Correlation Between Indicators of Intestinal Environment and Amount of Carbon Dioxide in Gas Excreted During Defecation

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Intestinal microbiota have marked metabolic activity and influences the host in both beneficial and harmful ways. In the present study, we developed an apparatus to measure the amount of CO₂ in the gas excreted during defecation and investigated whether this amount of CO₂ can be used as an indicator of the intestinal environment. The apparatus consists of a fan and a commercial CO₂ sensor attached to a toilet stool. Fecal pH, fecal water content, concentrations of short chain organic acids (SCFAs) and intestinal putrefactive products and composition of fecal microbiota were analyzed as indicators of the intestinal environment. The apparatus could measure the amount of CO₂ in the gas with good reproducibility, irrespective of the open area at the top of the toilet stool, position of gas injection and composition of the gas. In a volunteer study, the amount of CO₂ in defecation gas correlated with pH, water content and concentrations of SCFAs and intestinal putrefactive products in the feces, although correlation with the composition of intestinal microbiota was not observed. The results indicate that the amount of CO₂ in defecation gas can be measured with simple and sanitary procedures and is a good indicator of the intestinal environment.

Key words: CO₂; defecation gas; fecal water content; putrefactive products; short chain fatty acids

INTRODUCTION

About 10¹⁴ bacteria in total colonize the human intestine and have strong metabolic activities overwhelming the activities of the host itself (5). As a result of this active metabolism, microbiota are able to produce a wide range of compounds that have both beneficial and harmful effects on gut physiology as well as systemic influences. For instance, colonic bacteria produce short-chain fatty acids (SCFAs) through which the host may salvage energy from undigested carbohydrates and proteins (2, 3, 16). On the other hand, intestinal microbiota produces harmful metabolites including intestinal putrefactive products and carcinogens. Intestinal microbiota also deconjugate conjugated bile acids and convert primary bile acids to secondary bile acids, which show tumor promoter activity. Thus the metabolic activities of intestinal microbiota affect the health and diseases of the host (13). Therefore, it is important to maintain the composition and metabolic activities of the intestinal microbiota in a

healthy condition. However, a great deal of effort is required to analyze and monitor the intestinal environment.

In the present study, we developed an apparatus for analyzing the composition of the gas components that are excreted during defecation and investigated whether the defecation gas composition can be used as an indicator for the intestinal environment. CO₂ was selected as the measured gas component, because it is excreted by every individual and sensors with sufficient specificity and sensitivity are commercially available. As indicators of intestinal environment, pH, water content, concentrations of short chain organic acids and intestinal putrefactive products and composition of fecal microbiota were analyzed, and their correlations with the amount of CO₂ in defecation gas were investigated.

MATERIALS AND METHODS

Apparatus for the measurement of CO₂ in defecation gas

Air in the toilet stool was removed with a fan (90 l/min) and passed through a commercial CO₂ sensor which measures CO₂ using a non-dispersive infra-red (NDIR) technique (CO₂EngineK30, SenseAir AB, Sweden) (Fig. 1). The concentration of CO₂ was measured and the peak value of the output from the sensor was recorded as the

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Fig. 1. Apparatus for measuring the amount of CO₂ in gas excreted during defecation. Air in the toilet stool was removed with a fan (90 l/min) and passed through a commercial CO₂ sensor which uses a non-dispersive infrared technique to measure CO₂ in the air flow.

amount of CO₂ in defecation gas.

Confirmation of the specificity of the sensor

A gas mixture consisting of 3,500 ppm CO₂ and other components at different concentrations in N₂ gas was introduced at a flow rate of 10 l/min into a flow cell within which the CO₂ sensor was set, and the CO₂ sensor output was observed. The other gas components were H₂, NH₃ or H₂S at a concentration of 50–500, 5–50 or 5–50 ppm, respectively.

Evaluation of the effects of the open space at the top of the toilet stool and gas excretion position

To evaluate the efficacy of gas collection and the effects of the body habitus and position of subjects during defecation, the CO₂ concentration was measured with different open areas at the top of the toilet stool and positions of gas injection. The conditions tested are listed in Table 1. The injected gas composition was 15% of CO₂ in N₂ and 0.3 l of the mixed gas was injected in 2 seconds. When the mixed gas was injected at 7.25 cm from the posterior side, the open areas at the front, representing the space between the legs, were 0, 41 or 92 cm² and the open area at the rear, representing space behind the body, were 0, 11 or 29 cm². When the open area at the front was 92 cm² and that at the rear was 11 cm², the mixed gas was also injected at 10.5 and 13.75 cm from the posterior side. The measurements were repeated five times. The CO₂ concentration was also measured when the open area at the top of the toilet stool (450 cm²) was not covered at all.

