Received: 1 July 2021 | Revised: 10 August 2021 | Accepted: 10 August 2021

DOI: 10.1002/agm2.12174

ORIGINAL ARTICLE

Aging Medicine Open Access WILEY

# Influence of oral nutritional agents rich in soluble dietary fiber on intestinal flora of elderly men with malnutrition

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**Funding information**
This study was supported by funds from The Sixth Central Health Research Project (No. W2017BJ10).

## Abstract

**Objective:** Observe the influence of oral nutritional agents rich in soluble dietary (enteral nutritional suspension [TPF-DM]) on intestinal flora of elderly male subjects with malnutrition.

**Method:** Seventy-eight subjects with good nutrition were considered as the healthy control group. Twenty-eight male subjects who had malnutrition and were older than 70 years were included and randomly divided into the short-term (3 months) intervention group (n = 20) and the long-term (12 months) group (n = 8). They were provided with enteral nutritional suspension (TPF-DM) 500 mL/day or maximum tolerance dose, so as to observe the changes in nutrition-related indexes and intestinal flora after the elderly take enteral nutritional suspension (TPF-DM).

**Results:** (1) For elderly male subjects with malnutrition, their body weight, body mass index, hemoglobin, total protein, and albumin were significantly lower than the control group with favorable nutrition. (2) There were obvious differences in intestinal flora between healthy elderly male subjects and those with malnutrition. After the treatment of enteral nutritional suspension (TPF-DM), intestinal flora of the malnourished elderly subjects showed recovery toward the healthy elderly subjects. The obvious gradient changes of the flora were mainly in the bacteroidetes, firmicutes, and proteobacteria phyla, and the relative abundance of CAG2 clusters in the malnourished group was higher than that in the healthy control group, and the relative abundance decreased after long-term treatment, and the change approached the healthy control group. The relative abundance of CAG3 and CAG6 clusters in the malnourished group was lower than that in the healthy control group, and the relative abundance increased after long-term treatment, and the change approached the healthy control group.

**Conclusion:** Malnutrition has obvious impact on intestinal flora of the elderly. Enteral nutritional suspension (TPF-DM) not only prevents the further decline in the state of nutrition but also helps the recovery in intestinal flora of the elderly. Long-term application can produce better effects.

KEYWORDS
elderly male subjects, intestinal flora, malnutrition, oral nutritional agents

## 1 | INTRODUCTION

Since the end of the last century, our country has become an aging society. Due to health and economic reasons, such as illness, solitude, and declining income, elderly patients are more likely to suffer malnutrition. Nutrition and energy are essential to maintain vitality and organ functions. Malnutrition can severely damage their health and organ functions. It is not conducive to the prognosis of illness and the lifespan of the patients. Modern nutritional therapies are more than just meeting the need of elderly patients for energy. They are also concerned with regulation of blood glucose, protection of intestinal tract barriers, and adjusting intestinal microecology, etc.[1] Intestinal microorganism plays an important role in functions like promoting epithelium growth, intestinal tract barrier, immunity stimulation, anti-infection, steady metabolism, and digestive absorption, etc.[2] At present, multiple studies and research have proven that intestinal microecology of elderly patients is closely related to metabolism, immunity, sarcopenia, asthenia, and cognitive functions, etc.[3,4]

As intestinal microecology is connected to old age, nutrition, and other diseases, intestinal flora regulation may become the new target of nutritional intervention for the elderly. However, the influence of nutrition support on intestinal flora of the elderly who suffer malnutrition. Hence, this study aims to observe the influence of oral nutritional agents rich in soluble dietary fiber on intestinal microecology of elderly male subjects with malnutrition. It is expected to provide more evidence of intestinal nutrition support for the elderly.

## 2 | RESEARCH METHOD

### 2.1 | Objects of research

One hundred six subjects, who received treatments in the endocrinology and geriatrics departments of our hospital from October 2017 to June 2020, comply with the inclusion and exclusion criteria. After being approved by the ethics committee, the number of the study was 2017BJYYEC-118-02. It was also registered at the Chinese Clinical Trial Registration Center. The registration number was ChiCTR-OPB-17012750. All subjects took part in the study voluntarily and signed the informed consent form.

