Bioscience of Microbiota, Food and Health Vol. 40 (4), 168–175, 2021

Full Paper

The composition of the human fecal microbiota might be significantly associated with fecal SCFA levels under hyperbaric conditions

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Received August 8, 2020; Accepted March 18, 2021; Published online in J-STAGE April 3, 2021

The fecal microbiota and short-chain fatty acids (SCFAs) play important roles in the human body. This study examined how hyperbaric conditions affect the fecal microbiota and fecal SCFAs. Fecal samples were obtained from 12 divers at three points during deep-diving training (before the diving training, at 2.1 MPa, and after decompression). At 2.1 MPa, the changes in the frequency of *Clostridium* cluster IV and fecal iso-valerate levels were positively correlated, and the changes in the frequencies of *Bacteroides* and *Clostridium* subcluster XIVa were inversely correlated. After decompression, positive correlations were detected between the changes in the frequency of *Bifidobacterium* and fecal n-valerate levels and between the changes in the fecal levels of iso-butyrate and iso-valerate. On the other hand, inverse correlations were detected between the changes in the frequency of *Clostridium* cluster IX and fecal iso-butyrate levels, between the changes in the frequency of *Clostridium* cluster IX and fecal iso-valerate levels, and between the changes in the frequencies of *Bacteroides* and *Clostridium* cluster IV plus subcluster XIVa. During the study period, the changes in fecal iso-butyrate and iso-valerate levels were positively correlated, and inverse correlations were seen between the changes in the frequency of *Clostridium* cluster IV and fecal propionate levels and between the changes in the frequencies of *Prevotella* and *Clostridium* subcluster XIVa. These findings suggest that hyperbaric conditions affect the fecal microbiota and fecal SCFA levels and that intestinal conditions reversibly deteriorate under hyperbaric conditions.

Key words: short-chain fatty acid (SCFA), *Bifidobacterium*, *Lactobacillales*, *Clostridium* cluster, intestinal immunity, saturation diving

INTRODUCTION

Hyperbaric environments have various effects on the human body and are employed in the medical setting. The fecal microbiota is considered to play physiologically and pathologically important roles in the human body and is affected by various stressors, such as hyperbaric conditions or nervous/emotional stress [1–3]. We previously investigated the changes in the fecal microbiota that occur under hyperbaric conditions, although the factors associated with such changes could not be examined [4]. Short-chain fatty acids (SCFAs), which have various effects on the intestines, are produced as metabolites by intestinal bacteria [5]. Therefore, it is hypothesized that an association might exist between SCFA levels and the composition of the intestinal microbiota.

At the Maritime Self-Defense Force (MSDF) Undersea Medical Center (UMC), we regularly perform diving training under hyperbaric conditions using a deep-diving simulator. Deep-diving training is often called saturation diving, a diving technique that allows divers to remain safe underwater and under hyperbaric conditions for long periods of time [6]. During such training, the maximum pressure in the present training environment is 2.1 MPa, and the divers stay in a module for about two weeks. We collected feces from saturation divers at 0.1 MPa and 2.1 MPa and analyzed the microbiota and SCFAs in the samples. Hyperbaric condition-induced changes of intestinal microbiota and SCFAs can possibly alter the performance levels of the divers.

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doi: 10.12938/bmfh.2020-054

Table 1. Background data of the subjects

Subjects	Age	Sex	Height (cm)	Weight (kg)	BMI	Body fat (%)
A	30	Male	171	68	23.3	9.7
B	26	Male	168	89	31.5	13.5
C	40	Male	170	74	25.6	18.6
D	42	Male	168	68	24.1	24.0
E	27	Male	172	66	22.3	15.0
F	37	Male	163	68	25.6	10.8
G	34	Male	166	77	27.9	20.0
H	39	Male	178	68	21.5	22.0
I	43	Male	166	64	23.2	9.0
J	35	Male	177	71	22.7	10.0
K	25	Male	168	89	31.5	13.5
L	48	Male	171	73	25.0	27.0

Twelve healthy male Maritime Self-Defense Force (MSDF) divers (subjects A–L; age, 25 to 48 years old; mean age ± standard deviation, 35.5 ± 7.0; mean body fat percentage ± standard deviation, 16.1 ± 5.8; well trained and muscular) undergoing saturation diving training participated in this study. During this study, all of the subjects received the same food, and none of them received any medication.

Deep-diving training is performed annually at the UMC, and this provides a good opportunity to investigate the effects of hyperbaric environments on human performance. While there are some limitations to the medical experiments that can be performed during such training, it offers a chance to obtain precious data that cannot be acquired at many institutions. Such data may contribute to studies on human performance in special environments, such as salvage operations, pneumatic caisson construction, and aerospace activities. Thus, in this study, we attempted to investigate how a hyperbaric environment affects both the fecal microbiota and fecal SCFA levels.