Confirmation of linearity of the CO₂ sensor output

The linearity of the CO₂ sensor output was investigated when the constant amount (0.3 l) of mixed gases with different CO₂ concentrations (5, 10, 15 and 20%) were injected into the toilet stool. CO₂ sensor output was also investigated when different amounts of mixed gas (0.1, 0.3 and 0.5 l) with a fixed CO₂ concentration (15%) were injected. The open area was 92 cm² at the front and 0 cm² at the rear. The mixed gases were injected at 7.25 cm from the posterior side in 2 seconds.

Subjects

Ten healthy volunteers, aged 25 to 48 years, were thoroughly informed about the aim and procedure of the experiment. The Ethical Committee of TOTO Ltd. approved the experimental protocol. After 2 weeks of

Table 1. Influence of different open areas at the top of the toilet stool and positions of gas injection on CO₂ sensor output

Position of gas injection from the posterior side (cm)	Open area at rear (cm ²)	Open area at front (cm ²)	Mean of sensor output (ppm)	CV value (%)
7.25	Open ^a	Open	1675	2.8
7.25	29	92	1740	5.9
7.25	11	92	1656	6.0
7.25	0	92	1728	3.2
7.25	0	41	1614	3.2
7.25	0	0	1548	2.3
10.50	11	92	1737	4.5
13.75	11	92	1784	4.1

^a: Open area at the top of the toilet stool (450 cm²) was not covered at all.

usual life without any dietary restriction, the volunteers were given 250 g/day of commercial yoghurt (Meiji Bulgaria Yoghurt, Meiji Dairies Co., Ltd., Tokyo) for 2 weeks to manipulate the intestinal environment. In addition to yoghurt, the subjects were then given 40 g/day of cereal (All-Bran®, Kellogg (Japan) K. K., Tokyo) and 16 g/day of commercial oligosaccharides (Oligono Okage®, Ensui Sugar Refining Co., Ltd., Tokyo) for another 2 weeks. Feces were collected on a tray placed above the water surface in the toilet stool at each defecation during the experimental period and the amount of CO₂ in defecation gas and fecal pH were measured by the volunteers themselves. In addition to these measurements, fecal samples were collected into a container (a capacity of 1.2 l, about 9 cm in height) instead of a tray at weeks 2 and 6 of the experiment, and transported anaerobically with AnaeroPack-Anaero® (Mitsubishi Gas Chemical Co., Inc., Tokyo) on ice to a laboratory at The University of Tokyo for analysis of indicators of the intestinal environment.

Analysis of feces as an indicator of intestinal conditions

Fecal pH was measured with a pH meter (D-51, Horiba Ltd., Kyoto) by placing an electrode (type 6252, Horiba Ltd.) in the feces. The measurements were repeated three times at different locations of the feces and the average of the values was recorded as fecal pH. The composition of the fecal microbiota was analyzed immediately after receiving the samples and the samples were stored at -80°C until used in other analyses. Fecal water content was calculated from the difference of the weight before and after overnight freeze-drying. The composition of fecal microbiota was analyzed by the culture method described previously (8). The concentration of fecal SCFAs was measured by the post-column method (15) with HPLC and the concentration of intestinal putrefactive products was measured with HPLC (9).

Statistical analysis

Correlations and the significance of the correlations were calculated with Microsoft® Office Excel 2003.

RESULTS

Basic data of the apparatus for measurement of CO₂ in defecation gas

The CO₂ sensor output was not affected by the addition of H₂, NH₃ or H₂S to the mixture of N₂ and CO₂ (Fig. 2). Outputs of the CO₂ sensor with different open areas at the top of the toilet stool and different positions of gas injection are shown in Table 1. In all conditions tested, the coefficient of variation (CV value) of the outputs of

Fig. 2. Effects of H₂, NH₃ or H₂S added to the mixed gas of N₂ and CO₂ on CO₂ sensor output. A gas mixture consisting of 3,500 ppm CO₂ and one other component gas with different concentrations in N₂ gas was introduced at a flow rate of 10 l/min into a flow cell within which the CO₂ sensor was set, and the CO₂ sensor output was observed. The other component gases were H₂, NH₃ and H₂S, and their concentrations were 50–500, 5–50 and 5–50 ppm, respectively.

five repeated measurements was less than 6%. The CV value of all recorded outputs with different open areas and injection positions was 5.8%. The concentration of CO₂ in the mixed gases correlated well with the CO₂ sensor output ($r = 0.999$) and the amount of mixed gas also correlated well with the CO₂ sensor output ($r = 0.999$) (Fig. 3).

