Age-associated effect of kestose on Faecalibacterium prausnitzii and symptoms in the atopic dermatitis infants

Yasuhiro Koga1, Shouji Tokunaga2, Jun Nagano3, Fuyuhiko Sato4, Kenta Konishi4, Takumi Tochio4, Youko Murakami5, Natsuko Masumoto6, Jun-ichirou Tezuka6, Nobuyuki Sudo7, Chiharu Kubo8 and Rumiko Shibata5

BACKGROUND: Although Faecalibacterium prausnitzii is a major bacterium in the intestine of adults, which is known to have anti-inflammatory effects, the development in infants or the response to prebiotics remains unclear.

METHODS: The counts of F. prausnitzii in the feces were examined by real-time polymerase chain reaction (PCR). Fecal samples were obtained from 65 atopic dermatitis (AD) infants who participated in a randomized controlled clinical trial to investigate the therapeutic effect of kestose, the smallest fructooligosaccharide.

RESULTS: Although the F. prausnitzii count was undetectable level in most 0- to 1-y-old infants, the count reached a level comparable to that in adults in 2- to 5-y-old infants. The bacterial number increased about 10-fold by oral administration of kestose every day for 12 wk in the younger infants, but not so much in the older infants. This bacterial increase was significantly correlated with an improvement in the AD symptoms in the older infants.

CONCLUSION: The F. prausnitzii population in the intestine reaches a level comparable to that in adult at approximately 2 y of age. Kestose efficiently stimulates the growth of this bacterium in the intestine, which might lead to an improvement in AD symptoms in infants.
be elucidated precisely when this bacterium initially colonizes the intestine of the infants after birth and how this bacterium expands in the intestinal microbiota thereafter. Accumulating evidence clearly has shown that this bacterium is a major component of the healthy human microbiota. *F. prausnitzii* is reported to be depleted in AD (10) as well as inflammatory bowel diseases (11). Moreover, this bacterium has been shown to exhibit anti-inflammatory effects by secretion of butyrate, a predominant short-chain fatty acid produced by gut microbiota (8). Thus, an enlargement of the indigenous *F. prausnitzii* population by prebiotics may be a promising method for the treatment of AD.

In the present study, we first examined the colonization of *F. prausnitzii* in the hosts ranging from early to late infancy who suffered from AD, using *Bifidobacterium* as a control because this genus is well known to be predominant at early infancy and remains a major component of the intestinal microbiota during all infancy (12). We then investigated the prebiotic effect of kestose on bacteria colonizing the intestine and analyzed the correlation between the changes in the count of the bacteria and the improvement in AD symptoms.

### RESULTS

#### Study Population

A total of 65 AD patients were enrolled, and 33 and 32 of the patients were randomly allocated to the kestose- and placebo-treated groups, respectively. Seven patients dropped out of the trial before the completion of the treatment because they moved out of the region during the trial. Compliance estimated by interviews with guardians was >90% for all subjects. Finally, 29 subjects in the kestose group and 29 subjects in the placebo were analyzed in the present study. The baseline characteristics and demographics of the subjects for the analyses are summarized in Table 1. No significant differences were found in any of the items.

#### The Relationship Between the Age and the Bacterial Count in the Infants

To investigate the development of *F. prausnitzii* and *Bifidobacterium* in the gut microbiota of the infants, we first analyzed the relationship between the subject age and the bacterial count found in any of the items.

![Figure 1](image-url)

**Figure 1.** Bacterial count in the infants at different ages. Each circle represents the bacterial count of *F. prausnitzii* (left subpanel) or *B. catenulatum* group (right subpanel) in the feces obtained from a subject at 0 to 5 y of age. The counts are expressed as the logarithmic copy number per gram feces. Bars represent the median of the counts at each subject age.

