USEFULNESS OF THE SUPPLEMENT CONTAINING PROTEOGLYCAN FOR JAPANESE HEALTHY PEOPLE FEELING KNEE'S DISCOMFORT

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Abstract

Objectives: The objective of this study is to verify the knee joint pain relieving effect of ingesting food containing proteoglycan.
Methods: A randomized placebo-controlled, double-blind study was conducted in 36 subjects with symptoms of knee joint discomfort. The test food was a tablet containing proteoglycan. Subjects consumed three tablets every day for 12 weeks. Visual analog scale (VAS) for knee pain, the knee range of motion measurements and the modified J-KOOS (Japan-Knee injury and Osteoarthritis Outcome Score) was evaluated as primary outcomes.
Results: Out of the 36 subjects, 5 dropped due to personal reasons and the remaining 31 subjects were evaluated [the mean of the age is 55.0±7.6 (test food) and 52.1±11.1 (placebo)]. Pain Vas, significant differences were observed in the intragroup comparison of the test food (in repose, in walking and in stairs going up and down) and also in the intergroup comparison of changes from the baseline (in stairs going up and down). With respect to the right and left knee extension and flexion, there were significant differences in the intragroup comparison of the test food and in the intergroup comparison of changes from the baseline. Furthermore, several categories on the questionnaire of modified J-KOOS showed the significant differences of changes from the baseline in 10 items and the total score. In addition, no abnormal change had been triggered by the ingestion of the test food.
Conclusion: It is suggested that the ingestion of the supplement containing proteoglycan by Japanese healthy people feeling knee's discomfort for 12 weeks contributed to improving the joint support.

Key words: proteoglycan, locomotive syndrome, knee joint, salmon

1. INTRODUCTION

Articulation consists of cartilage cells, cartilage matrix, subchondral bone, tendons and ligaments around the articulation and muscles. These components undergo various changes with increasing age. The progression of these changes may trigger off the development of osteoarthritis (OA), the symptom associated with chronic pain[1]. Now in Japan, the number of patients who suffer from OA increases significantly due to the aging of population[2]. Once developed, there is no definite cure/therapy to inhibit the progression of OA, therefore it is crucially important to find out the means, other than drug intervention, for avoiding the development of and/or inhibiting the progression of OA.

Among the changes associated with the aging, the change of cartilage matrix is a key factor for developing OA. It is reported that the volume of articular cartilage of the knee joint, which mainly consists of cartilage matrix, decreases with age[3]. The cartilage matrix contains various types of collagen and proteoglycan in addition to a plenty of moisture. The change of cartilage matrix with the age is thought to be related to the fact that the turnover period for the protein of these collagen and proteoglycan is relatively longer for the aged people and therefore they are easily modified during the period[4].

Proteoglycan, on the other hand, is regarded as an edible in general, and is an ingredient which is extracted from the nasal cartilage of salmon. It is reported that the supplement containing glucosamine and chondroitin sulfate, which are components of salmon-derived proteoglycan[5], possibly contributes to the pain relief of and/or the inhibition of progression of OA[6)~8)]. However, there are very few numbers of reports that illustrate the relationship between the ingestion of proteoglycan itself by healthy aged person and the prevention of development of OA.

In this study, we examined the effect of a food containing proteoglycan to verify the joint support and the safety of the food. The test targets were Japanese healthy person who are feeling knee's discomfort, and the test method was a randomized, placebo-controlled, double-blind study.

2.METHOD

2.1. Trial Design

A randomized, placebo-controlled, double-blind study was conducted with the aid of a fund from Smile-Japan co.,ltd. (Fukushima) at two centers (OZ clinic, Tokyo and JACTA, Tokyo).

The study period was 12 weeks, from June 27th to September 19th, 2015.

This study was conducted in accordance with the ethical principles of the declaration of Helsinki. The study protocol was approved by the Institutional Review Board of LLP. Pharmaceutical Law Wisdoms (Tokyo). Written informed consent was obtained from all subjects.

The allocation of the test product to the subjects was carried out by the person in charge of allocation.

The allocation list was sealed and strictly controlled in a safe deposit box of JACTA until the end of the study.

The clinical trial was registered at UMIN clinical Trial Registration (Trial ID: UMIN000017839).

2.2. Subject

Healthy subjects participated in the present study.

All of the subjects in this study were public volunteers who had enrolled in the monitor bank of CROee Inc. (Tokyo).

2.2.1. Inclusion Criteria

(1) Healthy people aged between 40 and 69 years;
(2) Modified J-KOOS (Japan-Knee injury and Osteoarthritis Outcome Score)[9] : **Appendix**.

2.2.2. Exclusion criteria

(1) Individuals already undertaking medical treatment for knee pain or discomfort;
(2) Individuals on taking medication, including herbal medicines;
(3) Individuals judged to be unsuitable to participate in the trial by the doctor conducting present study.

2.2.3. Efficacy eligibility

With respect to the analysis of efficacy, we set the following criteria of exclusion:
(1) Participants who consumed less than 80% of the expected dose;
(2) Participants without adequate records;
(3) Participants who fell under the exclusion criteria after enrolment;
(4) Participants who had justifiable reason for exclusion.

