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Effects of Medium- and Long-Chain Structured Triacylglycerol on the Therapeutic Efficacy of Vitamin D on Ulcerative Colitis: A Consideration for Efficient Lipid Delivery Systems

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ABSTRACT: Due to intestinal malabsorption and poor water solubility, vitamin D (VitD) deficiency in ulcerative colitis (UC) continues to increase. Medium- and long-chain triacylglycerols (MLCT), as novel lipids, have been widely applied in the field of functional food and medicine nutrition. Our previous studies showed that the difference in MLCT structure could affect VitD bioaccessibility *in vitro*. In this study, our results further indicate that, although identical in fatty acid composition, structured triacylglycerol (STG) had a higher VitD bioavailability (AUC = 15470.81 μg/L × h) and metabolism efficacy [s-25(OH)D, $p < 0.05$] than physical mixtures of triacylglycerol (PM), which further affect the amelioration efficiency in UC mice. Compared with PM, the damage of colonic tissues, intestinal barrier proteins, and inflammatory cytokines in STG showed better amelioration at the same dose of VitD. This study provides a comprehensive understanding of the mechanism of nutrients in different carriers and a solution for developing nutrients with high absorption efficiency.

KEYWORDS: *fat-soluble vitamin, bioavailability, inflammatory bowel disease, medium- and long-chain triacylglycerol, delivery system*

1. INTRODUCTION

Ulcerative colitis (UC), one of the most commonly occurring inflammatory bowel diseases (IBD), is difficult to cure completely and easily develops into colon cancer.[1,2] Despite certain effects in amino salicylates, corticosteroids, and immunosuppressants, the side effects limit their therapeutic value.[3,4] Vitamin D (VitD), an essential fat-soluble compound, has received increasing attention due to its functions in regulating the dysfunction in the intestinal barrier, alleviating the inflammatory reaction and imbalance in immunology.[5] However, for UC, due to intestinal malabsorption, insufficient dietary intake and lack of long-term exposure to sunlight, up to 60−70% of patients have VitD deficiency or insufficiency.[6] Our previous meta-analysis also proved that having a low-VitD status can affect a patient's immune response and increase the disease activity.[7]

Due to the poor water solubility, high sensitivity to degradation, and low bioavailability,[8,9] VitD deficiency continues to increase despite the promotion of VitD-fortified foods.[10,11] As a carrier of fat-soluble nutrients, lipids influence the uptake, absorption patterns, and metabolic behavior of VitD.[12,13] Recently, there has been considerable interest in formulated lipids characterized by the more excellent characteristic of digestion, metabolism, and their benefits on health outcomes. Medium- and long-chain triacylglycerol (MLCT), as a novel formulated lipid, has been widely applied in the field of functional food and medicine nutrition.[14,15] MLCT combines the characteristic of long-chain triacylglycerol (LCT) and medium-chain triacylglycerol (MCT) that can quickly provide energy, overcome the disadvantage of LCT in metabolism and immune function, and make up for the deficiency of essential fatty acids in MCT. Notably, due to the special distribution, MLCT is thought to potentially promote lipid digestion and increase micelle and chylomicron content, which can favor the delivery and absorb fatty acid (FA) and fat-soluble nutrients.[16−18] The distribution of FA in triacylglycerol (TAG) can affect the digestion behavior of lipids. Previous study has shown that although the same fatty acid (FA) composition but different distributions of FA on TAG have the significantly difference in lipolysis behavior. These differences can further affect the ability to accommodate fat-soluble vitamins when forming micelles, that is, bioaccessibility. Previous work in our laboratory has demonstrated that STG was greater than PM in VitD bioaccessibility.[19]

Herein, we innovatively hypothesize that other therapeutic effects on UC might be achieved if VitD was delivered to the body by different absorption and metabolic characteristics *in vivo*. In this sense, as an improvement and extension of our previous work, fabrications of two other different TAG structures but identical FA composition MLCT, structured triacylglycerol (STG) and physical mixtures of triacylglycerol (PM), were performed to prove our hypothesis. The digestive fate of STG and PM, including pharmacokinetics behavior and

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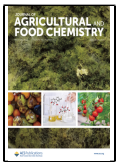

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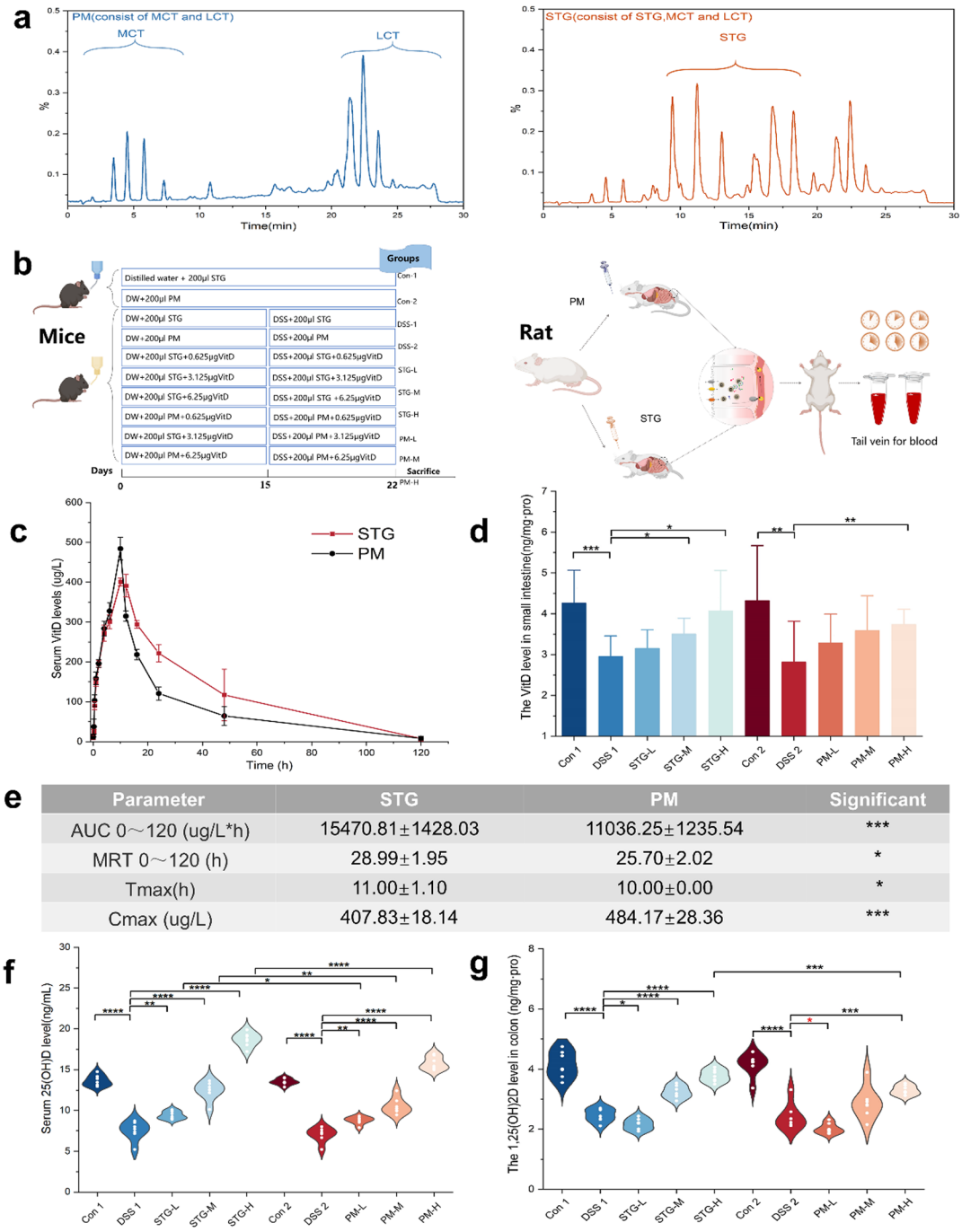

