— Research paper —

Prebiotic effect of lactulose on ammonia emanating from human skin surface

Yoshika SEKINE^{1*}, Shiori UCHIYAMA¹, Michihito TODAKA¹, Yohei SAKAI²,
Ryo SAKIYAMA², Hiroshi OCHI², Fumiaki ABE²,
Satomi ASAI³, Kazuo UMEZAWA⁴

¹Graduate School of Science, Tokai University, 4-1-1 Kitakaname, Hiratsuka, Kanagawa 259-1292, Japan

²Food Ingredients & Technology Institute, R&D Division, Morinaga Milk Industry Co., Ltd.,
5-1-83 Higashihara, Zama, Kanagawa 252-8583, Japan

³Department of Laboratory Medicine, School of Medicine, Tokai University,
143 Shimokasuya, Isehara, Kanagawa 259-1193, Japan

⁴Department of Emergency and Critical Care Medicine, School of Medicine, Tokai University,
143 Shimokasuya, Isehara, Kanagawa 259-1193, Japan

Ammonia emanating from the human skin surface is known to cause unpleasant body odour. Lactulose has been used as a bifidobacterial growth factor and a medicine for lowering blood ammonia because of its metabolization to lactic acid and short-chain fatty acids. However, no study has been previously conducted on the effect of lactulose on the dermal emission of ammonia at any dosage levels. This study aimed to investigate the prebiotic effect of lactulose at a food dosage level of 4 g d⁻¹ on the dermal emission flux of ammonia in 12 healthy volunteers using a passive flux sampler coupled with ion chromatography. A significant decrease in the dermal emission was found after 8 days, and the emission flux was found to decrease with an increase in the number of faecal bifidobacteria. Thus, the daily intake of lactulose at the studied food dosage level improved the colonic microflora and reduced the dermal emission of ammonia.

1. Introduction

Human skin gas is known as traces of gas emanating from the human skin surface. It has been attracting considerable attention because of its various roles such as a source of body odour^{1)~3)}, mosquito attractant⁴⁾, indicator of tobacco smoking⁵⁾, non-invasive medical biomarkers for emotional stress⁶⁾, acute chemical poisoning⁷⁾, and diabetes⁸⁾. When volatile compounds are formed by the internal metabolism and carried into the blood, they can rise to the skin surface with perspiration and/or travel directly from the blood through the dermal layers because of the presence of an expansive network of blood capillaries beneath the skin⁹⁾. Ammonia is a typical human skin gas, and its pungent odour is a possible cause of body odour when emanating from the human skin surface. Because the dermal emission of ammonia is known to increase with physical and/or psychological stress¹⁰⁾, the body odour caused by dermal ammonia is often called “fatigue odour”.

Lactulose (IUPAC name: 4-O- β -D-galactopyranosyl- β -D-fructofuranose) is a synthetic disaccharide composed of two simple sugars, galactose and fructose, and is made from the milk sugar lactose. Since lactulose was first found to increase the number of bifidobacteria in infant faeces¹¹⁾, it has been used as a bifidogenic growth factor (bifidus factor) in infant formula and various foods for health promotion¹²⁾. The prebiotic effect of lactulose at a clinical dosage level (19–39 g d⁻¹) is utilized for treating high blood ammonia which leads to hepatic encephalopathy. Lactulose is converted by gut microbiota to lactic acid and short-chain fatty acids (SCFAs)^{13),14)}, resulting in the acidification of colon contents¹⁵⁾. The acidic contents can trap ammonia in the colon by transforming the freely diffusible molecular ammonia (NH₃) to the ammonium ion (NH₄⁺) which will not diffuse back to the blood¹⁶⁾. Therefore, ingestion of lactulose may also decrease the amount of ammonia emanating from the human skin, because the dermal emission flux (emission rate per unit surface area) varies with blood concentration^{9),10)}.

*Corresponding author : E-mail : sekine@keyaki.cc.u-tokai.ac.jp

Fig. 1 Schedule of the open-label and before-after trial tests for investigating the prebiotic effect of lactulose at a dosage of 4 g d^{-1} on the dermal emission of ammonia of 12 healthy volunteers.

However, no study has been previously conducted on the effect of lactulose ingestion on the dermal emission of ammonia at any dosage levels.

