

## Effect of high dietary fiber low glycemic index diet on intestinal flora, blood glucose and inflammatory response in T2DM patients.

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### Abstract

**Aims:** To study the effect of high dietary fiber low glycemic index (GI) diet on intestinal flora, blood glucose and inflammatory response in T2DM patients.

**Methods:** 130 T2DM patients treated in our hospital were selected as the subjects from January 2016 to January 2017. The patients were divided into control group and observation group by random number table method. Each group has 65 patients. Patients in the control group received diabetes diet, exercise therapy, and oral hypoglycemic drugs. The observation group was treated with high dietary fiber and low glycemic index diet on the basis of the control group. The two groups were intervened for 6 months continuously. Before and after intervention, the changes of intestinal flora and blood glucose levels in the two groups were compared, and the changes of serum hs-CRP, IL-1 beta and IL-6 levels in the two groups were observed.

**Results:** There was no significant difference in intestinal flora indicators between two groups of *Enterococcus*, *Escherichia coli*, *Bifidobacterium* and *Lactobacillus* before intervention ( $P>0.05$ ). After intervention, the number of *Enterococcus*, *Escherichia coli* in the observation group was significantly lower than that in the control group, and the number of bacteria in *Bifidobacterium* and *Lactobacillus* was significantly higher than that in the control group ( $P<0.05$ ). There were no significant differences in FPG, HbA1c, FINS and HOMA-IR between the two groups before intervention ( $P>0.05$ ). After intervention, the FPG, HbA1c, FINS and HOMA-IR in the observation group were significantly lower than that in the control group ( $P<0.05$ ). Before intervention, there was no significant difference in the levels of hs-CRP, IL-1 $\beta$  and IL-6 between the two groups ( $P>0.05$ ). After intervention, the levels of hs-CRP, IL-1 $\beta$  and IL-6 in the observation group were significantly lower than that in the control group ( $P<0.05$ ).

**Conclusion:** High dietary fiber low glycemic index diet can effectively improve the intestinal flora imbalance and blood glucose level in T2DM patients, reduce serum inflammatory response and promote the rehabilitation of patients.

**Keywords:** Type 2 diabetes mellitus, Dietary fiber, Blood sugar, Intestinal flora.

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### Introduction

With the improvement of living standards and the changes in diet structure, prevalence of obesity, diabetes and other diseases is on the rise, and has become one of the prominent social problems in the world. Intestinal flora is closely associated with the incidence of obesity and diabetes mellitus [1]. *Lactobacillus* and *Bifidobacterium* are Probiotics while *E. coli* and *Enterococcus* are intestinal opportunistic bacteria. The intestinal flora is disordered in obese and diabetic patients and the number of probiotics decreases. Intestinal flora is involved in carbohydrate, lipid, amino acids and other nutrients in the body metabolism, affects the energy absorption, closely related with the occurrence and development of metabolic disease [2]. It has become a new target for the prevention and treatment of metabolic diseases. Relevant data show that [3] irrational dietary structure can cause imbalance of intestinal flora,

thereby affecting the occurrence and progress of diabetes. There is an imbalance of intestinal flora in diabetic patients [4]. The number of beneficial bacteria is reduced and the number of conditional pathogens is increased. Compared with normal people, there was a statistically significant difference. The intestinal flora is mainly influenced by diet. Unbalanced dietary composition disrupts intestinal flora structure, produces immunotoxin, destroys intestinal barrier function, causes toxins to enter blood, and causes chronic inflammation of the body. Ultimately, it will lead to insulin resistance, excessive fat accumulation, and promote the development of metabolic syndrome. Dietary fiber provides food for intestinal bifidobacteria and other probiotics. It enriches short chain fatty acid producing bacteria and butyrate synthesis, provides sufficient nutrients for intestinal cells [5]. On the other hand, it can inhibit the growth of pathogenic bacteria such as

*Escherichia coli* and reduce the formation of toxic fermentation products by reducing the intestinal pH value, ensure the normal physiological function of the intestinal tract, relieve chronic inflammation, improve insulin sensitivity and restore metabolic damage. Dietary fiber can also enhance satiety, delay and decrease carbohydrate absorption, balance and stabilize blood glucose levels [6]. At present, the high dietary fiber low glycemic index diet for clinical intervention in T2DM patients has attracted more and more attention. However, there are few reports about the effects of high dietary fiber low glycemic index diet on intestinal flora, blood glucose and inflammatory response in T2DM patients. In this study, dietary intervention was used to investigate the effects of high dietary fiber low glycemic index diet on intestinal flora, blood glucose and inflammatory response in T2DM patients.

