Original Article

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N-acetyl glucosamine and proteoglycan containing supplement improves the locomotor functions of subjects with knee pain

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Summary **The aim of this study was to investigate the effect of *N*-acetyl glucosamine and proteoglycan-containing supplement (NGPS) on knee pain and locomotor functions in middle-aged and elderly persons with knee pain. An open trial was conducted on 19 subjects suffering from knee pain. The subjects, aged (55.6 ± 6.9) years, were given the NGPS tablets, which they must take 3 times per day, that contain 526.5 mg of *N*-acetyl glucosamine (GlcNAc) and 33.6 mg of proteoglycan for 12 weeks. Subjective pain was evaluated using the Visual Analog Scale (VAS), while the function of the knee with regard to daily operation was evaluated using the Japanese Knee Osteoarthritis Score (JKOM). Walking, stair-climbing and swelling were evaluated using the Japanese Orthopedic Association Score (JOA). These items were evaluated at a baseline, and after 4, 8, and 12 weeks of NGPS treatment. The VAS scores at 8 ($p = 0.004$) and 12 ($p < 0.001$) weeks were significantly lower than that at the baseline. The JKOM total score was significantly lower at 8 and 12 weeks ($p = 0.001$) than that at the baseline. The JOA score in the more painful side of the leg was significantly higher at 12 weeks ($p = 0.002$) than that at the baseline. The present study reveals that intake of NGPS is effective for relieving knee pain and improving knee function when walking or climbing stairs, swelling and bending or stretching.**

Keywords: Oligosaccharide, gonalgia, osteoarthritis, alternative medicine

1. Introduction

Osteoarthritis (OA) is the most common form of arthritis and is a leading cause of disability (*1*). Current treatment of OA includes both non-pharmacologic and pharmacological therapies (*2*). Among pharmacological treatment, analgesic and non-steroidal anti-inflammatory drugs (NSAIDs) are the primary treatment methods. However, the use of NSAIDs is limited by their serious side effects on the gastrointestinal tract and cartilage metabolism (*3,4*). Therefore, attention has been focused on safe and causal treatment, but not supportive treatment, in response to clinical symptoms of OA. The causal treatment has been performed with glucosamine. Kongtharvonskul and colleagues have shown that NSAIDs and glucosamine are equally efficacious for symptom relief in knee OA but NSAIDs have more side effects as observed on meta-analysis (*5*).

Tsuji *et al*. reported that supplementation with *N*-acetyl glucosamine (GlcNAc), one of the components of cartilage, improved knee function (*6*). Since, with age, the body starts to decrease the production of glycosaminoglycans from glucose (*7*), oral intake of GlcNac may increase synthesis of cartilage glycosaminoglycans and improve the symptoms of the knee joint (*8*). Because glucosamine consists of glycosaminoglycan after being converted into GlcNac in the cells of the target tissue, GlcNac is considered to be more effective in small amounts than glucosamine for improving knee functions. However, to our knowledge, no study on the effect of small amounts of GlcNac on OA has been conducted so far. In the present study, we investigated the effects of 12 weeks of treatment with a supplement containing 526.5 mg of GlcNAc and 33.6 mg of proteoglycan on knee functions in subjects with knee pain but who were not diagnosed

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with OA.

The purpose of this study was to investigate the effectiveness of *N*-acetyl glucosamine and proteoglycan-containing supplement (NGPS) for 12 weeks of oral supplementation in middle aged subjects with chronic knee pain. Because the aim of this NGPS dosing is for primary prophylaxis, we recruited participants who have chronic knee pain but not diagnosed. In addition, we confirm the safety of NGPS.

2. Materials and Methods

2.1. *Subjects*

A total of 21 subjects, recruited from the Japanese Red Cross Society Iyama Hospital (Iyama hospital), expressed interest in participating in this study. Participants' eligibility was tested using a screening questionnaire. The criteria were the participants' age (40-69 years old) and conscious awareness of knee pain but not diagnosed. The exclusion criteria were knee pain due to an injury, accident or ligament damage within a year, progressive or possibility of rheumatoid arthritis, gout and calcium pyrophosphate dehydrate deposition disease, artificial joint, recent (within a month period) injected hyaluronan-modified in knee, routine use of supplements for knee pain (*e.g*., glucosamine, chondroitin, hyaluronan), cardiovascular disturbance, dyslipidemia, hepatic disease, kidney disease, circulatory condition, endocrine disease, alimentary disease, mental disorder, food allergy, improper lifestyle (*e.g*., dietary abnormality, alcohol dependence, night shift workers, takers of irregular holidays), participation in other clinical investigations within a month, pregnancy or lactation, or deemed unsuitable as per doctor's discretion.

