Dear professor Tang,

We would like to thank the editor and the reviewers for their conscientious reviews, and insightful comments and suggestions to improve the manuscript. In the response below, we have addressed all the concerns raised by the editor and the reviewers in the revised manuscript. We hope the editor and the reviewers will find that our revised manuscript has improved and is suitable for publication. All changes have been marked in blue.

I hope my paper could achieve the academic standards of your magazine and be published finally. Thank you very much.

Yours Sincerely,

Min Jiang and Lixuan Sang

Response to Reviewers Comments

Manuscript NO: 53772

Title: Probiotic mixture VSL#3: An overview of basic and clinical studies in chronic diseases

Authors: Fang-Shu Cheng, Dan Pan, Bing Chang, Min Jiang, Li-Xuan Sang

Reviewers' comments:

Reviewer #1: To me, the title, abstract, key words and core tip reflect closely what is in this review.

Major comments

1. In the abstract, authors said « We observed that VSL#3 had a therapeutic or preventive effect in various systemic diseases ». Even if authors previously published on VSL#3, this statement is not appropriated.

Response: Yes, thank you for pointing out the problem, our statement is not appropriated enough. It has been corrected as following:

We found that VSL#3 has a therapeutic or preventive effect in various systemic diseases per a large number of studies

2. In the Core Tip, authors said « The imbalance of intestinal microbiota is the causal factor and concurrent cause of multiple diseases. ». This is a very strong assumption which has not been yet demonstrated.

Response: Thanks for your reminding, our previous writing is a very strong assumption. Now we revise our statement as following:

The imbalance of intestinal microbiota is one of the important factors in multiple diseases.

All along the manuscript, mainly before page 11, some of the authors statements requires more precautions regarding the effects of the probiotic product.

Minor comments

1. - p.5 : « VSL#3 has a protective effect on intestinal barrier function (IBF), which is the major mechanism in treating all kinds of chronic diseases. »

Response: Yes, our statement is not accurate enough. Now we revise the statement as following:

VSL#3 has a protective effect on intestinal barrier function (IBF), which is one of the important functions for treating multiple chronic diseases.

2. - p.7 : « The positive effect of VSL#3 on intestinal biological barrier function was also mediated by SCFA acetate ». This sentence does not prove that acetate is responsible of the positive effects of VSL#3. Author statements require more precise and scientific explanations.

Response: Yes, our statements are lack of precise and scientific explanations. Now we add some explanations as following:

The positive effects of VSL#3 treatment were also mediated by SCFA acetate. Acetate significantly reduced colonic permeability by reducing colonic inflammation and promoting growth of the commensal *Lactobacillus*[11].

3. - p.8 : « Besides, a culture of HT-29 monolayer cells with VSL#3 DNA was shown to inhibit IL-8 secretion induced by Salmonella in the presence of proinflammatory stimuli ». The sentence does not mean that VSL#3 inhibits IL-8 secretion in stimulated HT-29 cells. The sentence has to be modified.

Response: Yes, thanks very much for your reminding, our statement is not accurate enough. Now we revise the sentence as following:

VSL#3 DNA was shown to inhibit epithelial IL-8 secretion in response to *Salmonella* DNA and proinflammatory stimuli (such as TNF-α)[20].

4. - p.9 : « Interestingly, Lactobacilli and Bifidobacteria from VSL#3 did not induce IL-8, and a high concentration of streptococcal strains induced IL-8 when HT29/19A monolayers were cultured with cell extract fractions from a single strain of VSL#3 ». This sentence is hard to understand, and authors need to discuss what does this observation mean.

Response: Yes, our expression is not clear. We think it is not appropriate to put this sentence here, so we delete it.

5. - p.10 : « Then, a study in IL 10-knock out (KO) mice showed that VSL#3 down-regulated the signaling pathway of toll-like receptors (TLR) ». This sentence is not clear when compared to the original publication. This has to be modified.

