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Effects of Isomaltodextrin Intake on the Skin Condition and QOL of Healthy Subjects:

A Randomized, Double-blind, Parallel-group, Placebo-controlled Study with Intake for 8 Consecutive Weeks

Yuki ISHIDA[1)] / Tsuyoshi SADAKIYO[1)] / Hikaru WATANABE[1)] / Hitoshi MITSUZUMI[1)] / Shimpei USHIO[1)] / Shuji NAKADA[2)]

● Abstract

Effects of the intake of isomaltodextrin (IMD), a water-soluble dietary fiber, on the skin condition and quality of life (QOL) of healthy adults were studied in a randomized, double-blind, parallel-group, placebo-controlled study. Forty healthy men and women with awareness of dry skin symptoms and a tendency toward constipation were administered IMD or placebo for 8 weeks, and 39 completed the study. The results showed that continuous intake of IMD had an improvement in skin condition with evaluation by a dermatologist in all subjects. Of these, in 15 of the 39 subjects who had dry skin probably caused by a disorder of intestinal environment, a significantly higher moisture content of the skin on the dorsal foot 8 weeks after intake of IMD versus placebo. Furthermore, the serum phenol concentration, an indicator of the composition of the intestinal environment, was significantly lower 8 weeks after intake, and the frequency of defecation and number of days of defecation, indicators of bowel movement, were significantly higher 4 to 8 weeks after intake compared with placebo. The above results suggest that IMD intake may be an effective way to moisturize the skin and promote QOL by improving the composition of the intestinal environment. The reason for this may be that continuous intake of IMD contributed to the continuous production of short-chain fatty acids in the intestine. IMD shows promise as an effective material for forming an intestinal environment that suppresses the production of phenols by intestinal bacteria.

Key words: isomaltodextrin, skin moisturization, skin condition, phenol, prebiotics, human study, QOL

1. INTRODUCTION

In general, constipation is a condition characterized by difficulty in having a smooth bowel movement due to decrease in intestinal peristaltic movement, caused by unhealthy dietary or lifestyle habits[12)13)]. According to the National Basic Survey[3)] conducted by the Ministry of Health, Labour and Welfare in 2016, 11% of Japanese adults have an awareness of constipation symptoms, and constipation is a serious issue for people in modern society due to multiple factors, such as increased stress, lack of exercise, and irregular habits, in addition to a simplified diet and nutritional intake biased by taste preference. Furthermore, constipation is said to be a sign of an imbalance in the intestinal flora, and repeated episodes of constipation increases the production of intestinal putrefactive products, including ammonia, amines, phenols, indoles, and hydrogen sulfate. These compounds, produced during the metabolism of proteins or amino acids by intestinal bacteria, are cytotoxic and are involved in aging or onset of lifestyle disease[11)12)]. Among them, phenols produced by the metabolism of tyrosine (an amino acid), were found to have harmful effects on the human body[10)16)]. Iizuka et al. reported the results of mouse and human studies showing that phenols had adverse effects on the formation of the epidermis[4)5)]. It is considered that phenols absorbed from the intestine are transferred to the bloodstream and are involved in the worsening of skin conditions. Thus, constipation has a great impact on quality of life (QOL). In general, rough skin is caused not only by external factors, such as ultraviolet light, dryness, or dirt on the skin, but also constipation, as one of internal factors, is noted. Several investigators have reported an association of constipation with skin conditions[1)8)13)14)], and an improvement in rough skin was reported as one of the beneficial effects of oral intake of fermented milk containing *Bifidobacterium* in humans[8)]. Moreover, Yamamoto et al.[20)] reported that about half of young women have an awareness of constipation symptoms, and 90% of them also have skin

1) R&D Division, Hayashibara Co., Ltd.
2) Medical Corporation Bokushinkai CLINTEXE Clinic

conditions, such as dryness or pimples. Thus, a great need for an improvement in rough skin exists among individuals with constipation.

We recently found that consecutive ad libitum intake of isomaltodextrin (IMD), a soluble dietary fiber, inhibited the production of phenols *in vivo* in a tyrosine load test in rats[17]. IMD, a highly branched α-glucan with a characteristic structure composed only of glucose units, is produced from starch by enzymatic reactions with α-glucosyltransferase and α-amylase derived from *Paenibacillus alginolyticus* PP710[19]. Since IMD has different binding profiles from indigestible dextrin or polydextrose, which are known as similar soluble dietary fibers, IMD is expected to have unique physiological effects. We have reported that consecutive intake of IMD improved bowel movements in humans[6)7)]. In addition, since Nishimura et al. reported that consecutive administration of IMD increased *Bifidobacterium* populations in rats[15], IMD is expected to improve rough skin caused by disorders of the intestinal environment. However, the effect of IMD intake on skin conditions in humans has not yet been reported. Therefore, we studied the effects of IMD intake on skin condition and QOL in healthy adult men and women with symptoms of dry skin and a tendency toward constipation.