After the pre-study screening, 4 eligible men and 15 eligible women volunteered to participate in this study. The participants' average [mean ± standard error (SE)] age, body weight, height, and heart rate were 55.6 ± 6.9 years, 64.6 ± 10.1 kg, 160.8 ± 5.9 cm, and 75.1 ± 10.0/min, respectively. They were informed about the possible risks and discomforts involved in the experiment prior to giving their written consent to participate in the study. Written consent forms were collected from all participants. The study design was approved by the Kenshokai Ethical Review Board and conducted in accordance with the principles of the amended Declaration of Helsinki.

2.2. *Test supplement*

NGPS containing N-acetyl glucosamine (526.5 mg) and proteoglycan (33.6 mg) as its main active ingredients in a tablet form (3 tablets a day) were supplied by CHARLE (CHARLE CO., LTD., Hyogo, Japan). The tablets also included Maltitose, Shark Fin Cartilage Extract (Type II Collagen and Chondroitin), Bosvvellia Serrata Extract, Ajuga Extract, crystalline cellulose, aroma chemical, Silicone dioxide (fine), calcium stearate and hyaluronan-modified.

2.3. *Procedures*

After the pre-study screening, the eligible subjects were assigned to a 12 week dietary intervention. During the intervention, the subjects ingested 3 tablets of NGPS per day. Every day, they recorded the time of ingestion and their physical condition in case they had a general feeling of unwellness. They underwent anthropometric tests, blood pressure tests, blood exam, analysis of urine and pain assessment before intake and every 4, 8, and 12 weeks after intake at the Japanese Red Cross Society Iyama Hospital. Moreover, as a posteriori survey, they underwent the same medical checks after 4 weeks of dietary intervention (at week 16).

2.4. *Anthropometric assessment and blood pressure*

The subjects' height, weight, and blood pressure were recorded at baseline and at the 4^{th}, 8^{th}, and 12^{th} week. Blood pressure was determined after 5 minutes of complete rest in a seated position. The Body Mass Index (BMI) was calculated based on the measurements of height and weight. All measurements were recorded by nurses at the Iyama Hospital.

2.5. *Hematological assessment and urinalysis*

Hematological assessment and urinalysis were performed to confirm the safety of NGPS. The following blood indices were analyzed: white blood cells, red blood cells, blood pigment, hemoglobin, hematocrit, leukocyte count, blood platelets, whole protein, albumin, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transferase (γ-GTP), urea nitrogen, creatinine, uric acid, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglyceride, natrium, kalium, calcium, and hemoglobin A1c. Food intake and beverages other than water were not allowed 8 hours prior to blood sampling. Blood samples were drawn from the antecubital vein. The samples were immediately stored in a cooler box, which was maintained at 4°C until centrifugation was done in a refrigerated centrifuge at 4°C. Samples were analyzed at a clinical laboratory in the Iyama Hospital.

Samples for urinalysis were collected from each participant and uric acid, creatinine, qualitative protein, qualitative sugar and urobilinogenuria were measured.

2.6. *Assessment of efficacy*

Subjects answered the following three questionnaires for rating knee pain during rest, while walking and while doing stepping exercises: (1) Visual Analog

Table 1. The mean characteristics of the subjects

Items	0 w, Mean ± SD	4 w, Mean ± SD	*p* (*vs*. 0 w)	8 w, Mean ± SD	*p* (*vs*. 0 w)	12 w, Mean ± SD	*p* (*vs*. 0 w)
Age (years)	55.6 ± 6.9	-		-		-	
Height (cm)	160.8 ± 5.9	-		-		-	
Weight (kg)	64.6 ± 10.1	64.7 ± 10.0	0.267	64.7 ± 10.1	0.370	64.9 ± 10.1	0.029*
BMI (kg/m^2)	25.0 ± 3.9	25.1 ± 3.9	0.258	25.1 ± 4.0	0.367	25.1 ± 4.0	0.027*
Systolic blood pressure (mmHg)	127.3 ± 10.7	127.2 ± 10.1	0.958	127.1 ± 14.1	0.940	125.2 ± 11.8	0.435
Diastolic blood pressure (mmHg)	78.3 ± 6.9	77.2 ± 5.7	0.508	78.3 ± 6.8	0.976	76.5 ± 6.4	0.249
Heart race (beats/min)	75.1 ± 10.0	72.5 ± 11.0	0.249	74.0 ±8.6	0.564	74.9 ± 8.4	0.934

Note: *n* = 19 (4 male and 15 female).