2.3. Randomization

Subjects who fulfilled the eligibility criteria were 36 persons. The inclusion was judged by the principle investigator. All subjects were sequentially assigned based on a random number table to one of the masked products and randomized to group P (placebo: 18) and group T (test sample: 18)

The allocation was pre-assigned on the basis of randomized numbers.

2.4. Description of test foods and blinding

The test food "Locomo Ace" ("LA") is a tablet containing salmon-derived proteoglycan. The amount of a daily intake is 3 tablets (750 mg). In the 3 tablets, salmon-derived proteoglycan is included over 10 mg, the placebo does not include proteoglycan. **Table 1**. shows the nutritional content of the sample. Both tablets were indistinguishable in shape, color or taste. Tablets were managed by the identification symbol. All involved were blinded.

2.5. Experimental procedures

2.5.1. Experimental protocol

Subjects consumed 3 tablets of the supplement with hot or cold water every day for 12 weeks. Subjects were instructed as follows: to take the assigned foods as indicated; to maintain their usual lifestyles and habits; to avoid excessive amounts of food, drink, or alcohol; to maintain a daily record of lifestyle factors such as all the food items they consumed during the test period; and to send the diary to the study coordinator on every Friday by mobile email.

2.5.2. Outcome

The objective of this study is to verify the knee joint pain relieving effect of ingesting food containing proteoglycan.

Table 1　Nutritional content of the sample per 100 g

Item	LA	Placebo
Energy	394 kcal	215 kcal
Protein	6.8 g	0.06 g
Lipid	2.6 g	2.77 g
Ash	5.6 g	1.48 g
Carbohydrates	85.8 g	95.11 g
Na	182 mg	0.706 mg

Table 2　Schedule for the study

Term / Item	Screening	Pre Trial Test	Test period		
			4 w	8 w	12 w
Modified J-KOOS	●		●	●	●
Informed consent	●				
Selection and/or allocation	●				
VAS, Knee extension and flexion		●	●	●	●
Biochemical analysis of blood		●			●
Urine analysis		●			●
Ingestion of test foods			↔	↔	↔
Log			↔	↔	↔

● : Implementation
↔ : Daily practice during the test period

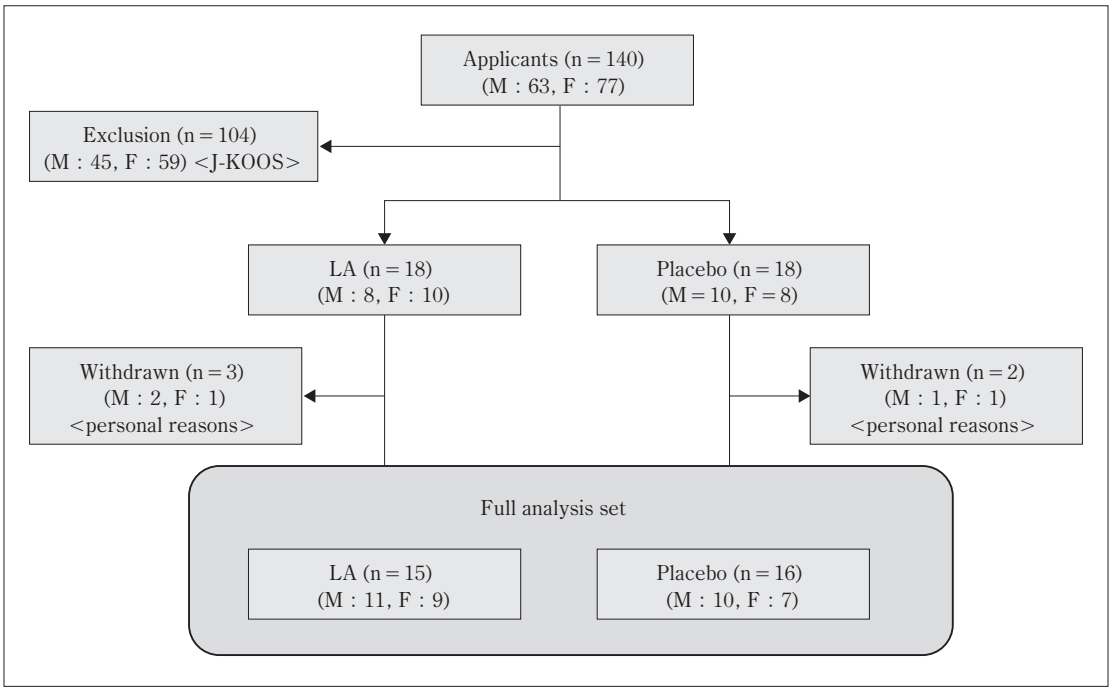

Figure 1 Flow diagram of subject disposition

Table 3 Subject demographics

Item	Unit	LA	Placebo
Subjects*	−	15	16
Male : Female*	−	9:6	7:9
Age	years	55.0 ± 7.6	52.1 ± 11.1
VAS (in repose)	mm	9.7 ± 11.2	18.0 ± 19.8
VAS (in walking)	mm	19.4 ± 11.3	28.6 ± 17.3
VAS (in going up and down stairs)	mm	35.4 ± 18.1	40.4 ± 19.2
Modified J-KOOS	point	52.9 ± 8.1	48.3 ± 11.7

* Number of subjects
mean ± SD

To evaluate this objective, visual analog scale (VAS)[10] for knee pain was conducted as the primary outcomes. The knee range of motion measurements and the modified J-KOOS was also observed as the primary outcome. The questionnaire of the modified J-KOOS covered: joint discomfort, pain, function and the quality of life. Blood biochemical and urine parameters were recorded to evaluate the safety of the test foods as the secondary outcome.