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Parameter	STG	PM	Significant
AUC 0～120 (ug/L*h)	15470.81±1428.03	11036.25±1235.54	***
MRT 0～120 (h)	28.99±1.95	25.70±2.02	*
Tmax(h)	11.00±1.10	10.00±0.00	*
Cmax (ug/L)	407.83±18.14	484.17±28.36	***

Figure 1. Total ion chromatograms of TAG of PM and STG (a); detail animal model experimental design of VitD short or long intervention (b); pharmacokinetic curves of STG and PM in rat (c); the level of VitD in small intestine (d); pharmacokinetic parameters in rat (e); the level of s-25(OH)D in mice (f); the level of 1,25$(OH)_2$D in colon (g). Asterisk represents a significant difference between different groups ($p < 0.05$).

the biodistribution profile in serum, was determined. In addition, we studied the effects of VitD delivered in different formulations on dextran sodium sulfate (DSS)-induced UC mice to illustrate the therapeutic efficiency of MLCT delivery systems on VitD. This study not only provides a comprehensive understanding of the mechanism of VitD in different MLCT structure lipids but also opens up a new door for developing a suitable delivery system in facilitating the UC beyond lifting the bioavailability of fat-soluble nutrients.

2. MATERIALS AND METHODS

2.1. Materials. VitD was purchased by Shanghai Macklin Biochemical Co., Ltd. (with a purity ≥98% $VitD_3$). STG and PM were prepared according to previous research.[19] The total ion chromatograms of TAG of PM and STG are shown in Figure 1a. The detailed information of FA and TAG composition is reported in a previous study.[19]

2.2. Designs of Animal Experiments and Diets. The detail protocol is listed in Figure 1b and in the Supporting Information. Briefly, 70 male C57BL/6 mice aged seven-weeks old and housed in the Animal Housing Unit of Jiangnan University (Gem Pharmatech Co., Ltd., Nanjing, China) were used. For one week to adapt, the mice were randomly divided into 10 groups ($n = 7$). Oil, VitD, or vehicle was given

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once a day by gavage from day 1 to day 21. From the 15th day, the DSS intervention groups were given 2.8% DSS (w/v) solution (MW: 36−50 kDa, MP Biomedicals) and oral gavage with 200 μL of STG or PM. Then, VitD was added to STG and PM at the same concentrations (0.625, 3.125, and 6.25 μg/mL). Afterward, the VitD pretreatment groups (low, medium, and high) were given 2.8% DSS (w/v) solution and different doses of VitD by oral gavage with 200 μL of STG or PM. After 21 days, all animals were euthanized by CO_2 asphyxiation followed by cervical dislocation and exsanguination using cardiac puncture. The Animal Protection Committee has approved the animal experiment protocol of Jiangnan University (JN. No20200710c0600901[134]).

The pharmacokinetics study of VitD was tested following protocol, which was also registered (JN. No20210930S0201206[356]). First, 12 male SD rats (Zhejiang Vital River Laboratory Animal Technology Co., Ltd., China) aged seven weeks were fed adaptively for a week and divided into two groups (n = 6). The rats fasted 12 h before the experiment but were allowed to drink water freely. The drug was administered in 1.5 mL STG or PM (the dose of VitD was 500 μg/kg). After administration, about 500 μL of blood samples was collected in 1.5 mL EP tubes through the tail vein at different times.

2.3. Pharmacokinetics Study of VitD. Pharmacokinetic parameters were determined using the drug and statistics 2.0 software to calculate the following: the total area under the curve (AUC), mean residence time (MRT), time to peak concentration (T_{max}), and peak concentration (C_{max}).

2.4. Concentrations of VitD, 25(OH)D, and 1,25(OH)$_2$D. The concentrations of VitD and 25(OH)D in serum (s-25(OH)), VitD in the small intestine, and 1,25(OH)$_2$D in colon were assessed by commercially available enzyme-linked immunosorbent assay (ELISA) kits (Shanghai Enzyme-linked Biotechnology Co., Ltd., China). First, the freshly isolated pieces of tissues were homogenized with PBS, and the 1% protease inhibitor cocktail was added. Second, the miscible liquids after homogenizing were further centrifuged at 10,000g at 4 °C. Then, the supernatants were taken out. The protein concentration was measured using a bicinchoninic acid (BCA) protein assay kit (Shanghai Enzyme-linked Biotechnology Co., Ltd., China). The results were expressed as pg/mg protein of the tissue.

2.5. Disease Activity Index and Histopathology. Each mouse disease activity index (DAI) during DSS treatment was assessed daily to determine stool consistency, hematochezia, and body weight changes according to the previous method (Table 1). The occult blood in the feces was measured using an occult blood kit (BaSo Biotechnology Co., Ltd., Zhuhai, China). The colon length of each mouse was measured after mercy killing. The colon tissues of each mouse were stained with hematoxylin and eosin (H&E) according to the previous study.[20]

Table 1. Standard for Evaluation of the Disease Activity Index and Histological Injury

weight loss (%)	occult blood or gross bleeding	stool	score[a]
none	negative (no bleeding)	normal#	0
1−5	negative	loose	1
6−10	hemoccult positive (slight)	loose	2
11−15	hemoccult positive	diarrhea (slight)	3
over 15	gross bleeding	diarrhea (water)	4

inflammation	extent	crypt damage	percent involvement	score
none	none	none	none	0
slight	mucosa	1/3	1−25%	1
moderate	mucosa and submucosa	2/3	26−50%	2
severe	transmural	only surface epithelium intact	51−75%	3
		entire crypt and epithelium lost	76−100%	4

[a]Disease activity index = (combined score of weight loss, stool constancy, and bleeding) / 3.