MATERIALS AND METHODS

Fecal specimens

Twelve healthy male MSDF divers (subjects A–L; age, 25 to 48 years old; mean age ± standard deviation (SD), 35.5 ± 7.0; mean body fat percentage ± SD, 16.1 ± 5.8%) undergoing saturation diving training participated in this study. The profiles of the subjects are shown in Table 1. They were selected for saturation diving training because they were well trained and muscular. This was the first time that the subjects had participated in such a study. None of the subjects regularly used any drugs or had been diagnosed with a digestive disease. All of the subjects stayed in the deep-diving module during the training period. The environmental pressure was gradually pressurized to 2.1 MPa. The subjects stayed in the 2.1-MPa environment for several days, during which they took excursions and performed various measurements. The excursions involved moving to an inundated area of the deep-diving module and performing various tasks, for example assembling a sign. A decompression period of almost one week was used to prevent decompression sickness. Fecal sample collection was conducted before the training (before), on day 4 (at 2.1 MPa), and on the last day of the training (after decompression). The subjects underwent medical checkups every day, and their health, including abdominal symptoms and stool conditions, was assessed. During this study, all of the subjects received the same food, and none of them received any medication. The energy profile of their diet was as follows: 63% carbohydrates, 14% protein, and 23% lipids. Their diet also contained almost 20 g of fiber. Therefore, all of the subjects ate the same amount of food during the training period, and their diets were identical. The study protocol was approved by the ethics committee of the Japan Self-Defense Force Hospital Yokosuka. Written informed consent was obtained from all subjects.

Analysis of the fecal microbiota and SCFAs

The subjects' fecal microbiotas were analyzed using terminal restriction fragment length polymorphism (T-RFLP) analysis, and their fecal SCFA levels were analyzed using gas chromatography with a flame ionization detector (GC-FID). The analyses were performed at TechnoSuruga Laboratory. The Friedman test with Bonferroni's correction was used for pairwise comparisons of the changes in the levels of each SCFA among the first (before training), second (at 2.1 MPa), and third (after decompression) samples.

The correlations between the changes in the frequency of each fecal microorganism, the correlations between the changes in the fecal levels of each SCFA, and the correlations between the changes in the frequency of each type of bacteria and the fecal levels of each SCFA were investigated using Fisher's exact test after correcting for multiple comparisons (Bonferroni's correction). The Friedman test with Bonferroni's correction was used for pairwise comparisons of the frequencies of each fecal microorganism and the levels of each SCFA between the first (before training), second (at 2.1 MPa), and third (after decompression) samples. Corrected p-values of <0.05 were considered to indicate statistically significant results.

RESULTS

Changes in the type of bacterial species in the fecal microbiota

No abdominal pain was observed during the training period in any subject, but subject E developed diarrhea at 2.1 MPa. The frequencies of each type of bacterial species in subjects A–L are shown in Fig. 1. The compositional changes in the fecal microbiota that occurred during the study period exhibited interindividual variability. When the samples that were collected at 2.1 MPa were compared with those collected before the training, it was found that the frequencies of *Bifidobacterium* and

doi: 10.12938/bmfh.2020-054

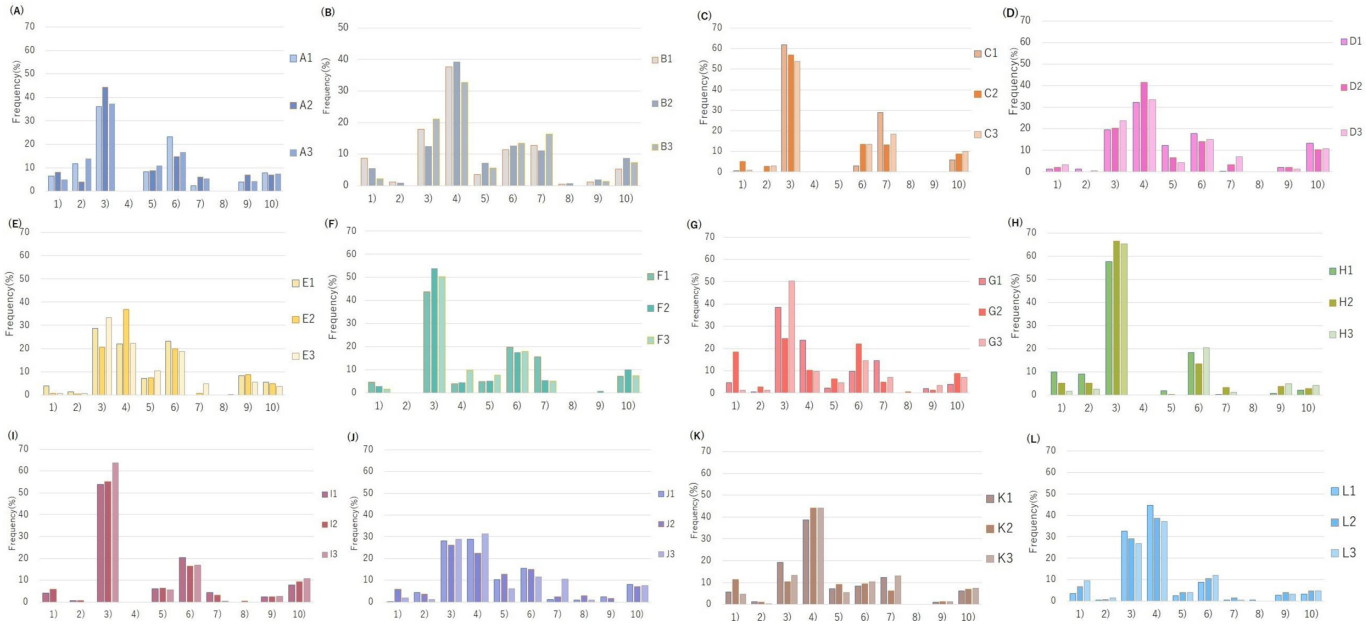

Fig. 1. The frequencies of each type of bacterial species in subjects A–L.

The left, middle, and right bars indicate the frequencies of each type of bacterial species before the training, at 2.1 MPa, and at the end of the training (after decompression), respectively.