2. MATERIALS AND METHODS

2.1. Subjects

A total of 40 healthy Japanese volunteers (4 men and 36 women) aged between 20 and 59 years with awareness of dry skin symptoms and a tendency toward constipation (stool frequency of ≤ 4 per week) were enrolled. Recruitment of the study candidates, an explanatory meeting, and investigation of their background characteristics (pre-examination) were conducted by TES Holdings Co., Ltd. (Tokyo, Japan). From 81 study candidates, 40 subjects had not received medications that would interfere with the results and no chronic physical disease including skin disease, and were judged suitable for enrollment by the investigator. Other exclusion criteria were as follows: 1) food or seasonal allergy; 2) smoker; 3) use of health foods or supplements that would interfere with the results; 4) pregnancy, lactation, or planning to become pregnant; 5) participation in another clinical study. The number of subjects in this study was determined on the basis of a similar study[8], in which the difference in skin moisture content between the test article and placebo was about 20% (about a 25% standard deviation). Applying the above results to this study, we converted these values to 4 for the difference between the study diets and 4.5 for the standard deviation, because the skin moisture content was determined with a Corneometer® CM825 (Courage + Khazaka electronic GmbH, Cologne, Germany). As a result, the number of subjects needed for the study was 17. Considering a dropout rate of about 15%, we set the number needed as 20 subjects per group.

2.2. Study diet

The study diet consisted of the test food and a placebo food. Five grams of the test food (IMD [Fibryxa®, Hayashibara Co., Ltd., Okayama, Japan]) and the placebo food (maltodextrin [Pinedex® #1, Matsutani Chemical Industry Co., Ltd., Hyogo, Japan]) on the dry solid basis was inserted into an aluminum sachet. Both the test and placebo foods were a white powder without odor or taste, and were confirmed to be indistinguishable in appearance or taste by subjects before they were used.

The dietary fiber content per sachet was calculated to be 4.29 g, based on the dietary fiber content of IMD (determined with an enzyme-HPLC method) of the lot used in this study.

2.3. Study design

This study was a randomized, double-blind, parallel-group, placebo-controlled study. An individual who was not directly involved in the study randomly assigned the 40 subjects to 2 groups of 20 subjects each (groups A and B) using random numbers, so that the groups had the same sex ratio and age range, based on the skin moisture content at pre-examination. Next, a study diet was assigned to each group. The Assignment Person sealed the assignment table for subjects and study diets and kept it confidential until the fixation of all data. We confirmed that there were no significant differences in sex ratio or age range between groups before starting the study.

The subjects received either the test or placebo food once daily (1 sachet) for 8 consecutive weeks, the intake timing was not limited. They visited the clinic for examination before intake (week 0), and at 4 and 8 weeks after intake (3 times in total). Each subject kept a diary to note the intake of the study diet and bowel movement data during the study period. The subjects were instructed not to work outdoors for a long time, and to carry on as usual during the study period. In particular, a shortage of sleep, overeating/overdrinking, or over-exercise was avoided to maintain quality and quantity in their daily lives. Furthermore, to unify the conditions of the subjects as much as possible, a pre-specified meal was given for supper on the day before each visit day (3 times). At each visit, the subjects had fasted from after supper (a pre-specified meal) on the night before until the next morning, for more than 12 hours, except for a small amount of water.

2.4. Endpoints and methods of measurement and assessment

The primary endpoints were dermatologist's findings (skin texture and skin quality) and skin measurements (skin moisture content and transepidermal water loss as indicators of skin condition). The secondary endpoints

were serum concentrations of putrefactive products (phenol and *p*-cresol) as indicators of the composition of the intestinal environment; and a questionnaire survey on bowel movements and skin condition as indicators of QOL.

2.5. Visual assessment by a dermatologist

A dermatologist assessed the texture and quality of the skin as objective indicators for dryness of skin condition. These assessments were performed in the same environment as that of the skin measurements.

The texture of the skin was assessed at the center of a line connecting the bottom of the earlobe and the edge of the lips on the left side of the face, and the 3 items including *sulci cuts*, *cristae cutis*, and *overall assessment* were scored on a 5-point scale (−2＝poor, −1＝slightly poor, 0＝average, 1＝slightly good, 2＝good) using a DermLite (DL100, J. Hewitt, CA, USA) and a Digital Microscope (KH-1300, Hirox Co. Ltd., Tokyo, Japan). The lower the score, the poorer the skin texture.

The entire face was assessed for skin quality, and 5 items, including *dryness*, *erythema*, *scaly skin*, *irritation*, and *itching* were scored on a 5-point scale by visual examination and interview (0＝none [no symptoms], 1＝minor [very slight symptoms], 2＝mild (slight symptoms), 3＝moderate [apparent symptoms], 4＝sever [severe symptoms]). The higher the score, the poorer the skin quality.

2.6. Skin measurements

As indicators of water retention and barrier function of the skin, the skin moisture content and transepidermal water loss were measured at the following 3 sites: the cheek (at the top of the cheekbone), the upper arm (at the medial side of the upper arm), and the foot (at the dorsal foot). After the measurement sites were washed, the subjects were acclimatized in a constant temperature and humidity room (set at 21℃ ± 1℃ and 50% ± 5% relative humidity) for more than 20 minutes prior to measurements.

