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Original article

Effects of mats with "A Distinctive 4-Layer 3-Dimensional Structure" on sleep quality and gut microbiota: A non-controlled open-label study

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Abstract

***Purpose*:** "Sleep quality" plays an important role in maintaining the homeostasis of the body, and deterioration in sleep quality causes various lifestyle-related diseases. In the present study, we verified the effects of a mat with a "Distinctive 4-Layer 3-Dimensional Structure" as the study product on sleep quality and gut microbiota through a non-controlled open-label study.

***Methods*:** The Pittsburgh Sleep Quality Index (PSQI-J) was used to assess 36 men and women who were not satisfied with their sleep quality, and 12 subjects (age 50.1 ± 4.9 years) with a marked decrease in "sleep quality" were included in the study. A non-controlled open-label study was conducted for changes in physical data when the study product (Nishikawa Co., Ltd., Chuo-ku, Tokyo) was used for 8 weeks. Analysis of gut microbiota by PSQI-J and T-RFLP, and measurement of plasma amyloid-β (Aβ) 40/42 ratio were conducted before commencement and 8 weeks after the start of the study. The present study was conducted with the approval of the ethics review committee.

***Result*:** PSQI-J showed a significant improvement in sleep quality, time to fall asleep, difficulty sleeping and daytime difficulty waking after 8 weeks. The PSQI global score improved significantly from 8.2 ± 1.4 to 4.2 ± 2.2 ($p < 0.01$). Gut microbiota analysis showed a significant increase in the percentage of genus *Bacteroides* and short-chain fatty acid (SCFA)-related bacteria. Plasma Aβ40 was slight but significantly elevated, and there were no changes in the Aβ40/42 ratio.

***Conclusion*:** The use of the study product improved "sleep quality," affecting the gut microbiota through the gut-brain connection, increasing bacteria belonging to genus *Bacteroides*, suggesting that the study product may contribute to the maintenance of homeostasis in glycolipid metabolism and immune defense mechanisms.

***KEY WORDS*:** mats with a "Distinctive 4-Layer 3-Dimensional Structure," sleep quality, gut microbiota, *Bacteroides*, amyloid-β

Preface

Sleep is essential for maintaining and improving health; however, the sleeping time of Japanese people is low when compared to other countries. Various studies have shown that poor sleep quality decreases glucose tolerance and increases the risk of developing obesity, metabolic syndrome and hypertension [1-4]. Our research group also verified whether using a mattress suited to the user improves the health index [5-7]. As a result, effects such as improvement in oxidative stress [6], improvement in glycolipid metabolism [5,7], and improvement in the secretion of growth hormone [5] and melatonin [7], and improvement of skin conditions [8] were observed in cases where the use of a comfortable mattress improved sleep quality. In the present study, we verified the effect of improved sleep quality by using a mat with a "Distinctive 4-Layer 3-Dimensional Structure," on gut microbiota, through a non-controlled open-label study.

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Method

Subjects

Thirty-six slightly overweight men and women between the ages of 50 and 65 years were enrolled as subjects for the study. Preliminary examination (SCR) was conducted for the potential enrollees, including anthropometric measurements, the Japanese version of the Pittsburgh Sleep Quality Index (PSQI-J), and a medical interview with a physician. Individuals who met the selection criteria, did not violate the exclusion criteria, and had a PSQI-J score of 6 or more were ranked in the order of increasing score, and 12 subjects were selected in the order starting from the subject with the highest overall ranking.

The selection criteria are given below.

1) Healthy men and women between 50 to 65 years old when the consent for participation in the study was obtained
2) Individuals whose body mass index (BMI) is more than 25.0 kg/m^2 and less 30.0 kg/m^2
3) Individuals who are aware of mild sleep disorders such as waking up in the middle of the night (nocturnal awakening), waking up early in the morning (early-morning awakening) and not feeling like they slept well (disturbed sleep).
4) Individuals whose work schedule is 3-5 days a week during the daytime and have holidays during the weekends
5) Individuals with regular bedtime (lights out) and wake-up time, bedtime (lights out) before midnight, and sleep habit of 4 hours or more
6) Individuals who sleep alone
7) Individuals who do not have the habit of drinking alcohol
8) Individuals with the ability to give consent after receiving an adequate explanation of the purpose and content of the study and who volunteer to participate of their own accord after sufficient understanding and provide written consent to participate in the present study
9) Individuals who can come on the designated examination date to undergo examination
10) Individuals determined to be suitable as a subject of the present study by the principal investigator

The exclusion criteria are given below.

1) Individuals currently suffering from an illness and receiving drug therapy
2) Individuals currently in the habit of taking or applying drugs
3) Individuals taking medications for the treatment of a disease for the past one month (excluding those with a history of taking temporary-relief medicines for headaches, menstrual pain and cold)
4) Individuals with a history of, or currently suffering from, mental illness, sleep disorders, hypertension, diabetes mellitus, dyslipidemia or a severe illness
5) Individuals suspected of, under treatment, or previously treated for Sleep Apnea Syndrome (SAS)
6) Individuals with nocturia, enlarged prostate gland, or overactive bladder
7) Individuals with a history of, or currently suffering from, a severe disease of the liver, kidney, heart, lungs, digestive organs, or blood
8) Individuals with comorbidities or a history of severe gastrointestinal disorders
9) Individuals with severe constipation or daily diarrhea symptoms
10) Individuals who cannot abstain from alcohol during the study period
11) Individuals who smoke
12) Individuals with the habit of consuming functional foods, food for specified health use, health foods, or dietary supplements in the past month, and those who plan to take them during the study period
13) Individuals who have donated 200 ml of blood in the past month, or over 400 ml within the past 3 months
14) Individuals who may have difficulty in using the test mat during the study period due to possible changes in lifestyle, or travel
15) Individuals who are currently participating in other human clinical trials and those who have participated in other human clinical trials within the past 3 months
16) Women who are pregnant, lactating or who might be pregnant
17) Individuals who have been determined by the principal investigator as not suitable to be a subject of the present study

Study Design

This is a non-controlled open-label study.

The study product was the mat "AiR SX" with a distinctive 4-layer 3-dimensional structure (Nishikawa Co., Ltd., Chuo-ku, Tokyo). It was a single-size mattress (9 x 97 x 200 cm) with special sheets provided by Nishikawa Co., Ltd. The mattresses used by the subjects were changed to the study product at the start of the study.

Before starting the study and 8 weeks after the commencement of the study, confirmation of subjective symptoms, PSQI-J, gut microbiota examination (Fecal T-RFLP Flora Analysis) and a medical interview with a physician were implemented. The study participants recorded the existence and degree of adverse events, and lifestyle, dietary, and exercise habits during the study period in a life journal. The study period was from October to December 2020.