(A1–L1, before the training; A2–L2, at 2.1MPa; A3-L3, after decompression)

1) *Bifidobacterium*, 2) *Lactobacillales*, 3) *Bacteroides,* 4) *Prevotella,* 5) *Clostridium* cluster IV, 6) *Clostridium* subcluster XIV, 7) *Clostridium* cluster IX, 8) *Clostridium* cluster XI, 9) *Clostridium* cluster XVIII, and 10) others.

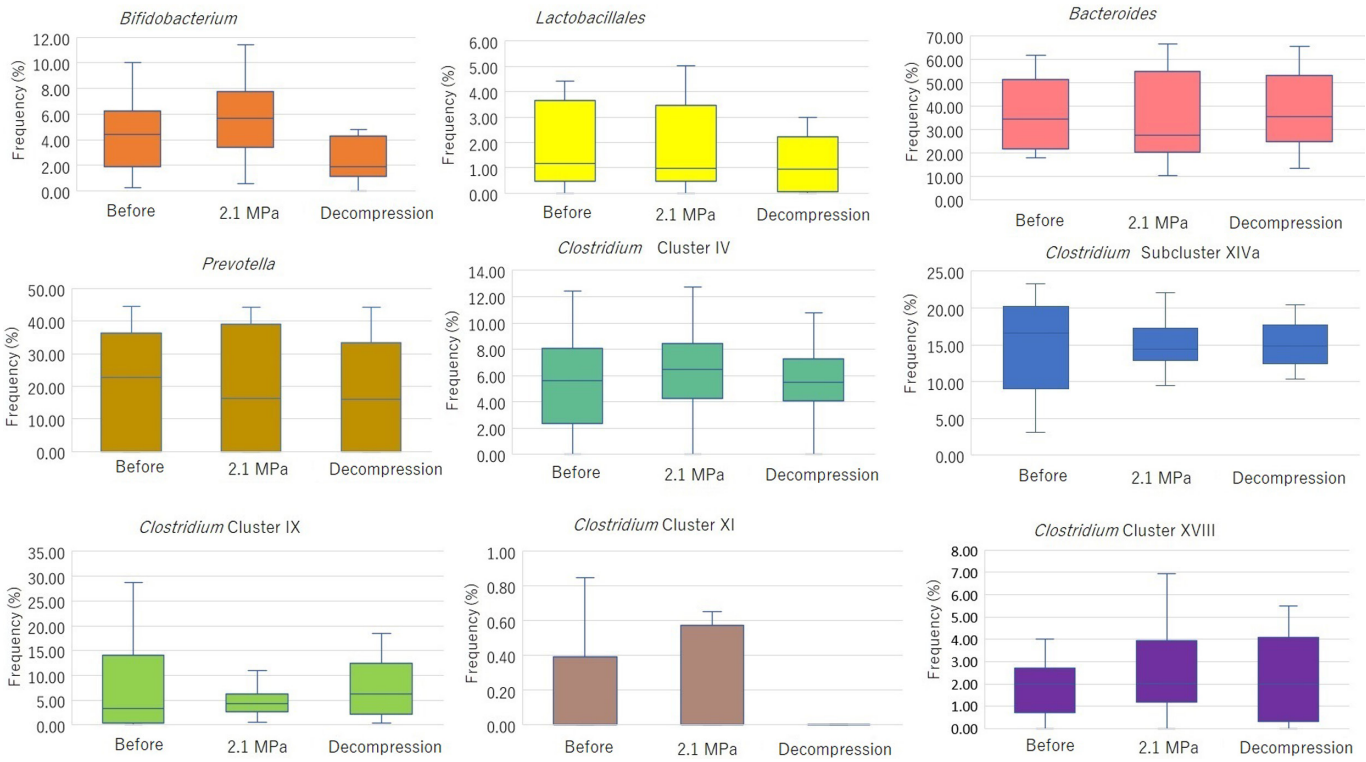

Fig. 2. Changes of the proportions of each fecal microbiota in all subjects at the three sample collection points. Each box-and-whisker plot contains the lower quartile (Q1/4), median, upper quartile (Q3/4), and distribution interval. The vertical axis shows the frequency of each fecal microbiota.

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Clostridium cluster IX were increased in 7 (58.3%) and 6 (50%) of the 12 subjects, respectively. The frequencies of *Bacteroides* and *Clostridium* subcluster XIVa were both decreased in 7 of the 12 (58.3%) subjects. The frequencies of *Clostridium* cluster XI, *Clostridium* cluster XVIII, *Clostridium* cluster IV, and *Prevotella* were increased in 5 of the 6 (83.3%), 9 of the 11 (81.8%), 8 of the 11 (72.7%), and 4 of the 8 (50%) subjects in which they were detected, respectively. The frequencies of *Lactobacillales* and *Clostridium* cluster IV plus subcluster XIVa were decreased in 8 of the 11 (72.7%) and 6 of the 11 (54.5%) subjects in which they were detected, respectively.

When the samples collected after decompression were compared with those collected at 2.1 MPa, it was found that the frequency of *Bifidobacterium* was decreased in 10 of the 12 (83.3%) subjects. On the other hand, the frequencies of *Clostridium* subcluster XIVa and *Clostridium* cluster IX were both increased in 7 of the 12 (58.3%) subjects. The frequencies of *Lactobacillales*, *Clostridium* cluster XI, *Clostridium* cluster XVIII, *Clostridium* cluster IV, *Prevotella*, and *Clostridium* cluster IV plus subcluster XIVa were decreased in 7 of the 11 (63.6%), 5 of the 6 (83.3%), 8 of the 11 (72.7%), 7 of the 11 (63.6%), 5 of the 8 (62.5%), and 6 of the 11 (54.5%) subjects in which they were detected, respectively.

