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REVIEW ARTICLE

A Potential Effect of Carbohydrate and Lipid Consumption towards Intestinal Mucus Production: *In vivo* Animal Studies and *In vitro* Studies

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A B S T R A C T

The intestinal mucus layer plays a crucial role in protecting the epithelium layer and acts as a barrier to separate the epithelium layer from pathogenic microorganisms. The mucus is synthesized by goblet cells located in the epithelium layer. The production of mucus inside the goblet cells is regulated by the expression of the mucin gene family, such as *MUC2*, for the mucus production in the jejunum, ileum, and colon. Recent studies have suggested the influence of macronutrient intake, such as carbohydrates and fat, in mucus production. A high fiber diet and resistant starch consumption were found to positively affect mucus production by upregulating mucin gene expression. Meanwhile, a high saturated fat diet was found to negatively affect mucus production by promoting ER stress and downregulating epithelial differentiation transcription factor (KLF4). Nonetheless, a low saturated fat diet was found to upregulate mucin expression. On the other hand, an unsaturated fat diet (oleic acid, linoleic acid, EPA, and DHA) decreased mucin expression by disrupting epithelial differentiation transcription factors (HATH1 and TLR4). However, studies on the effect of dietary intake on mucus production are still limited, especially in the underlying molecular pathway. Therefore, further research on the molecular pathway on the effect of dietary intake on mucus production needs to be performed.

K E Y W O R D S

Intestinal mucus; carbohydrate; dietary fat; MUC2.

H I G H L I G H T S

- Mucus is produced on the intestinal epithelium layer to protect it from pathogenic microorganisms.
- A high carbohydrate diet of fiber and resistant starch increases mucus production.
- A low saturated fat diet increases mucus production.
- A high unsaturated fat diet decreases mucus production.

INTRODUCTION

The intestinal mucus layer acts as the first barrier to protect the epithelial cells from being contacted and stimulated by intestinal microorganisms (Yao et al., 2021). Mucus is produced by goblet cells which are located in the epithelium layer of all parts of the intestinal tract (Corfield, 2018). Impairment of goblet cells' function and its secreted mucin strongly leads to inflammatory bowel disease, classified into Crohn's disease and ulcerative colitis (Grondin et al., 2020). The production of mucus inside the goblet cell is regulated by the

expression of the mucin gene family. The mucin family is divided into two groups based on its biological function: secreted mucins and membrane-associated mucins. The secreted mucins can be further divided into two groups: gel-forming mucin (essential for the mucus barrier production) and non-gel-forming mucin. Each mucin is responsible for the formation of mucus in different parts of the body. For example, gel-forming mucin MUC2 is responsible for mucus production in the jejunum, ileum, and colon. Meanwhile, MUC5AC is produced in the stomach, MUC5B is produced in submandibular and other salivary glands, and MUC6 is produced in the stomach and ileum. The non-gel-forming mucin MUC7 is produced in the sublingual and submandibular glands (Corfield, 2018). The mucus is derived from the lower part of intestinal crypt stem cells, and the formation is controlled by several transcriptional factors, such as HATH1, TLRs, HNF4α, and others, during cell differentiation (Benoit et al., 2015; Ma et al., 2018).

Gel-forming mucin is a heavily O-glycosylated protein that forms an intricate polymer network (Yamashita & Melo, 2018). Glycosylation is the most common post-translational modification with the addition of sugar chains to proteins. The two types of glycosylation are N-glycosylation (linked to asparagine) and O-glycosylation (linked to serine or threonine) (Hargett et al., 2021). MUC apo-proteins undergo N-glycosylation in the endoplasmic reticulum, followed by O-glycosylation in the Golgi apparatus. This protein modification is initiated by the addition of GalNac sugar to serine or threonine residues of the mucin protein backbone. Thus, the repeating domain rich in proline, threonine, and serine (PTS domain) could become heavily O-glycosylated (Maares et al., 2020). The addition of GalNac sugar extends the polypeptide backbone, arranged on the opposing sides of the backbone, stiffens the backbone, and becomes rod-like (Wandall et al., 2021). After O-glycans are attached to mucin, N-terminal properties sort mucin to the secretory pathway. Mucin is then condensed in the goblet cells' secretory granules and released together with the granules. The mucin domains have a high capability to bind water, thus expanding its volume significantly, and are resistant to protease due to the attachment of O-glycans (Johansson et al., 2010; Hansson, 2012). Secreted mucus layers act as defensive barriers from pathogen invasion by clearing and renewing multiple times a day, which prevents mucosal and systemic infection (Carlson et al., 2018). Therefore, glycosylation contributes to gel-forming ability and mucus layer formation.