### 2.2 | Criteria of inclusion

1. The control group with favorable nutritional status: less than 70 years old. Prebiotics/probiotics was not taken in recent 3 months. Favorable nutritional state (Mini-Nutritional Assessment Short-Form [MNA-SF] score 12 to 14 points).
2. The experimental group with malnutrition: more than 70 years old. Prebiotics/probiotics not taken in the recent 3 months. Favorable nutritional state (MNA-SF score 0 to 7 points).

### 2.3 | Criteria of exclusion

Subjects who comply with one of the following conditions cannot be included. Contraindications of enteral nutrition treatment: intestinal obstruction, active bleeding of the digestive tract; severe nausea and emesis that cannot be controlled through medication; malabsorption caused by chronic gastrointestinal diseases; congestive heart failure and respiratory failure; severe infection; patients who have used antibiotics within 2 weeks; terminal stages of chronic diseases, chronic kidney disease (CKD) 5 phase, estimated glomerular filtration rate (eGFR) <15 mL/min/1.73 $m^2$; chronic liver disease, level C liver function; malignant tumor; severe endocrine diseases, including thyroid dysfunction, but those with normal thyroid functions who also take medicine to cure thyroid gland malfunctions were not included; uncontrolled diabetes, etc.

### 2.4 | Methods of research

#### 2.4.1 | Subjects of the control group with favorable nutrition status

The patient's fasting blood was drawn to test blood routine and biochemical indicators. Personal information and medical history were gathered through questionnaire surveys. Stool samples were retained as well.

#### 2.4.2 | Experimental group with malnutrition

Subjects were given nutritional agents (enteral nutritional suspension [TPF-DM]; Nutricia Corp.) with soluble dietary fiber for nutrition support therapy. They were randomly divided into short-term intervention (3 months) and long-term intervention (12 months) and compared with the changes in nutrition-related blood indexes (blood routine examination and biochemistry) and intestinal flora before and after the use of enteral nutritional suspension (TPF-DM).

#### 2.4.3 | Stool sample retain, disposal and intestinal flora 16S rDNA sequencing

Approximately 10 g of stool sample with no urine, sanitizer, or sewage, was collected and was placed in the fridge of −80°C. QIAamp Fast DNA Stool Mini Kit (50) was used to extract the total DNA of

**TABLE 1** Subjects' baseline in some nutrition indicators

| | Well-nourished <70 (n = 41) | Well-nourished ≥70 (n = 37) | Malnourished ≥70 (n = 28) | *F* value | *P*-value |
|---|---|---|---|---|---|
| Weight (kg) | 76.89 ± 9.38 | 69.43 ± 11.08 | 64.33 ± 10.52 | 11.316 | 0.000 |
| BMI (kg/m$^2$) | 26.52 ± 2.38 | 25.05 ± 3.16 | 22.72 ± 3.43 | 11.382 | 0.000 |
| Hemoglobin (g/L) | 147.64 ± 9.97 | 130.67 ± 17.39 | 121.29 ± 13.58 | 33.529 | 0.000 |
| Lymphocyte (×10$^9$/L) | 2.16 ± 1.71 | 1.88 ± 0.59 | 2.2 ± 3.17 | 0.310 | 0.734 |
| Total protein (g/L) | 72.32 ± 3.11 | 66.86 ± 5.74 | 65.88 ± 6.93 | 16.970 | 0.000 |
| Albumin (g/L) | 45.95 ± 2.61 | 40.11 ± 7.25 | 40.53 ± 7.54 | 12.150 | 0.000 |
| 25(OH)D (ng/mL) | 24.92 ± 11.96 | 21 ± 10.26 | 19.51 ± 8.16 | 2.607 | 0.078 |
| Serum iron (μmol/L) | 21.12 ± 7.64 | 16.11 ± 5.97 | 19.48 ± 39.48 | 0.551 | 0.578 |

