ORIGINAL RESEARCH

Glucosamine-containing supplement improves locomotor functions in subjects with knee pain: a randomized, double-blind, placebo-controlled study

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Background: The aim of this study was to investigate the ability of a glucosamine-containing supplement to improve locomotor functions in subjects with knee pain.

Methods: A randomized, double-blind, placebo-controlled, parallel-group comparative study was conducted for 16 weeks in 100 Japanese subjects (age, 51.8±0.8 years) with knee pain. Subjects were randomly assigned to one of the two supplements containing 1) 1,200 mg of glucosamine hydrochloride, 60 mg of chondroitin sulfate, 45 mg of type II collagen peptides, 90 mg of quercetin glycosides, 10 mg of imidazole peptides, and 5 μg of vitamin D per day (GCQID group, n=50) or 2) a placebo (placebo group, n=50). Japanese Knee Osteoarthritis Measure, visual analog scale score, normal walking speed, and knee-extensor strength were measured to evaluate the effects of the supplement on knee-joint functions and locomotor functions.

Results: In subjects eligible for efficacy assessment, there was no significant group × time interaction, and there were improvements in knee-joint functions and locomotor functions in both groups, but there was no significant difference between the groups. In subjects with mild-to-severe knee pain at baseline, knee-extensor strength at week 8 (104.6±5.0% body weight vs 92.3±5.5% body weight, P=0.030) and the change in normal walking speed at week 16 (0.11±0.03 m/s vs 0.05±0.02 m/s, P=0.038) were significantly greater in the GCQID group than in the placebo group. Further subgroup analysis based on Kellgren–Lawrence (K–L) grade showed that normal walking speed at week 16 (1.36±0.05 m/s vs 1.21±0.02 m/s, $P<0.05$) was significantly greater in the GCQID group than in the placebo group in subjects with K–L grade I. No adverse effect of treatment was identified in the safety assessment.

Conclusion: In subjects with knee pain, GCQID supplementation was effective for relieving knee pain and improving locomotor functions.

Keywords: normal walking speed, knee-extensor strength, quercetin glycosides, imidazole peptides, vitamin D

Introduction

In Japan, people aged 65 years and older comprise >21% of the population, and the nation is becoming a super-aged society more rapidly than any other country. In 2007, the Japanese Orthopaedic Association (JOA) proposed the concept of "locomotive syndrome", which refers to those elderly individuals who require nursing-care services because of problems with the locomotor organs or because they are at risk for conditions for which they may need such services in the future.[1] Locomotive syndrome is caused mainly by age-related locomotor organ diseases such as osteoporosis and osteoarthritis

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(OA) or age-related locomotor dysfunctions such as decline in walking speed and loss of muscle strength.[2,3]

OA is the most common joint disease, which causes pain in elderly patients. The knee is most frequently affected by OA because it is a weight-bearing joint, and knee OA impairs locomotor functions such as walking speed.[4] According to the Research on Osteoarthritis Against Disability (ROAD) study, >25 million Japanese people aged 40 years and older have been estimated to have radiographically determined Kellgren–Lawrence (K–L) grade II or higher joint-space narrowing.[5,6] Previous studies reported that walking speed was associated with survival in older adults and that muscle weakness is the highest risk factor for falling, which can result in being bedridden.[7,8] Therefore, preventing these locomotor dysfunctions is considered essential to maintain quality of life.

We previously demonstrated that an orally administered supplement containing glucosamine hydrochloride, chondroitin sulfate, and quercetin glycosides (GCQ) was effective for relieving knee pain.[9,10] Supplements containing glucosamine and chondroitin sulfate have been widely used for the management of knee pain in OA.[11–13] Nonsteroidal anti-inflammatory drugs (NSAIDs) relieve knee pain associated with OA and improve locomotor functions in patients with knee OA.[14] These facts led us to speculate that glucosamine-containing supplements, as well as NSAIDs, could contribute to improved locomotor functions such as knee-extensor strength and walking speed.

Quercetin, one of the flavonoids, which is widely distributed in plants and fruits, has been reported to suppress the atrophy of skeletal muscle in vivo.[15] Imidazole peptides are distributed mainly in the muscles of animals and have been reported to attenuate muscle fatigue in humans.[16] Furthermore, vitamin D is a nutrient that supports bone metabolism, and several studies have demonstrated its benefit in maintaining musculoskeletal functions and reducing the risk of falls in the elderly.[17,18]

In the present study, we investigated the effects of 16 weeks of treatment with a supplement containing glucosamine hydrochloride, chondroitin sulfate, type II collagen peptides, quercetin glycosides, imidazole peptides, and vitamin D (GCQID) on locomotor functions in subjects with knee pain.

