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# The efficacy and safety of low-molecular-weight collagen peptides for joint pain in patients with osteoarthritis: A randomized, double-blind, placebo-controlled study

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ABSTRACT

Collagen is the main component of the articular cartilage. Collagen disruption is suggested to be an underlying cause of knee pain in osteoarthritis (OA). However, clinical guidelines and standard treatment methods for OA knee pain remain controversial. We investigated whether low-molecular-weight collagen peptide (LMWCP) can reduce the knee pain in patients with OA. A total of 78 patients were randomly assigned to the test (LMWCP 4 g/day) and placebo groups at a 1:1 ratio and treated for 12 weeks. At 6 or 12 weeks after randomization, pain was assessed using the 100 mm Visual Analog Scale (VAS), Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), knee X-ray, and laboratory tests. The group receiving LMWCP 4 g/day for 12 weeks showed a significant decrease in the WOMAC pain score and VAS than the placebo group. These results indicate that LMWCP significantly reduces the knee pain in patients with OA.

## 1. Introduction

Osteoarthritis (OA) is a degenerative arthropathy that causes structural and functional alterations in synovial joints (Dieppe and Lohmander, 2005; Hunter and Felson, 2006). It is characterized by the gradual deterioration of the articular joint cartilage, remodeling of the subchondral bone, and synovial inflammation (Bay-Jensen et al., 2018). Main clinical manifestations of OA include joint pain, stiffness after inactivity, limited mobility, crepitus, and varying degrees of local inflammation (Puigdellivol et al., 2019).

In the United States, OA affects nearly 25 million people, accounting for 25% of middle-aged and above visits to primary care physicians, and costs the North American economy approximately $60 billion annually, posing a huge economic burden (Crowley et al., 2009). In particular, knee is the most frequently affected region in approximately 83% of all patients with OA (Vos et al., 2012). OA is one of the most common painful chronic diseases (García-Coronado et al., 2019) that is often associated with significant disability and impaired quality of life (Crowley et al., 2009).

Although no curative therapies are currently available, non-surgical treatment methods for knee OA focus on relieving symptoms, minimizing functional impairment, and preserving the quality of life of the affected patients (García-Coronado et al., 2019). Initial pharmacological treatment mainly consists of oral non-steroidal anti-inflammatory drugs (NSAIDs), acetaminophen, or intra-articular corticosteroids, according to the clinical guidelines for OA treatment (Nelson et al., 2014). Although short-term therapies alleviate the symptoms of OA (Hunter, 2011); long-term use of these drugs is associated with considerable side effects; such as gastrointestinal bleeding, nephrotoxicity, and cardiovascular disease (Borja-Flores et al., 2020). Hence, many attempts have been made to replace or reduce the intake of currently used drugs with dietary supplements as alternatives (Lee et al., 2021).

*Abbreviations:* BMI, body mass index; C.I., confidence interval; ECM, extracellular matrix; ESR, erythrocyte sedimentation rate; hs-CRP, high-sensitivity C-reactive protein; IGA, investigator global assessment; KL grading, Kellgren–Lawrence grading; LMWCP, low-molecular-weight collagen peptide; NSAIDs, non-steroidal anti-inflammatory drugs; OA, osteoarthritis; PGA, patient global assessment; VAS, Visual Analog Scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

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Collagen is the main component of the articular cartilage (Verzijl et al., 2000). Collagen disruption is suggested to be one of the underlying causes of knee pain in patients with OA (Lee et al., 2021). Several clinical trials have revealed the potential beneficial effects of different nutraceuticals and dietary supplements, such as collagen peptide, for the treatment of OA (Lugo et al., 2016). They could serve as good alternatives with a low risk of serious adverse events for OA treatment (Verzijl et al., 2000). In particular, collagen hydrolysate (CH) derived from hydrolyzing collagen via enzyme engineering is beneficial for cartilage regeneration via extracellular matrix (ECM) synthesis in cells (Song and Li, 2017). Among the various peptide sequences included in CH, Gly–-Pro–Hyp and Pro–Hyp are the major functional components (Lee et al., 2021). Low-molecular-weight collagen peptide (LMWCP) is a form of CH derived from fish skin containing 3% Gly–Pro–Hyp, with tripeptide (Gly-X-Y) content > 15% (Lee et al., 2021). Oral LMWCP administration has been reported to ameliorate cartilage damage and reduce proteoglycan loss rabbit in an anterior cruciate ligament transection model of OA (Lee et al., 2021). In addition, LMWCP significantly increased the mRNA expression levels of collagen type II alpha-1 and aggrecan, components of the ECM of cartilage, in chondrocytes isolated from a patient with OA, suggesting that LMWCP can effectively promote ECM synthesis during OA induction (Lee et al., 2021). Therefore, we hypothesized that LMWCP can improve the knee joint pain in patients with OA. In this study, we aimed to evaluate the safety of LMWCP supplements and their effects on symptoms, including pain, in patients with OA using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and 100 mm Visual Analog Scale (VAS).

