Is a high-fiber diet able to influence ovalbumin-induced allergic airway inflammation in a mouse model?

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ABSTRACT

Background: More recently, a large amount of experimental and clinical discovered that dietary-fiber intake would decrease the susceptibility to allergic airway disease (AAD) and respiratory inflammation.

Objective: To investigate whether a fiber-supplement is able to influence the induction of AAD and to elucidate the interactive relationship.

Methods: AAD model mice and control mice were raised on a fundamental diet with standard 4% fiber content, whereas other mice were fed a 10% fiber-content diet in the high fiber-content group, along with a 25% fiber-content diet instead in very-high fiber-content group. All experimental mice were sensitized and challenged with ovalbumin to induce allergic inflammation in both the upper and lower airways. Hallmarks of AAD were examined in terms of eosinophil infiltration and goblet cell metaplasia in subepithelial mucosa, T-helper type 1 (Th1) to Th2 skewing of the immune response. Furthermore, to elucidate the interrelations, we generated 16S ribosomal DNA from fecal samples and further validated the variation of colony composition in each group.

Results: The excessive high-fiber supplement induced a promoting effect rather than a suppressive effect, including a rise in nasal rubbing and sneezing, an increase in eosinophil infiltration and goblet cell metaplasia in subepithelial mucosa, and promoted Th2 skewing of the immune response as well as the production of serum levels of ovalbumin-specific immunoglobulin E. Moreover, overconsumption of dietary fiber greatly altered the construction of bacterial flora in the intestinal tract, including an increased proportion of Firmicutes, Actinobacteria, and Proteobacteria, and a decreased proportion of Bacteroidetes.

Conclusion: Our work indicated that, instead of a protecting impact, excessive fiber intake preformed a negative influence on the induction of AAD. Therefore, we suspected that an excessive supplement of dietary fiber might not be an advisable method for the prevention and treatment of AADs.

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symptom appearances, pathologic morphology, and biologic index testing, we further evaluated its effective influence, after the analysis of the variation in microbial colony structure, and expected to obtain a deeper understanding of the underlying mechanisms. To the best of our knowledge, no study has been conducted to elucidate the consequences of a high-fiber diet on respiratory inflammation associated with allergic rhinitis complicated with asthma. Our purpose was to provide experimental evidence for a clearer recognition of the interaction between a high-fiber diet and AAD, as well as to offer references for the rational use of dietary fiber.

**METHODS**

**Animals and Reagents**

Female Balb/c mice (age, 3 weeks); weight range, (11–13 g) were purchased from the Animal Centre of Shandong University, Jinan, Shandong, P.R. China, and were housed under standard specific pathogen free conditions, with sterile food and autoclaved water supplied by the animal experiment center of the Provincial Hospital Affiliated with Shandong University. The animal care and experimental protocol were approved by the animal care committee of Shandong University (ECAESDUSM20123011). The authors’ contributions were the following: Z. Zhang, L. Shi, and G. Shi conceived, designed, and performed the experiments; Z. Zhang, L. Shi, W. Pang, and X. Wang, analyzed and discussed the data; J. Li and H. Wang contributed reagents, materials, and tools; Z. Zhang, L. Shi, and G. Shi wrote the manuscript; and all the authors read and approved the final version of the manuscript.

After assigned at random mice were fed diets with different fiber proportions as follows. The normal mice and the AAD model mice were fed standard-fiber chow (which contained 4% dietary-fiber contents), whereas the fiber-intervened mice were given normal fiber-chow supplemented with 10% or 25% of dietary fiber (Solka-Floc 200FCC; International Fiber Corporation, Urbana, OH). All the diets were purchased from Keaoxie Feed Co., Ltd. (Beijing, China). OVA (A5503, Sigma-Aldrich, St Louis, MO) and aluminum hydroxide (Imject Alum; Thermo Scientific, Waltham, MA) were used to induce T-helper type 2 (Th2) allergic responses in the respiratory tract. Wright-Giemsa and Alcian blue periodic acid–Schiff (AB-PAS) as staining reagents were purchased from Beijing Solarbio Science and Technology Co., Ltd., Beijing, China. Mice OVA-specific serum immunoglobulin E (IgE) levels were determined by using the IgE antibody assay kit (3010, Chondrex, Redmond, WA). The levels of interleukin 4 (IL-4), interferon γ (IFN-γ), and IL-10 in bronchoalveolar lavage fluid (BALF) and nasal lavage fluid (NALF) were tested with mouse enzyme-linked immunosorbent assay (ELISA) kits (88–7711, eBioscience, Santiago, CA). Total bacterial DNA was extracted from fecal samples by using the QIAamp DNA Stool Mini kit (Qiagen, 51504, Hilden, Germany). The specific 16S ribosomal DNA–targeted primers mentioned for quantitative reverse transcription polymerase chain reaction (PCR) were designed and synthesized by Sangon Biotech Co., Ltd., Shanghai, China.

