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Epithelial Transport in Inflammatory Bowel Diseases

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Abstract

The epithelium of the gastrointestinal tract is one of the most versatile tissues in the organism, responsible for providing a tight barrier between dietary and bacterial antigens and the mucosal and systemic immune system, while maintaining efficient digestive and absorptive processes to ensure adequate nutrient and energy supply. Inflammatory Bowel Diseases (IBD; Crohn's disease and ulcerative colitis) are associated with a breakdown of both functions, which in some cases are clearly interrelated. In this updated literature review, we focus on the effects of intestinal inflammation and the associated immune mediators on selected aspects of the transepithelial transport of macro- and micronutrients. The mechanisms responsible for nutritional deficiencies are not always clear and could be related to decreased intake, malabsorption and excess losses. We summarize the known causes of nutrient deficiencies and the mechanism of IBD-associated diarrhea. We also overview the consequences of impaired epithelial transport, which infrequently transcend its primary purpose to affect the gut microbial ecology and epithelial integrity. While some of those regulatory mechanisms are relatively well established, more work needs to be done to determine how inflammatory cytokines can alter the transport process of nutrients across the gastrointestinal and renal epithelia.

INTRODUCTION

The gastrointestinal tract is the most sophisticated immune organ of the human body. The intestinal epithelium, a single cell layer lining of the intestine, is evolutionarily the most ancient part of the innate immune system. It separates the essentially sterile host from the intestinal microbiota, which is one of the most densely populated microbial habitats known to science. The lack of a massive mucosal inflammatory response within this environment is not due to passive unresponsiveness, but rather due to an active process involving the gut-associated lymphoid tissue, lamina propria cells, and the intestinal epithelial cells (IECs). A tightly regulated immune balance has been shaped by the co-evolution between the host and gut microbiota and is essential for the integrity of the intestine and the overall health. Breakdown of the immune homeostasis can lead to inflammatory bowel disease (IBD) in one of its two main forms: Crohn's disease (CD) or ulcerative colitis (UC).

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Epithelial cell homeostasis is designed to provide a balance between efficient nutrient absorption while fending off potential pathogens and limiting the submucosal and systemic exposure to dietary and microbial antigens. Decreased barrier and transport function of epithelial cells has been observed in IBD and many other acute and chronic inflammatory diseases. Dysregulated epithelial barrier function and its role in the pathogenesis of IBD has been a subject of much attention and has been reviewed elsewhere (1, 2). We focus this review on alterations of transcellular nutrient transport, the involved carrier molecules and the consequences of their altered expression and/or activity in the course of chronic intestinal inflammation. In some instances, those alterations of transport protein complexes at the apical or basolateral membranes have consequences transcending their basal functions as nutrient or electrolyte transporters to modulate epithelial barrier function and gut microbiota, and may shape the immune response in the course of IBD.

EPITHELIAL TRANSPORT OF MACRONUTRIENTS IN IBD

Carbohydrates

Carbohydrate malabsorption has been associated with diarrhea in CD patients. Small intestinal inflammation and/or resection may lead to excessive entry of carbohydrates into the colon beyond the typical 25g observed in a healthy individual (3). Colonic bacterial fermentation products, including unabsorbed short-chain fatty acids (see later discussion in this review) and other organic anions and carbohydrates may cause osmotic diarrhea (4), and lead to accumulation of gas responsible for abdominal distension, pain, and flatulence. Malabsorption of lactose is often diagnosed in IBD patients (5), and lactose restriction frequently reduces symptoms. However, diarrhea usually does not subside completely, presumably because additional mechanisms remain active. Diagnosis of lactose malabsorption is usually confirmed by lactose hydrogen breath test, the presence of reducing substances, and fecal pH of less than 5.3. Since no primary effects of inflammation or inflammatory mediators on epithelial monosaccharide transport have been reported in IBD, the mechanisms involved may be limited to a reduction of hydrolase activity in the brush border membrane, bacterial overgrowth, and increased intestinal motility.

Amino acids

Data from experimental models of IBD evaluating pharmaconutrition with amino acids such as arginine, glutamine, glycine, cysteine, histidine, and taurine are promising and have been recently reviewed by Coëffier et al. (6). However, current clinical data do not support the use of amino acid supplementation in IBD. It remains to be investigated whether lack of clinical efficacy may be related to transport deficiencies or may simply require more thorough evaluation of dosage, amino acids combinations, better delivery and tissue targeting or their use in combination with standard medications. Here, we will briefly review the data on the epithelial transport of L-arginine and L-glutamine in relation to IBD pathogenesis and treatment.

L-Arginine (L-Arg)—L-Arg is a substrate for four enzymes, some of which exist as multiple isoforms: arginase, nitric oxide (NO) synthase (NOS), arginine: glycine amidinotransferase, and arginine decarboxylase. Similarly to glutamine discussed below, L-

Arg is a "conditionally essential" amino acid for which the demand may exceed the supply under condition of catabolic stress or organ dysfunction. L-Arg supplementation attenuated tissue damage during acute ischemic colitis and promoted healing of intestinal mucosa (7, 8), and improved the symptoms of DSS-induced colitis in mice (9). Interestingly, L-Arg treatment resulted in increased ex vivo migration of colonic epithelial cells, thus suggesting an increased capacity for wound repair. The protective effects of L-Arg were abolished in DSS-treated iNOS$^{-/-}$ mice (9).

Cellular uptake of L-Arg is mediated primarily by cationic amino acid transporter (CAT) family proteins, also known as y^+ transporters. CAT2 is the inducible transporter, while CAT1 is constitutively expressed. The role of CAT3 and CAT4 is poorly understood. In the study by Coburn et al. (9), DSS colitis resulted in increased tissue and serum levels of L-Arg, decreased expression of CAT1 and increased expression of CAT2, the latter further potentiated by L-Arg supplementation, which also improved the clinical parameters of DSS colitis (9). Moreover, in a more recent publication from this group, Singh et al. showed an increased susceptibility to DSS-induced mucosal injury and loss of the beneficial effects of L-Arg supplementation in CAT2$^{-/-}$ mice (10). It remains unclear what the relative contribution of CAT2 in macrophages and epithelial cells is. Although CAT2 is known as an inducible L-Arg transporter in macrophages, Singh et al. (11) also described that CAT2 can be upregulated in mouse colonic epithelial cells, and that the CAT2-mediated L-Arg uptake is essential for colonic epithelial wound repair. Although based on these and other pre-clinical studies, L-Arg has been considered an important component in the maintenance of immunophysiological homeostasis of the gut mucosa (12), its clinical efficacy in IBD patients remains to be established.

L-Glutamine (Gln)—L-Glutamine is a "conditionally essential" amino acid, which plays vital roles in the maintenance of mucosal integrity. In the small bowel mucosa, glutamine is a unique nutrient providing fuel for metabolism, regulating cell proliferation, repair and maintenance of the epithelial tight junctions and gut barrier (13). Its metabolism in the small bowel mucosa exceeds the rate of production during catabolic stress such as inflammation, trauma, sepsis, and post-surgery, and it has been suggested that glutamine may have beneficial effects during IBD, provided an adequate dose is established and the inflamed sites can be targeted (6). However, although some evidence from experimental models has been encouraging (14–16), the initial clinical studies with Gln supplementation in pediatric CD and adult CD and UC have been disappointing (17–20). However, glutamine mucosal content is decreased in non-inflamed, and more so in the inflamed ileum and colon from CD patients (21). Therefore it is plausible that reduced epithelial Gln transport may be impaired in Crohn's disease. While a small amount of Gln absorption in the mammalian enterocytes is mediated via Na^+-independent transport, the bulk of Gln is absorbed via the apical Na^+-dependent neutral amino acid co-transporters, a mechanism facilitated by the basolateral Na^+/K^+-ATPase. Most of these transporters, such as B(0)AT1 (SLC6A19) in villus cells and SN2 (SLC38A5) in crypt cells, have been cloned and functionally characterized. In a rabbit model of chronic ileitis induced by *Eimeria magna* oocytes, Na-dependent Gln uptake was inhibited in the villus cells, but enhanced in the crypts, and was normalized with anti-inflammatory treatment in both cases (22). In B(0)AT1-expressing IEC-6 cells, TNFα

inhibited Na^+-dependent Gln cotransport (23). Although the cytokine treatment did not affect the B(0)AT1 mRNA or protein expression, transport kinetics indicated TNF-dependent reduction of the cotransporter's affinity for Gln (23). It remains speculative whether a similar phenomenon takes place in the affected small intestine and/or ileum of CD patients.

Peptides—Since the original description of intact di- and tri-peptide transport in the intestinal and renal brush border membrane vesicles by Ganapathy and Leibach (24, 25), the SLC15 family of peptide transporters has been identified, cloned, thoroughly characterized, and recently comprehensively reviewed by Smith et al. (26). From among the four proton-coupled oligopeptide carriers - PepT1, PepT2, PhT1, and PhT2 -the high capacity, low affinity PepT1 trasporter plays a predominant role in the intestinal absorption of dipeptides and tripeptides, including zwitterionic, anionic and cationic peptides as well as a variety of peptide-like drugs. In the gut, PepT1 is abundantly expressed at the apical membrane of duodenal, jejunal, and ileal villous enterocytes, while negligible or no expression of PepT1 was described in the healthy colon (27–29). Although multiple studies demonstrated the importance of PepT1 in the epithelial peptide transport, PepT1 null knockout mice are phenotypically entirely normal, thus suggesting sufficient biological redundancy, presumably in form of excessive capacity for amino acid transport in the distal small intestine and colon (26). The activity of PepT1 depends on the proton gradient generated by epithelial apical Na^+/H^+ exchange. It can be modulated by indirect regulation of Na^+/H^+ exchange: stimulated by a calcium channel blocker (30) and inhibited by amiloride (31), and has been functionally linked to NHE3/NHX-2 in mammalian cells and *C. elegans* (32–34). A more detailed review on the various modes of PepT1 regulation has been recently published (35).