The purpose of this study is to investigate the prebiotic effect of lactulose at food a dosage level of $\leq 4\text{ g d}^{-1}$ on the dermal emission of ammonia from the skin surface of healthy volunteers. This is the first report on the successful reduction in the dermal emission of ammonia by lactulose ingestion.

2. Materials and Methods

2.1 Test food sample

Lactulose crystal-anhydrate powder (MLC-97, $\geq 97\%$, Morinaga Milk Industry Co., Ltd, Tokyo, Japan) was provided for test volunteers in an aluminium sachet.

2.2 Volunteer Study

Open-label and before-after trial tests were carried out at Tokai University, Japan from 10 July to 1 August 2018 by recruiting 12 healthy volunteers consisting of 7 males and 5 females (age: 22–52) with 164 ± 9.8 cm of height, 55 ± 6.9 kg of weight and 20 ± 1.1 of body mass index. This study was conducted in accordance with the principles of the Declaration of Helsinki. The trial protocol was approved by the Institutional Review Board of Shonan campus, Tokai University (No. 18065). A written informed consent was obtained from all participants. Exclusion criteria were as follows: (1) subjects with severe hepatic, renal, cardiac, gastrointestinal, cerebrovascular, endocrine, metabolic, or infectious diseases; (2) those with a history of gastrointestinal resection; (3) those with gastrointestinal dysfunction, such as irritable bowel syndrome or inflammatory bowel disease; (4) those regularly using medicines or supplements that could influence defaecation frequency (e.g., antibiotics, probiotics, prebiotics, laxatives, anti-diarrhoeals, and fibre); (5) those allergic to milk; (6)

those participating in another study; and (7) those judged inappropriate for the study by the investigator or physician.

Fig. 1 shows the test schedule. The volunteers ingested the lactulose powder for 2 weeks during the ingestion period at a dose of 4 g d^{-1} . The time of ingestion was not specified and the mode of ingestion (such as taking with water, drinking after dissolving into other beverages, and eating after topping on meals) was subjected to each volunteer's choice. During the research period, subjects were instructed in advance to avoid the use of pharmaceuticals and supplements (e.g., antibiotics, laxatives, antidiarrhoeals, probiotics, prebiotics, and fibre) that affect intestinal micro-organism and defaecation. Meanwhile, they were allowed to take usual meals without any restrictions. Faecal samples were collected before and after the ingestion period to investigate the number of bifidobacteria in faeces. Human skin gas was also collected in the morning (before breakfast) and night (1 hour before going to bed) of 5 days before, during, and after the ingestion period.

Since a household yogurt containing lactulose at less than 4 g cup^{-1} is commercially available at present, additional studies were carried out at Tokai University, Japan from 13 to 29 March 2019 by changing the dosage to lower levels, 2 g d^{-1} and 1 g d^{-1} , following the schedule shown in Fig. 1, in order to investigate the dose effect of the lactulose ingestion on the dermal emission of ammonia. The recruited 12 healthy volunteers were randomly divided into two groups: one for 2 g d^{-1} ($n=6$) and another for 1 g d^{-1} ($n=6$). Faeces sampling was not conducted in these additional studies.

2.3 Determination of ammonia emanating from human skin surface

Following the previous work by Furukawa et al.¹⁷⁾,

Fig. 2 Schematic of the passive flux sampler for human skin gas measurement and sampling at the forearm of a healthy volunteer

the dermal emission flux of ammonia was determined for healthy volunteers by a passive flux sampler (PFS) coupled with ion chromatography (IC). Fig. 2 illustrates a schematic view of the PFS when applied to human skin. The device simply consists of a stainless Petri dish, trapping filter, O-ring and polytetrafluoroethylene (PTFE) plate as support. The PFS is placed on the skin surface to create a headspace. Through the open face of the sampler, ammonia emanating from skin moved toward the trapping media within the headspace by molecular diffusion and the gas molecules were then collected on the filter. Diffusion length which is a distance between the skin surface and trapping media within the PFS was set at 0.95 cm. Since the PFS employs the molecular diffusion process and does not require power supply, an investigator can non-invasively apply the ubiquitous sampler and volunteers mostly do not experience sampling stress.

