REVIEW

Dietary Nutrients Mediate Intestinal Host Defense Peptide Expression

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ABSTRACT

The intestinal tract is the shared locus of intestinal epithelial cells, immune cells, nutrient digestion and absorption, and microbial survival. The gut in animals faces continuous challenges in communicating with the external environment. Threats from endogenous imbalance and exogenous feeds, especially pathogens, could trigger a disorder of homeostasis, leading to intestinal disease and even systematic disease risk. As a part of the intestinal protective barrier, endogenous host defense peptides (HDPs) play multiple beneficial physiological roles in the gut mucosa. Moreover, enhancing endogenous HDPs is being developed as a new strategy for resisting pathogens and commensal microbes, and to maintain intestinal health and reduce antibiotic use. In recent years, multiple nutrients such as branched-chain amino acids, SCFAs, lactose, zinc, and cholecalciferol (vitamin D_3) have been reported to significantly increase HDP expression. Nutritional intervention has received more attention and is viewed as a promising means to defend against pathogenic infections and intestinal inflammation. The present review focuses on current discoveries surrounding HDP expression and nutritional regulation of mechanisms in the gut. Our aim is to provide a comprehensive overview, referable tactics, and novel opinions. *Adv Nutr* 2020;11:92–102.

Keywords: nutrients, gut, pathogens, inflammation, host defense peptides, antibiotic alternative

Introduction

The gut is the junction and one of the most intimate zones between the body and the external environment. Many bacteria, fungi, viruses, and parasites live in the complex gut environment. As the largest immunity organ, the gut is at the forefront of the body's defense. Against this background, the gut has evolved multiple effective protection mechanisms to defend against endogenous and exogenous threats. However, a variety of factors inside and outside the gastrointestinal tract, such as diets (1), toxins (2), pathogens (3), and environmental factors (4), can seriously affect these defense mechanisms, upset the balance within the gut ecosystem, and destroy gut homeostasis. In turn, the damaged homeostasis further aggravates the unbalanced ecosystem and enhances the probability of infection by intestinal pathogens. Such infections eventually lead to local or systemic diseases such as inflammatory bowel disease, septicemia, and similar conditions (5).

Antibiotics are widely used in the treatment of illness and as feed supplements in animal husbandry because of their efficient antibacterial and somatotrophic properties. However, long-term, consistent heavy use of antibiotics has caused problems such as bacterial resistance, antibiotic residues, and environmental pollution, which have proved to be serious threats to human health. As a result of high selection pressure and development of resistance, ordinary antibiotics are losing their efficacy. Even though it is necessary to continue to develop new antibiotics with antimicrobial activities, host-directed therapies have emerged

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Abbreviations used: AMP, antimicrobial peptide; AvBD9, avian β-defensin 9; BCAA, branched-chain amino acid; CAMP, cathelicidin antimicrobial peptides; C/EBPε, CCAAT/enhancer binding protein ε; ERK, extracellular signal regulated protein kinase; FFAR, free fatty acid receptor; GPCR, G protein–coupled receptor; HAT, histone acetyltransferase; hBD, human β-defensin; HDAC, histone deacetylase; HDP, host defense peptide; JNK, c-Jun N-terminal kinase; LCFA, long-chain fatty acid; LL-37, cathelicidin antimicrobial peptide LL-37; LYZ, lysozyme; MAPK, mitogen-activated protein kinase; MCFA, medium-chain fatty acid; MEK, mitogen-activated protein kinase kinase; mTOR, mammalian target of rapamycin; NOD, nucleotide-binding oligomerization domain protein; pBD, porcine β-defensin; PR-39, cathelicidin proline-arginine-rich-39; TLR, Toll-like receptor; VDR, vitamin D receptor; $1,25(OH)_2$-D_3, 1,25-dihydroxycholecalciferol.

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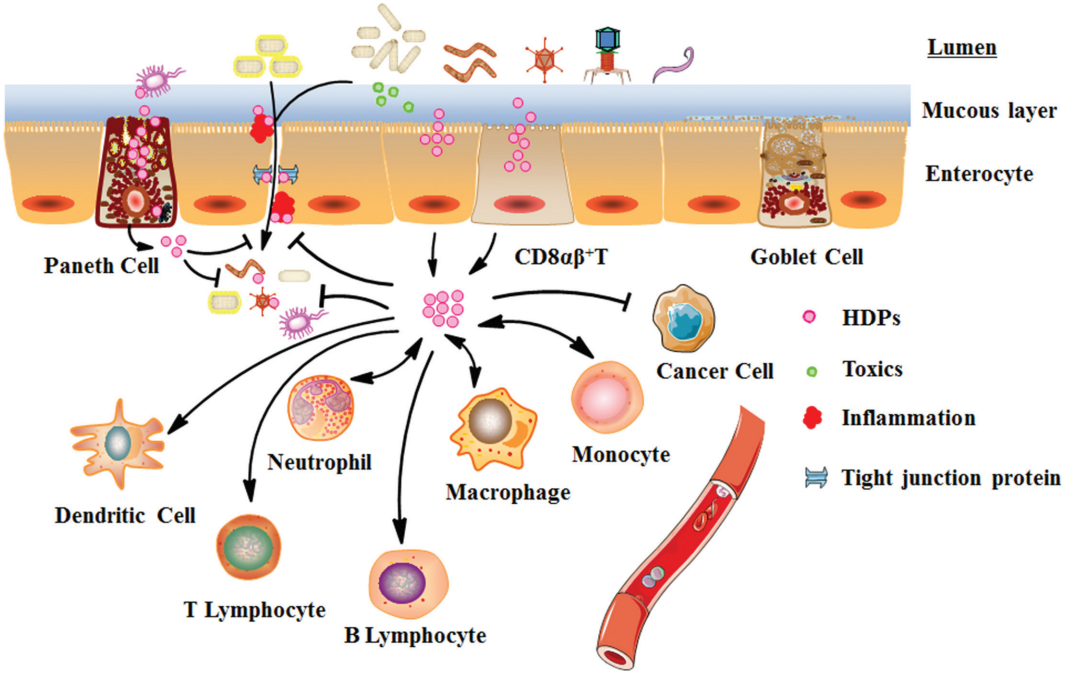