A genetic association was found between a functional SLC15A1 polymorphism (rs2297322, Ser117Asn) and disease susceptibility in two cohorts of Swedish and Finnish Crohn's patients (36). However, the genetic effects were opposite in the two populations (risk and protection in Swedish and Finnish patients, respectively), and the actual contribution of this SNP to the disease susceptibility remains questionable. The prevalent hypothesis about the role of PepT1 in the pathogenesis of IBD is related to its aberrantly induced colonic expression during inflammation (37, 38). This ectopic PepT1 expression may be driven by TNFα and IFN-γ (39, 40) and/or by leptin, also abnormally expressed in in inflamed tissues (41). Such upregulation could result in increased absorption of small bacterially-derived peptides, such as *N*-formylmethionyl-leucyl-phenylalanine (fMLP) (42), muramyl dipeptide (MDP) (43), or L-Ala-γ-D-Glu-meso-diaminopimelic acid (Tri-DAP) (44), and in the activation of inflammatory signaling pathways which lead to enhanced immune responses. Some of the described effects include increased MHC-I expression (37), NF-κB and AP-1 activation (45), and increased expression of neutrophil and monocyte chemoattractants - IL-8 and MCP-1 (CCL2) (43). Such enhanced innate response may exacerbate the symptoms of colitis in IBD. Indeed, transgenic mice overexpressing PepT1 in thee intestinal epithelial cells (*Villin-PepT1*) are more susceptible to DSS- (but not TNBS) induced colitis, an effect abolished by antibiotics and in transgenic mice on $NOD2^{-/-}$ background (46). The overall conclusion from these studies implicates increased transport of bacterial NOD2

ligands via the ectopically expressed PepT1 in the colonic epithelium to drive exacerbated inflammation in response to mucosal injury. Such an enhanced innate response, however, could prove protective in infectious colitis. Indeed, pathogenic bacteria such as enteropathogenic *E. coli* (EPEC) or *C. rodentium* (a murine attaching and effacing pathogen) induce expression of PepT1 in the colon (47), and *Villin-PepT1* transgenic mice showed reduced mucosal attachment of the pathogen, and attenuated symptoms of colitis. This finding was attributed to the modulation of lipid rafts as the site of bacterial adhesion via PepT1 rather than to the enhanced innate responses since PepT1 overexpression in colonocytes resulted to attenuated MAPK activation and IL-8 reduction in response to EPEC.

Interestingly, the concept of inducible expression of PepT1 in the colon has been recently challenged in a report by Wuensch et al. (48). This group demonstrated detectable apical expression of PEPT1 in the distal (but not proximal) colon in three strains of mice, in rats, and in humans under non-inflammatory conditions. In support of this, absorption of radiolabeled PepT1 substrate, Gly-Sar peptide, was higher in the everted sacs from the murine distal colon than in the proximal segment, and higher than in $PepT1^{-/-}$ mice. Moreover, the study also suggested an involvement of PepT1 in the colonic electrolyte and water transport in mice (48). The mechanism for increased water content in the stools of $PepT1^{-/-}$ mice, as well as the basis for these controversial findings remains to be elucidated. Nevertheless, due to the relatively broad array of dietary, microbial, and synthetic drug substrates for PepT1, the transporter continues to receive considerable attention as a potential therapeutic target (26, 49).

Short-chain fatty acids (SCFAs)—SCFAs produced by the bacterial fermentation of carbohydrate and fiber are the major regulators of colonic sodium transport and the preferred energy source for the colonic epithelium. Acetate, propionate, and butyrate account for approximately 95% of colonic SCFA contents, and butyrate alone has been estimated to provide 60% to 70% of the colonocyte's energy needs (50). SCFAs stimulate active electroneutral sodium absorption by enhancing Na^+/H^+ exchange via transcriptional and post-translational mechanisms (51–54). A wide spectrum of positive effects of butyrate on colonic functions, including inhibition of carcinogenesis, anti-inflammatory effects, modulation of oxidative stress, enhanced epithelial barrier function, and modulation of gene transcription via inhibition of histone deacetylase (HDAC) activity (55, 56), have made it an attractive target of investigation and a potential therapeutic in IBD. However, butyrate enemas have not become a mainstream treatment option in UC due in part to the inconsistent results in human UC intervention studies. These discrepancies may be partly explained by differences in treatment duration, differences in concentrations and volumes, and suboptimal study designs. Recent high profile studies demonstrating the effects of commensal-derived butyrate on FoxP3 promoter acetylation, on increased extrathymic regulatory T cell (Treg) differentiation, and on inhibition of T-cell mediated colitis via a GPR109a-dependent mechanism, may revive the field (57–59).

The supply and utilization of butyrate by the colonocytes in IBD may be affected at several levels. Butyrate synthesis by the commensal bacteria may be decreased secondary to changes in the gut microbial ecology (60, 61). Inhibition of SCFA oxidation by colonic

epithelial cells has been demonstrated *in vitro* and *in vivo* in UC, and was not only postulated to contribute to the chronicity of the disease (62), but also as a sufficient mechanism for induction of experimental colitis in rats (63). However, not all studies confirmed these observations (64, 65). Since the initial description of inhibited SCFA absorption in experimental colitis by Butzner et al. (66), a specific carrier protein involved in this process was identified as the monocarboxylate transporter 1 (MCT1; SLC5A8), which functions as an H^+-coupled electroneutral transporter (67, 68). A more recent study also suggested a significant contribution of the MCT4 isoform (SLC16A3), typically expressed at lower levels in the gut epithelium (69). MCT1 expression was found to be strongly downregulated in the intestinal mucosa of IBD patients (70), thus supporting the hypothesis of butyrate transport deficiency in IBD. In this study, MCT1 mRNA expression negatively correlated with the degree of inflammation and IL-1β mRNA expression, and a transcriptional regulation at the level of the basal gene promoter by inflammatory cytokines was implicated (70). Since MCT1 expression in the human intestine correlates with the epithelial cell capacity to oxidize butyrate, Thibault et al. have postulated that that the impairment of butyrate oxidation observed in IBD should be considered a consequence of the reduction in MCT1-mediated butyrate uptake (71). Butyrate normally leads to upregulation of MCT1 expression in colonocyte via a GPR109a-dependent sensing mechanism (72), it is therefore plausible that the relative loss of butyrate-producing commensal microorganisms in IBD may be at least in part responsible for decreased expression of MCT1, thus creating a compounded effect leading to exacerbated mucosal immune responses. Expression and activity of MCT4 in IBD has not been investigated.

EPITHELIAL ELECTROLYTE ABSORPTION: IBD-ASSOCIATED DIARRHEA AND BEYOND

The average daily luminal load of water and sodium in the gastrointestinal tract of an adult human amounts to ~9L and 800 mEq of Na^+. Less than 20% of it comes from ingestion, and over 80% represents secretory fluids (saliva, gastric and pancreatic juices, bile, and enteric secretions). A healthy gut is capable of absorbing more than 98% of this load, resulting in ~200g daily stool output. In healthy individuals, daily ileocecal flow is approximately 2 liters of electrolyte-rich fluid. Of this amount, 1.5–1.9 l is absorbed in the colon, although the maximal capacity of the human large intestine to absorb fluids may be as high as 5–6 l/day (73). Therefore, there is a large margin within which a healthy colon can compensate for increased ileocecal flow ensuing from defective small intestinal absorption. Exceeding this maximal capacity will result in diarrhea. On the other hand, in colonic disease, relatively small changes in water and electrolyte absorption will produce a significant increase in stool water output, emphasizing the relevance of fine-tuning of colonic transport processes. It is therefore not surprising, that diarrhea is one of the common symptoms in patients with IBD, occurring in about 50% of acute flare-ups of CD and in nearly all UC patients (74). The pathophysiology of diarrhea in IBD is multifactorial and involves both abnormal epithelial ion transport and altered intestinal motility. Several excellent reviews have been published in recent years covering the topic of IBD-associated diarrhea (75–77).

The development of diarrhea in IBD was initially believed to be caused by increased net secretion, a concept based on data from infectious models and in vitro observations that many inflammatory mediators stimulate active anion secretion. However, later observations demonstrated that epithelial transport is not stimulated but reduced. Sandle et al. (78) showed poor response to secretagogues and diminished Na^+ absorption in the colonic mucosa from IBD patients, in agreement with earlier studies with UC patients, where a profound decrease in the net absorption of Na^+ (and consequently of chloride and water) from the lumen of the colon was described (79, 80).

Several interconnected mechanisms are responsible for Na^+ (and water) absorption along the GI tract. Postprandial Na^+ absorption in the small intestine is driven by Na^+-dependent glucose and amino-acid symporters, while two other key non-nutrient dependent mechanisms in the small and large intestines involve electroneutral apical Na^+/H+ exchange (NHE) and epithelial Na^+ channels (ENaC). NHE, mediated primarily by the apical NHE3 isoform, is responsible for NaCl and bicarbonate absorption through coupled Na^+/H^+ and Cl^-/HCO_3^- (or Cl^-/OH^-) exchange, the latter mechanism mediated by PAT1 (SLC26A6) and down-regulated in adenoma (DRA; SLC26A3). Elelectrogenic Na^+ absorption carried by the epithelial Na^+ channels (ENaC, comprised of α,β,γ-ENaC subunits) takes place in the surface epithelium and upper crypts of the distal colon. All these transport mechanisms require a favorable electrochemical gradient maintained by the basolateral Na^+/K^+-ATPase, Cl^- channels (apical CFTR and basolateral CLC-2), and K^+ channels (Kir 7.1). Water passively follows the ion movement paracellularly, through the tight junctions, or transcellularly, through the cell membrane, in order to avoid the buildup of osmotic gradients.

