Evaluation of the effect of N-acetyl-glucosamine administration on biomarkers for cartilage metabolism in healthy individuals without symptoms of arthritis: A randomized double-blind placebo-controlled clinical study

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Abstract. The present study aimed to evaluate the effect of N-acetyl-glucosamine (GlcNAc) on the joint health of healthy individuals without arthritic symptoms. A randomized double-blind placebo-controlled clinical trial was performed to investigate the effect of oral administration of a GlcNAc-containing test supplement (low dose, 500 mg/day and high dose, 1,000 mg/day) on cartilage metabolism in healthy individuals with a mean age of 48.6±1.3 years (range, 23-64 years) by analyzing the ratio of type II collagen degradation to type II collagen synthesis using type II collagen degradation (C2C) and synthesis (PIICP) markers. The results indicated that the changes in C2C/PIICP ratios from the baseline were suppressed in the treated with low and high doses of GlcNAc, compared with the placebo group at week 16 during intervention. To further elucidate the effect of GlcNAc, subjects with impaired cartilage metabolism were evaluated. Notably, the changes in the C2C/PIICP ratios were markedly suppressed in the groups treated with low and high doses of GlcNAc at week 16. Finally, to exclude the effect of heavy body weight on joint loading, subjects weighing <70 kg with impaired cartilage metabolism were analyzed. Notably, the changes in the C2C/PIICP ratios were suppressed in the groups treated with low and high doses of GlcNAc at weeks 12 and 16. No test supplement-related adverse events were observed during or following the intervention. Together, these observations suggest that oral administration of GlcNAc at doses of 500 mg and 1,000 mg/day exhibits a chondroprotective effect on healthy individuals by reducing the C2C/PIICP ratio (relatively decreasing type II collagen degradation and increasing type II collagen synthesis) without any apparent adverse effects.

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Introduction

Frequency and severity of joint loading are important determinants for the development of joint destruction. Osteoarthritis, which develops due to the progressive destruction of articular cartilage, is the most common joint disease and is the leading cause of pain and physical disability in elderly individuals (1-3). Knee joints are particularly affected in patients with osteoarthritis, as they are weight-bearing joints. Previous studies investigating experimental osteoarthritis models have demonstrated that the early changes in the metabolic and chemical properties of cartilage matrix can be detected prior to the appearance of radiological changes (4). Therefore, various molecular markers have been developed as indicators of cartilage metabolism in patients with joint disorders (5-8). Furthermore, biomarkers are used for evaluating the effects of disease-modifying drugs, since they specifically reflect alterations in the metabolism of cartilage (9).

Type II collagen is one of the major constituents of cartilage and represents 90-95% of the total collagen in cartilage (7). Therefore, fragments of type II collagen have been targeted as biomarkers for cartilage metabolism. C-terminal crosslinking peptide II (CTX-II) is cleaved during the degradation of type II collagen (10), whereas a C2C neoepitope is generated by intrahelical cleavage at the C terminus of the 3/4 piece of degraded type II collagen (11). Both CTX-II and C2C are used as markers for type II collagen degradation. In contrast, a C-terminal type II procollagen peptide (CPII or PIICP), which is localized in newly formed type II collagen and cleaved during processing of synthesized type II procollagen, can be used as a marker for type II collagen synthesis (12).

Nutritional supplements, including glucosamine, N-acetylglucosamine, chondroitin and collagen, are used for 'joint health' to treat or prevent cartilage disorders, including osteoarthritis (13-15). Among these, glucosamine inhibits the degradation and stimulates the synthesis of the characteristic glycosaminoglycan polysaccharide chains of proteoglycans (16,17). Furthermore, glucosamine suppresses the expression of collagen-degrading enzymes, such as matrix metalloproteinases (MMPs), whereas it increases the expression of type II collagen in chondrocytes (18,19). Based on these observations, it has been hypothesized that glucosamine exerts a chondroprotective effect on cartilage disorders by retaining proteoglycans and type II collagen in the articular cartilage, thus glucosamine may be used to treat osteoarthritis in humans (20-22). A previous study evaluated the effect of glucosamine on cartilage metabolism in healthy bicycle racers (mean age, 20 years) with normal cartilage metabolism, as assessed by the levels of CTX-II and CPII (23). The results indicated that oral administration of glucosamine may exert a chondroprotective effect in healthy individuals by preventing type II collagen degradation and simultaneously maintaining type II collagen synthesis.

N-acetylglucosamine (GlcNAc), a derivative of glucosamine, has been reported to stimulate hyaluronan synthesis via the upregulation of hyaluronan synthase-2 in chondrocytes (24), whereas it inhibits the interleukin (IL)-1β-mediated expression of inducible nitric oxide (NO) synthase, cyclooxygenase-2 and IL-6 in chondrocytes (25). Therefore, it is hypothesized that GlcNAc exerts chondroprotective and antiinflammatory effects in cartilage disorders. In previous studies investigating experimental osteoarthritis models, intra-articular injection of GlcNAc has been demonstrated to exhibit a chondroprotective effect (26,27). Moreover, the administration of a GlcNAc-containing beverage has been shown to enhance type II collagen synthesis, and resolves the symptoms of patients with knee osteoarthritis (28). Therefore, GlcNAc is hypothesized to exhibit a chondroprotective effect in healthy individuals by improving cartilage metabolism.