FIGURE 1 Multiple enterocytes and phagocytes synthesize and secrete HDPs in the intestine. The HDPs in the intestine can attach to and kill bacteria. Simultaneously, HDPs can enhance innate and adaptive immune functions to reduce inflammatory reactions and enhance antimicrobial capacity, can kill cancer cells, and can repair damage to intestinal tissues. HDP, host defense peptide.

as an attractive alternative method to resist infectious diseases (6). Antimicrobial peptides (AMPs) are evolutionarily conserved components of the innate immune system and are widely distributed in animals, plants, and microbes. These peptides confer protection against infection. AMPs have long been considered ideal antibiotic substitutes based on their stable structure, wide-spectrum and high-efficiency antimicrobial activity, selective toxicity, and minimal side effects (7). Most of all, AMPs are less likely to contribute to drug resistance than are conventional antibiotics (8). In recent years, because they act as immunomodulators that mediate host immune responses, AMPs have been renamed host defense peptides (HDPs). The gastrointestinal tract is a major functional locus for HDPs. Recent research suggests that HDPs are a vital protective mechanism for the gut and their expression can be regulated to some degree.

Based on their properties and vital roles in the innate immune system, HDPs are being developed by genetic and fermentation engineering technologies to serve as drugs or additives. But the high cost and low production efficiency involved are temporarily limiting their widespread application. Furthermore, clinically therapeutic HDPs still bear the risk of drug resistance (9, 10) and may affect the protective function of endogenous HDPs in the innate immune system. However, many relevant studies have found some nutrients significantly contribute to the expression of endogenous HDPs in the gut. Generation of endogenous HDPs provides a more economical, effective, and promising pathway to protect intestinal health from invading pathogens and inflammatory responses.

Character and Functions of HDPs in the Gut

HDPs are short peptides of <50 amino acids that carry an average net charge of +3 because of the predominance of acidic amino acids (Arg, Lys, and His) (11). There are >2000 HDPs found in animals according to the antimicrobial peptide database, mainly including defensin, cathelicidin, lysozyme (LYZ), natural killer cell lysin, regenerating protein family, hepcidin, chemokines, and some RNA enzymes (12, 13). The types of HDPs present vary between different species. Defensin and cathelicidin are 2 major HDP families in vertebrate animals and there are multiple members in each family (14). Enterocytes, paneth cells, CD8$\alpha\beta$+T cells, and phagocytes in the gut can all synthesize and secrete HDPs (12, 15) (**Figure 1**). In healthy animals, HDPs produced by the gut mucosa take part in gut barrier functions to defend against pathogens. HDPs are active against a wide range of organisms covering bacteria, fungi, helminths, and viruses. HDPs kill bacteria by penetrating the cell wall and cytomembrane using a high proportion of acidic phospholipids or by inhibiting synthesis of biological macromolecules (16). In addition, HDPs can clear inflammation, suppress cancer cells, and promote wound healing in the gut (17). Without compromising the immune functions required to clear infections, HDPs simultaneously control inflammation responses (8). Therefore, HDPs are of special biological significance for intestinal protection by enhancing