Recent studies, with both *in vivo* (Chen et al., 2019; Duriancik et al., 2015; Escoula et al., 2019; Saqui-Salces et al., 2017) and *in vitro* (Benoit et al., 2015; Ma et al., 2018) approaches have demonstrated that diet could influence the production of intestinal mucus. In an *in vitro* study, carbohydrate intake was found to affect the composition of gut microbiota, thus affecting mucus thickness and increasing the strength of the mucus layer (Yamada et al., 2019). Different types of dietary fiber were also found to affect the expression of *MUC2*, which increases the production of mucus protein (Chen et al., 2019; Saqui-Salces et al., 2017). In addition, various dietary fatty acids (FAs) and dietary oils (DOs) were found to affect *MUC2* expression and production. Fish oil, oleic acid (OA), linoleic acid (LA), and high palmitic acid (PA) were found to decrease the expression of MUC2, resulting in a decrease of mucus production. Additionally, food intake might also affect goblet cell maturation, thus influencing intestinal mucus production (Benoit et al., 2015; Duriancik et al., 2015; Escoula et al., 2019; Ma et al., 2018). The objective of this review is to present current knowledge and findings regarding the effects of macronutrient intake on the production of intestinal mucus.

CARBOHYDRTATE EFFECTS ON INTESTINAL MUCUS PRODUCTION

Carbohydrate is the primary source of energy that can be found in many foods. Consumption of carbohydrates affects mucin gene expression, which also affects mucus production in the intestine (Chen et al., 2019; Ghosh et al., 2020; Qin et al., 2020; Saqui-Salces et al., 2017; Vila et al., 2018). Different types of carbohydrates affect mucin gene expression and mucus production in different parts of the gastrointestinal tract (Chen et al., 2019; Ghosh et al., 2020; Qin et al., 2020; Saqui-Salces et al., 2017; Vila et al., 2018). The main findings of multiple *in vivo* studies to various types of carbohydrates are reported in Table 1 below.

Table 1. Summary of studies investigating the effect of carbohydrates on mucus production.

Types of Carbohydrate	Method	Result	Reference
Dietary fiber	*In vivo* experiment using 54 pigs fed with six different diets consisting of: (1) control diet based on corn and soybean meal (CSB); (2) CSB containing 40% corn distillers dried grains with solubles (DDGS); (3) CSB containing 30% wheat middlings (WM); and (4,5,6) the same diet supplemented with exogenous NSP-degrading enzyme cocktail (E). Diet treatments were conducted for 28 days. Goblet cell quantification and gene expression were measured using ileal tissue samples.	Consumption of high fiber diets (DDGS and WM) significantly increased *MUC2* gene expression compared to the control diet (CSB). However, no differences were found in the number of goblet cells between diet treatments.	Vila et al. (2018)
	In vivo experiment using pigs. Four different dietary treatments were tested on 12 pigs each treatment. Diet treatment consists of: (1) control diet based on corn and soybean meal (NDF); (2) NDF containing 23% wheat straw (WS); (3) NDF containing 55% corn distillers grains with solubles (DDGS); and (4) NDF containing 30% soybean hull (SBH). Diet treatments were given for 14 days. Goblet cell quantification and gene expression were measured using ileal tissue samples.	DDGS and SBH diet treatment significantly increased *MUC2* gene expression compared to the control diet. However, no differences were found between the high fiber diet treatments (WS, DDGS, and SBH). Both WS and DDGS were found to increase goblet cell area compared to the control diet.	Saqui-Salces et al. (2017)
	In vivo experiment using 24 pigs, treated with 4 dietary treatments consisting of: (1) control maize-soybean diet; (2) 1% insoluble dietary fiber (IDF); (3) 1% soluble dietary fiber (SDF); and (4) 0.5% IDF + 0.5% SDF. IDF diet replaces 1% maize in the control diet with 1% ARBOCEL (lignocellulose), while SDF diet replaces 1% maize in the control diet with 1% inulin. Treatments lasted for 28 days. Gene expression analysis was conducted using RNA extracted from colonic mucosa.	Both SDF and IDF diet treatments increased gene expression levels of *MUC2.*	Chen et al. (2019)
Raw Potato Starch (RPS)	*In vivo* experiment using 3 groups of 8 Cherry Valley meat-type male ducks, fed with starter (1-14 days) and finisher (15-35 days) diets contained 0%, 12%, and 24% RSP. Caecal tissues were sampled after 35 days and analyzed for *MUC2* mRNA expression.	Supplementation of 12% and 24% RPS to the diet significantly increases *MUC2* mRNA expression.	Qin et al. (2020)
Galactooligosaccharides (GOS)	*In vivo* experiment using healthy mice, treated with four diet groups: (1) standard rodent diet; (2) western-type diet; (3) western-type diet containing 5% GOS; and (4) western-type diet	Western diets with galacto oligosaccharides were found to increase the MUC2 layer, increase goblet cell number,	Ghosh et al. (2020)