The trapping filter was prepared by dipping a commercially available cellulose filter paper (Advantec, Tokyo, Japan, No.51A, 32 mm ϕ) into 2% phosphoric acid – 1% glycerol in methanol solution and subsequently drying in a vacuum desiccator. Before use, the open end of the sampler was covered with a cap of the stainless Petri dish, sealed by a piece of ParafilmTM, enveloped in an aluminium bag.

The PFS was softly fixed on the skin surface of forearm by a piece of medical tape (Fig. 2) and sampling was conducted for 1.0 h. The ammonia gas emanating from skin surface was collected by the trapping media as NH_4^+ and subsequently determined by IC after extraction in 8.0 mL of Milli-Q water with mild shaking (150 rpm) for 2.0 h. The emission flux of ammonia at a sampling position, E ($\text{ng cm}^{-2} \text{ h}^{-1}$) was obtained by

$$E = \frac{W}{S \cdot t} \quad (eq1)$$

where W is the collection amount of ammonia (ng) by the PFS, S is the effective cross-section area of the trapping media (5.26 cm^2) and t is the sampling duration (1.0 h). No special treatment was conducted for the surface of the forearm before sampling.

The IC system consists of LC-20AD pump, conductivity detector, COD-10A vp, column oven, CTO-10A vp, and a recorder manufactured by Shimadzu corporation (Kyoto, Japan). The following conditions were used: column, 4.6 mm $\phi \times 150$ mm, IC-C4 (Shimadzu, Japan); eluent, 1.0 mM nitric acid at 1.0 mL min^{-1} (isocratic); automatic injection volume, $20 \mu\text{L}$; oven temperature, 313 K. The diluted solutions of NH_4^+ in Milli-Q water, 0.20, 0.50, 2.0, and 5.0 mg L^{-1} , were prepared from ammonium sulfate and used for calibration ($r = 0.996$ for ammonia concentration versus peak area). The limit of quantitation (LOD) of the PFS method was defined as 3-fold the standard deviation of multiple blank samplers and was calculated in $9.3 \text{ ng cm}^{-2} \text{ h}^{-1}$ of emission flux of ammonia for 1-h sampling duration following the analytical procedure described above.

2.4 Quantification of faecal bacterial cell numbers

Collected faecal samples were stored below -18°C until arrival at the laboratory and at -80°C thereafter. As described by Sugahara et al.¹⁸⁾, bacterial DNA was extracted from the faecal samples of tested volunteers and amplified using quantitative PCR. Briefly, faecal pellets of 20 mg were suspended in 0.45 mL of extraction buffer (100 mM Tris-HCl and 40 mM EDTA at pH 9.0) with $50 \mu\text{L}$ of 10% SDS. Glass beads (0.1 mm ϕ ,

300 mg) and 0.5 mL of TE buffer-saturated phenol were added to the suspension, and the mixtures were vigorously shaken at 2,700 rpm for 180 s with Multi-beads shaker device, MB801 (Yasui Kikai Corporation, Osaka, Japan). After centrifugation, 0.4 mL of the supernatant was extracted with phenol in chloroform, and 0.25 mL of the supernatant was precipitated with isopropanol. The precipitates were subsequently washed with 70% ethanol and dissolved in 0.2 mL of Tris-EDTA buffer at pH 8.0. Extracted bacterial DNA was subjected to real-time PCR. For the quantification of bifidobacterial cell number, genus-specific forward (5' CTCCTGGAAACGGGTGG3') and reverse (5' GGTGTTCTTCCCGATATCTACA 3') primers¹⁹ were used in this study. A stan-

dard curve was prepared using dilutions of *Bifidobacterium longum* ATCC 15707 cells²⁰.

3. Results

Though lactulose can be partially digested in the small intestine²¹, most of the disaccharide is not metabolised or absorbed in the upper gastrointestinal tract because of the 1,4 β -glycosidic bond in the lactulose molecule¹². Once it reaches the colon it is anaerobically fermented by colonic microflora, causing changes in the bacterial composition and metabolic activities of the colonic flora²². **Fig. 3** shows the changes in the number of colonial forming units (CFU) of bifidobacteria per gram of faeces as the arithmetic mean and standard error before and after lactulose ingestion for 2 weeks at a dose of 4 g d⁻¹. As estimated by the previous study¹², the number of bifidobacteria in the faeces (log CFU g⁻¹) significantly increased from 8.8 ± 0.10 to 9.2 ± 0.11 (paired t-test, $p = 0.0034$) after the ingestion.