Evaluation Items

Subjective Symptoms

PSQI-J was used to evaluate sleep quality [9]. The sleep quality, time to fall asleep, sleeping time, sleep efficiency, difficulty sleeping, use of sleep inducers and daytime difficulty waking were scored according to the scoring method tabulation table of PSQI and the PSQI global score (PSQIG) was calculated. Concerning the evaluation criteria, 5 points or less indicates that there is no sleep disorder, 6 points or more indicates a sleep disorder, 6 to 8 points indicates a mild disorder and 9 points or more indicates a severe disorder [10].

Anthropometric Measurements

Height, weight, body fat percentage, body mass index

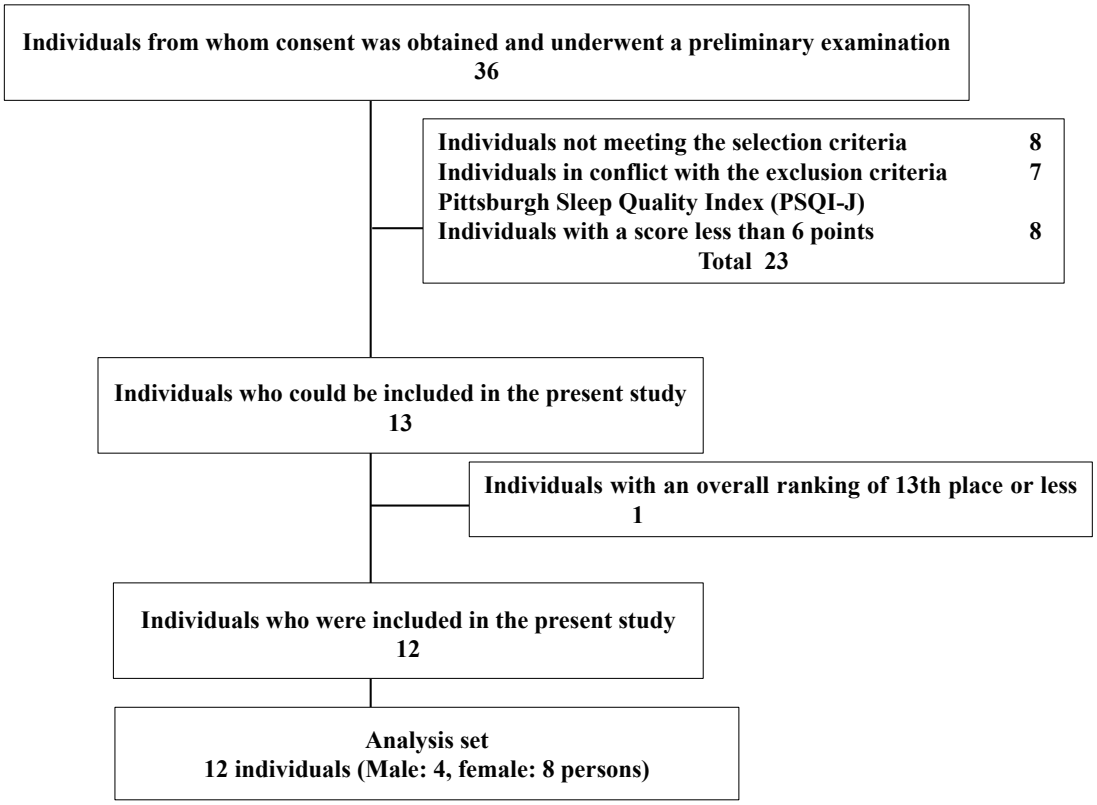

Fig. 1. **Changes in the number of test subjects.**

(BMI), systolic and diastolic blood pressure, and pulse rate were measured. The body composition test was performed using a body composition analyzer (DC-320; Tanita, Itabashi-ku, Tokyo).

Blood Tests

Plasma Aβ-40 and Aβ-42 were measured by LSI Medience Corporation (Chiyoda Ward, Tokyo) using blood samples. Blood tests were performed at the Ueno-Asagao Clinic (Director: Takahiro Ono, Taito-ku, Tokyo).

Gut Microbiota Examination

Fecal T-RFLP Flora Analysis (TechnoSuruga Laboratory Co., Ltd., Shimizu-ku, Shizuoka City) was conducted to examine the gut microbiota [11, 12]. The test item was the analysis of the gut microbiota (order to genus level (> 0.1-1%)) specific to the human intestine. The 16S ribosomal RNA gene is an essential gene for bacteria and is classified based on the similarity of the base sequences. When the base sequences of the 16S ribosomal RNA gene sequences of bacteria are classified by a computer using their similarity (generally 96-97%) as an index, the unit obtained is called OTU (Operational Taxonomic Unit). The major taxonomic groups of human gut microbiota (genus *Bifidobacterium*, and genus *Bacteroides*) in the samples were analyzed as OTUs. The results were expressed as the composition ratio (percentage) of the number of OTUs of each bacterium to the total number of bacteria.

Statistical Analysis

The values of the examination results were tabulated in a cumulative table using Microsoft Office Excel 2016 (Microsoft Corp.). Appropriate software such as SAS (SAS 9.4) and SPSS (Statistics 26) was used to perform the statistical analysis, and significance level for all tests was set at both sides as 5%, and 10% was set as the trend. For intra-group comparison of the analyzed data, the results of the examination performed before and 8 weeks after using the test product were compared by statistical analysis using an equivalent t-test. The scores obtained from the questionnaire survey were treated as non-parametric, and a Wilcoxon signed-rank test was performed for comparison between each group.

Ethical Review

The present study was conducted with the approval (September 28, 2020, Glycative Stress Research 2020 No. 005) of the Ethics Review Committee for "Research on Human Subjects" of the Society for Glycative Stress Research, in compliance with the Helsinki Declaration (Amended October 2013) and "Ethical Guidelines for Medical and Health Research Involving Human Subjects" (Ministry of Education, Culture, Sports, Science and Technology and Ministry of Health, Labour and Welfare, and Ministry of Education, December 22, 2014). The informed consent form was given to the study participants in advance, and voluntary consent was obtained in writing. The present study

was conducted after registration on the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) (registration number: UMIN 000041883). Due to the social situation under the new coronavirus epidemic, we decided to go ahead with the study only if the sponsor, consignee, principal investigator and the Ethics Review Committee determined that the study could be conducted safely. The study was conducted with sufficient consideration to prevent infectious diseases following the infection control measures of the medical institution conducting the study.

Results

An open study was conducted to determine the effects of using the test product for 8 weeks on sleep quality, alertness, quality of life, gut microbiota and special blood tests in slightly overweight men and women between the ages of 50 and 65.