The skin moisture content was measured 5 times at each site with a Corneometer® CM825. The highest and lowest values were eliminated, and the mean of the remaining 3 measurements was used. The transepidermal water loss was continuously measured every second for more than 60 seconds with a Tewameter® TM300 (Courage + Khazaka Electronic GmbH, Cologne, Germany). The mean of the measurements (of which standard deviation was minimum) for 30 seconds before the end of measurement was used.

2.7. Determination of serum concentrations of putrefactive products

Blood was collected from each subject to determine the circulating serum concentrations of phenols (phenol and *p*-cresol), putrefactive products, as indicators of the composition of the intestinal environment. Prior to blood collection, subjects did not eat or drink for more than 12 hours after supper (pre-specified meal) on the day before each visit day. Blood samples were centrifuged at 3000 rpm at 15℃ to 24℃ for 10 minutes, and serum was collected and stored at − 80℃ until analysis.

The serum concentrations of phenols (phenol and *p*-cresol) were analyzed by high-performance liquid chromatography (HPLC) according to the method of Kano et al.[8], with some modifications. A total of 25 μL of serum, 500 μL of ion-exchanged water (IEW), 250 μL of concentrated hydrochloric acid, and 10 μL of the internal standard (10 μg/mL in IEW) were mixed and heated to 95℃ for 60 minutes to hydrolyze the phenol conjugates. After cooling, the whole solution was loaded onto an Oasis HLB 1 cc (30 mg) reversed-phase cartridge (Waters, MA, USA), washed 2 times with IEW (1000 μL), and eluded with methanol (500 μL) and IEW (500 μL) to prepare a sample for HPLC analysis. The Oasis HLB 1 cc was previously conditioned and equilibrated with methanol (500 μL) and IEW (1000 μL) prior to use.

The HPLC system (Prominence, SHIMADZU, Tokyo, Japan) was equipped with API 3200TM LC-MS/MS (SCIEX, MA, USA) and Kinetex® Biphenyl (SHIMADZU, Tokyo, Japan; 2.1 mm (i.d.) × 100 mm (length) and 5 μm particle size) as an HPLC-APCI-MS apparatus. The sample (10 μL) was automatically injected onto the HPLC column and eluted with a linear gradient of acetonitrile-IEW at 40℃ at a flow rate of 0.2 mL/minute. The concentrations of phenols (phenol and *p*-cresol) were calculated according to calibration curves, which were obtained by determining the ratio of the peak area of phenol (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) or *p*-cresol (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) to that of the internal standard (4-chlorophenol, Tokyo Chemical Industry Co., Ltd., Tokyo, Japan).

2.8. Questionnaire survey on bowel movements and skin condition

Since this study was conducted in subjects with symptoms of dry skin and a tendency toward constipation, we carried out a questionnaire survey on bowel movements and skin condition to examine whether IMD intake contributes to the improvement of QOL.

The questionnaire on bowel movements was in the form of a diary: the number of bowel movements, number of days of defecation, and fecal amount (converted to a medium-sized egg unit: 4.0 cm in the minor axis and 5.5 cm in the major axis) were tallied every week during the study period.

The questionnaire on skin condition contained 3 questions about subject's perception, based on the Visual Analogue Scale (VAS), which was used for questions about the face and whole body. The subject was asked to

Table 1 The characteristics of subjects

	Selected subjects			All subjects			Subgroup analysis subjects		
	Total	Test food group	Placebo food group	Total	Test food group	Placebo food group	Total	Test food group	Placebo food group
Men (n)	4	2	2	4	2	2	1	0	1
Women (n)	36	18	18	35	17	18	14	6	8
Total subjects (n)	40	20	20	39	19	20	15	6	9
Age (years old)	40.3 ±10.1	39.7 ±9.0	40.9 ±11.2	40.8 ±9.7	40.7 ±8.0	40.9 ±11.2	44.0 ±9.6	40.7 ±8.6	46.2 ±10.0
Skin moisture content (cheek)	40.5 ±10.1	39.9 ±10.7	41.1 ±9.8	40.5 ±10.3	39.9 ±11.0	41.1 ±9.8	40.3 ±9.0	40.8 ±11.2	39.9 ±7.9

Mean ± SD

mark his/her current condition on the 100-mm line with anchor statements on the left end (*never better* or *not noticeable at all / insensitive / not concerned*) and on the right end (*never worse* or *very noticeable / sensitive / concerned*), and the examiner scored the VAS by measuring the distance from the left end. Higher scores reflected lower QOL.

Questions about the face

Overall satisfaction; *skin conditions*; *moisture (wetness) of the skin*; *rough skin around the eyes or mouth*; *wrinkles around the eyes*; *softness of the skin*; *transparency of the skin*; *firmness or elasticity of the skin*; *plump feeling of the skin*; *rough texture of the skin*; *redness of the skin*; *notable pores in the skin*; *clogged pores in the skin*; *sagging skin*; *pigmented spots*; and *make-up sitting on the skin* (only for women)

Questions about the whole body

Dryness of the skin; *redness of the skin*; *itching of the skin*; and *condition of the skin*

Specific perception

Free/open description by the subject

2.9. Safety

Adverse events based on signs and symptoms were recorded by the investigator or subinvestigators during the study period.

