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Effect of apple intake on fecal microbiota and metabolites in humans

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ABSTRACT

The effects of apple intake on the fecal flora, water content, pH, and metabolic activities in eight healthy volunteers and the utilization of apple pectin *in vitro* were investigated. Although several isolates of *Bifidobacterium*, *Lactobacillus*, *Enterococcus*, and the *Bacteroides fragilis* group utilized apple pectin, most isolates of *Escherichia coli*, *Collinsela aerofaciense*, *Eubacterium limosum*, and *Clostridium perfringens* could not. When fecal samples from healthy adults were incubated in liquid broth with apple pectin present or absent, the numbers of *Bifidobacterium* and *Lactobacillus* in the former were higher than those in the later. After the intake of apples (2 apples a day for 2 weeks) by eight healthy adult humans, the number of bifidobacteria in feces increased ($p < 0.05$ on day 7 and $p < 0.01$ on day 14 of the intake period), and the numbers of *Lactobacillus* and *Streptococcus* including *Enterococcus* tended to increase. However, lecithinase-positive clostridia, including *C. perfringens*, decreased ($p < 0.05$), and *Enterobacteriaceae* and *Pseudomonas* tended to decrease. Moreover, the concentrations of fecal acetic acid tended to increase on apple intake. The fecal ammonia concentration showed a tendency to reduce and fecal sulfide decreased ($p < 0.05$) on apple intake. These findings indicate that apple consumption is related to an improved intestinal environment, and apple pectin is one of the effective apple components improving the fecal environment.

1. Introduction

The intestinal flora of an individual is composed of 100 trillion viable bacteria, representing 100 or more different bacterial species. The intestinal microbiota contains a variety of enzymes that perform extremely varied types of metabolism in the intestine. These organisms live together in symbiotic or antagonistic relationships, growing on the food components ingested and biocomponents secreted into the alimentary tract by the host. It has been reported that bifidobacteria and lactobacilli, which are members of the intestinal microbiota, are beneficial bacteria [1]. The intestinal microbiota shows marked stability and constancy, but can be altered by many endogenous and exogenous factors, including the diet [2]. An optimal intestinal microbiota and intestinal environment are possibly achieved by the intake of a nutritionally well-balanced diet and functional foods, such as dietary fiber, oligosaccharides, and fermented milk, which promote beneficial or suppress harmful bacteria [1].

Recently, functional foods have attracted attention in Japan because the incidence of lifestyle-related disease is increasing. These functional foods are classified into 3 groups based on their mechanisms of action: probiotics, prebiotics, and biogenics. Prebiotics are defined as nondigestible food components exhibiting beneficial effects on the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already residing in the colon, and, thus, improve the host's health [3]. The effects of dietary fiber from fruit on intestinal microbiota, however, remain unclear. Therefore, it is important to elucidate the effects of dietary fiber contained in fruit on the intestinal microbiota.

Apples constitute a major proportion of the fruit supply throughout the year in Japan as a result of various factors such as market availability, the diversity of cultivars, and variety of processing forms (fresh fruit, juice, cider, mashed apples). Most of the investigations on the beneficial effects of apples have focused on their lipid-lowering effects [4,5] and anti-oxidative properties [6–8]. It has described that apple pectin, which is dietary fiber contained in apples, might strongly influence the intestinal microbiota because it has a strong bacteriostatic action on *Staphylococcus aureus*, *Streptococcus faecalis*, *Pseudomonas aeruginosa*, and *Escherichia coli* [9].

In the present study, we investigated the utilization of apple pectin by fecal bacteria *in vitro* and the effects of daily apple intake on the fecal microbiota, water content, pH, and metabolic activities in eight healthy volunteers.

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2. Materials and methods

2.1. Investigation of bacterial utilization of apple pectin on the bacteria

2.1.1. Bacterial strains

A total of 55 strains including *Bifidobacterium*, *Lactobacillus*, *Bacteroides*, *E. coli*, *Clostridium*, *Eubacterium*, *Collinsella*, and *Enterococcus* were tested in this study (Table 1). Bacterial strains were obtained from the Japan Collection of Microorganisms (JCM) and American Type Culture Collection (ATCC), and isolated from human feces and foods in our laboratory. Strains we isolated from human feces except for *E. coli* and *C. perfringens* were identified with the DNA–DNA hybridization test or investigation of the 16S rRNA gene. Isolates of *E. coli* and *C. perfringens* were identified by the investigation of phenotypic characters.