When the third samples were compared with the first samples, it was found that the frequency of *Bifidobacterium* was decreased in 8 of the 12 (66.7%) subjects. Conversely, the frequencies of *Bacteroides*, *Clostridium* subcluster XIVa, and *Clostridium* cluster IX were increased in 9 (75%), 6 (50%), and 7 (58.3%) of the 12 subjects, respectively. The frequencies of *Lactobacillales*, *Clostridium* cluster XI, *Clostridium* cluster XVIII, and *Clostridium* cluster IV plus subcluster XIVa were decreased in 7 of the 11 (63.6%), 3 of the 4 (75%), 7 of the 10 (70%), and 7 of the 11 (63.6%) subjects in which they were detected, respectively. The frequencies of *Clostridium* cluster IV and *Prevotella* were increased in 6 of the 11 (54.5%) and 4 of the 8 (50%) subjects in which they were detected, respectively.

Also, in 5 of the 12 (41.7%) subjects in which *Clostridium* cluster IV, the pattern of change in the frequency of *Clostridium* cluster IV between samples 1 and 3 was the opposite of that for *Bacteroides*. In 4 of the 12 (33.3%) subjects, the pattern of change in the frequency of *Clostridium* subcluster XIVa was the opposite of that for *Bacteroides*. The changes in the frequencies of each type of bacterial species seen among all subjects are summarized in Fig. 2. We observed significant changes in the frequency of *Bifidobacterium* (p value=0.0498). The frequency of *Bifidobacterium* increased at 2.1 MPa and decreased after decompression. We noted marginally significant changes in the frequencies of *Clostridium* cluster IV and cluster XVIII (p=0.0784 and 0.0757, respectively). Both the frequencies of *Clostridium* cluster IV and cluster XVIII increased at 2.1 MPa and decreased after decompression. However, no significant changes in the frequencies of *Lactobacillales*, *Bacteroides*, *Prevotella*, *Clostridium* subcluster XIVa, *Clostridium* cluster IX, or *Clostridium* cluster XI were observed during the diving training (p=0.336, 0.368, 0.607, 0.558, 0.779, and 0.212, respectively).

Relationships among the frequencies of bacteria under hyperbaric conditions

The results regarding the correlations among the changes in the fecal levels of each type of bacteria according to Fisher's exact test are shown in Table 2. Regarding the changes in bacterial frequencies seen after pressurization to 2.1 MPa, a marginally significant inverse correlation was detected between the changes in the frequencies of *Bacteroides* and *Clostridium* subcluster XIVa (p=0.081). As for the changes in bacterial frequencies seen after decompression, a marginally significant inverse correlation was detected between the changes in the frequencies of *Bacteroides* and *Clostridium* cluster IV plus subcluster XIVa (p=0.045). Concerning the changes in bacterial frequencies seen during the training period (between sample collection points 1 and 3), a significant inverse correlation was detected between the changes in the frequencies of *Prevotella* and *Clostridium* subcluster XIVa (p=0.042), and a marginally significant inverse correlation was detected between the changes in the frequencies of *Prevotella* and *Clostridium* cluster XVIII (p=0.087).

Changes in fecal SCFA levels

The fecal levels of each type of SCFA in subjects A–L are shown in Fig. 3. The changes in the fecal levels of SCFAs exhibited interindividual variability.

When the samples collected at 2.1 MPa were compared with those collected before the training, it was found that the fecal levels of acetate, propionate, n-butyrate, and iso-valerate were increased in 8 (66.7%), 7 (58.3%), 7 (58.3%), and 7 (58.3%) of the 12 subjects, respectively. On the other hand, the fecal levels of iso-butyrate were decreased in 6 of the 11 (54.5%) subjects in which it was detected, and the fecal levels of n-valerate were increased in 5 of the 10 (50%) subjects in which it was detected. When the samples collected after decompression were compared with those collected at 2.1 MPa, it was found that the fecal levels of acetate, propionate, and n-butyrate were all decreased in 7 of the 12 (58.3%) subjects, whereas the fecal levels of iso-valerate were increased in 7 of the 12 (58.3%) subjects. In addition, the fecal levels of iso-butyrate were decreased in 6 of the 10 (60%) subjects in which it was detected, and the fecal levels of n-valerate were increased in 8 of the 10 (80%) subjects in which it was detected.

During the training period (between sample collection points 1 and 3), the fecal levels of acetate and iso-valerate increased in 8 (66.7%) and 6 (50%) of the 12 subjects, respectively, whereas the fecal levels of propionate and n-butyrate both decreased in 8 of the 12 (66.7%) subjects. Furthermore, the fecal levels of iso-butyrate increased in 5 of the 10 (50%) subjects in which it was detected, and the fecal levels of n-valerate decreased in 5 of the 9 (45.5%) subjects in which it was detected.

However, no significant changes in the fecal levels of acetate, propionate, n-butyrate, iso-butyrate, n-valerate, or iso-valerate (p=0.338, 0.558, 0.558, 0.907, 0.301, and 0.667, respectively) were observed during the training period (between sample collection points 1 and 3).