Correlation between the amount of CO₂ in defecation gas and fecal pH

The amount of CO₂ in defecation gas and fecal pH were both measured throughout the experimental period, and the correlation between the amount of CO₂ and fecal pH was investigated (Fig. 4). There was a negative correlation between the amount of CO₂ and fecal pH ($r = -0.550$).

Correlation between CO₂ in defecation gas and indicators of the intestinal environment

The amount of CO₂ in defecation gas and various parameters of the intestinal environment, before and after ingestion of yoghurt, cereal and oligosaccharides, are shown in Tables 2 and 3. All of the parameters investigated were not significantly affected by the intake of these dietary factors. The correlations between the amount of CO₂ and parameters of the intestinal environment were calculated for all fecal samples

Fig. 3. Effects of concentration of CO₂ in mixed gas and amount of mixed gas on CO₂ sensor output. A) CO₂ was mixed with N₂ at a concentration of 5, 10, 15 or 20%, and a constant volume (0.3 l) was injected into the stool. B) CO₂ was mixed with N₂ at a concentration of 15% and 0.1, 0.3 or 0.5 l of the mixed gas was injected into stool. The open area was 92 cm² at the front and 0 cm² at the rear. The mixed gases were injected at 7.25 cm from the posterior side in 2 seconds.

obtained. Fecal water content and the amount of CO₂ in the defecation gas showed a strong and significant positive correlation ($r = 0.754$) and fecal concentration of acetic acid also showed a significant positive correlation with the amount of CO₂. The concentration of total SCFAs was also positively correlated with the amount of CO₂, whereas the concentrations of total intestinal putrefactive products, *i*-butyric acid and *i*-valeric acid, showed negative correlations with the amount of CO₂, although the correlations were not statistically significant (Table 4).

Fig. 4. Correlation between the amount of CO₂ in defecation gas and fecal pH. The correlation between the amount of CO₂ and fecal pH was analyzed when measurement of both the amount of CO₂ in defecation gas and fecal pH were both possible during the experimental period.

Table 2. Amount of CO₂ in defecation gas, fecal pH, fecal water content and composition of fecal microbiota before and after ingestion of yoghurt, cereal and oligosaccharides (n=10)

	Before	After
(ppm)		
CO ₂ (at fecal sampling)	263 ± 232 ^a	287 ± 172
CO ₂ (immediately before fecal sampling)	484 ± 232	476 ± 341
pH	6.27 ± 0.41	6.43 ± 0.50
Water (%)	76.5 ± 6.1	78.8 ± 5.8
(log ₁₀ /g)		
Total bacteria	10.3 ± 0.5	10.6 ± 0.5
Bacteroidaceae	9.8 ± 0.9 (10) ^b	10.2 ± 0.6 (10)
Bifidobacteria	9.6 ± 0.4 (10)	9.9 ± 0.7 (10)
Eubacteria	9.7 ± 0.4 (10)	9.8 ± 1.0 (10)
Clostridia	8.6 ± 0.8 (10)	8.9 ± 0.9 (10)
Veillonellaceae	4.9 ± 1.3 (6)	6.9 ± 2.3 (6)
Lactobacilli	5.7 ± 2.0 (7)	5.5 ± 1.7 (7)
Enterobacteriaceae	6.4 ± 1.7 (7)	6.1 ± 1.6 (9)
Streptococci	7.4 ± 1.5 (10)	7.8 ± 1.2 (10)
Direct microscopic count	11.2 ± 0.2	11.2 ± 0.4

a: Mean ± SD

b: Number detected.

The amount of CO₂ in the defecation gas might not have been measured correctly in some cases because of disturbance to air flow caused by the container used for fecal sampling. Accordingly, correlations between the intestinal environment and the amount of CO₂ at defecation immediately before the collection of feces were also analyzed. The amount of CO₂ at defecation immediately before the collection of feces showed a strong and significant negative correlation with fecal pH ($r = -0.720$) and significant negative correlations with concentrations of total intestinal putrefactive products,

Table 3. Concentrations of fecal SCFAs and intestinal putrefactive products before and after ingestion of yoghurt, cereal and oligosaccharides (n=10)