The present study was started with 12 subjects. All 12 subjects completed the study and were included in the analysis. The age of the 12 subjects in the study group (4 males and 8 females) was 55.0 ± 4.4 years (54.8 ± 5.9 years for males and 55.1 ± 4.0 years for females).

Evaluation of Subjective Symptoms

According to the Pittsburgh Sleep Questionnaire, subjective symptoms such as sleep quality ($p \leqq 0.01$), time to fall asleep ($p \leqq 0.01$), sleeping time ($p \leqq 0.01$), and sleep efficiency ($p < 0.05$) improved significantly after using the study product for 8 weeks compared to before commencement of the study. Difficulty sleeping ($p < 0.05$) and daytime difficulty waking ($p < 0.01$) also significantly improved after 8 weeks compared to before the study (***Table 1***). As a result, PSQIG significantly improved from 8.8 ± 1.9 before the study to 3.8 ± 1.3 after 8 weeks ($p < 0.01$).

Body Composition and Blood Pressure

There were no significant differences in each of the measurement items: Body weight, body fat percentage, fat mass, lean body mass, muscle mass, BMI and basal metabolic rate after 8 weeks compared to before use of the study product (***Table 2***).

Change in Gut Microbiota

Fecal T-RFLP Flora Analysis showed that the percentage of bacteria of genus *Bacteroides* was 33.5 ± 8.2% before use and 39.4 ± 7.3% after 8 weeks of use, a

Table 1. Sleep quality evaluation.

		Before	8 weeks	p-value
PSQI-J	Sleep quality	2.0 ± 0.4	0.8 ± 0.4	**0.002**
	Time to fall asleep	1.8 ± 0.8	0.8 ± 0.9	**0.008**
	Sleeping time	1.8 ± 0.4	1.1 ± 0.7	**0.007**
	Sleep efficiency	0.8 ± 0.9	0.0 ± 0.0	**0.023**
	Difficulty sleeping	1.2 ± 0.4	0.8 ± 0.4	**0.046**
	Use of sleep inducers	0.0 ± 0.0	0.0 ± 0.0	1.000
	Daytime difficulty waking	1.3 ± 0.9	0.3 ± 0.5	**0.010**
	PSQIG	8.8 ± 1.9	3.8 ± 1.3	**0.002**

Results are expressed as mean ± SD, Wilcoxon signed-rank test, n = 12. PSQI-J, Pittsburgh Sleep Quality Index (Japan version) questionnaire; PSQIG, PSQI global score; SD, standard deviation.

Table 2. Anthropometry.

	Before	8 weeks	p-value
Bodyweight (kg)	72.1 ± 10.9	72.1 ± 10.9	0.9821
Body fat (%)	32.8 ± 6.0	32.9 ± 6.3	0.7112
BMI	26.9 ± 1.6	26.9 ± 1.8	0.9498
Blood pressure			
systolic (mmHg)	125.7 ± 12.2	121.9 ± 11.3	0.2118
diastolic (mmHg)	79.8 ± 7.9	77.3 ± 7.4	0.1718
Pulse (/min)	69.9 ± 9.3	69.1 ± 9.7	0.6319

Results are expressed as mean ± SD, Wilcoxon signed-rank test, n = 12. BMI, body mass index; SD, standard deviation.

significant increase after 8 weeks ($p < 0.001$). There was a substantial decrease in uncategorized bacteria (other) from 14.5 ± 3.8% before use to 10.6 ± 3.2% after 8 weeks. ($p < 0.01$, ***Fig. 2***).

Plasma Aβ Test

Change in Aβ-40 was slight after 8 weeks, but had significantly increased ($p < 0.05$). There were no significant changes in Aβ-42 or Aβ-40/Aβ-42 ratios before and after using the test product (***Table 3***). There was no correlation with the number of bacteria belonging to the genus *Bacteroides*.

Discussion

A total of four clinical studies have been conducted for the study product[5-8], and this is the fifth study. According to the PSQJ-I, sleep quality and time to fall asleep improved in all five studies, daytime difficulty waking improved in four out of five studies, difficulty sleeping and sleeping time improved significantly in three out of five studies. The global score PSQIG significantly improved in all five studies. Similar results were obtained from all five studies, indicating that the improvement effect of subjective symptoms in the present study was highly reproducible and that "sleep quality" improved with the use of the

Table 3. Plasma Aβ examination

		Before	8 weeks	p-value
Aβ40	(pg/mL)	294.1 ± 37.6	313.0 ± 25.0	**0.040**
Aβ42	(pg/mL)	31.7 ± 5.9	33.6 ± 3.0	0.255
Aβ40/42		9.53 ± 1.77	9.35 ± 0.74	0.752

Results are expressed as mean ± SD, paired t-test, n = 12. Aβ, amyloid-beta; SD, standard deviation.

