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Original article

A study of the health actions of consuming a mature extract of brown rice, consisting of the sub-aleurone layer, germ blastula, and crushed cells.

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Abstract

***Objective*:** The bran layer of brown rice contains a variety of nutritional components, which have been suggested to be useful in maintaining homeostasis of body functions and improving health. However, its effect on subjective symptoms in humans is still unclear. In this study, we investigated the effect of oral intake of a test food made from the sub-aleurone layer (part of the bran layer) of brown rice on subjective symptoms.

***Methods*:** A total of 1,023 healthy men and women (313 men and 710 women) were included in the study group. They consumed the test food (3.5 g per package) for one month, and subjective symptoms were investigated using the Anti-Aging QOL Common (53 items in total). A total of 3,002 age- and sex-adjusted cases (930 men and 2,072 women) from the Doshisha University Anti-Aging Research Center' (AARC) data were used as the hypothetical control.

***Results*:** In the test group, the items with the highest improvement rates were (a) "constipation" (45.5%), (b) "liable to catch cold" (35.6%), (c) "lethargy" (33.7%), and (d) "skin problems" (33.3%) ($p < 0.001$ by χ-square test, Cramer's $V > 0.2$ except (b)). The prevalence of these four symptoms was significantly higher in the tset group than in the hypothetical group; after one month, the prevalence of "constipation," "susceptibility to catch cold," and "tiredness" was lower than in the control group, and the prevalence of "skin problems" decreased to the same level as in the control group. There were no dropouts in the test group and no adverse events were observed.

***Conclusion*:** The consumption of the test food may be an effective and safe functional food for the improvement of subjective symptoms such as "constipation," "susceptibility to catching colds," "tiredness," and "skin problems".

***KEY WORDS*:** brown rice, bran layer, sub-aleurone layer, lipopolysaccharide (LPS), anti-ageing quality of life questionnaire, hypothetical control

Introduction

In order to achieve the goal of maintaining homeostasis of body functions, it is important to keep good lifestyle habits in terms of diet and exercise. Rice is a staple food for many Japanese people. Brown rice, which is a whole grain, is richer in dietary fiber and nutrients than polished rice, which is a refined grain, and is said to be preferable for maintaining good health. Brown rice retains the bran, germ, and other parts of the grain that are removed in the milling process, and contains high levels of dietary fiber, the vitamin B group, and other nutrients. However, many people find brown rice difficult to eat because it contains a water-repellent, hard-to-degrade part called the wax layer and a part with a bran flavor called the bran layer [1, 2]. According to an epidemiological survey conducted by the National Cancer Center, only about 3% of Japanese eat brown rice as a staple food (Cancer Center Cohort Study Report) [3]. For this reason,

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processed brown rice that takes advantage of the advantages of brown rice and improves its eating difficulty has been commercialized.

Brown rice/processed brown rice has been reported in clinical trials to inhibit the increase in blood triglycerides/total cholesterol [4-6], to improve postprandial hyperglycemia [6-8] and fasting glucose [9], lower HbA1c [10], to prevent bone density loss in old age [11], to reduce internal fat in metabolic syndrome [12], to improve bowel movements [13, 14], bone density [15], vascular endothelial function [6, 16], and to maintain cognitive function in the elderly [17]. We have also reported the improvement of skin condition [1, 2] and the reduction of medical cost [18] by eating processed brown rice.

The bran layer of brown rice contains a variety of nutritional components. In recent years, functional foods that utilize the nutritional components derived from brown rice have been developed [19, 20]. The "mature extract consisting of brown rice bran layer, germ blast and crushed cell group" used as a test food in this study is one of the functional foods derived from brown rice. Here, an open-label study was conducted on more than 1,000 healthy subjects to investigate the effects of the test food on subjective symptoms after one month of intake.

Method

Subjects

The subjects were 1,023 healthy men and women (313 men and 710 women) between the ages of 18 and 100 years old who met the following selection criteria and did not meet the exclusion criteria, and who ingested a mature extract (sub-aleurone layer residual rinse-free [Kinmemai] rice extract) consisting of the sub-aleurone layer, blastema of the germ, and crushed cell group extracted from brown rice as the test food for one month.