Na^+/H^+ Exchange

In mammalian cells, tight pHi regulation is crucial for cell function and survival, as its subtle changes may significantly affect cellular physiology. Variations in pHi mediated by the NHE mechanism have been associated with basic biological processes such as proliferation, cell adhesion, and migration; cell volume regulation; and transepithelial electrolyte and water transport. From among the nine members of the mammalian Na+/H+ exchanger family, all except for NHE5 have been detected in the gastrointestinal tract, with segmental differences, crypt-villus gradients of expression, and different cellular localizations. Of those, NHE2 (SLC9A2), NHE3 (SLC9A3), and NHE8 (SLC9A8) have been detected on the apical membrane of the small intestinal and colonic epithelial cells (81). Gene targeting in mice implicated NHE2 primarily in the function of the gastric oxyntic mucosa (82), while deletion of NHE3 profoundly affected intestinal and renal Na^+ and water (re)absorption (83). Although NHE8 knockout mice showed no discernible diarrhea, potentially due to a compensatory increase in epithelial NHE2 and NHE3 expression, decreased colonic expression of MUC2 and mucin production as well as DRA expression was shown by Xu et al. (84).

IFN-γ leads to a decreased expression and function of NHE3 and NHE2 in vitro and in vivo (85). Also, enteropathogenic bacteria inhibit (86), whereas commensal *Lactobacillus acidophilus* upregulates intestinal NHE3 expression and function (87). Inhibition of NHE3

expression and activity has been described in several experimental models of colitis, including IL-2$^{-/-}$ mice (88), and in DSS- and TNBS-induced colitis (89). The latter study also described decreased NHE3 protein expression in sigmoid mucosal biopsies from most cases of active UC and/or CD, in ileal mucosal biopsies of active CD, as well as in ~50% of sigmoid biopsies from inactive UC or CD. In a study by Siddique et al. (90), NHE3 protein and activity were also reduced in both the untreated and treated patients with CD and UC. NHE3 mRNA was reduced only in CD, but not in patients with UC. Interestingly, in IL-10$^{-/-}$ mice, NHE3 activity, measured fluorometrically in apical enterocytes within isolated colonic crypts, was significantly decreased despite unaltered NHE3 expression and localization(91). The authors speculated that decreased expression of two key NHE3-regulatory PDZ adaptors NHERF2 and PDZK1 may be responsible for decreased NHE3 activity (91). Two other studies by Yeruva et al. (92) and Farkas et al. (93) demonstrated significant inhibition of NHE3 activity in patients with UC despite preserved protein expression and cellular localization.

While it is clear that the NHE3-mediated apical Na^+/H^+ exchange and epithelial Na^+ absorption are inhibited in IBD, the exact mechanism, which may depend on the specific disease, segment involved, and/or the severity of inflammation, remains unclear. On the other hand, work from our laboratory and by others has identified several consequences of NHE3 inhibition extending beyond epithelial electrolyte transport. We described that conventionally housed NHE3$^{-/-}$ mice maintained on the original mixed genetic background develop bacterially mediated, spontaneous distal colitis, which could be ameliorated by the oral administration of broad-spectrum antibiotics (94) and display remarkably high susceptibility to DSS-induced mucosal injury (95). We described increased bacterial adhesion by in situ hybridization and bacterial translocation by tissue gram staining in the distal colon of NHE3$^{-/-}$ mice (94), and Johansson et al. (96) recently confirmed changes in the mucous structure and bacterial penetrance in NHE3-deficient mice. In a recent study, we expanded upon the modulatory role of NHE3 in colitis by demonstrating that NHE3−/− mice develop microbial colonic dysbiosis reminiscent of that described in IBD patients, with increased *Proteobacteria* and decreased *Firmicutes*, including *Clostridia* clusters IV and XIVa (97). Similar changes were observed in the ileum of NHE3-deficient mice by Engevik et al. (98). In the latter study, expansion of *Bacteroides thetaiotaomicron* (*B. theta*), a commensal strongly immunogenic in Crohn's disease (99, 100), could be attributed to elevated luminal Na^+ concentration. Changes in the intestinal microbial ecology may be a prerequisite to the development of spontaneous colitis in NHE3$^{-/-}$ mice as their rederivation from conventional to an ultra-clean barrier facility eliminated the signs of colitis and decreased DSS susceptibility, whereas reintroduction of the conventional microflora resulted in the restoration of the symptoms initially described in the conventional environment (97).

Although the expression of NHE8 has not yet been analyzed in IBD patients, our group has shown that colonic mRNA expression is downregulated in vivo in TNBS-treated or LPS-treated rats and by TNFα in vitro in Caco-2 cells (101). Moreover, bacterial adherence in the distal colonic mucosa of NHE8$^{-/-}$ mice was increased, and NHE8 siRNA-mediated knockdown in Caco-2 cells increase the adherence of the pathogenic strain of *Salmonella typhimurium* but not of the probiotic strain *Lactobacillus plantarum* (102). Although the role

of NHE8 in IBD-associated diarrhea remains to be clearly established, the collective data from NHE3- and NHE8-deficient mice strongly suggest an important role that epithelial Na^+/H^+ exchange plays in the maintenance of the gut microbial ecology, microbial-host interactions, and ultimately in the pathogenesis of IBD in general.

Cl^-/HCO_3^- exchange

Luminal HCO_3^- is essential for mucosal protection by modulating the release and proper function of intestinal mucus and the intestinal wound healing. It is also crucial for intestinal electrolyte absorption. DRA (SLC26A3) is involved in duodenal HCO_3^- secretion and, coupled with NHE3, in jejunal NaCl absorption. DRA is also the dominant anion exchanger on the apical side of colonic epithelial cells, and its allelic variants have been implicated in the pathogenesis of congenital chloride diarrhea. In colonic crypts isolated from UC patients, Farkas et al. (93) observed significantly lower Cl^-/HCO_3^- exchange activity as compared to healthy controls. This was accompanied by ~50% reduction of SLC26A3 mRNA in UC. This observation was followed up in a mouse model of ileitis (TNFα overexpressing $TNF^{\Delta ARE}$ mice) by Xiao et al. (103). Decreased epithelial Cl^-/HCO_3^- exchange corresponded with downregulation of DRA mRNA and protein expression both in the ileum, and mildly inflamed mid-colon. In this model, the authors observed no significant changes in CFTR, NHE3, Na^+/HCO_3^- cotransporter NBC, or ENaC expression (103). Interestingly, an additional mechanism for the loss of HCO_3^- has been postulated by the same group (104), whereby cytokine-mediated increase epithelial permeability, irrespective of DRA expression, is sufficient for increased loss of HCO_3^- into the lumen. Molecular mechanisms in the transcriptional inhibition of DRA by IFN-γ have been exploited in more detail by Alrefai et al. (105) and Saksena et al. (106).

PAT1 (SLC23A6) is primarily expressed in the proximal small intestine, with very low or no expression in the proximal and distal colon, respectively (107). Although it has been shown to be transcriptionally inhibited by IFN-γ (108), there have been no reports of its inhibition in human CD or UC.

ENac

Expression and function of ENac was decreased in the distal colon of UC (93, 109, 110) and CD patients (89, 111). The mechanism implicated involves transcriptional inhibition of β and γ subunit expression by IFN-γ, TNFα, and IL-1β (109, 110, 112). In experimental models of IBD, reduction of EnaC subunit expression and/or colonic electrogenic sodium absorption was described in IL-$2^{-/-}$ mice (113), CCR$7^{-/-}$ mice (114), and in DSS- and TNBS-induced colitis (89).

Na^+/K^+ ATPase

Basolateral Na^+/K^+ ATPase couples the hydrolysis of 1 molecule of ATP to the translocation of 2 K^+ into and 3 Na^+ out of the cell against their electrochemical gradient. The pump complex consists of ubiquitous and essential α and β subunits, and an optional regulatory γ subunit (FXYD2), which is expressed throughout the GI tract with the exception of esophagus and anal mucosa. Na^+/K^+ ATPase is expressed all along the crypt surface–cell axis and is involved in a multitude of cellular functions. These include not only

the facilitation of membrane transport (through stabilization of the membrane potential and providing a driving force for Na^+-dependent transport) and cell volume (115), but also the assembly and maintenance of apical junction complexes and the cortical actin ring (116, 117), and the regulation of cell migration and signaling (118). Decreased Na^+/K^+ ATPase activity in the large intestine of pediatric and adult IBD patients has been described (119–121). Greig and Sandle (122) demonstrated that the decreased Na^+/K^+ ATPase-mediated basolateral Na^+ extrusion from the surface epithelial cells in the inflamed distal colon of UC patients is due to diminished basolateral expression of the $_1$ subunit protein without concomitant changes in its mRNA level. A follow-up study from this group extended this finding for both α_1 and β_1 subunits in the colonic mucosa of patients with mild to moderate UC (109). Similarly, Sullivan et al. (89) showed a decreased expression of the α subunit in colonic biopsies from patients with active UC or CD. Sugi et al. (123) showed that changes in the barrier function in IFN-γ-treated T-84 cells were accompanied with inhibition of Na^+/K^+ ATPase and decreased expression of the α- but not β-subunit protein. Similar data were obtained with SCID mice bearing human intestinal xenografts, in which human recombinant IFN-γ injection resulted in inhibition of the pump activity and decreased expression of the α- but not β-subunit protein expression (124). The exact mechanism behind the decreased basolateral expression of the α subunit is not known. Studies by Magro et al. (125) and Musch et al. (126) added an additional dimension to this phenomenon by demonstrating that Na^+/K^+ ATPase can be inhibited without altering the expression of the α or β subunit by IFN-γ in vitro, or by anti-CD3 antibody-induced T cell activation and TNFα in vivo, respectively. Although the mucosal expression of the α subunit of Na^+/K^+ ATPase was not altered in DSS- or TNBS-induced colitis (89), its activity has not been investigated in murine IBD models.