Responses: Yes, thank you for your suggestion. The statement is not complete and we add some information as following:

Then, a study showed that VSL#3 down-regulated the signaling pathway of toll-like receptors (TLR), mainly TLR4, and the related effector pathways such as T-cell and B-cell receptor signaling in IL 10-knock out (KO) mice.

6. - p. 10 : « In the review, VSL#3 was proven to be effective in gastric ulcer, diarrhea-predominant enteritis, irritable bowel syndrome (IBS), ulcerative

colitis (UC), pouchitis, colitis and so on. ». This review has not proven any effect of VSL#3 in these diseases. Authors should revise the sentence carefully.

Response: Yes, the sentence is not correct enough and has been corrected as following:

Studies have indicated that VSL#3 provides benefits in gastric ulcer, diarrhea-predominant enteritis, irritable bowel syndrome (IBS), ulcerative colitis (UC), pouchitis, and colitis in animals and humans.

7. - p.11 : « The use of antibiotics generally suppresses the normal intestinal microbiota, which leads to the overgrowth of resistant microbiota (Clostridium difficile). » This is a strong statement and fortunatelly not every single people using antibiotics had CD infection.

Response: Yes, thank you for your reminding and our statement is not accurate enough. Now we revised our statement as following:

The occurrence of diarrhea is probably due to disruption of the normal gastrointestinal microbiota.

8. - p.14 : « They are a group of heterogeneous diseases caused by an overreaction of intestinal immune systems to intestinal commensal bacteria ». Are authors sure of this statement ? What about food components?

Response: Thanks very much for pointing out the problem and this statement about the etiology of inflammatory bowel disease is not comprehensive enough. We improved the statement as following:

It is currently thought that possible factors include: environmental factors, genetic susceptibility, infection factors, and a dysregulated immune system. An improper immune response to the intestinal microbiota probably contributes to the occurrence of IBD in genetically susceptible hosts. Dietary factors among environmental factors might influence immune function and could also be some of the risk factors for the occurrence of IBD[61,62]. One

study indicated that milk and fried food were associated with increased risk of IBD[63].

9. - p.15 : « The fecal microbiota of patients with UC was not significantly different from that of controls » This is the statement from only one study from 1991 on the fecal microbiota. Is this the same conclusion in more recent studies? Many studies have since demonstrated that the composition and function of intestinal microbiota in UC patients are compromised.

Response: Yes, thank you for pointing out an important problem and it is an excellent advice. There were some studies indicating that the composition of intestinal microbiota in IBD patients recently. We adopt the viewpoint from a recent journal in the paragraph of UC and CD as following:

In a systemic review, *Eubacterium rectale* and *Akkermansia* were decreased in all three studies, and *Escherichia coli* (*E coli*) was increased in four of nine studies for patients with UC[64].

In a systemic review, *Christensenellaceae* and *Coriobacteriaceae* in all three studies and *Faecalibacterium prausnitzii* in 6 of 11 studies were decreased compared with controls, and *Actinomyces*, *Veillonella*, and *E coli* were increased in two studies for patients with CD[64].

10. - p.16 : « Several studies have provided evidence about the effect of VSL#3 in dextran sulphate sodium (DSS)-induced murine colitis, which is similar to human UC. » This statement is not appropriated.

Response: Yes, we revised the sentence and a similar sentence in the paragraph of CD as following:

Several studies have provided evidence about the effect of VSL#3 in dextran sulphate sodium (DSS)-induced colitis.

Moreover, several studies elucidated the protective effect of VSL#3 in 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis from different mechanisms.

11. - p.16-17 : « The different results indicated that the efficacy of VSL#3 was dependent on the dosage of VSL#3. ». Authors cannot compare two different studies from different labs and conclude that the difference is only due to the dosage of the probiotic, unless the negative controls of the two studies have been compared.