### Table 1. Baseline characteristics of the subjects

| Species                  | Detection ratio (%) | Rs   | P      |
|--------------------------|---------------------|------|--------|
| *F. prausnitzii*         | 78                  | 0.62 | <0.001 |
| *Bifidobacterium*        | 100                 | −0.12| 0.37   |
| *B. adolescentis*        | 34                  | 0.19 | 0.15   |
| *B. bifidum*             | 31                  | −0.01| 0.96   |
| *B. breve*               | 72                  | −0.14| 0.29   |
| *B. catenulatum group*   | 86                  | 0.38 | 0.004  |
| *B. dentium*             | 21                  | −0.18| 0.17   |
| *B. infantis*            | 59                  | −0.23| 0.08   |
| *B. longum*              | 93                  | 0.08 | 0.56   |

### Table 2. Correlation value between age and fecal bacterial number in the infants (at 0 wk = before the treatment)

| Species                  | Correlation coefficient | P      |
|--------------------------|-------------------------|--------|
| *F. prausnitzii*         | 0.38                    | 0.004  |
| *B. catenulatum*         | 0.38                    | 0.004  |
| *B. infantis*            | 0.38                    | 0.004  |
| *B. longum*              | 0.38                    | 0.004  |

P = 0.004) but not the genus *Bifidobacterium* or other *Bifidobacterium* species (Table 2). The *B. catenulatum* group bacterial count was lower than the detectable level in 7 and 2 subjects at 0 and 1 y of age, respectively (Figure 1, right...
subpanel). The median count remained low at 0 y of age (5.9) but plateaued at 1 y of age (9.1). This finding suggests that the *B. catenulatum* group population expands from birth and plateaus in the gut microbiota at around 1 y of age.

### Effect of Kestose on the Number of Fecal Bacteria

All subjects were treated with kestose or placebo daily for 12 wk and fecal samples were collected to measure the bacterial count at 0, 6, 12, and 18 wk after the start of the treatment.

#### Table 3. Effect of kestose on the number of fecal bacteria

| Treated with   | Bacterial counts; median (interquartile range) (% detection ratio) | Weeks after the start of treatment |
|---------------|-------------------------------------------------------------------|-----------------------------------|
|               | 0 (*n* = 58) | 6 (*n* = 56) | 12 (*n* = 57) | 18* (*n* = 52) |
| **F. prausnitzii** | Kestose | 7.4 (5.2–8.7) (76) | 7.9 (6.5–8.7) (83) | 8.3 (6.5–8.9)* (89) | 8.0 (6.6–8.5) (83) |
|               | Placebo | 7.6 (5.3–8.7) (79) | 8.0 (6.0–8.4) (78) | 8.3 (7.6–8.4) (83) | 8.0 (7.2–8.4) (93) |
| **Bifidobacterium** | Kestose | 10.1 (9.6–10.7) (100) | 10.5 (9.8–10.7) (100) | 10.5 (9.8–11.0)* (100) | 9.9 (9.7–10.5) (100) |
|               | Placebo | 10.1 (9.7–10.7) (93) | 10.2 (9.7–10.5) (100) | 9.9 (9.5–10.5) (90) | 9.9 (9.5–10.4) (96) |
| **B. adolescentis** | Kestose | ND (ND–8.4) (34) | ND (ND–9.0) (41) | ND (ND–9.6) (46) | ND (ND–8.5) (42) |
|               | Placebo | ND (ND–6.8) (34) | ND (ND–7.4) (37) | ND (ND–8.2) (38) | ND (ND–8.3) (36) |
| **B. bifidum** | Kestose | ND (ND–8.4) (31) | ND (ND–7.5) (31) | ND (ND–8.8) (43) | ND (ND–8.0) (38) |
|               | Placebo | ND (ND–8.4) (38) | ND (ND–7.8) (33) | ND (ND–7.7) (31) | ND (ND–5.6) (25) |
| **B. breve** | Kestose | 6.5 (ND–8.2) (72) | 7.4 (6.6–8.1) (76) | 7.5 (6.0–8.6) (79) | 6.7 (6.1–7.8) (88) |
|               | Placebo | 7.2 (5.7–8.3) (79) | 8.0 (7.0–8.6) (93) | 7.0 (5.8–8.0) (79) | 7.2 (5.6–8.1) (75) |
| **B. catenulatum group** | Kestose | 8.8 (8.0–9.7) (86) | 8.9 (8.2–9.7) (79) | 9.1 (8.3–9.9)* (89) | 8.9 (7.5–9.3) (79) |
|               | Placebo | 8.7 (6.9–9.4) (76) | 8.3 (6.0–9.4) (78) | 8.3 (5.0–8.7) (69) | 8.4 (7.6–8.8) (79) |
| **B. dentium** | Kestose | ND (ND–ND) (21) | ND (ND–7.0) (34) | ND (ND–7.1) (36) | ND (ND–7.5)** (46) |
|               | Placebo | ND (ND–ND) (10) | ND (ND–6.8) (30) | ND (ND–6.9) (34) | ND (ND–5.2) (25) |
| **B. infantis** | Kestose | 5.8 (ND–7.4) (59) | 6.5 (ND–8.3) (69) | 6.7 (ND–7.9) (68) | 6.6 (ND–7.2) (67) |
|               | Placebo | 7.3 (ND–8.2) (72) | 8.2 (ND–8.6) (70) | 6.7 (ND–8.0) (62) | 6.7 (ND–8.2) (68) |
| **B. longum** | Kestose | 9.6 (8.6–10.3) (93) | 9.7 (9.3–10.2) (97) | 9.6 (9.1–10.3)* (89) | 9.3 (8.8–9.8) (92) |
|               | Placebo | 9.4 (7.8–10.0) (83) | 9.5 (9.0–10.0) (96) | 9.0 (8.4–9.8) (83) | 9.3 (7.8–9.8) (83) |