OTHER MICRONUTRIENTS

Micronutrient and vitamin deficiencies are relatively common among IBD patients, especially Crohn's Disease with active small bowel disease, or CD patients undergoing resection. Those deficiencies have been recently reviewed by Hwang et al. (127) and Alkhouri et al. (128), and Vavricka and Rogler (129). Recognizing the importance and the scope of micronutrient and vitamin transport in IBD, in this article, we focus only on selected micronutrients with more defined mechanisms of altered epithelial transport during disease.

Iron absorption in Inflammatory Bowel Disorders

Iron deficiency is the leading cause of anemia in IBD patients and presents in approximately 36 to 90 percent of patients (130). Iron deficiency anemia could be secondary to decreased dietary intake, blood loss in the gastrointestinal tract (specifically in patients with ulcerative colitis), and/or impaired absorption from the gastrointestinal tract. Iron absorption occurs primarily in the proximal duodenum and is driven by divalent metal transporter 1 (DMT1; SLC11A2). It is believed that inflammation, per se, may decrease the transport of iron across the duodenum, albeit no data indicating negative effects of inflammatory cytokines on DMT1 expression and activity have been published. Contrary to this hypothesis, in the bronchial epithelium, IFN-γ, TNFα, and LPS increased expression of DMT1 (131). Another

plausible, and not mutually exclusive hypothesis for iron deficiency anemia in IBD, is the anemia of inflammation (AI). This hypothesis implicates hypoferremia due to iron sequestration in the enterocytes, macrophages, and monocytes, driven by inflammatory cytokines – the most prominent of which is IL-6. Elevated concentration of inflammatory cytokines in the portal or systemic circulation results in upregulation of a hepcidin, a small antimicrobial peptide from the defensin family secreted by hepatocytes. Hepcidin binds to ferroportin, which leads to the internalization and lysosomal degradation of the latter. This results in a defect in the efflux of iron from the cell (including enterocytes and macrophages) to the systemic circulation (132). Experimental induction of hepcidin expression by turpentine injection in mice leads to profound hypoferremia, an effect abolished in hepcidin-deficient mice (133).

Confirming the role of hepcidin in IBD patients has not been straightforward. One potential confounding element is the fact that hepcidin expression drops in established hypoferremia. Indeed, serum concentration of hepcidin has been shown to be elevated in IBD patients in several studies, while others published contradictory results [recently reviewed by Dudkowiak et al. (134)]. Those discrepant findings may be related to the assay specificity and patient heterogeneity and iron status (135).

Zinc

Zinc is an essential trace mineral required for the catalytic activity of a number of enzymes including metalloproteinases, as well as for DNA synthesis required during wound healing. A number of studies have reported low zinc levels in patients with IBD (136–138), although contradicting data have also been reported (139). However, it is not clear whether the decrease in plasma zinc levels is related to a decreased dietary intake, increased losses, or a defect in the absorption of zinc throughout the gastrointestinal tract. ZIP4, encoded by the *SLC39A4* gene, is believed to be the major zinc transporter in the gastrointestinal tract responsible for adequate zinc balance and homeostasis in humans. However, its expression and activity has not been investigated in IBD patients or in experimental models. Recently, Suwendi et al. (140) showed that in DSS colitis in rats, serum zinc level was reduced regardless of dietary Zn concentration. Moreover, rats on a zinc-deficient diet showed exacerbated symptoms of colitis with increased TNFa production (140). Current knowledge, however limited, suggests that plasma Zn levels should be measured in IBD patients and used as a guide for supplementation with zinc sulfate.

Calcium

Calcium is an essential mineral required for a significant number of cellular functions and is a critical element in bone formation. Indeed, in patients with IBD, osteopenia and osteoporosis have been described in 40% to 50% of patients and the etiology of metabolic bone disease in IBD has been under investigation by several groups. The results implicate poor dietary intake of calcium and vitamin D, the negative effects of steroids (which are utilized in patients with IBD), the role of inflammatory cytokines on the intestinal and renal epithelial calcium transport and the resulting calcium losses, and the direct effects of inflammatory mediators on bone metabolism (141). It is recognized that calcium is transported throughout the small and large intestine via two mechanisms: a transcellular

process that predominates in the duodenum during low luminal calcium concentration and is strongly regulated by $1,25(OH)_2D_3$, and a paracellular process that occurs throughout the small and large intestine and is weakly dependent on $1,25(OH)_2D_3$.

Two members of the transient receptor potential family of calcium channels, TRPV6 and TRPV5 have been demonstrated as responsible for the majority of apical transcellular calcium absorption in the gastrointestinal tract and in the renal distal convoluted tubules. Calbindin D_{9K} and calcium pump PMCA1b serve as an intracellular buffer/shuttle and basolateral extrusion mechanisms in the enterocytes. In the renal epithelial cells, similar functions are provided by calbindin D_{28K} and Na^+/Ca^{2+} exchanger, NCX1. Joost Hoenderop's group in the Netherlands has shown decreased mRNA expression of TRPV6, calbindin D_{9K} and PMCA1b in the duodenal mucosa of $TNF^{\Delta ARE}$ mice, which develop chronic ileitis (142). In this model, renal calbindin D_{28K} and NCX1 transcripts were also significantly decreased, concomitantly with diminished serum $1,25(OH)_2D_3$ levels and reduced trabecular and cortical bone thickness (142). The authors postulated that decreased expression of calcium transporters combined with unchanged serum Ca^{2+} levels implicates Ca^{2+} loss from bone to compensate for the systemic negative Ca^{2+} balance. Our group has shown that the expression of Klotho, a protein that supports renal calcium re-absorption by sialic acid cleavage and stabilization of the TRPV5 channel on the apical membranes of the distal tubule epithelial cells is markedly decreased in several models of experimental colitis via a cytokine mediated transcriptional mechanism (143). This decrease in Klotho resulted in a markedly increased fractional urinary calcium excretion and reduced levels of TRPV5 protein, but not mRNA in distal convoluted tubules (144). We further showed that loss of Klotho expression leads to increased TRPV5 sialylation, endocytosis, UBR4-medated ubiquitination, and protein degradation. In vivo and in vitro experiments suggested that membrane-bound, but not soluble Klotho, is sufficient for the reversal of the cytokine effects on TRPV5 protein activity and expression (144). More recent studies from our lab also conformed the negative effects of intestinal inflammation on renal expression of NCX1 mRNA via a transcriptional mechanism, and also implicate Klotho in the mechanism of protection (Kiela & Ghishan; unpublished data).

Phosphorus

Inorganic phosphorus is a major component of the skeleton and a mediator of cell signaling and energy transfer, and it is intimately involved in a number of metabolic reactions within the cells. Intestinal phosphate absorption in mammals occurs primarily in the small intestine where it is dependent on the activity of the type 2b Na^+- dependent co-transporter, NaPi-IIb (SLC34A2). In the kidney, phosphate is re-absorbed primarily in the proximal convoluted tubules via a concerted action of NaPi-IIa (SLC34A1) and NaPi-IIc (SLC34A3). We have shown that the expression and activity of NaPi-IIb in the small intestine of TNBS-treated mice is reduced (145). We observed a similar reduction in TNFα-treated Caco-2 cells, and postulated that the TNFα-induced decrease of NaPi-IIb expression is mediated via the cytokine's interaction with the EGF receptor, activation of ERK1/2-dependent MAPK pathway, and NF1 transcription factor-dependent inhibition of the NaPi-IIb gene promoter activity (145). Renal expression and activity of NaPi-IIa or NaPi-IIc has not been

investigated, although our observations do not indicate increased fractional urinary phosphate excretion (Kiela & Ghishan; unpublished data).