Colonic tissue pathology pieces were scanned by a Pannoramic MIDI Digital Slide Scanner (3D-Hitech Co., Ltd., Hungary). The damage of colonic histological was scored using the modified scoring methods according to a previous study (Table 1).[21]

2.6. Alcian Blue Stain, Mucin2, Adherents Protein, and Tight Junction Proteins. Alcian blue staining was used to evaluate the area of mucin in the colon tissue (All from Vector, Burlingame, CA).[22] The concentrations of recombinant Mucin2 (MUC_2), adherens junction, (AJ) and tight junction (TJ) protein in colon tissues were detected by ELISA kits (SenBeiJia Biotechnology Co., Ltd., Nanjing, China). The pretreatment methods of tissues and the BCA method are the same as in Section 2.4.

2.7. Inflammatory Markers. The concentrations of inflammatory cytokines were measured by ELISA kits (Shanghai Enzyme-linked Biotech Co., Ltd., China). The pretreatment methods of tissues and the BCA method are the same as in Section 2.4.

2.8. Statistical Analysis. Statistical product service solutions (SPSS) 22.0 and Origin 8.0 (Electronic Arts Inc., USA) were used to analyze the data. One-way analysis of variance (Tukey's test) was used to evaluate the significant difference. The study of Spearman correlational was used to evaluate the relevance between TJ/AJ proteins, inflammatory cytokines, and apparent indicators and presented by "correlation heatmap".

3. RESULTS AND DISCUSSION

3.1. Pharmacokinetic Studies of VitD. To compare the difference of MLCT structures in VitD metabolism, we explored the characteristic of pharmacokinetic parameters after a single-dose VitD intervention. As shown in Figure 1c, the serum VitD concentration increased from baseline to maximum during the first 10 h, decreased gradually, and then reached a plateau, similar to the previous study.[23] After entering the blood circulation, VitD is hydroxylated to 25(OH)D in the liver and, subsequently, change to 1,25(OH)$_2$D after a second hydroxylation in the kidney. Finally, 1,25(OH)$_2$D performs various physiological functions in target organs.

In contrast, the T_{max} of PM was 10.00 h earlier than the STG (11.00 h), and the level of C_{max} in the PM group was 484.17 μg/L, significantly higher than STG (Figure 1e). These results suggested that the absorption rate of VitD in PM was faster, and the VitD peak concentration was higher than STG. Fat-soluble metabolism is thought to follow the same fate as lipids in the upper gastrointestinal tract (GIT).[24] Thus, fat-soluble compound bioavailability highly depends on TAG digestion, absorption, and metabolism characteristics. Generally, the digestion and metabolism processes are faster in medium-chain triacylglycerol (MCT) compared with long-chain triacylglycerol (LCT). This could be due to two reasons. Compare with long-chain fatty acid(LCFA), the medium-chain fatty acid (MCFA) molecules have a higher water dispersibility, which causes MCFA to be quickly removed from the surface of the droplets. This characteristic promotes the lipase to continue performing hydrolysis functions.[14] However, the LCFA molecules prefer to accumulate on the droplets, inhibiting lipase's ability to digest further.[18] On the other hand, after lipolysis, MCT is no longer esterified in small intestinal epithelial cells, which tend through directly to the cells and enter the portal vein. Subsequently, they are transported to the systemic system via the liver.[25] For VitD, the PM system is a physical mixture of MCT and LCT; MCT performs a higher hydrolyzed degree and faster metabolism rate. The lipid digestion degree has been proven to play an essential role in the bioavailability of fat-soluble compounds. Digestible lipids increase the bioaccessibility of hydrophobic bioactive in the gastrointestinal tract because they contribute to their release and prefer to form more

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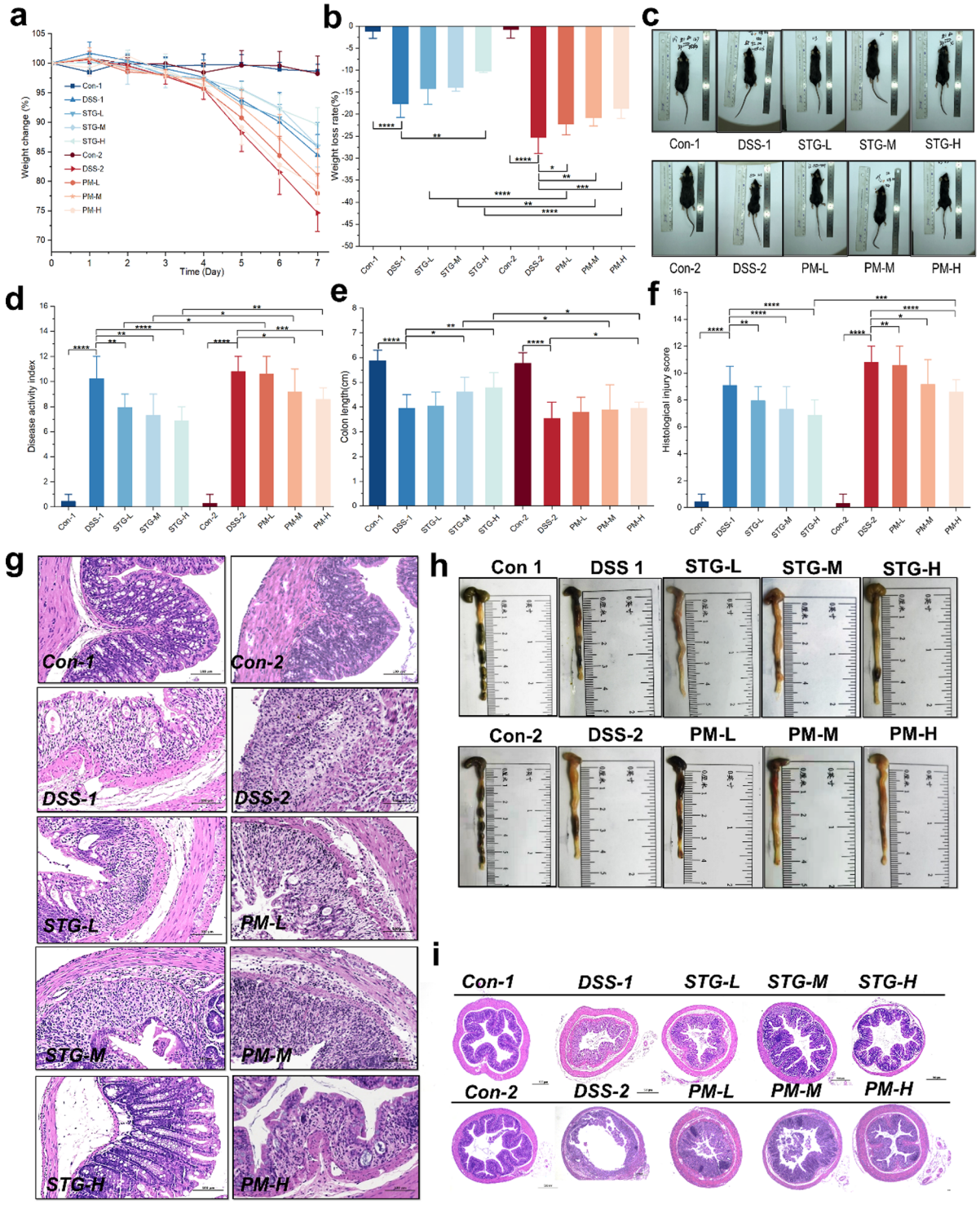