**TABLE 2** Effect of TPF-DM on relevant indicators of the malnourished elderly

| | Short-term intervention (n = 20) | | | Long-term intervention (n = 8) | | |
|---|---|---|---|---|---|---|
| | Baseline | Terminal value | *P* value | Baseline | Terminal value | *P* value |
| Weight (kg) | 69.00 ± 8.85 | 66.08 ± 9.21 | 0.014 | 66.57 ± 7.77 | 66.77 ± 9.03 | 0.867 |
| BMI (kg/m$^2$) | 24.2 ± 2.68 | 23.22 ± 3.10 | 0.012 | 23.57 ± 2.37 | 23.42 ± 2.72 | 0.731 |
| Hemoglobin (g/L) | 129.38 ± 9.77 | 121.88 ± 12.26 | 0.037 | 118.05 ± 13.72 | 124.15 ± 16.05 | 0.109 |
| Lymphocyte (×10$^9$/L) | 1.54 ± 0.33 | 1.48 ± 0.48 | 0.434 | 2.47 ± 3.74 | 1.75 ± 1.16 | 0.430 |
| Total protein (g/L) | 68.64 ± 2.96 | 66.01 ± 4.79 | 0.182 | 64.66 ± 8.04 | 66.94 ± 9.37 | 0.436 |
| Albumin (g/L) | 43.5 ± 2.19 | 39.53 ± 3.05 | 0.004 | 39.34 ± 8.59 | 38.02 ± 4.77 | 0.375 |
| 25(OH)D (ng/mL) | 20.97 ± 10.63 | 15.18 ± 6.22 | 0.082 | 18.56 ± 7.17 | 20.57 ± 5.28 | 0.143 |
| Serum iron (μmol/L) | 17.22 ± 4.74 | 10.47 ± 2.62 | 0.106 | 10.09 ± 4.73 | 10.35 ± 4.10 | 0.380 |

the fecal microbiota. The V3-V4 region of 16S rDNA were amplified with primers of was F_5′-CCTACGGGNGGCWGCAG-3′ and R_5′-GGACTACHVGGGTATCTAATCC-3′. The 16S rDNA library of 600 bp was sequenced on the Miseq sequencing platform.

### 2.4.4 | Bioinformatic methods

QIIME 2 (version 2020.11.0) was used for quality control of the sequencing data, including sequencing separation (was "qiime cutadapt demux-paired" was parameter: -p-error-rate 0), eliminating primer sequence ("qiime cutadapt trim-paired" parameter: --p-match-adapter-wildcards --p-match-read-wildcards --p-discard-untrimmed), denoising ("qiime dada2 denoise-paired," was parameter:-p-trunc-len-f 270 --p-trunc-len-r 228 --p-n-threads 20 --p-min-fold-parent-over-abundance 4), and driving the feature sequences ("qiime feature-table rarefy" parameter:--p-sampling-depth 4000). A total of about 4000 sequences was obtained for each sample; Amplicon Sequence Variants (ASVs) feature table was obtained; and was finally annotated by comparing it to the Silva database. The α diversity was estimated by observed ASVs, Chao1 index, Shannon index, and Simpson index; the β diversity of principle coordinates analysis (PCoA) was analyzed based on was unweighted UniFrac distance.

Analysis of the co-abundance groups (CAG) was performed as follows: (1) calculating the Pearson correlation coefficients based on ASVs relative abundance; and (2) hierarchical clustering was based on the Ward-linkage method and the Euclidean distance. Pearson correlation coefficients were calculated based on category levels. The threshold value of correlation coefficient was 0.4. This experiment conducts significantly different analysis among levels, categories, and classification grade of ASVs. For the Pearson correlation, an $P$ value < 0.05 was considered statistically significant after the adjustment with the Benjamini-Hochberg method.

### 2.4.5 | Statistical methods

Continuous variables were described as mean value with standard deviation, or median value with interquartile range (IQR). Categorial variables were descript by the frequency and percentage. For categorical variables, the Chi-square or the Fisher exact test were carried out to test the group differences; for continuous variables, variance, or analysis of covariance, or nonparametric methods were used. All the tests were two-tailed and a $P$ value < 0.05 was considered statistically significant.