**Design for Dietary Intervention and OVA Induction**

To assay the effects of dietary fiber intervention on OVA-induced allergic inflammation, Balb/c mice were divided into four groups (Fig. 1): (A) the normal group of mice fed standard-fiber chow, sensitized, and challenged with phosphate-buffered saline (PBS) solution, as the control group; (B) the AAD model group, of mice fed standard 4% fiber-content chow, sensitized, and challenged with OVA; (C) the high fiber-content group, of mice fed 10% fiber-content chow, sensitized, and challenged with OVA; and (D) the very-high fiber-content group, of mice fed 25% fiber-content chow, sensitized, and challenged with OVA. All experimental and control groups consisted of 10 animals. During the phase of allergen induction, the mice were sensitized and challenged with OVA to induce allergic inflammation in the respiratory tract. On days 22, 24, 26, 28, 30, 32, and 34, all the mice, apart from the normal group, were sensitized by intraperitoneal injections with 40 μg of OVA and 2 mg of aluminum hydroxide in a
200-μL volume of PBS solution. Hereafter, on days 42 to 48, the mice were challenged with 5% of aero-solized OVA for 30 minutes by inhalation and then were instilled intranasally with 20 μL of OVA (40 mg/mL) each day. Mice in the normal group were sensitized and challenged with PBS solution only. After 24 hours of the final challenge, samples from the mice were collected and detected as described in the following sections.

Assessment of Allergic Symptoms
After the last OVA atomization, the frequencies of nose rubbing and sneezing behaviors per mice were counted, in a blinded way, immediately for 10 minutes, as previously described.9

Cell Counts for NALF and BALF
Mice were killed after 24 hours of the final challenge, and the nasal tissues and the lungs were perfused with 1.2 mL of PBS that contained 1% fetal bovine serum for the collection of NALF and BALF. Lavage fluids were centrifuged at 2500 rpm for 7 minutes at 4°C, and the lavage supernatant was separated and stored at −80°C for further analysis. Lavage cells were resuspended in 150 μL of PBS and counted by using a hemocytometer. Resuspension liquid was used for smear preparation staining with Wright-Giemsa. For classification and counting, lavage cells were differentiated into monocytes, eosinophils, lymphocytes, and neutrophils according to standard morphology and counted at least 300 cells at ×400 magnification under a light microscope (Leica, Oskar-Barnack, Germany) in a randomly ordered, blinded fashion.10

Hematoxylin and Eosin Staining for the Nasal Mucosa and Lung
After 24 hours of the final OVA challenge, all the animals were killed. The nasal tissues and lungs were removed from each mouse and fixed in 10% neutral buffered formalin for 36 hours. After fixation, the nasal tissues still needed to be decalcified with 10% EDTA solution for 1 week. The sections were all prepared at a thickness of 4 μm. The sections of the nasal tissues, which were coronally at a distance of 5 μm from the nasal vestibule, were stained with hematoxylin and eosin to calculate the number of eosinophils in subepithelial mucosa of the nasal septum by using a light microscope at ×400 magnification. The scores of lung inflammation conducted by using a reproducible scoring system, as previously described.11 Briefly, the scores were ranged from 0 to 3, based on the levels of peribronchial and perivascular inflammation across the large bronchus. The values were given as follows: a value of 0 was adjudged for no inflammation, 1 for occasional cuffing with inflammatory cells, 2 for thin layer of inflammatory cells surrounding most bronchi or vessels, and 3 for a thick layer of inflammatory cells surrounding most bronchi or vessels. At least five tissue sections per mouse were selected and assessed in a randomly ordered, blinded fashion.