To evaluate the safety of the test foods, adverse events were collected by means of a written questionnaire during the study.

According to the schedule shown in **Table 2**, we measured parameters on efficacy and safety.

2.6. Data Analysis

All analyses were performed on the on-treatment population in the study.

Data were expressed as mean ± SD. For pain VAS, knee extension, flexion, and biochemical analyses of blood and urine, changes from the baseline in the same group were assessed using the paired t-test. Student's t-test was used for intergroup comparisons of changes from the baseline. For the modified J-KOOS, changes from baseline in the same group were assessed using the Wilcoxon signed-rank test. The Mann–Whitney U test was used for intergroup comparisons of changes from the baseline. Student's t-test was used to compare subject backgrounds between groups. Statistical analyses were performed using Statcel 3 (Yanai, 2011). The results were considered significant at the < 5% level in the two-sided test.

3. RESULT

3.1. Participant Demographics

From all of 140 applicants, 104 were eliminated according to the modified J-KOOS score. The 36 subjects were randomly assigned to intervention groups and made a start with ingestion. 5 were withdrawn due to personal

Table 4　Results of test analyses

Item <unit>	Group	Baseline	4 weeks			8 weeks			12 weeks		
		Mean ± SD	Mean ± SD	P-value[1)]	P-value[2)]	Mean ± SD	P-value[1)]	P-value[2)]	Mean ± SD	P-value[1)]	P-value[2)]
VAS (in repose) <mm>	LA	9.7 ± 11.2	11.3 ± 15.1	0.707	0.117	5.1 ± 7.3	0.182	0.236	1.9 ± 3.3	0.017*	0.066‡
	P	18.0 ± 19.8	8.0 ± 11.0	0.105		18.1 ± 16.9	0.938		15.5 ± 13.1	0.453	
VAS (in walking) <mm>	LA	19.4 ± 11.3	19.9 ± 15.9	0.913	0.938	18.3 ± 7.8	0.728	0.702	11.1 ± 11.9	0.040*	0.109
	P	28.6 ± 17.3	28.6 ± 17.4	0.984		25.9 ± 15.3	0.298		26.6 ± 17.4	0.549	
VAS (in stairs going up and down) <mm>	LA	35.4 ± 18.1	31.4 ± 14.5	0.464	0.866	21.1 ± 10.2	0.024*	0.121	10.5 ± 5.3	< 0.001**	0.041#
	P	40.4 ± 19.2	37.5 ± 19.8	0.402		36.5 ± 20.5	0.217		31.4 ± 19.5	0.162	
knee extension (right) <degree>	LA	23.0 ± 7.1	16.6 ± 8.5	0.033*	0.013#	16.4 ± 5.9	0.003**	0.005##	12.2 ± 5.3	< 0.001**	< 0.001##
	P	17.0 ± 10.4	18.8 ± 10.7	0.169		17.8 ± 9.3	0.510		17.2 ± 5.5	0.843	
knee extension (left) <degree>	LA	23.7 ± 7.5	14.9 ± 10.3	0.009**	0.011#	14.5 ± 4.4	< 0.001**	0.070‡	10.1 ± 3.4	< 0.001**	< 0.001##
	P	20.4 ± 12.1	21.2 ± 7.7	0.404		16.1 ± 7.7	0.117		17.5 ± 4.9	0.299	
knee flexion (right) <degree>	LA	128.3 ± 8.9	124.5 ± 8.1	0.085†	0.721	129.3 ± 8.4	0.726	0.417	131.8 ± 8.8	0.192	0.020#
	P	129.0 ± 9.3	125.7 ± 8.5	0.104		127.2 ± 8.2	0.336		125.1 ± 8.9	0.013*	
knee flexion (left) <degree>	LA	125.0 ± 6.0	124.1 ± 8.9	0.721	0.557	122.7 ± 11.2	0.493	0.417	129.2 ± 6.3	0.029*	0.025#
	P	125.3 ± 13.0	126.9 ± 9.2	0.640		126.5 ± 10.3	0.671		124.4 ± 11.7	0.313	

n = 31

1)*: P < 0.05, **: P < 0.01 intragroup differences versus baseline

2)#: P < 0.05, ##: P < 0.01 intergroup differences between changes from baseline

reasons (3; occupational conflict, 1; cold, 1; tendovaginitis) and the remaining 31 subjects completed the study. Thus, data obtained from the 31 subjects (test group; 15, placebo group; 16) were used for efficacy analysis (**Figure 1**.). There were no significant differences in the mean age, gender ratios, VAS or modified J-KOOS observed between groups (**Table 3**.).