The results regarding the correlations among the changes in the fecal levels of each SCFA according to Fisher's exact test are shown in Table 2. Regarding the changes induced by pressurization to 2.1 MPa, a significant positive correlation was detected between the changes in the fecal levels of acetate and n-butyrate (p=0.030). Concerning the changes induced by decompression, significant positive correlations were detected between the changes in the fecal levels of acetate and n-butyrate (p=0.003) and between the changes in the fecal levels of iso-butyrate and iso-valerate (p=0.039). As for the changes seen during the training period (between sample collection points 1

doi: 10.12938/bmfh.2020-054

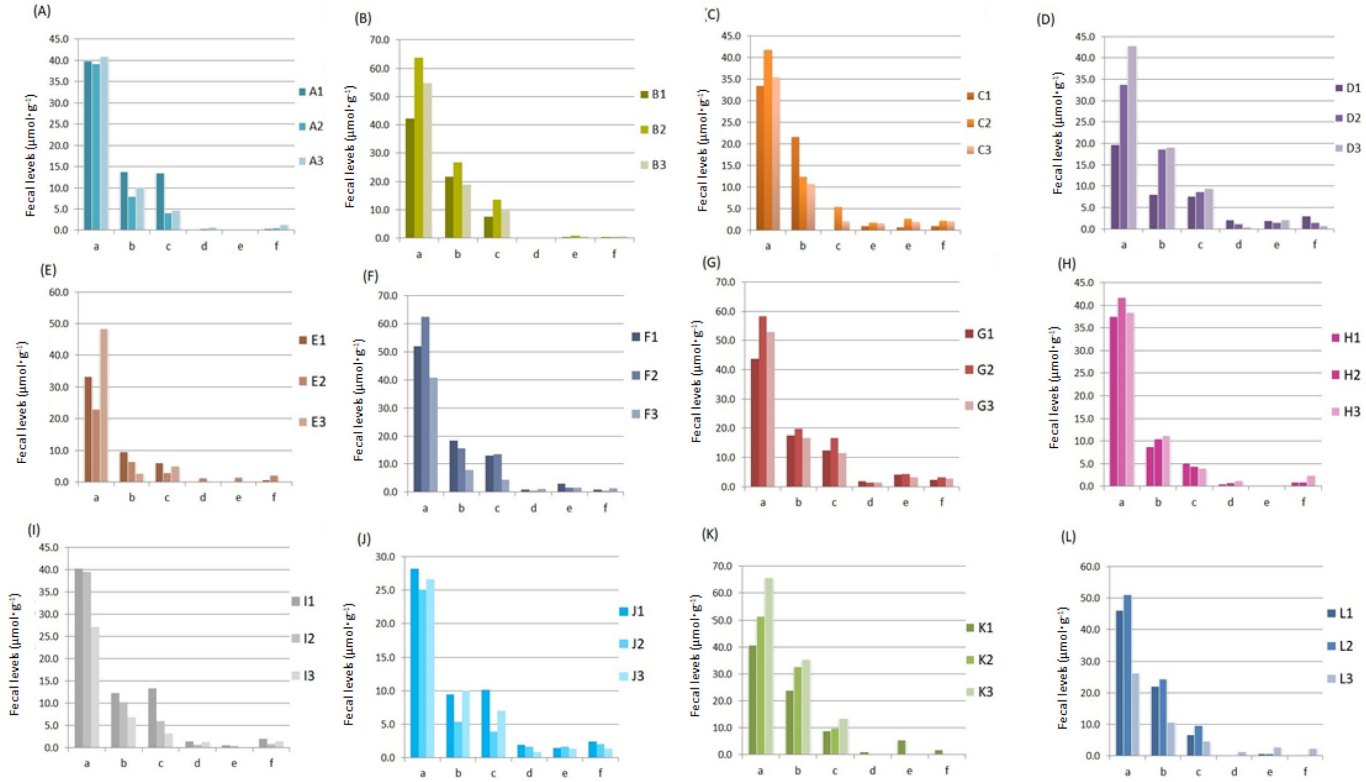

Fig. 3. The levels of each type of short-chain fatty acid (SCFA) in subjects A–L.
The left, middle, and right bars indicate the fecal levels of each SCFA before the training (A1–L1), at 2.1 MPa (A2–L2), and at the end of the training (after decompression; A3–L3), respectively.
a: Acetate, b: Propionate, c: n-Butyrate, d: Iso-butyrate, e: n-Valerate, f: Iso-valerate.

Table 2. Associations (p-values) among the changes in the frequency of each type of bacteria and the fecal level of each SCFA

		At 2.1 MPa	After decompression	During the training period
Bacteroides	*Clostridium* subcluster XIVa	0.081	1	0.273
	Clostridium cluster IV plus subcluster XIVa	0.138	0.045	0.327
Prevotella	*Clostridium* subcluster XIVa	0.729	1	0.042
	Clostridium cluster XVIII	0.642	1	0.087
Acetate	propionate	0.09	0.138	1
	n-butyrate	0.03	0.003	0.423
Iso-butyrate	iso-valerate	0.738	0.013	0.012
Bifidobacterium	n-valerate	1	0.066	0.501
Clostridium cluster IV	propionate	0.363	1	0.045
	iso-valerate	0.072	1	0.525
Clostridium cluster IX	iso-butyrate	0.786	0.006	0.933
	iso-valerate	0.765	0.081	1

P-values were calculated using Fisher's exact test. Only the combinations that demonstrated statistically significant associations are shown. None of the other combinations exhibited statistically significant associations. SCFA: short-chain fatty acid.