The selection criteria are as follows:

(1) Men and women between 18 and 100 years of age
(2) Healthy and free from chronic physical diseases.
(3) Patients who have received sufficient explanation of the purpose and content of the study, have the ability to consent, understand the study well, volunteer to participate, and agree to participate in the study in writing.
(4) Those who have been approved by the principal investigator to participate in the study.

Exclusion criteria are listed below:

(1) Persons who have a history of or are currently suffering from serious disorders of the liver, kidneys, heart, lungs, or blood.
(2) Persons who may have allergic symptoms to the test food, or who may have serious allergic symptoms to other foods or medicines.
(3) Other persons who are judged by the investigator to be inappropriate for this study.

Test food

The test food, sub-aleurone layer residual rinse-free rice extract, was supplied by Toyo Rice (Tokyo, Japan). The test food is made by extracting this rare part of brown rice. It is rich in enzymes and a wide variety of nutrients in the basal part of the embryo, *i.e.* the blastoderm and blastoderm, and in crushed cells of the boundary between the blastoderm and endosperm. Only 1% of the brown rice is extracted using a unique milling technique and matured without any additives, making it a 100% rice-derived health food. The only ingredient is rice. Each packet (3.5 g) contains 292.3 mg of phytic acid, 7.95 mg of γ-amino butyric acid (GABA), 6.65 mg of γ-oryzanol and 149.1 μg (estimated value) of lipopolysaccharide (LPS).

Test method

This was an open-label study. After explaining consent to the subjects, quality of life questionnaires were administered and collected via the internet or by post.

Assessment items

Anti-Aging QOL Common questionnaire

The Anti-Aging QOL Common questionnaire (AAQol) was used to investigate subjective symptoms related to QOL [21, 22]. The subjects were asked to rate their physical symptoms (32 items) and mental symptoms (21 items) on a four-point scale (1. originally no symptoms, 2. improved, 3. neither can be said, 4. worsening).

As a hypothetical control, we used 3,002 age- and sex-adjusted cases (930 men and 2,072 women) from the AAQol data (5,999 cases, 3,355 men and 2,644 women) stored at the Anti-Aging Research Center (AARC), Doshisha University. This data has been used as control data in the past as anonymous, unlinked data containing no personal information [23-25].

Statistical analysis

Statistical analysis was carried out using Excel statistics (Social Information Service, Tokyo), with two-tailed tests, with a risk rate of less than 5% ($p < 0.05$) being considered a significant difference.

Ethical Review

This study was approved by the Ethical Review Committee for Research on Human Subjects of the Japan Society for the Study of Glycation and Stress (22 March, 2021, #GSE2021006) and was conducted in accordance with the Declaration of Helsinki (revised October 2013) and the Ethical Guidelines for Medical Research Involving Human Subjects (Ministry of Education, Culture, Sports, Science and Technology and Ministry of Health, Labour and Welfare, 22 December 2014). Informed consent was given to the study participants in advance and free consent was obtained in writing. The study was conducted after pre-registration in the University Hospital Medical Information Network Clinical Trial Registration System (UMIN-CTR) (registration number: UMIN #000043778).

Results

The composition of the 1,023 participants in the study group is given in ***Table 1***. There were no drop-outs (those who did not continue to consume the test food for one month) in this study.

Table 1. Composition of subjects: test group.

	n	Average age	Standard deviation
Male	313	58.20	14.75
Female	710	52.67	13.08
Total	1,023	54.36	13.84

The AAQol scores of the test group are shown in ***Table 2***. Among the items in AAQol, the prevalence rate of symptoms was more than 50%, excluding the subjects who answered "1. originally no symptoms" from all subjects. 12 physical symptoms were "tired eyes," "blurry eyes," "stiff shoulders," "muscle pain/stiffness," "tendency to gain weight," "lethargy," "no feeling of good health," "skin problems," "constipation," "gray hair," "lumbago," and "cold skin." In terms of mental symptoms, there were three symptoms: "irritability," "easily angered," and "shallow sleep."

The highest percentage of subjects whose symptoms "2. improved" after one month of consumption of the test food were "constipation" (45.5%), "liable to catch cold" (35.6%), "lethargy" (33.7%) and "skin problems" (33.3%).

In terms of safety, there were no adverse events during or after the study.