## 3 | RESEARCH RESULTS

### 3.1 | The baseline of subjects

The average age of the 116 male subjects who were involved in this experiment was 75.7 ± 14.9 years. They were divided into three groups by nutritional status and age – group 1 had 41 people who were well-nourished and under 70 years old, group 2 had 37 cases who were well-nourished and above or equal to 70 years old, and group 3 were 28 people who were malnourished and above or equal to 70 years old. The comparing baseline was the relevant indicators of nutrition (see Table 1). This research finds that the subjects in group 3 have significant poorer performance than the other two groups in weight, body mass index (BMI), total protein, and albumin. The difference among the three groups was statistically significant ($P < 0.05$).

### 3.2 | Effect of TPF-DM on relevant indicators of the malnourished elderly

The subjects in group 3 were treated with TPF-DM from small amounts to 500 mL per day or the maximum dose (<500 mL). Twenty people were short-term (3 months) subjects of TPF-DM treatment, and eight people were long-term (12 months) subjects. Then, some nutrition indicators of these patients before and after treatment were compared. The results are shown in Table 2.

The results demonstrate that, compared with the baseline, the indicators like weight, BMI, hemoglobin, and albumin still decrease after short-term TPF-DM intervention. The difference between groups was statistically significant ($P < 0.05$). However, there was no significant difference in subjects treated long term.

### 3.3 | Effect of malnutrition on intestinal flora in elderly men

PCoA analysis (Figure 1A) shows that the first and second principal components contributed to 15.45% and 8.2% of the variations, respectively, between the malnourished and control subjects. The performance between the two groups was significantly different. The indicators of the malnourished elderly (group 3) after treatment approached the control group (group 2), and the intestinal flora of some healthy elderly men also reaches those dystrophic aged people. At the level of ASVs, the heatmap (Figure 1B) shows that the dystrophic and healthy groups can be, to a large extent, clustered. After the treatment of TPF-DM, the malnourished people's flora becomes more similar to the healthy ones, and the phyla had a gradient change among the groups. There were significant differences in bacteroidetes, firmicutes, and proteobacteria phyla between healthy control group, malnutrition group, short-term treatment, and long-term treatment groups ($P < 0.05$; Table S1).

### 3.4 | Changes of intestinal microflora in malnourished old man after TPF-DM treatment

Figure 2 shows the changes of gut microbiota at the genus level before and after TPF-DM treatment in malnourished old men. Similar with the results obtained at ASVs, the genus of microbiota was clearly clustered for the malnutrition and healthy subjects, respectively. By the horizontal clustering, the genera were divided into six clusters, namely, CAG1, CAG2, CAG3, CAG4, CAG5, and CAG6. Bacteroidota and firmicutes were the two mostly affected phyla; among the six CAGs, CAG2 was the largest group containing 15 genera and six families (Table S2). Microbial network