and 3), a significant positive correlation was detected between the changes in the fecal levels of iso-butyrate and iso-valerate (p=0.012). No significant correlations were detected between any of the other combinations. The changes in the levels of each SCFA seen among all participants are summarized in Fig. 4. The subjects' acetate levels tended to remain stable despite the changes in pressure. On the other hand, their propionate and n-butyrate levels tended to be decreased at 2.1 MPa and after decompression. As for their levels of iso-butyrate, they tended to be decreased at 2.1 MPa. The levels of propionate and n-butyrate observed at decompression tended to be lower than those seen before compression. However, the fecal levels of n-valerate fluctuated little during the training, and we observed the tendency for a slight increase at 2.1 MPa and decrease after decompression. The iso-valerate levels of the samples obtained after decompression tended to be higher than those of the samples obtained at 2.1 MPa. Thus, the levels of iso-butyrate, n-valerate, and iso-valerate seen after decompression tended to be higher than those observed

doi: 10.12938/bmfh.2020-054

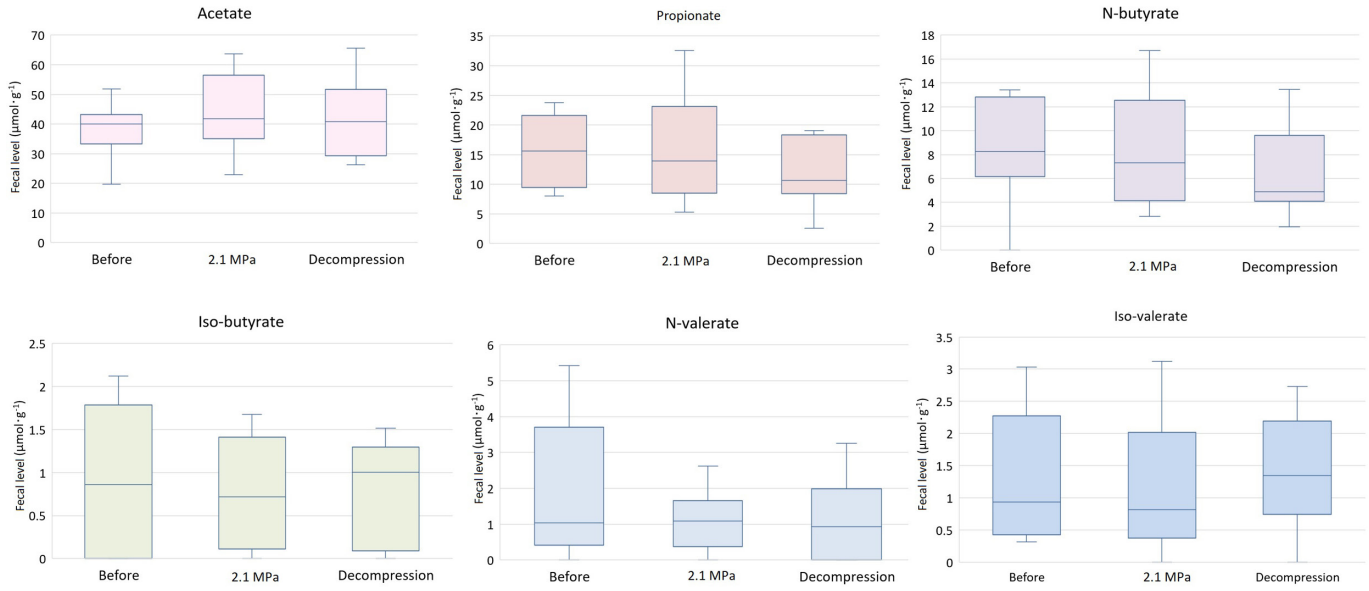

Fig. 4. Changes of each short-chain fatty acid (SCFA) in all subjects at the three sample collection points.

Each box-and-whisker plot contains the lower quartile (Q1/4), median, upper quartile (Q3/4), and distribution interval. The vertical axis shows the level of each SCFA.

before compression. However, no significant changes in the levels of acetate, propionate, n-butyrate, iso-butyrate, n-valerate, or iso-valerate were detected during the diving training (p value=0.338, 0.558, 0.558, 0.907, 0.301, and 0.667, respectively).

Associations between the changes in the fecal levels of SCFAs and the frequencies of bacteria

There was little consistency in the observed patterns of change in the subjects' fecal SCFA levels; that is, they exhibited marked variability during the study period (Fig. 3).

The results regarding the correlations between the changes in the frequencies of each type of bacteria and the changes in the fecal levels of each SCFA according to Fisher's exact test are shown in Table 2. Regarding the changes induced by pressurization to 2.1 MPa, a marginally significant positive correlation was detected between the change in the frequency of *Clostridium* cluster IV and the change in the fecal level of iso-valerate (p=0.072).

Concerning the changes observed after decompression, a significant inverse correlation was detected between the changes in the frequency of *Clostridium* cluster IX and the fecal level of iso-butyrate (p=0.006). A marginally significant inverse correlation was also detected between the changes in the frequency of *Clostridium* cluster IX and the fecal level of iso-valerate (p=0.081). Furthermore, a marginally significant positive correlation was detected between the changes in the frequency of *Bifidobacterium* and the fecal level of n-valerate (p=0.066). As for the changes that occurred during the training period (between sample collection points 1 and 3), a significant inverse correlation was detected between the changes in the frequency of *Clostridium* cluster IV and the fecal level of propionate (p=0.045). No significant correlations were detected for any other combination.