Table 2. Assessment of subjective symptoms: test group.

Physical symptoms	n	1. Originally no symptoms	Persons with symptoms	The prevalence rate of symptoms	2. Improved	3. Neither can be said	4. Worsening	Rate of symptomatic improvement
Tired eyes	1,009	332	677	67.1%	94	576	7	13.9%
Blurry eyes	1,008	459	549	54.5%	65	474	10	11.8%
Eye pain	1,004	686	318	31.7%	32	279	7	10.1%
Stiff shoulders	1,009	281	728	72.2%	121	586	21	16.6%
Mascular pain/stiffness	1,006	353	653	64.9%	92	547	14	14.1%
Palpitations	1,006	758	248	24.7%	32	212	4	12.9%
Shortness of breath	1,005	743	262	26.1%	32	224	6	12.2%
Tendency to gain weight	1,011	389	622	61.5%	86	502	34	13.8%
Weight los/; thin	1,007	614	393	39.0%	55	328	10	14.0%
Lethargy	1,010	398	612	60.6%	206	390	16	33.7%
No feeling of good health	1,007	425	582	57.8%	164	408	10	28.2%
Thirst	1,009	671	338	33.5%	35	285	18	10.4%
Skin problems	1,009	381	628	62.2%	209	399	20	33.3%
Anorexia	1,007	769	238	23.6%	70	161	7	29.4%
Early satiety	1,008	702	306	30.4%	66	219	21	21.6%
Epigastralgia	1,008	757	251	24.9%	42	197	12	16.7%
Liable to catch cold	1,010	695	315	31.2%	112	197	6	35.6%
Coughing and sputum	1,008	679	329	32.6%	49	265	15	14.9%
Diarrhea	1,008	715	293	29.1%	81	195	17	27.6%
Constipation	1,010	480	530	52.5%	241	267	22	45.5%
Gray hair	1,006	535	471	46.8%	59	405	7	12.5%
Hair loss	1,008	245	763	75.7%	31	712	20	4.1%
Headache	1,005	603	402	40.0%	54	332	16	13.4%

Dizziness	1,008	747	261	25.9%	26	229	6	10.0%
Tinnitus	1,009	741	268	26.6%	24	231	13	9.0%
Lumbago	1,011	705	306	30.3%	14	281	11	4.6%
Arthralgia	1,011	451	560	55.4%	55	480	25	9.8%
Edematous	1,009	628	381	37.8%	43	328	10	11.3%
Easily breaking into a sweat	1,006	572	434	43.1%	79	342	13	18.2%
Frequent urination	1,010	603	407	40.3%	26	371	10	6.4%
Hot flush	1,009	536	473	46.9%	46	404	23	9.7%
Cold skin	1,006	814	192	19.1%	18	172	2	9.4%
Dizziness	1,010	466	544	53.9%	92	439	13	16.9%

Mental symptoms	n	1. Originally no symptoms	Persons with symptoms	The prevalence rate of symptoms	2. Improved	3. Neither can be said	4. Worsening	Rate of symptomatic improvement
Irritability	1,007	436	571	56.7%	94	459	18	16.5%
Easily angered	1,007	495	512	50.8%	73	424	15	14.3%
Loss of motivation	1,006	507	499	49.6%	100	386	13	20.0%
No feeling of happiness	1,006	630	376	37.4%	60	308	8	16.0%
Nothing to look forward to in life	1,007	682	325	32.3%	42	275	8	12.9%
Daily life is not enjoyable	1,007	652	355	35.3%	59	289	7	16.6%
Loss of confidence	1,007	706	301	29.9%	28	269	4	9.3%
Reductance to talk with others	1,007	671	336	33.4%	29	297	10	8.6%
Depressed	1,008	703	305	30.3%	39	252	14	12.8%
No Feeling of usefulness	1,006	725	281	27.9%	21	255	5	7.5%
Shallow sleep	1,007	411	596	59.2%	144	424	28	24.2%
Difficulty in falling asleep	1,007	554	453	45.0%	123	317	13	27.2%
Pessimism	1,010	632	378	37.4%	33	335	10	8.7%
Lapse of memory	1,007	510	497	49.4%	19	455	23	3.8%
Inablity to concentrate	1,008	561	447	44.3%	45	389	13	10.1%
Inability to solve problems	1,008	648	360	35.7%	23	328	9	6.4%
Inability to make judgments readily	1,008	665	343	34.0%	26	311	6	7.6%
Inability to sleep because of worries	1,010	656	354	35.0%	46	292	16	13.0%
A sense of tension	1,008	594	414	41.1%	34	371	9	8.2%
Feeling of anxiety for no special reason	1,007	669	338	33.6%	42	283	13	12.4%
Vague feeling of fear	1,008	770	238	23.6%	26	204	8	10.9%