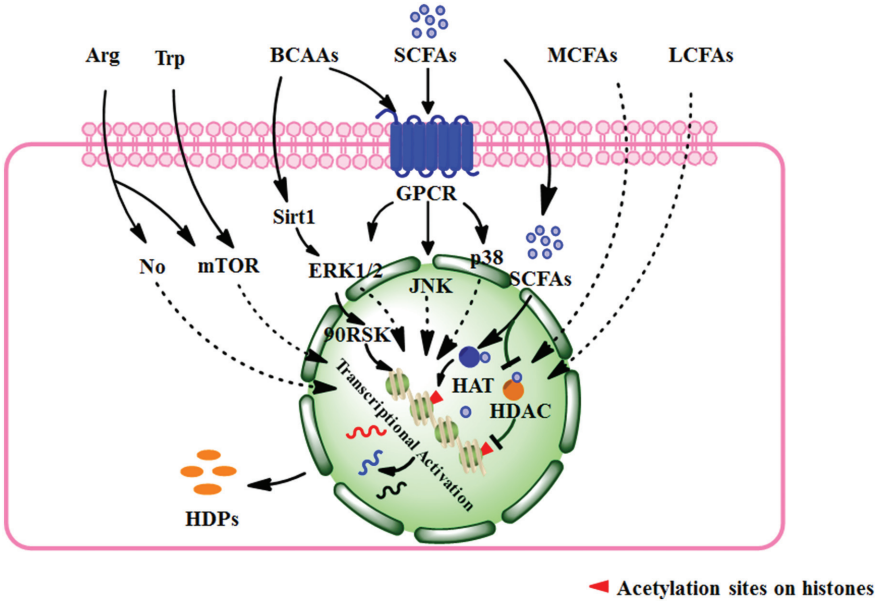

FIGURE 2 Multiple amino acids and fatty acids upregulate HDP expression in the gut. The BCAAs enhance HDP expression by the Sirt1–ERK1/2–90RSK and GPCR–MAPK pathways. Arg significantly enhances HDP expression and may be mediated by the NO signal or mTOR pathway. Trp may enhance HDP expression by the mTOR pathway. SCFAs, MCFAs, and LCFAs can all contribute to HDP expression and SCFAs affect the HDP expression conducted by directly influencing histone acetylation and the GPCR–MAPK signal pathway. BCAA, branched-chain amino acid; ERK, extracellular regulated protein kinase; GPCR, G protein-coupled receptor; HAT, histone acetyltransferase; HDAC, histone deacetylase; HDP, host defense peptide; JNK, c-Jun N-terminal kinase; LCFA, long-chain fatty acid; MAPK, mitogen-activated protein kinase; MCFA, medium-chain fatty acid; mTOR, mammalian target of rapamycin; Sirt1, sirtuin-1; 90RSK, 90-kDa ribosomal S6 kinase.

the innate and adaptive immune systems (17). This ability is particularly important for infants or other young of various species because of their immature immune systems. Corresponding to these views, decreased HDP expression is involved in pathogenesis of digestive diseases and bacterial translocation (7, 18).

Multiple Amino Acids Contribute to HDP Expression in Enterocytes

Amino acids have obvious first-pass metabolic effects in the intestinal tract. Long-term studies have revealed that a large number of amino acids are used in intestinal tissue metabolism and microbial utilization (19). Malnutrition, especially lack of amino acids, significantly restrains the immune function of gut tissue. The branched-chain amino acids (BCAAs), Arg, and Trp are important functional amino acids. Their metabolism boosts intestinal immune capacity in several ways, including enhancement of HDP expression (**Figure 2**).

BCAAs induce HDPs in enterocytes by the G protein-coupled receptor–extracellular regulated protein kinase 1/2 and sirtuin-1–extracellular regulated protein kinase–90-kDa ribosomal S6 kinase pathways

In humans, the co-incubation of isoleucine and IL-1α induces human β-defensin-2 (hBD2) expression in colonic epithelial cells, which is mediated by the G protein–coupled receptor (GPCR)–extracellular regulated protein kinase-1/2 (ERK1/2) signaling pathway (20). This provides a method to mediate endogenous HDP expression. Considering their similarity in structure and immune function, leucine and valine may have similar induction capacity. Another piece of research more completely investigated the induction capacity and possible regulation mechanism of β-defensins gene expression by BCAAs (21). They found that leucine, isoleucine, and valine all significantly increased intestinal epithelial porcine β-defensin-1 (*pBD1*), *pBD2*, *pBD3*, *pBD114*, *pBD129*, and epididymis protein 2 splicing variant C (*pEP2C*) in vivo and in vitro. Of these 3 amino acids tested, isoleucine appeared to be the most effective. Further investigation revealed that activation of the sirtuin-1–ERK–90-kDa ribosomal S6 kinase pathway was an important mechanism for the expression of β-defensins (21). Therefore, BCAAs, especially isoleucine, can promote expression of β-defensins to enhance intestinal immune function.

Arg may enhance HDP expression in enterocytes by the NO and mammalian target of rapamycin pathways

An experiment exploring immune-enhancing formulas found that Arg, Ile, and albumin all contributed to *hBD1* expression in human colonic cells (22). By the same token, dietary Arg supplementation evidently enhanced *pBD2*

and *pBD3* expression in ileum (23). Such trials in vitro and in vivo indicate Arg can protect intestinal epithelial cells through upregulation of HDPs. Growing evidence indicates that Arg and its metabolite NO are critical to maintain normal physiology, mucosal integrity, and defense of the gastrointestinal tract (24). The NO produced via NO synthase can prevent mucosal injury and reduce inflammatory responses (25). The NO signal pathway may be one of the functional mechanisms of Arg in the expression of HDPs. Moreover, protein synthesis in intestinal epithelial cells induced by Arg is mammalian target of rapamycin (mTOR)-dependent and independent of NO (26). Therefore, HDP expression induced by Arg may also be mediated by mTOR.