## Materials and Methods

### Normal information

A total of 130 T2DM patients treated in our hospital were selected as the research objects from January 2016 to January 2017, included 70 males and 60 females, Aged 56-71 years, mean age ( $56.7 \pm 3.5$ ) years; diabetes course of 1 to 8 years, the average duration of diabetes ( $6.3 \pm 1.2$ ) years, as shown in Table 1. Inclusion criteria: (1) in line with the 1999 WHO T2DM diagnostic and classification criteria; (2) no serious liver and kidney damage, no infection and ketosis, stable condition, no antibiotics, no prebiotics and probiotics within 1 month (3) only accept oral hypoglycemic agents, no use of insulin, can carry out mild or moderate intensity of physical activity. A total of 130 patients were divided into observation group and control group by random number table method. Each group has 65 cases. There was no significant difference in sex and age in the two groups ( $P > 0.05$ ). All patients were informed of informed consent. The study was approved by the hospital medical ethics committee.

**Table 1.** Comparison of intestinal flora before and after intervention in two groups.

| Characteristics      | Control group  | Study group    | T     | P     |
|----------------------|----------------|----------------|-------|-------|
| Sex                  |                |                |       |       |
| Male                 | 34             | 36             | 0.352 | >0.05 |
| Female               | 26             | 24             | 0.268 | >0.05 |
| Age                  | $56.4 \pm 3.7$ | $56.9 \pm 3.9$ | 0.376 | >0.05 |
| Duration of diabetes | $6.1 \pm 1.4$  | $6.5 \pm 1.6$  | 0.198 | >0.05 |

### Treatment methods

**Intervention measures:** Patients in the control group received diabetes diet that without high dietary fiber and low glycemic index, exercise therapy, and oral hypoglycemic agents, the intervention lasted 6 months.

The observation group was treated with high dietary fiber and low glycemic index diet on the basis of the treatment in the

control group. The specific measures are as follows: professional nutritionists calculate the total energy and intake of nutrients according to the labor intensity, age and body mass of the patients, then formulate individual nutrition recipes. Recipes are based on physical exchange. In the range of total calorie supply, we provide soluble dietary fiber and low glycemic index coarse grains nutrition recipe intervention food: fruit and vegetable fiber meal (Yongan Kangjian Pharmaceutical Co., Ltd.), 10 g/d; snow buckwheat coarse grain surface (Shenzhen snow buckwheat Food Co., Ltd.), 50 g/d, directed and urged to use. Patients of two groups received food on the 1<sup>st</sup> and 15<sup>th</sup> of each month, and got the "Food Nutrition Recipe Food Record Form" which wrote by researchers. The patient would return the form the next time he received the food. The intervention lasted for 6 months.