To the best of our knowledge, no previous studies have demonstrated the effect of GlcNAc on cartilage metabolism in healthy individuals. Therefore, in the present study, to evaluate the effect of GlcNAc on the joint health of healthy individuals without symptoms of joint disorders, the effect of oral administration of low (500 mg/day) and high (1,000 mg/day) dose GlcNAc on cartilage metabolism in healthy middle-aged adults (mean age,48.6±1.3 years; range, 23-64 years) by analyzing the ratio of type II collagen degradation to synthesis using type II collagen degradation (C2C) and synthesis (PIICP) markers.

Materials and methods

Study design. The present prospective randomized double-blind placebo-controlled, parallel-group comparative study was designed to assess the effects of a GlcNAc-containing test supplement and placebo on cartilage metabolism (by proxy of type II collagen synthesis and degradation) in healthy individuals without any joint disorder symptoms. The safety of the test supplement was also evaluated. This study was performed from January 2014 to August 2014, and involved three clinical service organization centers in Japan. The study protocol was approved by the local ethics committee of Tana Orthopedic Surgery and was conducted in accordance with the Declaration of Helsinki and the Ethical Guidelines for Epidemiological Research outlined by the Japanese Government in 2008. Written informed consent was obtained from all participants prior to their enrollment in the study. The design of the study consisted of a 4-week run-in (screening) period, a 16-week intervention period and a subsequent 4-week follow-up period without intervention. Subjects were screened for baseline values at a run-in visit, which included a physical examination, a knee radiograph according to a standardized method (29), a symptom questionnaire and routine laboratory tests. In total, medical examinations and laboratory tests were performed at baseline, weeks 4, 8, 12 and 16 during the intervention, and 4 weeks after the intervention for all enrolled subjects.

Subjects. Exclusion criteria were as follows: Gout/hyperuricemia or rheumatoid arthritis; surgical treatment of joint(s) or its necessity; clinical history of bone or cartilage disorders (including fracture and distortion) within one year of enrollment; routine use of health foods containing glucosamine, chondroitin sulfate, collagen peptides or any other constituents of the test supplement within 3 months of enrollment; hypersensitivity or allergy to constituents of the test supplement; previous or current medication for malignancies, hypertension, cardiac diseases, renal diseases, thyroid diseases or cerebral infarction; intra-articular injections of either corticosteroids or hyaluronic acid within one year of enrollment; severe exercise with excessive motion and exposure which places load on the joints; intake of >60 g alcohol/day; pregnancy, nursing mothers or women of child-bearing potential during the study period; participation in other clinical studies within one month of enrollment; and the presence of any clinically significant conditions judged by the medical investigator to preclude the subject's participation in the present study.

Based on the criteria outlined, 75 male and female Japanese adults (mean age, 49.3±1.3 years; range, 23-64 years) without clinical or radiographic evidence of knee osteoarthritis (Kellgren and Lawrence grades 0-1, predominantly 0) (29) were enrolled as eligible subjects. Subjects were randomly assigned to high (n=25) or low (n=25) dose GlcNAc, or placebo (n=25) groups (Fig. 1). However, during the intervention period, five subjects discontinued the study of their own accord or the onset of ulcerative colitis (placebo group, n=3; high dose GlcNAc group, n=2). Therefore, 70 subjects completed the present study. Five subjects were subsequently excluded from the study analysis by the medical investigator due to treatment with antidepressants for depressive disorders and antiinflammatory agents for ankle and Achilles-tendon pains, and increased body weight (>4 kg) during the intervention period, as these may affect the efficacy of the test supplement (placebo group, n=2; high dose GlcNAc group, n=3). Therefore, 65 subjects (mean age, 48.6±1.3 years) in the placebo (n=20), low dose GlcNAc (n=25) and high dose GlcNAc (n=20) groups were eligible for the assessment of the efficacy of the test supplement (Table I).

Intervention and subject assignment. The test supplement was manufactured by Q'sai Co., Ltd (Fukuoka, Japan) in a powdered preparation containing 1,000 mg GlcNAc for the