2.1.2. Preparation of bacterial culture

The purity of all strains was verified by sub-culturing them anaerobically for 48 h at 37 °C on glucose-liver-blood (BL) agar [10] using AnaeroPack · Anaero (Mitsubishi Gas Chemical Co., Inc., Tokyo, Japan).

The bacteria were pre-cultured in 3 ml of peptone-yeast extract (PY) broth [11] with 4% Fildes peptic digest of horse blood (Fildes solution [12]) (designated as PYF broth) and 0.3% glucose. The inoculated media were incubated anaerobically at 37 °C for 24 h by the gas pack method. After incubation, cultured bacteria were pre-cultured in PYF broth with 0.3% glucose. Three milliliters of PYF broth with 0.5% apple pectin (Sigma Chemical Co., St. Louis, MO, USA) (designated as PYFP broth) and PYF broth were used in this study.

2.1.3. Utilization of apple pectin in vitro

Three milliliters of PYF broth, PYF containing 0.5% glucose (designated as PYFG), and PYFP broth were inoculated with 0.05 ml of the test organisms pre-cultured in PYF broth with 0.3% glucose. The inoculated media were incubated anaerobically at 37 °C for 48 h using AnaeroPack · Anaero. PYF and PYFG broths were used as controls. After incubation, the pH of each medium was measured using a pH meter model, D-25 (Horiba Ltd., Kyoto, Japan), and scored in the following manner: ;,≧pH 6.0; (+), pH 5.5–5.9; +, pH 5.0–5.4; ++, ≦pH 4.9.

2.2. Influence of apple pectin on the growth of fecal bacteria of humans in vitro

Freshly voided fecal samples were collected from two healthy male volunteers. One gram of feces was dissolved in 9 ml of diluent [10] and mixed thoroughly, and then a 10^{-5} dilution was prepared. Subsequently, 0.3 ml of a 10^{-5} dilution was used to inoculate 3 ml of PYF broth and 3 ml of PYFP broth, respectively. The inoculated broth was incubated anaerobically at 37 °C for 48 h using AnaeroPack · Anaero. After incubation, bacterial analysis was carried out using the methods and media of Mitsuoka et al. [10,13] and described previously [14], and the heat treatment method of Terada et al. [15]. One milliliter of cultured broth was dissolved in 9 ml of diluent [10] and mixed thoroughly, a series of 10-fold dilutions (10^{-1}–10^{-8}) was prepared, and 0.05 ml aliquots were spread onto three non-selective agars: modified Eggerth–Gagnon (EG) agar for anaerobes [10]; BL agar for anaerobes; and trypticase soy blood (TS) agar (BBL Microbiology System, Cockeysville, Md., USA) for aerobes [13], as well as eleven selective agars: bifidobacteria selective (BS) agar for bifidobacteria [10]; eubacteria selective (ES) agar for eubacteria [13]; neomycin-brilliant green-taurocholate-blood (NBGT) agar for bacteroides [10]; neomycin-Nagler (NN) agar for lecithinase-positive clostridia [10]; modified veillonella selective (VS) agar for veillonellae and megasphaerae [10]; modified lactobacilli selective (LBS) agar for lactobacilli [10], triphenyltetrazolium chloride–acridine orange-thallous sulfate-aesculin-crystal violet (TATAC) agar for enterococci and streptococci [10]; phenylethyl alcohol egg yolk suspension (PEES) agar for staphylococci and micrococci [10]; potato dextrose (P) agar for yeasts and molds [10]; deoxycholate hydrogen sulfide lactose (DHL) agar (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) for enterobacteria [10]; and NAC agar (Nissui) for *P. aeruginosa*. Eight agars (EG, BL, NBGT, BS, ES, NN, VS, and LBS) were incubated at 37 °C for 3 days in an anaerobic steel wool jar filled with an atmosphere of oxygen-free CO_2. In addition, diluents (10^{-1}, 10^{-3}, and 10^{-5}) of the fecal specimens were heated at 80 °C for 10 min to select clostridial spores, 0.05 ml of each dilution was used to inoculate CW agar (Nissui) containing egg yolk emulsion, and this was incubated at 37 °C for 3 days anaerobically using an AnaeroPack · Anaero. Four agars (TATAC, PEES, NAC, and P) were incubated aerobically at 37 °C for 48 h, and TS and DHL agars were incubated at 37 °C for 24 h. Then, dilutions of the fecal specimens were heated at 80 °C for 10 min to select clostridial spores, 0.05 ml of each dilution (10^{-1}–10^{-5}) was used to inoculate CW agar containing egg yolk emulsion, and this was incubated at 37 °C for 3 days anaerobically using AnaeroPack · Anaero. After incubation, each plate was examined for bacterial colonies. The identification of bacterial groups was performed by examining the gram reaction, colonial and cellular morphologies, spore formation, aerobic growth, and selected biochemical characteristics. The bacterial count per ml of broth was calculated and converted to a logarithmic equivalent.