Response: Yes, thank you for your reminding. Our conclusion is so arbitrary and there is not enough evidence to support it. Now we revised the statement as following:

The above results are not exactly consistent, and the negative result may be related to the sex of mice and the induced pattern of DSS colitis. The dose, the mode of administration, and the treatment course of VSL#3 also impact the result to some extent.

12. - p.20 : « One study showed that VSL#3 could retard the development of colonic inflammation to dysplasia and cancer, accompanied by an increase of antiangiogenic factor vitamin D receptor (VDR) ». Authors need to mention that this study has been done on rats (moreover, the use of rats instead of mice in CRC models is discussable).

Response: Yes, I am sorry to forget to mention the experimental objects. The sentence has been corrected as following:

One study showed that VSL#3 could retard the development of colonic inflammation to dysplasia and cancer, accompanied by an increase in antiangiogenic factor vitamin D receptor (VDR) in a rat model of colitis-associated CRC[103].

And we also mentioned the use of mice in CRC model as following:

However, in terms of murine colitis-associated CRC, two studies showed contrasting results.....

13. - p.22 : « It encompasses a system of histopathological change from simple liver steatosis to cirrhosis or fibrosis, and NAFLD might be caused by intestinal dysbiosis, which increases intestinal permeability to bacterial products and harmful substances. » Dysbiosis might participate to NAFLD, but the authors of reference 107 never assumed that dysbiosis is the cause.

Response: Yes, the reference is not accurate enough. We correct the reference as following:

It encompasses a system of histopathological changes from simple liver steatosis to steatohepatitis, advanced fibrosis, and cirrhosis, and NAFLD may be caused by intestinal dysbiosis, which increases intestinal permeability to bacterial products and harmful substances[107,108].

14. - p.28 : « Atherosclerosis is caused by bacterial translocation from the oro-gastrointestinal tract to circulation ». Authors cannot make this statement. First atherosclerosis is multifactorial and secondly, the reference 155 never reported this information. I would suggest to authors to revise the writting of their paper according to the « Radiation-induced enteritis » (p.11) paragraph which has been well written.

Response: Yes, our statement is not complete enough and I am very sorry to make a wrong reference. Now we improved the paragraph as following:

Atherosclerosis is a chronic disease that might be associated with the bacteria from the oral cavity and even the gut[156]. Two studies assessed the ability of VSL#3 to protect against atherosclerosis in ApoE $^{-}$ / $^{-}$ mice. They demonstrated that VSL#3 had a beneficial effect on AS and perhaps worked by significantly reducing the development of atherosclerotic or aortic plaques and biomarkers of vascular inflammation. Vascular cell adhesion molecules

(VCAM) and inter cellular adhesion molecules (ICAM) were shown to be reduced by VSL#3 in both two studies[157,158]. One study indicated that VSL#3 (2.78×10^{11} CFU/day for 12 weeks) was able to significantly lower gelatinase matrix metalloproteinase-9 (MMP-9), but another plasma atherosclerotic biomarker, total plasminogen activator inhibitor-1 (tPAI-1), was significantly elevated by the combination of telmisartan (positive control drug) and VSL#3[157]. The reason for this is not clear. The administration of VSL#3 (2×10^{10} CFU/kg/day, 6 days per week for 12 weeks) effectively decreased the percentage of CD36 positive cells among circulating macrophages[158].

15. A proper definition of the term « probiotic » could be appreciated in the introduction section.

Response: Yes, thank you for reminding us that important aspect to enrich our review. We add the definition to the introduction section as following:

The internationally endorsed definition of probiotics is live microorganisms which, when administered in adequate amounts, confer a health benefit to the host[1].

16. The use of the expression « a kind of » to refer to a certain type of probiotics or strains does not mean what authors want to say and is not appropriated in a scientific paper.

Response: Yes, the expression is not appropriated. We revise the expression as following:

VSL#3 is a commercial probiotic mixture consisting of eight bacterial strains: four strains of *Lactobacillus* (*Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus paracasei,* and *Lactobacillus delbrueckii ssp. Bulgaricus*), three strains of *Bifidobacterium* (*Bifdobacterium breve,*

Bifdobacterium longum, and *Bifdobacterium infantis*), and one strain of *Streptococcus* (*Streptococcus thermophilus*).