	supplemented with curcumin.	partially restore mucin layer continuity, and reduce neurogenin-3 gene expression. No changes were observed in *MUC2* mRNA expression.

Dietary fiber plays a crucial role in maintaining the composition of the gut microbiota, in which many studies have found that a low fiber diet was disadvantageous to the mucus layer. A low fiber diet might cause mucus layer thinning, making it more susceptible to infection caused by pathogenic bacteria, such as *Citrobacter rodentium*, *Bacteroides caccae*, and *Akkermansia muciniphila*. Low dietary fiber intake also resulted in a higher level of sulfatase and alpha-fucosidase enzyme production from bacteria, such as *Bacteroides thetaiotaomicron, Bifidobacterium longum,* and *Bifidobacterium bifidum,* which are involved in mucin degradation (Desai et al., 2016). The thinning of the mucus layer could increase the risk of gastrointestinal disorders, such as Crohn's Disease and Ulcerative Colitis (Yamada et al., 2019).

A high fiber diet, both soluble and insoluble fiber, was found to increase intestinal mucin expression. A study showed that high insoluble fiber consumption in pigs increased ileal *MUC2* gene expression but not the number of goblet cells (Vila et al., 2018). In contrast, another study in pigs found that a high fiber diet consumption increased ileal *MUC2* gene expression as well as the number of goblet cells (Saqui-Salces et al., 2017). According to Vila et al. (2018), the increase of *MUC2* gene expression is not always associated with the increase in the number of goblet cells due to specific chemical characteristics of fiber given or species-specific responses to fiber, such as different fiber compositions used in different studies, inclusion levels, and intakes used in the studies. Besides, different animals used in the experiment, either rats or pigs, might have different mechanisms in response to fiber (Vila et al., 2018). A study by Chen et al. (2019) showed overexpression of colonic *MUC2* as the impact of both soluble and insoluble dietary fiber consumption in pigs. On the other hand, studies on rats showed that high-molecular konjac mannan soluble fiber was not affecting *MUC2* gene expression (Hino et al., 2013; Ito et al., 2009). Meanwhile, chronic ingestion of insoluble fiber in rats showed lasting enhancement of luminal mucin secretion (Morita et al., 2008).