Magnesium

Magnesium is the fourth most abundant cationic element in the body. It plays a fundamental role in most cellular reactions, mainly as a cofactor in enzymatic reactions involving ATP. 50%–60% of body magnesium is incorporated in the hydroxypatite crystals in the bone and may be important in bone metabolism, as implicated by several epidemiological studies suggesting that dietary magnesium and hypomagnesemia may be weakly associated with osteoporosis. Moreover, magnesium deficiency is known to induce hypocalcemia via impaired parathyroid gland function and inappropriately low PTH levels, which leads to lower intestinal calcium absorption. This mechanism may further potentiate the negative effects of inflammatory mediators on calcium absorption and bone metabolism. The primary sites of intestinal magnesium absorption vary between humans and animals. Indeed, in humans it has been shown that magnesium is absorbed primarily in the ileum and in the jejunum, whereas the colon was shown to be the major site for magnesium absorption in rats. Magnesium is absorbed by two mechanisms – by an active, saturable transport and by passive transcellular diffusion. The active process is driven by magnesium channels, which belong to the same transient receptor potential ion channel family as the above discussed epithelial Ca^{2+} channels. In the gut, TRPM6 and TRPM7 appear to be essential for the absorption of Mg^{2+}, while TRPM7 appears to also contribute to the pacemaker activity of the interstitial cells of Cajal, and TRPC4 transduces smooth muscle contraction evoked by muscarinic acetylcholine receptor activation. TRPM6 and TRPM7 can form heteromeric ion channels, and it remains unclear whether TRPM6 can also function on its own and whether TRPM7 is required for trafficking of TRPM6 to the plasma membrane. In humans, mutations in TRPM6 are associated with hypomagnesemia with secondary hypocalcemia, whereas $TRPM6^{-/-}$ mice do not survive beyond weaning (146, 147). TRPM7 gene deletion is embryonically lethal in mice (148)and TRPM6 deletion mice.

Magnesium deficiency has been reported to occur in 13%–88% of IBD patients (149–151) and is believed to be the result of a combination of decreased dietary intake, gastrointestinal losses from chronic diarrhea and fistula output, and malabsorption (149). It remains to be investigated whether there is a true epithelial Mg^{2+} transport defect in intestinal inflammation, and whether the TRPM6/7 expression and/or activity are affected by inflammatory mediators. Clinically, determining magnesium status (generally assessed by random serum magnesium level) should be carried out in patients with IBD followed by dietary supplementation as clinically indicated.

CONCLUSIONS

Patients with inflammatory bowel disorders suffer from numerous nutritional deficiencies, including generalized total calorie malnutrition and specific nutrient losses such as mineral, trace mineral, and vitamin deficiencies. The mechanisms responsible for these deficiencies could be related to decreased intake, malabsorption and excess losses. This review detailed many of these factors, however, it is clear that more work needs to be done to determine

how inflammatory cytokines can alter the transport process of nutrients across the gastrointestinal and renal epithelia.

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REFERENCES

1. Pastorelli L, De Salvo C, Mercado JR, et al. Central Role of the Gut Epithelial Barrier in the Pathogenesis of Chronic Intestinal Inflammation: Lessons Learned from Animal Models and Human Genetics. Frontiers in immunology. 2013; 4:280. [PubMed: 24062746]
2. Rescigno M. The intestinal epithelial barrier in the control of homeostasis and immunity. Trends in immunology. 2011; 32:256–264. [PubMed: 21565554]
3. Caspary WF. Diarrhoea associated with carbohydrate malabsorption. Clinics in gastroenterology. 1986; 15:631–655. [PubMed: 3742842]
4. Hammer HF, Fine KD, Santa Ana CA, et al. Carbohydrate malabsorption. Its measurement and its contribution to diarrhea. The Journal of clinical investigation. 1990; 86:1936–1944. [PubMed: 2254453]
5. Barrett JS, Irving PM, Shepherd SJ, et al. Comparison of the prevalence of fructose and lactose malabsorption across chronic intestinal disorders. Alimentary pharmacology & therapeutics. 2009; 30:165–174. [PubMed: 19392860]
6. Coeffier M, Marion-Letellier R, Dechelotte P. Potential for amino acids supplementation during inflammatory bowel diseases. Inflammatory bowel diseases. 2010; 16:518–524. [PubMed: 19572337]
7. Fotiadis C, Adamis S, Misiakos EP, et al. The prophylactic effect of L-arginine in acute ischaemic colitis in a rat model of ischaemia/reperfusion injury. Acta chirurgica Belgica. 2007; 107:192–200. [PubMed: 17515270]
8. Cross RK, Wilson KT. Nitric oxide in inflammatory bowel disease. Inflammatory bowel diseases. 2003; 9:179–189. [PubMed: 12792224]
9. Coburn LA, Gong X, Singh K, et al. L-arginine supplementation improves responses to injury and inflammation in dextran sulfate sodium colitis. PloS one. 2012; 7:e33546. [PubMed: 22428068]
10. Singh K, Coburn LA, Barry DP, et al. Deletion of cationic amino acid transporter 2 exacerbates dextran sulfate sodium colitis and leads to an IL-17-predominant T cell response. American journal of physiology Gastrointestinal and liver physiology. 2013; 305:G225–G240. [PubMed: 23703655]
11. Singh K, Coburn LA, Barry DP, et al. L-arginine uptake by cationic amino acid transporter 2 is essential for colonic epithelial cell restitution. American journal of physiology Gastrointestinal and liver physiology. 2012; 302:G1061–G1073. [PubMed: 22361732]
12. Fritz JH. Arginine cools the inflamed gut. Infection and immunity. 2013; 81:3500–3502. [PubMed: 23897606]
13. Rao RK, Samak G. Role of Glutamine in Protection of Intestinal Epithelial Tight Junctions. Journal of Epithelial Biology and Pharmacology. 2012; 5:47–54.
14. Xue H, Sufit AJ, Wischmeyer PE. Glutamine therapy improves outcome of in vitro and in vivo experimental colitis models. JPEN Journal of parenteral and enteral nutrition. 2011; 35:188–197. [PubMed: 21378248]
15. Vicario M, Amat C, Rivero M, et al. Dietary glutamine affects mucosal functions in rats with mild DSS-induced colitis. The Journal of nutrition. 2007; 137:1931–1937. [PubMed: 17634266]
16. Ameho CK, Adjei AA, Harrison EK, et al. Prophylactic effect of dietary glutamine supplementation on interleukin 8 and tumour necrosis factor alpha production in trinitrobenzene sulphonic acid induced colitis. Gut. 1997; 41:487–493. [PubMed: 9391247]

17. Akobeng AK, Miller V, Stanton J, et al. Double-blind randomized controlled trial of glutamine-enriched polymeric diet in the treatment of active Crohn's disease. Journal of pediatric gastroenterology and nutrition. 2000; 30:78–84. [PubMed: 10630444]

18. Akobeng AI, Clayton PE, Miller V, et al. Low serum concentrations of insulin-like growth factor-I in children with active Crohn disease: effect of enteral nutritional support and glutamine supplementation. Scandinavian journal of gastroenterology. 2002; 37:1422–1427. [PubMed: 12523592]

19. Akobeng AK, Richmond K, Miller V, et al. Effect of exclusive enteral nutritional treatment on plasma antioxidant concentrations in childhood Crohn's disease. Clinical nutrition. 2007; 26:51–56. [PubMed: 17161887]

20. Ockenga J, Borchert K, Stuber E, et al. Glutamine-enriched total parenteral nutrition in patients with inflammatory bowel disease. European journal of clinical nutrition. 2005; 59:1302–1309. [PubMed: 16077744]

21. Sido B, Seel C, Hochlehnert A, et al. Low intestinal glutamine level and low glutaminase activity in Crohn's disease: a rational for glutamine supplementation? Digestive diseases and sciences. 2006; 51:2170–2179. [PubMed: 17078002]

22. Arthur S, Saha P, Sundaram S, et al. Regulation of sodium-glutamine cotransport in villus and crypt cells by glucocorticoids during chronic enteritis. Inflammatory bowel diseases. 2012; 18:2149–2157. [PubMed: 22508450]

23. Talukder JR, Boyd B, Griffin A, et al. Inflammatory cytokine TNF-alpha inhibits Na(+)-glutamine cotransport in intestinal epithelial cells. Canadian journal of physiology and pharmacology. 2013; 91:275–284. [PubMed: 23627839]

24. Ganapathy, Leibach FH. Is intestinal peptide transport energized by a proton gradient? The American journal of physiology. 1985; 249:G153–G160. [PubMed: 2992286]

25. Ganapathy V, Leibach FH. Carrier-mediated reabsorption of small peptides in renal proximal tubule. The American journal of physiology. 1986; 251:F945–F953. [PubMed: 3538905]

26. Smith DE, Clemencon B, Hediger MA. Proton-coupled oligopeptide transporter family SLC15: physiological, pharmacological and pathological implications. Molecular aspects of medicine. 2013; 34:323–336. [PubMed: 23506874]

27. Groneberg DA, Doring F, Eynott PR, et al. Intestinal peptide transport: ex vivo uptake studies and localization of peptide carrier PEPT1. American journal of physiology Gastrointestinal and liver physiology. 2001; 281:G697–G704. [PubMed: 11518682]

28. Jappar D, Wu SP, Hu Y, et al. Significance and regional dependency of peptide transporter (PEPT) 1 in the intestinal permeability of glycylsarcosine: in situ single-pass perfusion studies in wild-type and Pept1 knockout mice. Drug metabolism and disposition: the biological fate of chemicals. 2010; 38:1740–1746. [PubMed: 20660104]

29. Walker D, Thwaites DT, Simmons NL, et al. Substrate upregulation of the human small intestinal peptide transporter, hPepT1. The Journal of physiology. 1998; 507(Pt 3):697–706. [PubMed: 9508831]

30. Wenzel U, Kuntz S, Diestel S, et al. PEPT1-mediated cefixime uptake into human intestinal epithelial cells is increased by Ca2+ channel blockers. Antimicrobial agents and chemotherapy. 2002; 46:1375–1380. [PubMed: 11959571]

31. Westphal JF, Jehl F, Brogard JM, et al. Amoxicillin intestinal absorption reduction by amiloride: possible role of the Na(+) -H+ exchanger. Clinical pharmacology and therapeutics. 1995; 57:257–264. [PubMed: 7697943]