Rate of symptomatic improvement is defined as the percentage of people whose symptoms have improved. The prevalence rate of symptoms are red-highlighted when > 50%. Rate of symptomatic improvement are red-highlighted when > 30%.

Comparison with control group

The compositionof the 3,002 patients in the hypothetical control group is shown in ***Table 3***. The AAQol score is based on a five-point scale (score 1: no symptoms, 2: few symptoms, 3: some symptoms, 4: moderate symptoms, 5: severe symptoms), so that in this study (score 1: no symptoms, 2: few symptoms) was counted as "originally no symptoms." The results are shown in ***Table 4***.

The prevalence of symptoms in the test group was significantly higher than that in the control group for the following ten physical symptoms: "eye pain," "weight loss/thin," "lethargy," "no feeling of good health," "skin problems," "anorexia," "liable to catch cold," "diarrhea,""constipation," and "frequent urination." In terms of mental symptoms, there were 14 symptoms: "loss of motivation," "no feeling of happiness," "daily life is not enjoyable", "reductance to talk with others," "depressed," "no feeling of usefulness," "shallow sleep," "difficulty in falling asleep," "inability to solve problems," "inability to make judgments readily," "inability to sleep because of worries," "feeling of anxiety for no special reason," and "vague feeling of fear".

Of the items listed above, five physical symptoms were significantly lower in the test group than in the control group: "weight loss/thin," "lethargy", "liable to catch cold," "diarrhoea," and "constipation," and none of the mental symptoms.

In the pre- and post-test analysis by Chi-square test, the three items with Cramer's V of 0.2 or higher were "lethargy," "skin problems," and "constipation." Cramer's V is a measure of association in chi-square analysis, and it ranges from 0 to 1, with higher values indicating greater association.

Table 3. Composition of subjects: control.

	n	Average age	Standard deviation
Male	930	58.04	14.35
Female	2,072	53.21	13.14
Total	3,002	54.70	13.71

The hypothetical control group is used as control.

Table 4. Assessment of subjective symptoms: comparison of test and control groups.

	Control				Test group			Compariosn	
Physical symptoms	n	1. Originally no symptoms	Persons with symptoms	The prevalence rate of symptoms (%)	n	The prevalence rate of symptoms: Pre (%)	Post (%)	p values	Cramer's V
Tired eyes	3,002	782	2,220	74.0	1,009	67.1	57.8	**<0.001**	0.0962
Blurry eyes	3,002	1,430	1,572	52.4	1,008	54.5	48.0	**0.0038**	0.0645
Eye pain	3,002	2,282	720	24.0	1,004	31.7	28.5	0.1194	0.0347
Stiff shoulders	3,002	661	2,341	78.0	1,009	72.2	60.2	**<0.001**	0.1267
Mascular pain/ stiffness	3,002	1,131	1,871	62.3	1,006	64.9	55.8	**<0.001**	0.0935
Palpitations	3,002	2,154	848	28.2	1,006	24.7	21.5	0.0903	0.0378
Shortness of breath	3,002	2,130	872	29.0	1,005	26.1	22.9	0.0969	0.0370
Tendency to gain weight	3,002	1,102	1,900	63.3	1,011	61.5	53.0	**<0.001**	0.0860
Weight los / thin	3,002	2,649	353	11.8	1,007	39.0	33.6	**0.0108**	0.0568
Lethargy	3,002	1,395	1,607	53.5	1,010	60.6	40.2	**<0.001**	0.2040
No feeling of good health	3,002	1,729	1,273	42.4	1,007	57.8	41.5	**<0.001**	0.1629
Thirst	3,002	2,026	976	32.5	1,009	33.5	30.0	0.0942	0.0373
Skin problems	3,002	1,721	1,281	42.7	1,009	62.2	41.5	**<0.001**	0.2073
Anorexia	3,002	2,649	353	11.8	1,007	23.6	16.7	**<0.001**	0.0866
Early satiety	3,002	2,180	822	27.4	1,008	30.4	23.8	**<0.001**	0.0737
Epigastralgia	3,002	2,262	740	24.7	1,008	24.9	20.7	**0.0258**	0.0496
Liable to catch cold	3,002	2,227	775	25.8	1,010	31.2	20.1	**<0.001**	0.1270
Coughing and sputum	3,002	2,021	981	32.7	1,008	32.6	27.8	**0.0175**	0.0529