2.10. Ethics

The study was approved by the ethics committee of Hayashibara Co., Ltd. (December 19, 2017, registration No. 211) and by the ethical review committee of Oriental Ueno Health Checkup Center (Tokyo, Japan; January 23, 2018, Protocol No. HR-2018-HN03), and thereafter conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and ethical guidelines for medical and health research involving human subjects (The Ministry of Health, Labour and Welfare Ministerial Notification, Ministry of Education, Culture, Sports, Science and Technology) under the supervision of Medical Corporation Bokushinkai CLINTEXE Clinic (Tokyo, Japan). The study was pre-registered in the Clinical Trial Registration System operated by the University Hospital Medical Information Network.

All subjects received complete information on the content, purpose, and significance of the study, and signed a consent form about participation in the study and about possible publication of the study results, in which all information was recorded in such a manner that subjects could not be identified.

2. 11. Statistical analysis

All data were expressed as mean ± standard deviation (SD) or standard error (SE) of the mean. For efficacy assessment, the comparison before and after intake of each study diet was performed with a paired t-test or Wilcoxon rank sum test, and the comparison between intake groups was performed with an unpaired t-test or Wilcoxon rank sum test, with a P value < 0.05 (two-sided) being considered statistically significant. SAS® 9.4 (SAS Institute Inc., NC, USA) or IBM SPSS Statistics19 (International Business Machines Corporation, NY, USA) was used for statistical analysis.

In addition, to assess the results in more detail, a subgroup analysis was performed by serum phenol concentration, as an indicator of the composition of the intestinal environment, according to the protocol.

3. RESULTS

3.1. Subjects

The study started with 40 subjects; 1 subject in the test food group withdrew before the examination at 4 weeks after intake, because the subject could not be present for the examination due to schedule reasons. The intake rate of the study diet was 99.9% ± 0.6% for the 39 subjects who completed the study, and 100% until the day before the one subject withdrew. The 40 subjects who received

Table 2　Visual assessment by a dermatologist

		Item	Group	week 0	4 weeks	8 weeks
Skin texture	All subjects	Crista cutis	Test	− 0.5 ± 0.1	− 0.2 ± 0.1 ‡	− 0.1 ± 0.2
			Placebo	− 0.7 ± 0.2	− 0.7 ± 0.1 ‡	− 0.1 ± 0.1 *
		Sulci cutis	Test	− 0.8 ± 0.1	− 0.8 ± 0.1	− 0.6 ± 0.1
			Placebo	− 1.0 ± 0.1	− 0.9 ± 0.1	− 0.8 ± 0.1 †
		Overall assessment	Test	− 0.8 ± 0.1	− 0.6 ± 0.1	− 0.4 ± 0.2 *
			Placebo	− 0.9 ± 0.1	− 0.9 ± 0.1	− 0.4 ± 0.2 *
	Subgroup analysis subjects	Crista cutis	Test	− 0.8 ± 0.2	− 0.3 ± 0.3	− 0.3 ± 0.3
			Placebo	− 0.6 ± 0.2	− 0.6 ± 0.2	0.0 ± 0.2 †
		Sulci cutis	Test	− 0.8 ± 0.2	− 0.8 ± 0.2	− 0.7 ± 0.2
			Placebo	− 0.9 ± 0.1	− 0.9 ± 0.1	− 0.7 ± 0.2
		Overall assessment	Test	− 1.0 ± 0.0	− 0.7 ± 0.2	− 0.7 ± 0.2
			Placebo	− 0.8 ± 0.1	− 0.9 ± 0.1	− 0.2 ± 0.2 †
Skin quality	All subjects	Dryness	Test	1.7 ± 0.2	1.3 ± 0.2 * ‡	1.2 ± 0.2 *
			Placebo	1.9 ± 0.2	1.9 ± 0.2 ‡	1.5 ± 0.2
		Erythema	Test	0.3 ± 0.1	0.1 ± 0.1	0.1 ± 0.1 *
			Placebo	0.5 ± 0.2	0.3 ± 0.2	0.2 ± 0.1
		Scaly skin	Test	1.3 ± 0.2	0.9 ± 0.2 * ‡	0.8 ± 0.2 *
			Placebo	1.5 ± 0.2	1.7 ± 0.2 ‡	1.2 ± 0.2
		Irritation	Test	0.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
			Placebo	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
		Itching	Test	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
			Placebo	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0
	Subgroup analysis subjects	Dryness	Test	1.8 ± 0.4	1.5 ± 0.2	1.0 ± 0.3
			Placebo	1.7 ± 0.2	1.7 ± 0.2	1.1 ± 0.3 †
		Erythema	Test	0.5 ± 0.2	0.2 ± 0.2	0.0 ± 0.0 †
			Placebo	0.3 ± 0.2	0.2 ± 0.2	0.2 ± 0.2
		Scaly skin	Test	1.5 ± 0.2	1.0 ± 0.4 †	0.5 ± 0.3 *
			Placebo	1.3 ± 0.3	1.3 ± 0.3	0.9 ± 0.2
		Irritation	Test	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
			Placebo	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
		Itching	Test	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
			Placebo	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0