AB-PAS Staining for the Nasal Mucosa and Lung
To accurately evaluate the severity of goblet cell metaplasia in nasal mucous and bronchial epithelium, tissue sections were stained with AB-PAS. The percentage of AB-PAS–stained goblet cells versus the total cells were counted by using a microscope, and the percentages were assayed from at least five tissue sections per mouse in a blinded fashion.12,13

ELISA for Serum OVA-specific IgE
After 24 hours of the final OVA challenge, a blood sample was withdrawn by heart puncture to extract serum for further determination of OVA-specific IgE levels by using the ELISA kit.

ELISA for Cytokines IL-4, IFN-γ, IL-10, and IL-2 in Lavage Fluid
The expression of cytokines IL-4, 1L-10, and IFN-γ in both NALF and BALF were assessed by ELISA testing by following the kit’s brochure.

Bacterial DNA Isolation from Mice Feces
Mice feces were harvested with 2 mL clean Eppend-dorf tube under sterile conditions 2 hours before they were killed and were stored immediately in liquid nitrogen until processing for DNA isolation. Total microbacteria DNA was isolated from the feces by using the Qiamp DNA Mini kit according to the manufacturer’s instructions, and then used directly for quantitative PCR.

Quantitative PCR for Analyzing the Changes in Intestinal Bacteria
To ascertain the composition of the bacterial phyla present in feces of the mice that were fed the different diets, isolated bacterial DNA was submitted for quantitative PCR and amplified by using previously described primers8:

- Pan-bacteria: forward: 5’-GCAGGCCTAACACATGCAAAGTCC-3’; and reverse: 5’-CTGCTGCTCCTCCCCGTAGGAGT-3’
- Bacteroidetes: forward: 5’-CRAACAGGATTACCTACCCCT-3’; and reverse: 5’-GGTAAGGTTCCTCCTGCGTAT-3’
The 25-μL PCR reactions were set up and contained 2 μg of template DNA, 12.5 μL SYBR Green reaction mix (TaKaRa, Shiga, Japan), 0.5 μL of each primer at a concentration of 10 μM, and 9.5 μL of nuclease-free water. Quantitative PCR was performed on the CFX96 Touch Real-Time PCR Detection System by using the following conditions: one cycle at 95°C for 3 minutes, then 40 cycles at 95°C for 15 seconds, 61.5°C for 30 seconds, and 70°C for 20 seconds, followed by a dissociation stage at 65°C for 31 seconds and cycles of 5 seconds starting at 65°C, increasing 0.5°C per cycle, to obtain melting curves for specificity analysis.

Statistical Analyses
Statistical analyses were performed by using SPSS v.19.0. Graph generation, and statistical analyses were performed by using Prism version 5.0. One-way analysis of variance, followed by the Student-Newman-Keuls test and the Mann-Whitney U test were applied for analysis of experimental data; p < 0.05 was considered significant.

RESULTS
Excessive High-Fiber Intake Aggravated Allergic Symptoms
The occurrences of nasal rubbing and sneezing in the high fiber-content groups were more frequent than in the AAD group (p < 0.01), especially in the 25% fiber-content group (p < 0.01), as shown in Fig. 2. Analysis of these data indicated that excessive high-fiber intake aggravated allergic symptoms.

Excessive High-Fiber Intake Increased OVA-induced Inflammation Cells in Both NALF and BALF
To evaluate the effect of a high-fiber diet on AAD, we next counted the inflammation cells obtained from NALF and BALF. Analysis of the results indicated that there was an obvious increase in the numbers of the total and four main inflammation cells (eosinophils, monocytes, lymphocytes, and neutrophils) in both NALF and BALF (all p < 0.01, compared with the standard-fiber diet) by excessive fiber supplement (Fig. 3), with a more-robust increase with the 25% fiber-content diet (p < 0.01). Analysis of all the data indicated that the excessive high-fiber supplement induced negative effect to allergic inflammation.

Excessive High-Fiber Intake Influenced OVA-induced Upper and Lower Allergic Airway Inflammation
As shown in Figs. 4 and 5, the AAD model mice had obvious allergic inflammation (Fig. 4), such as a significant aggravation of eosinophil infiltration (all p < 0.01) (Fig. 4) and a goblet cell metaplasia (all p < 0.01) (Fig. 5) in the nasal mucosa and lung compared with the control mice. The numbers of the eosinophil infiltration (Fig. 4) and goblet cell (Fig. 5) metaplasia in the nasal mucosa and the lung were higher in the mice fed a supplemented high-fiber diet than in the mice fed a standard-fiber diet (all p < 0.01). Interestingly, it even seemed to express a rising tendency with the consumption of dietary fiber (p < 0.01). This work indicated that excessive high-fiber intake exacerbated OVA-induced allergic inflammation in both the upper and lower airways.