Table 2. Assessment of efficacy

Items	0 w Mean ± SD	4 w Mean ± SD	*p* (*vs*. 0 w)	8 w Mean ± SD	*p* (*vs*. 0 w)	12 w Mean ± SD	*p* (*vs*. 0 w)	16 w Mean ± SD	*p* (*vs*. 12 w)
VAS (mm)	50.5 ± 24.7	33.6 ± 22.3	0.003**	30.6 ± 26.7	0.003**	18.8 ± 18.4	0.000**	28.9 ± 27.1	0.205
JKOM	43.6 ± 7.9	38.6 ± 5.9	0.156	35.9 ± 6.0	0.001**	35.8 ± 6.5	0.000**	36.5 ± 9.0	0.717
JOA*[1]	83.9 ± 8.6	90.5 ± 0.0	0.035*	88.9 ± 8.4	0.360	91.8 ± 6.3	0.001**	92.9 ± 7.7	0.508
Each evaluated items in JKOM									
Pain and stiffness in the knee	17.3 ± 4.4	13.9 ± 3.6	0.001**	13.1 ± 3.4	< 0.001**	13.2 ± 3.6	0.001**	13.5 ± 4.9	0.925
State of daily life	14.6 ± 4.0	13.3 ± 3.4	0.030*	13.1 ± 3.0	0.029*	12.9 ± 3.2	0.009**	12.9 ± 3.9	0.751
Daily activities	7.2 ± 1.9	7.6 ± 1.9	0.291	6.3 ± 1.0	0.106	6.2 ± 1.4	0.030*	6.6 ± 1.3	0.101
Condition of health	4.5 ± 1.3	3.8 ± 1.4	0.103	3.5 ± 1.2	0.003**	3.5 ± 1.1	0.009**	3.4 ± 1.3	0.603

*[1]: JOA score evaluated more painful side of the legs. *$p < 0.05$ and **$p < 0.01$.

Scale (VAS): respondents specified their pain level on a continuous scale from 0 to 10, (2) Japanese Knee Osteoarthritis Measure (JKOM) respondents evaluated the pain and stiffness suffered, the state of their daily life, daily activities, and the condition of their health with a total score of 125 (*9*), and (3) Japanese Orthopedic Association score (JOA score) respondents evaluated their ability to walk (30 points), ability to climb up and down stairs (25 points), range of motion (ROM; 35 points), and joint swelling (10 points) (*10*).

2.7. *Statistical analysis*

Statistical tests were carried out using SPSS ver. 20.0 (SPSS, IBM). A significance level of $p < 0.05$ was used.

For the assessment of efficacy, a non-parametric multiple comparison test was performed using the significant findings (Friedman test) of each pain assessment (JKOM, JOA, and VAS) questionnaire at each measurement point (0, 4, 8, and 12 weeks). For safety examination, hematological assessment, biochemical tests, urinalysis, and physical measurement other than qualitative tests, paired *t*-test using Excel 2013 (Microsoft) were used. All data are expressed as mean ± standard error, unless otherwise specified.

3. Results

The mean characteristics of the subjects are shown in Table 1. Body weight and BMI were significantly higher at 12 weeks ($p = 0.029$ and $p = 0.027$) than at the baseline. However, such a change is not significant to the study that it does not figure at all in the clinical data.

The knee pain assessment showed improvement in knee function for all evaluated items at the 12th week in Table 2. VAS scores at the 8th ($p = 0.004$) and 12th week ($p < 0.001$) were significantly lower than at the baseline in Figure 1A.

JKOM total scores at the 8th week ($p = 0.003$) and 12th week ($p < 0.001$) were significantly lower than at the baseline in Figure 1B. Pain-and-stiffness-score at the 4th week ($p < 0.001$), 8th week ($p < 0.001$), and 12th week ($p < 0.001$) were significantly lower than at the baseline in Figure 2A. The state-of-daily-life scores at the 4th week ($p = 0.024$), 8th week ($p = 0.033$), and 12th week ($p = 0.007$) were significantly lower than at the baseline in Figure 2B. Soreness due to daily activities score at the 12th week ($p = 0.049$) was significantly lower than at the baseline in Figure 2C. The Condition-of-health-scores at the 8th week ($p < 0.001$) and 12th week ($p = 0.009$) were significantly lower than at the baseline in Figure 2D. The JOA score at the 12th week ($p = 0.002$) was significantly higher than at the baseline for the more painful side of the leg in Figure 1C. However, these scores returned to the baseline level after 4 weeks of washout period (16 weeks); no significant difference was observed compared to the one on the 12th week.