3.2. VAS

Pain VAS was conducted in three situations. All of which showed significant differences from the baseline of LA group after 12 weeks of ingestion. First and foremost, the significant Pain VAS recorded in repose showed a change of p = 0.017. Subsequently, the measurement of change on the Pain VAS of walking came to p = 0.040 and the Pain VAS of going up and down the stairs recorded at p < 0.001. Overall the intergroup comparison of changes between LA group and placebo group came to p = 0.041 from going up and down the stairs (**Table 4**.).

3.3. Knee extension and flexion

Table 4. summarizes the average knee extension and flexion changes over time for subjects supplemented with either LA or placebo.

For the results of the right and left extension, the right knee extension changed significantly from the baseline of LA group after 4,8 and 12 weeks of ingestion (p = 0.033, p = 0.003, p < 0.001, respectively). Furthermore, significant differences were observed between the changes from the baseline of two groups after 4, 8 and 12 weeks of ingestion (p = 0.013, p = 0.005, p < 0.001, respectively). The left knee extension showed significant differences from the baseline of LA group after 4, 8, 12 weeks of ingestion (p = 0.009, p < 0.001, p < 0.001, respectively). Moreover there were significant differences between the change from baseline of two groups after 4 and 12 weeks of ingestion (p = 0.011, p < 0.001, respectively).

For the results of the right and left knee flexion, a significant difference was observed in changes from the baseline of the two groups after 12 weeks of ingestion. The right knee showed p = 0.020, while the left knee demonstrated p = 0.025.

3.4. Modified J-KOOS

Table 5. reveals the Modified J-KOOS. Stiffness, Pain, Function (daily living) and Quality of Life were asked as Modified J-KOOS.

The result based on Modified J-KOOS criteria, showed that a change of the aggregate scores was significantly improved at week 4 and 12 in the LA group compared to the placebo group. In the intergroup difference of changes from baseline, Question S1. ("Stiffness"; How severe is your knee joint stiffness after first wakening in the morning?), S2. (How severe is your knee stiffness after sitting, lying or resting later in the day?), P2. ("Pain"; Walking on flat surface), A1. ("Function, daily living"; Descending stairs), A3. (Rising from sitting), A5. (Bending to floor/ picking up an object), A6 (Walking on flat surface), A12. (Sitting), Q1. ("Quality of life"; Due to pain, do you restrict your hobbies or recreational activities?) and Q3. (Is your overall health condition affected by your knee condition?) illustrated significant differences.

3.5. Blood and Urine test

Table 6. and **7**. show the blood biochemical and urine parameters.

The change of Total Protein, Neutral Fat (TG) and Creatinine in LA group, revealed a significant difference after 12 weeks of ingestion. However the change of Total Protein and Creatinine was within the standard value. 4 subjects with Neutral Fat changed over the standard value after 12 weeks of ingestion, but the investigator

Table 5 Results of questionnaire (Modified J-KOOS)