Comparing Figs. 1 and 3, although marked interindividual variability was seen in the changes in the frequencies of each type of bacteria and the fecal levels of SCFA, the patterns of change in the frequency of *Lactobacillales* and the fecal levels of acetate, propionate, and n-butyrate induced by pressurization to 2.1 MPa were very similar (7 [58.3%], 7 [58.3%], and 9 [75%] of the 12 subjects, respectively). Furthermore, the patterns of change in the frequency of *Clostridium* subcluster XIVa and the fecal levels of acetate, propionate, n-butyrate, and iso-valerate induced by pressurization to 2.1 MPa tended to be very similar (9 [75%], 9 [75%], 10 [83.3%], and 8 [66.6%] of the 12 subjects, respectively). There was, however, no significant correlation between the patterns of change in the frequency of *Clostridium* subcluster XIVa and the fecal levels of these SCFAs. The patterns of change in the frequency of *Clostridium* cluster XVIII and the fecal levels of acetate, propionate, n-butyrate, and iso-valerate induced by pressurization to 2.1 MPa also tended to be very similar (7 of the 10 [70%], 7 of the 10 [70%], 6 of the 10 [60%], and 7 of the 8 [87.5%] subjects in which they were detected, respectively).

DISCUSSION

This study was carried out during deep-diving training at the UMC. The training was undertaken by 6 divers at a time, and the training schedule was designed to achieve efficient diving. For these reasons, the study had some limitations, including the collection of only a small number of samples, the lack of a control group, and the difference in ages among the divers. Thus, further study with a larger number of samples is necessary to reduce the effects of these limitations. In addition, there are a number of factors that affect the fecal microbiota and fecal SCFA levels, including the effects of a closed environment, diet, helium gas, changes in oxygen partial pressure, and changes in the environmental pressure per se. Therefore, it will be necessary for us to determine the effects of these factors on the fecal microbiota and fecal SCFA levels in the future.

doi: 10.12938/bmfh.2020-054

In this study, the fecal frequencies of each of the examined types of bacteria exhibited marked variability in their patterns of change during the study period, which was also seen in our previous study. At 2.1 MPa, a reduction in the frequency of *Lactobacillales* and increases in the frequencies of *Clostridium* cluster XI and cluster XVIII were observed. In this regard, we were able to obtain results that were consistent with those of our previous study. In our previous study, the changes in the frequencies of *Bacteroides* and *Clostridium* cluster IV plus subcluster XIVa induced by pressurization to 2.1 MPa seemed to be inversely correlated [4]. In the current study, we did not detect a significant inverse correlation between the changes in the frequencies of *Bacteroides* and *Clostridium* subcluster XIVa induced by pressurization to 2.1 MPa; however, we did detect a marginally significant inverse correlation between these parameters. The frequency of *Clostridium* subcluster XIVa tended to increase during decompression in the previous study, but it tended to decrease in the current study. However, a marginally significant inverse correlation was detected between the changes in the frequencies of *Bacteroides* and *Clostridium* subcluster XIVa induced by pressurization to 2.1 MPa, and we detected a significant inverse correlation between the changes in the frequencies of *Bacteroides* and *Clostridium* subcluster XIVa induced by decompression. Both *Bacteroides* and *Clostridium* subcluster XIVa play important roles in the activation of regulatory T cells and intestinal immunity [7]. The results of the current study suggest that there might be a weak inverse correlation between the variations in the frequency of *Bacteroides* and *Clostridium* subcluster XIVa induced by changes in environmental pressure. Since *Bacteroides* and *Clostridium* subcluster XIVa have similar functions, it is considered that bacterial species that are less susceptible to changes in environmental pressure might compensate for the roles of bacterial species that are susceptible to such changes.

In addition, increases in the frequencies of *Clostridium* cluster XI and cluster XVIII were observed in >80% of the subjects at 2.1 MPa in this study. *Clostridium* clusters XI and XVIII have been reported to be associated with a greater risk of colonic cancer, Crohn's disease, obesity, and fatty liver [8]. The frequency of *Clostridium* cluster XI tended to decrease after decompression, and the frequency of *Clostridium* cluster XVIII increased marginally significantly after pressurization and decreased after decompression. Therefore, it is suggested that the intestinal environment deteriorates in terms of the composition of the fecal microbiota during pressurization.

We also detected a significant inverse correlation between the changes in the frequencies of *Prevotella* and *Clostridium* subcluster XIVa seen during the training period (between sample collection points 1 and 3), and a marginally significant inverse correlation was detected between the changes in the frequencies of *Prevotella* and *Clostridium* cluster XVIII observed during the training period. Larsen [9] reported that *Prevotella* mediates chronic inflammatory disease via various immune cells in the intestinal immune system. Also, it was reported that some *Prevotella* species suppress arthritis by regulating the activation of regulatory T cells in the intestinal immune system to reduce inflammatory reactions [10]. Taking these findings together with those of our study, it is suggested that interactions between *Prevotella* and *Clostridium* subcluster XIVa might influence intestinal immunity. *Prevotella* and *Clostridium* subcluster XIVa might act complementarily, or *Prevotella* might affect the activity of *Clostridium* cluster XVIII.

Significant positive correlations were detected between the changes in the fecal levels of acetate and n-butyrate induced by pressurization to 2.1 MPa and those induced by decompression. Acetate has beneficial effects on the human body; for example, it has anti-inflammatory properties and improves glycometabolism. n-Butyrate has similar beneficial effects and activates regulatory T cells and intestinal immunity [7]. Since acetate and n-butyrate have similar functions and the expression of both molecules changes in a similar manner under pressurized conditions, their effects might be strengthened under hyperbaric conditions.