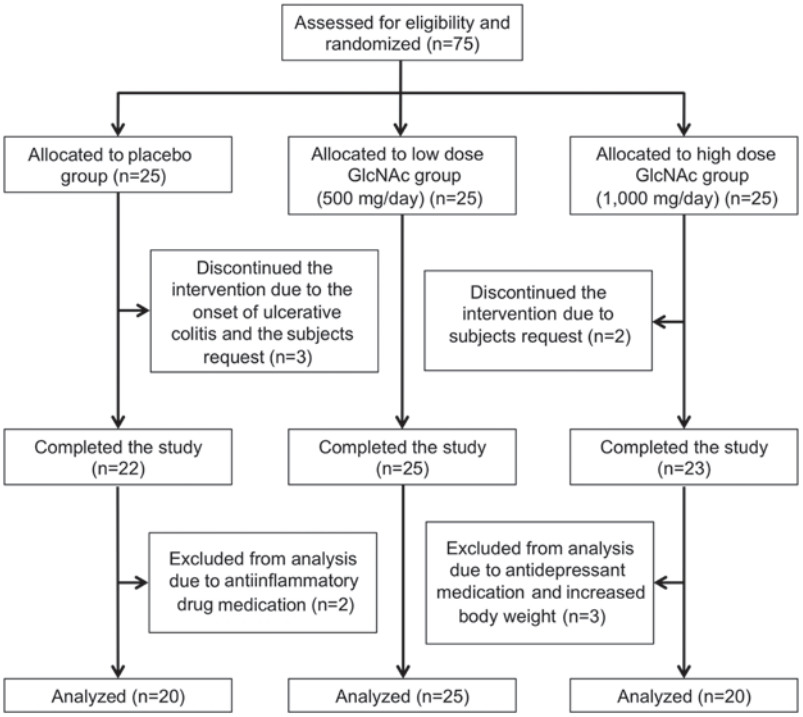

Figure 1. Schematic flow diagram of the subjects who participated in the present study. GlcNAc, N-acetyl-glucosamine.

high dose group, and 500 mg GlcNAc and 500 mg maltodextrin (vehicle) for the low dose group. Subjects were randomly assigned to either the high (1,000 mg GlcNAc) or low (500 mg GlcNAc and 500 mg maltodextrin) dose GlcNAc groups, or the placebo group, (1,000 mg maltodextrin vehicle). All subjects were instructed to take the test supplement or placebo (dissolved in 100 ml water) once daily at any time of the day. The daily dose of the test supplement in the present study was determined according to the results of the previous study (16). Intervention was continued for 16 weeks, and adherence to the intervention protocol was evaluated based on the consumption record in the study diary; <80% adherence was considered a protocol violation.

Serum and second morning void of urine samples were collected from subjects in a fasting state, at baseline, weeks 4, 8, 12 and 16 during intervention, and 4 weeks post-intervention. Aliquots of serum and urine samples were stored at -80˚C until use, whereas the remaining aliquots of serum and urine samples were immediately used for routine laboratory tests.

Evaluation of efficacy and safety. To evaluate the effect of the GlcNAc-containing test supplement on cartilage metabolism, serum samples collected at baseline, weeks 8, 12 and 16 during intervention, and 4 weeks after intervention, were analyzed by assays for type II collagen degradation and synthesis markers (C2C and PIICP, respectively). Serum C2C and PIICP were measured using Collagen Type II Cleavege (IBEX Pharmaceuticals Inc., Montreal, Canada) and Procollagen II C-Terminal Propeptide (USCN Life Science Inc., Wuhan, China) ELISA kits, respectively. In addition, the C2C/PIICP ratio was calculated and compared among the test supplement and placebo groups.

Safety and tolerability were assessed throughout the study on the basis of the incidence and severity of intervention-related adverse events, as well as abnormal changes in blood pressure, pulse rate and laboratory tests, including hematology, biochemical profiling and urinalysis.

Statistical analysis. Values are expressed as the mean ± standard error of the mean. Using the baseline characteristics of enrolled subjects, the sex distribution and Kellgren and Lawrence grades were analyzed by χ^2 test, whereas other parameters were analyzed by one-way analysis of variance among the placebo and low and high dose GlcNAc test supplement groups. In addition, the changes in biomarker levels from the baseline during and after intervention were compared among the placebo and low and high dose GlcNAc test supplement groups, and between the placebo and test supplement groups by Student's t-test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Characterization of study groups. Table I presents the baseline characteristics of the 65 subjects (placebo group, n=20; test supplement groups: low dose GlcNAc, n=25 and high dose GlcNAc, n=20), who fulfilled the eligibility criteria and completed the present study. Baseline characteristics included demographic characteristics (age and sex), physiological characteristics (body height, body weight, body mass index, systolic and diastolic blood pressure, and pulse rate), distribution of Kellgren and Lawrence grades, and the levels of biomarkers for type II collagen metabolism (C2C, PIICP and C2C/PIICP ratio). No significant differences in baseline characteristics were detected among the placebo and test supplement (low and high dose GlcNAc) groups, with the exception of systolic blood pressure. Although systolic blood pressure values were significantly different among the groups ($P<0.05$), they

Table I. Baseline characteristics of the subjects in the placebo and low and high dose GlcNAc test supplement groups.