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Excessive High-Fiber Intake Increase Levels of Serum OVA specific IgE

Results of our study indicated that levels of serum OVA-specific IgE in AAD mice were significantly higher than in the mice in the control group \((p < 0.01)\) (Fig. 6). Notably, serum IgE levels of mice fed a high-fiber diet were further elevated by especially 25% content fiber diet (all \(p < 0.01\) compared with the standard diet).

Excessive High-Fiber Intake Influenced Cytokine Production in Both NALF and BALF

We found that levels of the Th2 cytokine IL-4 in both NALF and BALF from the AAD model mice were significantly detectable \((p < 0.01)\). However, all the mice housed with a high-fiber diet, especially the 25% fiber-content diet, contained relative higher levels of the cytokine IL-4 than did the AAD model mice \((p < 0.05\) or \(p < 0.01)\), as shown in Fig 7. In contrast with Th2 cytokines IL-4 levels, the levels of Th1 cytokines IFN-\(\gamma\) in both NALF and BALF were significantly lower in the mice fed a high-fiber diet than levels in the AAD model mice (all \(p < 0.01\) for IFN-\(\gamma\) in both NALF and BALF in mice fed a 10% fiber diet or a 25% fiber diet). Furthermore, we observed an obvious decrease in the production of the cytokine IL-10 in the mice supplemented with dietary fiber, \((p < 0.05\) for NALF and \(p < 0.01\) for BALF from the 10% fiber-content group compared with the AAD mice; \(p < 0.01\) for both NALF and BALF from the 25% fiber-content group compared with the AAD mice).

Excessive High-Fiber Intake Impacted Microbial Community Structure

To better reveal the interaction between dietary fiber and AAD, we extracted total DNA of microbes from fecal samples and then performed quantitative analysis of bacterial populations by using real-time PCR technology (Fig. 8). As illustrated in Fig 8, compared with mice fed a standard-fiber diet, the proportions of Bacteroidetes in fecal bacteria from mice supplemented with dietary fiber were reduced relatively (Bacteroidetes, a 0.99-fold change for a 10% fiber-content diet; Bacteroidetes, a 0.88-fold change for a 25% fiber diet). In contrast, the populations of Firmicutes, Actinobacteria, and Proteobacteria were proportionally increased (Firmicutes, a 1.07-fold change; Actinobacteria, a 2.23-fold increase; Proteobacteria, a 1.82-fold increase for a 10% fiber-content diet, along with Firmicutes, a 1.36-fold increase; Actinobacteria, a 2.69-fold increase; Proteobacteria, a 1.38-fold increase for a 25% fiber-content diet). Analysis of all the data illustrated that excessive high-fiber intake influenced the microbial community structure.

DISCUSSION

Over the past decades, the incidence rates of allergic rhinitis and asthma have been increasing worldwide and seriously affect the quality of life levels in patients.\(^\text{14,15}\) Despite of the experimental research from multiple perspectives, including variation in fermentation metabolites,\(^\text{8,16}\) modulation of intestinal bacterial flora,\(^\text{17,18}\) and even induction of immune tolerance,\(^\text{12,19}\) and so forth, these internal relations and the influential
mechanism remain, to a wide extent, unclear. Current dietary studies held that the increased epidemic of AAD was closely related to the modification of lifestyle, including the reduced intake of dietary fiber.3–6 A related epidemic survey observed that the incidences of allergic rhinitis and/or asthma show an apparent negative correlation with the consumption of dietary fiber over the past decades.7,20 To evaluate whether a high-fiber supplement could be used to prevent or cure AAD as an alternative therapy, we adjusted the diet structure for the experimental mouse with a fiber supplement and examined the effect of a high-fiber diet on OVA-induced allergic airway inflammation. Also, to eliminate the difference in susceptibility to OVA allergen between female and male mice, all mice in this study were female Balb/c mice. Interestingly, our study found that excessive fiber supplementation did not suppress the progress of allergic airway inflammation in AAD and even induced more-severe inflammatory responses, which augmented the increase of fiber consumption. As pathology section shown (Figs. 4 and 5), the high-fiber intake markedly aggravated eosinophil infiltration and goblet cell metaplasia in the nasal mucosa and lung as well as the remarkable symptoms of nasal rubbing and sneezing among groups, especially with the 25% fiber-content intake.