In the human colon, dietary fiber is fermented by gut bacteria, producing end-products of short-chain fatty acids (SCFA), including acetate, propionate, and butyrate (Jha et al., 2019; Wang et al., 2019). Soluble dietary fiber diets increased the number of SCFAs (propionate, acetate, and butyrate), while insoluble dietary fiber diets increased only acetate concentration (Chen et al., 2019). In maintaining the mucus layer, butyrate acts as the principal source of metabolic energy for colonocytes, differentiating colonocytes and inducing apoptosis to remove dysfunctional cells as means of protection against colon cancer (Conlon & Bird, 2015). In the intestinal epithelial cell, the anti-inflammatory properties of butyrate modulate colonic inflammation by reducing the expression of IL-8 and inhibiting inducible NO synthase expression (Fung et al., 2012). Furthermore, butyrate can increase mucin production and the proportion of mucin-secreting goblet cells in a macrophage-dependent manner. Gut microbiota-derived butyrate also regulates gut mucus barrier repair (Liang et al., 2022). An *in vivo* study in mice by Gaudier et al. (2009) showed a butyrate effect in increasing the expression of *the MUC3* gene, as well as stimulating *MUC1* and *MUC2* gene expressions in the proximal and distal colon. The result of this study suggested *that MUC2* gene expression was the most affected by butyrate, thus increasing by 6-fold. Gaudier et al. (2009) showed a 2.5-fold decrease in mucus thickness due to butyrate application, contradictory to Kleessen et al. (2013), who found that butyrate increased mucus thickness. Nevertheless, Gaudier et al. (2009) also stated that they could not discriminate the effect of butyrate itself from the modification of colonic microorganisms, which also occurs during carbohydrate fermentation.

In vivo study was conducted by Qin et al. (2020) on three groups of ducks with 0%, 12%, and 24 raw potato starch (RPS) diets. Supplementation of RPS into the diet was found to significantly increase *MUC2* mRNA expression compared to the control diet (0% RPS) (Qin et al., 2020). Elevation of *MUC2* expression was suspected to be stimulated by butyrate. In addition, Trachsel et al. (2018) reported that the RPS diet increased butyrate concentration and *MUC2* expression in the caecum. This finding demonstrates that consumption of RPS might help to improve mucus production of the host by elevating *MUC2* expression through butyrate stimulation (Qin et al., 2020).

Another *in vivo* study in 2020 found that the disruption of the mucus layer of the mice was found to be restored in the Western diet supplemented with GOS fiber compared to the regular Western diet. An increase in the MUC2 layer was observed after a Western diet supplemented with GOS treatment, but no changes were observed in the *MUC2* expression in the ileum and colon of the mice. Downregulation of *neurogenin-3* expression in the colon of the mice due to GOS treatment was suggested to be the reason for the improvement in the mucin layer (Ghosh et al., 2020). Neurogenin-3 is known to be the key gene control towards endocrine cell development by redirecting potential secretory progenitors to endocrine rather than mucin-secreting goblet cells. The number of goblet cells was observed to decrease in neurogenin 3-expressing transgenics corresponding to the increase of endocrine cells (Lopez-Diaz et al., 2007). Additionally, multiple studies showed that deletion of intestine-specific *neurogenin-3* was found to increase goblet cell number (Ye & Kaestner, 2009; Mellitzer et al., 2010; Li et al., 2021). Thus, GOS consumption may increase MUC2 secretion and improve the mucin layer through the downregulation of *neurogenin-3*.

FAT EFFECTS ON INTESTINAL MUCUS PRODUCTION

Various studies using *in vivo* and *in vitro* approaches on different types of FAs, such as saturated and unsaturated fatty acids, and DOs had been found to have distinct effects on mucus production (Benoit et al., 2015; Duriancik et al., 2015; Escoula et al., 2019; Ma et al., 2018). Some FAs and DOs may decrease *MUC2* synthesis through alteration of goblet cells differentiation due to the disruption in transcription factors expression, while the others may enhance the MUC2 synthesis. These effects are strongly influenced by the chemical structure of FAs and composition of DOs. The main findings of multiple *in vivo* or *in vitro* studies on various FAs and DOs are reported in Table 2 below.