Methods
Study design and participants
A randomized, double-blind, placebo-controlled, parallel-group comparative study was designed to assess the efficacy and safety of GCQID supplementation in Japanese women and men aged 40–74 years. Inclusion criteria were the presence of knee pain, confirmed using the walking subscale of the JOA criteria (≤25 points for either the left or right knee joint);[19] visual analog scale (VAS) score for knee pain (≥20 mm using the first (I) item of the Japanese Knee Osteoarthritis Measure [JKOM]);[20] and K–L grades 0–II.[5] All participants received an explanation about the study from the medical investigators, and written informed consent was obtained prior to enrollment in the study. Exclusion criteria were the following: faster walking speed (≥1.6 m/s); presence of hyperuricemia, diabetes, cardiovascular disease, hepatic disease, renal disease, or heart disease; treatment with exercise, diet, or medicine that may have an effect on the weight; presence of rheumatic arthritis that may cause joint pain; surgical treatment of knee joint(s) undergone or its necessity; needs to undergo pharmacological articular treatments during the study period; intra-articular hyaluronic acid within 2 weeks or corticosteroids within 3 months before inclusion; a history of osseous or articular diseases other than OA within the past 3 months; routine use of health food or medicine that may have an effect on the evaluation of the study; daily use of a cane; performing daily or occasional vigorous exercise; treatment with warfarin; bronchial asthma, respiratory disease, or potential for developing allergy to the test supplement; pregnant women; nursing mothers or women of child-bearing potential; and presence of any medical condition judged by the medical investigator.

One hundred participants were enrolled in the study, which was performed from February 2012 to September 2012 at a clinical services center in Japan. All subjects, who had never taken the same or similar type of supplement before, were recruited in and around Osaka, Kyoto, Japan, through a volunteer bank managed by HUMA R&D Co, Inc. (Tokyo, Japan). Once radiographs were obtained, each subject's K–L grade was determined by an orthopedic surgeon. The study protocol was approved by the institutional ethics committee of East One Medical Clinic (Tokyo, Japan) and was conducted in accordance with the principles of the amended Declaration of Helsinki and Ethical Guidelines for Epidemiological Research (issued by the Japanese government in 2008).

Treatment and group assignment
The GCQID contained 1,200 mg of glucosamine hydrochloride, 300 mg of shark cartilage extract (60 mg as chondroitin sulfate, 45 mg as type II collagen peptides), 90 mg of quercetin glycosides, 100 mg of fish meat extract (10 mg

as imidazole peptides [anserine and carnosine]), and 5 μg (200 IU) of vitamin D in eight tablets. Placebo just contained base material such as dextrin, and it did not contain components such as glucosamine hydrochloride, chondroitin sulfate, type II collagen peptides, quercetin glycosides, imidazole peptides, and vitamin D in eight tablets. Subjects were randomly distributed to the two treatment groups in terms of normal walking speed, age, sex, average steps walked in a week, body mass index, and the aggregate scores for the JOA criteria and sequentially assigned to receive either GCQID (GCQID group) or placebo (placebo group). The randomization codes for enrolled subjects were held by an appointed person who was not involved in the study. The GCQID and placebo tablets were manufactured by Suntory Wellness Ltd specifically for the purpose of the present study to ensure that the placebo tablet was indistinguishable from the GCQID tablet in appearance, taste, and packaging. Allocation was preassigned on the basis of randomization numbers and was concealed from the subjects, the investigators, and the researchers who recruited and assessed participating subjects. All subjects were instructed to take eight tablets once a day and to record in their study diary whether they took the tablets or not.

Efficacy assessment

The efficacy of GCQID supplementation was assessed on the basis of measures of knee-joint functions and locomotor functions. Data on knee-joint functions were collected at baseline and at weeks 4, 8, 12, and 16. Data on locomotor functions were collected at baseline and at weeks 8 and 16. Serum 25-hydroxy vitamin D (25-OHD) levels were measured at baseline and week 16 as an assessment of efficacy. Exclusion criteria based on efficacy assessment were as follows: taking <80% of the test supplement, performing actions that affected the reliability of the efficacy assessment (subjects who had too great a difference [≥±0.25 m/s] in measured walking speed between the screening period and baseline), and noncompliance with the clinical protocol.

Knee-joint functions were measured as score for JKOM item I (VAS score for JKOM knee pain), total score for JKOM items II–V (JKOM total score),[20] and VAS score for pain on walking. VAS score was measured on a scale from 0 to 100, where 0 indicated no pain and 100 indicated the worst pain ever experienced. Subjects self-reported these measures on a website available only to the study participants. JKOM total score is a self-administered, disease-specific measure, consisting of 25 items that cover four different categories: "II: pain and stiffness in knees", "III: conditions

in daily life", "IV: general activities", and "V: health conditions". An overall result was assessed by summing the scores from the 25 items, with results ranging from 0 (no complaint) to 100 (most severe condition possible).