Trp may enhance HDP expression in enterocytes by the mTOR pathway

Trp is an essential amino acid and its metabolites from multiple metabolic pathways mediate crosstalk between the intestinal mucosal immune system and microflora. Trp may be a promising nutrient target for regulation of mucosal immune functions (27). The activated mTOR can promote the expression of HDPs (28). Several studies have found that Trp can directly activate the mTOR pathway but independently of the PI3K–AKT pathway (29, 30). Hence, there is speculation that dietary Trp can enhance HDP induction in gut tissue. More experiments are necessary to investigate the ability of Trp to alleviate intestinal inflammation through HDP expression. What is noteworthy is that in trials of amino acids inducing HDP expression, appropriate dose is the key element. In growing and adult pigs, it has been investigated whether moderate dietary protein restriction can optimize the gut microbiota and mucosal barrier (31–33). These suggest that an excessive concentration of amino acid may have the opposite effect.

Various Types of Fatty Acids Promote Intestinal HDP Expression

Repeated trials have found that fatty acids of differing lengths are closely connected with intestinal HDPs (Figure 2). According to their aliphatic chain length, fatty acids are broadly classified into 3 categories: SCFAs (1–5), medium-chain fatty acids (MCFAs, 6–11), and long-chain fatty acids (LCFAs, $\geq$12).

SCFAs, MCFAs, and LCFAs promote intestinal epithelial and phagocytic HDP expression

Besides possessing direct antimicrobial characteristics, lauric acid (12), palmitic acid (16), and oleic acid (18) in human sebum also disinfected by inducing hBD2 expression through CD36 and NF-κB (34). These findings suggest LCFAs can protect the epithelium from infection by inducing HDP expression. Similarly, in addition to direct antimicrobial effects, MCFAs also have HDP induction effects. MCFAs can prevent inflammation and intestinal barrier dysfunction, and reduce diarrhea triggered by pathogenic *Escherichia coli* (35). Exploring reasons for the beneficial effects of MCFAs, it was found that the *pBD1*, *pBD2*, and *pBD3* significantly increased (35). In colonic epithelial cells and monocytic cells of humans, hexanoate (6:0) and heptanoate (7:0) were more potent in promoting gene expression of cathelicidin antimicrobial peptides (*CAMP*), which encodes the human cathelicidin antimicrobial peptide LL-37 (LL-37). However, free fatty acids with chains longer than 7 or shorter than 4 carbons showed only a marginal effect on *CAMP* expression (36). Therefore, MCFAs may be more active in individual types of HDP expression in the gut. However, long-term studies conclude that the category of fatty acids with the most potential to modulate HDP expression is SCFAs. Under normal conditions, SCFAs exist in the small and large intestines, but are most abundant in the cecum and colon (37). In the distal intestine, SCFA concentrations range from 70 to 140 mM in the proximal colon and from 20 to 70 mM in the distal colon. Acetate, propionate, and butyrate are present in an approximate molar ratio of 60:20:20 (38). In addition to providing energy to epithelial and peripheral tissues, SCFAs also promote HDP expression in the intestine. SCFA-induced synthesis of endogenous HDPs is a phylogenetically conserved innate host defense mechanism. In mammals and aves, SCFAs are able to remarkably induce the expression of genes of multiple HDPs in intestinal epithelial cells and phagocytes (39, 40).

To evaluate the effects of fatty acid chain length on induction of HDPs, SCFAs, MCFAs, and LCFAs were used to treat macrophages and monocytes of chickens. Results showed that these 3 groups of fatty acids can all significantly contribute to the expression of multiple HDP genes including avian β-defensin 9 (*AvBD9*) and cathelicidin B1 in a dose-dependent manner. But butyric acid seemed to have the greatest potential (41). Similar results occurred in a porcine intestinal epithelial cell model (40). Therefore, butyric acid is confirmed as the most powerful inducer of SCFAs. Moreover, sodium butyrate shows excellent capacity to modulate intestinal permeability and change the bacterial community to decrease postweaning diarrhea in animals (41). Supplementing chickens with a combination of acetate, propionate, and butyrate in water resulted in a further reduction of *Salmonella enteritidis* in the cecum (39). Combinations of SCFAs seemed to exert a synergistic effect on HDP induction. This observation indicates the possibility to augment HDP expression by the synergy of combinations of different inducers. As for the level of fatty acid saturation, when comparing acids of the same carbon chain length, the oleic acid (18:1), linoleic acid (18:2), conjugated linoleic acid (18:2), and α-linoleic acid (18:3) treatment groups showed higher *AvBD9* mRNA levels than the stearic acid (18:0) group in monocytes of chicken (42). But PUFAs do not increase cathelicidin expression (43). It is possible that the species of HDPs evoked by different inducers are selective. These studies suggest that SCFAs and unsaturated fatty acids may

have stronger capacity to induce synthesis of multiple but not all HDPs. Similar effects are widespread in animals including mammals and birds.