Item	Group	Baseline	4 weeks			8 weeks			12 weeks		
		Mean ± SD	Mean ± SD	P-value[1)]	P-value[2)]	Mean ± SD	P-value[1)]	P-value[2)]	Mean ± SD	P-value[1)]	P-value[2)]
Total	LA	52.9 ± 8.1	42.7 ± 10.7	0.002**	0.034#	41.9 ± 10.9	0.003**	0.093‡	38.9 ± 10.2	0.002**	0.001##
	Placebo	48.3 ± 11.7	44.6 ± 7.0	0.079†		43.4 ± 8.3	0.015*		46.4 ± 8.6	0.235	
S1.	LA	2.9 ± 0.7	1.7 ± 0.7	0.001**	< 0.001##	1.6 ± 0.6	0.001**	0.236	1.7 ± 0.7	0.001**	< 0.001##
	Placebo	2.3 ± 0.6	2.9 ± 0.6	0.006**		2.5 ± 0.5	0.310		2.7 ± 0.7	0.123	
S2.	LA	2.7 ± 0.7	2.2 ± 1.0	0.173	0.363	2.1 ± 0.7	0.033*	0.048#	1.8 ± 0.9	0.011*	0.017#
	Placebo	2.3 ± 0.5	2.1 ± 0.3	0.225		2.3 ± 0.5	1.000		2.3 ± 0.4	0.686	
P1.	LA	3.9 ± 1.0	3.1 ± 1.1	0.114	0.514	3.2 ± 1.2	0.131	0.984	2.7 ± 1.2	0.025*	0.323
	Placebo	3.5 ± 0.8	3.1 ± 0.6	0.018*		2.8 ± 0.7	0.008**		2.9 ± 0.9	0.059†	
P2.	LA	2.2 ± 0.4	1.7 ± 0.7	0.033*	0.031#	1.6 ± 0.6	0.022*	0.514	1.5 ± 0.6	0.013*	0.004##
	Placebo	1.9 ± 0.7	2.1 ± 0.3	0.401		1.5 ± 0.5	0.142		2.1 ± 0.3	0.237	
P3.	LA	2.5 ± 0.7	2.2 ± 0.7	0.401	0.859	1.9 ± 0.6	0.074†	0.477	2.0 ± 0.5	0.076†	0.567
	Placebo	2.4 ± 0.5	2.3 ± 0.5	0.361		2.1 ± 0.3	0.043*		2.3 ± 0.6	0.374	
P4.	LA	1.2 ± 0.4	1.1 ± 0.4	0.317	1.000	1.3 ± 0.5	0.317	0.252	1.2 ± 0.4	1.000	0.406
	Placebo	1.2 ± 0.4	1.1 ± 0.3	0.593		1.0 ± 0.0	0.109		1.0 ± 0.0	0.109	
P5.	LA	1.5 ± 0.6	1.4 ± 0.6	0.529	0.353	1.3 ± 0.6	0.208	0.635	1.3 ± 0.5	0.262	0.678
	Placebo	1.6 ± 0.9	1.2 ± 0.4	0.0499*		1.2 ± 0.4	0.0499*		1.4 ± 0.5	0.401	
A1.	LA	2.3 ± 0.7	1.9 ± 0.6	0.018*	0.874	1.7 ± 0.6	0.019*	0.040#	1.7 ± 0.6	0.022*	0.024#
	Placebo	2.3 ± 0.6	2.1 ± 0.3	0.273		2.2 ± 0.4	0.686		2.3 ± 0.6	1.000	
A2.	LA	2.3 ± 0.7	1.8 ± 0.8	0.063†	0.385	1.8 ± 0.8	0.086†	0.385	1.7 ± 0.7	0.033*	0.185
	Placebo	2.3 ± 0.6	2.1 ± 0.3	0.345		2.1 ± 0.3	0.225		2.1 ± 0.3	0.225	
A3.	LA	2.8 ± 1.0	1.9 ± 0.6	0.005**	0.055‡	2.0 ± 0.8	0.008**	0.161	1.8 ± 0.7	0.003**	0.002##
	Placebo	2.7 ± 0.8	2.5 ± 0.6	0.374		2.4 ± 0.6	0.262		2.8 ± 0.7	0.735	
A4.	LA	1.9 ± 0.8	1.3 ± 0.6	0.033*	1.000	1.3 ± 0.5	0.018*	0.813	1.1 ± 0.4	0.012*	0.937
	Placebo	1.9 ± 0.9	1.3 ± 0.7	0.012*		1.3 ± 0.7	0.008**		1.3 ± 0.7	0.008**	
A5.	LA	2.7 ± 0.8	1.9 ± 0.7	0.005**	0.220	1.9 ± 0.7	0.013*	0.236	1.8 ± 0.6	0.005**	0.033#
	Placebo	2.1 ± 0.9	1.8 ± 0.8	0.110		1.8 ± 0.8	0.248		2.0 ± 0.5	0.575	
A6.	LA	2.0 ± 0.0	1.3 ± 0.5	0.005**	0.0101#	1.4 ± 0.5	0.008**	0.018#	1.3 ± 0.5	0.005**	0.002#
	Placebo	1.6 ± 0.5	1.5 ± 0.5	0.180		1.6 ± 0.5	0.593		1.8 ± 0.4	0.361	
A7.	LA	2.1 ± 0.6	1.7 ± 0.6	0.076†	0.664	1.7 ± 0.7	0.110	0.607	1.5 ± 0.5	0.022*	0.0504‡
	Placebo	1.8 ± 0.6	1.4 ± 0.5	0.068†		1.4 ± 0.5	0.068†		1.7 ± 0.7	0.753	
A8.	LA	1.6 ± 0.6	1.6 ± 0.6	1.000	0.514	1.5 ± 0.5	0.594	0.906	1.5 ± 0.5	0.594	0.906
	Placebo	1.7 ± 0.8	1.5 ± 0.6	0.225		1.6 ± 0.7	0.735		1.6 ± 0.7	0.735	
A9.	LA	2.2 ± 0.4	1.7 ± 0.5	0.012*	0.465	1.9 ± 0.6	0.139	0.567	1.7 ± 0.6	0.025*	0.053‡
	Placebo	2.2 ± 1.0	1.8 ± 0.5	0.076†		1.9 ± 0.8	0.178		2.1 ± 1.0	0.686	
A10.	LA	1.3 ± 0.6	1.2 ± 0.6	0.593	0.797	1.3 ± 0.5	1.000	0.580	1.2 ± 0.4	0.317	0.553
	Placebo	1.3 ± 0.4	1.1 ± 0.3	0.361		1.1 ± 0.3	0.463		1.1 ± 0.3	0.225	
A11.	LA	1.7 ± 0.9	1.6 ± 0.6	0.686	0.921	1.5 ± 0.5	0.500	0.828	1.4 ± 0.5	0.237	0.333
	Placebo	1.5 ± 0.7	1.3 ± 0.5	0.345		1.3 ± 0.5	0.345		1.6 ± 0.7	0.800	
A12.	LA	1.9 ± 0.7	1.2 ± 0.4	0.151	0.060‡	1.3 ± 0.5	0.018*	0.236	1.3 ± 0.5	0.012*	0.034#
	Placebo	1.7 ± 0.8	1.6 ± 0.6	0.500		1.4 ± 0.5	0.068†		1.6 ± 0.7	0.593	
A13.	LA	1.3 ± 0.5	1.2 ± 0.4	0.686	0.953	1.5 ± 0.5	0.034*	0.089‡	1.3 ± 0.5	1.000	0.580
	Placebo	1.3 ± 0.5	1.3 ± 0.4	0.317		1.2 ± 0.4	0.180		1.2 ± 0.4	0.180	
A14.	LA	2.1 ± 0.6	2.0 ± 0.8	1.000	0.937	2.1 ± 0.8	1.000	0.514	1.7 ± 0.7	0.076†	0.0504‡
	Placebo	2.3 ± 1.1	2.0 ± 0.5	0.333		2.1 ± 0.9	0.374		2.4 ± 0.7	0.310	
A15.	LA	2.0 ± 0.5	2.2 ± 0.9	0.401	0.813	2.1 ± 0.8	0.735	0.363	1.7 ± 0.7	0.237	0.874
	Placebo	2.0 ± 1.0	2.1 ± 1.0	0.594		2.1 ± 0.8	0.767		1.8 ± 0.8	0.310	
Q1.	LA	2.1 ± 0.3	1.3 ± 0.6	0.0096**	0.020#	1.3 ± 0.5	0.002**	0.016#	1.2 ± 0.4	0.002**	0.005##
	Placebo	1.4 ± 1.0	1.3 ± 0.8	0.686		1.3 ± 0.5	1.000		1.2 ± 0.4	0.500	
Q2.	LA	1.3 ± 0.6	1.2 ± 0.4	0.554	0.453	1.1 ± 0.3	0.178	0.179	1.0 ± 0.0	0.068†	0.101
	Placebo	1.1 ± 0.3	1.2 ± 0.4	0.361		1.2 ± 0.4	0.361		1.2 ± 0.4	0.361	
Q3.	LA	2.6 ± 0.7	2.1 ± 1.0	0.083†	0.027#	1.8 ± 0.8	0.021*	0.138	1.7 ± 0.7	0.012*	0.027#
	Placebo	2.1 ± 1.1	1.9 ± 0.8	0.529		2.0 ± 0.8	0.859		2.2 ± 0.8	0.575	