## 2. Materials and methods

### 2.1. Ethics

The study protocol was approved by the institutional review board of Severance Hospital (Seoul, Republic of Korea; IRB number 4-2020-0320). It is also registered with the Clinical Research Information Service (Identifier: KCT0007584). This study was performed in accordance with the Declaration of Helsinki and Korean Good Clinical Practice guidelines. All subjects provided written informed consent before enrolment in the study (Kim et al., 2019).

### 2.2. Participants

We recruited 78 volunteers from the Severance Hospital (Seoul, Republic of Korea). Subjects between 40 and 75 years of age, diagnosed with OA for more than 6 months, with grade I or II Kellgren–Lawrence (KL) grading of one or both knee joints on simple X-ray, knee arthritis pain score ≥ 30 mm evaluated using VAS (100 mm), and body mass index (BMI) < 30 kg/m$^2$ were included in this study. In addition, subjects who met the following criteria were excluded: (1) patients presenting clinically significant cerebrovascular, cardiovascular, immune, respiratory, hepatobiliary, renal, urinary, neurologic, musculoskeletal, psychiatric, infectious, hematologic, or oncologic disease; (2) patients who had knee replacement or plan to receive it during the clinical trial; (3) patients diagnosed with inflammatory arthritis, such as rheumatoid arthritis and lupus arthritis, or secondary osteoarthritis due to systematic disease; (4) patients with gout or recurrent pseudogout; (5) patients with infection or severe inflammation in the knee joint, such as septic arthritis; (6) patients with a history of lower extremity fracture within the last 3 months; (7) patients with a history of clinically significant hypersensitivity to collagen components; (8) patients who received the following drugs before clinical trial: hyaluronic acid or steroid to the knee joint within 3 months, systemic steroid within 1 month (excluding topical application and inhalation), and NSAIDs, glucosamine, and chondroitin sulfate that affect knee joint pain within 2 weeks; (9) patients who consistently took drugs or health functional foods affecting the knee joint pain; (10) patients with uncontrolled hypertension (systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 100 mmHg); (11) patients with abnormal laboratory results (creatinine level ≥ 2.0 × upper normal limit and aspartate aminotransferase or alanine aminotransferase level ≥ 2.5 × upper normal limit); (12) subjects with < 80% treatment compliance during the study.

### 2.3. Study design and intervention

This was a randomized, double-blinded, and placebo-controlled study, and the overall study schedule is shown in Fig. 1. All participants registered in this study underwent the following baseline evaluation: vital signs, anthropometric measurements, pain assessment using questionnaires, laboratory tests, including erythrocyte sedimentation rate (ESR), high-sensitivity C-reactive protein (hs-CRP) levels, and knee X-ray. After the baseline evaluation, participants were randomly assigned to the test and placebo groups at a 1:1 ratio. Participants in the test group received four capsules of LMWCP (4 g/day) twice a day for 12 weeks and those in the placebo group received placebo for 12 weeks in the same regimen as the test group. Placebo capsules had a similar color, flavor, and form as LMWCP capsules. LMWCP and placebo capsules were supplied by NEWTREE (Songpa-gu, Seoul, Republic of Korea) and LMWCP was prepared from fish skin.

Participants visited the clinic to evaluate the effects of the study treatment (LMWCP or placebo) 6 and 12 weeks after randomization. Vital signs and body weight were measured and pain was assessed using a questionnaire at 6 and 12 weeks. They were also subjected to knee X-ray and laboratory tests for assessment of knee joint width and inflammatory marker levels at 12 weeks. At every visit, they were asked about the first and last dates of LMWCP or placebo administration, and the remaining treatment capsules provided during the previous visit were returned. During the study period, all participants were prohibited from taking collagen-related food, drugs affecting pain, such as NSAIDs, steroids, and hyaluronic acid, and dietary supplements, such as glucosamine and chondroitin. In addition, physical therapy and herbal treatment, such as acupuncture, buhwang, and moxibustion, aimed at relieving pain were prohibited.

### 2.4. Pain Scale questionnaire

Baseline pain score was measured using the VAS (100 mm), WOMAC, patient global assessment (PGA), and investigator global assessment (IGA) on the first visit, and the treatment efficacy was measured on subsequent visits (2, 3, and 4).

### 2.5. Social history questionnaire

Alcohol intake was classified as non-drinker and current drinker depending on current drinking status, and smoking status was classified as non-smoker, ex-smoker, and current smoker depending on current cigarette use.

### 2.6. Anthropometric measurement and body composition

For the screening tests (visit 1), body weight (kg) and height (cm), approximated to the first decimal, were measured using an automatic extensometer (BSM 330; Biospace, Seoul, Republic of Korea) while the participants were wearing light clothes, and BMI was calculated as the ratio of body weight (kg) to height$^2$ (m$^2$) (Kim et al., 2019). Only body weight was assessed from the second visit.