The mechanisms of SCFAs on HDP expression in enterocytes

Based on their superior ability to induce HDP expression, many studies have investigated the mechanisms of SCFAs. SCFA-mediated regulation is mainly achieved by inhibition of histone deacetylase (HDAC), activation of GPCRs, stimulation of histone acetyltransferase (HAT) activity, and stabilization of the hypoxia-inducible factors (44–47). In humans, one of the main mechanisms for SCFAs to maintain intestinal health is through inhibition of HDACs (48); butyrate is the most potent HDAC inhibitor, whereas valerate and propionate are moderate and acetate is the least potent (49). HDAC inhibition enhances histone acetylation and is closely related to the transcriptionally active chromatin. However, the effects of HAT are contrary. Elevated levels of histone H2, H3, and H4 acetylation at particular sites such as H3K9 are active marks and often associated with ongoing transcription (50, 51). The individual treatment of chickens with acetate, propionate, or butyrate can inhibit the HDAC activity. In line with the synergy for HDP expression, cotreatment with 3 SCFAs showed the greatest HDAC inhibition (42). In vivo or in vitro trials with piglets demonstrated that butyrate upregulated the expression of multiple HDPs to promote clearance of pathogens and alleviate inflammation by depending on HDAC inhibition in the gut (51). Therefore, the acetylation of the histones is one of the key control factors.

However, besides directly inhibiting HDACs, the exact signal transduction mechanism contributing to HDP gene transcription is still unclear. The GPCR signaling pathway is another main mechanism of SCFAs. GPCR41 [free fatty acid receptor (FFAR)3], GPCR43 (FFAR2), GPCR109, and olfactory factor 78 are normally expressed in intestinal epithelial cells, adipocytes, and immune cells (52, 53). GPCRs are involved in SCFA-induced alleviation of inflammation (46). These receptors present specificities for different SCFAs, for example butyrate preferentially binds to GPCR41, yet GPCR43 has higher affinity for acetate and propionate (47, 54). In the colon of neonatal piglets, butyric acid–induced HDP expression was accompanied by increase in the mRNA level of *GPCR41* (55). GPCRs and the mitogen-activated protein kinase (MAPK) pathway present a close correlation. Combined treatment with butyrate and cAMP showed a stronger effect on promoting *AvBD9* expression in macrophages and primary jejunal explants by MAPK pathways (56). Inhibition of the mitogen-activated protein kinase kinase (MEK)–ERK pathway augmented *AvBD9* expression, whereas blocking c-Jun N-terminal kinase (JNK) or the p38 MAPK pathway diminished expression (56). However, the upregulation of cathelicidin gene expression depended on the activation of ERK1/2 (57). In some reports, the role of ERK1/2 in induction of HDPs is contradictory. Maybe, the regulatory roles of ERK1/2 in different tissue cells and HDP types are inconsistent. Therefore, the GPCR–MAPK pathway plays an important role in mediating SCFA-induced HDP expression in the gut. Next, we still need further research to clarify SCFA induction mechanisms, including the GPCR, MAPK, and other pathways.

Saccharides of Different Sources Facilitate the Expression of HDPs in the Intestine

Lactose and polysaccharides from plants enhance intestinal HDP expression

A study reported that lactose can upregulate the transcript of the *CAMP* gene encoding the LL-37 in human colon cancer cell line (HT-29) and human monocytic cell line (THP-1) in a dose- and time-dependent manner. This active substance from milk protected the gut of infants from invasion of pathogens (58), and indicated the importance of bioactive compounds in breast milk for the intestinal health of newborn animals. Some kinds of active polysaccharides in plants have broad areas of bioactivity involving immune adjunction; antibacterial, antivirus, and antitumor effects; and reducing heart and cerebral vessel diseases, in which several may act by HDP expression. For example, astragalus polysaccharides were found to enhance *LYZ* expression in spleen of carp (59). A lot of immune cells in the intestinal mucosa originate from bone marrow and may have similar HDP induction capacity. Besides immune cells, the astragalus polysaccharides significantly increased respiratory epithelial cells' expression of LL-37, which was attributed to activation of the p38 MAPK, JNK, and NF-κB pathways (60). Some dietary fiber, such as the macromolecules polysaccharides and prebiotics, can be fermented into SCFAs by intestinal flora together with other undigested carbohydrates. The induction capacity of SCFAs on HDP expression was discussed in the previous section. In different intestinal regions of the same or different species, the same or different types of fermentable fiber can produce various contents and components of SCFAs (61). Divergent SCFA combinations may have differential effects on HDP expression. This implies that different types of fermentable fiber may induce divergent expressions of HDP. Whether there are other mechanisms from dietary fiber on HDP expression is unclear.