*aSix weeks after the termination of treatment.

*Significantly greater (*P* < 0.05) than 0 wk. †Significantly greater (*P* < 0.05) than placebo at the same week.

ND, not detected.
In the kestose-treated group, the number of *F. prausnitzii* at 12 wk was about 10-fold higher than that at 0 wk, and the increase was statistically significant ($P < 0.05$). No such increase was observed in the placebo-treated group. Regarding bifidobacteria, the kestose group had a significantly higher *Bifidobacterium* count than the placebo group at 12 wk, a significantly higher *B. catenulatum* group count at 12 wk, a significantly higher *B. dentium* count at 18 wk and a significantly higher *B. longum* count at 12 wk. Next, to determine whether the stimulating effect of kestose on *F. prausnitzii* is age-dependent, the change in the bacterial count was compared between two subgroups including 0- to 1-y olds and 2- to 5-y olds. The basal count remained far lower in the former than in the latter subgroup (see Figure 1). In the 0- to 1-y-old subgroup, an exclusive increase, but no decrease, in the differential count was found in all subjects after kestose treatment at 6 and 12 wk, whereas both an increase and decrease in the count were observed after placebo treatment (Figure 2, left subpanel). Indeed, the median differential bacterial counts were considerably greater in the kestose group than in the placebo group, although the differences were not significant. Considering that a decrease was observed even in the kestose group at 18 wk, 6 wk after the termination of kestose treatment, the exclusive increase of the *F. prausnitzii* in this subgroup during kestose treatment may have been directly exerted by kestose itself. In the 2- to 5-y-old subgroup, however, an increase and decrease was observed in the kestose- and placebo-treated groups at 6 and 12 wk after the treatment. Thus, no significant decrease was observed. Therefore, the stimulating effect of kestose on *F. prausnitzii* may vary depending on age.

![Figure 3](image)

**Figure 3.** Changes in the number of *B. catenulatum* group after the treatment in the subgroups of younger and older infants. Left and right subpanels show the subgroups composed of subjects at 0 to 1 y of age and 2 to 5 y of age, respectively. Each symbol represents the differential change in the number of bacteria in a subject at 6, 12, and 18 wk (= 6 wk after the finish of the treatment) after the start of the treatment. Filled and open symbols indicate the subjects treated with kestose and placebo, respectively. Bars represent the median of the differential bacterial numbers. *$P < 0.05$ value in the inter-group difference < 0.1.