**Observe indicators:** The intestinal flora indicators of *Enterococcus*, *Escherichia coli*, *Bifidobacterium* and *Lactobacillus* were compared in two groups before and after intervention. We retained the stool specimens before and after intervention, stored it in a closed fecal storage box within 2 h, and placed it in  $-80^{\circ}\text{C}$  low temperature refrigerator quickly. The fecal samples were thawed on ice. We operated them according to the specifications of the TIANamp Stool DNA Kit (TIANGEN) product. The stool of each subject was weighed 200 mg. Genomic DNA of the fecal flora was extracted and stored at  $-20^{\circ}\text{C}$ . Design and synthesize the bacterial primer sequences: *Enterococcus*: upstream: 5'-CCCTTATTGTTAGTTGCCATCATT-3', downstream: 5'-ACTCGTTGTACTTCCCATTGT-3'; *Escherichia coli*: upstream: 5'-GTTAATACCTTTGCTCATTGA-3', downstream: 5'-ACCAGGGTACTAATCCTGTGA-3'; *Bifidobacterium*: upstream: 5'-CTCCTGGAAACGGGTGG-3', downstream: 5'-GGTGTCTTCCCAGATCTACA-3'; *Lactobacillus*: upstream: 5'-CTGATGTGAAAGCCCTCG-3', downstream: 5'-GAGCCTCAGCGTCAGTTG-3'. The primer synthesis was performed by Shanghai Gongda Biology Co. Ltd. and the amplified product fragment was about 200 bp. We amplified the gene with the above specific primers. The PCR reaction system was 60  $\mu\text{L}$ , included 6  $\mu\text{L}$  PCR buffer 6  $\mu\text{L}$ , 1.5 mmo/L  $\text{MgCl}_2$  solution 4.8  $\mu\text{L}$ , dNTP 4.8  $\mu\text{L}$ , upstream and downstream primers 1.2  $\mu\text{L}$ , template DNA 2.4  $\mu\text{L}$ , Taq DNA polymerase 4.8  $\mu\text{L}$ , and finally filled it by adding sterile double distilled water. The amplification reaction was performed using a Perkin Elmer Model 970 PCR apparatus. The first 20 cycles:  $94^{\circ}\text{C}$  pre-denaturation 5 min,  $94^{\circ}\text{C}$  denaturation 30 s,  $65^{\circ}\text{C}$  annealing 45 s; the annealing temperature drop  $0.5^{\circ}\text{C}$  after each cycle,  $72^{\circ}\text{C}$  extension 1 min, for 40 cycles. After 10 cycles:  $94^{\circ}\text{C}$  denaturation 30 s,  $65^{\circ}\text{C}$  annealing 45 s,  $72^{\circ}\text{C}$  extension 1 min. The last 1 cycle:  $72^{\circ}\text{C}$  extension 10 min. The polyacrylamide gel electrophoresis of the PCR product with mass fraction of 8% was carried out using Dcode<sup>TM</sup> Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, Calif. The variation range is 30%-60%, and the loading amount is 20  $\mu\text{L}$  PCR amplification product. In 1X TAE electrophoresis buffer, it was operated under the conditions of temperature  $60^{\circ}\text{C}$  and voltage 85 V for 16 h constantly. After electrophoresis, silver nitrate stained for 20 min, deionized

## Effect of high dietary fiber low glycemic index diet on intestinal flora, blood glucose and inflammatory response in T2DM patients

water rinsed 2 times, each for 10 min. The electrophoretic bands of each sample were observed and photographed using an ultraviolet gel imaging analysis system and compared with the primer specificity in the BLAST gene pool.

Compare the blood glucose indexes in the two groups before and after intervention. The fasting venous blood was extracted before and after intervention. We used 7170A automatic biochemical analyzer to measure fasting blood glucose (FPG), glycosylated hemoglobin (HbA1c) and fasting insulin (FINS) levels of patients, and calculated the steady state model of insulin resistance index (HOMA-IR),  $HOMA-IR = FINS \times FPG / 22.5$ .

Compare serum high-sensitivity C reactive protein (hs-CRP), interleukin-1 beta (IL-1 beta) and interleukin-6 (IL-6) levels in the two groups before and after intervention. Serum levels of inflammatory factors hs-CRP, IL-1 beta and IL-6 were determined by enzyme linked immunosorbent assay (ELISA). The kit was purchased from Shanghai Hengyuan Biotechnology Co. Ltd. Briefly, Blood samples were collected into tubes containing EDTA, and then centrifuged at 3000 rpm at 4°C for 10 min to separate the plasma, which was stored at -80°C until ELISA testing. Corresponding ELISA kits were prepared to analyze the activity of hs-CRP, IL-1 beta and IL-6 in accordance with the kit instructions strictly.

### Statistical methods

All statistical analyses were performed using SPSS 21.0 software. Intestinal flora, blood glucose, inflammatory factors and other measurement data were used student's t-test. The values were compared using a  $\chi^2$  test. A p-value of <0.05 was considered statistically significant.

**Table 2.** Comparison of intestinal flora before and after intervention in two groups.

| Groups        | N  | <i>Enterococcus</i> |                    | <i>Escherichia coli</i> |                    | <i>Bifidobacterium</i> |                    | <i>Lactobacillus</i> |                    |
|---------------|----|---------------------|--------------------|-------------------------|--------------------|------------------------|--------------------|----------------------|--------------------|
|               |    | Before intervention | After intervention | Before Intervention     | After intervention | Before intervention    | After intervention | Before intervention  | After intervention |
| Control group | 65 | 6.45 ± 0.23         | 5.67 ± 0.41        | 4.16 ± 0.11             | 2.97 ± 0.35        | 4.02 ± 0.32            | 5.28 ± 0.56        | 3.17 ± 0.41          | 4.68 ± 0.91        |
| Study group   | 65 | 6.46 ± 0.21         | 4.97 ± 0.15        | 4.17 ± 0.18             | 2.36 ± 0.13        | 4.03 ± 0.34            | 5.84 ± 0.37        | 3.18 ± 0.53          | 5.01 ± 0.85        |
| T             |    | 0.316               | 6.792              | 0.258                   | 6.542              | 0.163                  | 7.005              | 0.102                | 7.139              |
| P             |    | >0.05               | <0.05              | >0.05                   | <0.05              | >0.05                  | <0.05              | >0.05                | <0.05              |