Table 2. Summary of studies investigating the effect of dietary fats on MUC2 production

Dietary Fats	Method	Result	Reference
***In Vitro* Studies**			
PA	*In vitro* using HT29-MTX human colon carcinoma-derived mucin-secreting goblet cell line, treated for 21 days through apical pole (with ethanol as control).	*In vitro* PA (150 µM) enhanced mucin-like glycoprotein in the apical media, *MUC2* gene expression, MUC2 released, and MUC2 intracellular content. Western blotting showed an increase in *HNF4α* gene expression.	Benoit et al. (2015)
	In vitro using LS174T well-differentiated human colonic goblet cells, treated for 3, 6, and 24h (with ethanol and MEM-BSA as control).	*In vitro* PA (300 µM) was observed to downregulate *MUC2* gene expression after 6h treatment and *KLF4* gene expression after 3h. Additionally, it also reduced MUC2 release.	Escoula et al. (2019)

Stearic acid	*In vitro* using HT29-MTX human colon carcinoma-derived mucin-secreting goblet cell line, treated for 21 days through apical pole (with ethanol as control).	*In vitro* stearic acid (150 µM) was found to increase mucin-like glycoprotein in the apical media, *MUC2* gene expression, and MUC2 release. Western blotting also showed an increased *HNF4α* gene expression.	Benoit et al. (2015)
OA		*In vitro* OA (150 µM) was found to reduce *MUC2* gene expression, synthesis, and release. Additionally, decreased *HATH1* gene expression was also observed.	
LA		*In vitro* LA (150 µM) was found to reduce *MUC2* gene expression and synthesis. Additionally, decreased *HATH1* gene expression was also observed.	
EPA		*In vitro* EPA (150 µM) was found to reduce *MUC2* gene expression, synthesis, and release. Additionally, decreased *HATH1* gene expression was also observed.	
***In Vivo* Studies**			
PO	Male Wistar rat. 10 µL/g body weight oral administration through gastric gavage for 6 days (with water as control).	Administration of PO resulted in higher LCFAs, PA, and OA content in the colon. The colonic goblet cell number was not affected, while goblet cell mucus granules were observed to increase.	Benoit et al. (2015)
RO		Administration of RO resulted in lower PA content in the colon and colonic goblet cells. Higher OA content in the colon was also observed.	
SO		Administration of SO resulted in higher OA content in the colon. Meanwhile, a reduced number of colonic goblet cells was observed.	
Fish oil	Male Sprague-Dawley rat. Formulated diet for 5 weeks. Analysis of colonic morphology, RT-PCR (*MUC2* and *TLRs*), and western blotting (*MUC2*) with soybean oil as a control.	Administration of fish oil resulted in decreased mucosal thickness and crypt depth. Additionally, a lower number of goblet cells and decreased *MUC2* (RT-PCR and Western blotting) and *TLR4* (RT-PCR) gene expression were observed.	Ma et al. (2018)
	Female and male SMAD3 pups. Treated for 5 weeks with either AIN-93G-based as control or 6% fish oil (contained 1% corn oil).	Administration of fish oil was found to reduce goblet cell numbers in the colon and cecal. Additionally, reduced mucus thickness in the cecal was also observed as a result.	Duriancik et al. (2015)

PA: Palmitic acid; OA: Oleic acid; LCFAs: Long-chain fatty acids; EPA: Eicosapentaenoic acid; PO: Palm oil; RO: Rapeseed oil; SO: Sunflower oil

Saturated fatty acids

Administration of DOs was found to affect MUC2 production. *In vivo* study done on male Wistar rat pups fed with 10 µL/g body weight of palm oil (PO) through gastric gavage for 6 days showed a hypertrophic change in goblet cells in rats' colon receiving PO. Although there was no effect on colonic goblet cell amount, there was an increase in goblet cell mucus granules. Additionally, an increase in MUC2 production and a high amount of palmitic acid (PA) in the colon was also observed, as PA is the predominant FA in PO. Beneficial effects of PO might be justified by an *in vitro* study, in which low concentration PA treatment (150 µM) towards HT29-MTX cell line (human colon carcinoma-derived-from-secreting goblet cell) increased *MUC2* expression and the release of mucin-like glycoprotein, which identified as MUC2, in the apical media. Also worth noting is that a low concentration of stearic acid (SA; 150 µM), another predominant FA in PO, showed an increase in *MUC2* expression and MUC2 production in the HT29-MTX cell line (Benoit et al., 2015; Rooijen & Mensink, 2020).