We also detected significant positive correlations between the changes in the fecal levels of iso-butyrate and iso-valerate induced by decompression and those observed during the training period (between sample collection points 1 and 3). Kish *et al.* [11] reported that interleukin 10-deficient mice exposed to particulate matter displayed increased fecal iso-butyrate and iso-valerate levels and developed intestinal inflammation due to enhanced histological damage. Cardona *et al.* [12] reported that the fecal levels of iso-butyrate and iso-valerate exhibited very strong correlations, irrespective of species, age, diet, and living conditions, due to the microbial breakdown of sloughed intestinal cells. Wang *et al.* [13] reported that mice with dysbiotic fecal microbiotas displayed increased fecal levels of iso-butyrate and iso-valerate. Although we did not examine the damage caused to intestinal cells in the present study, it is likely that environmental pressure changes cause such damage. It could be said that the correlations between the changes in the fecal levels of iso-butyrate and iso-valerate induced by hyperbaric conditions in the present study were similar to those described in previous studies.

A marginally significant positive correlation was detected between the changes in the frequency of *Clostridium* cluster IV and the fecal level of iso-valerate induced by pressurization to 2.1 MPa. As stated above, iso-valerate acts as a virulence factor in the human body, but *Clostridium* cluster IV has been reported to have important anti-inflammatory properties [14] and might improve glucose metabolism [15]. These functions of *Clostridium* cluster IV might help to compensate for reductions in iso-valerate activity. During decompression, the fecal level of iso-butyrate and frequency of *Clostridium* cluster IX tended to decrease and increase, respectively, and a significant inverse correlation was detected between the changes in the frequency of *Clostridium* cluster IX and the fecal level of iso-butyrate. Changes in environmental pressure might cause an increase in the frequency of virulent fecal bacteria followed by increases in the levels of virulent SCFAs, and increases in the intestinal levels of virulent SCFAs might lead to an increase in the frequency of beneficial fecal bacteria. A marginally significant positive correlation was detected between the changes in the frequency of *Bifidobacterium* and the fecal level of n-valerate. *Bifidobacterium* plays an important role in the production of acetate, propionate, and butyrate in the intestinal tract [16]. The frequency of *Bifidobacterium* increased marginally significantly after pressurization and decreased after decompression. Thus, our study suggested that *Bifidobacterium* might somehow be involved in the reduction in fecal n-valerate levels observed during decompression. It is possible that the recovery of the intestinal flora induced by decompression overlaps with the timing of the increases in the fecal levels of virulent SCFAs. A

doi: 10.12938/bmfh.2020-054

significant inverse correlation was detected between the changes in the frequency of *Clostridium* cluster IV and the fecal levels of propionate induced by decompression. Beneficial changes in the fecal microbiota might occur during decompression that might delay the increases in the production of propionate and improve the intestinal environment.

Some earlier studies have reported that supplementation with SCFAs, such as with acetate, propionate, and/or n-butyrate, reduced the effects of psychosocial or oxidative stress [17, 18]. Based on these reports and the results of the present study, it is suggested that the effects of such supplementation might not be limited to the direct effects of particular products but might also involve interactions with bacterial species and SCFAs. Therefore, the marked changes in SCFA levels induced by environmental pressure might be due to the activity of individual bacterial species rather than interactions among various bacterial species.

Diet is one of the most important determinants of the composition of the fecal microbiota [19]. During the training, the composition of the divers' diet was as follows: 63% carbohydrates, 14% protein, and 23% lipids, with almost 20 g of fiber. Basically, they ate rice as a staple food along with miso soup and some side dishes. All of the food eaten by the divers was cooked. All of the divers ate the same menu at each mealtime (6:30, 12:00, and 17:00) during the training. However, the diets that the divers ate before the training may also have affected the results of this study. Unfortunately, we do not have detailed data about their previous diets or about the exact compositions of their diets during the training. The relationships among diet, environmental pressure, and intestinal microbiota should be investigated further.

In vitro studies have also demonstrated that hyperbaric environments affect bacterial growth. Thom and Marquis [20] demonstrated that subjecting a culture medium to hydrostatic pressure inhibited bacterial growth, and He and N_2 reversed the growth-inhibiting effects of hydrostatic pressure. On the other hand, an *in vitro* study showed that the susceptibility of Gram-negative bacilli to antibiotics was enhanced in an He-O_2 atmosphere that mimicked that found during 60-m saturation diving [21]. Oxygen is known to inhibit some bacterial growth, and hyperbaric oxygen therapy is effective against infectious diseases, such as bacterial osteomyelitis and gas gangrene [22]. These earlier studies strongly suggest that environmental pressure and gas composition affect bacterial growth and viability *in vivo* and *in vitro*. In this study, the intestinal microbiota may also have been affected by environmental pressure and the environmental partial pressure of helium gas.

In this study, we evaluated the changes in the compositions of the subjects' fecal microbiotas and fecal SCFA levels that occurred under hyperbaric conditions. The patterns of change in the fecal microbiota and fecal SCFA levels induced by hyperbaric conditions exhibited marked interindividual variations. The subjects' fecal microbiota and fecal SCFA profiles tended to deteriorate under hyperbaric conditions. It is necessary to further evaluate whether the changes in the fecal microbiota and fecal SCFA levels induced by hyperbaric conditions are beneficial.

CONFLICTS OF INTEREST

The authors have no conflicts of interest that are directly related to the content of this article.

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