Figure 2. Weight change in mice (a); weight loss rate in mice (b); representative appearance of mice from each group (c); DAI (d); colon length (e); histological injury score (f); histological examination (the scale bars are 100 and 500 μm) (g, i); macroscopic pictures of colons (h).

content of mixed micelles. Similar results about 5-demethylnobiletin have been shown in the Yao et al. study.[26] Thus, the absorption rate of VitD in PM was faster, which caused the VitD peak concentration to be higher than STG.

Interestingly and notably, the AUC_{0-120} of the STG group is 15470.81 μg/L × h, significantly higher than 11036.25 μg/L × h of the PM group. As mentioned above, the MCFA and LCFA of MLCT are simultaneously on the same glycerol skeleton and digested by pancreatic lipase. This promotes the STG system to transport MCFA and LCFA synchronously.[27] Yang et al. showed that LCT bioavailability of fat-soluble compounds is markedly higher than MCT.[28] This phenomenon may be explained by the fact that the LCFA molecule can produce a much larger hydrophobic area than MCFA, which can contain more hydrophobic compounds of VitD. Because the hydrolysis sequence of LCT and MCT does not exist, the higher content LCFA molecules produced by STG hydrolysis can form mixed micelles with high VitD solubility. However, lipase was

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preferentially used by MCT in PM delivery system, which competitively inhibits the digestion and metabolism of LCT. Our previous study also showed that, compared with PM, the higher LCFA molecules in STG were released at the end of the small intestine stage.[19] Overall, the total bioavailability of VitD in the STG group is significantly higher than in the PM group.

3.2. Level of VitD in Small Intestine, s-25(OH)D and 1,25(OH)$_2$D in Colon. We were considering the differences of VitD bioavailability between STG and PM, which may further affect the amelioration of UC. Therefore, we administered the same dose of VitD but a different oil base to mice for two weeks before DSS treatment and continued supplementation during DSS treatment. We first tested the level of VitD in the small intestine. The DSS intervention caused the level of VitD in the colon to decrease compared with the Con group. Previous data showed that mucosal disease and surgical resection could cause lipid malabsorption, further promoting the loss of fat-soluble nutrients.[29] VitD treatment in different doses could significantly increase VitD concentration in the small intestine (Figure 1d). Compared with other MLCT groups, the level of VitD in the STG group was higher (L, M, and H; 3.15, 3.50, and 4.07 ng/mg·pro, respectively), but no statistical difference (p = 0.616, 0.818, 0.377, respectively).

The level of s-25(OH)D in different groups was measured, which is considered the best indicator of body stores and the circulatory status of VitD.[29] As shown in Figure 1f, when fed with DSS, the level of s-25(OH)D in mice decreased compared with the Con group, whatever STG or PM groups. Intervention by VitD significantly increased s-25(OH)D in all VitD groups. Notably, the s-25(OH)D levels in STG-L, STG-M, and STG-H were 0.62, 1.87, and 2.88 ng/mL higher than in the PM groups, respectively. These data suggested that, as a carrier oil, STG could promote VitD metabolism and improve the s-25(OH)D level more effectively than PM during the long-intervention experiment. The level of colon 1,25(OH)$_2$D showed a similar trend in the STG high-dose group, which was significantly higher than PM (p < 0.001, Figure 1g). Specifically, as mentioned above, MCFA and LCFA enter systemic circulation differently. The former tends to enter directly into the intestinal epithelial cells, along with the portal vein, and finally enters into systemic circulation through the liver. Meanwhile, the LCFA prefers to reconstitute TAG in the intestinal epithelial cells and repackage them into lipoproteins, which would enter systemic circulation through the lymphatic system.[25] However, although VitD can be delivered to the liver and join in the first hydroxylation faster, the smaller hydrophobic structure prevents MCT from encapsulating more VitD. Thus, significant differences can be seen in the s-25(OH)D level between STG and PM, which may lead to differences in the amelioration of colitis. In previous epidemiological studies, the higher s-25(OH)D level is associated with lower disease activity, lower intestinal inflammation, better life quality, and lower risk of clinical recurrence in IBD patients.[30] Thus, the index of colitis apparent symptoms, inflammation cytokines, and an intestinal barrier was further evaluated in the next section.

3.3. Improved the Colitis Symptoms. The weight change, colonic length, disease activity index, and histopathological injury score are important apparent evaluation indexes to evaluate the degree of UC in mice.[31] As we can see from Figure 2a−c, from the 4th day, due to the effect of drugs, the body weight of mice showed a significant downward trend, and the body weight of mice in the model group continued to decrease with time. The mice lost 17.7−25.4% of their initial body weight at the process's end. Compared with the DSS group, VitD intervention can effectively reduce the weight loss caused by colitis in mice. However, it is worth noting that, under the intervention of low, medium, and high doses of VitD, the average weight loss rates of the STG group were 14.11, 13.97, and 10.29%, respectively. The average weight loss rates of the PM group were 22.2%, 20.81, and 18.69%, respectively. This indicated that the VitD intervention group with STG as carrier oil had a lower rate of weight loss (p < 0.01). Yeung et al. showed that, in mice fed with VitD deficient (0 IU) or adequate diet (37.8 IU/d/mouse) for seven weeks, VitD-deficient mice gained significantly less weight than VitD-sufficient mice.[32]

In addition, with the increase of the days of membrane making, the formation degree of mouse feces became lower and lower, and the amount of bleeding gradually increased, from occult blood at the beginning of stool to blood visible to the naked eye.[33] Therefore, the DAI value of the model group continued to rise and was higher than that of the other groups. On the 7th day, the DAI value of the model group reached 9.07−10.79 (Figure 2d). Compared with the DSS group, different doses of VitD treatment alleviated the symptoms of a loose stool and bloody stool, except for STG-L and PM-L groups. That is, medium-dose VitD and high-dose VitD significantly relieve colitis. In addition, at the same quantity of VitD intervention, the level of DAI in STG groups was lower than in PM groups. The significant differences between STG and PM in low, medium, and high VitD dose groups were p < 0.05, p < 0.05, and p < 0.01, respectively.