**Table 4.** Correlation between changes in the SCORAD score and the bacterial count in AD patients treated with kestose

| Duration of treatment | Treated with (n) | Decrease in the SCORAD score; median (IQR) | Correlation between the improvement in the SCORAD and the increase in bacterial count |
|-----------------------|------------------|------------------------------------------|-----------------------------------------------------------------------------------|
| 0- to 1-y old         |                  |                                          |                                                                                   |
| Δ (0–6 wk)            | Kestose (12)     | 0.00 (−8.50~3.50)                        | $-0.55$ > 0.05 $-0.07$ > 0.05                                                   |
|                       | Placebo (17)     | 2.26 (−3.03~8.22)                        | $-0.31$ > 0.05 0.05                                                             |
| Δ (0–12 wk)           | Kestose (13)     | −1.87 (−4.50~4.49)                       | $-0.13$ > 0.05 $-0.03$ > 0.05                                                   |
|                       | Placebo (17)     | 3.10 (0.56~9.40)                         | 0.01 > 0.05 0.36                                                             |
| 2- to 5-y old         |                  |                                          |                                                                                   |
| Δ (0–6 wk)            | Kestose (16)     | 4.05 (0.90~9.86)                         | 0.52 0.044 0.33 > 0.05                                                          |
|                       | Placebo (12)     | 3.99 (−4.67~9.18)                        | 0.11 > 0.05 0.55                                                                |
| Δ (0–12 wk)           | Kestose (16)     | 4.67 (−0.53~10.05)                       | $-0.18$ > 0.05 $-0.19$ > 0.05                                                   |
|                       | Placebo (12)     | 8.00 (0.30~12.65)                        | 0.17 > 0.05 0.20 > 0.05                                                        |
Bifidobacterium and different beginning of supplementation and the cessation of breast-feeding after the birth; phase II, the remaining period of solely breast-development of intestinal microbiota in infants into four separating which feeding shifts from breast-feeding to weaning to the gut microbiota of the hosts at 0 to 1 y of age. Regarding the B. catenulatum group, however, no age-dependent stimulating effect by kestose was observed. Both an increase and decrease in the differential bacterial count were observed in the 0- to 1-y-old and 2- to 5-y-old subgroups at 6, 12, and 18 wk after the treatment (Figure 3). Additionally, there was no significant difference between the kestose- and placebo-treated groups at any time point after the start of the treatment.

Relationship Between the Differential Bacterial Count and the SCORAD Score

To determine whether the increase in the F. prausnitzii count by kestose was correlated with an improvement in the SCORAD score in the subgroup, this relationship was analyzed using Spearman's test (Table 4). In the 2- to 5-y-old subgroup, there was a significant positive correlation between an increase in the F. prausnitzii count and an improvement in the SCORAD score when subjects were treated with kestose for 6 wk (rs = 0.52, P = 0.044). However, no such positive correlation was observed in the 0- to 1-y-old subgroup treated with kestose, although there was a considerable increase in the F. prausnitzii count in this subgroup (see Figure 2). Additionally, there was no significant correlation between the changes in the B. catenulatum group count and the SCORAD score in any subgroup.