Locomotor functions were measured as walking speed and knee-extensor strength. For the measurement of walking speed, subjects were instructed to walk 10 m two times at their usual speed. The time it took to walk the intermediate 6 m was measured, and the average of two times was calculated as a subject's walking speed. We measured the knee-extensor strength (torque) using a procedure previously described.[21] For the measurement of knee-extensor strength, subjects performed isometric knee extensions on a custom dynamometer mounted force transducer (LU-100KSE; Kyowa Electronic Instruments, Tokyo, Japan). During the contraction of muscle, both knee joint and hip angles were flexed at 90° (180° was fully extended). For the maximal voluntary contractions of subjects, knee extension force exerted by knee extensor muscles was gradually increased from baseline to maximum in 2–3 seconds and then sustained at maximum for 2 seconds. Subjects carried out three trials with each leg, and the highest of the three was used. The sum of the values for both legs per unit body weight represented knee-extensor strength. The number of steps walked weekly, performed in leisurely fashion, and associated with activities of daily living was monitored using a pedometer.

A stratified analysis of subjects with mild-to-severe knee pain (VAS score for JKOM knee pain ≥20 at baseline) was also performed. Using the OA criteria from the ROAD study,[6] subgroups with or without radiographic OA (K–L grade ≥II or ≤I, respectively) were then analyzed further.

Safety assessment

The safety of GCQID supplementation was assessed on the basis of the incidence and severity of treatment-related adverse events reported throughout the 16-week intervention period. Furthermore, enrolled subjects were requested to visit a clinic to undergo laboratory tests (hematology, blood biochemistry, and urinalysis) and physical examinations at baseline and week 16 as part of the safety assessment. Blood samples were obtained with the subjects in a fasting state, and urine samples from the morning's second void were collected.

Statistical analysis

All data are expressed as mean ± standard error unless otherwise specified. Baseline data were compared between the groups using the unpaired *t*-test for quantitative variables and

χ^2-test for qualitative variables. Two-way repeated-measures analysis of variance was used for analyzing the differences in the effect of intervention, and post hoc analysis was conducted. In case of no significant group×time interaction, comparisons between groups at each time point were performed using the Mann–Whitney U-test for JKOM total score and the unpaired t-test for physical and laboratory test variables, VAS score for JKOM knee pain, VAS score for pain on walking, normal walking speed, and knee-extensor strength. For reference, changes in measurements during the intervention were compared with baseline using a paired t-test for physical and laboratory test variables; the Steel test for JKOM total score; and Dunnett's test for VAS score for JKOM knee pain, VAS score for pain on walking, normal walking speed, and knee-extensor strength. P-values <0.05 were considered significant. Effect sizes (d values for parametric variables and r values for nonparametric variables) were calculated in the measures in which there were significant differences between the groups. All statistical analyses were carried out using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corporation, Armonk, NY, USA) and Ekuseru-Toukei 2010 for Windows (Social Survey Research Information Co, Ltd, Tokyo, Japan).

Results

Table 1 presents the baseline characteristics for all enrolled subjects. No significant difference was observed between the groups for any characteristic. The 100 subjects who took the test supplement were eligible for safety assessment.

Three subjects dropped out of the study because of adverse events (GCQID: 1, placebo: 1) or personal reasons (GCQID: 1), and 15 subjects were excluded from efficacy assessment because of efficacy-assessment exclusion criteria: taking <80% of the test supplement (GCQID: 1); performing actions that affected the reliability of the efficacy assessment (too great a difference [≥±0.25 m/s] in measured walking speed between the screening period and baseline) (GCQID: 1, placebo: 2); and noncompliance with the clinical protocol (GCQID: 5, placebo: 6). Thus, 41 subjects in the GCQID group and 41 in the placebo group were deemed eligible for efficacy assessment.

Table 2 shows the changes in knee-joint functions and locomotor functions during the 16-week intervention period. There was no significant group × time interaction in all measures for efficacy assessment. There was no significant difference between the groups in all measures for efficacy assessment except for serum 25-OHD levels. Serum 25-OHD levels were significantly greater in the GCQID group than in the placebo group at week 16 (31.9±0.8 ng/mL vs 28.5±1.1 ng/mL, P<0.05, d=0.49).

A stratified analysis of subjects with mild-to-severe knee pain (VAS score for JKOM knee pain ≥20 at baseline) was performed. No significant difference was observed between the groups for any characteristic (Table 3). There was no significant group × time interaction in all measures for efficacy assessment except for serum 25-OHD levels, and knee-extensor strength was significantly greater in the GCQID group than in the placebo group at week 8 (P<0.05, d=0.65; Table 4).