### 2.7. Blood collection and analysis

Blood and urine samples were collected to assess the efficacy and safety of treatment. Blood samples were collected after an 8 h overnight fasting period between visits 1 and 4. Complete blood count was composed of white blood cells, red blood cells, hemoglobin, hematocrit,

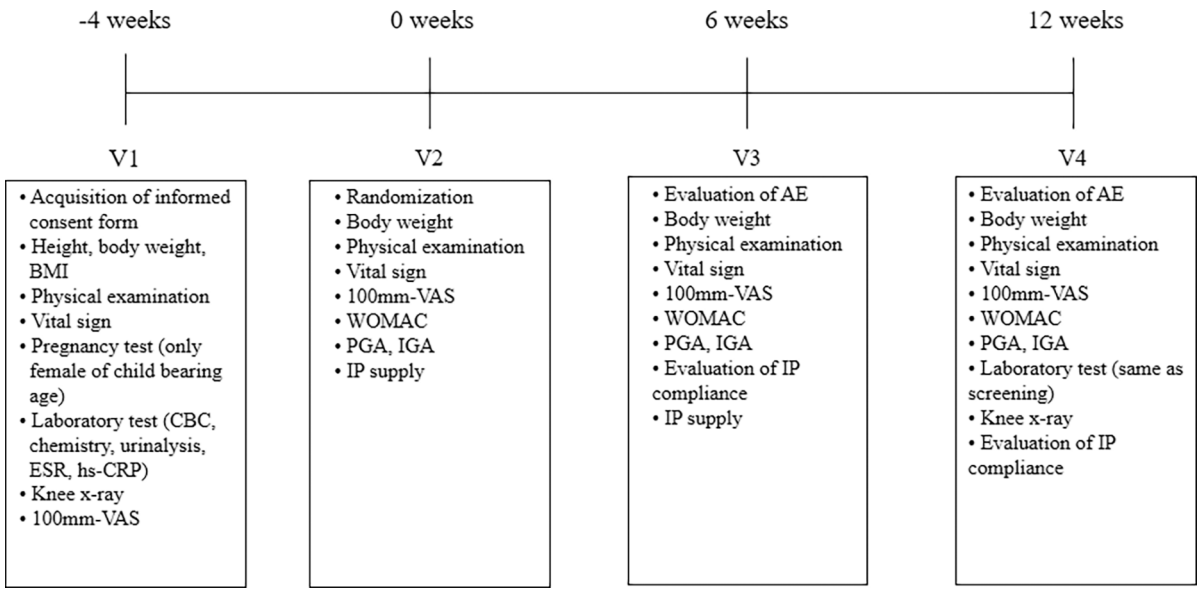


**Fig. 1.** Overview of the study design.

and platelets. Levels of calcium, inorganic phosphate, uric acid, fasting glucose, blood urea nitrogen, creatinine, total protein, albumin, total bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, creatine kinase, and total cholesterol were also measured (Kim et al., 2019). In addition, levels of inflammatory markers (ESR and hs-CRP) were assessed to determine the treatment efficacy. Urinalysis was performed to assess the treatment safety, and urine pregnancy tests were performed on women of childbearing age during screening. White blood cell counts were quantified using an XN-9000 Hematology Analyzer (Sysmex, Kobe, Japan) (Kim et al., 2019). Fasting glucose, hs-CRP, and total cholesterol levels were measured using the ADVIA 1650 Clinical Chemistry System (Siemens Medical Solutions, Tarrytown, NY, USA) (Kim et al., 2019). Levels of calcium, inorganic phosphate, uric acid, blood urea nitrogen, creatinine, total protein, albumin, total bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, creatine kinase, and total cholesterol were measured using an ADVIA 1800 Clinical Chemistry System (Siemens Healthcare Diagnostic, Inc., Tarrytown, NY, USA) (Kim et al., 2019). Urinalysis was performed using an AU680 chemistry analyzer (Beckman Coulter, Brea, CA, USA) (Kim et al., 2019).

### 2.8. Knee joint X-ray

Joint space width was determined via X-ray of the knee joints of patients on visits 1 and 4.