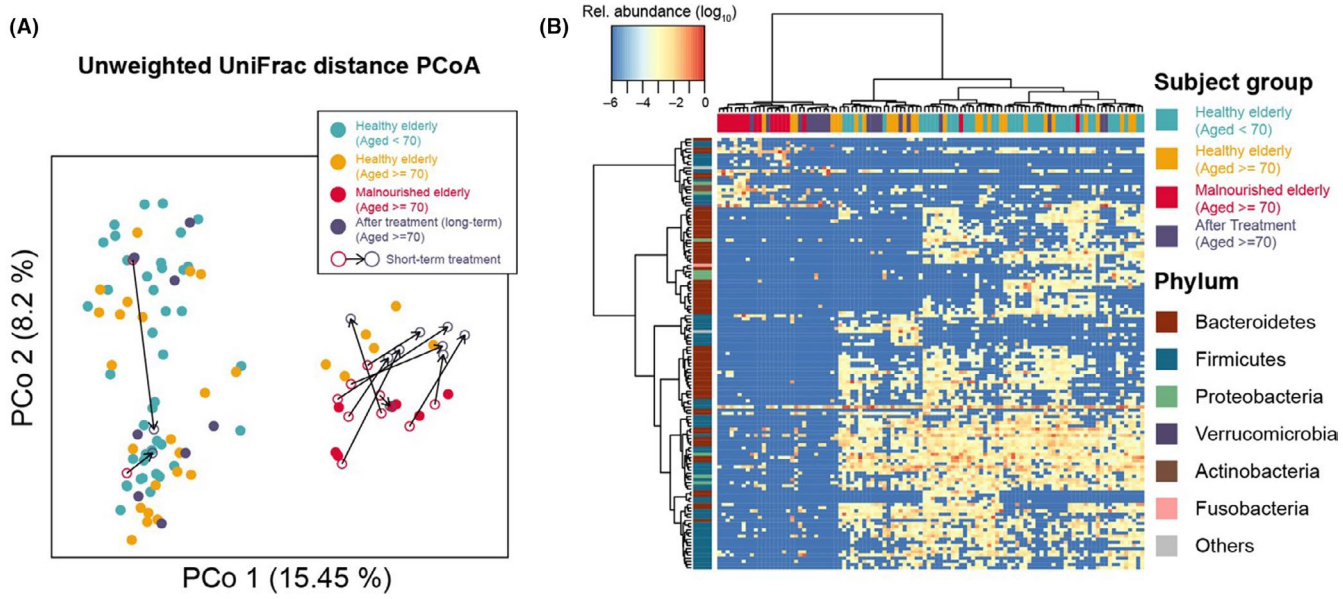


**FIGURE 1** Effect of malnutrition on intestinal flora in elderly men. PCoA, principle coordinates analysis

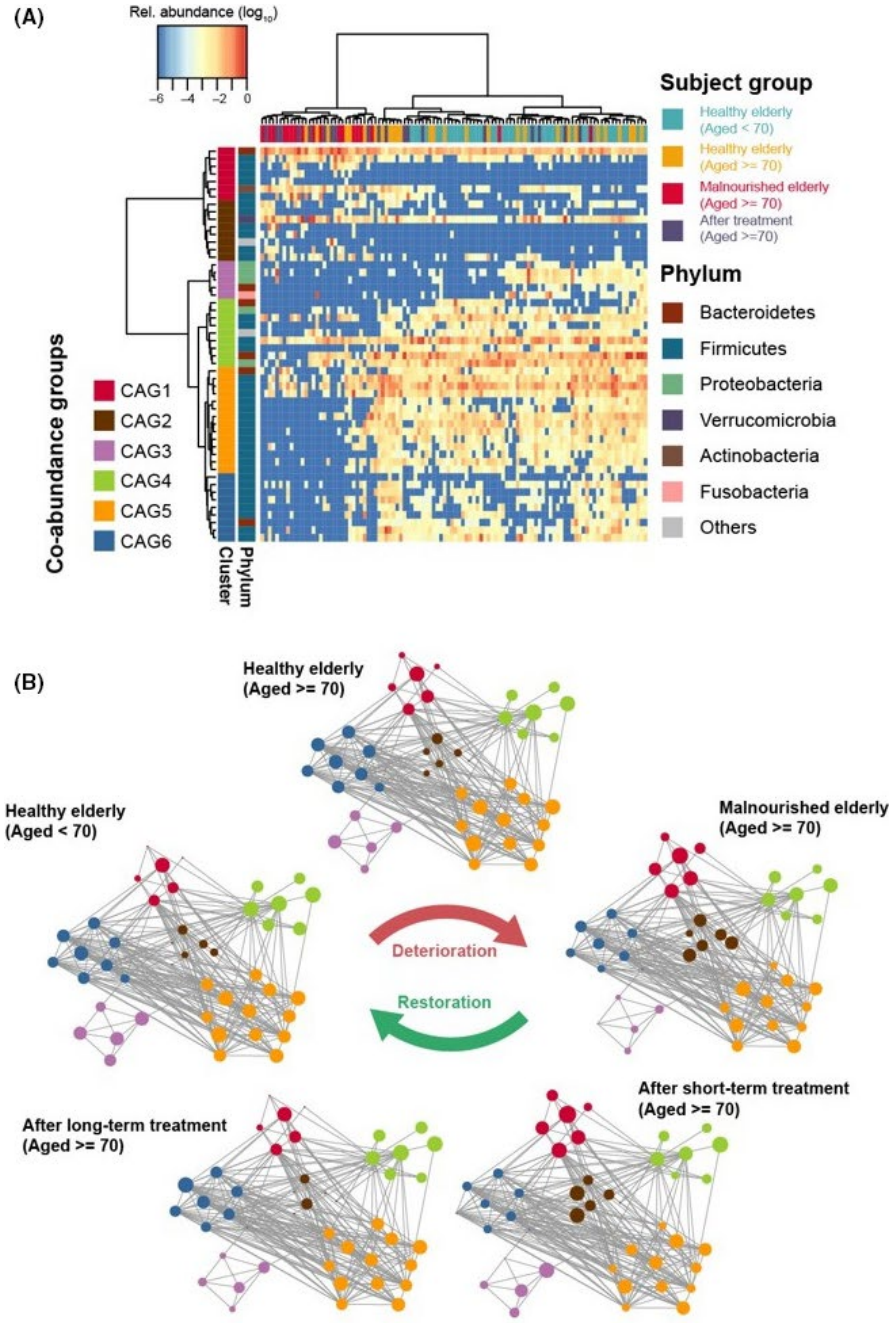


**FIGURE 2** Changes of intestinal microflora in malnourished old man after enteral nutritional suspension TPF-DM treatment

analysis shows that the relative abundance of CAG2 in the malnutrition group was higher than that in the healthy group at the level of genus; in parallel, it decreased, after long-term treatment, to what was a similar level of the healthy group. Opposite trends were found for the CAG3 (contained 9 genera and 6 families) and CAG6 (contained 8 genera and 8 families), the relative abundance of which was lower in the malnutrition group was than that in the healthy group, and increased to a level similar to the healthy subjects after long-term treatment. There were no significant changes among the healthy subjects, malnourished subjects, and the TPF-DM treated subjects for the clusters of CAG1, CAG4, and CAG5.

## 4 | DISCUSSION

In the important part of the human body, intestinal microecology, the mucosal space-occupying protection of intestinal probiotics can build an immune defense line and resist colonization of pathogenic bacteria, which is the first barrier of the human body.[5] Intestinal microorganisms are basically formed around the age of 3 years and remain relatively stable. The dominant bacteria in healthy adults are firmicutes and bacteroides, accounting for 80% to 90% of the total flora.[6] Over these years, with the development of high-throughput gene sequencing technology, more and more researches were conducted on intestinal microecology. Studies have found that aging,