**Table 3.** Comparison of blood glucose levels between two groups before and after intervention.

| Groups        | N  | FPG (mmol/L)        |                    | HbA1c (%)           |                    | FINS (U/L)          |                    | HOMA-IR             |                    |
|---------------|----|---------------------|--------------------|---------------------|--------------------|---------------------|--------------------|---------------------|--------------------|
|               |    | Before intervention | After intervention | Before intervention | After intervention | Before intervention | After intervention | Before intervention | After intervention |
| Control group | 65 | 7.44 ± 0.23         | 6.52 ± 0.57        | 8.02 ± 0.54         | 6.22 ± 0.21        | 11.27 ± 0.86        | 9.84 ± 0.79        | 5.77 ± 0.81         | 4.42 ± 0.78        |
| Study group   | 65 | 7.45 ± 0.21         | 6.13 ± 0.36        | 8.04 ± 0.62         | 5.38 ± 0.14        | 11.28 ± 0.63        | 8.51 ± 0.65        | 5.78 ± 0.54         | 3.43 ± 0.61        |

## Results

### Comparison of intestinal flora before and after intervention in two groups

There was no significant difference in intestinal flora indicators between two groups of *Enterococcus*, *Escherichia coli*, *Bifidobacterium* and *Lactobacillus* before intervention ( $P > 0.05$ ). After intervention, the number of *Enterococcus*, *Escherichia coli* in the observation group was significantly lower than that in the control group, and the number of bacteria in *Bifidobacterium* and *Lactobacillus* was significantly higher than that in the control group ( $P < 0.05$ ) (Table 2).

### Comparison of blood glucose levels between two groups before and after intervention

There were no significant differences in FPG, HbA1c, FINS and HOMA-IR between the two groups before intervention ( $P > 0.05$ ). After intervention, the FPG, HbA1c, FINS and HOMA-IR in the observation group were significantly lower than that in the control group ( $P < 0.05$ ) (Table 3).

### Comparison of the levels of inflammatory factors before and after intervention

There was no significant difference in the levels of hs-CRP, IL-1 $\beta$  and IL-6 between the two groups before intervention ( $P > 0.05$ ). After intervention, the levels of hs-CRP, IL-1 $\beta$  and IL-6 in the observation group were dramatically lower than that in the control group ( $P < 0.05$ ) (Table 4).

|   |       |       |       |       |       |       |       |       |
|---|-------|-------|-------|-------|-------|-------|-------|-------|
| T | 0.405 | 6.063 | 0.211 | 6.081 | 0.182 | 7.442 | 0.173 | 7.096 |
| P | >0.05 | <0.05 | >0.05 | <0.05 | >0.05 | <0.05 | >0.05 | <0.05 |

**Table 4.** Comparison of the levels of inflammatory factors before and after intervention.

| Group         | N  | hs-CRP (mg/L)       |                    | IL-1 $\beta$ (pg/mL) |                    | IL-6 (pg/mL)        |                    |
|---------------|----|---------------------|--------------------|----------------------|--------------------|---------------------|--------------------|
|               |    | Before intervention | After intervention | Before intervention  | After intervention | Before intervention | After intervention |
| Control group | 65 | 8.03 ± 0.72         | 5.01 ± 0.32        | 12.07 ± 0.46         | 9.02 ± 0.53        | 12.26 ± 1.57        | 9.01 ± 0.83        |
| Study group   | 65 | 8.04 ± 0.75         | 3.68 ± 0.29        | 12.09 ± 0.51         | 7.38 ± 0.71        | 12.29 ± 1.44        | 7.97 ± 0.86        |
| T             |    | 0.247               | 6.981              | 0.204                | 6.937              | 0.162               | 7.005              |
| P             |    | >0.05               | <0.05              | >0.05                | <0.05              | >0.05               | <0.05              |