### 2.9. Statistical analysis

Data were analyzed using the SAS statistical software version 9.3 (SAS Institute Inc. Cary, NC, USA). Efficacy and baseline analysis set included randomized participants who were compliant with the study protocol and received treatment of at least > 80% of the study period (Kim et al., 2019). Baseline characteristics between the two treatment groups were compared using independent two-sample *t*-tests or Wilcoxon rank-sum tests for continuous data and chi-square tests for categorical data (Kim et al., 2019). Differences in parameters between the two groups after intervention were analyzed using an independent two-sample *t*-test or Wilcoxon rank sum test, and within-group differences were analyzed using a paired *t*-test or Wilcoxon signed rank test (Kim et al., 2019). In case of rejection of the Shapiro–Wilk normality test, Wilcoxon tests were used to compare the differences (Kim et al., 2019). In addition, changes in each parameter from the baseline between the two groups were analyzed using analysis of covariance, which was adjusted for its baseline value (sex, age, BMI, and baseline WOMAC pain scale). All data are expressed as the mean ± standard deviation, median (interquartile range), or number (%) (Kim et al., 2019). Statistical tests were two-sided, and statistical significance was defined as $P < 0.05$ (Kim et al., 2019). This study was not confirmatory, but exploratory in nature; therefore, we did not consider any correction for multiple comparisons (Kim et al., 2019).

## 3. Results

A total of 78 subjects were enrolled in this study and randomized into the test and placebo groups at a ratio of 1:1 (Fig. 2). Two participants in the placebo group voluntarily withdrew from the study for personal reasons. A total of 76 participants completed the study; however, two subjects with < 80% treatment compliance during the study were excluded from the test group. Therefore, 37 participants each in the test and placebo groups were included in the final data analysis.

### 3.1. Baseline characteristics

Baseline characteristics of each treatment group are presented in Table 1. No significant differences were observed with respect to age, sex, anthropometric measurements, KL grading scale, serum inflammatory marker levels, pain scores, smoking status, and alcohol intake between the two groups.

### 3.2. WOMAC, VAS, and other Efficacy-Related parameters

Fig. 3 shows the WOMAC scores for each group throughout the study period. Compared with the baseline, WOMAC pain decreased in the test and placebo groups (–3.4 ± 3.9 and –0.6 ± 4.1, respectively) after 6 weeks, but there was no significant difference between the two groups ($P > 0.05$). However, after 12 weeks of treatment, WOMAC pain was significantly decreased only in the test group (–3.9 ± 4.1, $P < 0.01$). In addition, the decrease in WOMAC physical function and total WOMAC score after 12 weeks was significantly different between the test and placebo groups (WOMAC physical function: –13.6 ± 14.9 and –2.3 ± 16.6, respectively; total WOMAC score: –19.0 ± 20.3 and –2.8 ± 22.3, respectively; both $P < 0.05$). However, there were no significant differences in WOMAC joint stiffness 6 and 12 weeks after the study ($P >$