17. All bacterial names are supposed to be in italic.

Response: Yes, thanks for your reminding. Now all bacterial names are revised in italic.

18. p.7 « Furthermore, the production of short-chain fatty acids (SCFAs) by intestinal mucosal cells reduces the intestinal pH and plays an important role in maintaining intestinal biological barrier function[5]. » This statement is partially wrong. SCFA are not produced by intestinal cells but by bacterial fermentation only.

Response: Yes, thank you for pointing out the mistake. Now we revise the opinion as following:

The production of short-chain fatty acids (SCFAs) by gut microbial fermentation reduces intestinal pH and plays an important role in maintaining intestinal biological barrier function[3,11].

19. p.11 : « CDAD » has to be defined.

Response: Yes, thank you for your reminding and i am very sorry. We defined "CDAD" as following:

Clostridium difficile-associated diarrhea (CDAD) is the most serious form of AAD.

20. p.16 : « DAI » has to be defined.

Response: We defined DAI as "disease activity index" as following:

In two trials, VSL#3 was both capable of reducing the ulcerative colitis disease activity index (DAI) scores and ameliorating clinical symptoms significantly compared with placebo[67,68].

21. p.21 : « The reason for the two different results may be that the administration time of VSL#3 was after the onset of inflammation and dysbiosis in the second study ». What does this conclusion mean in regard of the use of VSL#3 in CRC patients? Authors should discuss more this conclusion.

Response: Yes, we should discuss more about the conclusion. At present, there is a lack of clinical trials of VSL#3 in CRC patients, which needs further studies. We delete the statement which is not accurate and add one sentence as following:

The different results may be associated with the type of mice and the induced pattern of murine CRC in the two studies. The dose, the mode of administration, and the treatment course of VSL#3 may also have impacted the results.

22. p.31 : « Apart from this, we also demonstrated that VSL#3 had a benefit on obesity and diabetes, allergic diseases, nervous systemic diseases, AS, bone diseases and female reproductive systemic diseases. » Authors did not demonstrated all these potential effects, but reported and participated to it. In their manuscript and Tables, authors should talk about probiotic intake in terms of CFU per day when possible, rather than volumes or masses.

Response: Yes, thank you very much for pointing out the problem, our statement is not accurate enough. Now we revised the sentence as following:

Apart from this, many studies demonstrated that VSL#3 has a benefit on obesity and diabetes, allergic diseases, nervous systemic diseases, AS, bone diseases, and female reproductive systemic diseases.

Besides, we add the intake of VSL#3 in terms of CFU per day when the original publications mentioned it in these paragraphs. But in describing some studies, we do not mention the specific dose of VSL#3

when the original publications did not mention it. We add them as following: In overweight (body mass index, BMI > 25) adults without diabetes, VSL#3 (one capsule every day before any meal or breakfast, 1.125 × 10^{11} CFU/capsule) was able to significantly reduce total cholesterol, triglyceride (TG), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL) concentrations and increase high-density lipoprotein (HDL) concentration[141].

The administration of VSL#3 (1.5 × 10^{10} CFU in 250 µL of PBS daily) inhibited the b-lactoglobulin (BLG)-induced allergic reaction, mainly by increasing intestinal sIgA in BLG-sensitized mice[151].

One study indicated that VSL#3 (2.78 × 10^{11} CFU/day for 12 weeks) was able to significantly lower gelatinase matrix metalloproteinase-9 (MMP-9), but another plasma atherosclerotic biomarker, total plasminogen activator inhibitor-1 (tPAI-1), was significantly elevated by the combination of telmisartan (positive control drug) and VSL#3[157].

The administration of VSL#3 (2 × 10^{10} CFU/kg/day, 6 days per week for 12 weeks) effectively decreased the percentage of CD36 positive cells among circulating macrophages[158].