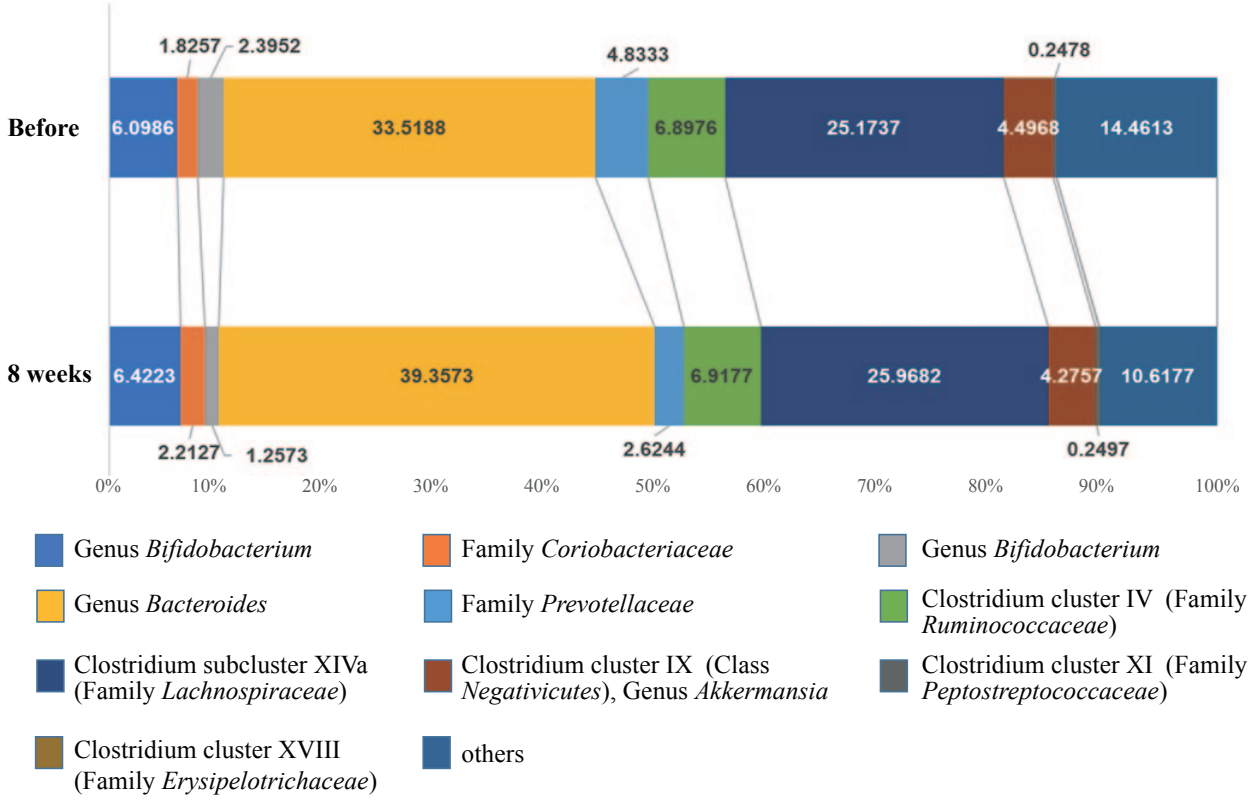

Fig. 2. **Distribution of the gut microbiota.**

Results are expressed as average values, n = 12. Terminal restriction fragment length polymorphism (T-RFLP) analysis.

study product. In the present study, we examined the gut microbiota changes brought about by improved sleep quality.

Human Gut Microbiota

More than 100 trillion bacteria of about 1,000 species in the human intestinal tract are known to form a stable community (gut microbiota, microflora, microbiota) in a symbiotic antagonistic relationship. The bacteria that make up the gut microbiota interact with the host, either directly or through their metabolites, influencing the physiology and immune system of the host [13-16] and bidirectionally influencing the central nervous system via the gut-brain connection [17, 18].

There are regional, individual and ethnic differences in gut microbiota, which are often influenced by diverse cultures and dietary habits. A project was launched in 2009 under the auspices of the Asian Federation of Societies for Lactic Acid Bacteria. The "Asian Microbiome Project (AMP)" (http://www.agr.kyushu-u.ac.jp/lab/microbt/AMP_HP.html), which investigates the gut microbiota of Asians, aims at building a database of the basic gut microbiota of Asians covering all regions and age groups. A total of 10 countries in East and Southeast Asia are participating at present in the AMP project. To date, data on the gut microbiota of more than 1,000 people, ranging from newborns to the elderly, have been collected.

Next-generation DNA sequencing technology is being used in these projects to perform 16S rRNA amplicon analysis and whole shotgun metagenomic sequencing. The 16S ribosomal RNA gene is an essential gene for bacteria, classified based on the similarity of the base sequences. When the base sequences of the 16S ribosomal RNA gene sequences of bacteria are classified by a computer using their similarity (generally 96-97%) as an index, the unit obtained is called OTU (Operational Taxonomic Unit). The number of OTUs represents the number of bacterial groups/species that make up the bacterial flora. The number of reads belonging to the same OTU represents the relative abundance of that species. A representative sequence can be selected from the number of reads belonging to each OTU, and the genus and species name can be identified by comparison to a database of known bacterial genetic data. However, some of the OTUs may not strictly correspond to the traditional taxonomic classification in bacteriology.

Even in the present study, the major taxonomic groups of human gut microbiota (genus *Bifidobacterium*, and genus *Bacteroides*) were analyzed as OTUs. The present study aims to verify the effect of improved sleep quality on gut microbiota by using the study product for 8 weeks. The study product used in the present study was confirmed to improve "sleep quality," through previous clinical studies and the current PSQI-J. As a result of the improved sleep quality, there was a significant increase in genus *Bacteroides* and a significant decrease in bacteria belonging to other genera (others).

The main constituents of the gut microbiota change from facultative anaerobic bacteria in the neonatal period to genus *Bifidobacterium* in infancy and class Clostridia and phylum Bacteroidetes in adults [19]. However, there are individual differences, and many infants deviate from this pattern [20]. Facultative anaerobic bacteria produce ATP through aerobic respiration in the presence of oxygen to obtain energy. These bacteria can switch their metabolism to get energy through fermentation in the absence of oxygen. *Lactobacillus, Escherichia coli*, and *Enterococcus* usually fall under this category.

Bacteroides

The relationship of *Bacteroides* with immunity and allergies has been well studied [21]. During infancy, food-specific IgE antibodies that cause the onset of food allergies are easily produced, but the barrier function of the gastrointestinal mucosa and oral immune tolerance, which are functions to suppress this allergic reaction, are weak, due to which production of digestive enzymes and secretory IgA is low. In infants with IgE-dependent allergies, a decrease in *Bacteroides* and *Clostridium* cluster XVIII, specifically an increase in *Clostridium sensu stricto* and *Anaerobacter*, have been shown [22]. During infancy and later, genus *Bacteroides* are less common, and family *Enterobacteriaceae* is more common in allergic groups.

A difference based on age was observed in the relationship between the gut microbiota and the onset of allergies, and the genus *Bacteroides* was more common in allergic groups among newborns [23]. A difference based on the region was observed in the relationship between gut microbiota and the onset of allergies. A cohort study in three countries, Russia, Finland and Estonia, found that genus *Bacteroides* was more prominent in the infants of Finland and Estonia, where allergies are more common, than in the infants of Russia [21].

Bacteroides are gram-negative bacteria, like phylum Proteobacteria, having an extracellular membrane that produces lipopolysaccharide (LPS) as its component. LPS induces innate immunity through Toll-like Receptors (TLRs). However, compared to *Escherichia coli* LPS, Bacteroidetes phylum LPS has weaker immunostimulation, and the innate immunity cannot be sufficiently acquired by *Escherichia coli*. It has been pointed out that the stimulation of the immune system by gut microbiota may be weak in children of Finland and Estonia during infancy leading to the inadequate acquisition of innate immunity.

Short-chain fatty acids (SCFAs) produced by gut microbiota also have immune regulatory functions. SCFAs such as acetic, butyric and propionic acids are involved in maintaining the homeostasis of the host through the G Protein-Coupled Receptors (GPCRs), which are seven-transmembrane receptors on the cell membrane. GPCRs include GPR43, GPR41 and GPR109A. Acetic acid enhances the barrier function of colon epithelial cells and inhibits pathogenic bacterial infections [24, 25]. This acid promotes IgA secretion in the intestinal tract by regulating the Immunoglobulin A (IgA) class switch of B cells by intestinal dendritic cells via GPR43 [26]. Propionic acid regulates airway epithelial inflammation through GPR41 in pulmonary dendritic cells [27].