2.3. Influence of apple on the fecal microbiota and fecal physiological properties of humans

2.3.1. Subjects and diet

Eight subjects were chosen from healthy male volunteers (Laboratory of Food Hygiene, Nippon Veterinary and Life Science University, Tokyo, Japan), ranging in age from 21 to 60 years. They consumed a normal non-restricted diet for 1 week before and after the experiment. In addition to their free-choice diet, the volunteers consumed 2 apples/day for 2 weeks during the experiment. The experimental design is shown in Table 2. None of the subjects received medications or foods with abundant viable cultures 4 weeks prior to and during the experiment. Apples were purchased from retail stores in Japan.

This study was performed in accordance with the Helsinki Declaration as updated in Tokyo, 1975.

2.3.2. Collection of specimens

Freshly voided fecal specimens were collected on day 0 (end of control diet period before apple consumption (the normal free-choice diet), days 7 and 14 during consumption, and day 7 after consumption. The specimens were immediately transported at 4 °C to the laboratory for analysis. The fecal flora, moisture content, and pH were analyzed within 3 h. The remainder of samples was frozen at −80 °C for later analysis of metabolites.

2.3.3. Analysis of fecal microbiota

The fecal microbiota were analyzed using the methods and media of Mitsuoka et al. [10,13] and described previously [14], and the heat treatment method of Terada et al. [15]. One gram of feces was dissolved in 9 ml of diluent [13] and mixed thoroughly, a series of 10-fold dilutions (10^{-1}–10^{-8}) was prepared, and 0.05 ml of each dilution was used to inoculate four non-selective media (EG, BL, TS, and modified Medium 10 [13]) and 11 selective media (NBGT, BS, ES, NN, VS, LBS, TATAC, PEES, NAC, DHL, and P). Media except for modified Medium 10 were incubated as described above, and

Table 1
Utilization of apple pectin by various bacteria.[a]