A possible mechanism of the beneficial effect of PA and SA treatment was through the upregulation of *HNF4α* gene expression, which is an important regulator for intestinal regeneration and maturation of mucin-producing goblet cells (Benoit et al., 2015; Lv et al., 2021; Montenegro-Miranda et al., 2020). Garrison et al. (2006) showed that HNF4α-null mouse embryo colons had disrupted colon crypt and goblet cell maturation. In humans, the loss of the *HNF4α* gene may trigger mucosal barrier dysfunction and has been associated with IBD (Dubois et al., 2020). Because of the drawback of deletion of the *HNF4α* gene, it may implicate the beneficial effect of PA and SA on MUC2 production by upregulating *HNF4α* (Benoit et al., 2015).

In contrast, a moderate concentration of PA (300 µM) treatment on LS174T cell line (well-differentiated human colonic goblet cell) was found to downregulate *MUC2* mRNA expression. Possible mechanisms of reduction in MUC2 secretion were explained by the reduction in the mature fully-glycosylated MUC2 amount and increase in the immature non-glycosylated MUC2. Due to mucin's large size and complexity, mucins are prone to misfold, resulting in immature mucin (Escoula et al., 2019). Excessive mucin secretion results in an accumulation of misfolded mucin in the endoplasmic reticulum (ER) and triggers the unfolded protein response (UPR), aiming to return ER homeostasis (Xu et al., 2021). Failure in UPR disrupts the ER protein-folding environment and leads to ER stress (Coleman & Haller, 2019). This evidence may explain the mechanism of PA to decrease MUC2 secretion.

Another possible mechanism is a disruption in goblet cell differential transcription factor KLF4. Moderate PA (300 µM) treatment towards the LS174T cell line was found to downregulate *KLF4* mRNA expression after 3 hours of treatment. This downregulation of *KLF4* mRNA expression was also accompanied by a reduction in MUC2 secretion (Escoula et al., 2019). This supports the possible association between KLF4 with MUC2 production. In the mice's intestine, *KLF4* was reported to regulate the goblet cell terminal differentiation (Yang & Yu, 2021). Deletion of *KLF4* in mice led to the loss of mature goblet cells (Chen et al., 2018). An increased number of immature goblet cells leads to unstable or immature mucin production (Bankole et al., 2021).

Unsaturated fatty acids

Dietary oils administration in *vivo* study of male Wistar rat pups, fed with 10 µL/g body weight of RO and SO through gastric gavage for 6 days, both showed a reduction of colonic goblet cell number and an increase of OA in the colonic content, as OA is one of the predominant FAs in RO and SO. *In vitro* study supports the finding that low concentration of OA (150 µM) treatment on HT29-MTX cell line (human colon carcinoma-derived-from-secreting goblet cell) decreased *MUC2* expression and MUC2 production. LA, another predominant fatty acid in both RO and SO, was also found to reduce *MUC2* expression and MUC2 production in the treatment on HT29-MTX cell lines (Awatif & Shaker, 2014; Benoit et al., 2015; Matthaus et al., 2016). A possible mechanism for the reduction of MUC2 production by OA and LA may be explained

through the downregulation of *HATH1* mRNA expression, a basic helix-loop-helix transcription factor that plays a key role in the differentiation of goblet cells (Benoit et al., 2015; Melhem et al., 2021). Downregulation of *HATH1* mRNA expression was suggested to be the regulator for *MUC2* expression. Furthermore, mutation on the *HATH1* binding site was also found to reduce the stimulatory effect of HATH1 on *MUC2* expression (Yamashita & Melo, 2018; Yang & Yu, 2021). Thus, the presence of OA and LA may reduce MUC2 production through the downregulation of *HATH1* in the epithelial cell.