Bacteroides have an immunomodulatory effect on the intestinal immune system, and their ability to induce IgA production in the small intestinal Peyer's patches is stronger than *Lactobacillus* [28]. In aseptic mice with immature intestinal lymphoid tissue formation, administration of *Bacteroides* induces the formation of germinal center in the

lymph nodes of the small intestine and cecum, increasing IgA production in the lamina propria of the intestinal mucosa [29]. *Bacteroides* exhibit a modulatory effect on antigen-presenting cells during immune sensitization in the intestinal immune system. The bacterial components of *Bacteroides* (mainly LPS) show immunomodulatory effects such as activation of regulatory T cell (Treg) responses through antigen-presenting cells, regulation of inflammatory responses, and immune tolerance. Smith reported that short-chain fatty acids produced by gut microbiota are involved in the induction of intestinal regulatory T cell (Treg) differentiation [30].

Lifestyle and Bacteroides

In the gut microbiota of infants, bacteria of genus *Bifidobacterium*, which predominantly utilize (assimilate) the oligosaccharides in human breast milk, proliferate significantly [31, 32]. As baby foods are started, and weaning progresses, the "Bifidus flora," which mainly consists of genus *Bifidobacterium*-dominant bacterial flora, is replaced by adult-type intestinal bacteria of phyla Bacteroidetes and Firmicutes [33-35].

Bacteroides can assimilate indigestible sugars, including oligosaccharides. *Bacteroides* proliferate in the intestine using sugars, especially fructooligosaccharides and their constituent sugars (1-kestose [GF2] and nystose [GF3]). Since the increase of indigestible sugars in the intestinal tract can sometimes induce abnormal fermentation in the intestine, assimilation action of indigestible sugars by *Bacteroides* may play a role in maintaining a healthy intestinal environment.

Vitamin K, which is produced by specific enteric bacteria, plays a role in maintaining bone quality. A report has analyzed the relationship between bone metabolism index and gut microbiota in 38 postmenopausal women (mean age 62.9 years) [36]. Genus *Bacteroides* was more common in the high vitamin K2 group (blood Vit K ≥ 0.06 ng/mL). Incidence of fracture was significantly higher in the low *Bacteroides* group, and the risk ratio associated with previous bone fractures was 5.6 times higher than the high *Bacteroides* group. There was no significant difference in Bone Mineral Density (BMD) between the groups.

Diet also affects gut microbiota. People with a prevalence of genus *Bacteroides* consume more proteins and fat, while those with a prevalence of genus *Prevotella* tend to consume more dietary fiber [37]. Children in Italy and Burkina Faso (a country in Africa, where the staple food is grain such as sorghum) have the bacterial flora of the *Bacteroides* type and *Prevotella* type, respectively [14]. The modern diet consists of high fat and low carbohydrates, which causes a shift from enterotype P (high *Prevotella* content) to type BB (high *Bacteroides* and *Bifidobacterium* content). Obese individuals are characterized by lower expression of Bacteroidetes and higher expression of Firmicutes compared to healthy individuals [38]. The impact of a high-fat diet is considered to be significant.

Gastric bypass surgery (Roux-en-Y gastric bypass; RYGB) is bariatric surgery for highly obese individuals. By changing the passage of ingested food, insulin resistance is improved by decreasing the secretion of ghrelin from the stomach and increasing GLP-1 and GIP secretion from the small intestine. Glucagon-Like Peptide-1 (GLP-1) is secreted from the L cells of the small intestine, which binds to the GLP-1 receptors on the surface of the pancreatic β-cells and works to help secrete insulin from within the β-cells. Glucose-Dependent Insulinotropic Polypeptide (GIP) is a hormone secreted in K-cells present on the upper part of the small intestine in response to food ingestion, which acts on the pancreatic β-cells to promote insulin secretion. Changes in gut microbiota observed after the RYGB surgery is an increase in phylum Bacteroidetes and a decrease in phylum Firmicutes [39]. Even when RYGB surgery is performed on patients with type 2 diabetes mellitus, phylum Bacteroidetes and *Escherichia coli* increase, making the gut microbiota more diverse [40]. However, lactic acid bacteria and *Bifidobacteria* decrease.

There have been no reports on the relationship between sleep and *Bacteroides*, and it is a novel finding that improvement in sleep quality increases *Bacteroides* abundance. Although it is expected that the gut-brain connection is involved, further research is required to elucidate the detailed mechanism.

Bacteroides, Dementia and Parkinson's Disease

The relationship between the gut microbiome and various forms of cognitive decline has been the focus of much research. Murine models of Alzheimer's disease (AD) have revealed the impact of Bacteroidetes and its subtaxa on the disease's pathogenesis. In a transgenic mouse model of AD, human amyloid-β precursor is expressed in the gut as well as the brain, and Bacteroidetes are significantly reduced at 9 weeks of age [41]. In another transgenic study [42], amyloid pathology was improved by altering the gut microbiome with post-natal antibiotic treatment. In this model, the treatment group demonstrated an increased abundance of family *Bacteroidaceae*. Diet induced low abundance of Bacteroidales (the order containing *Bacteroides* and *Prevotella*) has also been associated with poorer outcomes on cognitive tests [43].

These alterations in *Bacteroide*s have also been observed in human studies. Saji N *et al.* analyzed the relationship between gut microbiota and dementia [44]. They collected stool samples from 128 patients (mean age 74 years) who visited the outpatient clinic for memory loss and analyzed the relationship between gut microbiota and cognitive function. Dementia was diagnosed in 34 patients, while the remaining 94 patients did not have dementia. The patients were classified into three types: enterotype I (30% or more *Bacteroides*), enterotype II (15% or more Prevotella) and enterotype III (rich in other bacteria), based on the percentage of bacteria. The results indicated that patients with dementia had fewer *Bacteroides* (Enterotype I) and more "other" bacteria (Enterotype III).

Similar results have been reported in studies of AD and mild cognitive impairment, with patients having reduced *Bacteroides* compared to healthy controls [45, 46]. However, in another study by Saji *et al.* [47] regarding mild cognitive impairment, *Bacteroides* abundance was increased in patients showing early signs of cognitive decline compared to the control group, in contrast to their findings with diagnosed dementia patients. Liu *et al.* [48] also found that Bacteroidetes were increased in patients with mild cognitive

impairment, but not in patients with full AD. It may be that as dementia progresses, the microbial profile of the gut changes over time with the progression of cognitive decline.