DISCUSSION

The initial acquisition and subsequent succession of microbes colonizing the intestinal tract is marked in the infancy, during which feeding shifts from breast-feeding to weaning to the introduction of solid food. Cooperstock et al. (13) divided the development of intestinal microbiota in infants into four separate phases: phase I, the initial acquisition phase a few weeks after the birth; phase II, the remaining period of solely breast-feeding around the first 6 mo; phase III, the time between the beginning of supplementation and the cessation of breast-feeding; and phase IV, the period of conversion to adult microbiota beginning after completion of weaning. In the present study, the development of F. prausnitzii and different Bifidobacterium species in the gut microbiota was investigated using fecal samples from infants suffering from AD. The analysis of the relationship between the subject age and the bacterial counts demonstrated a significant positive correlation in F. prausnitzii and the B. catenulatum group. A further analysis of the age-associated increase of F. prausnitzii revealed that this bacterium initially appeared at 0 y of age and plateaued at around 2 y of age. The bacterial count at plateau using the qPCR method was about 10^8 to 10^9 copies/g feces, which was comparable to the bacterial count in 45 healthy Japanese adults (mean age 48 y) examined by the same method in our previous study (9). A study conducted in Spain composed of 31 healthy adults (mean age 32 y) also showed that the median F. prausnitzii count was 6.5 x 10^6 copies/g feces (14). These studies thus indicated that the F. prausnitzii population in the gut microbiota in infants reaches that in adults at around 2 y of age. A previous study on the development of microbiota by the amount of short-chain fatty acids in the feces reported that butyrate levels in infants reached that in adult level at 9 to 18 mo of age (15). Considering that F. prausnitzii is the major bacterial source of butyrate in the intestine, our finding that the count of this bacterium became the adult level around 2 y old is nearly compatible to the result analyzed by such microbiota-associated characteristic as the amount of butyrate produced by the gut microbiota. According to the phases mentioned above, it is considered that this bacterial population reaches its full development in the intestine at around phase III. In a comparative study using atopic and healthy children who were 4 to 14 y of age, the number of fecal F. prausnitzii in atopic subjects was about one-third of that in healthy subjects (10). As the F. prausnitzii count in the present study was measured in AD patients at ages ranging from 0 to 5 y, the difference in the number between these AD patients and age-matched healthy infants remains to be examined.

Throughout the breast-feeding period (phase II), a predominance of bifidobacteria is generally already established and no more increase or rather a decrease in the bacterial count often follows after this period. In fact, six out of seven kinds of Bifidobacterium species examined in the present study exhibited no age-associated increase in the bacterial count during infancy. The B. catenulatum group, on the other hand, showed a significant age-associated increase similar to that of F. prausnitzii, although the bacterial count of the B. catenulatum group plateaued at about 1 y earlier than the F. prausnitzii count. In a study aimed to determine the influence of an obesity treatment program on the gut microbiota in adolescents (16), the counts of both the B. catenulatum group and the Clostridium leptum group which is phylogenetically closely related to F. prausnitzii were significantly higher in the high-weight loss group than in the low-weight loss group before and after intervention. Conversely, the counts of B. breve and B. bifidum, which showed no significant age-associated increase in the count in the present study, were significantly lower in the former group than in the latter group. These results suggested that there is a close association between the B. catenulatum group and F. prausnitzii with regard to nutritional and ecological requirements in the intestinal tract of the host, which are considerably different from those required by the phase II-dominant Bifidobacterium species such as B. breve and B. bifidum.

Although modulation of indigenous F. prausnitzii abundance using probiotic strains of this bacterium might have therapeutic applications in inflammatory and allergic diseases, an extremely oxygen-sensitive characteristic of this bacterium...
renders industrial cultivation of this bacterium very difficult. Alternatively, the introduction of prebiotics to modulate the indigenous *F. prausnitzii* population in the intestine might be possible. Indeed, Ramirez-Farias et al. reported the effect of inulin on the number of *F. prausnitzii* in the gut (17). In their study, fecal samples obtained from 12 healthy adults who were given 10 g/day inulin for 16 d showed a significant increase in the abundance of the bacterium, however, the degree of increase was only about 50%. In the present study using infants, on the other hand, the degrees of increase in the count by 1~3 g/day kestose treatment were significantly greater that 3-fold and 10-fold at 6 and 12 wk, respectively, after the start of treatment.