Organism	Control	Glucose	Apple pectin	Isolated from	Obtained from
Bifidobacterium					
B. longum JCM1217^T	−	++	(+)	Intestine of adult	JCM
B. longum 120	−	++	(+)	Feces of adult	Own isolate
B. bifidum JCM1255^T	−	++	−	Feces of infant	JCM
B. breve JCM1192^T	−	++	−	Intestine of infant	JCM
B. breve 33	−	++	+	Feces of adult	Own isolate
B. breve 103	−	++	+	Feces of adult	Own isolate
B. breve 108	−	++	+	Feces of adult	Own isolate
B. breve 138	−	++	(+)	Feces of adult	Own isolate
B. adolescentis JCM1275^T	−	++	−	Intestine of adult	JCM
B. adolescentis 113	−	++	(+)	Feces of adult	Own isolate
B. adolescentis S-10	−	++	(+)	Feces of adult	Own isolate
B. adolescentis F-10	−	++	−	Feces of adult	Own isolate
B. catenulatum JCM1194^T	−	++	−	Human feces	JCM
B. pseudocatenulatum JCM1200^T	−	++	(+)	Feces of infant	JCM
B. pseudocatenulatum 126	−	++	(+)	Feces of adult	Own isolate
B. pseudocatenulatum F-11	−	++	−	Feces of adult	Own isolate
Lactobacillus					
L. gasseri JCM1131^T	−	++	+	Human intestine	JCM
L. gasseri L-3	−	++	+	Food	Own isolate
L. gasseri F191	−	++	+	Feces of adult	Own isolate
L. johnsonii ATCC332	−	++	+		ATCC
L. salivarius subsp. *salivarius* JCM1231^T	−	++	+	Human saliva	JCM
L. casei L-4	−	++	+	Food	Own isolate
L. casei L- 1	−	++	+	Food	Own isolate
L. plantarum L-2	−	++	+	Food	Own isolate
L. crispatus F199	−	++	+	Feces of adult	Own isolate
L. ruminis B-2-34	−	++	(+)	Feces of adult	Own isolate
Bacteroides					
B. fragilis ATCC25285^T	−	+	(+)	Appendix abscess	ATCC
B. uniformis ATCC8492^T	−	+	(+)		ATCC
B. uniformis F-4	−	+	(+)	Feces of adult	Own isolate
B. vulgatus ATCC8482^T	−	+	(+)		ATCC
B. vulgatus S-18	−	++	++	Feces of adult	Own isolate
B. ovatus S-17	−	++	++	Feces of adult	Own isolate
Clostridium					
C. perfringens U-1	−	+	−	Feces of adult	Own isolate
C. perfringens S-79	−	+	−	Feces of adult	Own isolate
C. perfringens NC	−	+	−	Feces of adult	Own isolate
C. innocuum C-1	−	++	(+)	Feces of adult	Own isolate
Eubacterium					
E. limosum E−1	−	++	−	Feces of adult	Own isolate
Collinsella					
C. aerofaciens A-1	−	++	−	Feces of adult	Own isolate
Escherichia					
E. coli ATCC8739	−	++	(+)	Feces	ATCC
E. coli ATCC25922	−	++	−	Clinical isolate	ATCC
E. coli ATCC11775^T	−	++	−	Urine	ATCC
E. coli N-I	−	(+)	−	Feces of adult	Own isolate
E. coli 5	−	++	−	Feces of adult	Own isolate
E. coli 123	−	++	(+)	Feces of adult	Own isolate
E. coli M-1	−	+	−	Feces of adult	Own isolate
Enterococcus					
E. faecalis ATCC29212	−	++	(+)	Urine	ATCC
E. faecalis 1-2	−	++	(+)	Feces of adult	Own isolate
E. faecalis SE-1	−	++	(+)	Feces of adult	Own isolate
E. faecalis SE-2	−	++	(+)	Feces of adult	Own isolate
E. faecalis SE-4	−	++	(+)	Feces of adult	Own isolate
E. faecalis SE-5	−	++	(+)	Feces of adult	Own isolate
E. faecalis SE-6	−	++	(+)	Feces of adult	Own isolate
E. faecalis SE-7	−	++	(+)	Feces of adult	Own isolate
E. faecalis SE-8	−	++	(+)	Feces of adult	Own isolate
E. faecium SF-1	−	++	(+)	Feces of adult	Own isolate

[a] Judgement of bacterial growth: −:pH ≧ 6.0, (+):5.5 ≦ pH ≦ 5.9, +:5.0 ≦ pH ≦ 5.4, ++:pH ≦ 4.9. JCM: Japan Collection of Microorganisms. ATCC: American Type Culture Collection.

modified Medium 10 was incubated anaerobically by the plate-in-bottle method [16] at 37 °C for 3 days. Furthermore, dilutions of the fecal specimens were also heated and incubated using CW agar containing egg yolk emulsion for the isolation of clostridia, as described above. After incubation, each plate was examined for bacterial colonies. The identification of bacterial groups, yeasts, and molds was performed by examining the gram reaction, colonial and cellular morphologies, spore formation, aerobic growth, and

Table 2
Experimental schedule.

Experimental period Weeks	Before intake ← 1 →	During intake ← 2 →		After intake ← 1 →
Sampling day	0	7	14	7
Items investigated:				
Fecal microbiota	○[a]	○	○	○
Fecal short-chain fatty acids	○	○	○	○
Fecal ammonia	○	○	○	○
Fecal sulfide	○	○	○	○
Fecal pH	○	○	○	○
Fecal water content	○	○	○	○
Fecal hardness	○	○	○	○

[a] ○: Investigation was performed.