lifestyle, diet structure, antibiotics, nonsteroidal anti-inflammatory drugs, and other drugs, pathogenic bacteria infection, etc., can change the intestinal microecology,[7] and reduce intestinal microbial diversity. With the decrease of intestinal probiotics, conditional pathogenic bacteria increased and intestinal probiotic metabolite short-chain fatty acids (SCFAs) decreased, also. In addition, the changes in intestinal microecology are closely related to diabetes, obesity, cardiovascular diseases, autoimmune diseases, Alzheimer's disease, etc.[8,9] How to improve the intestinal microecology of the elderly, especially the malnourished, deserves the attention of medical staff.

This study found that the nutrition-related indicators of the malnourished elderly, including body weight, BMI, hemoglobin, albumin, and flora structure, are significantly different from those of the healthy subjects, and the flora of some elderly subjects over 70 years also began to develop toward the direction of malnutrition. Therefore, this study suggests that nutritional status is an important factor affecting intestinal flora in the elderly. Due to the loss of appetite, chewing, and digestion ability, the elderly, especially the senile, show a significant decrease in the intake of protein and dietary fiber.[10] Both nutritional deficiency and decreased intake of specific nutrients can lead to changes in the composition and function of intestinal flora in the elderly, including the decrease of microbial diversity, butyric acid-producing bacteria, and SCFA level of intestinal flora, whereas harmful substances, such as lactic acid, methane, and branched-chain fatty acid increased significantly.[11]