Of note, there was a significant difference in the degree of kestose-induced increase in *F. prausnitzii* between the 0- to 1-y-old and 2- to 5-y-old subgroups in the present study. A greater increase in this bacterium was found in the younger subgroup after kestose treatment than in the older subgroup. In the younger subgroup, the primary source of nutrients was breast-feeding, while solid foods including dietary nondigestible carbohydrates were rarely consumed in the subgroup. As a result, bifidobacteria were dominant, while *F. prausnitzii* remained the minority in the microbiota of these subjects. Thus, we speculate that kestose, a nondigestible FOS, exerted a marked stimulating effect on the growth of *F. prausnitzii* in younger infants because the intestinal environment was devoid of substrates to promote the growth of this bacterium. However, in the older subgroup, the nutritional source was primarily solid foods, which nearly led to a plateau in the number of *F. prausnitzii*; thus further intake of kestose exerted no marked stimulating effect on this bacterium. By contrast, no such difference in the stimulating effect of kestose on the *B. catenulatum* group count was observed between the younger and older subgroups, which had a low count in the younger subgroup. Moreover, in a clinical study to examine the effect of inulin in the gut microbiota (17), no increase was found in the *B. catenulatum* group, while a significant increase in other *Bifidobacterium* species, including *B. adolescentis* and *B. bifidum*, was observed. Taken together, fructans such as inulin and kestose might not be utilized so efficiently by the *B. catenulatum* group.

Allergies such as AD are dependent on T helper 2 (Th2)-derived immune responses characterized by the production of cytokines IL-4, -5, and -13, which promote the production of various mediators to induce allergic responses (18). The Foxp3+ regulatory T cell (Treg) is characterized by the production of IL-10, one of the major immunoregulatory cytokines, and considered to suppress the excessive activation of Th2 cells thereby ameliorating allergic responses. Some species of *Clostridium*, a dominant genus of commensal microbes in the gut, are known to induce Treg cells (19,20). Furthermore, Furusawa et al. (21) demonstrated that butyrate can induce the differentiation of Treg cells. Considering that *F. prausnitzii* is one of the main butyrate-producing bacteria in the intestinal microbiota of the human, it is reasonable that the kestose-induced improvement of the AD symptoms was correlated with the increased number of *F. prausnitzii* in the present study. Additionally in their study, acetate, the main short-chain fatty acid produced by bifidobacteria, could not induce Treg cell differentiation, suggesting a potential explanation for the lack of a correlation between the improvement in kestose-induced AD symptoms and the number of bifidobacteria observed in our previous clinical trial (7). However, in the present study, a significant correlation between the increase in the *F. prausnitzii* count and improvement in the AD symptoms was found only in the 2- to 5-y-old subgroup after 6-wk treatment with kestose but not in the 0- to 1-y-old subgroup in which a marked increase in the number of this bacterium was observed. One of the reasons for poor response to kestose in the younger subgroup may be that the basal count remained very low in this subgroup therefore even marked stimulation by kestose treatment could not increase the size of the *F. prausnitzii* population to an adequate level to have a significant effect on the host. However, in the older subgroup, the basal count was so great that even a small increase by kestose treatment might have resulted in a significant improvement in the AD symptoms.

In conclusion, while prebiotic oligosaccharides have thus far almost exclusively targeted bifidobacteria, the present study has demonstrated that kestose, the smallest FOS, was able to strongly activate *F. prausnitzii*, another major indigenous bacterium that has a beneficial effect and helps to maintain good health in infants.

**METHODS**

**Ethical Consideration**

This study protocol was approved by the Committee on Ethical Practice in Tokai University School of Medicine. Written informed consent was obtained from all the patients’ guardians.

**Research Design**

Fecal samples were collected from all subjects who participated in a randomized double-blind placebo-controlled trial named “A clinical study to investigate the therapeutic effect of 1-kestose on atopic dermatitis” which was carried out from October 2012 to December 2013 (Registered in UMIN-CTR www.umin.ac.jp/ctr TEST 000000488). In brief, the subjects were recruited from outpatient clinics in two hospitals in Fukuoka Prefecture, Japan. The inclusion criteria for the subjects were (i) children < 6 y of age and diagnosed with AD of which the severity grading of skin symptoms were Mild or Moderate by Japanese Dermatologist Association, (ii) no use of antibiotics or probiotic/prebiotic preparations during 1 mo before the start of the trial. Patients were randomly allocated to either the kestose- or placebo-treated group. The kestose-treated group was orally given 1 g (< 1 y old of age), 2 g (1 to 3 y old of age), or 3 g (4 to 5 y old of age) 1-kestose daily for 12 wk according to the protocol in the previous study (7). The 1-kestose-lot used in the present study was supplied from B Food Science Co. Ltd., Tokyo, and contained >98% 1-kestose. The placebo-treated group was given maltose (glucose-glucose) in the same way as the kestose-treated group. Fecal sampling from the both groups was performed four times at 0, 6, 12, and 18 wk (=6 wk after the termination of the treatment). The fecal samples were immediately sent to the laboratory and frozen until analysis.
Clinical Evaluation