Fig. 4 shows the variations in the dermal emission flux of ammonia measured for 12 volunteers from their forearm in the morning (a) and at night (b). The arithmetic mean and standard deviation are shown in the figures; zero is used for the samples whose readings are below LOD. Paired t-test was conducted between the values after ingestion and those at Day 2. For the morning samples shown in **Fig. 4 (a)**, the dermal emission fluxes at Day 1 and 2 were $2.0 \times 10^2 \pm 1.1 \times 10^2$ ng cm⁻² h⁻¹ and $2.7 \times 10^2 \pm 2.5 \times 10^2$ ng cm⁻² h⁻¹, respectively. After the ingestion of the lactulose powder,

Fig. 3 Increase in number of bifidobacteria after daily ingestion of 4 g d⁻¹ of lactulose for 2 weeks ($n=11$ due to a lack of datum of one volunteer).

Fig. 4 Variations in the emission flux of ammonia emanating from the skin surface of 12 healthy volunteers before (Day 1 and 2) and after (Day 6, 10 and 17) daily ingestion of 4 g d⁻¹ of lactulose (sampling position: forearm, sampling duration 1 h, morning and night). Paired t-test was conducted for the values after ingestion against those at Day 2.

Fig. 5 Relationship between the dermal emission flux of ammonia and number of bifidobacteria in the faeces (Morning data at Day 2 and Day 17 were used for the dermal emission flux in this figure)

the emission flux significantly decreased to $1.3 \times 10^2 \pm 94 \text{ ng cm}^{-2} \text{ h}^{-1}$ at Day 10, eight days after the ingestion, and subsequently to $46 \pm 49 \text{ ng cm}^{-2} \text{ h}^{-1}$ at Day 17, one day after the termination of ingestion. Similar results were found for the night samples shown in Fig. 4 (b). The dermal emission fluxes at Day 1 and 2 were $2.0 \times 10^2 \pm 1.6 \times 10^2 \text{ ng cm}^{-2} \text{ h}^{-1}$ and $2.0 \times 10^2 \pm 1.5 \times 10^2 \text{ ng cm}^{-2} \text{ h}^{-1}$, respectively. After ingestion of the lactulose powder, the emission flux significantly decreased to $98 \pm 1.2 \times 10^2 \text{ ng cm}^{-2} \text{ h}^{-1}$ at Day 10 and finally to $79 \pm 1.0 \times 10^2 \text{ ng cm}^{-2} \text{ h}^{-1}$ at Day 17.

Fig. 5 shows the relationship between the number of bifidobacteria in the faeces and dermal emission flux of ammonia of all individual volunteers. Data on the dermal emission flux in the morning at Day 2 and Day 17 were used in these plots. A remarkable decrease in the emission flux of ammonia was found with an increase in the number of bifidobacteria. This indicates that the daily intake of lactulose at the food dosage level improved the colonic microflora and reduced the amount of ammonia emanating from the skin surface.

To investigate the dose effect of lactulose ingestion, additional studies were conducted by changing the dosage at lower levels, 2 g d^{-1} and 1 g d^{-1} , following the schedule shown in Fig. 1. The number of participants were six for each test. Fig. 6 shows the results. In the case of 2 g d^{-1} , the dermal emission flux of ammonia decreased only at Day 17 (one day after the termination of ingestion). However, no significant change was found at the dosage of 1 g d^{-1} . Although a signifi-

cant increase in the number of bifidobacteria was reported for healthy Japanese women even at the dosage of 2 g and 1 g per day¹²⁾, the prebiotic effect of lactulose on the dermal emission of ammonia was not apparent at these levels.

4. Discussions

A prebiotic is defined as a substrate that is selectively utilized by host microorganisms conferring a health benefit²³⁾. According to the meta-analysis conducted by Shukla et al.²⁴⁾, lactulose appeared to have the most effect on the improvement in minimal hepatic encephalopathy rather than by probiotics and synbiotics at the clinical dosage levels. Lactulose is converted by a gut microflora to lactic acid and SCFAs, which acidify colon contents by increasing the H_3O^+ concentration in the gut. When ammonia (NH_3) is dissolved in water, certain portion of ammonia converts to ammonium ions (NH_4^+) and reaches to equilibrium of electrolytic dissociation.