It has been reported that the oral administration of VSL#3 (twice a week with 1 × 10^{9} CFU) could protect against ovariectomy (OVX)-induced bone loss in sex steroid-deficient mice by increasing intestinal barrier integrity and inhibiting intestinal and bone marrow inflammation[159].

23. Are Figure 2 and «3 original figures made by authors ?

Response: Yes, Figure 2 and 3 are original figures made by us.

24. A recent publication by Razafindralambo H et al. (Probiotics Antimicrob Proteins. 2019) showed variability in the probiotic formulation VSL#3 which could impact effects of the product. Do authors can comment this work ?

Response: Yes, we comment this work in the paragraph of side effects and limits related to VSL#3 as following:

One study indicated that proteomic analyses showed differences in protein abundances, identities, and origins of VSL#3 from different sites[167]. Therefore, we suggest that the protein abundances should be identified through proteomic analyses in the clinical and basic studies of VSL#3 in the future.

25. Authors should not only discuss the « side effects related to VSL#3 » (p.30) but also discuss the limits of this formulation in their review.

Response: Yes, thank you very much for reminding us the important aspect to enrich our review. We add a new paragraph about the limits of VSL#3 as following:

SIDE EFFECTS AND LIMITS RELATED TO VSL#3

..... The use of VSL#3 also has some limits. VSL#3 contains only eight bacterial strains, and the intestinal microecosystem of each patient is complex, so these strains may not be suitable for all patients. Achieving the one-to-one treatment of formula probiotics and individuals is difficult. The results mentioned above of VSL#3 in the treatment of various diseases are not exactly consistent, which may have been caused by different sources of VSL#3. One study indicated that proteomic analyses showed differences in protein abundances, identities, and origins of VSL#3 from different sites[167]. Therefore, we suggest that the protein abundances should be identified through proteomic analyses in the clinical and basic studies of VSL#3 in the future.

26. The conclusion is a bit long and should fit into one paragraph. Some informations in the Conclusion could move to Introduction section.

Response: Yes, thanks for your reminding, we modify the introduction and the conclusion as following:

INTRODUCTION

.....VSL#3 is a kind of formula probiotic with sufficient evidence-based medical evidence in some digestive systemic diseases, but evidence is insufficient in many other systemic diseases. We need to observe whether VSL#3 is effective in these diseases in the future.

CONCLUSION

In the past, it has been reported that VSL#3 has a profound effect on digestive systemic disorders, especially gastrointestinal disorders. Apart from this, many studies demonstrated that VSL#3 has a benefit on obesity and diabetes, allergic diseases, nervous systemic diseases, AS, bone diseases, and female reproductive systemic diseases. In most cases, the use of VSL#3 is safe and well-tolerated, but more precise mechanisms and effects of VSL#3 still need to be studied. To achieve one-to-one individualized treatment, we need to prepare exclusive probiotics according to the real-time monitoring of patients' intestinal microbiota. However, the current detection technology is not accurate enough. The study of the intestinal microbiota is challenging since most cannot be cultivated[10]. Thus, the solutions to the above problems will hopefully provide a new direction for precise medicine in the future.

27. The language and grammar is not accurate all along the manuscript. The language requires a deep revision. A strong review of the English is required before publication of the review.

Response: Yes, thanks for your reminding, we have already made a second language editing.

Reviewer #2:

The manuscript is a systematic review of the literature on the therapeutic use of VSL#3, a probiotic mixture of bacterial strains, for treatment of various chronic diseases. Many studies have reported that VSL#3 can decrease the severity of chronic diseases, including but not limited to those of the gastrointestinal tract. The mechanisms of action of VSL#3 include the synthesis of small molecules and reduced colonization of the gastrointestinal tract by armful bacteria, which have beneficial effects on the function of the intestinal barrier and of gut immune cells.