Diarrhea	3,002	2,263	739	24.6	1,008	29.1	21.0	**< 0.001**	0.0927
Constipation	3,002	1,857	1,145	38.1	1,010	52.5	28.6	**< 0.001**	0.2430
Gray hair	3,002	1,659	1,343	44.7	1,006	46.8	41.0	**< 0.001**	0.1878
Hair loss	3,002	673	2,329	77.6	1,008	75.7	72.6	0.1148	0.0351
Headache	3,002	1,830	1,172	39.0	1,005	40.0	34.6	**0.0128**	0.0555
Dizziness	3,002	2,191	811	27.0	1,008	25.9	23.3	0.1788	0.0299
Tinnitus	3,002	2,269	733	24.4	1,009	26.6	24.2	0.2195	0.0273
Lumbago	3,002	1,149	1,853	61.7	1,011	55.4	50.0	**0.0143**	0.0545
Arthralgia	3,002	1,854	1,148	38.2	1,009	37.8	33.5	**0.0456**	0.0445
Edematous	3,002	1,833	1,169	38.9	1,006	43.1	35.3	**< 0.001**	0.0804
Easily breaking into a sweat	3,002	1,538	1,464	48.8	1,010	40.3	37.7	0.2356	0.0264
Frequent urination	3,002	1,808	1,194	39.8	1,009	46.9	42.3	**0.0394**	0.0459
Hot flush	3,002	2,355	647	21.6	1,006	19.1	17.3	0.2982	0.0232
Cold skin	3,002	1,424	1,578	52.6	1,010	53.9	44.8	**< 0.001**	0.0911
Mental symptoms									
Irritability	3,002	1,320	1,682	56.0	1,007	56.7	47.4	**< 0.001**	0.0934
Easily angered	3,002	1,479	1,523	50.7	1,007	50.8	43.6	**0.0011**	0.0726
Loss of motivation	3,002	1,873	1,129	37.6	1,006	49.6	39.7	**< 0.001**	0.1000
No feeling of happiness	3,002	2,362	640	21.3	1,006	37.4	31.4	**0.0049**	0.0628
Nothing to look forward to in life	3,002	2,425	577	19.2	1,007	32.3	28.1	0.0415	0.0454
Daily life is not enjoyable	3,002	2,402	600	20.0	1,007	35.3	29.4	**0.0049**	0.0626
Loss of confidence	3,002	2,194	808	26.9	1,007	29.9	27.1	0.1669	0.0308
Reductance to talk with others	3,002	2,425	577	19.2	1,007	33.4	30.5	0.1657	0.0309
Depressed	3,002	2,388	614	20.5	1,008	30.3	26.4	0.0539	0.0429
No Feeling of usefulness	3,002	2,453	549	18.3	1,006	27.9	25.8	0.2910	0.0235
Shallow sleep	3,002	1,688	1,314	43.8	1,007	59.2	44.9	**< 0.001**	0.1431
Difficulty in falling asleep	3,002	2,006	996	33.2	1,007	45.0	32.8	**< 0.001**	0.1066
Pessimism	3,002	1,855	1,147	38.2	1,010	37.4	34.2	0.1256	0.0341
Lapse of memory	3,002	730	2,272	75.7	1,007	49.4	47.5	0.3969	0.0189
Inability to concentrate	3,002	1,684	1,318	43.9	1,008	44.3	39.9	**0.0424**	0.0452
Inability to solve problems	3,002	2,202	800	26.6	1,008	35.7	33.4	0.2815	0.0240
Inability to make judgments readily	3,002	2,152	850	28.3	1,008	34.0	31.4	0.2172	0.0275
Inability to sleep because of worries	3,002	2,153	849	28.3	1,010	35.0	30.5	**0.0292**	0.0485
A sense of tension	3,002	1,586	1,416	47.2	1,008	41.1	37.7	0.1212	0.0345
Feeling of anxiety for no special reason	3,002	2,356	646	21.5	1,007	33.6	29.4	**0.0439**	0.0449
Vague feeling of fear	3,002	2,585	417	13.9	1,008	23.6	21.0	0.1643	0.0310