Polysaccharides from bacteria trigger intestinal self-protection by enhanced HDPs

To some extent, specific components carried by some pathogens such as *Citrobacter*, *Giardia*, and *Salmonella* can contribute to intestinal HDP expression (62). For self-protection, the gut can respond to the stimulation of pathogenic bacteria polysaccharides by significantly enhancing HDP expression. For example, peptidoglycan can induce a protease cascade reaction through peptidoglycan recognition proteins-SA and lead the Toll-like receptors (TLRs) to form dimers that promote the expression of HDPs (63). Normal expression of the regenerating islet-derived protein 3γ gene depends on the TLRs (64). LPS can stimulate TLRs and nucleotide-binding oligomerization domain protein (NOD) signaling. And activation of the TLR and NOD

signaling pathway can enhance HDP expression (63–65). The upregulation of HDPs neutralizes exotoxins and endotoxins (66). But, when the influence from bacterial polysaccharides lasts for a long time or is overly severe, the body cannot resist the excessive stimulation and endogenous defense elements are downregulated (67). So even though some components of pathogens can induce the HDPs in the gut, it is not desirable to stimulate HDP expression by special pathogenic molecular structures because of the possible concomitant inflammatory response and other side effects. However, exopolysaccharides from bacteria and fungus, mostly the probiotics, have beneficial bioactivity in modulating innate antiviral responses (68), pathogen-induced inflammatory responses (69), and microbial diversity in the gut with no toxic effects (70). By feeding of exopolysaccharides, functional immunity of the gut is enhanced. But whether HDP expression is one of their functions is still unknown and worthy of attention.

Micronutrients Augment Intestinal HDP Expression to Inhibit Infection

Multiple trace elements like zinc and iron, and vitamins such as cholecalciferol (vitamin D_3), vitamin A, and niacin, are recognized as regulators of both innate and adaptive immune responses in intestinal mucosa, and command the intestinal immune defensive capacity (**Figure 3**). Improper micronutrients contribute to decreased mucosal immune responses against infection in the intestine (71).

Some kinds of trace elements augment HDP expression to inhibit intestinal infection

Mineral elements play a major role in nearly all metabolism processes and some mineral elements have beneficial effects for the intestinal mucosa barrier. Some studies have found mineral elements have positive impacts in regulating HDP expression. In the Caco-2 cell line, zinc $>20\ \mu$M was found to significantly increase LL-37 secretion in a dose- and time-dependent manner, and the regulation was mediated by the ERK1/2 and p38 MAPK pathways (72). A high level of zinc supplementation in the diet shows effective mitigation of diarrhea in young animals. For weaned pigs, dietary ZnO at the concentration of 164 mg/kg induced the greatest expression of *pBD3* and a decreased diarrhea ratio (73). These trials suggest that at least part of the intestinal protection effects of zinc is mediated by contributing to HDP expression. However, an exorbitant concentration of dietary ZnO (2425 mg Zn/kg) decreased *pBD3* expression (73). Lactoferrin, a nonheme iron binding glycoprotein in milk, effectively contributes to the development of the gut and defends against intestinal infections. In weaned pigs, 1% lactoferrin in diets increased the cathelicidin proline-arginine rich-39 (*PR-39*) and Protegrin-1 mRNA levels (74). This once more demonstrates the importance and potential value of milk-derived bioactive substances to intestinal health. In addition, enhanced expression of HDPs can compete with bacteria for essential trace elements such as iron, manganese, and zinc to restrain the proliferation of bacteria in the gut (12).

Vitamin D_3 and $1,25(OH)_2$-D_3 enhance HDP expression in the gut by the vitamin D receptor and histone acetylation

Vitamin D_3 is a sterol that can be obtained from feed. After absorption into the body, vitamin D_3 gets 2 hydroxylations in the liver and kidney, respectively, and is metabolized into 1,25-dihydroxycholecalciferol [$1,25(OH)_2$-D_3]. It is the most biologically active form of vitamin D_3. Previous studies focused on its major functions in maintenance of calcium and phosphorus homeostasis. However, it is also able to promote HDP expression in many animal species. In humans, vitamin D_3 or $1,25(OH)_2$-D_3 increases the expression of multiple HDPs in multiple cells including enterocytes and immune cells (75). In bovines and chickens, vitamin D_3 or $1,25(OH)_2$-D_3 stimulates the production of many kinds of HDPs such as cathelicidins and β-defensin in gut and other tissues (76, 77).

Mechanistic research indicated that $1,25(OH)_2$-D_3 can increase histone acetylation level, and activation of the histone acetylation enzyme is mediated by the vitamin D receptor (VDR) (78). The VDR is a valid target to modulate HDP expression by histone acetylation. However, 1 report showed that the VDR did not respond to the induction of $1,25(OH)_2$-D_3 in the intestinal epithelial cells of chickens (77). The response may be diverse across different doses. In different tissues and species, there may be other mechanisms involving $1,25(OH)_2$-D_3. Some new mechanisms have recently been discovered and researched, such as the PPAR-γ–mediated induction of $1,25(OH)_2$-D_3 in hBD3 and cathelicidin expression through the regulation of AP-1 and p38 MAPK activity in human keratinocytes (79). Moreover, IL-17A can enhance induction by $1,25(OH)_2$-D_3 of cathelicidin through activation of Act1 and the MEK–ERK pathway in keratinocytes (80). In contrast, the glucocorticoid, dexamethasone, downregulated induction in human monocytes and bronchial epithelial cells (81). In addition, butyrate synergized with $1,25(OH)_2$-D_3 to contribute to LL-37 expression and enhance the antimicrobial function of keratinocytes (78). Similarly to butyrate, 4-phenylbutyrate also enhanced vitamin D in LL-37 induction possibly through the VDR pathway in human bronchial epithelial cells and macrophages (82). The mechanisms of butyrate and vitamin D_3 in modulation of HDP synthesis show potential value in antibiotic-free livestock production (83).