Table 1 The baseline characteristics of the study population

Variables	GCQID group[a] (n=50)	Placebo group[a] (n=50)	*P*-value
Age (years)	51.9±1.2	51.6±1.1	0.852
Sex (male/female)	22/28	22/28	1.000
Height (cm)	162.0±1.0	163.0±1.2	0.507
Body weight (kg)	60.3±1.3	62.3±1.3	0.397
Body mass index (kg/m²)	22.9±0.5	23.3±0.5	0.500
Systolic blood pressure (mmHg)	129.7±2.1	125.7±2.2	0.198
Diastolic blood pressure (mmHg)	81.1±1.5	80.2±1.5	0.704
Heart rate (beats/min)	71.8±1.6	75.4±1.7	0.131
Normal walking speed (m/s)	1.28±0.02	1.29±0.02	0.912
Knee-extensor strength (% body weight)[b]	100.0±5.2	93.1±5.3	0.360
JOA criteria aggregate scores (points)	180.4±1.3	177.9±2.0	0.919
VAS score for pain on walking (mm)	25.5±3.0	21.3±2.7	0.297
Average daily steps walked in a week (steps)	6,511±465	6,192±446	0.622
K–L grades (0, I, II)	14, 25, 11	16, 23, 11	0.897

Notes: [a]All values except sex and K–L grade are expressed as mean ± standard error. [b]Knee-extensor strength was measured in both knees and expressed as total strength of both knees.
Abbreviations: GCQID, glucosamine hydrochloride, chondroitin sulfate, type II collagen peptides, quercetin glycosides, imidazole peptides, and vitamin D; JOA, Japanese Orthopaedic Association; K–L, Kellgren–Lawrence; VAS, visual analog scale.

Table 2 Changes in knee-joint functions and locomotor functions in subjects eligible for efficacy assessment[a]

Variables	Values at each time point[b]					Two-way ANOVA (group × time) P-value
	Baseline	Week 4	Week 8	Week 12	Week 16	
JKOM						
(I) VAS score for knee pain (mm)						
GCQID	27.0±3.4	24.2±3.1	17.1±2.5*	14.0±3.0**	9.8±2.3**	0.939
Placebo	28.5±3.0	24.2±3.3	18.7±3.2*	13.0±2.5**	11.4±2.1**	
(II–V) Total score (points)						
GCQID	14.0±1.6	11.8±1.5	9.2±1.0*	9.1±1.2**	8.5±1.1**	0.362
Placebo	13.0±1.0	12.6±1.1	11.0±1.1	10.5±1.2	9.3±0.9*	
VAS score for pain on walking (mm)						
GCQID	23.7±3.3	16.2±2.8	12.5±2.5**	9.6±2.3**	7.1±2.2**	0.216
Placebo	18.6±2.2	16.1±2.9	15.0±3.1	11.6±2.3*	7.3±1.6**	
Normal walking speed (m/s)						
GCQID	1.28±0.03		1.32±0.03*		1.35±0.02**	0.524
Placebo	1.28±0.02		1.32±0.02*		1.33±0.02**	
Knee-extensor strength (% body weight)[c]						
GCQID	95.9±5.0		104.6±5.0*		104.5±4.9*	0.546
Placebo	88.1±5.8		92.3±5.5		92.6±5.6	

Notes: [a]GCQID group (n=41) and placebo group (n=41). [b]Values expressed as mean ± standard error. [c]Knee-extensor strength was measured in both knees and expressed as the total strength of both knees. *$P<0.05$ and **$P<0.01$ compared with baseline value.
Abbreviations: ANOVA, analysis of variance; GCQID, glucosamine hydrochloride, chondroitin sulfate, type II collagen peptides, quercetin glycosides, imidazole peptides, and vitamin D; JKOM, Japanese Knee Osteoarthritis Measure; VAS, visual analog scale.

The change in JKOM total score at week 8 ($P<0.05$, r=0.37) and the change in normal walking speed at week 16 ($P<0.05$, d=0.62) were significantly greater in the GCQID group than in the placebo group (Figure 1). There was a significant group × time interaction in serum 25-OHD levels, and the change in serum 25-OHD levels at week 16 was significantly greater in the GCQID group than in the placebo group (11.9±1.8 ng/mL vs 6.9±1.3 ng/mL, $P<0.05$, d=0.80).