General comment

This is an interesting review. In its present form, the manuscript requires substantial editorial correction of the English. Several sentences need to be revised throughout the manuscript. Instead of using the words "improve" or "ameliorate" at all sauces, it would be preferable to use other words (reduce, decrease, dampen) depending on the context. Treatment does not improve tissue damage or disease severity but can improve tissue functions or decrease (reduce, dampen) disease severity.

Response: Yes, thank you for pointing out the problem, it is an excellent advice. We have already made a second language editing, and have revised these words and sentences as following:

Another study showed that VSL#3 was able to dampen clinical symptoms by improving the mechanical distensions of the colonic wall in patients with diarrhea-predominant IBS[49].

Besides, one placebo-controlled study showed an obvious decrease in inflammation and a reduction of epithelial permeability upon administration of a high-dose VSL#3 (15 mg/day for 7 days) to patients[76].

Both heat-killed and live VSL#3 could reduce DSS-induced rat colitis by reducing IL-23, IL-6, STAT3 and phosphorylated-STAT3 (P-STAT3) expression in colonic tissue[78,79].

Furthermore, a significant decrease of iNOS scores in the VSL#3 group suggested a decrease in the inflammatory status of the disease[95].

The combination of metformin and VSL#3 was able to decrease inflammation and tumor progression by inhibiting macrophage infiltration, and the positive effect of VSL#3 on murine ulcerative colitis-associated carcinogenesis was achieved by adjusting the proportion of beneficial and harmful bacteria in feces and intestinal mucosa[100,101].

In contrast, the second study showed that VSL#3 was not able to protect against colitis-associated colorectal cancer.

One study in Table 5 showed that VSL#3 could decrease liver damage in NAFLD and hepatitis C virus (HCV)-related hepatitis patients[109].

VSL#3 could decrease the severity of NAFLD, mainly by increasing the levels of glucagon-like peptide 1 (GLP-1) and activated GLP-1 (aGLP-1)[110,111].

VSL#3 was not able to prevent liver steatosis or inflammation but was able to reduce hepatic fibrosis and the accumulation of collagen and α-smooth muscle actin (α-SMA) in methionine choline-deficient (MCD) diet-induced murine NASH[120].

One trial indicated that VSL#3 could mitigate liver damage in patients with alcoholic liver cirrhosis (AC) by decreasing the plasma levels of malondialdehyde (MDA), 4-hydroxynonenal (4-HNE), and S-Nitrosothiols (S-NO).

And we add a sentence in the paragraph of other diarrhea:
An improvement may have been induced by VSL#3 in tissue functions, but this requires confirmation.

Specific comment
The following sentence (underlined words) should be revised since the current literature suggests that PTPN2 has anti-inflammatory properties.
1. “Sometimes, the influence of VSL#3 on tight junction protein is achieved by increasing the protein level and enzymatic activity of T-cell protein tyrosine phosphatase (TCPTP), an inflammatory bowel disease (IBD) candidate gene PTPN2 protein product.”

Response: Yes, thank you for your reminding, our statement is not clear. Now we revised the sentence as following:
The influence of VSL#3 on tight junction protein is achieved by increasing the protein level and enzymatic activity of T-cell protein tyrosine phosphatase (TCPTP), which is the protein product of the tyrosine-protein phosphatase non-receptor type 2 (*PTPN2*) gene, which has a protective effect on inflammatory bowel disease (IBD)[5,8].

The following sentences should be revised/rewrote:
Response: Yes, thank you for pointing out the problems. Now we revised these sentences as following:

2. -The stimulation effect required the temporary colonization of high-dose VSL#3 in the intestinal lumen[26].
Response: The stimulation effect required the temporary colonization of VSL#3 in the intestinal lumen[25].