Mean ± SE
Comparison within a group (* $p < 0.05$, † $p < 0.10$)
Comparison between groups (‡ $p < 0.05$)

at least one serving of the study diet were included in the safety analysis data set (Full Analysis Set [FAS]), and the 39 subjects who completed the study were included in the efficacy analysis data set (Per Protocol Set [PPS]). For a more detailed efficacy assessment of IMD, a subgroup analysis was performed for 15 subjects whose serum phenol concentrations were higher than the mean value (0.83 ng/mL in serum), in addition to the analysis of all 39 subjects. The characteristics of the subjects analyzed are shown in **table 1**.

3.2. Visual assessment by a dermatologist

Table 2 shows the scores for skin texture and skin quality, with each item evaluated by a dermatologist.

In the comparison of skin texture between groups, there was a significant improvement in the crista cutis score in the test food group compared with that in the placebo food group at 4 weeks after intake. Meanwhile, compared with before intake, there were significant improvements in the overall assessment score in both groups and a significant improvement in the crista cutis score in the

Table 3 Skin measurements

		Site	Group	week 0	4 weeks	8 weeks	Percent change (%)	
							4 weeks	8 weeks
Skin moisture content	All subjects	Cheek	Test	39.9 ± 2.5	52.4 ± 3.0 *	52.6 ± 3.1 *	135.9 *	140.3 *
			Placebo	41.1 ± 2.2	49.3 ± 2.3 *	51.4 ± 2.2 *	122.9 *	129.8 *
		Upper arm	Test	25.8 ± 1.9	27.0 ± 1.2	25.1 ± 1.2 §§	110.4 †	103.0
			Placebo	24.9 ± 1.3	26.6 ± 1.7	28.7 ± 1.5 * §§	108.6	117.3 *
		Foot	Test	22.1 ± 1.1	23.8 ± 1.4	24.0 ± 1.4	108.6 †	110.0 †
			Placebo	22.2 ± 1.6	22.7 ± 1.4	21.1 ± 1.3	108.3	100.9
	Subgroup analysis subjects	Cheek	Test	40.8 ± 4.6	55.7 ± 4.8 *	52.5 ± 5.5 †	146.0 †	137.9
			Placebo	39.9 ± 2.6	48.0 ± 2.0 *	51.5 ± 2.9 *	122.7 *	131.9 *
		Upper arm	Test	29.1 ± 2.6	28.9 ± 1.7	27.3 ± 2.1	102.4	98.1
			Placebo	25.6 ± 2.1	25.1 ± 1.7	27.8 ± 1.7	100.5	110.5 †
		Foot	Test	22.3 ± 1.7	25.0 ± 1.8	24.8 ± 2.0 ‡‡	113.2	112.8
			Placebo	21.2 ± 2.3	21.7 ± 2.2	19.2 ± 1.6 ‡‡	105.1	96.8
Transepidermal water loss (g/hm^2)	All subjects	Cheek	Test	18.6 ± 0.9	19.2 ± 1.4	18.4 ± 1.1	103.9	100.5
			Placebo	17.8 ± 1.0	19.4 ± 1.5	16.9 ± 1.1	109.8	95.6
		Upper arm	Test	8.9 ± 0.5	8.5 ± 0.6	8.6 ± 0.5	97.3	98.2
			Placebo	9.2 ± 0.6	7.5 ± 0.7 *	7.3 ± 0.6 *	83.8 *	86.6
		Foot	Test	10.4 ± 0.8	11.1 ± 0.6	9.0 ± 0.7 †	114.1	90.6
			Placebo	10.4 ± 0.6	10.3 ± 0.5	9.7 ± 0.7	103.1	95.8
	Subgroup analysis subjects	Cheek	Test	17.1 ± 1.7	18.9 ± 1.5	18.5 ± 2.4	113.3	110.5
			Placebo	15.6 ± 1.1	17.7 ± 1.8	16.0 ± 1.8	113.6	101.2
		Upper arm	Test	8.1 ± 0.6	8.2 ± 1.1	8.1 ± 0.9	102.2	102.2
			Placebo	8.2 ± 0.7	7.4 ± 0.5	6.9 ± 1.0	95.7	93.0
		Foot	Test	9.3 ± 1.1	10.4 ± 1.3	8.7 ± 1.7	119.1	91.7
			Placebo	10.2 ± 0.8	9.3 ± 0.9	10.4 ± 1.3	92.7	106.5

Mean ± SE

Comparison within a group (* $p < 0.05$, † $p < 0.10$)

Comparison between groups (‡ $p < 0.05$, § $p < 0.10$)

The percent change shows the rate of change on skin moisture content or transepidermal water loss from week 0 (before intake).

placebo food group at 8 weeks after intake. In the comparison of skin quality between groups, there were significant improvements in the dry skin score and scaly skin score in the test food group compared with those in the placebo food group 4 weeks after intake. Meanwhile, compared with before intake, there were significant improvements in the erythema score as well as in the dry skin score and scaly skin score in the test food group at 4 or 8 weeks after intake.