Another PUFAs dietary source is fish oil, which has been known for its high levels of omega-3 PUFAs, particularly eicosapentaenoic acid (EPA). Ma et al. (2018) found that fish-oil-fed male Sprague-Dawley rats showed a lower abundance of *MUC2* expression and a smaller number of goblet cells. These findings indicated that fish oil intake might have an adverse effect on the colonic barrier by lowering mucus production (Ma et al., 2018). Similar to OA and LA, EPA was also found to downregulate *HATH1* mRNA expression, which may suggest the reduction in mucus production after fish oil intake (Benoit et al., 2015; Ma et al., 2018). Another possible mechanism of alteration in MUC2 production by dietary fish oil intake might be due to the significant weakening of *TLRs* signaling, particularly *TLR4* (Ma et al., 2018). *TLR4* has a role in the regulation of normal intestinal epithelial development (Hackman & Sodhi, 2018). *TLR4* may also regulate the development of mucin-producing goblet cells in the small intestine (McKernan, 2019). Activation of *TLR4* was found to upregulate *MUC2* expression (Grondin et al., 2020). Thus, fish oil treatment may reduce MUC2 production through the downregulation of *HATH1* and *TLR4.*

POSSIBLE MECHANISMS OF MACRONUTRIENTS AFFECTING INTESTINAL MUCUS PRODUCTION

Intestinal mucus production can be affected by macronutrient intake through alteration in several processes. As seen in Figure 1 below, a diagram has been constructed to summarize all macronutrient effects on intestinal mucus production, as mentioned by the authors above.

Two types of carbohydrates explored in this review are dietary fiber and raw potato starch. High consumption of dietary fiber, either soluble or insoluble fiber, was found to increase mucin gene expression, especially *MUC2* (Saqui-Salces et al., 2017; Vila et al., 2018). While there were conflicting results on whether high fiber consumption affects goblet cell number due to specific chemical characteristics, both fibers did increase the mucin gene expression, and mucus excretion also may be improved (Chen et al., 2019). Butyrate from dietary fiber fermentation was also found to enhance *MUC2* expression (Gaudier et al., 2009). However, the molecular pathway on how butyrate influences glycosylated mucin formation is still unknown (Yamada et al., 2019). In addition, GOS were found to reduce the neurogenin-3 expression in mice treated with a Western diet supplemented with GOS. The neurogenin-3 expression has been known to have an antagonist effect on goblet cells. Thus, increased expression of neurogenin-3 may result in the reduction of goblet cell number and lowering mucin gene expression (Ghosh et al., 2020).

High saturated fatty acids SFA intake, especially palmitic acid (PA), was found to cause ER stress that negatively affects mucin gene expression and secretion (Escoula et al., 2019). However, a low intake of PA (150 µM) was found to positively affect mucin expression and mucus secretion through the upregulation of transcription factor HNF4α (Benoit et al., 2015). This transcriptional factor (HNF4α) has been known to play an important role in intestinal epithelial regeneration and goblet cell maturation, and the deletion of this gene may trigger mucosal barrier dysfunction (Dubois et al., 2020; Lv et al., 2021; Montenegro-Miranda et al., 2020). Unsaturated fatty acids exposure, such as OA and LA, might decrease mucus secretion through downregulation of *HATH1* mRNA expression (Benoit et al., 2015). EPA and docosahexaenoic acid (DHA) from fish oil intake were also found to decrease mucin expression through the weakening of *TLR4* signaling (Duriancik et al., 2015; Ma et al., 2018). Both *HATH1* and *TLR4* work by regulating epithelium development and goblet cell differentiation (Hackman & Sodhi, 2018; McKernan, 2019; Melhem et al., 2021).