32. Kennedy DJ, Leibach FH, Ganapathy V, et al. Optimal absorptive transport of the dipeptide glycylsarcosine is dependent on functional Na+/H+ exchange activity. Pflugers Archiv : European journal of physiology. 2002; 445:139–146. [PubMed: 12397398]

33. Watanabe C, Kato Y, Ito S, et al. Na+/H+ exchanger 3 affects transport property of H+/oligopeptide transporter 1. Drug metabolism and pharmacokinetics. 2005; 20:443–451. [PubMed: 16415530]

34. Benner J, Daniel H, Spanier B. A glutathione peroxidase, intracellular peptidases and the TOR complexes regulate peptide transporter PEPT-1 in C. elegans. PloS one. 2011; 6:e25624. [PubMed: 21980510]

35. Spanier B. Transcriptional and functional regulation of the intestinal peptide transporter PEPT1. The Journal of physiology. 2013
36. Zucchelli M, Torkvist L, Bresso F, et al. PepT1 oligopeptide transporter (SLC15A1) gene polymorphism in inflammatory bowel disease. Inflammatory bowel diseases. 2009; 15:1562–1569. [PubMed: 19462432]
37. Merlin D, Si-Tahar M, Sitaraman SV, et al. Colonic epithelial hPepT1 expression occurs in inflammatory bowel disease: transport of bacterial peptides influences expression of MHC class 1 molecules. Gastroenterology. 2001; 120:1666–1679. [PubMed: 11375948]
38. Wojtal KA, Eloranta JJ, Hruz P, et al. Changes in mRNA expression levels of solute carrier transporters in inflammatory bowel disease patients. Drug metabolism and disposition: the biological fate of chemicals. 2009; 37:1871–1877. [PubMed: 19487253]
39. Buyse M, Charrier L, Sitaraman S, et al. Interferon-gamma increases hPepT1-mediated uptake of di-tripeptides including the bacterial tripeptide fMLP in polarized intestinal epithelia. The American journal of pathology. 2003; 163:1969–1977. [PubMed: 14578196]
40. Vavricka SR, Musch MW, Fujiya M, et al. Tumor necrosis factor-alpha and interferon-gamma increase PepT1 expression and activity in the human colon carcinoma cell line Caco-2/bbe and in mouse intestine. Pflugers Archiv : European journal of physiology. 2006; 452:71–80. [PubMed: 16328452]
41. Nduati V, Yan Y, Dalmasso G, et al. Leptin transcriptionally enhances peptide transporter (hPepT1) expression and activity via the cAMP-response element-binding protein and Cdx2 transcription factors. The Journal of biological chemistry. 2007; 282:1359–1373. [PubMed: 16963449]
42. Merlin D, Steel A, Gewirtz AT, et al. hPepT1-mediated epithelial transport of bacteria-derived chemotactic peptides enhances neutrophil-epithelial interactions. The Journal of clinical investigation. 1998; 102:2011–2018. [PubMed: 9835627]
43. Vavricka SR, Musch MW, Chang JE, et al. hPepT1 transports muramyl dipeptide, activating NF-kappaB and stimulating IL-8 secretion in human colonic Caco2/bbe cells. Gastroenterology. 2004; 127:1401–1409. [PubMed: 15521010]
44. Dalmasso G, Nguyen HT, Charrier-Hisamuddin L, et al. PepT1 mediates transport of the proinflammatory bacterial tripeptide L-Ala-{gamma}-D-Glu-meso-DAP in intestinal epithelial cells. American journal of physiology Gastrointestinal and liver physiology. 2010; 299:G687–G696. [PubMed: 20558765]
45. Buyse M, Tsocas A, Walker F, et al. PepT1-mediated fMLP transport induces intestinal inflammation in vivo. American journal of physiology Cell physiology. 2002; 283:C1795–C1800. [PubMed: 12419711]
46. Dalmasso G, Nguyen HT, Ingersoll SA, et al. The PepT1-NOD2 signaling pathway aggravates induced colitis in mice. Gastroenterology. 2011; 141:1334–1345. [PubMed: 21762661]
47. Nguyen HT, Dalmasso G, Powell KR, et al. Pathogenic bacteria induce colonic PepT1 expression: an implication in host defense response. Gastroenterology. 2009; 137:1435–1447. e1431–e1432. [PubMed: 19549526]
48. Wuensch T, Schulz S, Ullrich S, et al. The peptide transporter PEPT1 is expressed in distal colon in rodents and humans and contributes to water absorption. American journal of physiology Gastrointestinal and liver physiology. 2013; 305:G66–G73. [PubMed: 23660505]
49. Ingersoll SA, Ayyadurai S, Charania MA, et al. The role and pathophysiological relevance of membrane transporter PepT1 in intestinal inflammation and inflammatory bowel disease. American journal of physiology Gastrointestinal and liver physiology. 2012; 302:G484–G492. [PubMed: 22194420]
50. Bergman EN. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiological reviews. 1990; 70:567–590. [PubMed: 2181501]
51. Kiela PR, Kuscuoglu N, Midura AJ, et al. Molecular mechanism of rat NHE3 gene promoter regulation by sodium butyrate. American journal of physiology Cell physiology. 2007; 293:C64–C74. [PubMed: 17344314]

52. Krishnan S, Rajendran VM, Binder HJ. Apical NHE isoforms differentially regulate butyrate-stimulated Na absorption in rat distal colon. American journal of physiology Cell physiology. 2003; 285:C1246–C1254. [PubMed: 12878493]
53. Kiela PR, Hines ER, Collins JF, et al. Regulation of the rat NHE3 gene promoter by sodium butyrate. American journal of physiology Gastrointestinal and liver physiology. 2001; 281:G947–G956. [PubMed: 11557515]
54. Musch MW, Bookstein C, Xie Y, et al. SCFA increase intestinal Na absorption by induction of NHE3 in rat colon and human intestinal C2/bbe cells. American journal of physiology Gastrointestinal and liver physiology. 2001; 280:G687–G693. [PubMed: 11254495]
55. Hamer HM, Jonkers D, Venema K, et al. Review article: the role of butyrate on colonic function. Alimentary pharmacology & therapeutics. 2008; 27:104–119. [PubMed: 17973645]
56. Davie JR. Inhibition of histone deacetylase activity by butyrate. The Journal of nutrition. 2003; 133:2485S–2493S. [PubMed: 12840228]
57. Arpaia N, Campbell C, Fan X, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature. 2013; 504:451–455. [PubMed: 24226773]
58. Furusawa Y, Obata Y, Fukuda S, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature. 2013; 504:446–450. [PubMed: 24226770]
59. Singh N, Gurav A, Sivaprakasam S, et al. Activation of gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. Immunity. 2014; 40:128–139. [PubMed: 24412617]
60. Wang W, Chen L, Zhou R, et al. Increased Proportion of Bifidobacterium and the Lactobacillus group and Loss of Butyrate-producing Bacteria in Inflammatory Bowel Disease. Journal of clinical microbiology. 2013
61. Machiels K, Joossens M, Sabino J, et al. A decrease of the butyrate-producing species Roseburia hominis and Faecalibacterium prausnitzii defines dysbiosis in patients with ulcerative colitis. Gut. 2013
62. Roediger WE. The colonic epithelium in ulcerative colitis: an energy-deficiency disease? Lancet. 1980; 2:712–715. [PubMed: 6106826]
63. Roediger WE, Nance S. Metabolic induction of experimental ulcerative colitis by inhibition of fatty acid oxidation. British journal of experimental pathology. 1986; 67:773–782. [PubMed: 3099821]
64. Jorgensen J, Mortensen PB. Substrate utilization by intestinal mucosal tissue strips from patients with inflammatory bowel disease. American journal of physiology Gastrointestinal and liver physiology. 2001; 281:G405–G411. [PubMed: 11447021]
65. Finnie IA, Taylor BA, Rhodes JM. Ileal and colonic epithelial metabolism in quiescent ulcerative colitis: increased glutamine metabolism in distal colon but no defect in butyrate metabolism. Gut. 1993; 34:1552–1558. [PubMed: 8244143]
66. Butzner JD, Meddings JB, Dalal V. Inhibition of short-chain fatty acid absorption and Na+ absorption during acute colitis in the rabbit. Gastroenterology. 1994; 106:1190–1198. [PubMed: 8174882]
67. Hadjiagapiou C, Schmidt L, Dudeja PK, et al. Mechanism(s) of butyrate transport in Caco-2 cells: role of monocarboxylate transporter 1. American journal of physiology Gastrointestinal and liver physiology. 2000; 279:G775–G780. [PubMed: 11005765]
68. Tamai I, Takanaga H, Maeda H, et al. Participation of a proton-cotransporter, MCT1, in the intestinal transport of monocarboxylic acids. Biochemical and biophysical research communications. 1995; 214:482–489. [PubMed: 7677755]
69. Kekuda R, Manoharan P, Baseler W, et al. Monocarboxylate 4 mediated butyrate transport in a rat intestinal epithelial cell line. Digestive diseases and sciences. 2013; 58:660–667. [PubMed: 23344966]
70. Thibault R, De Coppet P, Daly K, et al. Down-regulation of the monocarboxylate transporter 1 is involved in butyrate deficiency during intestinal inflammation. Gastroenterology. 2007; 133:1916–1927. [PubMed: 18054563]