n = 31

1) **: P < 0.01 intragroup diffierences versus baseline

2) # : P < 0.05, ##: P < 0.01 intergroup differences between changes from baseline

Table 6　Changes in biochemical blood test

Item	Unit	Std. Value	Gender	Group	Baseline Mean ± SD	12 weeks Mean ± SD
Total Bilirubin	mg/dL	0.2–1.2	M/F	LA	0.72 ± 0.40	0.67 ± 0.35
				Placebo	0.81 ± 0.48	0.84 ± 0.52
Total Protein	g/dL	6.5–8.3	M/F	LA	7.2 ± 0.4	7.5 ± 0.4**
				Placebo	7.4 ± 0.4	7.5 ± 0.4†
Albumen	g/dl	3.8–5.3	M/F	LA	4.4 ± 0.2	4.4 ± 0.2
				Placebo	4.6 ± 0.2	4.5 ± 0.2
AST (GOT)	U/L	8–38	M/F	LA	20.1 ± 5.1	22.1 ± 6.0†
				Placebo	20.3 ± 4.4	21.4 ± 4.6†
ALT (GPT)	U/L	4–43	M/F	LA	15.5 ± 5.6	15.8 ± 6.2
				Placebo	17.1 ± 5.9	17.6 ± 7.6
ALP	U/L	110–354	M/F	LA	207.9 ± 59.2	219.6 ± 70.2
				Placebo	224.9 ± 74.6	227.0 ± 70.0
LD (LDH)	U/L	121–245	M/F	LA	190.6 ± 37.9	202.6 ± 49.2
				Placebo	174.1 ± 27.4	175.0 ± 22.8
γ-GT (γ GTP)	U/L	86 and under	M	LA	20.5 ± 7.7	24.3 ± 7.8
				Placebo	42.6 ± 44.4	49.9 ± 59.4†
		48 and under	F	LA	20.6 ± 12.8	20.0 ± 9.4
				Placebo	68.9 ± 138.1	58.0 ± 109.5
CK (CPK)	U/L	38–196	M	LA	156.7 ± 71.8	199.8 ± 63.8
				Placebo	114.8 ± 55.4	126.8 ± 59.7
		30–172	F	LA	110.8 ± 42.6	140.0 ± 71.9
				Placebo	101.1 ± 26.9	90.1 ± 29.3
Total Cholesterol	mg/dL	130–219	M/F	LA	207.9 ± 38.2	209.3 ± 38.5
				Placebo	218.1 ± 39.9	219.3 ± 46.9
Neutral Fat (TG)	mg/dL	30–149	M/F	LA	99.1 ± 46.7	128.1 ± 57.0*
				Placebo	143.8 ± 63.8	141.3 ± 73.0
Sodium	mEq/L	135–150	M/F	LA	142.9 ± 2.2	142.5 ± 2.0
				Placebo	142.9 ± 2.0	142.6 ± 2.5
Chloride	mEq/L	98–110	M/F	LA	105.7 ± 2.4	105.2 ± 1.9
				Placebo	104.2 ± 2.1	103.2 ± 1.9†
Potassium	mEq/L	3.5–5.3	M/F	LA	4.3 ± 0.3	4.3 ± 0.2
				Placebo	4.1 ± 0.3	4.1 ± 0.3
Calcium	mg/dL	8.4–10.2	M/F	LA	9.6 ± 0.3	9.7 ± 0.3
				Placebo	9.6 ± 0.3	9.8 ± 0.4*
Inorganic Phosphorus	mg/dL	2.5–4.5	M/F	LA	3.5 ± 0.6	3.6 ± 0.4
				Placebo	3.4 ± 0.4	3.4 ± 0.6
Urea Nitrogen	mg/dL	8.0–22.0	M/F	LA	14.1 ± 4.1	14.6 ± 3.9
				Placebo	14.4 ± 3.8	15.1 ± 4.1
Creatinine	mg/dL	0.61–1.04	M	LA	0.81 ± 0.10	0.76 ± 0.08*
				Placebo	0.90 ± 0.19	0.88 ± 0.22
		0.47–0.79	F	LA	0.69 ± 0.09	0.65 ± 0.09*]#
				Placebo	0.66 ± 0.09	0.61 ± 0.09†
Blood Sugar (Serum)	mg/dL	60–109	M/F	LA	70.1 ± 6.1	72.3 ± 7.0
				Placebo	73.8 ± 9.8	73.8 ± 14.3