However, there are also studies presenting more contradictory results. Haran *et al.*[49] and Vogt *et al.*[50] find significantly higher abundance of *Bacteroides* in AD patients. In a study of healthy older adults, Manderino *et al.*[51] also report poorer scores on tests of cognitive functions in those with increased *Bacteroidetes* abundance. The differences in microbial profile observed in these studies may be due to factors such as population and diet, as they examine American participants. Another factor that may need to be taken into account for more accurate analysis is the presence of different species of the genus *Bacteroides* which are not captured at the genus level. Some species of *Bacteroides*, such as *B. fragilis* are anti-inflammatory[52], and abundance is negatively correlated with amyloidosis[53]. Conversely, species like *B. dorei* and *B. uniformis* have been associated with dementia[54], despite beneficial roles in other disease models such as atherosclerosis[55] and high-fat-diet induced obesity[56].

The overall bacterial metabolite levels of the gut are also a useful biomarker for dementia[57]. It has been reported that both an elevated concentration of faecal ammonia (basic) and low lactic acid concentration are associated with increased risk for dementia. The predictive strength of the two metabolites is similar to that of traditional dementia biomarkers. Independently of the effects of specific species, the pH of the gut as influenced by resident bacteria may also contribute to the development of dementia.

Parkinson's disease (PD) is a neurodegenerative disease that increases with age. It has been revealed that the onset of PD does not originate from the central nervous system but with the abnormal accumulation of α-synuclein (Lewy bodies) in gastrointestinal epithelial cells, which gradually spreads to the central nervous system. The permeability of the intestinal tract increases with PD, and excessive oxidative stress in the intestinal wall is considered to cause accumulation of α-synuclein in the intestinal mucosa[58]. The results of analyzing the gut microbiota of 30 pairs (total 60 subjects) consisting of PD patients and their spouses (control) showed that *Clostridium* (*C. coccoides* group and *C. leptum* group) and *B. fragilis* group abundance was significantly reduced in PD patients, while *Lactobacillus* was elevated, without significant differences in carriage rates. Genus *Prevotella* abundance and carriage were also reduced in the PD patients, however the difference was not significant. Overall, there was a significant decrease in abundance of Gram-negative bacteria reported.

The Firmicute/Bacteroidetes ratio appears to be somewhat variable between studies involving PD[59] and may not be a reliable metric on its own without greater context. Unger *et al.*[60] found that the gut of PD patients was characterized by reduced SCFA production and *Bacteroidetes* abundance compared to controls. At lower taxonomic levels, reductions in several *Bacteroides* species have been reported[61]. Additionally, a two-year observational study of gut dysbiosis in PD[62] found that *B. fragilis* group was significantly reduced in deteriorated patients compared to stable patients, and that low *B. fragilis* abundance at the start of the study was associated with increased severity of disease progression after 2 years.

While the complex interactions of the numerous species which inhabit the gut are not yet fully understood, it is clear that their influence on SCFA production, gut permeability and acidity, and other factors have a significant role to play in neurodegenerative disease.

The results of the present study showed the following changes in the 12 patients.

Enterotype I 8 subjects → 12 patients
Enterotype II 2 subjects → 1 patient (*Bacteroides* 30% or more)
Enterotype III 2 subjects → 0 patients

Aβ40/42 ratio was used as the indicator for dementia in the present study. Since there was no change in the Aβ40/42 ratio despite a significant increase in Aβ40, the Aβ40 clearance could have increased, leading to increased transfer from the brain to the bloodstream. We have studied the age-related changes of the Aβ40/42 ratio in our laboratory and found that the ratio increases with age[63, 64]. Since the independent elderly in the Yurin district have been practicing walking exercise for nearly 10 years and belong to a population with low glycative stress, their Aβ40/42 ratio has shifted downwards compared to other populations. Although the subjects of the present study belong to a population with a tendency of deterioration in "sleep quality," there are some subjects with a higher Aβ40/42 ratio compared to other populations. Further verification is required to determine whether "sleep quality" is a factor that increases the Aβ40/42 ratio. In the present study, Aβ40/42 ratio did not change even though there was an increase in "sleep quality." We infer that the change in the Aβ40/42 ratio could not be detected as the study duration of 8 weeks was too short to observe this phenomenon.

Bacteroides and Short-Chain Fatty Acids

In recent years, the development of omics analysis such as metagenomics, proteomics and metabolomics, and analytical technologies have not only made identification of single bacterial strains along with their variations in the gut microbiota possible but also shown that metabolites derived from the intestinal bacteria are actively involved in the energy metabolism of the host. SCFAs are typical intestinal bacteria-derived metabolites produced by the intestinal bacteria through fermentation, using dietary fiber as a substrate. They affect the energy metabolism and immune functions, and even epigenome regulation of the host. SCFAs exhibit their functions after being recognized by fatty acid receptors and G Protein-Coupled Receptors (GPCRs). Among GPCRs, GPR41 and GPR43 are actively involved in maintaining the homeostasis of energy metabolism in the host[8]. The GPCR signals induce an increase in basal metabolism, elevation of body temperature, improvement in insulin sensitivity and promotion of lipolysis.

A large-scale cohort study of type 2 diabetic patients in Europe and China indicated a lower percentage of the butyric acid-producing genus *Clostridium* and a higher percentage of the non-butyric acid-producing genus *Clostridium* in the gut microbiota of patients with type 2 diabetes mellitus[65, 66]. The amount of short-chain fatty acids was found to have decreased in the stool of patients with type 2 diabetes mellitus[67].

Bacteroides are closely related to SCFAs. The production of SCFAs depends on the utilization of ingested nutrients, bacterial composition of gut microbiota and transit time through the intestine. Although a significant portion of the SCFAs in the intestine are derived by the bacterial decomposition of carbohydrates in the proximal intestine, digestion products of proteins and peptides have an effect of promoting SCFA production[68]. Among carbohydrates, Fructooligosaccharides (FOS) are indigestible sugars that cannot be easily decomposed by the digestive enzymes of the host when orally ingested by humans. The majority of oligosaccharides reach the lower intestinal tract and are decomposed into glucose and fructose by β-fructosidase produced mainly by *Bifidobacterium* in the colon. This causes changes to the gut microbiota and increases the production of SCFAs and lactic acid by the enteric bacteria [69-71]. As a result, a slightly acidic state is maintained in the intestinal tract, and homeostasis of fecal properties and frequency of bowel movements is maintained. FOS activates the growth of not only *Bifidobacterium* but also *Bacteroides* in the intestine[72]. The addition of HMO Sialyl-Lactose (SL) and galactooligosaccharides (GOS) has been shown to promote the growth of *Bacteroides* and *Bifidobacterium*, increase the production of SCFA, promote the healing of intestinal epithelial wounds and enhance the epithelial barrier function. The two genera can also cooperate to promote further SCFA production, as exopolysaccharides produced by *Bifidobacterium* increase propionate and acetate production in *B. fragilis*[73].