Pre and post comparisons were analysed using the χ-square test. The prevalence of symptoms in the test group is shown in red if it is significantly higher than in the control group. Cramer's V > 0.2 is shown in red (Cramer's V = Cramer's coefficient of association). The hypothetical control group is used as control. Rate of symptomatic improvement is defined as the percentage of people whose symptoms have improved.

Discussion

Results of the evaluation using the AAQol

An open-label study was conducted in which 1,023 healthy subjects consumed test foods containing brown rice (especially rice bran) nutrients for one month. For the analysis of the results, 3,002 age- and sex-adjusted subjects from the AARC data were used as a hypothetical control group.

The AAQol questionnaire [21, 22] was used to assess subjective symptoms. The AAQol showed a significant improvement in the following four physical symptoms: "constipation" (45.5%), "liable to catch cold" (35.6%), "lethargy" (33.7%) and "skin problems" (33.3%). The prevalence of these symptoms was significantly higher in the pre-test group than in the hypothetical control group. After the test, the prevalence of "constipation," "liable to catch cold," and "lethargy" were lower than in the control group. The prevalence of "skin problems" decreased to the same level as in the control group.

As this is an open-label study, the level of evidence is not high. However, it is an important pilot study to obtain information on the efficacy and adverse effects of new foods, to find out to what extent the test foods improve physical symptoms and whether any of the symptoms worsen. As the AAQol contains 54 items of 'physical symptoms' and 'mental symptoms,' it is unclear to respondents which symptoms are being tested. The AAQol is therefore suitable for an initial pilot study.

Setting up a control group

The four items shown here, "constipation," "liable to catch cold," "lethargy," and "skin problems," were the items that more than 30% of the subjects felt had improved. Next, the relationship with the control group is discussed.

In the study, 3,002 age- and sex-adjusted AARC data were used as a control group. These data are based on the results of anti-aging health examinations and clinical trials over the past 20 years and are not linked to personal data. In the past, we have used age- and sex-adjusted AARC data as hypothetical controls when measuring blood levels of DHEA-s and IGF-I in patients with non-alcoholic fatty liver disease (NAFLD) [23, 24].

In post-marketing functional food studies, there has also been experience of comparing a group of subjects who had consumed the test food for a long period of time with an age- and sex-adjusted hypothetical control. This showed which items showed significant changes and which did not as a result of consuming the test food [25]. In this way, we are willing to collaborate with other centers if they require hypothetical controls.

A major advantage of using hypothetical controls for comparative analysis is that differences in characteristics between the test population and the control can be recognised. For example, a test population with poor sleep will show differences in subjective symptoms and physical information. In the present study, there were also differences in pre-test symptom rates between the test group and the control. In particular, the prevalence of mental symptoms was higher in the test group than in the control. This may be due to the social context of the Covid-19 outbreak, which may have had a strong impact on the psychological stress load of this group.

When conducting a clinical trial, there should obviously be a control. However, due to budgetary constraints or because it is an early pilot study, the control may not be established in some studies. Experience has shown that even in a control group, there can be a range of changes after four or eight weeks of participation in a clinical trial. There may be placebo effects, blood sampling effects and lifestyle changes associated with participation in the trial. In order to improve the accuracy and quality of the results of the study, we would like to construct hypothetical controls for the changes after four and eight weeks and try to perform comparative analyses.

Improvement items

The improvement items "constipation," "liable to catch cold," "lethargy," and "skin problems" are discussed in terms of the ingredients contained in the test foods.