n = 31

†: $P < 0.1$, *: $P < 0.05$, **: $P < 0.01$ intragroup differences versus baseline

#: $P < 0.05$ intergroup differences between changes from baseline

Table 7 Transition of Urinalysis

Item	Unit	Std. Value	Gender	Group	Baseline Mean ± SD	12 weeks Mean ± SD
Specific Gravity	mg/dL	1.010–1.025	M/F	LA	1.016 ± 0.005	1.017 ± 0.005
				Placebo	1.019 ± 0.006	1.019 ± 0.006
pH	g/dL	4.5–8.0	M/F	LA	5.8 ± 0.8	5.8 ± 0.9
				Placebo	5.9 ± 0.8	5.8 ± 0.8

n = 31

judged it as within the range of physiological variation. No significant change was detected in the urine test.

4. DISCUSSION

We conducted a randomized, placebo-controlled, double-blind study to verify the joint support effects of the supplement containing proteoglycan. As the primary outcome, the study showed significant differences in the scores of VAS (Visual Analogue Scale), in the intragroup comparison of LA group (in repose, in walking and in stairs going up and down) and also in the intergroup comparison of changes from the baseline (in stairs going up and down). In addition, as for the right and left knee extension and flexion, there were significant differences in the intragroup comparison of LA group and in the intergroup comparison of changes from the baseline. Furthermore, several categories on the questionnaire of modified J-KOOS (Japanese Knee Injury and Osteoarthritis Outcome Score) showed the significant differences of changes from the baseline in 10 items and the total score. At the same time, as the secondary outcome the observation of clinical findings such as medical interview, blood and urine test revealed no abnormal change had been triggered by the ingestion of test products.

Evaluating the above findings comprehensively, it is suggested that the ingestion of the test product containing proteoglycan contributes to the improvement of the knee condition.

Proteoglycan contained in this test product is one of the glycoproteins which are the complex of sugar and protein, and it is a biogenic substance people have by nature. It excels in moisture holding ability and contributes to the maintenance of body tissue by producing an extracellular matrix with collagen and hyaluronic acid. Especially in articulation (joints), it is contained in the cartilage and helps the smooth movement of joints by its buffering function.

Intravitally, proteoglycan is produced in the Golgi body, by adding a number of sugar chains to the core-protein (i.e. glycosylation)[11]. On the other hand, the usage of proteoglycan for food and cosmetic product has started after the technological establishment of extracting it from the nasal cartilage of salmon. Although the mechanism underlying proteoglycan absorption in the intestine is not clear, the recent study showed even by the oral ingestion, it is directly absorbed in the intestine by the intermediary of clathrin-mediated endocytosis[12].

Historically, for the maintenance of joint function, glucosamine and chondroitin sulfate (both of which are the components of proteoglycan) have been used as a supplement in expectation of their production of cartilage matrix intravitally[7][8]. Glucosamine reportedly owns functions such as protection of cartilage and anti-inflammatory power for cartilage[13], and an oral ingestion of chondroitin, on the other hand, has a functionality of alleviating arthritic pain[6][14]. Since proteoglycan is directly absorbed in the intestine, it can be expected that the functionality from the mechanism totally different from the ingestion of its components should be achieved. There is a study which reports proteoglycan owns growth-stimulating effect and differentiation- maintaining of cartilage cells[15], and this study encourages the expectation that proteoglycan can improve the condition of knee-joint by regeneration and protection against scuffing of cartilage. In addition, another study illustrates the anti-inflammatory power of proteoglycan[16][17], therefore it suggests proteoglycan achieves a suppression of inflammation, which is a cause of pain.

In this study, the improvement effect of knee-joints was mainly seen in the situations when the subjects experienced a low-impact on their knees, and this finding is suggested by the results such as VAS of “in repose” or J-KOOS of “walking on flat surface”. It is thought that this finding has some relations to the function of proteoglycan as explained above. On the other hand, the situations such as “up/down stairs” and “sports” when the subjects experienced a relatively high-impact did not show the improvements; this result indicates that the test product may gradually improve the condition of knee-joints and therefore the longer ingestion period is recommended.

Based upon the above discussion, it is suggested that in this study, the ingestion of the test product containing proteoglycan showed a trend toward improvement in the condition of knee-joints.