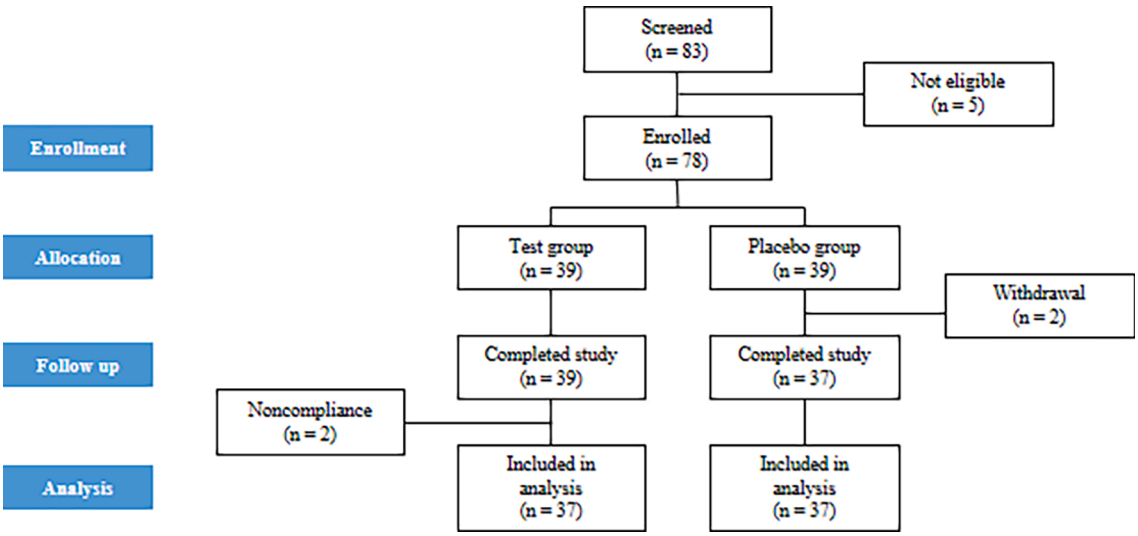


**Fig. 2.** Flowchart illustrating the inclusion and exclusion criteria for the study participants.

**Table 1**
Demographic and baseline characteristics of the study population.

| Demographic variable | Test (n = 37) | Placebo (n = 37) | *P*-value |
|---|---|---|---|
| Age (years) | 51.1 ± 8.0 | 53.4 ± 7.9 | 0.145* |
| Female (%) | 32 (86.5) | 32 (86.5) | 1.000 |
| Body weight (kg) | 62.5 ± 11.1 | 64.6 ± 9.9 | 0.385 |
| BMI (kg/m$^2$) | 23.8 ± 3.0 | 25.1 ± 2.8 | 0.061 |
| Kellgren–Lawrence grading scale | | | |
| Low grade | | | 0.513 |
| Grade I (%) | 33 (89.2) | 30 (81.1) | |
| Grade II (%) | 4 (10.8) | 7 (18.9) | |
| ESR (mm/h) | 10.5 ± 6.2 | 11.4 ± 10.6 | 0.539* |
| hs-CRP (mg/L) | 0.8 ± 1.0 | 0.9 ± 0.9 | 0.652* |
| WOMAC score | | | |
| Pain | 7.2 ± 3.4 | 5.6 ± 2.7 | 0.076* |
| Joint stiffness | 3.1 ± 1.7 | 3.3 ± 1.7 | 0.806* |
| Physical function | 25.8 ± 14.1 | 23.3 ± 13.1 | 0.474* |
| Total | 36.1 ± 18.8 | 32.2 ± 16.8 | 0.417* |
| VAS (100 mm) | 50.1 ± 15.3 | 45.1 ± 13.6 | 0.177* |
| PGA | 48.2 ± 18.1 | 48.8 ± 18.4 | 0.918* |
| IGA | 42.7 ± 16.3 | 44.1 ± 13.4 | 0.744* |
| Current smoker | 2 (5.4) | 1 (2.7) | 1.000 |
| Current drinker | 18 (48.6) | 15 (40.5) | 0.640 |

BMI, body mass index; ESR, erythrocyte sedimentation rate; hs-CRP, high-sensitivity C-reactive protein; WOMAC, Western Ontario and McMaster Universities; VAS, Visual Analog Scale; PGA, patient global assessment; IGA, investigator global assessment.
Categorical variables are presented as numbers (percentages), and continuous variables are presented as the mean ± standard deviation.
*P*-values were calculated using an independent two-sample *t*-test (*calculated via Wilcoxon rank sum test for non-parametric tests) for continuous variables and the chi-square or Fisher's exact test for categorical variables.

0.05).

VAS score decreased after 6 weeks of treatment compared to the baseline in both the test and placebo groups, but there was no significant difference between the two groups. However, the VAS score after 12 weeks was significantly lower in the test group than in the placebo group (–25.5 ± 25.4 and –8.3 ± 21.9, respectively; $P < 0.05$).

Table 2 presents PGA, IGA, laboratory parameters, and knee joint space width for each group throughout the study period. There were no significant differences in PGA and IGA 6 and 12 weeks after treatment ($P > 0.05$). Moreover, there were no significant differences in ESR, hs-CRP levels, and knee joint space width 12 weeks after treatment ($P > 0.05$).

### 3.3. Subgroup analysis for WOMAC pain

Significant correlations were observed between sex, age, BMI, and baseline VAS score and WOMAC pain; therefore, we conducted a subgroup analysis (Table 3). For sex, the mean difference (90% confidence interval [CI]) was –4.2 (–5.7 to –2.7) and –1.3 (–1.8 to –0.9), respectively, showing a greater decrease in males. For age, a significant decrease was observed in the mean difference (90% CI) under 50 years of age, –2.3 (–3.0 to –1.7). For BMI, the mean difference (90% CI) of the obese group over 25 kg/m$^2$ was significantly reduced to –2.4 (–3.0 to –1.8). For VAS score at baseline, the mean difference (90% CI) of the subject group with more than 50 mm was significantly reduced to –2.6 (–3.2 to –1.9).

### 3.4. Safety

Seven of 78 subjects (9.0%, nine cases) exhibited adverse events after ingesting LMWCP or placebo supplements. In the test group, three adverse events occurred in three subjects (7.7%), and in the control group, six adverse events occurred in four subjects (10.3%). All adverse events in the test group were mild, with no serious adverse events. All adverse events resolved without any complications or sequelae.

One adverse drug reaction was observed in one subject in the control group (elevated gamma-glutamyl transferase levels), but no adverse drug reactions were found in the test group ingesting LMWCP.

There was no significant difference in the occurrence of adverse events or adverse drug reactions between the two groups. No clinically significant adverse events were observed during the laboratory tests, physical examinations, or vital sign measurements.

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## 4. Discussion

In our study, which evaluated the efficacy and safety of LMWCP in patients with knee OA, the group that received 4 g/day of LMWCP for 12 weeks showed a significant decrease in WOMAC pain and VAS (100 mm) scores compared to the placebo group. In addition, WOMAC total score and physical function were significantly increased in the treatment group than the placebo group. Safety evaluation revealed no significant differences in the occurrence of adverse events and adverse drug reactions between the two groups, confirming the safety of the treatment.