The subgroup analysis showed no significant differences in skin texture or skin quality between groups. However, compared with before intake, there was a significant improvement in skin quality in the test food group at 8 weeks after intake.

3.3. Skin measurements

Table 3 shows the data on skin moisture content and transepidermal water loss and the percent change in these parameters from week 0 (before intake) at 3 sites.

In the comparison of skin moisture content between groups, there was no significant difference at any site. However, compared with before intake, there were significant increases at 4 and 8 weeks after intake in the cheek in both groups, and at 8 weeks in the upper arm in the placebo food group. On the foot, there were no changes in either group. However, there was a significant percent increase or a trend toward an increase at all 3 sites where skin measurements were performed in the test food group 4 or 8 weeks after intake. In the comparison of transepidermal water loss between groups, there was no significant difference at any site. However, compared with before intake, there were significant decreases in the upper arm in the placebo food group at 4 and 8 weeks after intake, and there was a decreasing

Table 4　Serum phenols

		Item	Group	4 weeks	8 weeks
Changes in serum concentrations of putrefactive products	All subjects	Phenol (ng/mL Plasma)	Test	− 0.1 ± 0.2	− 0.7 ± 0.1 *
			Placebo	0.0 ± 0.1	− 0.6 ± 0.1 *
		p-Cresol (ng/mL Plasma)	Test	0.7 ± 0.3 †	0.0 ± 0.4
			Placebo	0.0 ± 0.2	− 0.4 ± 0.3
	Subgroup analysis subjects	Phenol (ng/mL Plasma)	Test	− 0.7 ± 0.4	− 1.3 ± 0.1 * ‡
			Placebo	− 0.1 ± 0.1	− 0.8 ± 0.1 * ‡
		p-Cresol (ng/mL Plasma)	Test	0.5 ± 0.3	− 0.4 ± 0.5
			Placebo	0.0 ± 0.3	− 1.2 ± 0.4 *

Mean ± SE
Comparison within a group（* $p < 0.05$, † $p < 0.10$）
Comparison between groups（‡ $p < 0.05$）

trend on the foot in the test food group at 8 weeks after intake. In the cheek, there were no changes in either group.

The subgroup analysis showed that the skin moisture content on the foot in the test food group was significantly higher than that in the placebo food group at 8 weeks after intake. However, compared with before intake, there was a significant increase or increasing trend at 4 and 8 weeks after intake in the cheek in both groups. In the upper arm, there were no changes in either group. In addition, there was a significant percent increase or increasing trend at 4 and 8 weeks after intake in the cheek and the upper arm in the placebo food group, and there was an increasing trend at 4 weeks after intake in the cheek in the test food group. With regard to transepidermal water loss, there were no significant changes including percent changes.

3.4. Changes in serum phenol and *p*-cresol concentrations

Table 4 shows the changes in serum phenol and *p*-cresol concentrations from before intake, using blood samples collected from the subjects.

In the comparison of serum phenol concentration changes, there were no significant differences between groups. However, compared with before intake, there were significant decreases in both groups at 8 weeks after intake. Also, in the comparison of serum *p*-cresol concentration changes, there were no significant changes in either group and no significant differences between groups.

The subgroup analysis showed that there were significant decreases in serum phenol concentrations in both groups at 8 weeks after intake compared with those before intake, and at that time, there was a significantly lower value in the test food group than in the placebo food group. With regard to serum *p*-cresol concentration, there were significant decreases in the placebo food group compared with that before intake, but there were no significant differences between groups.

3.5. Questionnaire survey on bowel movements and skin condition

The number of bowel movements, number of days of defecation, and fecal amount were tallied every week. In comparisons of the number of bowel movements, number of days of defecation, or fecal amount, there were no significant differences between groups. However, compared with before intake, there was a significant increase or a trend toward an increase in the number of bowel movements, number of days of defecation, and fecal amount from week 1 to week 8 after intake in both groups (data not shown).

Table 5 shows the data of the subgroup analysis. There was a significantly higher number of bowel movements at 6 weeks after intake and a significantly higher number of days of defecation at 4, 6, and 8 weeks after intake in the test food group than in the placebo food group. Furthermore, compared with before intake, there was a significant increase or a trend toward an increase in the number of bowel movements, number of days of defecation, and fecal amount from week 3 to week 8 after intake in the test food group; however, there were no significant changes in the placebo food group.