Through nutrition intervention on the malnourished elderly, the results showed that, compared with the baseline, the nutrition-related clinical indexes of the elderly, including body weight, BMI, hemoglobin, and albumin, still decreased after short-term TPF-DM intervention. However, there was no obvious change in nutrition-related indexes after long-term TPF-DM intervention. TPF-DM is rich in nutrients, including protein, fat, carbohydrate, dietary fiber, vitamins, minerals, and trace elements. However, due to the low energy density (0.75 kcal/mL), TPF-DM was mostly used in patients with diabetes in previous studies. Some research results have found that, after 3 months of treatment of TPF-DM, the albumin, blood sugar, and blood lipid metabolism of patients with diabetes over the age of 60 years with malnutrition or high risk of malnutrition (MNA-SF score of approximately 0 to 11) can be effectively improved. The reason for the inconsistent results is considered to be related to the worse nutritional status of the subjects of all elderly subjects over the age of 70 years in this study (MNA-SF score was approximately 0 to 7). We had only observed changes in the flora, which may be due to the shorter intervention time in this study, and it may take longer to see clinical changes.

In addition, this study also found that after short-term treatment of TPF-DM, the intestinal flora of malnourished elderly men shows a recovery tendency to the state of healthy elderly people over the age of 70 years. After long-term treatment, the intestinal flora may become healthier, mainly manifested in obvious flora gradient changes in phyla B, F, and P, and abundance changes in CAG2/3/6 clusters. This study suggests that the mechanism of intestinal flora improvement may be related to the rich dietary fiber and monounsaturated fatty acids of TPF-DM. Different from other nutritional preparations, TPF-DM features a high content of monounsaturated fatty acids (70% of total fat) and rich dietary fiber. As the nutritional preparation with the highest soluble fiber content today, TPF-DM contains three soluble dietary fibers and three insoluble dietary fibers, and the content of the former one is as high as 80%. Due to failing to be decomposed by human enzymes, dietary fibers can strengthen the barrier of the intestinal tract against pathogens, accelerate the intestinal movement, and reduce potential inflammatory state, thus improving the health state.[13] In addition, soluble dietary fiber is an indigestible carbohydrate rich in polysaccharides and oligosaccharides, which can be fermented by one or a few intestinal florae in the intestinal tract to produce nutrient substrates (mainly SCFAs) of probiotics, thus stimulating the growth and activity of probiotics.[14] Meanwhile, monounsaturated fatty acids can improve the biodiversity of intestinal microorganisms, increase the content of bacteroides and bifido bacteria, and decrease the content of sclerenchyma. The increase of probiotics is related to the increase of intestinal SCFA by mono-unsaturated fatty acids.[15]

To sum up, this study suggests that attention should be paid to the assessment and nutritional support of nutritional status and intestinal flora of the elderly, especially the senile. Nutritional supplements for the malnourished senile elderly using TPF-DM can not only prevent the declined nutritional status, but improve the intestinal flora. Moreover, the long-term use effect is better. However, the shortcomings of this study lie in the small cases as well as the single-center study. There still needs to be a larger sample size and a longer time for further verification in the later period.

## CONFLICTS OF INTEREST

Nothing to disclose.

## AUTHOR CONTRIBUTIONS

Y.D.N. and Q.H.M. supervised the project and designed the workflow and performed the statistical analysis. C.M.Y., G.L.X., L.W.L., L.Y., N.K., T.Y.G., Y.X.J., and Q.L. performed material preparation and data collection. Yu wrote the first draft. C.M.Y. prepared the figures and tables. All authors commented on the manuscript. All authors read and approved the final manuscript.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

**How to cite this article:** Yu D, Cheng M, Guo L, et al. Influence of oral nutritional agents rich in soluble dietary fiber on intestinal flora of elderly men with malnutrition. *Aging Med*. 2021;4:162–168. https://doi.org/10.1002/agm2.12174