71. Thibault R, Blachier F, Darcy-Vrillon B, et al. Butyrate utilization by the colonic mucosa in inflammatory bowel diseases: a transport deficiency. Inflammatory bowel diseases. 2010; 16:684–695. [PubMed: 19774643]
72. Borthakur A, Priyamvada S, Kumar A, et al. A novel nutrient sensing mechanism underlies substrate-induced regulation of monocarboxylate transporter-1. American journal of physiology Gastrointestinal and liver physiology. 2012; 303:G1126–G1133. [PubMed: 22982338]
73. Debongnie JC, Phillips SF. Capacity of the human colon to absorb fluid. Gastroenterology. 1978; 74:698–703. [PubMed: 631507]
74. Seidler U, Lenzen H, Cinar A, et al. Molecular mechanisms of disturbed electrolyte transport in intestinal inflammation. Annals of the New York Academy of Sciences. 2006; 1072:262–275. [PubMed: 17057206]
75. Binder HJ. Mechanisms of diarrhea in inflammatory bowel diseases. Annals of the New York Academy of Sciences. 2009; 1165:285–293. [PubMed: 19538318]
76. Tang Y, Forsyth CB, Keshavarzian A. New molecular insights into inflammatory bowel disease-induced diarrhea. Expert review of gastroenterology & hepatology. 2011; 5:615–625. [PubMed: 21910579]
77. Wenzl HH. Diarrhea in chronic inflammatory bowel diseases. Gastroenterology clinics of North America. 2012; 41:651–675. [PubMed: 22917170]
78. Sandle GI, Higgs N, Crowe P, et al. Cellular basis for defective electrolyte transport in inflamed human colon. Gastroenterology. 1990; 99:97–105. [PubMed: 2344946]
79. Harris J, Shields R. Absorption and secretion of water and electrolytes by the intact human colon in diffuse untreated proctocolitis. Gut. 1970; 11:27–33. [PubMed: 5435265]
80. Rask-Madsen J, Hammersgaard EA, Knudsen E. Rectal electrolyte transport and mucosal permeability in ulcerative colitis and Crohn's disease. The Journal of laboratory and clinical medicine. 1973; 81:342–353. [PubMed: 4631401]
81. Kiela PR, Xu H, Ghishan FK. Apical NA+/H+ exchangers in the mammalian gastrointestinal tract. Journal of physiology and pharmacology : an official journal of the Polish Physiological Society. 2006; 57(Suppl 7):51–79. [PubMed: 17228096]
82. Schultheis PJ, Clarke LL, Meneton P, et al. Targeted disruption of the murine Na+/H+ exchanger isoform 2 gene causes reduced viability of gastric parietal cells and loss of net acid secretion. The Journal of clinical investigation. 1998; 101:1243–1253. [PubMed: 9502765]
83. Schultheis PJ, Clarke LL, Meneton P, et al. Renal and intestinal absorptive defects in mice lacking the NHE3 Na+/H+ exchanger. Nature genetics. 1998; 19:282–285. [PubMed: 9662405]
84. Xu H, Zhang B, Li J, et al. Impaired mucin synthesis and bicarbonate secretion in the colon of NHE8 knockout mice. American journal of physiology Gastrointestinal and liver physiology. 2012; 303:G335–G343. [PubMed: 22575219]
85. Rocha F, Musch MW, Lishanskiy L, et al. IFN-gamma downregulates expression of Na(+)/H(+) exchangers NHE2 and NHE3 in rat intestine and human Caco-2/bbe cells. American journal of physiology Cell physiology. 2001; 280:C1224–C1232. [PubMed: 11287336]
86. Hecht G, Hodges K, Gill RK, et al. Differential regulation of Na+/H+ exchange isoform activities by enteropathogenic E. coli in human intestinal epithelial cells. American journal of physiology Gastrointestinal and liver physiology. 2004; 287:G370–G378. [PubMed: 15075254]
87. Singh V, Raheja G, Borthakur A, et al. Lactobacillus acidophilus upregulates intestinal NHE3 expression and function. American journal of physiology Gastrointestinal and liver physiology. 2012; 303:G1393–G1401. [PubMed: 23086913]
88. Barmeyer C, Harren M, Schmitz H, et al. Mechanisms of diarrhea in the interleukin-2-deficient mouse model of colonic inflammation. American journal of physiology Gastrointestinal and liver physiology. 2004; 286:G244–G252. [PubMed: 14715519]
89. Sullivan S, Alex P, Dassopoulos T, et al. Downregulation of sodium transporters and NHERF proteins in IBD patients and mouse colitis models: potential contributors to IBD-associated diarrhea. Inflammatory bowel diseases. 2009; 15:261–274. [PubMed: 18942765]
90. Siddique I, Hasan F, Khan I. Suppression of Na+/H+ exchanger isoform-3 in human inflammatory bowel disease: lack of reversal by 5'-aminosalicylate treatment. Scandinavian journal of gastroenterology. 2009; 44:56–64. [PubMed: 18785066]

91. Lenzen H, Lunnemann M, Bleich A, et al. Downregulation of the NHE3-binding PDZ-adaptor protein PDZK1 expression during cytokine-induced inflammation in interleukin-10-deficient mice. PloS one. 2012; 7:e40657. [PubMed: 22848392]
92. Yeruva S, Farkas K, Hubricht J, et al. Preserved Na(+)/H(+) exchanger isoform 3 expression and localization, but decreased NHE3 function indicate regulatory sodium transport defect in ulcerative colitis. Inflammatory bowel diseases. 2010; 16:1149–1161. [PubMed: 20027604]
93. Farkas K, Yeruva S, Rakonczay Z Jr, et al. New therapeutic targets in ulcerative colitis: the importance of ion transporters in the human colon. Inflammatory bowel diseases. 2011; 17:884–898. [PubMed: 20722063]
94. Laubitz D, Larmonier CB, Bai A, et al. Colonic gene expression profile in NHE3-deficient mice: evidence for spontaneous distal colitis. American journal of physiology Gastrointestinal and liver physiology. 2008; 295:G63–G77. [PubMed: 18467500]
95. Kiela PR, Laubitz D, Larmonier CB, et al. Changes in mucosal homeostasis predispose NHE3 knockout mice to increased susceptibility to DSS-induced epithelial injury. Gastroenterology. 2009; 137:965–975. 975 e961-910. [PubMed: 19450596]
96. Johansson ME, Gustafsson JK, Holmen-Larsson J, et al. Bacteria penetrate the normally impenetrable inner colon mucus layer in both murine colitis models and patients with ulcerative colitis. Gut. 2014; 63:281–291. [PubMed: 23426893]
97. Larmonier CB, Laubitz D, Hill FM, et al. Reduced Colonic Microbial Diversity is Associated with Colitis in NHE3-deficient mice. American journal of physiology Gastrointestinal and liver physiology. 2013
98. Engevik MA, Aihara E, Montrose MH, et al. Loss of NHE3 alters gut microbiota composition and influences Bacteroides thetaiotaomicron growth. American journal of physiology Gastrointestinal and liver physiology. 2013; 305:G697–G711. [PubMed: 24072680]
99. Edwards LA, Lucas M, Edwards EA, et al. Aberrant response to commensal Bacteroides thetaiotaomicron in Crohn's disease: an ex vivo human organ culture study. Inflammatory bowel diseases. 2011; 17:1201–1208. [PubMed: 21484962]
100. Hansen JJ, Huang Y, Peterson DA, et al. The colitis-associated transcriptional profile of commensal Bacteroides thetaiotaomicron enhances adaptive immune responses to a bacterial antigen. PloS one. 2012; 7:e42645. [PubMed: 22880065]
101. Xu H, Chen H, Dong J, et al. Tumor necrosis factor-{alpha} downregulates intestinal NHE8 expression by reducing basal promoter activity. American journal of physiology Cell physiology. 2009; 296:C489–C497. [PubMed: 19109523]
102. Liu C, Xu H, Zhang B, et al. NHE8 plays an important role in mucosal protection via its effect on bacterial adhesion. American journal of physiology Cell physiology. 2013; 305:C121–C128. [PubMed: 23657568]
103. Xiao F, Juric M, Li J, et al. Loss of downregulated in adenoma (DRA) impairs mucosal HCO3(−) secretion in murine ileocolonic inflammation. Inflammatory bowel diseases. 2012; 18:101–111. [PubMed: 21557395]
104. Juric M, Xiao F, Amasheh S, et al. Increased epithelial permeability is the primary cause for bicarbonate loss in inflamed murine colon. Inflammatory bowel diseases. 2013; 19:904–911. [PubMed: 23502355]
105. Alrefai WA, Wen X, Jiang W, et al. Molecular cloning and promoter analysis of downregulated in adenoma (DRA). American journal of physiology Gastrointestinal and liver physiology. 2007; 293:G923–G934. [PubMed: 17761837]
106. Saksena S, Singla A, Goyal S, et al. Mechanisms of transcriptional modulation of the human anion exchanger SLC26A3 gene expression by IFN-{gamma}. American journal of physiology Gastrointestinal and liver physiology. 2010; 298:G159–G166. [PubMed: 19940027]
107. Wang Z, Petrovic S, Mann E, et al. Identification of an apical Cl(−)/HCO3(−) exchanger in the small intestine. American journal of physiology Gastrointestinal and liver physiology. 2002; 282:G573–G579. [PubMed: 11842009]
108. Saksena S, Dwivedi A, Singla A, et al. Characterization of the 5'-flanking region and regulation of expression of human anion exchanger SLC26A6. Journal of cellular biochemistry. 2008; 105:454–466. [PubMed: 18655181]