	Before	After
($\mu\text{mol/g}$)		
Total organic acids	132.1 $\pm$ 28.0 ^a	130.5 $\pm$ 34.7
Formate	1.2 $\pm$ 0.5	0.1 $\pm$ 0.2
Acetate	69.7 $\pm$ 17.6	67.8 $\pm$ 19.4
Propionate	32.3 $\pm$ 9.2	29.3 $\pm$ 10.0
<i>i</i> -Butyrate	1.7 $\pm$ 0.9	1.9 $\pm$ 1.2
<i>n</i> -Butyrate	17.6 $\pm$ 3.8	17.8 $\pm$ 8.2
<i>i</i> -Valerate	2.3 $\pm$ 2.0	2.4 $\pm$ 1.7
<i>n</i> -Valerate	3.2 $\pm$ 1.6	3.4 $\pm$ 2.2
Succinate	4.2 $\pm$ 7.7	7.7 $\pm$ 18.1
Lactate	0.0 $\pm$ 0.0	0.1 $\pm$ 0.2
($\mu\text{g/g}$)		
Total putrefactive products	75.5 $\pm$ 39.7	63.3 $\pm$ 40.5
Phenol	7.0 $\pm$ 13.0	2.7 $\pm$ 5.4
Cresol	38.9 $\pm$ 29.8	29.2 $\pm$ 24.5
Indole	27.4 $\pm$ 11.8	28.0 $\pm$ 14.2
Skatole	2.3 $\pm$ 4.4	3.4 $\pm$ 5.8

^a: Mean $\pm$ SD

indole, *p*-cresol and *i*-valeric acid ($r = -0.692$, -0.660 , -0.490 and -0.619 , respectively), and significant positive correlations with fecal water contents and concentrations of total SCFAs, acetic acid and lactic acid ($r = 0.550$, 0.485 , 0.594 and 0.449 , respectively) were observed (Table 4). Significant negative correlations were also observed with direct microscopic counts ($r = -0.449$) (Table 4). The amounts of CO₂ measured at defecation immediately before fecal collection and at fecal collection showed a significant positive correlation ($r = 0.490$).

DISCUSSION

Intestinal microbiota have marked metabolic activity and their metabolism influences the health and diseases of the host in both beneficial and harmful ways. Nutrients that pass undigested from the small intestine into the large intestine are fermented by colonic bacteria. Fermentation products include SCFAs, intestinal putrefactive products and gases, e.g. carbon dioxide, methane and hydrogen (1, 4, 5). SCFAs are absorbed and utilized as an energy source by the host or directly by colonic epithelial cells (12). SCFAs also influence the metabolism and growth of endogenous and exogenous bacteria by reducing the pH of intestinal contents (5, 14). Intestinal microbiota also produce metabolites harmful to the host's health. For example, metabolites produced by intestinal bacteria from amino acids, including indole, phenol and *p*-cresol, are thought to relate to carcinogenesis (23, 24). It is

Table 4. Correlations between the amounts of CO₂ in defecation gas at fecal sampling or at defecation immediately before fecal sampling and fecal pH, fecal water content, composition of fecal microbiota and concentrations of fecal SCFAs and intestinal putrefactive products in fecal samples from 10 healthy volunteers

	CO ₂ at fecal sampling	CO ₂ at defecation immediately before fecal sampling
CO ₂ at defecation immediately before fecal sampling	0.490 *	
pH	-0.329	-0.720 ***
Water (%)	0.754 ***	0.550 *
Total bacteria	-0.145	-0.376
Bacteroidaceae	0.013	-0.270
Bifidobacteria	-0.144	-0.174
Eubacteria	-0.254	-0.428
Clostridia	0.261	0.131
Veillonellaceae	0.185	0.038
Lactobacilli	-0.156	0.232
Enterobacteriaceae	0.265	0.225
Streptococci	0.216	0.052
Direct count	-0.263	-0.449 *
Total organic acids	0.417	0.485 *
Formate	-0.073	-0.146
Acetate	0.546 *	0.594 **
Propionate	0.015	0.127
<i>i</i> -Butyrate	-0.425	-0.407
Butyrate	0.320	0.333
<i>i</i> -Valerate	-0.433	-0.619 **
Valerate	-0.101	-0.344
Succinate	0.154	0.223
Lactate	0.241	0.449 *
Total putrefactive products	-0.402	-0.692 ***
Phenol	-0.281	-0.156
Cresol	-0.308	-0.660 **
Indole	-0.262	-0.490 *
Skatole	-0.255	-0.344

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

reported that the production of these intestinal putrefactive products are significantly reduced by ingestion of probiotics and prebiotics (5, 18). Probiotic bacteria or indigenous bifidobacteria are stimulated by probiotics and prebiotics produce SCFAs as their metabolic products and these SCFAs reduce the intestinal pH. A low pH environment is considered to suppress the proliferation of harmful bacteria and to inhibit the production of putrefactive products (5–7, 19, 21).