Subgroup analysis based on K–L grade in subjects with mild-to-severe knee pain at baseline was also performed. There was no significant group × time interaction, and there was no significant difference between the groups in JKOM total score and normal walking speed both in subjects with K–L grades ≥II and in subjects with K–L grade ≤I (Table 5). In subjects with K–L grades ≥II, the change in JKOM total score at week 8 was significantly greater in the

Table 3 Baseline characteristics of subjects selected for stratified analyses

Variables	GCQID group[a] (n=23)	Placebo group[a] (n=25)	P-value
Age (years)	51.5±1.7	50.6±1.4	0.702
Sex (male/female)	9/14	12/13	0.536
Height (cm)	162.3±1.7	163.8±1.7	0.527
Body weight (kg)	63.1±2.8	62.9±2.3	0.956
Body mass index (kg/m²)	23.8±0.7	23.4±0.7	0.696
Systolic blood pressure (mmHg)	131.1±3.6	126.6±3.2	0.352
Diastolic blood pressure (mmHg)	82.7±2.5	82.4±2.5	0.924
Heart rate (beats/min)	72.0±2.3	76.7±2.3	0.153
Normal walking speed (m/s)	1.26±0.04	1.25±0.03	0.957
Knee-extensor strength (% body weight)[b]	93.4±6.7	81.5±7.6	0.248
JOA criteria aggregate scores (points)	178.9±2.0	175.4±2.9	0.522
VAS score for pain on walking (mm)	33.3±4.3	24.5±3.0	0.103
Average daily steps walked in a week (steps)	6,221±510	6,386±599	0.837
K–L grades (0, I, II)	6, 14, 3	7, 12, 6	0.563

Notes: [a]All values except sex and K–L grade are expressed as mean ± standard error. [b]Knee-extensor strength was measured in both knees and expressed as total strength of both knees.
Abbreviations: GCQID, glucosamine hydrochloride, chondroitin sulfate, type II collagen peptides, quercetin glycosides, imidazole peptides, and vitamin D; JOA, Japanese Orthopaedic Association; K–L, Kellgren–Lawrence; VAS, visual analog scale.

Table 4 Changes in knee-joint functions and locomotor functions at each time point in subjects with mild-to-severe knee pain at baseline[a]

Variables	Values at each time point[b]					Two-way ANOVA (group × time) P-value
	Baseline	Week 4	Week 8	Week 12	Week 16	
JKOM						
(I) VAS score for knee pain (mm)						
GCQID	41.5±4.0	31.2±4.3	19.9±2.9**	15.4±4.4**	9.9±3.4**	0.490
Placebo	40.0±3.1	28.6±3.9*	22.4±3.5**	18.5±3.7**	16.4±3.0**	
(II–V) Total score (points)						
GCQID	17.6±2.5	14.3±2.5	10.3±1.5*	10.7±2.0**	9.2±1.7**	0.169
Placebo	15.2±1.3	13.7±1.6	13.3±1.6	12.2±1.7	10.6±1.2	
VAS score for pain on walking (mm)						
GCQID	33.3±4.3	22.2±4.3*	14.3±3.4**	12.0±3.9**	8.7±3.8**	0.067
Placebo	24.5±3.0	20.0±3.7	17.9±3.4*	16.6±3.4*	9.6±2.4**	
Normal walking speed (m/s)						
GCQID	1.26±0.04		1.32±0.04*		1.37±0.04**	0.095
Placebo	1.25±0.03		1.28±0.03		1.30±0.03*	
Knee extensor strength (% body weight)[c]						
GCQID	93.4±6.7		108.4±5.3**,§		109.8±5.6**	0.330
Placebo	81.5±7.6		88.0±7.3		92.0±7.8**	

Notes: [a]GCQID group (n=23) and placebo group (n=25). [b]Values are expressed as mean ± standard error. [c]Knee-extensor strength was measured in both knees and expressed as total strength of both knees. *$P<0.05$ and **$P<0.01$ compared with values at baseline. §$P<0.05$ compared with placebo.
Abbreviations: ANOVA, analysis of variance; GCQID, glucosamine hydrochloride, chondroitin sulfate, type II collagen peptides, quercetin glycosides, imidazole peptides, and vitamin D; JKOM, Japanese Knee Osteoarthritis Measure; VAS, visual analog scale.

GCQID group than in the placebo group (−20.3±12.5 points vs 0.5±1.5 points, $P<0.05$, $r=0.74$). The change in normal walking speed tended to be greater in the GCQID group than in the placebo group at week 16, but the difference was not significant ($P=0.08$). Similarly, in subjects with K–L grade ≤I, the change in normal walking speed tended to be greater in the GCQID group than in the placebo group at week 16 ($P=0.08$).