Colonic length is an important index to characterize the degree of colonic inflammation.[34] As illustrated in Figure 2e,h, the colon lengths of the Con groups were 5.76−5.87 cm, respectively, and the colon was ordinarily red. The colonic lengths of DSS group treated mice were 3.54−4.24 cm, which showed a dark red colonic color and intestinal wall swelling and bleeding. Consistent with the above results, the colonic length treated with high-dose VitD was significantly decreased. In contrast, mice with STG as a carrier had longer colon lengths and a better form in VitD-M and H groups. Previous studies have shown that VitD receptor agonists (BXL-62) can dose-dependently ameliorates weight loss and shorten colon length of DSS-induced colitis.[35]

3.4. Recovered the Damage in Colonic Tissues. As shown in Figure 2g,i, all layers of colon tissue have a clear structure, intact mucosal epithelium, and healthy crypt structures in normal mice. However, the mice in the DSS groups showed a large area of ulcer in colon tissue, missing mucosal epithelium, and large inflammatory cell infiltration. In contrast, VitD intervention attenuated colon tissue ulcers, effectively protected most mucosal and glandular structures, and reduced inflammatory cell infiltration. Further, the tissue damage was quantified by performing histological scores on scanned images of H&E-stained sections. As shown in Figure 2f, VitD treatment in different doses could significantly improve colonic inflammatory response. Compared with other MLCT groups, the STG group's histological injury score was higher. The colonic protection effect was better: the crypts were intact, and goblet cells did not significantly disappear, although the difference was statistically significant in the high-dose group (STG-H group vs PM-H group, p < 0.001). Yoo et al. also reported that the histology score of the colon tended to be lower for VitD supplementation (10,000 IU/kg/day of diet) in DSS-induced colitis high-fat mice for 14 weeks.[36]

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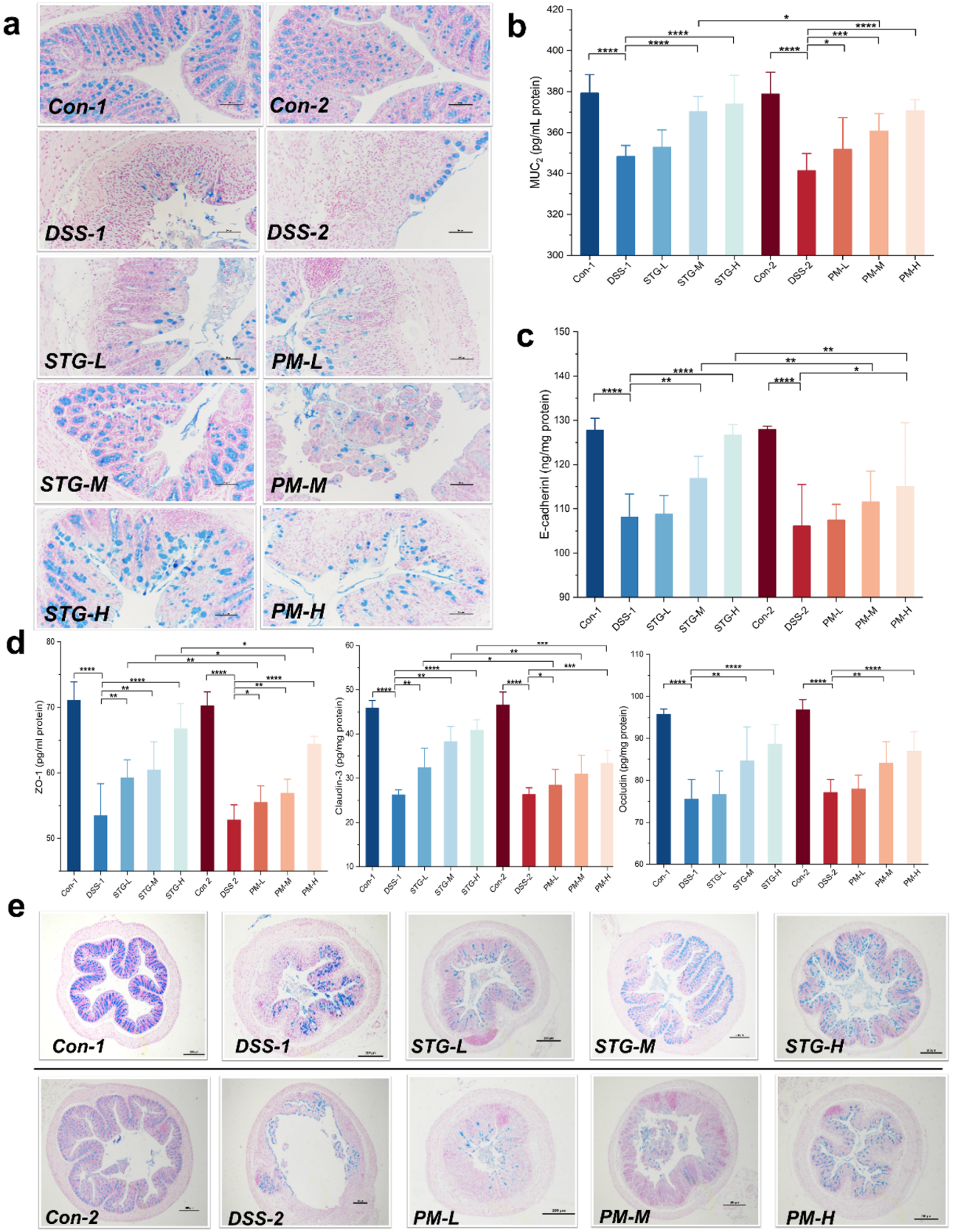

Figure 3. Alcian blue staining (the scale bars are 100 and 500 μm) (a,e). Effects on AJ and TJ proteins in the colon and apoptosis of colonic epithelial cells: MUC_2 (b); E-cadherin 1 (c); ZO-1, claudin-3, occludin (d).