109. Greig ER, Boot-Handford RP, Mani V, et al. Decreased expression of apical Na+ channels and basolateral Na+, K+-ATPase in ulcerative colitis. The Journal of pathology. 2004; 204:84–92. [PubMed: 15307141]
110. Amasheh S, Barmeyer C, Koch CS, et al. Cytokine-dependent transcriptional down-regulation of epithelial sodium channel in ulcerative colitis. Gastroenterology. 2004; 126:1711–1720. [PubMed: 15188166]
111. Zeissig S, Bergann T, Fromm A, et al. Altered ENaC expression leads to impaired sodium absorption in the noninflamed intestine in Crohn's disease. Gastroenterology. 2008; 134:1436–1447. [PubMed: 18355814]
112. Barmeyer C, Amasheh S, Tavalali S, et al. IL-1beta and TNFalpha regulate sodium absorption in rat distal colon. Biochemical and biophysical research communications. 2004; 317:500–507. [PubMed: 15063785]
113. Barmeyer C, Horak I, Zeitz M, et al. The interleukin-2-deficient mouse model. Pathobiology : journal of immunopathology, molecular and cellular biology. 2002; 70:139–142.
114. Schumann M, Winter S, Wichner K, et al. CCR7 deficiency causes diarrhea associated with altered ion transport in colonocytes in the absence of overt colitis. Mucosal immunology. 2012; 5:377–387. [PubMed: 22395421]
115. Bachmann O, Juric M, Seidler U, et al. Basolateral ion transporters involved in colonic epithelial electrolyte absorption, anion secretion and cellular homeostasis. Acta physiologica. 2011; 201:33–46. [PubMed: 20528802]
116. Vagin O, Dada LA, Tokhtaeva E, et al. The Na-K-ATPase alpha(1)beta(1) heterodimer as a cell adhesion molecule in epithelia. American journal of physiology Cell physiology. 2012; 302:C1271–C1281. [PubMed: 22277755]
117. Rajasekaran SA, Rajasekaran AK. Na, K-ATPase and epithelial tight junctions. Frontiers in bioscience. 2009; 14:2130–2148.
118. Rajasekaran SA, Barwe SP, Rajasekaran AK. Multiple functions of Na, K-ATPase in epithelial cells. Seminars in nephrology. 2005; 25:328–334. [PubMed: 16139688]
119. Rachmilewitz D, Karmeli F, Sharon P. Decreased colonic Na-K-ATPase activity in active ulcerative colitis. Israel journal of medical sciences. 1984; 20:681–684. [PubMed: 6088428]
120. Allgayer H, Kruis W, Paumgartner G, et al. Inverse relationship between colonic (Na+ + K+)-ATPase activity and degree of mucosal inflammation in inflammatory bowel disease. Digestive diseases and sciences. 1988; 33:417–422. [PubMed: 2832138]
121. Ejderhamn J, Finkel Y, Strandvik B. Na, K-ATPase activity in rectal mucosa of children with ulcerative colitis and Crohn's disease. Scandinavian journal of gastroenterology. 1989; 24:1121–1125. [PubMed: 2556782]
122. Greig E, Sandle GI. Diarrhea in ulcerative colitis. The role of altered colonic sodium transport. Annals of the New York Academy of Sciences. 2000; 915:327–332. [PubMed: 11193595]
123. Sugi K, Musch MW, Field M, et al. Inhibition of Na+, K+-ATPase by interferon gamma down-regulates intestinal epithelial transport and barrier function. Gastroenterology. 2001; 120:1393–1403. [PubMed: 11313309]
124. Bertelsen LS, Eckmann L, Barrett KE. Prolonged interferon-gamma exposure decreases ion transport, NKCC1, and Na+-K+-ATPase expression in human intestinal xenografts in vivo. American journal of physiology Gastrointestinal and liver physiology. 2004; 286:G157–G165. [PubMed: 12958023]
125. Magro F, Fraga S, Ribeiro T, et al. Intestinal Na+-K+-ATPase activity and molecular events downstream of interferon-gamma receptor stimulation. British journal of pharmacology. 2004; 142:1281–1292. [PubMed: 15277314]
126. Musch MW, Clarke LL, Mamah D, et al. T cell activation causes diarrhea by increasing intestinal permeability and inhibiting epithelial Na+/K+-ATPase. The Journal of clinical investigation. 2002; 110:1739–1747. [PubMed: 12464679]
127. Hwang C, Ross V, Mahadevan U. Micronutrient deficiencies in inflammatory bowel disease: from A to zinc. Inflammatory bowel diseases. 2012; 18:1961–1981. [PubMed: 22488830]

128. Alkhouri RH, Hashmi H, Baker RD, et al. Vitamin and mineral status in patients with inflammatory bowel disease. Journal of pediatric gastroenterology and nutrition. 2013; 56:89–92. [PubMed: 22832510]
129. Vavricka SR, Rogler G. Intestinal absorption and vitamin levels: is a new focus needed? Digestive diseases. 2012; 30(Suppl 3):73–80. [PubMed: 23295695]
130. Gasche C, Lomer MC, Cavill I, et al. Iron, anaemia, and inflammatory bowel diseases. Gut. 2004; 53:1190–1197. [PubMed: 15247190]
131. Wang X, Garrick MD, Yang F, et al. TNF, IFN-gamma, and endotoxin increase expression of DMT1 in bronchial epithelial cells. American journal of physiology Lung cellular and molecular physiology. 2005; 289:L24–L33. [PubMed: 15749739]
132. Nemeth E, Rivera S, Gabayan V, et al. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. The Journal of clinical investigation. 2004; 113:1271–1276. [PubMed: 15124018]
133. Nicolas G, Chauvet C, Viatte L, et al. The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation. The Journal of clinical investigation. 2002; 110:1037–1044. [PubMed: 12370282]
134. Dudkowiak R, Neubauer K, Poniewierka E. Hepcidin and its role in inflammatory bowel disease. Advances in clinical and experimental medicine : official organ Wroclaw Medical University. 2013; 22:585–591. [PubMed: 23986220]
135. Bergamaschi G, Di Sabatino A, Albertini R, et al. Serum hepcidin in inflammatory bowel diseases: biological and clinical significance. Inflammatory bowel diseases. 2013; 19:2166–2172. [PubMed: 23867871]
136. Vagianos K, Bector S, McConnell J, et al. Nutrition assessment of patients with inflammatory bowel disease. JPEN Journal of parenteral and enteral nutrition. 2007; 31:311–319. [PubMed: 17595441]
137. Ojuawo A, Keith L. The serum concentrations of zinc, copper and selenium in children with inflammatory bowel disease. The Central African journal of medicine. 2002; 48:116–119. [PubMed: 14562534]
138. Matsui T. Zinc deficiency in Crohn's disease. Journal of gastroenterology. 1998; 33:924–925. [PubMed: 9853577]
139. Ringstad J, Kildebo S, Thomassen Y. Serum selenium, copper, and zinc concentrations in Crohn's disease and ulcerative colitis. Scandinavian journal of gastroenterology. 1993; 28:605–608. [PubMed: 8362213]
140. Suwendi E, Iwaya H, Lee JS, et al. Zinc deficiency induces dysregulation of cytokine productions in an experimental colitis of rats. Biomedical research. 2012; 33:329–336. [PubMed: 23268956]
141. Ghishan FK, Kiela PR. Advances in the understanding of mineral and bone metabolism in inflammatory bowel diseases. American journal of physiology Gastrointestinal and liver physiology. 2011; 300:G191–G201. [PubMed: 21088237]
142. Huybers S, Apostolaki M, van der Eerden BC, et al. Murine TNF(DeltaARE) Crohn's disease model displays diminished expression of intestinal Ca2+ transporters. Inflammatory bowel diseases. 2008; 14:803–811. [PubMed: 18266230]
143. Thurston RD, Larmonier CB, Majewski PM, et al. Tumor necrosis factor and interferon-gamma down-regulate Klotho in mice with colitis. Gastroenterology. 2010; 138:1384–1394. 1394 e1381–1394 e1382. [PubMed: 20004202]
144. Radhakrishnan VM, Ramalingam R, Larmonier CB, et al. Post-translational loss of renal TRPV5 calcium channel expression, Ca(2+) wasting, and bone loss in experimental colitis. Gastroenterology. 2013; 145:613–624. [PubMed: 23747339]
145. Chen H, Xu H, Dong J, et al. Tumor necrosis factor-alpha impairs intestinal phosphate absorption in colitis. American journal of physiology Gastrointestinal and liver physiology. 2009; 296:G775–G781. [PubMed: 19196948]
146. Walder RY, Landau D, Meyer P, et al. Mutation of TRPM6 causes familial hypomagnesemia with secondary hypocalcemia. Nature genetics. 2002; 31:171–174. [PubMed: 12032570]
147. Walder RY, Yang B, Stokes JB, et al. Mice defective in Trpm6 show embryonic mortality and neural tube defects. Human molecular genetics. 2009; 18:4367–4375. [PubMed: 19692351]

148. Jin J, Desai BN, Navarro B, et al. Deletion of Trpm7 disrupts embryonic development and thymopoiesis without altering Mg2+ homeostasis. Science. 2008; 322:756–760. [PubMed: 18974357]

149. Hessov I. Magnesium deficiency in Crohn's disease. Clinical nutrition. 1990; 9:297–298. [PubMed: 16837375]

150. Geerling BJ, Badart-Smook A, Stockbrugger RW, et al. Comprehensive nutritional status in patients with long-standing Crohn disease currently in remission. The American journal of clinical nutrition. 1998; 67:919–926. [PubMed: 9583850]

151. Galland L. Magnesium and inflammatory bowel disease. Magnesium. 1988; 7:78–83. [PubMed: 3294519]