The clinical diagnosis of AD was made according to the criteria issued by the Japanese Dermatologist Association (22). In these criteria, the diagnosis of AD requires the presence of all three features including pruritus, typical morphology and distribution of eczematous dermatitis, and a chronically relapsing course. The severity grading of skin symptoms was rated using the following four-point scale: absence, mild, moderate, and severe. A clinical evaluation of the subjects before and after the treatment was performed using the Severity Scoring of Atopic Dermatitis (SCORAD) (23). The SCORAD index used in the present study includes objective items only (0–83 point scale).

DNA Extraction

Bacterial DNA was extracted from the feces using a Ultra Clean Soil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA) according to the manufacturer’s instruction with some modifications (24,25). Briefly, feces (0.1 g) were added to a tube that contained lysozyme (25 mg/ml) and N-acetylmuramidase (0.3 mg/ml) and then incubated for 30 min at 37 °C for cell lysis. Next, the extracted DNA was purified using a High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer’s instructions.

Real-Time Quantitative PCR

Real-time PCR amplification and detection were performed using an ABI PRISM 7700 sequence detection system (Applied Biosystems, Foster, CA). Bacterial DNA from the samples was subjected to real-time PCR assays. The primer set FpCRAU 07 (CCA TGA ATT GCC TTC AAA ACT GTT) and FpCRAU 02 (GAG CCT CAG CGT CAG TTG GT) described by Sokol et al. (11) was used to detect F. prausnitzii in the present study. Amplification and detection were carried out according to the method reported previously. Briefly, each reaction was performed in a final volume of 25 μl containing 12.5 μl SYBR GREEN Master Mix (Applied Biosystems) together with 0.2 μmol/l final concentration of each primer, and 10 μl of appropriate dilutions of DNA template. Amplifications were performed as follows: 95 °C for 10 min, to denature DNA and activate Ampli-Taq Gold Polymerase, followed by 40 cycles of 95 °C for 30 s and 60 °C for 1 min. The values of log_{10} cells/g feces for F. prausnitzii were calculated for each sample from the threshold cycle values using a constructed standard curve. The B. catenulatum group consists of B. catenulatum and B. pseu-docatenulatum. The primer pairs used to detect these groups were BiCATg-1 (CGG ATG CTC CGA CTA CT) and BiCATg-2 (CGA AGG CTT GCT CCC GAT) (26). The primer pairs used to detect the genus Bifidobacterium and other species of Bifidobacterium including B. adolescentis, B. bifidum, B. breve, B. dentium, B. infantis, and B. longum were described by Matsuki et al. (26) For the detection of bifidobacteria, amplification was performed in a total volume of 25 μl containing 12.5 μl SYBR GREEN Master Mix and 0.25 μl of each specific primer. The amplification program consisted of one cycle of 94 °C for 5 min, 40 cycles of 94 °C for 20 s, 55 °C for 30 s, and 72 °C for 1 min, followed by a final step of 94 °C for 15 s.

Statistics

All the values were initially examined to determine whether they had a normal distribution before the comparative analysis. The bacterial counts in the same group at different time points were first analyzed by the Friedman test. Then, the difference in the values was examined by the Wilcoxon signed-rank test. The Mann-Whitney U-test was applied to compare the values at the same time point or interval between the kestose and placebo groups. The relationship between the values stratified by the subject age and fecal bacterial count was analyzed by Spearman’s correlation coefficient using the rank test. The relationship between the change in the SCORAD scores and the change in the bacterial count after the treatment was also analyzed by Spearman’s test. All P-values were two-sided and considered to be significant at P < 0.05. The SPSS for Windows version 23 software package (IBM, New York, NY) was used for all statistical analyses.

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