3.5. Protected the Intestinal Barrier. The gut mucosal epithelial barrier separates the body from the luminal micro-organisms and toxic substances, which protects the surface of the healthy intestinal tract from adhesion and invasion by luminal

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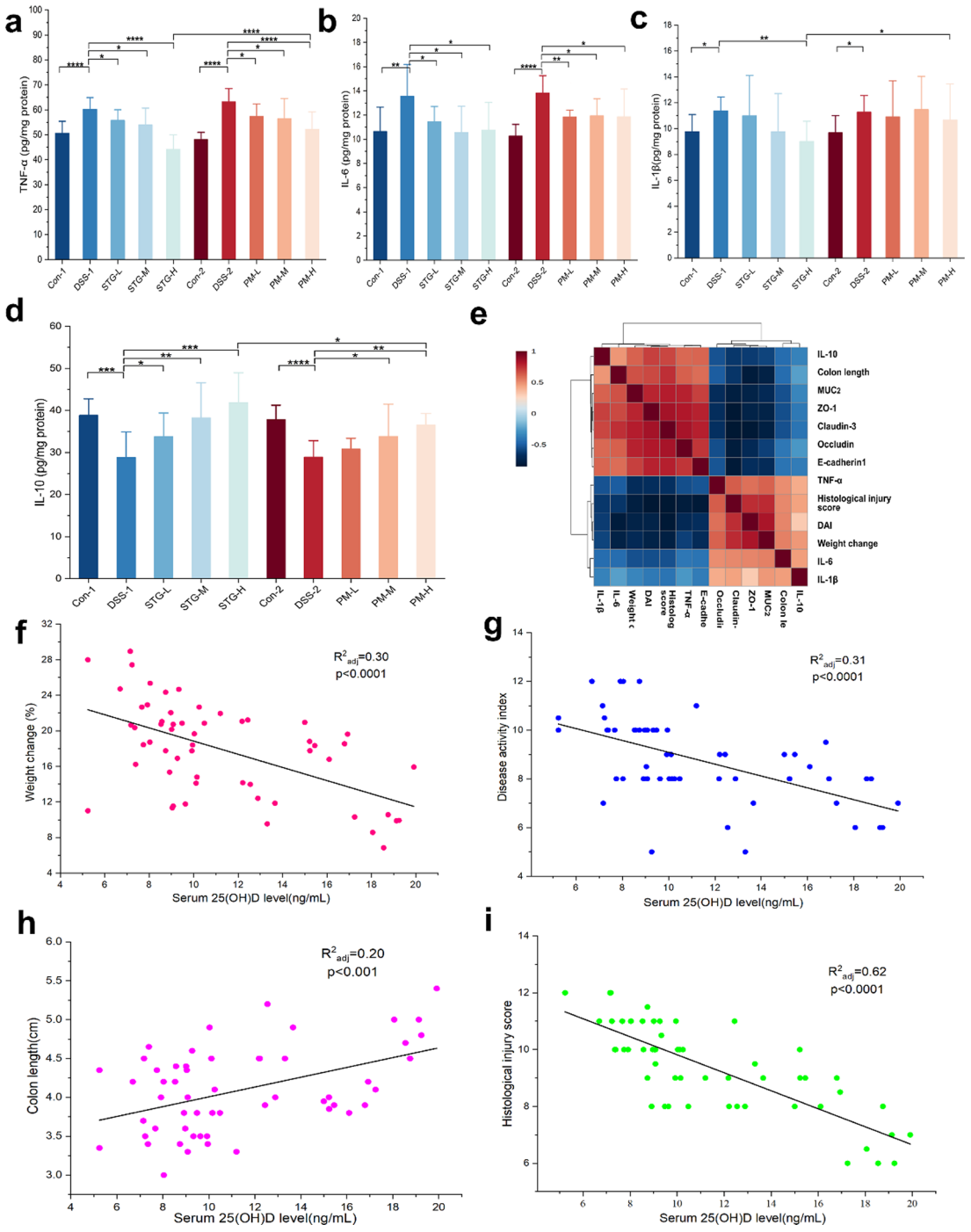

Figure 4. Effects on inflammatory cytokines in colonic tissues: TNF-α (a); IL-6 (b); IL-1β (c); IL-10 (d); correlation heatmap analysis (e); the correlation between s-25(OH)D concentration and apparent index of colitis: Weight change (f); DAI (g); colon length (h); histological injury score (i).

microorganisms.[37] Alcian blue staining solution could stain mucin blue, and the larger the blue area, the higher the integrity of the mucus layer. It can be observed from Figure 3a,e that the colon tissue of mice in the control group contains many goblet cells, which are tightly arranged and orderly, with normal shape and abundant mucin. Compared with the control group, goblet cells in the colon tissue of the model mice were almost destroyed, and only a small amount of mucin was retained. For UC mice, mucosal inflammation results in excess production of proinflammatory cytokines, which can further increase mucosal permeability by altering the intercellular tight junction structure and inducing apoptosis of cells.[38] The colon tissue of mice in the VitD intervention group contained normal goblet cells with orderly and abundant mucins, indicating that VitD could effectively protect goblet cells and ameliorate the destruction of the mucous layer caused by DSS. In addition, we found that, under the intervention of the same dose of VitD, the integrity of the mucus layer in the colon tissue of mice in the STG group was higher. The larger blue area indicated that the delivery of VitD by STG could effectively protect goblet cells and improve the damage to the mucosal layer.

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MUC_2, the main component of the mucus layer secreted by goblet cells, is the better indicator to evaluate and quantify the effect of the MLCT structure on the intestinal barrier. Data in Figure 3b suggest that, compared with the model group, VitD intervention effectively alleviated mucin loss caused by colitis and increased MUC_2 protein concentration (351.71−378.80 pg/mL protein). In contrast, the concentration of MUC_2 in STG groups was 370.10 pg/mL protein higher than in PM groups at the medium dose of VitD ($p < 0.05$). However, no significant difference was observed in the MUC_2 among STG and PM groups in low- or high-dose VitD ($p > 0.05$).

TJ, AJ, gap junctions, and desmosomes are four important junction types in animal cells that maintain the integrity of the intestinal epithelial structure.[39] Occludin, zonula occludins (ZO), claudin, and cadherin family proteins are essential components of TJ and AJ in the epithelial barrier.[40] According to Figure 3c,d, our results showed that the ZO-1, occluding, claudin-3, and E-cadherin 1 levels in the DSS group were significantly decreased compared to the control group. After the VitD supplement, the TJ and AJ protein level was substantially higher than in DSS groups, except for PM-M in claudin-3 and E-cadherin 1, suggesting the intervention of VitD can significantly delay the injury of the intestinal barrier. In contrast, STG as carrier oil had a more significant ameliorate function on TJ and AJ proteins. The proteins of ZO-1, claudin-3, and E-cadherin1 levels were higher than PM at the same dose VitD intervention, except for low-dose VitD in E-cadherin. In addition, no significant difference was observed in occludin protein between STG and PM.