Improvement in "constipation" means improvement in bowel movements. The common drug "laxative" improves bowel movements but may induce diarrhoea as an adverse event. In this study, however, the prevalence of diarrhoea improved significantly from 29.1% to 21.0%. This is reminiscent of the changes observed when taking prebiotics or probiotics to regulate the intestinal microflora.

Brown rice and processed brown rice are known to help regulate disruptive intestinal microflora (dysbiosis), which is thought to result in improved bowel movements. Animal studies in rodents have shown an increase in lactic acid bacteria [26] and a decrease in the ratio of Firmicutes/Bacteroidetes [27]. This results in an increase in organic acids, including short chain fatty acids. The test food contains ingredients derived from brown rice, which may have exerted a similar effect. Dietary fiber, LPS and phytic acid are assumed to be the components involved.

In terms of "skin problems," clinical trials have been conducted to evaluate the skin condition after consuming processed brown rice [1, 2]. This study was conducted mainly on university students, and skin age based on the degree of blemishes and wrinkles was assessed by skin image analysis using a skin measurement device (Clreo-Pro), and a significant improvement in skin age was observed after one month of consumption of processed brown rice compared to the control group. The study also showed an improvement in bowel movements, which is not in conflict with the present results.

A double-blind study of a functional food supplemented with rice bran and rice bran oil reported an improvement in skin elasticity [28]. In vitro studies have shown that rice bran components have an inhibitory effect on AGE formation [29], and clinical studies have shown a decrease in CML (a type of AGEs) [28]. Therefore, improvement of glycation stress may contribute to improvement of skin. It is possible that the improvement of glycation stress may contribute to the improvement of skin function.

In addition, the involvement of vitamins and other ingredients in brown rice, the improvement of intestinal bacterial dysbiosis, and the improvement of bowel movements are thought to be comprehensively involved in

the improvement of skin function.

The improvement in "liable to catch cold" may reflect immune function. As immune function is a complex mechanism involving a variety of immune response cells, it is not easy to discuss the relationship between individual cell function and the nutritional content of brown rice. There have been reports of LPS in brown rice activating macrophages and stimulating innate immunity [30]. Considering the disbiosis-improving effect of brown rice, it is possible that it may improve intestinal immune function and contribute to the improvement of immunocompetence. The effects of brown rice nutrients on immune function and on the intestinal bacterial layer will be investigated in the near future.

We have reported a study of a reduction in public health care costs per capita of nearly 40% due to a diet of processed brown rice [18]. Although the breakdown of diseases reduced is not known, it is expected that the actual reduction in the number of colds alone would contribute to a reduction in health care costs.

The "lethargy" category encompasses a wide range of physical symptoms. Some of them are associated with anti-fatigue effects. It is not at all possible to predict which ingredients acted in which way.

Masuzaki H *et al*. at the University of the Ryukyus have confirmed that a high-fat diet induces hypothalamic endoplasmic reticulum (ER) stress, leading to animal fat dependence and reduced glucose-responsive insulin secretion in pancreatic beta cells [31]. These findings are alleviated by γ-oryzanol [21, 32]. The effects of a high-fat diet may affect other cells, and it is possible that the accumulation of ER stress on cells may lead to fatigue. In a clinical study conducted on mature male patients with metabolic syndrome, an eight-week replacement of a polished rice diet with a brown rice diet was associated with weight loss, improvement in postprandial hyperglycaemia, and a change in preference to avoid meals containing animal fat [6]. These findings were attributed to a reduction in ER stress in neurons of the metabolic reward system in the brain. It remains to be seen how and to what extent the activation of the metabolic reward system influences behavioural change.

Safety

The test product is made from rice (part of the bran layer), with no other additives. Rice (including rice bran) has been consumed for many years and its safety is well established. In this study, no adverse events were observed, confirming the safety of the test product.

Conclusion

After 1,023 healthy subjects took the test food containing brown rice nutrients for one month, a questionnaire survey showed that the physical symptoms of "constipation," "liable to catch cold," "lethargy," and "skin problems" improved. The safety of the test food was confirmed without any drop-out cases or adverse events, indicating the usefulness and safety of the test food. The mechanism of action of the test foods can be attributed to the nutritional composition of the brown rice, the details of which will be the subject of further research.

Declaration of conflict of interest

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