Degree of the dissociation depends on the pH of the solution. Since ammonia is absorbed into the blood by non-ionic diffusion¹⁶⁾, the increases in the H_3O^+ concentration in the colon favours the formation of NH_4^+ . Lactulose is therefore effective in reducing plasma NH_3 concentration^{25), 26)}. The significant increase of the acids from lactulose was also found at subclinical dosages of $2\text{--}5 \text{ g}$ in a computer-controlled *in vitro* model of human

Fig. 6 Variations in the emission flux of ammonia emanating from the skin surface of healthy volunteers before (Day 1 and 2) and after (Day 6, 10 and 17) the daily ingestion of 1 and 2 g d⁻¹ of lactulose (sampling position: forearm, sampling duration 1 h, morning and night, number of volunteers: 6 for each test). Paired t-test was conducted for the values after ingestion against those at Day 2.

large intestine¹⁵⁾. Bothe et al.¹⁵⁾ also reported the increasing bacterial counts of *Bifidobacterium*, *Lactobacillus*, and *Anaerostipes*, and a decrease in branched-chain fatty acids, pH, and ammonia in the in vitro model.

Meanwhile, the dermal emission of ammonia was found to vary with its blood concentration in healthy volunteers⁹⁾ and a liver-disease patient¹⁰⁾. This is because the highly volatile molecular ammonia in the form of NH₃ rises directly to skin surface from blood capillaries⁹⁾ and/or emerges as a component of sweat¹⁷⁾. The pH of the blood and sweat also affects the form of ammonia species because of the equilibrium of electrolytic dissociation (eq.(2)). However, effect of the acids in the colon on the pH of the blood and sweat has not been identified. Thus, the significant decrease in the dermal emission flux of ammonia shown in Fig. 4 might be a result of reduction of absorbable ammonia in the colon by the formation of lactic acid and SCFAs at the dosage of 4 g d⁻¹.

The minimal dose of lactulose required for a prebiotic effect on the dermal emission of ammonia, however, remains to be determined considering the duration of

ingestion.

5. Conclusions

The prebiotic effect of lactulose at food dosage level of 4 g d⁻¹ was investigated on the dermal emission flux of ammonia for 12 healthy volunteers using a PFS-IC methodology. The results showed a significant decrease in the dermal emission of ammonia in the samples collected in the morning and at night. The emission flux was found to decrease with an increase in the number of faecal bifidobacteria. This indicated that the daily intake of lactulose at the studied food dosage level improved the colonic microflora and reduced the amount of ammonia emanating from the skin surface by the formation of lactic acid and SCFAs in the colon.

Acknowledgment

Authors awfully thank volunteers for their participation.