On the safety testing, no variation of the value on clinical importance for safety was observed in hematological assessment and urinalysis.

4. Discussion

This investigation showed that 12 weeks of NGPS supplementation was effective for pain relief and improvement of the function of the knee.

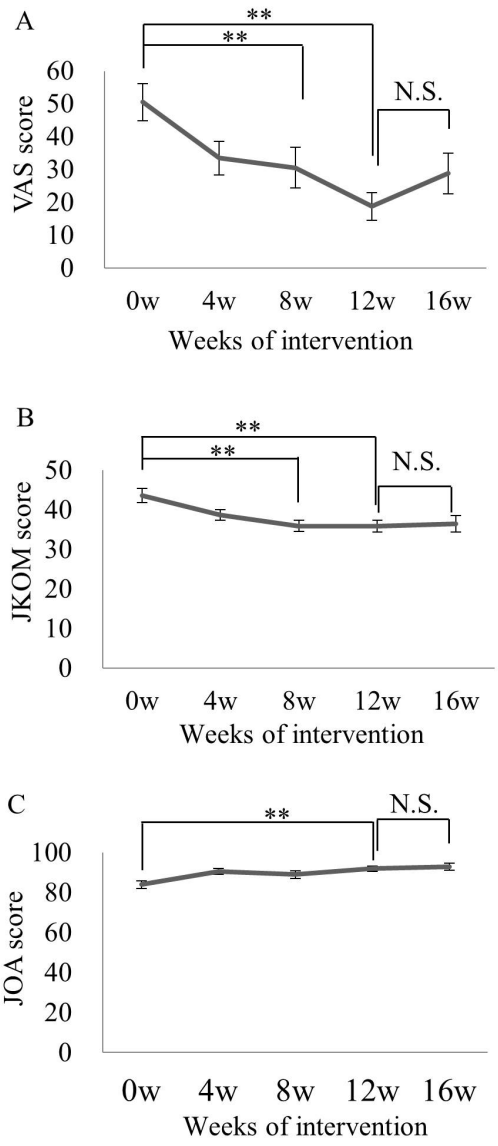

Figure 1. Effects of oral administration of NGPS on knee pain and function on time dependent change. A; the score of visual analog scale, **B**; Total scores of Japanese Knee Osteoarthritis, **C**; Total scores of Japan Orthopedic Association. Values represent mean ± SD.