Vitamin A enhances HDP expression in the gut

Vitamin A plays a crucial role in maintaining the function of epithelial tissue of the digestive tract and genital tract, and retinoic acid is its active metabolite (84). In intestine, retinoic acid plays a special role in maintaining immune homeostasis. Some studies reported that retinoic acid can induce gene expression of porcine *PR-39*and enhance human cathelicidin antimicrobial protein 18 promoter activity in bone marrow cells (85). Using stably integrated reporter constructs, retinoic acid was found to activate

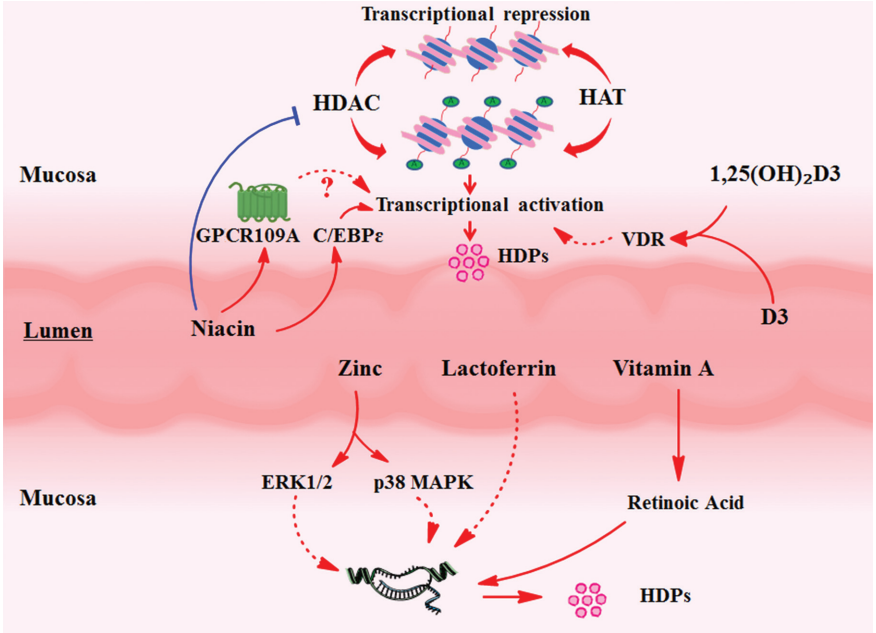

FIGURE 3 Multiple trace elements and vitamins enhance HDP expression in intestinal mucosa. Zinc contributes to HDP expression by the ERK1/2 and p38 MAPK pathways. Lactoferrin can increase intestinal HDP expression, but the study about its mechanism of action is limited. Cholecalciferol (vitamin D_3) and its metabolite 1,25-dihydroxycholecalciferol [1,25$(OH)_2$-D_3] and vitamin B-3 increase histone acetylation levels to enhance transcription of HDP genes. Vitamin D_3 and 1,25$(OH)_2$-D_3 regulate increased histone acetylation level via the VDR. Vitamin A can contribute to HDP expression by activating the retinoic acid response element. Niacin can restrain the activity of class III HDACs and increase C/EBPε to enhance antibacterial capacity. GPCR109A also mediates the regulation of niacin. C/EBPε, CCAAT/enhancer binding protein ε; ERK, extracellular regulated protein kinase; GPCR109A, G protein-coupled receptor 109A; HAT, histone acetyltransferase; HDAC, histone deacetylase; HDP, host defense peptide; p38 MAPK, p38 mitogen-activated protein kinase; VDR, vitamin D receptor.

α-defensin 1 gene expression directly through a proximal and distal element within a minimal promoter in a dose-dependent manner (86). In some HDP promoter regions, there is a retinoic acid response element (87). Considering the origin of immune cells in the intestinal mucosa, phagocytes in gut tissue may have similar functions. However, 1 report found retinoic acid inhibited the expression of hBD2 in human keratinocytes (88). This unexpected result may result from the dose of retinoic acid and specificity in the induction of HDP species.