Variable	Placebo (n=20)	GlcNAc-containing test supplement		P-value
		Low dose (n=25)	High dose (n=20)	
Age (years)	48.8±2.1	48.0±2.2	49.2±2.7	0.927
Male/female (N)	10/10	10/15	9/11	0.779
Height (cm)	164.33±1.58	162.22±1.73	163.51±2.04	0.695
Weight (kg)	57.68±2.47	57.48±2.36	62.39±2.87	0.327
Body mass index (kg/m^2)	21.23±0.66	21.63±0.56	23.22±0.80	0.107
Systolic blood pressure (mmHg)	120.0 ± 3.1	110.2±2.0	114.5±2.33	**0.020**
Diastolic blood pressure (mmHg)	76.8±2.1	71.7±1.5	75.1±1.8	0.126
Pulse rate (beats/min)	68.5±2.0	70.1±2.1	71.3±1.9	0.632
Kellgren and Lawrence grade (0:1)[a]				
Right knee	18:2	22:3	18:2	1.000
Left knee	18:2	21:4	18:2	0.803
C2C (ng/ml)[b]	226.57±11.08	224.47±7.55	226.08±9.07	0.985
PIICP (ng/ml)[c]	48.37±2.25	48.47±1.99	47.99±2.16	0.986
C2C/PIICP ratio	4.89±0.34	4.87±0.29	4.95±0.33	0.983

Values are expressed as the mean ± standard error of the mean, except where indicated otherwise. Signficant P-values (P<0.05) are indicated in bold. [a]Number of knees; [b]collagen degradation marker; [c]collagen synthesis marker. GlcNAc, N-acetyl-glucosamine.

Table II. Baseline characteristics of subjects with ≥220 ng/ml C2C and <60 ng/ml PIICP in the placebo and low and high dose GlcNAc test supplement groups.

Variable	Placebo (n=7)	GlcNAc-containing test supplement		P-value
		Low dose (n=12)	High dose (n=10)	
Ages (years)	51.7±3.8	46.5 ± 4.0	50.0 ± 3.8	0.647
Male/female (N)	4/3	3/9	5/5	0.393
Height (cm)	165.39±3.32	161.40±2.52	166.26±2.86	0.408
Weight (kg)	55.01±3.72	58.68±4.04	60.79±4.74	0.689
Body mass index (kg/m^2)	19.98±0.77	22.25±1.01	21.71±1.02	0.318
Systolic blood pressure (mmHg)	122.9±5.2	108.5±2.8	117.4±3.5	**0.032**
Diastolic blood pressure (mmHg)	77.9±4.3	71.8±2.0	77.6±2.5	0.196
Pulse rate (beats/min)	68.9±2.4	68.1±2.6	73.4±3.2	0.356
Kellgren and Lawrence grade (0:1)[a]				
Right knee	7:0	11:1	9:1	1.000
Left knee	7:0	10:2	9:1	0.770
C2C (ng/ml)[b]	277.63±16.09	249.77±8.58	255.79±10.93	0.248
PIICP (ng/ml)[c]	46.43±3.25	44.58±2.45	43.12±1.66	0.678
C2C/PIICP ratio	6.16±0.55	5.80±0.37	6.02±0.35	0.825

Values are expressed as the mean ± standard error of the mean, except where indicated otherwise. Signficant P-values (P<0.05) are indicated in bold. [a]Number of knees; [b]collagen degradation marker; [c]collagen synthesis marker. GlcNAc, N-acetyl-glucosamine.

remained within the normal range (<130 mmHg). Adherence to the allotted dietary supplement exceeded 96% in all of the who completed the study (n=70).

Assessment of cartilage metabolism using type II collagen degradation and synthesis markers. It has been suggested that the ratio of type II collagen degradation to synthesis is suitable for the prediction of joint damage progression in patients with knee osteoarthritis (30,31). Therefore, to evaluate the effect of a GlcNAc-containing test supplement on cartilage metabolism, the C2C/PIICP ratio was assessed using serum samples collected at baseline, weeks 8, 12 and 16 during intervention,