In the present study, fecal pH showed strong negative correlations with total SCFAs and acetic acid concentration ($r = -0.713$ and -0.728 , respectively) and negative correlations with propionic and *n*-butyric acid concentrations ($r = -0.467$ and -0.511 , respectively), as well as positive correlations with *i*-valeric acid, total

putrefactive products and *p*-cresol concentrations ($r=0.547$, 0.594 and 0.583 , respectively) (data not shown). Our results are in basic agreement with those of a previous study (10) in respect of the relationships among fecal pH, water content, SCFAs concentration and concentrations of intestinal putrefactive products. On the other hand, we did not find any significant changes in the amount of CO₂ and indicators of intestinal environment in response to dietary manipulation. A better controlled study will be needed to investigate the effects of pro- and prebiotics on defecation gas and intestinal environment.

Intestinal microbiota have an active role in carcinogenesis via conversion of bile acids to secondary bile acids, more active tumor promoters, and the suppression of this conversion would be beneficial for the prevention of colorectal cancer (11). It is thought that the production of SCFAs and the reduction of pH by intestinal microbiota may suppress the conversion of bile acids to secondary bile acids (17, 20). Epidemiological studies have also reported that increase of intestinal pH is correlated with colon cancer risk (22).

It is important to maintain the composition and metabolic activities of the intestinal microbiota in a healthy condition for health and prevention of diseases. Regular monitoring of the intestinal environment is useful for understanding and improving the intestinal environment through dietary habits and lifestyles. However, it is quite difficult and requires a great deal of work to directly analyze and monitor environmental conditions in the intestine. Even measurement of fecal pH, one of the easiest methods, causes sanitary and psychological problems based on collection and handling of fecal samples. In the present study, we employed gas, which is excreted at each defecation and can be collected without any contact with feces, as a parameter of the intestinal environment. Because carbohydrates in the large intestine are fermented by bacteria to form organic acids and gases including carbon dioxide, hydrogen and methane (1, 4, 5), we performed a preliminary analysis of carbon dioxide, hydrogen and methane for which sensors are commercially available. Based on the results of the preliminary analysis, an apparatus for the measurement of CO₂ in defecation gas was prepared, because CO₂ was excreted by all the subjects tested and the sensitivity and specificity of the sensor was sufficient. On the other hand, H₂ showed large inter-individual differences of concentration and was not excreted by some subjects and the sensor for methane had a specificity problem.

The results indicate that the apparatus used in the present study is able to measure the amount of CO₂ in defecation gas with good reproducibility, irrespective of

body position and body habitus of the subjects, and the presence of other gases in defecation gas (Table 1 and Figs. 2 and 3). The amount of CO₂ in defecation gas was measured by the apparatus and showed a negative correlation with fecal pH under experimental conditions with human volunteers (Fig. 4). The correlations between the amount of CO₂ in defecation gas and indicators of intestinal environment were further investigated and the amount of CO₂ measured was found to correlate with pH, water content, concentrations of SCFAs and intestinal putrefactive products of the feces (Table 4). Our results indicate that the apparatus used in the present study can measure the amount of CO₂ in defecation gas with easy and sanitary procedures, and also that the amount of CO₂ in defecation gas measured by the apparatus is a good indicator of the intestinal environment, although no correlation between the amount of CO₂ in defecation gas and the composition of intestinal microbiota was found. These results suggest that the metabolism of intestinal microbiota can be modified without significant changes in composition. It is possible that a more detailed bacteriological investigation including molecular techniques would reveal a correlation between the amount of CO₂ in defecation gas and the composition of intestinal microbiota.

The amount of CO₂ at defecation immediately before the collection of feces correlated well with the indicators of intestinal environment, while the amount of CO₂ measured at fecal collection showed fewer significant correlations. This is probably because the air flow in the toilet stool was disturbed by the container for fecal sampling used instead of the tray. However, the amount of CO₂ measured at defecation immediately before fecal collection significantly correlated with that measured at fecal collection. This result indicates that the intestinal environment is quite stable in the same individual over a short period of time and suggests that the amount of CO₂ in defecation gas is a good indicator of the intestinal environment.

In conclusion, the apparatus used in this study would be very useful for regular monitoring of the intestinal environment. Further studies with various types of subjects, e.g. subjects with abnormal intestinal environments, such as diarrhea and constipation, should be performed for evaluation of this apparatus.

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