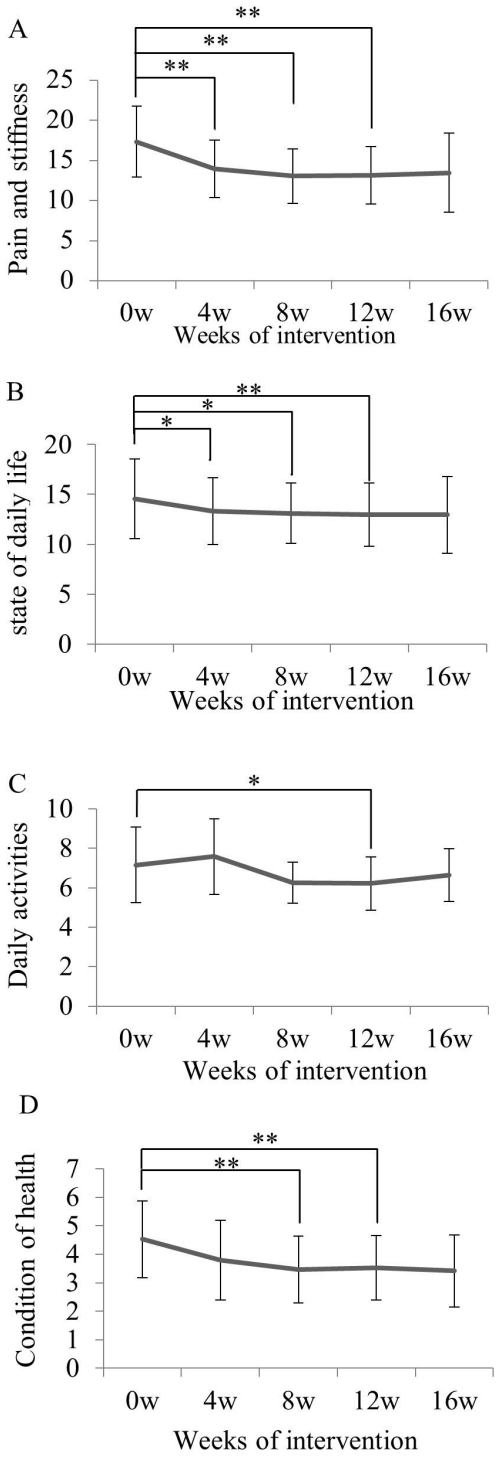

Figure 2. Effects of oral administration of NGPS on time dependent change of the each evaluated item in JKOM. Evaluated scores shows **A**; pain and stiffness, **B**; state of daily life, **C**; daily activities, **D**; condition of health in JKOM. Values represent mean ± SD.

The efficacy assessment revealed that NGPS supplementation decreased in VAS point and JKOM total scores and improved JOA total score after 12 weeks. However, 4 weeks after discontinuing supplementation, both VAS point and JKOM total score improved compared to the scores at 12 weeks. Similarly, at 16 weeks, JOA score decreased from that at 12 weeks. Giordano and colleagues showed 12 weeks of carry over effect of glucosamine sulfate in a randomized, double blind, placebo-controlled trial (*11*). They revealed that VAS scores tended to increase even after 4 weeks between the placebo group at 8 weeks and from the baseline. Since the results of this study are consistent with their report, we suggest that supplementation of NGPS is effective for improving knee-joint function.

A previous study has shown the effects of glucosamine hydrochloride supplementation on knee pain (*11*). The relationship of glucosamine and knee pain may be explained by the anti-inflammatory and chondroprotective activities of glucosamine hydrochloride (*13,14*), GlcNAc (*15*), chondroitin sulfate (*16*) and quercetin (*17,18*). Chan and colleagues

reported that glucosamine and chondroitin showed complementary anti-inflammatory effects when compared with glucosamine or chondroitin alone (*19,20*). Since in the result of JKOM, pain-and-stiffness-score showed the highest change ratio among the other evaluated items, the structure of the knee joint may have changed. The main components of this test supplement are a compound of GlcNAc and proteoglycan; the results that structural modification in the knee joint can be considered to be the composite effect of such anti-inflammatory reactions and chondroprotective activities. Ozkan and colleagues demonstrated that intraarticular *N*-acetyl glucosamine and intraarticular hyaluronate play a role in slowing the degenerative process and protecting the cartilage surface during the early stages of osteoarthritis in rabbits (*21*). Because test supplements also included hyaluronic acid as proteoglycan in addition to the GlcNAc, it was suggested to be effective for the improvement of the knee joint by chondroprotective and anti-inflammatory effects. In addition, because all participants in this study were not diagnosed with OA despite having knee pain, they might have only mild symptoms of knee pain, which is considered to be a factor influencing the improvement of knee function. These results suggest that glucosamine and proteoglycan intake in the early phase of knee pain inhibit the deformation of the knee cartilage, thus preventing OA.

In the safety assessment, all hematological evaluation items showed do not figure at all in the clinical data and no treatment-related adverse effects were experienced during the intervention periods. Moreover, there was no controversial weight gain likely to progress knee OA. These results demonstrated that NGPS can be taken safely. However, Hathcock and colleagues revealed that the safety only applies to intakes of up to 2,000 mg/day for glucosamine, and 1,200 mg/day for chondroitin sulfate (*22*); therefore intake beyond that which is stated above should be avoided.

There are some limitations to the present study. First, because no comparison control group was used, it is impossible to evaluate the placebo effect. Therefore, setting a placebo control group may be needed to clarify the actual effect of NGPS supplementation on knee function. Second, since there was no physiological endpoint, it is impossible to assess the state of the knee cartilage. To assess the structural conditions of the knee, metabolism markers, such as collagen in blood samples should have been evaluated. Third, NGPS had several components, further studies should be conducted to clarify the role of each component of NGPS on knee function.

The present results revealed that GlcNAc and proteoglycan containing supplement is effective for relieving knee pain and the improvement of knee function when walking or climbing stairs, swelling and bending or stretching. Moreover, the safety of this supplement was confirmed.

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Conflict of Interest

This study was funded by the CHARLE CO., LTD., Kobe, Japan. Haruo Yamamura is an employee of the Charle CO., LTD. No other authors declare any potential conflict of interest.

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