Table 3
Comparison of the numbers of fecal bacteria in the incubation of human feces using PYF broth with and PYF broth without apple pectin.[a]

Fecal sample from	Subject A		Subject B	
	PYF broth	PYFP broth	PYF broth	PYFP broth
Bacteroidaceae	8.76	8.34	8.35	8.70
Eubacteria	7.78	7.30	6.53	5.60
Bifidobacteria	5.30	5.87	4.89	6.30
Anaerobic gram-positive cocci	ND	ND	ND	6.78
Lactobacilli	3.31	6.30	ND	ND
Enterobacteriaceae	8.41	8.15	8.43	8.26
Streptococci	7.60	7.30	7.60	7.60

[a] Data are expressed the as $\log_{10}$ number per ml of broth. PYF broth: Peptone-yeast extract containing 0.4% of Fildes solution broth. PYFP broth: PYF broth with 0.5% apple pectin, ND: Not detected.

selected biochemical characteristics. The bacterial count per gram wet weight of fecal material was calculated and converted to a logarithmic equivalent.

2.3.4. Measurement of fecal pH and water content

Fecal pH values were measured with a pH meter, model D-25 (Horiba). The fecal water content was determined using 1-g samples, which were weighed before and after drying in a vacuum oven at 105 °C by an infrared moisture gauge of the FD-240 type (Ketto Science Lab., Tokyo, Japan).

2.3.5. Analysis of fecal metabolites

Fecal levels of short-chain fatty acids (succinic, lactic, formic, acetic, propionic, iso-butyric, butyric, iso-valeric, and valeric) were analyzed using a high-performance liquid chromatography organic acid analysis system (HPLCOA, Shimadzu Co., Ltd., Kyoto, Japan) following the methods of Hara et al. [17].

Fecal concentrations of ammonia and sulfide were determined by employing an ion meter IM-55G (DKK · TOA Co., Ltd., Tokyo, Japan) with the ammonia ion selective electrode AE-2041 (DKK · TOA Co., Ltd.) and sulfide electrode S-2021 (DKK · TOA Co., Ltd.), respectively, as described previously [14].

2.3.6. Statistical analysis

Student's *t*-test was used for statistical analysis.

3. Results

3.1. Utilization of apple pectin by isolates

The results of the *in vitro* utilization test of apple pectin are shown in Table 1. Apple pectin was utilized by several bifidobacterial isolates, and all lactobacilli, enterococci, *Bacteroidaceae* and *C. innocuum* tested. Other bacteria, such as *C. perfringens*, *E. limosum*, *C. aerofaciens*, and most isolates of *E. coli* did not utilize it.

3.2. Influence of apple pectin on the growth of fecal bacteria of humans in vitro

Fecal samples from healthy adults were incubated in PYFP and PYF broths, respectively. The numbers of *Bifidobacterium* and *Lactobacillus* in the former were higher than those in the latter, as shown in Table 3.

3.3. Influence of apple intake on human fecal microbiota

The effects of apple intake on the composition of fecal microbiota in the eight volunteers are shown in Table 4. The number of bifidobacteria ($p < 0.05$ on day 7 and $p < 0.01$ on day 14) were significantly increased during apple intake, while the number of lecithinase-positive clostridia, including *C. perfringens*, was significantly decreased ($p < 0.05$) on day 14 of apple intake. The numbers of lactobacilli and streptococci including enterococci tended to increase during apple intake, and *Enterobacteriaceae* were significantly decreased ($p < 0.05$) on day 14 of intake. The number of *Pseudomonas* tended to decrease on day 14 of apple intake.

3.4. Influence of apple intake on fecal water content, pH, and metabolites in humans

Fecal short-chain fatty acids tended to increase with the apple intake period, as shown in Table 5. Acetic acid was increased on days 7 and 14 of the intake period, although there was no significant difference. No marked changes were noted in other fecal short-chain fatty acids during intake.

Changes in the concentrations of fecal ammonia, sulfide, the pH value, and moisture are also shown in Table 5. Fecal contents of ammonia and sulfide were decreased with apple intake, and this was particularly marked on day 14 of the intake period. Fecal contents of sulfide and ammonia were decreased significantly ($p < 0.05$) on day 14 of intake. There were no marked changes in fecal moisture and fecal pH throughout the experiment.