Collagen supplementation suppresses the development of joint damage in experimentally induced arthritis (Nagler-Anderson et al., 1986). Collagen peptides provide a pool of amino acids in the body and

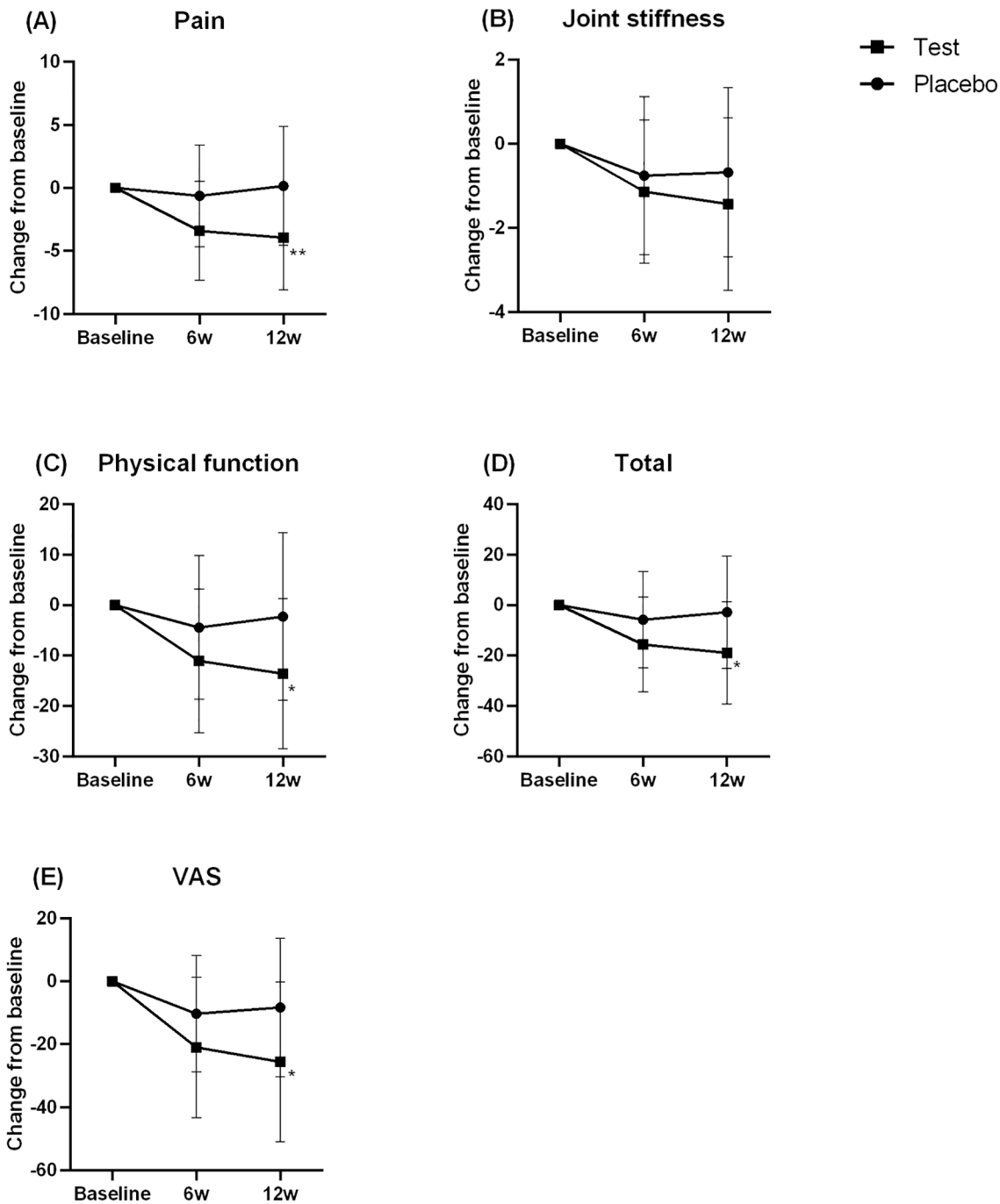


**Fig. 3.** Changes in the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) parameters and Visual Analog Scale (VAS) scores from baseline to 6 and 12 weeks after treatment between the two groups.

significantly improve the matrix structure (Moskowitz, 2000). Preclinical experiments have demonstrated that specific bioactive collagen peptides stimulate type II collagen and proteoglycan synthesis in the articular cartilage (Ng et al., 2007) and promote the synthesis of hyaluronic acid from synovial cells (Ohara et al., 2010). LMWCP shows a protective effect against OA progression by promoting chondrocyte function (Lee et al., 2021). Decreased ECM degradation reduces the pro-inflammatory and pain-stimulating processes. However, the exact mechanism underlying the clinical efficacy of collagen peptide supplementation remains unknown. Moreover, the mechanisms involved in the improvement of knee OA symptoms by oral collagen supplementation require further elucidation.