Since the results of the present study showed an increase in the percentage of genus *Bacteroides*, it can be presumed that the percentage of SCFAs derived from genus *Bacteroides* has increased.

Variations in bacteria other than genus *Bacteroides* will be discussed from the perspective of SCFA production.

Genus *Bifidobacterium* produces acetic and lactic acids[74]. Strictly speaking, lactic acid does not belong to SCFAs. Still, lactic acid maintains a slightly acidic state in the intestinal tract, which helps the growth of other SCFA producing bacteria and promotes the growth of acetic acid-producing bacteria that assimilate the lactic acid. Although a significant increase in the percentage of genus *Bifidobacterium* was not observed in the present study, there was no decrease.

Collinsella aerofacien of the *Coriobacteriaceae* family is widely distributed in the intestine of 90% of humans or more, and a high bacterial count of one-billion cells is detected per gram of stool. These bacteria assimilate glucose and lactose to produce organic acids and hydrogen gas[75]. *Collinsella tanakaei* of the same family metabolizes glucose to produce lactic, acetic and formic acids[76]. Although a significant increase in the percentage of the *Coriobacteriaceae* family was not observed, there was no decrease. In the present study, the percentage change was from 1.8% to 2.2% of the total bacterial count in the gut microbiota, which is a small proportion of the total.

Order Lactobacillales is one of the orders of bacteria in phylum Firmicutes, which mainly includes lactic acid bacteria[77,78]. Most lactic acid bacteria are included in order Lactobacillales. As the name suggests, lactic acid bacteria produce lactic acid. In the present study, the percentage of order Lactobacillales appears to have reduced slightly but not significantly. The percentage change was from 2.4% to 1.3% of the total bacterial count in the gut microbiota, which is a small proportion of the total.

Family *Prevotellaceae* helps to decompose foods containing proteins and carbohydrates in the human intestinal tract[79]. Bacteria of family *Prevotellaceae* may be opportunistic pathogens that are also involved in periodontal diseases[80]. There is little association of these bacteria with SCFAs. In the present study, although no significant decrease was observed in the percentage of family *Prevotellaceae*, there was no increase. *Faecalibacterium prausnitzii* belonging to *Clostridium* cluster IV (Family *Ruminococcaceae*) are commensal bacteria abundantly present in the human gut microbiota[81]. This bacterium accounts for about 5% of the enteric bacteria. This bacterium assimilates acetic acid to produce butyric acid. *Butyricicoccus faecihominis* belonging to the family *Ruminococcaceae* assimilates mucin, a mucous component secreted by the intestinal epithelial cells and produces butyric acid[82]. Although this bacterium is widely distributed in the human intestine, viable cell count in the stool is low. Although a significant increase was not observed in the percentage of family *Ruminococcaceae* in the present study, there was no decrease.

Clostridium subcluster XIVa (family *Lachnospiraceae*) assimilates various plant-derived polysaccharides and produces SCFAs (acetic acid and butyric acid)[83]. A part of the SCFAs are converted into ethanol by fermentation. Even in the present study, it is evident that the family *Lachnospiraceae* is an abundant bacterial group accounting for approximately 25%. Although a significant increase was not observed in the percentage of this family, there was no decrease.

Clostridium cluster IX (class Negativicutes) bacteria of genus *Akkermansia* are mucinolytic and use mucin as their only energy source. Mucin degradation is considered a pathogenetic factor since it leads to disruption of the intestinal barrier. However, at the same time, there is also an opinion that mucin degradation products may provide nutrition for other resident bacteria[84]. There is little association with SCFAs. Although a significant change was not observed in the present study, there was a decrease in 8 of the 12 subjects.

Clostridium cluster XI (family *Peptostreptococcaceae*) is often observed in the intestinal tract of colorectal cancer patients[85]. There is little association of these bacteria with SCFAs. *Clostridium difficile* belonging to this family produces toxin A and toxin B and is also known to be the causative bacteria of pseudomembranous colitis. When dysbiosis of the gut microbiota occurs due to the administration of antibacterial drugs, multi-drug-resistant *C. difficile* bacteria proliferate[86]. In the present study, these bacteria were detected in 6 of the 12 subjects. During the study period the bacteria was observed to decreased in 4 subjects, becoming non-detectable in three them. This change is considered to be a positive effect associated with the improvement in “sleep quality”.

Clostridium cluster XVIII (family *Erysipelotrichaceae*) are detected even in humans as enteric bacteria, some of which cause infections in humans and animals such as *Erysipelothrix rhusiopathiae*, which causes swine erysipelas[87]. There is little association of these bacteria with SCFAs. The bacteria of this family increase in mice fed with a high-fat

diet [88]. In the present study, bacteria were detected in 2 of the 12 subjects. After 8 weeks, the bacteria disappeared in one subject and increased in the other subject. One of the 10 subjects in whom the bacteria were non-detectable with the previous value showed positive results after 8 weeks.

When the total percentage of genus *Bifidobacterium*, genus *Coriobacteriaceae*, order Lactobacillales, genus *Bacteroides*, *Clostridium* cluster IV (family *Ruminococcaceae*) and *Clostridium* subcluster XIVa (family *Lachnospiraceae*) that contribute to the production of SCFAs is compared, a significant increase ($p = 0.029$, added to ***Table 4***) is observed after 8 weeks (82.1%) compared to the previous value (75.9%). This finding is considered to suggest that SCFA production increased with the improvement in "sleep quality". LPS and SCFA produced by enteric bacteria may be involved in a part of the mechanism of action in improving immunocompetence [6] and glucose tolerance [7] associated with improved "sleep quality" shown in past clinical trials.

Conclusion

It was reconfirmed with the PSQI-J evaluation that "sleep quality" improved after using the study product for 8 weeks. Although there was no significant change in the plasma Aβ40/42 ratio, which is measured as an index of cognitive function, gut microbiota analysis showed a significant increase in the percentage of bacteria of family *Bacteroides* and SCFA-related bacterial groups. These findings suggest that improvements in "sleep quality" may induce changes in the gut microbiota via the gut-brain connection and have a positive effect on immunocompetence and maintenance of homeostasis in glucose and energy metabolism through bacterial metabolites (LPS and SCFA). Sleep dysregulation has recently been shown to increase the risk of dementia [89], and sleep research will become increasingly important in the future.

Conflict of Interest Statement

We received research support from Nishikawa Co., Ltd. to conduct the present study.