Key words : body odour, human skin gas, ammonia, lactulose, bifidobacteria

References

- 1) Mochalski, P., King, J., Unterkofler, K., Hinterhuber, H. and Amann, A. : Emission rates of selected volatile organic compounds from skin of healthy volunteers, *J. Chromatogr. B.*, **959**, 62-70, (2014).
- 2) Gallagher, M., Wysocki, C.J., Leyden, J.J., Spielman, A. I., Sun, X. and Preti, G. : Analyses of volatile organic compounds from human skin, *British J. Dermatol.*, **159**, 780-791, (2008).
- 3) Kimura, K., Sekine, Y., Furukawa, S., Takahashi, M. and Oikawa, D. : Measurement of 2-nonenal and diacetyl emanating from human skin surface employing passive flux sampler-GCMS system, *J. Chromatogr. B*, **1028**, 181-185, (2016).
- 4) Bernier, U.R., Kline, D., Barnard, D.R., Schreck, C.E. and Yost, R.A. : Analysis of human skin emanations by gas chromatography/mass spectrometry. 2. Identification of volatile compounds that are candidate attractants for the yellow fever mosquito (*Aedes aegypti*), *Anal. Chem.*, **72**, 747-756, (2009).
- 5) Sekine, Y., Sato, S., Kimura, K., Sato, H., Nakai, S. and Yanagisawa, Y. : Detection of tobacco smoke emanating from human skin surface of smokers employing passive flux sampler - GCMS system, *J. Chromatogr. B*, **1092**, 394-340, (2018).
- 6) Zamkah, A., Hui, T., Andrews, S., Dey, N., Shi, F. and Dheratt, S. : Identification of Suitable Biomarkers for Stress and Emotion Detection for Future Personal Affective Wearable Sensors, *Biosensors*, **10**, (2020). doi:10.3390/bios10040040
- 7) Umezawa, K., Sekine, Y., Kimura, K., Asai, S., Saito, T., Inokuchi, S. and Miyachi, H. : Emanation of fenitrothion from skin surface of patient who attempted to suicide by acute poisoning, *Rinsho Byori*, **66**, 949-956, (2018).
- 8) Yokokawa, T., Sato, T., Suzuki, S., Oikawa, M., Yoshihisa, A., Kobayashi, A., Yamaki, T., Kunii, H., Nakazato, K., Ishida, T. and Takeishi, Y. : Feasibility of skin acetone analysis in patients with cardiovascular diseases, *Fukushima J. Med. Sci.*, **64**, 60-66, (2018).
- 9) Nose, K., Mizuno, T., Yamane, N., Kondo, T., Ohtani, H., Araki, S. and Tsuda, T. : Identification of ammonia in gas emanated from human skin and its correlation with that in blood, *Anal. Sci.*, **21**, 1471-1474, (2005).
- 10) Sekine, Y., Kimura, K. and Umezawa, K. : What does human skin gas work for ?. *J. Jpn. Assoc. Odor Environ.*, **48** (6), 410-417, (2017). (in Japanese).
- 11) Petuely, F. : Lactobacillus bifidus flora produced in artificially-fed infants by bifidogenic substances (bifidus factor), *Z. Kinderheilkd*, **79**, 174-179, (1957).
- 12) Sakai, Y., Seki, N., Hamano, H., Ochi, H., Abe, F., Shimizu, F., Masuda, K. and Iino, H. : A study of the prebiotic effect of lactulose at low dosages in healthy Japanese women, *Biosci. Microb. Food Health*, **38**, 69-72, (2019).
- 13) Vince, A., Killingley, H. and Wrong O.M. : Effect of lactulose on ammonia production in a faecal incubation system, *Gastro-enterology*, **74**, 544-549, (1978).
- 14) Vince, A.J., McNeil, N.I., Wager, J.D. and Wrong, O.M. : The effect of lactulose, pectin, arabinogalactan and cellulose on the production of organic acids and metabolism of ammonia by intestinal bacteria in a faecal incubation system, *British J. Nutr.*, **63**, 17-26, (1990).
- 15) Bothe, M.K., Maathuis, A.J.M., Bellmann, S., van der Vossen J.M.B.M., Berressem, D., Koehler, A., Schwejda-Guettes, S., Gaigg, B., Kuchinka-Koch, A. and Stover, J.F. : Dose-Dependent Prebiotic Effect of Lactulose in a Computer-Controlled In Vitro Model of the Human Large Intestine, *Nutrients*, **9** (767), 1-14, (2017).
- 16) Patil, D.H., Westaby, D., Mahida, Y.R., Palmer, K.R., Rees, R., Clark, M.L., Dawson, A.M. and Silk D.B. : Comparative modes of action of lactitol and lactulose in the treatment of hepatic encephalopathy, *Gut*, **28**, 255-259, (1987).
- 17) Furukawa, S., Sekine, Y., Kimura, K., Umezawa, K., Asai, S. and Miyachi, H. : Simultaneous and multi-point measurement of ammonia emanating from human skin surface for the estimation of whole body dermal emission rate, *J. Chromatogr. B*, **1053**, 60-64, (2017).
- 18) Sugahara, H., Odamaki, T., Hashikura, N., Abe, F. and Xiao, J.Z. : Differences in folate production by bifidobacteria of different origins, *Biosci. Microbiota. Food Health*, **34**, 87-93, (2015).
- 19) Matsuki, T., Watanabe, K., Fujimoto, J., Kado, Y., Takada, T., Matsumoto, K. and Tanaka, R. : Quantitative PCR with 16S rRNA-gene-targeted species-specific primers for analysis of human intestinal bifidobacteria, *Appl. Environ. Microbiol.*, **70**, 167-173, (2004).
- 20) Satoh, T., Odamaki, T., Namura, M., Shimizu, T., Iwatsuki, K., Nishimoto, M., Kitaoka, M. and Xiao, J.Z. : *In vitro* comparative evaluation of the impact of lacto-N-biose I, a major building block of human milk oligosaccharides, on the fecal microbiota of infants, *Anaerobe*, **19**, 50-57, (2013).
- 21) Oswald, H.-H. : In vitro Gastrointestinal models for prebiotic carbohydrates: a critical review, *Current Pharmaceutical Design*, **25** (32), 3478-3483, (2019).
- 22) Bouhnik, Y., Attar, A., Joly, F.A., Riottot, M., Dyard, F. and Flourié, B. : Lactulose ingestion increases faecal bifidobacterial counts: a randomised double-blind study in healthy humans, *Eur. J. Clin. Nutr.*, **58**, 462-466, (2004).
- 23) Gibson, G.R., Hutkins, R., Sanders, M.E., Prescott, S.L., Reimer, R.A., Salminen, S.J., Scott, K., Stanton, C., Swanson, K.S., Cani, P.D., Verbeke, K. and Reid, G. : Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics, *Nat. Rev. Gastroenterol Hepatol.*, **14**, 491-502, (2017).
- 24) Shukla, S., Shukla, A., Mehboob, S. and Guha, S. : Meta-analysis: the effects of gut flora modulation using prebiotics, probiotics and synbiotics on minimal hepatic encephalopathy, *Alim. Pharm. Therap.*, **33**, 662-671, (2011).