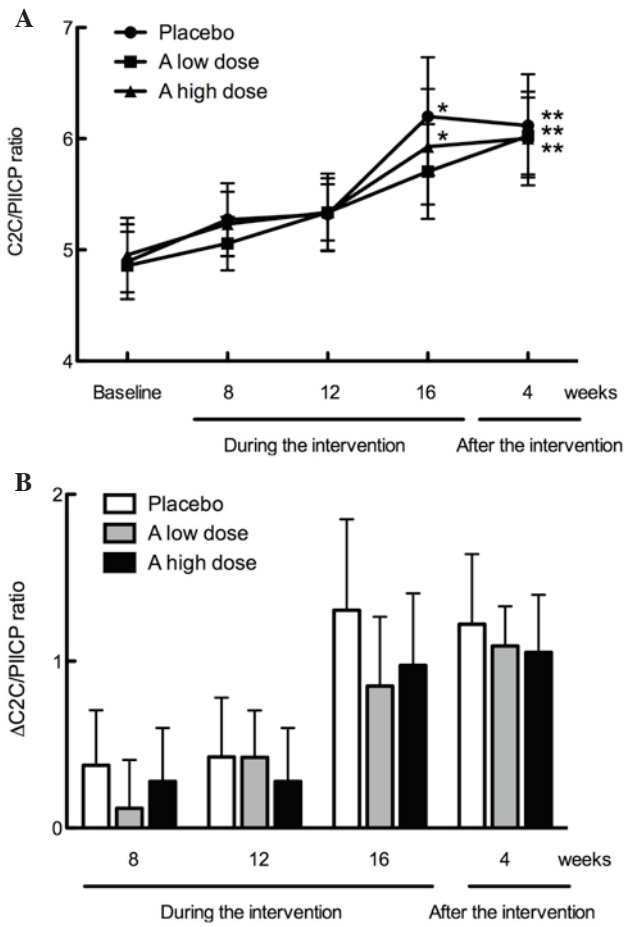

Figure 2. C2C/PIICP ratios of the subjects in the placebo and low and high dose GlcNAc test supplement groups during and after intervention. (A) C2C and PIICP were analyzed and the C2C/PIICP ratios were calculated using serum samples collected from subjects in the placebo (n=20; closed circles) and low (n=25; closed squares) and high (n=20; closed triangles) dose GlcNAc groups at baseline, weeks 8, 12 and 16 during intervention, and 4 weeks after intervention. (B) Subsequently, the changes of the C2C/PIICP ratios from the baseline were calculated and expressed as ΔC2C/PIICP ratio in the placebo (white) and low (gray) and high (black) dose GlcNAc groups at baseline, weeks 8, 12 and 16 during intervention, and 4 weeks after intervention. Values are expressed as the mean ± standard error of the mean. *P<0.05 and **P<0.01 vs. baseline.

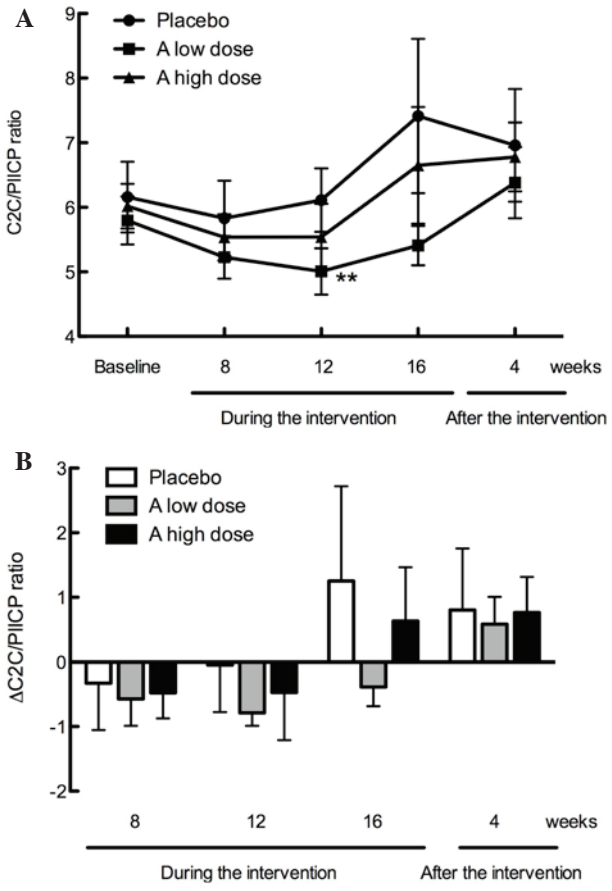

Figure 3. C2C/PIICP ratios of the subjects with ≥220 ng/ml C2C and <60 ng/ml PIICP in the placebo and low and high dose GlcNAc test supplement groups during and after intervention. (A) C2C and PIICP were analyzed and the C2C/PIICP ratios were calculated using serum samples collected from subjects in the placebo (n=7; closed circles) and low (n=12; closed squares) and high (n=10; closed triangles) dose GlcNAc groups at baseline, weeks 8, 12 and 16 during intervention, and 4 weeks after intervention. (B) Subsequently, the changes of the C2C/PIICP ratios from the baseline were calculated and expressed as ΔC2C/PIICP ratio in the placebo (white) and low (gray) and high (black) dose GlcNAc groups at baseline, weeks 8, 12 and 16 during intervention, and 4 weeks after intervention. Values are expressed as the mean ± standard error of the mean. **P<0.01 vs. baseline.

and 4 weeks after intervention. As demonstrated in Fig. 2A, C2C/PIICP ratios peaked at 16 weeks during intervention in the placebo group and high dose of GlcNAc (P<0.05), and maintained almost the same level 4 weeks after intervention (P<0.01). By contrast, the C2C/PIICP ratios gradually increased in the low dose of GlcNAc group during the 16-week intervention and 4 weeks post-intervention (P<0.01; 4 weeks after the intervention). However, there was no significant difference among the three groups. Following intervention, the C2C/PIICP ratios were maintained at almost the same level (P<0.01 in the C2C/PIICP ratios in the low and high doses of GlcNAc and placebo groups) 4 weeks after intervention, compared with the baseline. Notably, the ΔC2C/PIICP ratios from the baseline were markedly suppressed in the low and high dose GlcNAc test supplement groups (+0.81 and +0.97, respectively), compared with the placebo group (+1.31) at week 16 during the intervention. Furthermore, 4 weeks post-intervention, the ΔC2C/PIICP ratio in the test supplement groups (low and high dose GlcNAc) returned to the same level as the placebo group (Fig. 2B).