Further analysis of subjects with K–L grade I (14 in the GCQID group and 12 in the placebo group) showed that there was no significant group × time interaction in JKOM total score and normal walking speed, and the change in JKOM total score was significantly greater in the GCQID group than in the placebo group at week 8 (−6.2±1.5 points vs −1.8±0.9 points, $P<0.05$, $r=0.42$). Normal walking speed in the GCQID group was significantly greater than in the placebo
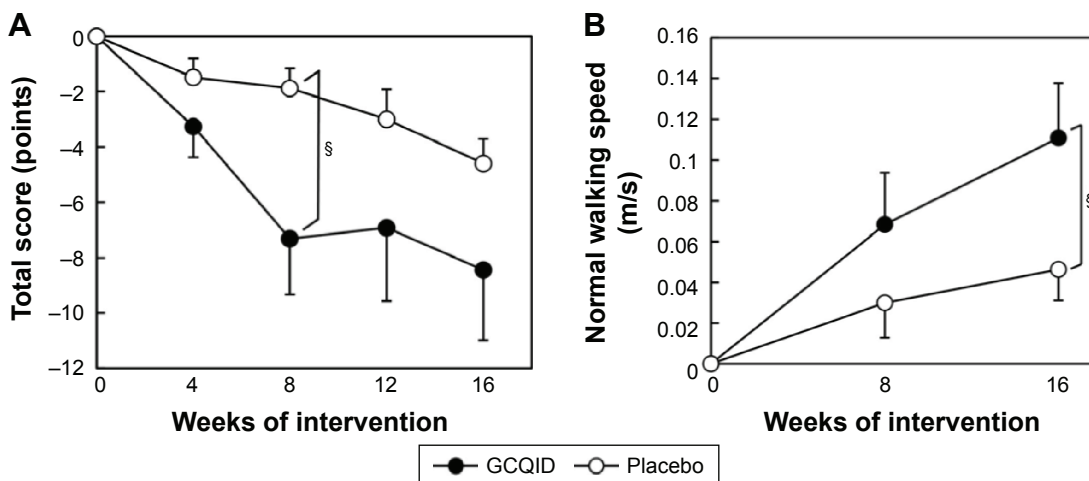

Figure 1 Changes in magnitude of (**A**) JKOM total score (items II–V) and (**B**) normal walking speed.
Notes: GCQID group (n=23) and placebo group (n=25). §$P<0.05$ compared with placebo group. Values are expressed as mean ± standard error.
Abbreviations: GCQID, glucosamine hydrochloride, chondroitin sulfate, type II collagen peptides, quercetin glycosides, imidazole peptides, and vitamin D; JKOM, Japanese Knee Osteoarthritis Measure.

Table 5 Changes in JKOM total score (items II–V) and normal walking speed at each time point in subjects with mild-to-severe knee pain and baseline K–L grade ≥II or ≤I[a]

| Variables | K–L grades ≥II | | | | K–L grades ≤I | | | |
| | JKOM total score (points) | | Normal walking speed (m/s) | | JKOM total score (points) | | Normal walking speed (m/s) | |
Values at each time point[b]	GCQID	Placebo	GCQID	Placebo	GCQID	Placebo	GCQID	Placebo
Baseline	29.7±14.8	13.7±2.6	1.16±0.07	1.28±0.04	15.8±1.8	15.7±1.5	1.27±0.04	1.25±0.04
Week 4	24.3±14.9	12.0±3.4			12.9±2.0	14.3±2.0		
Week 8	9.3±2.4	14.2±3.8	1.31±0.13	1.30±0.05	10.5±1.7	13.1±1.8	1.33±0.05	1.28±0.04
Week 12	9.3±1.5	13.0±5.3			10.9±2.3	11.9±1.6		
Week 16	8.0±2.6	9.3±2.8	1.35±0.08	1.35±0.06*	9.4±1.9*	11.0±1.4	1.37±0.04**	1.28±0.04
Two-way ANOVA (group × time) P-value	0.082		0.064		0.634		0.193	

Notes: [a]GCQID group (n=3), placebo group (n=6) for K–L grade ≥II. GCQID group (n=20), placebo group (n=19) for K–L grade ≤I. [b]Values expressed as mean ± standard error. *$P<0.05$ and **$P<0.01$ compared with baseline values.
Abbreviations: ANOVA, analysis of variance; GCQID, glucosamine hydrochloride, chondroitin sulfate, type II collagen peptides, quercetin glycosides, imidazole peptides, and vitamin D; JKOM, Japanese Knee Osteoarthritis Measure; K–L, Kellgren–Lawrence.

group at week 16 (1.36±0.05 m/s vs 1.21±0.02 m/s, $P<0.05$, d=0.68). There was no significant difference between the groups in subjects with K–L grade 0 (data not shown).

Significant changes in several blood biochemical and hematological variables were observed in both groups during the 16-week intervention (Table S1), but the values were judged by the investigators to have remained within the normal range and to be medically unrelated to the treatment. There were also no abnormal changes in physical parameters and urinalysis, including proteinuria, glucosuria, and hematuria (data not shown). Some subjects in both groups reported experiencing one or more adverse events during the intervention. However, there was no between-group difference in frequency or pattern of events (Table S2). All self-reported adverse events were transient and of mild or intermediate intensity. Moreover, no adverse effect of treatment was identified when these results were analyzed on an individual-subject basis.

Discussion

The present study was conducted to evaluate effects of a glucosamine-containing supplement (GCQID) on locomotor functions in subjects with knee pain.