Niacin enhances HDP expression in the gut

Niacin, also called vitamin B-3, can relieve pellagra characterized by inflammation of the skin and digestive system (89). In epithelial and nervous tissue, nicotinamide can be generated from niacin. The nuclear transcription factor CCAAT/enhancer binding protein ε (C/EBPε) family is closely associated to prevention of bacterial infection. C/EBPε deficiency led to severe infections by *Staphylococcus aureus* in mice, and C/EBPε deficiency in neutrophils contributed to the infectious phenotype. In contrast, exposure to nicotinamide significantly increased expression of C/EBPε in myeloid cells (90). Therefore, it can be sure that niacin has an important antibacterial capacity in relation to immune cells. Nicotinamide acts as a competitive inhibitor of class III HDACs, also called sirtuins (91). As an epigenetic modulator, nicotinamide enhances protein expression of a select number of its downstream targets in human neutrophils, such as cathelicidin (92). Moreover, activation of the receptor GPCR109A by nicotinamide suppressed intestinal inflammation and enhanced mechanical barrier functions similarly to butyric acid and β-hydroxybutyric acid (93). Thus, inhibition of HDAC and activation of GRCP109A may be additional mechanisms of the effects of niacin on HDP expression in the gut. However, excessive vitamin supplementation may lead to elevated intestinal inflammation as observed with vitamin D (94).

Additional Active Substances

Natural or synthetic nonnutrients are found to enhance intestinal HDP expression

There are some nonnutrients that can play a positive role in HDP induction. For example, bile salts, sulforaphane, curcumin, forskolin, resveratrol, pterostilbene, and polydatin can enhance HDP induction (95). Interestingly, some of these nonnutritive activators are able to amplify nutritive HDP induction capacity. A report has indicated that foskolin synergized butyric acid to enhance *AvBD9* expression in macrophages and primary jejunal explants (56). Lithocholic acid, metabolized from bile acid by bacteria in the gut, enhanced transcription of the *CAMP* gene and synergized

butyrate in colonic epithelial cells (96). Stilbenoids can synergistically induce human *cathelicidin* gene expression with vitamin D in monocytes and keratinocytes (97).

Probiotics and their metabolites enhance intestinal HDP expression

In a time- and dose-dependent manner, some probiotics significantly promote HDP expression in intestinal epithelial cells. *Escherichia coli* Nissle 1917 contributed to hBD2 expression in human Caco-2 cells by flagellin and JNK pathway (98). Not only a single probiotic, but incubation of a mixture of multiple lactobacillus strains obviously increased hBD2 expression via the NF-κB, AP-1, and MAPK pathways in Caco-2 cells (99). Besides the probiotic bacteria themselves, metabolites from these probiotic strains may be another reason for enhanced HDP expression. *Lactobacillus reuteri* I5007 increased the expression of multiple β-defensins in intestinal epithelial cells of neonatal piglets through increased butyric acid (55). HDP expression is partly responsible for the immunomodulatory function of probiotics.

Research Prospects

Currently, we lack sufficient data on the spatiotemporal expression rules of HDPs in the gut of humans and economically important animals. This information will guide when to intervene, which nutrients are to be used, and which species of HDPs will perform the main defensive functions. Previous studies showed that in the short term, HDPs were not easy to develop drug resistance against because they possessed multiple mechanisms to kill bacteria. But drug resistance may still occur in the long term. If so, high doses and direct supplements of exogenous HDPs through feed may be adverse. Some bacteria have evolved resistance mutations to HDPs, which can resist HDP expression (100). Moreover, another report showed that enteric α-defensin 5 can promote the adhesion and invasion of *Shigella* to host in humans (101). There is no proof explaining whether this is an innate characteristic or a mutational result of *Shigella*. However, these results indicate that the security and use-pattern of HDPs need further evaluations. Several researches confirmed that HDPs are found as novel antibiofilm agents to inhibit the growth of bacterial communities (102). The precise mechanism is not clear. Otherwise, previous reports have suggested endogenous HDPs may be insufficient at physiological concentration to kill bacteria, and their function is mainly based on an anti-inflammatory effect (103). This evidence shows that present knowledge about endogenous HDPs is still limited. A comprehensive study of HDP properties and functions is necessary.

Previous studies have found some highly effective HDP inducers. Most inducers functionally behave as HDAC inhibitors. However, it is surprising that some stronger HDAC inhibitors are less efficient in the induction of HDPs. Even stronger HDAC inhibition induced by higher concentrations of HDAC inhibitors conversely shows decreased HDP induction (83). Moreover, transcriptional genes of HDPs mediated by different inducers are selective. The mechanism is indistinct. Besides HDAC inhibition, the research about other functional mechanisms is insufficient. Other epigenetic modifications such as the histone modifications and accurate signal transduction mechanisms in the gut need to be researched. Comprehensively clarifying the networks governing HDP expression is necessary.

Conclusions

Intestinal endogenous HDP expression can be modulated by specific nutrients in the diet. As an important part of disease-resistant nutrition research, enhanced expression of endogenous HDPs could fortify immune defensive capacity, alleviate tissue inflammation, and shape gut microbes to maintain intestinal homeostasis. Some amino acids, fatty acids, saccharides, trace elements, vitamins, and other nonnutritional active substances show excellent potential to induce HDPs and may have crucial value in clinical application. The synergy of multiple inducers may be leveraged. Intestinal health ensures nutrient conversion and growth in humans and in animals of economic importance. Regulation of endogenous HDPs to maintain intestinal health by dietary nutrients may effectively promote replacement of antibiotics in the future.

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