A VAS score for each question about the subject's perception of skin condition and changes in skin condition was tallied. Compared with before intake, there was a significant improvement or a trend toward improvement in perception on many questions at 4 or 8 weeks after intake in both groups. Of these questions, the scores and changes in score for *redness of the skin* of the face and whole body were significantly improved at 4 or 8 weeks after intake in the test food group, but no significant changes were observed in the placebo food group. Furthermore, in a comparison between groups, there was a significant improvement in the score for *pigmented*

Table 5 Bowel movements

	Item	Group	week 0	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	7 weeks	8 weeks
Subgroup analysis subjects	Defecation frequency (number/week)	Test	3.7 ± 0.7	5.2 ± 1.3	5.8 ± 1.6	7.0 † ± 2.0	5.8 * ± 1.1	4.7 ± 0.7	6.3 * ± 1.1 ‡	5.3 ± 1.0	5.8 * ± 1.3
		Placebo	3.2 ± 0.2	4.0 ± 0.6	3.9 ± 0.7	4.4 ± 1.0	3.9 ± 0.8	3.8 ± 0.5	3.8 ± 0.4	3.8 ± 0.5	3.7 ± 0.6
	Number of days of defecation (number of days/week)	Test	3.2 ± 0.4	3.5 ± 0.4	4.2 ± 0.6	4.7 ± 0.8	4.8 * ± 0.5 ‡	4.2 † ± 0.6	5.2 * ± 0.5 ‡	4.5 ± 0.7	4.8 * ± 0.5 ‡
		Placebo	2.9 ± 0.1	3.7 ± 0.6	3.4 ± 0.5	3.8 ± 0.5	3.2 ± 0.4	3.6 ± 0.4	3.3 ± 0.3	3.3 ± 0.3	3.3 ± 0.4
	Fecal amount (number of egg unit/week)	Test	7.0 ± 1.9	6.8 ± 1.3	8.9 ± 1.4	10.1 ± 1.6	8.8 ± 1.2	7.9 ± 1.2	10.1 * ± 1.6	8.7 ± 1.6	9.1 * ± 1.7
		Placebo	5.3 ± 0.7	7.6 ± 1.6	7.1 ± 1.5	8.4 ± 2.2	7.8 ± 1.7	7.5 ± 1.4	7.3 ± 1.1	7.2 ± 1.2	6.3 ± 1.1

Mean ± SE
Comparison within a group (* $p < 0.05$, † $p < 0.10$)
Comparison between groups (‡ $p < 0.05$)

spots on the face at 8 weeks after intake in the test food group compared with the placebo food group (data not shown).

The stratified analysis also showed a significant improvement or a trend toward improvement in the VAS score and changes in score on many questions at 4 or 8 weeks after intake in both groups, compared with before intake. However, in comparisons between groups, there was a significant improvement or a trend toward improvement in the change in score for *clogged pores* on the face at 4 weeks after intake and in the score for *pigmented spots* on the face at 4 or 8 weeks after intake in the test food group compared with the placebo food group (data not shown).

3.6. Safety

Regardless of the type of study diet consumed, transient loose stool or abdominal distension developed, but disappeared in a short time in both the test and placebo food groups. There were no serious adverse events or adverse reactions based on the symptoms reported in the questionnaire for all subjects who received the study diet during the study period, including the subject who withdrew, in this study. No abnormal changes in laboratory tests were noted before and 8 weeks after intake.

4. DISCUSSION

The results showed that there was a significant improvement dry skin condition with evaluation by a dermatologist after intake of IMD versus placebo in all healthy subjects with awareness of dry skin symptoms and a tendency toward constipation. While, there were no significant difference between IMD and placebo in skin measurements with instruments because of both groups showed an improvement dry skin condition. Since the purpose of this study was to examine the improvement of skin condition through improvement of the intestinal environment, we selected those subjects who were likely to have a disordered intestinal environment and who had awareness of dry skin and a tendency toward constipation. However, it was likely that we included subjects who had an awareness of constipation tendency but whose intestinal environment was not very disordered. Dry skin is caused by several factors other than a disordered intestinal environment. Yamamoto et al.[20] reported that about 70% of young women with an awareness of healthy bowel movements, although significantly fewer than those with an awareness of constipation, had skin troubles, and they indicated that psychological stress was the major cause. Accordingly, we cannot rule out the possibility of including subjects whose dry skin was caused by factors other than a disordered intestinal environment.

Therefore, we conducted a subgroup analysis in 15 of the 39 subjects who had dry skin probably caused by a disorder of intestinal environment, whose serum phenol concentrations before intake were higher than the mean value in all subjects. As the results, although there was no statistically significant difference between IMD and placebo in visual assessment by a dermatologist, there was a significantly higher moisture content in the foot skin 8 weeks after intake of IMD versus placebo. Compared with before intake, there was also a significant increase in cheek skin moisture content in the test food group, showing an improvement in dry skin.