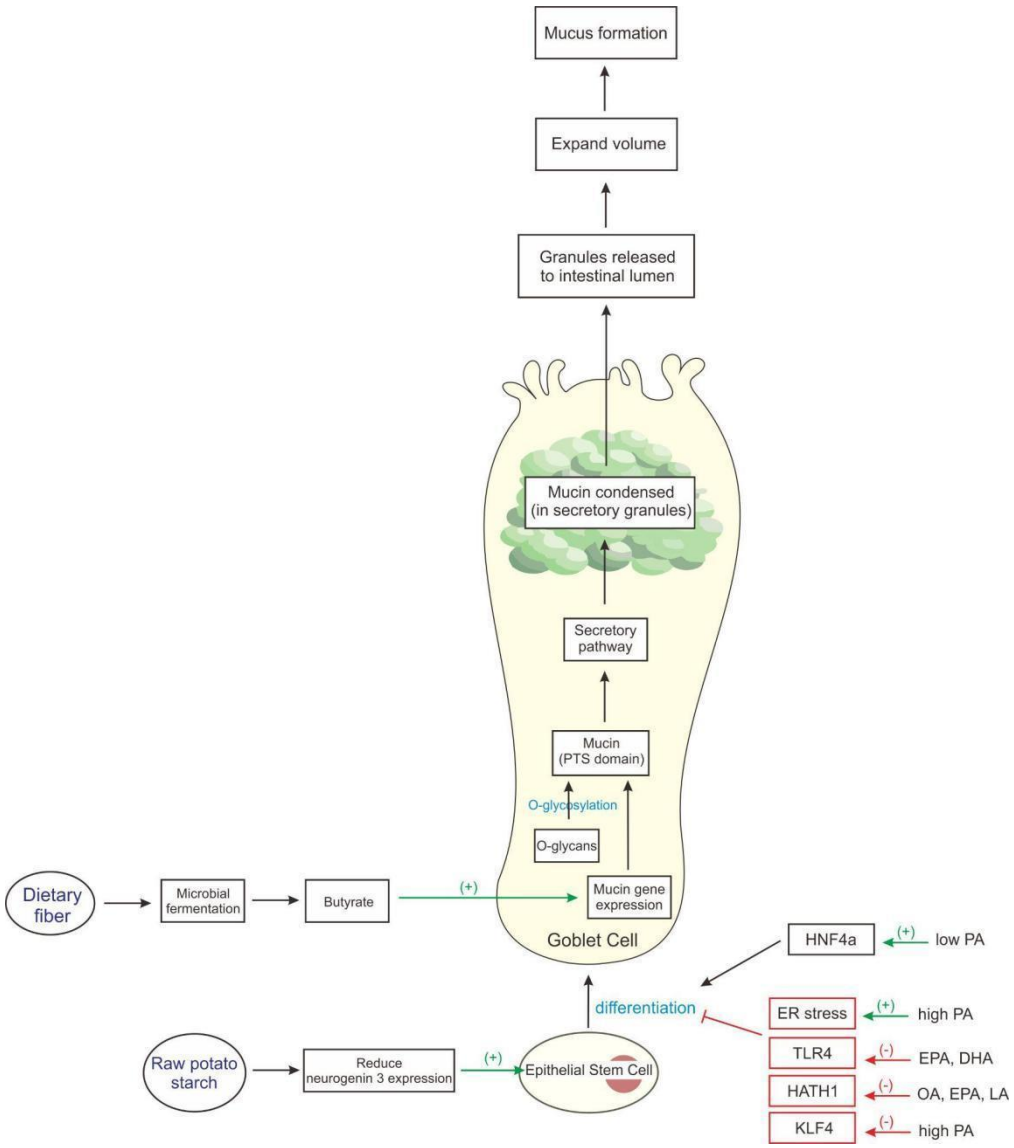

Figure 1. A summary of the possible mechanisms of macronutrients affecting the production of mucus and several microorganisms influencing the mucus layer. PA: palmitic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; OA: oleic acid; LA: linoleic acid. (+) showed beneficial effects, (-) showed detrimental effects.

CONCLUSION

Macronutrient intake was found to play roles in intestinal mucus production alteration, especially in mucin gene expression and secretion. Carbohydrate intake, such as a high fiber diet and resistant starch, is associated with enhanced mucin expression and increased mucus production. In contrast, a low fiber diet was found to harm the mucus layer. Consumption of high saturated fatty acids, especially palmitic acid, was found to negatively affect mucin expression and mucus secretion. Nonetheless, lower PA consumption could increase mucin expression and mucus production. Consumption of unsaturated fatty acids, such as oleic acid, linoleic acid, and fish oil, was found to negatively affect mucin expression by disrupting important transcriptional factors in epithelial cell differentiation.

GAPS IN UNDERSTANDING AND FUTURE RESEARCH

Studies on the effect of macronutrient intake on mucus production are still limited, especially in the molecular pathway of dietary intake, altering mucin expression and mucus production. Nevertheless, some possible mechanisms have been suggested, and further research will be needed to clarify the underlying mechanisms. In addition, thorough studies on other parts of intestinal epithelial cells are also needed to see the effect of macronutrient intake on various mucus production.

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