4. Discussion

It is known that intestinal bacteria play an important role in host health [1,9]. It has also been reported that an increase of *Bifidobacterium* in the intestinal flora brings about beneficial effects on the host [1], and so it has been used as a probiotic [18]. *Bifidobacterium* is a predominant member of the intestinal microbiota of humans [1]. Pectin is fermented by *Bacteroides* and *Eubacterium eligens*, but not *Bifidobacterium in vitro* [19,20]. Suzuki et al. [9] studied intestinal bacterial utilization of arabino-oligosaccharides derived from arabinan, a component of apple pectin. As a result, it was reported that only *Bifidobacterium adolescentis*, *Bifidobacterium longum*, and *Bacteroides vulgatus* utilized the arabino-oligosaccharides, and *C. perfringens* did not. Although feeding high-methoxylated pectin to rats resulted in an increase of fecal *Bacteroides* [21] and feeding 5% pectin to rats led to a decrease of cecal lactobacilli and streptococci [22], human ingestion of 35 g of pectin per day for more than 2 weeks [23] did not affect the intestinal microbiota. One of the reasons why the effects of pectin on intestinal microbiota are not clear is that the composition and structure of pectin vary depending on its source [9]. Our results showed that apple pectin was utilized by several isolates of *Bifidobacterium*. The results obtained from the present study indicate that apple pectin was used by *Bifidobacterium*, *Bacteroides*, *Lactobacillus*, and *Enterococcus* in the intestine. In this examination, in which fecal samples from healthy adults were incubated in liquid broth with and without

Table 4
Effect of apple intake on fecal microbiota in eight human subjects.[a]

Organism	Before intake	During intake		After intake
	Day 0	Day 7	Day 14	Day 7
Total bacteria	10.89 ± 0.15	10.83 ± 0.17	10.93 ± 0.11	10.71 ± 0.22
Bifidobacterium	10.17 ± 0.12 (100)	10.31 ± 0.10* (100)	10.42 ± 0.13** (100)	10.12 ± 0.23 (100)
Bacteroidaceae	10.77 ± 0.21 (100)	10.62 ± 0.22 (100)	10.69 ± 0.14 (100)	10.56 ± 0.31 (100)
Eubacterium	9.41 ± 0.49 (100)	9.28 ± 0.57 (100)	9.47 ± 0.47 (100)	9.50 ± 0.20 (100)
Anaerobic gram-positive cocci	9.36 ± 0.32 (100)	9.25 ± 0.38 (100)	9.35 ± 0.43 (100)	9.34 ± 0.38 (100)
Veillonellae	7.13 ± 0.69 (38)	6.88 ± 0.43 (38)	7.07 (25)	7.28 ± 0.62 (50)
Clostridium				
Lecithinase-positive	3.90 ± 0.87 (88)	3.57 ± 1.11 (63)	2.50 ± 0.28* (38)	3.51 ± 0.98 (63)
Lecithinase-negative	8.65 ± 0.55 (100)	8.45 ± 0.28 (70)	8.27 ± 0.29 (50)	8.74 ± 0.63 (100)
Lactobacillus	6.88 ± 1.54 (100)	7.59 ± 1.75 (88)	7.38 ± 1.29 (100)	8.74 ± 0.63 (100)
Enterobacteriaceae	8.33 ± 0.77 (100)	7.71 ± 0.97 (100)	7.35 ± 0.71* (100)	7.98 ± 0.61 (100)
Streptococcus and *Enterococcus*	7.29 ± 1.14 (100)	8.08 ± 0.78 (100)	8.11 ± 0.62 (100)	7.68 ± 1.03 (100)
Staphylococcus	3.26 ± 0.75 (63)	2.98 ± 0.36 (50)	3.20 ± 0.60 (50)	3.16 ± 0.53 (75)
Bacilli	2.73 ± 0.19 (38)	2.84 ± 0.24 (50)	2.73 ± 0.31 (38)	3.43 ± 1.21 (50)
Pseudomonas	3.84 ± 0.99 (75)	3.00 ± 0.47 (50)	2.66 ± 0.26 (38)	3.28 ± 0.38 (75)
Yeasts	4.38 ± 0.89 (50)	3.80 ± 1.13 (88)	3.42 ± 0.75 (88)	4.35 ± 0.93 (63)

*Significantly different ($p < 0.05$) from the value on day 0 (before intake). ** Significantly different ($p < 0.01$) from the value on day 0 (before intake).
[a] Values are expressed as the mean of the $\log_{10}$ number ±SD (CFU)/g wet feces. Figures in parentheses are frequency of occurrence (%).

apple pectin, the numbers of *Bifidobacterium* and *Lactobacillus* in the former were higher than those in the latter. Apple pectin evades digestion by intestinal enzymes. Thus, it is not absorbed in the small intestine, and passes directly into the colon where it is metabolized by apple pectin-utilizing bacteria such as bifidobacteria, which produce short-chain fatty acids.