The efficacy assessment revealed that GCQID supplementation improved JKOM total score, normal walking speed, and knee-extensor strength in subjects with mild-to-severe knee pain at baseline better than the placebo (Table 4 and Figure 1). Using OA criteria used in the ROAD study,[6] subgroup analysis based on K–L grade was performed to investigate the efficacy of GCQID supplementation with or without radiographic OA. We found that GCQID supplementation

only tended to improve normal walking speed more than placebo in subjects with K–L grade ≥II or ≤I, and it significantly improved both JKOM total score and normal walking speed more than placebo in subjects with K–L grade I. These results suggest that GCQID supplementation can be effective at improving knee-joint functions and locomotor functions in subjects with mild-to-severe knee pain, especially those with K–L grade I joint-space narrowing.

The ability of GCQID supplementation to relieve knee pain may be explained mainly by the anti-inflammatory and chondroprotective activities of glucosamine hydrochloride,[22,23] chondroitin sulfate,[24] and quercetin,[25,26] as described in a previous study on GCQ supplementation.[10] Pain signals suppress muscle performance, and knee OA impairs locomotor functions such as walking speed.[4] Najm et al[14] showed that NSAIDs improved knee-joint functions and locomotor functions at the same time in patients with knee OA. Similarly, the improvement in knee-joint functions observed with GCQID supplementation in the present study may partially contribute to improved locomotor functions in subjects with knee pain. Mukai et al[15] confirmed that quercetin can prevent atrophy caused by muscle disuse by attenuating the expression of ubiquitin ligases, and Horii et al[27] revealed that a type of imidazole peptide increased muscle blood flow via changes in muscle sympathetic nerve activity, suggesting that quercetin glycosides and imidazole peptides in GCQID can directly affect muscle functions. Lower serum 25-OHD levels are associated with impaired locomotor functions.[28] The 16-week intervention with GCQID supplementation elevated serum 25-OHD levels compared with the placebo group in subjects with mild-to-severe knee pain, suggesting

that the elevated serum 25-OHD levels in the GCQID group may partially contribute to improved locomotor functions, although there was a significant group × time interaction. In Japan, serum 25-OHD levels are lowest at the end of winter and highest at the end of summer,[29] and the present study was conducted from winter to summer. Therefore, the time effect in elevated serum 25-OHD levels can be partly explained by seasonal changes, which may also partially contribute to improved locomotor functions.

In the present study, GCQID supplementation improved locomotor functions. An increase in muscle strength with GCQID supplementation might be expected to lower the risk of falling because muscle weakness is the highest risk factor for falling.[6] It has been reported that walking speed is associated with survival in older adults[5] and that the ingestion of glucosamine or chondroitin is associated with decreased total mortality.[30] Therefore, GCQID supplementation may have a beneficial effect on survival rate. Further research is needed to elucidate the effect of supplements such as GCQID, which improve locomotor functions, on survival rate.

In the safety assessment, no abnormalities in any laboratory tests were observed and no treatment-related adverse events were experienced during the intervention period, which suggests that GCQID is a safe supplement.

There are some limitations to the present study. First, the study was conducted over a relatively short period and the sample size was relatively small. Therefore, a long-term study with a large sample size may be needed to clarify the effect of GCQID supplementation on locomotor functions. Second, because subjects' daily dietary intake, which might have affected knee-joint functions or locomotor functions, was not monitored, it must be acknowledged that daily diet might have partially affected the efficacy observed in the present study. Third, because the GCQID had several components, further studies should be conducted to clarify the role of each component of the GCQID on locomotor functions.

Conclusion

In conclusion, our findings suggest that GCQID supplementation was safe and effective in improving knee-joint functions and locomotor functions in subjects with knee pain.