To further elucidate the effects of the GlcNAc-containing test supplement, the subjects with impaired cartilage metabolism were assessed. For this purpose, subjects with reduced type II collagen degradation (<220 ng/ml C2C) and enhanced type II collagen synthesis (≥60 ng/ml PIICP) were excluded, and subjects with ≥220 ng/ml C2C and <60 ng/ml PIICP were evaluated. Table II presents the baseline characteristics of these subjects, including demographic and physiological characteristics, Kellgren and Lawrence grade distribution, and the levels of biomarkers for type II collagen metabolism. Among the placebo (n=7) and low (n=12) and high (n=10) dose GlcNAc test supplement groups, these parameters were not significantly different, with the exception of systolic blood pressure. As shown in Fig. 3A, the C2C/PIICP ratios were not significantly different among the three groups during intervention (16 weeks) and after intervention (4 weeks); however, the C2C/PIICP ratio was significantly decreased at week 12

Table III. Baseline characteristics of subjects weighing <70 kg with ≥220 ng/ml C2C and <60 ng/ml PIICP in the placebo and low and high dose GlcNAc test supplement groups.

Variable	Placebo (n=7)	GlcNAc-containing test supplement		P-value
		Low dose (n=10)	High dose (n=7)	
Ages (years)	51.7±3.8	44.1±4.3	48.9±5.1	0.472
Male/female (N)	4/3	2/8	2/5	0.319
Height (cm)	165.39±3.32	159.53±2.64	161.51±2.24	0.337
Weight (kg)	55.01±3.72	54.29±3.28	51.91±1.65	0.791
Body mass index (kg/m^2)	19.98±0.77	21.16±0.80	19.91±0.54	0.400
Systolic blood pressure (mmHg)	122.9±5.2	106.3±2.3	114.7±3.9	**0.013**
Diastolic blood pressure (mmHg)	77.9±4.3	69.7±1.7	75.4±3.2	0.139
Pulse rate (beats/min)	68.9±2.4	69.6±2.8	77.3±2.8	0.100
Kellgren and Lawrence grade (0:1)[a]				
Right knee	7:0	9:1	6:1	1.000
Left knee	7:0	8:2	7:1	0.315
C2C (ng/ml)[b]	277.63±16.09	238.62±5.00	258.00±11.63	**0.048**
PIICP (ng/ml)[c]	46.43±3.25	44.23±2.95	42.73±2.14	0.702
C2C/PIICP ratio	6.16±0.55	5.64±0.43	6.13±0.41	0.654

Values are expressed as the mean ± standard error of the mean, except where indicated otherwise. Signficant P-values (P<0.05) are indicated in bold. [a]Number of knees; [b]collagen degradation marker; [c]collagen synthesis marker. GlcNAc, N-acetyl-glucosamine.

of intervention in the low dose GlcNAc group (P<0.01). As shown in Fig. 3B, the ΔC2C/PIICP ratios from the baseline were markedly suppressed in the low and high dose GlcNAc groups (week 12, -0.79 and -0.47; and week 16, -0.39 and +0.63), as compared with the placebo group (week 12, -0.05 and week 16, +1.25). The ΔC2C/PIICP ratios in the test supplement groups returned to the same levels as the placebo group 4 weeks after intervention (Fig. 3B).

Notably, the change in the ΔC2C/PIICP ratios from the baseline was markedly suppressed in the low dose GlcNAc group (-0.79 and -0.39, respectively), as compared with the high dose GlcNAc group (-0.47 and +0.63, respectively) at weeks 12 and 16 during intervention. This may be due to the difference in the mean body weight between the two groups, since heavy body weight may place an increased load on the joints, thereby affecting the response of cartilage metabolism to the test supplement. Based on this hypothesis, subjects with a body weight of ≥70 kg were excluded, and subjects weighing <70 kg were subsequently evaluated. Table III presents the baseline characteristics of the subjects who weighed <70 kg and exhibited ≥220 ng/ml C2C and <60 ng/ml PIICP. No significant differences in demographic and physiological characteristics, Kellgren and Lawrence grade distribution, and the levels of biomarkers for type II collagen metabolism were detected among the placebo (n=7) and test supplement (low dose GlcNAc, n=10 and high dose GlcNAc, n=7) groups, with the exception of systolic blood pressure and C2C.

As demonstrated in Fig. 4A, the C2C/PIICP ratios were not significantly different among the three groups during intervention (16 weeks) and after intervention (4 weeks); however, the C2C/PIICP ratio was significantly decreased at week 12 during the intervention in the low dose GlcNAc group (P<0.01). As shown in Fig. 4B, the ΔC2C/PIICP ratios from the baseline were similarly suppressed in the low and high dose GlcNAc groups (week 12, -0.83 and -0.98; and week 16, -0.43 and -0.05, respectively), as compared with the placebo group (week 12, -0.05 and week 16, +1.25). The ΔC2C/PIICP ratios in the test supplement groups returned to the same levels as the placebo group 4 weeks after intervention (Fig. 4B).