**Conclusion:** After intervention, the number of *Enterococcus*, *Escherichia coli* in the observation group was significantly lower than that in the control group, and the number of bacteria in *Bifidobacterium* and *Lactobacillus* was significantly higher than that in the control group ( $P < 0.05$ ). The FPG, HbA1c, FINS and HOMA-IR in the observation group were significantly lower than that in the control group ( $P < 0.05$ ). Before intervention, there was no significant difference in the levels of hs-CRP, IL-1 $\beta$  and IL-6 between the two groups ( $P > 0.05$ ). After intervention, the levels of hs-CRP, IL-1 $\beta$  and IL-6 in the observation group were dramatically lower than that in the control group ( $P < 0.05$ ) after intervention. High dietary fiber low glycemic index diet can effectively improve the intestinal flora imbalance and blood glucose level in T2DM patients, reduce serum inflammatory response and promote the rehabilitation of patients.

## Discussion

Intestinal flora plays an important role in metabolism and energy absorption [7,8]. The imbalance of intestinal flora is closely related to the occurrence and development of metabolic diseases such as diabetes mellitus. There is an imbalance of intestinal flora in diabetic patients. The number of beneficial bacteria is reduced and the number of conditional pathogens is increased [9]. The structure of intestinal flora is mainly affected by diet, and the unreasonable diet structure causes the destruction of intestinal flora structure. The purpose of this study is to explore the effect of high dietary fiber low glycemic index diet on intestinal flora, blood glucose in T2DM patients. Probiotics (*Lactobacillus* and *Bifidobacterium*) decrease in diabetic patients, while *Enterococcus* sp. and other intestinal opportunistic bacteria increased [10,11]. It indicates that there is imbalance of intestinal flora in diabetic patients. This study found that before intervention, there was no statistically significant difference in *Enterococcus*, *Escherichia coli*, *Bifidobacterium*, *Lactobacillus* and other intestinal flora index

of two groups ( $P > 0.05$ ). After intervention, the number of *Escherichia coli* and *Escherichia coli* in the observation group was significantly lower than that in the control group. The number of bacteria in *Bifidobacterium* and *Lactobacillus* was significantly higher than that in the control group ( $P < 0.05$ ). This suggests that T2DM patients can regulate intestinal flora imbalance after a certain degree of intervention by high dietary fiber and low glycemic index diet. The reason may be that dietary fiber can provide the substrate for anaerobic glycolysis for bifidobacteria, and can enrich the short chain fatty acid producing bacteria in the intestine, providing nutrition for the intestinal cells. Meanwhile, anaerobic glycolysis of *Bifidobacterium* and *Lactobacillus* can reduce the intestinal pH value, thereby inhibiting the growth of *Escherichia coli*, *Enterococcus* and other opportunistic bacteria, and improving the intestinal flora imbalance [12,13]. In addition, we found that after high dietary fiber low glycemic index diet intervention, blood glucose indicators and insulin resistance in patients improved significantly. The results suggest that high dietary fiber and low glycemic index diet can not only improve the intestinal flora structure of diabetic patients, but also play a certain role in reducing blood sugar. The mechanism of dietary intervention in reducing hypoglycemia is not clear yet. Some scholars have studied the correlation between intestinal bacteria and blood glucose levels in patients with diabetes mellitus, and found that the number of enterococci is positively correlated with FPG, while the number of *Bacteroides* is negatively correlated with FPG [14].

Opportunistic bacteria in the gut can promote the progression of hyperglycemia and insulin resistance in diabetic patients by producing endotoxin to participate in the early inflammatory response of metabolic diseases. Hs-CRP, IL-1 beta and IL-6 have been confirmed to be involved in the pathogenesis of type 2 diabetes in the past [15]. This study compared the two groups of patients with inflammatory factors in serum and found, before intervention, there was no significant difference in the levels of hs-CRP, IL-1 $\beta$  and IL-6 between the two groups ( $P > 0.05$ ) after intervention, the levels of hs-CRP, IL-1 $\beta$  and IL-6 in the observation group were significantly lower than that in the control group ( $P < 0.05$ ). The results showed that high dietary fiber and low glycemic index diet intervention can reduce the degree of micro inflammation in varying degrees. The mechanism may be that high dietary fiber and low GI diet intervention can promote the recovery of intestinal flora in patients, and relieve the degree of response that endotoxin involved in metabolic disease early inflammatory response and systemic inflammation of, thereby inhibiting the release of inflammatory factors in patients with the body.

In conclusion, high dietary fiber low glycemic index diet can effectively improve the intestinal flora imbalance and blood glucose level in T2DM patients, reduce serum inflammatory response and promote the rehabilitation of patients.

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