The skin consists of the epidermis, the dermis and the subcutaneous tissue. The horny layer of the epidermis, the outermost layer of the skin, provides a physical

barrier and has a biological defense mechanism. It also plays an important role in retention of water within the skin, and the intercellular lipid lamellae located between the horny cells contributes to water retention and skin moisturization. The barrier function of the horny layer is affected by various external factors (including temperature, humidity, and ultraviolet light), and is also reported to maintain skin homeostasis internally through the intake of various foods[2)9)18)]. Phenols are putrefactive products produced by specific intestinal bacteria from some amino acids in protein in foods, and can be indicators of the composition of the intestinal environment. It was reported that phenols absorbed from the intestine were transferred to the bloodstream and involved in worsening of skin conditions[4)5)]. We have recently found in rats that phenol and *p*-cresol concentrations in the body were increased by tyrosine load, and co-administration of IMD remarkably reduced these concentrations[17)]. In addition, we have confirmed in hairless rats that the differentiation of epidermal cell was delayed and the corneocyte size was decreased by tyrosine load, and co-administration of IMD maintained the differentiation of epidermal cell and the corneocyte size remained normal (unpublished data). Kano et al. reported that intake of fermented milk containing *Bifidobacterium* reduced the blood concentration of phenol, maintained skin moisture, and likely improved keratinocyte differentiation in healthy adult women. According to their conclusions, suppression of the production of phenols in the intestine by intake of fermented milk containing *Bifidobacterium* inhibited the migration of phenols into the blood and skin, which led to an improvement in the condition of the epidermis and the maintenance of skin structure, resulting in an enhancement of moisture retention[8)]. Therefore, in subjects who has dry skin probably caused by a disorder of intestinal environment intake of IMD may also have suppressed the production of phenols in the intestine and inhibited their migration into the blood and skin. As a result, it is speculated that the improvement of keratinocyte differentiation and maintenance of the skin structure enhanced the moisture retention in the skin. Furthermore, since the test food group showed remarkable improvement in bowel movements in this study, facilitated excretion of phenols by IMD may be one of the reasons for the improvement in dry skin and the occurrence of a moisturizing effect.

Skin measurements were performed on the cheek, the upper arm, and the foot, and showed a remarkable moisturizing effect of IMD intake on the foot in the subgroup analysis. No superiority of IMD to the placebo was found except for the foot skin, although there was a tendency toward improvement in the skin moisture content of the cheek. One of the reasons for this seems to be the effects of weather. This study was conducted from the winter to spring (between February and April 2018), when the skin is said to be dry. However, the weather record in Tokyo by the Japan Meteorological Agency (Tokyo, Japan), where the study facility was located, shows that the temperature and humidity in 2018 remained higher than usual in terms of the annual average; in particular, the average humidity in March was higher by about 10％. Therefore, the unexpectedly not-dry-weather seemed to be a reason for the difficulty in detecting an effect of IMD intake. Nevertheless, IMD intake had an improving effect on the dry skin of the foot in the subgroup analysis. Of the 3 sites measured, the foot had the lowest skin moisture, an indicator of the water retention function of the skin. Moreover, compared with the other 2 sites, the foot is the site with the lowest sebum secretion, and is easily affected by dryness due to rough skin. The foot may have been the site where the moisturizing effect by oral intake of the study diet can be seen easily compared with other sites, because the foot is unlikely to be affected by external moisturizing factors such as cosmetics. From these results, the moisturizing effect of IMD, looks promising not only on the foot that was found to be effective in this study, but also for whole body skin.

Furthermore, the placebo food group also showed significant improvement in bowel movements compared with before intake. This seems to be a placebo effect; however, the improvement of bowel movements in subjects with an awareness of a tendency toward constipation may have led not only to the decrease in intestinal putrefactive products due to an increase in defecation frequency, but also to the reduction in psychological stress, and thus, this placebo effect may have greatly affected the study results. However, all subjects in the test food group showed an improvement in skin condition with evaluation by a dermatologist (objective assessment), including improved perception. This suggests that IMD is involved not only in bowel movements but also in inhibition of the production of substances that have adverse effects on the skin by altering the intestinal flora, as we found in the rat studies. The mechanism is speculated as follows: continuous intake of IMD provides a carbon source, reduces pH in the intestine by contributing to short-chain fatty acid production in the intestine, and thereby restores the balance of intestinal flora. In particular, having a characteristic structure, IMD is effective for forming an intestinal environment in which the production of phenols by intestinal bacteria is inhibited.

Consequently, continuous intake of IMD can be an effective measure to enhance daily QOL by improving the disordered intestinal environment, including constipation, which often occurs in modern life, leading to mitigation/improvement of dry skin or discomfort.

5. CONCLUSIONS

The results of this study indicate that continuous intake of IMD improves skin condition, including skin moisture, in subjects with dry skin symptoms likely caused by a disordered intestinal environment. It is speculated that the effects are the result of changes induced in the intestinal flora by continuous intake of IMD. Having a characteristic structure, IMD seems to contribute to the formation of an intestinal environment in which production of phenols is inhibited.

CONFLICT OF INTEREST

In this study, the sponsor, Hayashibara Co., Ltd. provided the appropriate funding and entrusted TES Holdings Co., Ltd. to execute and manage the study. Among the authors, Y. I., T. S., H. W., M. H. and S. U. belong to Hayashibara Co., Ltd. The investigator, S. N. belongs to Medical Corporation Bokushinkai CLINTEXE Clinic.

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