of the ZO-2 and occludin proteins, by reducing expression of the claudin-2 protein, and by increasing transcription of the occludin genes. Furthermore, Karczewski et al.[222] have recently shown that administration of *Lactobacillus plantarum* WCFS1 into the duodenum of healthy human volunteers increases expression of ZO-1 and occludin in duodenal biopsies and protects against phorbol ester-induced dislocation of ZO-1 and occludin in vitro.

Studies investigating the role of the intestinal microbiota in ethanol-induced intestinal barrier dysfunction have been performed in rats and have reported that pretreatment with antibiotics can ameliorate ethanol-induced intestinal barrier dysfunction and the associated endotoxemia.[164,223] Modulation of gut microbiota by probiotics or prebiotics[13,224] in animals[127,225] and in humans[226] has been demonstrated to improve the intestinal barrier function. Therefore, the effects of probiotic or prebiotic treatment on intestinal barrier function after moderate and long-term ethanol consumption merit further investigation.

CONCLUSION

On the basis of currently existing knowledge, ethanol and its metabolites, including acetaldehyde, PEth, and FAEEs,

are considered to reach the entire GI tract, including the large intestine. The effects of ethanol and its metabolites on the intestine depend on various factors such as the food consumption pattern and host factors, e.g., sex, and the presence of gene polymorphisms for the enzymes involved in ethanol metabolism. Although ethanol by itself can be injurious, scientific evidence strongly points towards a very important role of its oxidative metabolite acetaldehyde, especially in intestinal barrier disruption and induction of colorectal cancers associated with ethanol consumption. In addition, ethanol has been shown to act synergistically with acetaldehyde, *E. coli*, and burn injury, resulting in more pronounced intestinal barrier dysfunction.

In vitro studies have led to exciting new information on the mechanisms of ethanol- and acetaldehyde-induced TJ disruption. However, detailed information about the precise mode of interaction between the TJ proteins complex and the mechanisms by which this TJ disruption can be (therapeutically) modulated or prevented is still lacking. So far, information on the effects of the nonoxidative metabolites, i.e., PEth and FAEEs, on intestinal epithelial barrier function is limited and has received little attention. The evidence so far, however, indicates that nonoxidative metabolites could be major modulators of epithelial permeability. Moreover, information on the effects of ethanol and acetaldehyde on other components of intestinal epithelium, such as mucin and mucin-secreting cells (i.e., goblet cells), is largely lacking. Since the intestinal microbiota plays a crucial role in the generation and accumulation of intracolonic acetaldehyde, and since ethanol and its metabolites can reach the colon via blood, mechanistic research on ethanol-induced colonic barrier dysfunction, taking into account the role of the gut microbiota, is warranted. Understanding the cellular and molecular mechanisms that mediate the effects of ethanol and its metabolites on intestinal barrier dysfunction may provide leads for therapeutic targets that can prevent or reverse ethanol-induced intestinal failure and subsequent liver injury.

Acknowledgments

Funding. This study was funded by Top Institute Food and Nutrition, Wageningen, The Netherlands.

Declaration of interest. The authors have no relevant interests to declare.

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