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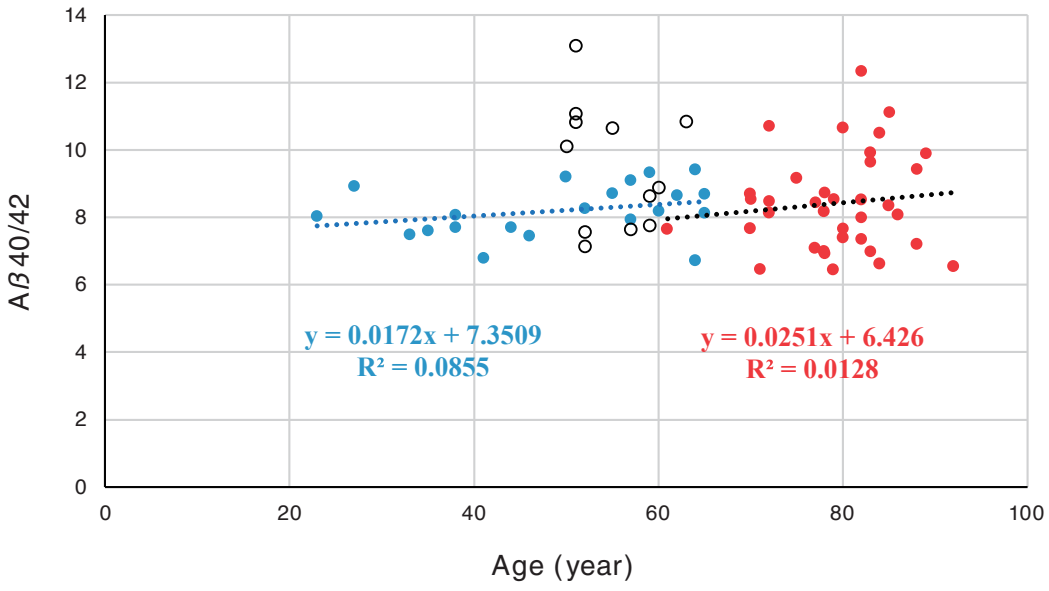

Fig. 3. **Aging transition of Aβ40/42.**

●University faculty and staff (n = 21), ●Independent elderly in the Yurin area (n = 36), ○Subjects with sleep disorders (n = 12 in the present study). The plasma Aβ40/42 ratio shows a gradual upward trend with aging. The elderly in the Yurin area is a group of people who walk about 7,000 steps a day more than the average of the same generation and have less glycative stress. Data quoted from References 63, 64).

Table 4. Changes of the gut microbiota by T-RFLP analysis.

Age	Gender	Genus *Bifidobacterium*		Family *Coriobacteriaceae*		Order Lactobacillales		Genus *Bacteroides*		Family *Prevotellaceae*		*Clostridium* cluster IV (Family *Ruminococcaceae*)		*Clostridium* subcluster XIVa (Family *Lachnospiraceae*)		*Clostridium* cluster IX (Class *Negativicutes*), Genus *Akkermansia*		*Clostridium* cluster XI (Family *Peptostreptococcaceae*)		*Clostridium* cluster XVIII (Family *Erysipelotrichaceae*)		others		SCFA related	
		%		%		%		%		%		%		%		%		%		%		%		%	
		Pre	8 week	Pre	8 week	Pre	8 week	Pre	8 week	Pre	8 week	Pre	8 week	Pre	8 week	Pre	8 week	Pre	8 week	Pre	8 week	Pre	8 week	Pre	8 week
50	Male	1.0	7.6	1.3	3.2	14.1	8.1	18.4	30.4	22.4	0.0	0.8	6.7	13.3	21.1	12.1	4.3	0.2	0.0	0.0	0.0	16.5	18.5	48.8	77.2
52	Female	11.4	6.9	2.8	1.8	1.2	0.4	42.4	44.9	0.0	0.0	3.0	1.9	17.2	14.0	8.7	20.7	0.0	0.0	0.0	0.0	13.5	9.4	77.8	69.9
51	Female	2.4	8.2	1.9	2.1	0.6	0.3	28.7	30.6	0.0	0.3	15.0	11.3	35.7	35.3	1.9	1.9	0.7	0.5	0.0	0.0	13.2	9.6	84.2	87.7
51	Male	9.2	20.1	1.8	1.8	1.3	1.3	35.3	35.5	0.0	0.0	4.5	5.7	28.5	20.6	3.4	1.8	0.3	0.0	0.0	0.7	15.7	12.7	80.5	84.9
63	Male	9.4	0.7	1.3	1.7	0.8	0.0	51.9	56.9	0.0	0.0	0.9	2.5	16.4	28.6	1.6	1.5	0.0	0.0	0.0	0.0	17.7	8.1	80.7	90.5
59	Female	1.8	7.1	1.3	1.4	1.5	0.4	33.6	33.9	0.0	0.0	8.6	9.9	24.6	30.9	4.6	3.1	0.3	0.0	0.0	0.0	23.6	13.4	71.5	83.5
55	Male	10.9	8.8	3.3	2.8	1.2	0.7	32.9	40.8	0.0	0.0	1.5	3.2	29.3	25.6	8.9	9.7	0.0	0.0	0.0	0.0	12.1	8.4	79.0	81.9
51	Female	0.5	0.6	1.7	1.9	4.0	0.5	27.1	37.4	30.4	24.7	1.1	0.8	22.4	23.9	1.5	1.2	0.0	0.0	0.0	0.0	11.3	9.0	56.8	65.1
59	Female	8.0	2.2	2.0	2.0	1.2	1.4	29.1	39.4	5.2	6.5	10.3	11.0	26.9	21.7	2.7	3.0	0.0	0.8	0.3	0.5	14.3	11.5	77.6	77.7
52	Female	3.8	0.2	1.8	2.1	0.6	0.0	36.3	44.1	0.0	0.0	13.0	8.2	29.7	34.5	1.7	1.5	0.7	0.8	0.3	0.0	12.0	8.5	85.2	89.2
57	Female	10.0	8.7	0.8	1.0	1.7	0.2	34.4	41.8	0.0	0.0	6.2	5.8	29.2	29.9	2.9	1.5	0.0	0.0	0.0	0.0	14.7	11.2	82.4	87.3
60	Female	4.9	6.1	2.0	4.6	0.5	1.8	32.1	36.6	0.0	0.0	17.9	16.1	28.9	25.5	4.1	1.3	0.7	0.9	0.0	0.0	8.8	7.1	86.4	90.7

T-RFLP, Terminal restriction fragment length polymorphism analysis; SCFA, short-chain fatty acid.

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