The numbers of *Bifidobacterium* increased significantly, and *Lactobacillus* and *Streptococcus* including *Enterococcus* tended to increase during apple intake in the examination of fecal microbiota using human volunteers. Since these bacteria utilized apple pectin, it is suggested that these effects are related to the presence of pectin. Suzuki et al. [9] reported that the beneficial effects of the daily consumption of apple on intestinal microbiota are principally due to its pectin content. Incidentally, the dietary fiber content of apples is relatively low (2–3 g/100 g) and soluble fibers such as pectin represent less than 50% of this fiber [5]. Further studies on the effect of apple pectin involving a large number of human volunteers are necessary to confirm that the beneficial effect of apple consumption is related to the presence of pectin. Simultaneously, the search for ingredients other than pectin to improve the intestinal environment is also needed. *Bacteroides* did not increase during apple intake, although it utilized apple pectin. It seems that the increase of intestinal short-chain fatty acids brought about by the increase in the numbers of *Bifidobacterium* and *Lactobacillus* is related to inhibition of the growth of *Bacteroides*. However, the specific reason is not clear.

Table 5
Effect of apple intake on fecal properties in eight humans.[a]

Item	Before intake	During intake		After intake
	Day 0	Day 7	Day 14	Day 7
pH	5.9 ± 0.5	5.9 ± 0.4	5.8 ± 0.3	5.8 ± 0.4
Water content (%)	75.9 ± 4.6	77.3 ± 5.5	76.8 ± 6.4	76.0 ± 3.5
Short-chain fatty acids				
Succinic acid (mg/g wet feces)	2.4 ± 2.8	3.1 ± 3.0	3.1 ± 4.3	2.9 ± 3.1
Lactic acid (mg/g wet feces)	2.1 ± 3.1	2.2 ± 2.6	1.8 ± 3.2	2.3 ± 1.9
Formic acid (mg/g wet feces)	1.0 ± 1.3	1.5 ± 1.6	0.8 ± 1.3	1.1 ± 0.9
Acetic acid (mg/g wet feces)	6.1 ± 1.5	7.7 ± 2.6	7.5 ± 1.6	7.0 ± 2.2
Propionic acid (mg/g wet feces)	3.2 ± 1.4	2.7 ± 1.6	3.0 ± 1.0	2.5 ± 1.0
Iso-butyric acid (mg/g wet feces)	0.3 ± 0.1	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2
Butyric acid (mg/g wet feces)	2.2 ± 1.2	1.7 ± 0.3	2.1 ± 0.4	2.2 ± 1.6
Iso-valeric acid (mg/g wet feces)	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	0.2 ± 0.1
Valeric acid (mg/g wet feces)	0.4 ± 0.3	0.5 ± 0.1	0.5 ± 0.3	0.6 ± 0.5
Ammonia (μg/g wet feces)	666.5 ± 134.2	539.9 ± 130.7	518.9 ± 88.0*	670.5 ± 81.0
Sulfide (μg/g wet feces)	4.9 ± 2.3	3.4 ± 2.0	2.3 ± 1.1*	3.1 ± 1.8

* Significant difference from the value on day 0 (before intake) at $p < 0.05$.
[a] Values are expressed as mean ± SD.

The putrefactive products in the human intestine are mainly composed of harmful substances [1] such as ammonia, sulfide, indole, and phenols produced by intestinal bacteria. It has been described that *p*-cresol, containing some phenol and 4-methylphenol groups, exhibits a tumor-promoting activity, and high concentrations of ammonia may be associated with certain toxic events *in vivo* [24]. The fermentation of apple pectin resulted in greater short-chain fatty acid production than did fermentation of either oat fiber or corn bran by human fecal bacteria *in vitro* [25]. In the present study, fecal acetic acid increased while ammonia and sulfide decreased during intake.

The results of this study suggest that the regular consumption of apple, one of the most popular fruits in the world, improves the intestinal environment and has beneficial effects.