There are few studies on the protective effect of VitD on the intestinal barrier of IBD. Hector et al. explored the impact of $1,25(OH)_2D_3$ on human colon carcinoma cells expressing variable levels of the VitD receptor. $1,25(OH)_2D_3$ induced the expression of E-cadherin and other adhesion proteins.[41] Liu et al. reported that VitD greatly regulated the mRNA expression in claudin-1, ZO-1, and cadherin 1 under a lipopolysaccharide challenge in yellow catfish.[42] Assa et al. showed that $1,25-(OH)_2D_3$ altered transepithelial electrical resistance, decreased permeability, and preserved barrier integrity.[43] Lee et al. confirmed that VitD could upregulate the expressions of occludin in the small intestine, claudin-1 and ZO-1 in the colon of cirrhotic rats.[44,45] Overall, although VitD in regulating immunity and improving the epithelial barrier provides new thinking to UC, more high-quality research is needed to identify the efficacy of VitD supplementation in improving the intestinal barrier's damage.

3.6. Regulated Inflammatory Cytokines. In addition to their differences in the lesion site and tissue morphology, DSS is involved in immune response-induced mouse UC response. This study explored the modulation of VitD intervention of the inflammatory factors associated with type Th2 immune response in colon tissues.[31] TNF-α can activate NF-κB signaling in nuclear factor-activated B cells and promote the production of inflammatory factors such as IL-1β, TNF-α, and IL-6.[34]

According to Figure 4a−d, treatment with DSS increased TNF-α, IL-1β, and IL-6 in the colon tissue of modeled mice and decreased IL-10 levels compared with control groups. These results indicated that VitD-feeding could reduce the concentrations of colonic tissue proinflammatory cytokines and increase anti-inflammatory cytokines. Compared with the DSS treatment group, different-dose VitD treatments significantly decreased the concentration of proinflammatory cytokines TNF-α and IL-6 in STG groups. A similar result was observed in the PM groups. However, only high-dose VitD treatment markedly decreased the concentration of proinflammatory cytokines IL-1β. In addition, compared with the different MLCT structure groups, the proinflammatory cytokines of colon tissue were lower in STG groups. However, significant differences were only observed in TNF-α and IL-1β of VitD high-dose groups ($p < 0.001$). For anti-inflammatory cytokines, compared with the DSS group, VitD treatment can significantly increase the concentration of IL-10 in all VitD treatment groups (Figure 4e, $p < 0.05$). In addition, the concentration of IL-10 in the STG-H group was higher than in the PM group ($p < 0.05$). These results suggest that inflammation severity in mice with UC varies according to their VitD absorption rate. Researchers reported that dietary $1,25(OH)_2D$ supplementation may prevent and limit intestinal inflammation in hosts with high susceptibility to chronic inflammation (IL-6 and IL-1β).[46,47] Yeung et al. found a marked increase in serum proinflammatory cytokine levels demonstrated in VitD deficiency mice, while the intervention VitD could effectively improve the proinflammatory levels.[32] Also, few clinical studies showed a correlation between VitD dose or levels and inflammation severity. Pappa et al. showed that the higher VitD doses were associated with lower levels of IL-6.[48] Dadiei et al. demonstrated that the TNF-α level was also associated significantly with VitD whenever before and after intervention.[49]

3.7. Correlation between TJ and AJ Proteins, Inflammatory Cytokines, and Apparent Indicators Regulated by VitD and Colitis Indexes. To analyze the correlation between VitD supplementation and intestinal barrier proteins, inflammatory cytokines, and apparent markers of enteritis, the correlation was investigated in this study. As shown in Figure 4f−i, different concentrations of s-25(OH)D had different effects on the apparent indicators. The s-25(OH)D concentrations showed negative correlations with a weight change, DAI, and histological tissue scores. Then, the Spearman correlation analysis of the heatmap showed that the concentrations of inflammatory cytokines and TJ/AJ proteins showed different significant correlations with colitis symptoms (Figure 4e). The colon length showed a highly negative correlation with IL-6, IL-1β, and TNF-α, while it showed a highly positive correlation with IL-10, MUC_2, ZO-1, claudin-3, occludin, and E-cadherin1. In addition, the DAI, histological injury score, and weight change showed a highly negative correlation with IL-10, MUC_2, ZO-1, claudin-3, occludin, and E-cadherin1, while they showed a highly positive correlation with IL-6, IL-1β, and TNF-α.

To summarize, the various structures of MLCT can have a significant impact on the absorption and metabolism of lipid-soluble substances. Our findings indicate that, although identical in fatty acid composition, STG had a higher VitD bioavailability (15470.81 μg/L × h) than PM ($p < 0.001$). Notably, the differences in the absorption of VitD will further affect the metabolism efficacy, which improves the level of VitD liver metabolite-25(OH)D. In a DSS-induced mice model of acute colitis, we found that, when DSS entered the intestinal tissue of mice, it destroyed the intestinal mucus layer and cell junction proteins between intestinal epithelial cells, leading to severe damage to epithelial barrier function. The damaged intestinal mucosal tissue loses its original function, gradually penetrating various bacteria and other antigenic substances in the intestinal tract. It stimulates immune cells to produce immune responses, thereby regulating the levels of various inflammatory cytokines. However, VitD intervention increases the s-25(OH)D, which is