Acknowledgments

Disclosure

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Supplementary materials

Table S1 Changes in laboratory values between baseline and week 16

Variables[a]	Group	Values at time point	
		Baseline	**Week 16**
AST (IU/L)	GCQID	21.3±0.8	20.6±0.9
	Placebo	22.1±1.1	19.5±0.8*
ALT (IU/L)	GCQID	20.7±1.5	19.7±2.0
	Placebo	22.0±1.8	20.2±1.7
ALP (IU/L)	GCQID	204.9±8.5	201.3±8.4
	Placebo	204.8±8.2	208.8±7.6
LDH (IU/L)	GCQID	185.3±3.9	190.9±3.8*
	Placebo	184.6±5.4	187.1±3.8
γ-GTP (IU/L)	GCQID	30.8±4.3	29.1±4.0
	Placebo	30.1±3.4	28.1±3.4
T-Bil (mg/dL)	GCQID	0.75±0.05	0.82±0.05*
	Placebo	0.72±0.05	0.74±0.06
TP (g/dL)	GCQID	7.33±0.05	7.19±0.05*
	Placebo	7.26±0.06	7.15±0.05*
Alb (g/dL)	GCQID	4.29±0.03	4.31±0.03
	Placebo	4.27±0.04	4.29±0.03
CPK (IU/L)	GCQID	124.5±12.6	121.3±9.9
	Placebo	103.0±6.3	112.7±7.3
Triglyceride (mg/dL)	GCQID	105.2±8.0	97.5±8.4
	Placebo	109.2±9.0	120.0±11.9
TC (mg/dL)	GCQID	220.7±4.7	214.3±4.4
	Placebo	223.3±5.0	213.4±5.2**
HDL-C (mg/dL)	GCQID	68.7±2.9	65.0±2.7**
	Placebo	68.2±2.7	62.5±2.3**
LDL-C (mg/dL)	GCQID	128.3±3.5	126.0±3.7
	Placebo	130.5±4.5	125.1±4.6*
BUN (mg/dL)	GCQID	14.0±0.5	13.3±0.6*
	Placebo	13.4±0.4	13.1±0.4
UA (mg/dL)	GCQID	4.53±0.18	4.95±0.19**
	Placebo	4.82±0.16	5.13±0.17**
Cr (mg/dL)	GCQID	0.69±0.02	0.70±0.02
	Placebo	0.66±0.02	0.68±0.02*
Na (mEq/L)	GCQID	140.1±0.2	139.9±0.2
	Placebo	140.0±0.2	140.5±0.3
K (mEq/L)	GCQID	4.45±0.05	4.28±0.05**
	Placebo	4.48±0.05	4.26±0.05**
Cl (mEq/L)	GCQID	103.2±0.3	103.9±0.2*
	Placebo	103.3±0.3	104.2±0.3**
Glucose (mg/dL)	GCQID	91.3±1.5	90.5±1.8
	Placebo	91.4±1.2	91.3±1.4
HbA$_{1c}$ (%)	GCQID	5.00±0.05	4.97±0.05
	Placebo	5.07±0.04	5.06±0.04
CRP (mg/dL)[b]	GCQID	0.10±0.02	0.10±0.02
	Placebo	0.10±0.02	0.07±0.01
Insulin (μU/mL)[b]	GCQID	5.83±0.58	5.92±0.69
	Placebo	5.55±0.65	7.23±0.84
WBC (×10³/μL)	GCQID	5.74±0.17	5.60±0.16
	Placebo	5.94±0.20	5.80±0.19
RBC (×10⁴/μL)	GCQID	463.1±4.3	449.0±4.6**
	Placebo	466.1±5.4	454.7±5.3**

(Continued)

Table S1 (*Continued*)

Variables[a]	Group	Values at time point	
		Baseline	**Week 16**
Hb (g/dL)	GCQID	13.9±0.2	13.5±0.2**
	Placebo	14.0±0.2	13.7±0.2**
Hct (%)	GCQID	42.4±0.4	41.1±0.4**
	Placebo	42.8±0.5	41.5±0.5**
Plt (×10⁴/μL)	GCQID	25.1±0.9	24.2±0.9*
	Placebo	25.6±0.9	24.2±0.8**

Notes: All values are expressed as mean ± standard error for GCQID group (n=48) and placebo group (n=49). [a]Of the 100 subjects eligible for safety assessment, three (two in the GCQID group and one in the placebo group) dropped out of the study and did not undergo laboratory examinations at week 16. [b]Values below the detectable limit for CRP and insulin are calculated as 0.05 mg/dL and 2.0 μU/mL, respectively. *$P<0.05$ and **$P<0.01$ compared with baseline.
Abbreviations: Alb, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Cl, chloride; CPK, creatinine phosphokinase; Cr, creatinine; CRP, C-reactive protein; GCQID, glucosamine hydrochloride, chondroitin sulfate, quercetin glycosides, imidazole peptides, and vitamin D; γ-GTP, gamma-glutamyl transpeptidase; Hb, hemoglobin; HbA$_{1c}$, hemoglobin A1c; Hct, hematocrit; HDL-C, high-density lipoprotein cholesterol; K, potassium; LDH, lactate dehydrogenase; LDL-C, low-density lipoprotein cholesterol; Na, sodium; Plt, platelet; RBC, red blood cell; T-Bil, total bilirubin; TC, total cholesterol; TP, total protein; UA, uric acid; WBC, white blood cell.

Table S2 Incidence of adverse events[a]

Variables	GCQID group	Placebo group	*P*-value
Number of subjects eligible for safety assessment	50	50	
Number of subjects with adverse events	10	10	1.000
Incidence of adverse events[a] (%)	20	20	1.000
Number of adverse events	14	14	1.000

Note: [a]Incidence of adverse events (%) =100× number of subjects with adverse events/number of subjects eligible for safety assessment.
Abbreviation: GCQID, glucosamine hydrochloride, chondroitin sulfate, quercetin glycosides, imidazole peptides, and vitamin D.