3. However, VSL#3 (10^5 organisms/ml) did not change the immature phenotype and costimulatory molecules expression of DC, and high-dose VSL#3 (10^7 organisms/ml)…
Response: However, VSL#3 (10^5 organisms/mL) did not change the immature phenotype and costimulatory molecules expression of DC, and VSL#3 (10^7 organisms/mL)…

4. At the earliest stage of DC antigen presentation, VSL#3 potentially induced IL-10 by DC from blood and intestinal tissue[31].
Response: VSL#3 also decreased IL-12 (p40) production induced by LPS, and was a potent inducer of IL-10 by DC from blood and intestinal tissue[30].

5. Additionally, a placebo-controlled study showed that VSL#3 expressed an anti-inflammatory effect…
Response: Additionally, one study showed that VSL#3 had an anti-inflammatory effect…

6. One study of rats demonstrated that VSL#3 was effective at high concentrations (1.2×10^{10} bacteria) at enhancing acetic acid-induced gastric ulcer healing by stimulating the production of angiogenesis-promoting growth factors…

Response: A study demonstrated that VSL#3 is effective at high concentrations (1.2×10^{10} bacteria) at enhancing acetic acid-induced gastric ulcer healing by stimulating the expression and production of angiogenesis promoting growth factors, mainly vascular endothelial growth factor (VEGF) in rats[34].

7. The up-regulation of the innate immune response against invasive microbiota was the main mechanism underlying the therapeutic effect of VSL#3 in patients with enteritis submitted to radiotherapy[40].
Response: The up-regulation of the innate immune reaction against invasive microbiota is probably one of the important mechanisms underlying the therapeutic effect of VSL#3 in patients with radiation-induced enteritis[39].

8. It is possible to treat IBD through a variety of the mechanisms mentioned above, such as by altering…
Response: The treatment of IBD may be associated with multiple mechanisms, such as by altering the intestinal microbial composition to improve intestinal barrier function.

9. …. which accelerated the recovery of colitis in Muc2 mucin-deficient mice[15].
Response:In DSS-induced murine colitis, VSL#3 was shown to reduce the production of proinflammatory chemokine KC and macrophage inflammatory protein-2 (MIP-2) and up-regulate transforming growth factor-β (TGF-β), fibroblast growth factor-1, and vascular endothelial growth factor-A (VEGF-A) in Muc2 mucin-deficient mice[11].

10. Besides, a significant reduction of iNOS scores in the VSL#3 group suggested the ameliorative inflammatory status of the disease, and the trial had limitations due to a small sample size and a short therapeutic time[94].
Response: Furthermore, a significant decrease of iNOS scores in the VSL#3 group suggested a decrease in the inflammatory status of the disease[95]. The trial had limitations probably due to a small sample size.

11. However, the trial had such a small number of patients enrolled that it could not clarify the conclusion completely.
Response: However, the trial had such a small number of patients enrolled that the conclusion might not be stable or reliable.

12. Thus, VSL#3 can correct glucose intolerance and obesity in $Lep^{ob/ob}$ mice, and significantly increased proopiomelanocortin (POMC) levels and decreased neuropeptide Y (NpY) and agouti-related protein (AgRP) levels (were observed) in the hypothalamus of VSL#3-treated mice.

Response: VSL#3 could protect against glucose intolerance and obesity in $Lep^{ob/ob}$ mice, and significantly increase proopiomelanocortin (*POMC*) levels and decrease neuropeptide Y (*NpY*) and agouti-related protein (*AgRP*) levels in the hypothalamus.....

13. Moreover, Th2-secreted cytokines IL-5 and IL-13 reduced and Treg/Th1 cytokines IL-10 and IFN-γ increased, and VSL#3 supplementation could induce TGF-β, which reduced the Th2 inflammation through inducing or maintaining regulatory T cells expressing FOXP3[148,149].

Response: Th2-secreted cytokines IL-5 and IL-13 decreased and Treg/Th1 cytokines IL-10 and IFN-γ increased in the presence of VSL#3[149]. TGF-β, induced by VSL#3 supplementation, was capable of reducing the Th2 inflammation related to food anaphylaxis in a mouse model of peanut sensitization. It acted through the induction or maintenance of regulatory T cells expressing FOXP3[150].