These findings suggest that the oral administration of the test supplement with low and high doses of GlcNAc exhibited a protective effect on cartilage metabolism in healthy individuals without any symptoms of joint disorders, by improving the C2C/PIICP ratio (relatively reduced type II degradation and increased type II collagen synthesis).

Assessment of safety and tolerability. Among the 75 subjects enrolled, seven subjects (28%) in the low dose GlcNAc group (n=25), eight subjects (32%) in the high dose GlcNAc group (n=25) and eight subjects in the placebo group (n=25) experienced one or more adverse events during the intervention period. The total number of adverse events reported was eight in the low dose GlcNAc group, 31 in the high dose GlcNAc group, and 18 in the placebo group, No significant differences in the frequency of adverse events were detected among the three groups. Adverse events reported from the study subjects predominantly included respiratory symptoms (sore throat, cough, rhinorrhea and/or fever), joint pain (shoulder or elbow) and neck stiffness. All adverse events were of mild intensity and were judged by an independent medical investigator who was blinded to the intervention.

Furthermore, no significant differences in physical measurement parameters (body weight and body mass index),

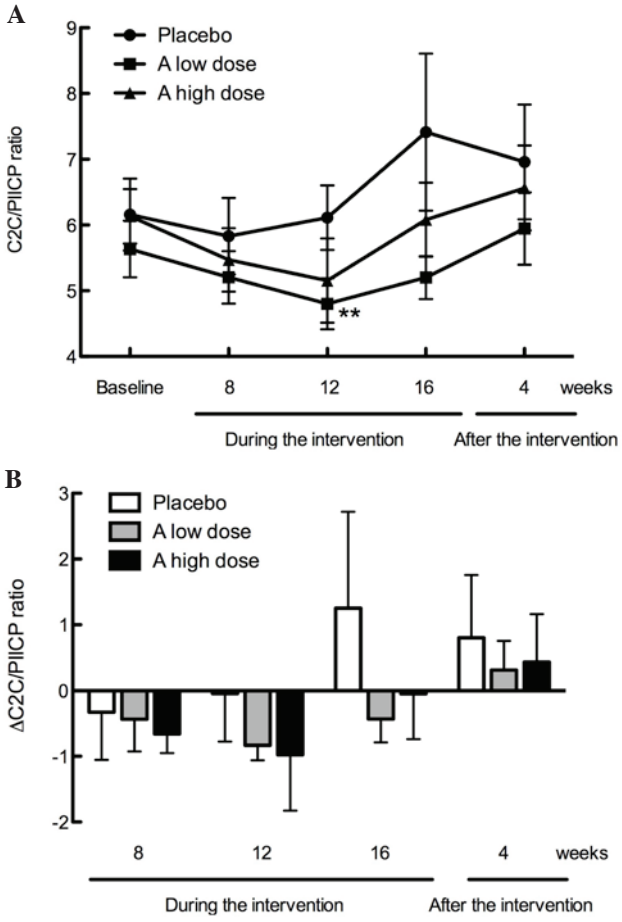

Figure 4. C2C/PIICP ratios of the subjects weighing <70 kg with ≥220 ng/ml C2C and <60 ng/ml PIICP in the placebo and low and high dose GlcNAc test supplement groups during and after intervention. (A) C2C and PIICP were analyzed and the C2C/PIICP ratios were calculated using serum samples collected from subjects in the placebo (n=7; closed circles) and low (n=10; closed squares) and high (n=7; closed triangles) dose GlcNAc groups at baseline, weeks 8, 12 and 16 during intervention, and 4 weeks after intervention. (B) Subsequently, the changes of the C2C/PIICP ratios from the baseline were calculated and expressed as ΔC2C/PIICP ratio in the placebo (white) and low (gray) and high (black) dose GlcNAc groups at baseline, weeks 8, 12 and 16 during intervention, and 4 weeks after intervention. Values are expressed as the mean ± standard error of the mean. **P<0.01 vs. baseline.

physiological examinations (systolic and diastolic blood pressures, and pulse rate) and laboratory tests (urinalysis, hematology and blood chemistry) were detected between the baseline and during and after the intervention in the three groups.

Discussion

Accumulating evidence indicates that biomarkers for cartilage metabolism, particularly type II collagen metabolism, can be used to screen for individuals at risk of progressive joint destruction, and for monitoring the effects of structure-modifying agents or therapies on osteoarthritis (8). For example, previous studies have demonstrated the use of type II collagen degradation biomarkers, including CTX-II and C2C, to evaluate the effects of chondroprotective agents such as glucosamine (32,33) and chondroitin sulfate (34). Subsequently, type II collagen synthesis biomarkers, such as CPII (PIICP), have been used alone or in combination with type II collagen degradation biomarkers (CTX-II and C2C) to monitor the disease state and progression of osteoarthritis, since the ratio of type II collagen degradation to synthesis has been demonstrated to be more effective than measuring a single biomarker for monitoring the effect of chondroprotective agents (30,31). Based on these findings, in the present study, in order to evaluate the effect of GlcNAc on joint health of healthy individuals without symptoms of arthritis, a randomized double-blind placebo-controlled clinical trial was performed to investigate the effect of oral GlcNAc administration (low dose, 500 mg/day and high dose, 1,000 mg/day) on cartilage metabolism in healthy middle-aged adults (mean age, 48.6±1.3 years) by analyzing the ratio of type II collagen degradation to synthesis using type II collagen degradation (C2C) and synthesis (PIICP) markers.