To further research this phenomenon, we accurately assayed the expression of the Th2 cytokine IL-4, the production of the Th1 cytokine IFN-γ, and the immunoregulatory cytokine IL-10 in NALF and BALF as
well as the total serum levels of OVA-specific IgE in the AAD mice by using ELISA. Consequently, high-fiber intake significantly upregulated the Th2 cytokine IL-4 level as well as downregulated the Th1 cytokine IFN-γ level, which indicated that there was an obvious skewing of immune inflammation responses in the allergic mice. The Th2 cytokine IL-4, always considered to be a proinflammatory cytokine, recruits more inflammatory cells, such as eosinophil and macrophage, to involve in inflammatory responses,\textsuperscript{21–23} whereas Th1 cytokines IFN-γ production has a beneficial effect on the suppression of the inflammatory gene expression of the proinflammatory factor and then inhibits Th2-specific immune response.\textsuperscript{24–27} Therefore, from the perspective of a Th1 to Th2 immune balance, it is reasonable to deem that excessive high-fiber intake exacerbated eosinophil inflammation, probably partly via shifting to a Th2 from a Th1 immune response to the OVA allergen. Moreover, high-fiber intake significantly increased the levels of serum OVA-specific IgE in the allergic mice ($p < 0.01$), which further indicated that the promoting effect conferred by the high-fiber diet on the Th2 immune response.\textsuperscript{28,29} A further observation from our study was that high-fiber intake remarkably inhibited the production of IL-10, especially the 25% fiber-content supplementation. Note that IL-10, as an important immunoregulatory cytokine, had biologic functions of restraining and even terminating the inflammation reactions.\textsuperscript{30–32}

**Figure 5.** Goblet cell metaplasia assessed on Alcian blue periodic acid–Schiff (AB-PAS) stained tissue sections of the nasal mucosa and lung. (A) Original magnification was $\times 400$ for nose and $\times 200$ for lung. Goblet cells were counted as the blue cells stained positive by AB-PAS. The percentages of goblet cell metaplasia were calculated from the total numbers of cells counted around the nasal mucosa (B) and the lung (C). Goblet cell metaplasia was relatively major in the mice that were fed high-fiber chows and seemed consistent with the increased consumption of dietary fiber. Data are expressed as mean $\pm$ SE; $n = 10$. *$p < 0.05$, **$p < 0.01$. 

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What are the underlying mechanisms that link dietary fiber with AAD? Previous studies, including experimental, preliminary clinical, and epidemiology studies, found that a conspicuous difference exists, whether between patients with allergic diseases and control people, or between allergic animals and control
animals, indicating the close correlation between occurrence of allergic diseases and variation of dietary contents. Therefore, we suspected that the aggravation of allergic inflammation in the AAD mice might be induced by impairment of a high-fiber–influenced intestinal flora structure. Through a contrastive analysis of the variation in the intestinal microflora structure among the groups, we noted that a high-fiber supplement significantly increased the proportion of Bacteroidetes and Actinobacteria as well as decreased Firmicutes and Proteobacteria in mice feces. Interestingly, the increased relative ratio of Firmicutes to Bacteroidetes has been shown to be closely associated with the occurrence of allergic airway inflammation in previous reports. However, due to the complexity and the diversity of intestine microenvironment, current works are still unable to give a clear explanation. Nevertheless, it is clear that we cannot blindly rely on a dietary-fiber supplement for the beneficial optimization of the intestinal flora structure or effective for the prevention and treatment of allergic disease.

CONCLUSION

For the first time, to our knowledge, we detected that a long-term high-fiber diet significantly increased the susceptibility and severity of AAD instead of having an inhibitory effect on inflammation as displayed in previous animal experiments. Although our experiment could not deny the conclusions of the anti-inflammation effect of dietary fiber in previous studies, any nutrient supplement should not exceed a certain limit, including fiber supplement. Therefore, we recommend that fiber supplementation should not be performed in a blindly manner, but fostered strengths and circumvented weakness from perspective of a balanced diet.

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