During the test period five subjects discontinued the test. Four of them stopped the test because of their personal reasons such as impossibility of continuing the test due to their business or diseases (i.e. cold). As for the remaining one subject in the placebo group, on the other hand, the measurement after 12-week ingestion

showed an abnormal value of CK, but after the medical interview the doctor determined the abnormity was due to the tendovaginitis and ruled out any relationship with the test itself. Therefore, based upon the medical interview, blood test and urine test, we observed no harmful influence against biochemical and/or physiological matters of the subjects which seem to have causal relationship with LA.

These results indicated the safety of the ingestion of the test product (LA) for the 12-week test period.

In Japan, as the society faces aging of population, it is now a big issue to prevent the elderly people from the loss of self-support. The fourth cause for the elderly care is an articular disease. There is also a report that prevalence rate of OA increases with advancing age[18]. The progression of its symptom makes it more difficult for the patients to walk. However, a full-scale treatment for OA has not yet been established and this fact strongly supports the importance of the prevention of symptom. Although it is inevitable for the articular cartilage to be changed with aging, achieving the improvement of their knee-joint condition by the ingestion of foods may contribute to the self-support of elderly people and to the prevention of the degradation of QOL.

Until several years ago it was difficult to use proteoglycan as a food because of its high price, but after the establishment of extraction method of it from salmon it became possible to use it as a food. Therefore, its functionality is worth noting from now on.

However, the study of proteoglycan was mainly based upon the in-vitro settings or the research using animals, and the functional mechanism in the human body has not yet been deeply speculated so far. There is also a report that concludes the ingestion of glucosamine and chondroitin sulfate (both of which are the components of proteoglycan) is not related to the improvement of joint pain[12].

In addition, since articular cartilage does not have many blood vessels, it is recognized that its capacity for repair is limited[19]. It is not clear that the improvement effect of knee-joints we have observed in this study was either the result of inflammatory suppression or because of the repair of articular cartilage, and its functional mechanism is a matter of speculation. These points should be further scrutinized in the future.

In conclusion, we found out that the ingestion of the supplement LA containing proteoglycan by Japanese healthy people feeling knee's discomfort for 12 weeks contributed to improving the joint support. In addition, no safety-related matter occurred during 12-week test period.

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Appendix

<Modified J-KOOS>

“Stiffness”

The following questions concern the amount of joint stiffness you have experienced during the last week in your knee. Stiffness is a sensation of restriction or slowness in the ease with which you move your knee joint.

S1. How severe is your knee joint stiffness after first wakening in the morning?

None, Mild, Moderate, Severe, Extreme

S2. How severe is your knee stiffness after sitting, lying or resting later in the day?

None, Mild, Moderate, Severe, Extreme

“Pain”

P1. How often do you experience knee pain?

None, Mild, Moderate, Severe, Extreme

What amount of knee pain have you experienced the last week during the following activities?

P2. Walking on flat surface

None, Mild, Moderate, Severe, Extreme

P3. Going up or down stairs

None, Mild, Moderate, Severe, Extreme

P4. At night while in bed

None, Mild, Moderate, Severe, Extreme

P5. Standing upright

None, Mild, Moderate, Severe, Extreme

“Function, daily living”

The following questions concern your physical function. By this we mean your ability to move around and to look after yourself. For each of the following activities please indicate the degree of difficulty you have experienced in the last week due to your knee.

A1. Descending stairs

None, Mild, Moderate, Severe, Extreme

A2. Ascending stairs

None, Mild, Moderate, Severe, Extreme

For each of the following activities please indicate the degree of difficulty you have experienced in the last week due to your knee.

A3. Rising from sitting

None, Mild, Moderate, Severe, Extreme

A4. Standing

None, Mild, Moderate, Severe, Extreme

A5. Bending to floor/ picking up an object

None, Mild, Moderate, Severe, Extreme

A6. Walking on flat surface

None, Mild, Moderate, Severe, Extreme

A7. Getting in/ out of car

None, Mild, Moderate, Severe, Extreme

A8. Going shopping

None, Mild, Moderate, Severe, Extreme

A9. Putting on/ off socks/ stockings

None, Mild, Moderate, Severe, Extreme

A10. Lying in bed (turning over, maintaining knee position)

None, Mild, Moderate, Severe, Extreme

A11. Getting in/ out of bath

None, Mild, Moderate, Severe, Extreme

A12. Sitting

None, Mild, Moderate, Severe, Extreme

A13. Getting on/ off toilet

None, Mild, Moderate, Severe, Extreme

For each of the following activities please indicate the degree of difficulty you have experienced in the last week due to your knee.

A14. Heavy domestic duties (moving heavy boxes, scrubbing floors, etc.)

None, Mild, Moderate, Severe, Extreme

A15. Activities such as sports, jogging, swimming, gateball, dancing, etc.

None, Mild, Moderate, Severe, Extreme

“Quality of Life”

Q1. Due to pain, do you restrict your hobbies or recreational activities?

Not at all, Mildly, Moderately, Severely, Totally

Q2. Due to pain, do you stop going out?

Not at all, Mildly, Moderately, Severely, Totally

Q3. Is your overall health condition affected by your knee condition?

Not at all, Mildly, Moderately, Severely, Totally