The results indicated that the changes in the C2C/PIICP ratios from the baseline were slightly suppressed in the low and high dose GlcNAc groups (+0.81 and +0.97, respectively), as compared with the placebo group (+1.31) at week 16 during intervention. The ΔC2C/PIICP ratios in the test supplement groups returned to the same level as the placebo group 4 weeks after intervention. To further elucidate the effect of the GlcNAc-containing test supplement, subjects with impaired cartilage metabolism (≥220 ng/ml C2C and <60 ng/ml PIICP) were evaluated. Notably, the changes in the C2C/PIICP ratios from the baseline were markedly suppressed in the low and high dose GlcNAc groups (week 12, -0.79 and -0.47; week 16, -0.39 and +0.63, respectively), as compared with the placebo group (week 12, -0.05 and week 16, +1.25). The ΔC2C/PIICP ratios in the test supplement groups returned to the same levels as the placebo group 4 weeks after intervention. Finally, to exclude the effect of heavy body weight on joint loading, subjects weighing <70 kg with ≥220 ng/ml C2C and <60 ng/ml PIICP were analyzed. Notably, the changes in the C2C/PIICP ratios from the baseline were markedly suppressed in the low and high dose GlcNAc groups (week 12, -0.83 and -0.98; week 16, -0.43 and -0.05, respectively), as compared with the placebo group (week 12, -0.05 and week 16, +1.25), and the ΔC2C/PIICP ratios in the test supplement groups returned to the same level as the placebo group 4 weeks after intervention. Moreover, no test supplement-related adverse events were observed during or after the intervention. Together, these observations suggest that oral administration of GlcNAc at doses of 500 mg and 1,000 mg/day induces a chondroprotective effect on the healthy individuals without any apparent adverse effect, by lowering the C2C/PIICP ratio (relatively reducing type II degradation and increasing type II collagen synthesis) and improving cartilage metabolism. However, this effect is reversible and disappears after withdrawal of the administration.

The mechanism by which the GlcNAc-containing test supplement exerts a protective effect on the cartilage metabolism remains to be clarified. In this context, it is interesting to note that GlcNAc stimulates hyaluronan synthesis via the upregulation of hyaluronan synthase-2 in chondrocytes (24). Hyaluronan is reported to inhibit IL-1β-induced MMP-13 expression via its principal receptor, CD44, and subsequent signaling of p38 mitogen-activated protein kinase (MAPK) in arthritic chondrocytes (35). In addition, hyaluronan suppresses

aggrecan degradation by downregulating IL-1α-induced expression a disintegrin-and metalloproteinase with thrombospondin motifs (ADAMTS)-4, which is an aggrecanase, through the CD44 signaling in osteoarthritic chondrocytes (36). Hyaluronan also suppresses the IL-1β-induced expression of MMP-3, MMP-13, ADAMTS-4 and ADAMTS-5 in osteoblasts (37). Notably, GlcNAc inhibits the IL-1β-mediated expression of inducible NO synthase, cyclooxygenase-2 and IL-6 via the inhibition of MAPKs including c-jun N-terminal kinase, extracellular signal-related kinase and p38MAPK activation in chondrocytes (25). Therefore, GlcNAc may improve cartilage metabolism by reducing the C2C/PIICP ratio (relatively reducing type II collagen degradation and increasing type II collagen synthesis) due to its chondroprotective and antiinflammatory effects based on the suppression of cartilage degrading enzymes, such as MMPs and ADAMTSs, potentially via the production of hyaluronan. However, a detailed mechanism outlining how GlcNAc effects cartilage metabolism via type II collagen degradation and synthesis remains to be elucidated.

The present study had a limitation. The number of subjects enrolled in study was small; thus, it was difficult to detect significant differences among the three groups, particularly when subjects with impaired cartilage metabolism were selected and analyzed. In future studies, the number of subjects enrolled should be increased when demonstrating the potential of a test supplement to improve cartilage metabolism in healthy individuals.

To the best of our knowledge, this study was first to evaluate the effect of oral GlcNAc administration on cartilage metabolism in healthy individuals. However, it has previously been demonstrated that intra-articular injection of GlcNAc exhibits chondroprotective effects on experimental osteoarthritis models (26,27), and the administration of a GlcNAc-containing beverage improved the symptoms of patients with knee osteoarthritis in a previous study, possibly by relatively increasing type II collagen synthesis and reducing the ratio of CTX-II/CPII (28). The efficacy and safety of GlcNAc demonstrated in the present study indicates that this GlcNAc-containing supplement can be safely administered, as it potently exerts a chondroprotective effect on healthy individuals by improving the type II collagen metabolism in the cartilage without any major adverse effects. Therefore, GlcNAc-containing supplements may be a potential candidate for improved joint health in healthy individuals without arthritic symptoms.

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