In our study, after 12 weeks, the test group taking LMWCP showed a significant decrease in the amount of change in both the WOMAC pain score and the VAS for knee pain compared to the placebo group. These results are similar to those of other studies that used collagen in patients with OA (Lugo et al., 2016). However, other studies using collagen have reported different results. In one study of intra-articular injection of collagen in OA patients, VAS showed a significant decrease, but WOMAC pain scores did not (Lee et al., 2021). Another study investigating the effectiveness of collagen supplements for 12 weeks in healthy middle-aged to elderly individuals did not show any significant improvement in pain (Bongers et al., 2020). This may be due to differences in the collagen dosing regimen and study period, differences in sample number, and discrepancies in the views and time points of the standard questions of VAS and WOMAC. Moreover, the difference in the intensity

**Table 2**
Effects of low-molecular-weight collagen peptides on patient global assessment (PGA), investigator global assessment (IGA), laboratory parameters, and knee joint space width in this study.

| Variable | | Test (n = 37) | Placebo (n = 37) | *P*-value |
|---|---|---|---|---|
| PGA | Baseline | 48.2 ± 18.1 | 48.8 ± 18.4 | 0.918* |
| | 6 weeks | 37.5 ± 20.7 | 38.7 ± 19.5 | 0.800 |
| | 12 weeks | 31.5 ± 19.3 | 42.4 ± 24.9 | 0.084* |
| | Δ6week† | −10.6 ± 24.9 | −10.1 ± 15.9 | 0.738 |
| | Δ12week‡ | −16.7 ± 22.1 | −6.4 ± 23.3 | 0.142 |
| IGA | Baseline | 42.7 ± 16.3 | 44.1 ± 13.4 | 0.744* |
| | 6 weeks | 35.5 ± 20.3 | 37.0 ± 18.0 | 0.660* |
| | 12 weeks | 32.0 ± 20.0 | 40.8 ± 21.7 | 0.096* |
| | Δ6week† | −7.2 ± 23.8 | −7.0 ± 15.4 | 0.755 |
| | Δ12week‡ | −10.7 ± 22.5 | −3.2 ± 22.0 | 0.327 |
| ESR (mm/h) | Baseline | 10.5 ± 6.2 | 11.4 ± 10.6 | 0.539* |
| | 12 weeks | 11.7 ± 8.3 | 12.7 ± 9.8 | 0.846* |
| | Δ12week‡ | 1.2 ± 7.3 | 1.3 ± 5.6 | 0.865 |
| hs-CRP (mg/L) | Baseline | 0.8 ± 1.0 | 0.9 ± 0.9 | 0.652* |
| | 12 weeks | 1.0 ± 2.2 | 0.8 ± 0.8 | 0.644* |
| | Δ12week‡ | 0.2 ± 2.3 | −0.1 ± 0.7 | 0.376 |
| Joint space width (left) | Baseline | 6.5 ± 1.3 | 6.7 ± 1.0 | 0.349 |
| | 12 weeks | 7.1 ± 1.2 | 7.2 ± 1.3 | 0.687 |
| | Δ12week‡ | 0.6 ± 1.1 | 0.5 ± 0.8 | 0.639 |
| Joint space width (right) | Baseline | 6.3 ± 1.2 | 6.1 ± 1.0 | 0.440 |
| | 12 weeks | 6.6 ± 1.3 | 6.6 ± 1.1 | 0.767* |
| | Δ12week‡ | 0.4 ± 1.1 | 0.5 ± 0.9 | 0.908 |
| Joint space width (mean) | Baseline | 6.4 ± 1.1 | 6.4 ± 0.9 | 0.898 |
| | 12 weeks | 6.9 ± 1.1 | 6.9 ± 1.1 | 0.914* |
| | Δ12week‡ | 0.5 ± 1.0 | 0.5 ± 0.8 | 0.762 |

PGA, patient global assessment; IGA, investigator global assessment; ESR, erythrocyte sedimentation rate; hs-CRP, high-sensitivity C-reactive protein.
Data are presented as the mean ± standard deviation.
*P*-values were calculated using an independent two-sample *t*-test (*calculated via Wilcoxon rank sum test for non-parametric tests).
† Changes in parameters between baseline and 6 weeks after treatment.
‡ Changes in parameters between baseline and 12 weeks after treatment.