References

[1] Mitsuoka T. Significance of dietary modulation of intestinal flora and intestinal environment. Biosci Microflora 2000;19:15–25.
[2] Mitsuoka T. Recent trends in research on intestinal flora. Bifidobacteria Microflora 1982;1:3–24.
[3] Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 1995;125:1401–12.
[4] Sable-Amplis R, Sicart R, Abadie D. Metabolic changes associated with adding apples to the diet in golden hamsters. Nutr Rep Int 1979;19:723–32.
[5] Aprikian O, Levrat-Verny MA, Besson C, Busserolles J, Rémésy C, Demigné C. Apple favorably affects parameters of cholesterol metabolism and of antioxidative protection in cholesterol-fed rats. Food Chem 2001;75:445–52.
[6] van der Sluis AA, Dekker M, Jongen WMF. Flavonoids as bioactive components in apple products. Cancer Lett 1997;114:107–8.
[7] Pearson DA, Tan CH, German JB, Davis PA, Gershwin ME. Apple juice inhibits human low density lipoprotein oxidation. Life Sci 1999;64:1913–20.
[8] Eberhardt MV, Lee CY, Liu RH. Antioxidant activity of fresh apples. Nature 2000;405:903–4.
[9] Suzuki Y, Tanaka K, Amano T, Asakura T, Muramatsu N. Utilization by intestinal bacteria and digestibility of arabino-oligosaccharides *in vitro*. J Japan Soc Hort Sci 2004;73:574–9.

[10] Mitsuoka T, Sega T, Yamamoto S. Eine verbesserte Methodik der qualitativen und quantitativen Analyse der Darmflora von Menschen und Tieren. Zentralbl Bakteriol Hyg Orig 1965;195:455–69.
[11] Holdeman LV, Cato EP, Moore WEC, editors. Anaerobe laboratory manual. 4th ed. Blacksburg: Virginia Polytechnic Institute and State University; 1977.
[12] Fildes P. New medium for the growth of *B. influenza*. Br J Exp Pathol 1920;1:129–30.
[13] Mitsuoka T, Ohno K, Benno Y, Suzuki K, Namba K. Die Faekalflora bei Menschen. IV. Mitteilung: Vergleich des neu entwickelten Verfahrens mit dem bisherigen üblichen Verfahren zur Darmfloraanalyse. Zentralbl Bakteriol Hyg Abt Orig 1976;A234:219–33.
[14] Fujisawa T, Shinohara K, Kishimoto Y, Terada A. Effect of miso soup containing Natto on the composition and metabolic activity of the human faecal flora. Microb Ecol Health Dis 2006;18:79–84.
[15] Terada A, Hara H, Ikegame K, Sasaki M, Mitsuoka T. Recommended method for enumeration of lecithinase-positive clostridia in human feces. Bifidobacteria Microflora 1994;13:27–30.
[16] Mitsuoka T, Morishita Y, Terada A, Yamamoto S. A simple method ("plate-in-bottle method") for the cultivation of fastidious anaerobes. Jpn J Microbiol 1969;13:383–5.
[17] Hara H, Li ST, Sasaki M, Maruyama T, Terada A, Ogata Y, et al. Effective dose of lactosucrose on fecal flora and fecal metabolites of humans. Bifidobacteria Microflora 1994;13:51–63.
[18] Fuller R. Probiotics in man and animals. J Appl Bacteriol 1989;66:365–78.
[19] Salyers AA, Vercellotti JR, West SEH, Wilkins TD. Fermentation of mucins and plant polysaccharides by strains of *Bacteroides* from the human colon. Appl Environ Microbiol 1977;33:319–22.
[20] Salyers AA, West SEH, Vercellotti JR, Wilkins TD. Fermentation of mucins and plant polysaccharides by anaerobic bacteria from human colon. Appl Environ Microbiol 1977;34:529–33.
[21] Aoe S, Ohta F, Ayano Y. Effect of water-soluble dietary fiber on intestinal microflora in rats. J Japan Soc Nutr Food Sci 1988;41:203–11 [in Japanese with English summary].
[22] Wise A, Mallett AK, Rowland IR. Dietary fiber, bacterial metabolism and toxicity of nitrate in the rat. Xenobiotica 1982;12:111–8.
[23] Drasar BS, Jenkins DJA. Bacteria, diet, and large bowel cancer. Am J Clin Nutr 1976;29:1410–6.
[24] Rowland IR, Mallett AK, Wise A. The effect of diet on the mammalian gut flora and its metabolic activities. CRC Crit Rev Toxicol 1985;16:31–103.
[25] Titgemeyer EC, Bourquin LD, Fahey Jr GC, Garleb KA. Fermentability of various fiber sources by human fecal bacteria in vitro. Am J Clin Nutr 1991;53:1418–24.