- 25) Weber, F.L. : The use of lactulose in the treatment of portal-systemic encephalopathy, In "Nutritional Pharmacology", ed. by Spiller G.A, Alan R. Liss, New York, p217-253, (1981).
- 26) Crossley, I.R. and Williams, R. : Progress in the treatment of chronic portasystemic encephalopathy, *Gut*, **25**, 85-98, (1984).

ヒト皮膚表面から放散するアンモニアに及ぼすラクチュロース摂取の影響

関根嘉香^{1*}, 内山汐里¹, 戸高惣史¹, 境 洋平², 崎山 亮²,
越智 浩², 阿部文明², 浅井さとみ³, 梅澤和夫⁴

¹ 東海大学大学院理学研究科化学専攻, 〒259-1292 神奈川県平塚市北金目 4-1-1

² 森永乳業株式会社研究本部素材応用研究所, 〒252-8583 神奈川県座間市東原 5-1-83

³ 東海大学医学部基盤診療学系臨床検査学, 〒259-1193 神奈川県伊勢原市下糟屋 143

⁴ 東海大学医学部外科学系救急救命医学, 〒259-1193 神奈川県伊勢原市下糟屋 143

要旨：ヒト皮膚表面から放散するアンモニアは体臭の原因となり，ヒトの快・不快感に影響する．難消化性のラクチュロースは大腸に直接届き，ビフィズス菌増殖因子として働く．ビフィズス菌が産生する乳酸や短鎖脂肪酸の作用などにより，肝性脳症患者では血中アンモニア濃度が低減することが知られている．しかしながら，ラクチュロース摂取が皮膚からのアンモニア放散に及ぼす影響については報告例がなかった．そこで本研究では，健常者12名を対象にパッシブ・フラックス・サンプラー-イオンクロマトグラフ法を用いて，ラクチュロース摂取に伴う皮膚アンモニア放散フラックスの変化を調べた．ラクチュロース摂取量は食品用量である4g/日とした．その結果，摂取8日目後から皮膚アンモニア放散フラックスは有意に減少した．また便中ビフィズス菌数は，ラクチュロース摂取後に有意に増加し，便中ビフィズス菌数と皮膚アンモニア放散量との間に負の相関が見いだされ，皮膚アンモニアの放散には，腸内環境も関わっていることがわかった．

キーワード：体臭，皮膚ガス，アンモニア，ラクチュロース，ビフィズス菌

(受稿 令和2年5月28日)

(受理 令和2年9月1日)