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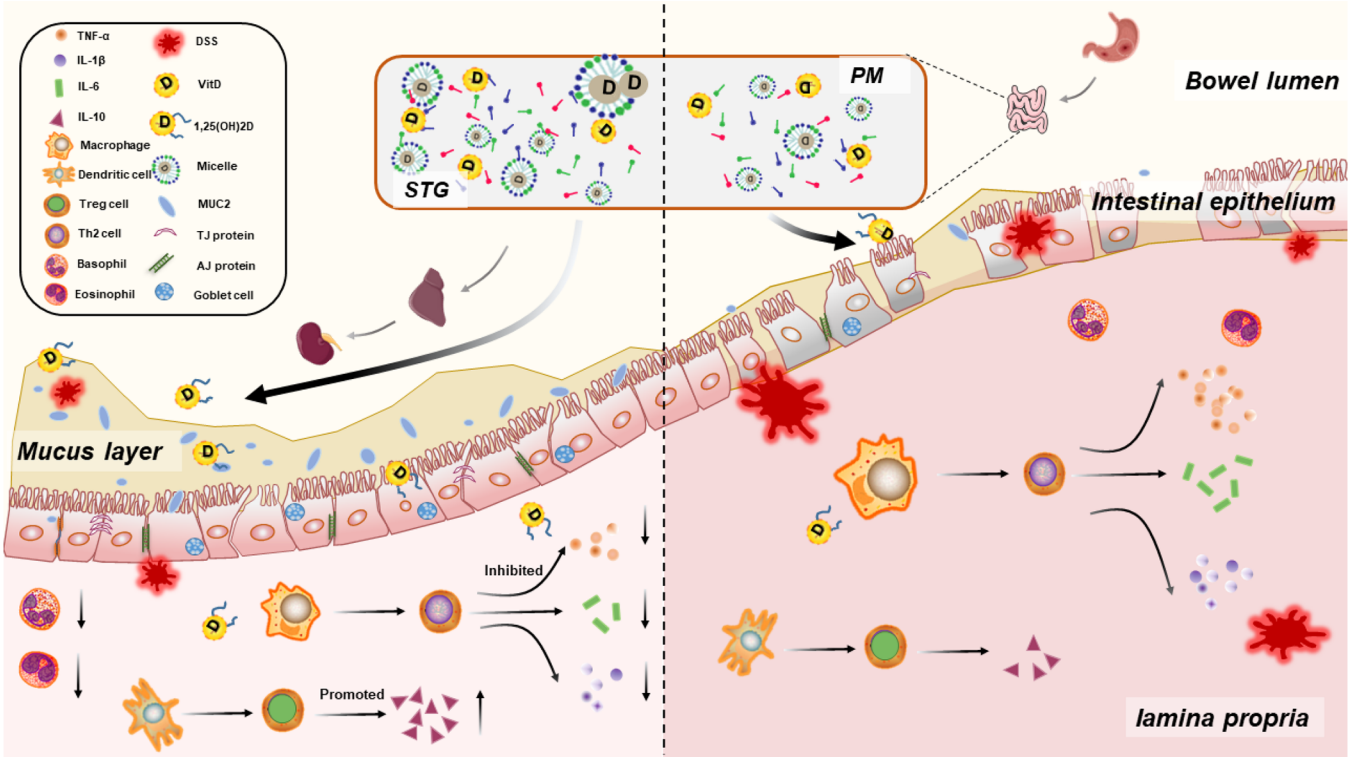

Figure 5. Possible schematic representation of medium- and long-chain structured TAG ameliorate DSS-induced colitis in mice by enhancing VitD bioavailability.

further converted to the active form $1,25(OH)_2D$ and used in various target organs to exert different efficacy. First, VitD and its active form can reduce the body's inflammatory response by regulating the secretion of proinflammatory and anti-inflammatory factors by the relevant immune cells. Second, VitD and its active form can directly regulate the levels of MUC_2 and cell connexin in the mucus layer of the intestinal lumen, protect the intestinal barrier from the invasion of foreign antigens, and maintain the body's homeostasis. The apparent indexes were to delay the weight loss caused by intestinal disease, decrease the DAI score, increase the colon length, and reduce the damage to colon tissue. Due to the higher bioavailability of VitD, STG can play a better preventive and therapeutic role in the remission of colitis. The illustration of the possible mechanism of STG ameliorating colitis induced by DSS in mice by improving VitD bioavailability is also explained in Figure 5.

Up to now, many researchers have devoted themselves to improving the bioaccessibility or bioavailability of VitD.[50,51] However, most studies focus on choosing and designing a suitable carrier to enhance bioavailability. Hardly any studies focus on whether these bioavailability differences further affect *in vivo* nutrition efficacy. Our findings indicate that different MLCT structures can lead to significant differences in the absorption and metabolism of lipid-soluble substances, which can further affect their nutritional effectiveness. The conclusions in the work provide a unique application for designing novel lipids with adequate delivery capacity for fat-soluble compounds. Meanwhile, it is noteworthy that several improvements can be considered for future studies. This paper's improvement of enteritis after VitD intake under different MLCT types was primarily investigated from serum bioavailability and pharmacokinetics. The effects of the MLCT structure on the transition of carriers in the digestive tract and the subsequent absorption (intestine), metabolism (liver and kidney), and distribution (target organ) behavior of embedded VitD are worth further exploring.

Recently, researchers have carried out relevant studies. Chen et al. identified that, if different carriers are used to deliver fat-soluble substances, there are significant differences in the composition and distribution of fat-soluble nutrient metabolite.[52,53] The nanoparticle beta-carotene tends to be stored in adipocytes. In contrast, the nanoemulsion beta-carotene tends to be metabolized into retinol and stored in the liver, leading to different effects on high-fat stimulation. *In vitro* studies by Xiao et al. have demonstrated that lipid-soluble substance' mode and site of action should be considered when considering bioavailability enhancement.[54] High concentrations of natural active nutrients mitigate UC by regulating immune homeostasis or inflammatory responses in the systemic circulation. In the colon, they improve UC using reparation of the intestinal mucosal barrier or regulation of intestinal microflora. Thus, in further reducing the loss in GIT, considering the function mechanism and target, achieving precise nutrition is our main challenge and trend.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.2c07437.

The specific details of Section 2.2-related experiments (PDF)

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Y.G.: data curation and analysis, writing—original draft; T.Z.: suggesting and manuscript revision; Y.X.: experiments, data curation; E.K.: investigation, manuscript language revision; M.C.: suggesting, writing-review and editing; X.S.: Experiments and software technology; R.L.: funding acquisition, project administration; M.C. and X.W.: suggesting and conceptualization.

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ABBREVIATIONS USED

AJ, adherens junction; BCA, bicinchoninic acid; DAI, disease activity index; DSS, dextran sulfate sodium; ELISA, enzyme-linked immunosorbent assay; FA, fatty acid; GIT, gastrointestinal tract; H&E, hematoxylin−eosin staining; LCT, long-chain triacylglycerol; LCFA, long-chain fatty acid; IBD, inflammatory bowel disease; IL-6, interleukin-6; MCT, medium-chain triacylglycerol; MCFA, medium-chain fatty acid; MLCT, medium- and long-chain triacylglycerol; MRT, mean residence time; MUC_2, recombinant mucin 2; PM, physical mixtures of MCT and LCT; SPSS, statistical product service solutions; STG, structured medium- and long-chain triglycerides; s-25(OH)D, 25(OH)D in serum; TAG, triacylglycerol; TJ, tight junction; UC, ulcerative colitis; VitD, vitamin D; ZO, zonula occludins

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