**Table 3**
Subgroup analysis for Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain.

| Subgroup | WOMAC pain | Test (n = 37) | Placebo (n = 37) | Mean difference* (90% C.I.) |
|---|---|---|---|---|
| Sex | Female | | | |
| | 0 week | 7.0 ± 3.5 | 6.0 ± 2.6 | |
| | 12 weeks | 3.6 ± 3.1 | 6.0 ± 5.2 | |
| | Δ12week† | −3.4 ± 4.0 | 0.0 ± 4.9 | −1.3 (−1.8 to −0.9) |
| | Male | | | |
| | 0 week | 8.4 ± 3.4 | 3.2 ± 1.9 | |
| | 12 weeks | 1.0 ± 1.2 | 4.4 ± 3.6 | |
| | Δ12week† | −7.4 ± 3.6 | 1.2 ± 3.6 | −4.2 (−5.7 to −2.7) |
| Age | <50 years old | | | |
| | 0 week | 6.8 ± 3.1 | 5.5 ± 2.5 | |
| | 12 weeks | 2.1 ± 1.8 | 6.8 ± 4.7 | |
| | Δ12week† | −4.7 ± 3.3 | 1.3 ± 4.4 | −2.3 (−3.0 to −1.7) |
| | ≥50 years old | | | |
| | 0 week | 7.7 ± 3.8 | 5.7 ± 2.9 | |
| | 12 weeks | 4.6 ± 3.6 | 5.4 ± 5.2 | |
| | Δ12week† | −3.1 ± 5.0 | −0.4 ± 4.8 | −1.4 (−2.0 to −0.8) |
| BMI | <25 kg/m$^2$ | | | |
| | 0 week | 7.3 ± 3.4 | 5.8 ± 3.1 | |
| | 12 weeks | 3.0 ± 2.9 | 3.5 ± 3.9 | |
| | Δ12week† | −4.3 ± 4.0 | −2.3 ± 4.0 | −0.7 (−1.3 to −0.1) |
| | ≥25 kg/m$^2$ | | | |
| | 0 week | 7.1 ± 3.8 | 5.5 ± 2.4 | |
| | 12 weeks | 3.8 ± 3.2 | 7.8 ± 5.1 | |
| | Δ12week† | −3.3 ± 4.4 | 2.3 ± 4.3 | −2.4 (−3.0 to −1.8) |
| VAS at 0 week | <50 mm | | | |
| | 0 week | 6.0 ± 2.7 | 4.8 ± 2.4 | |
| | 12 weeks | 3.1 ± 2.5 | 4.5 ± 4.3 | |
| | Δ12 week† | −2.9 ± 3.4 | −0.2 ± 4.5 | −0.9 (−1.4 to −0.4) |
| | ≥50 mm | | | |
| | 0 week | 8.2 ± 3.8 | 6.9 ± 2.7 | |
| | 12 weeks | 3.4 ± 3.4 | 7.7 ± 5.6 | |
| | Δ12week† | −4.9 ± 4.6 | 0.7 ± 5.2 | −2.6 (−3.2 to −1.9) |

WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index; C.I., confidence interval; BMI, body mass index; VAS, Visual Analog Scale.
Data are presented as the mean ± standard deviation.
*Mean change difference (test group–placebo group) was based on a two-sample *t*-test ($P < 0.05$).
†Changes in parameters between baseline and 12 weeks after treatment.

of knee pain at the time of the study may have affected the results.

Studies have shown increased proteoglycan content as part of the ECM of cartilage tissue after 10 g of collagen peptide intake in patients with mild OA (McAlindon et al., 2011) and showed improvement in pain symptoms (Moskowitz, 2000). In most clinical trials on OA patients, a daily dosage of 10 g collagen peptides over 2 to 3 months was effective in significantly reducing pain and improving mobility compared with placebo (Bruyere et al., 2012). In contrast, our study used an LMWCP of 4 g. Molecular weight distribution of the CP and the specific amino acid sequences might be important because its efficacy plays a major role (Kumar et al., 2015); and there has been a report that the stimulation of ECM synthesis is probably caused by specific Gly-Pro-Hyp containing peptides with a molecular size of <10 kDa (Ng et al., 2007); suggesting an advantage of LMWCP. In addition, our study targeted KL grading stages I to II, suggesting that the reduction of functional joint pain probably requires a shorter intervention period and a lower dose of collagen peptides in the early stages of OA (Crowley et al., 2009). In addition, the stimulatory effects of different collagen hydrolysates differ in their physicochemical properties, which could have an impact on the interaction of the peptides with certain integrin receptors depending on the specification of the collagen peptides administered (Stotzel et al., 2012). It is necessary to discuss the optimal and recommended dosages and duration of treatment.

In a study of patients with the most advanced KL grades II to III, a significant reduction in radiographic joint space width was shown (Lee et al., 2021), suggesting that they may have structural benefits implying preservation of the hyaline cartilage (Conaghan et al., 2011). However, in our study, KL grading stages I to II were targeted, and there was no significant difference in the joint space width of the knee joint X-ray observed after ingestion of LMWCP compared with the placebo group. Extent of cartilage loss depends on the severity of OA and varies greatly depending on the severity of OA (Crowley et al., 2009). A long follow-up period may be required to show radiographic results. In addition, there were no significant differences in ESR and hs-CRP changes observed after administration of LMWCP for 12 weeks compared to the placebo group in our study. A previous study showed that polymerized-type I collagen induces the downregulation of inflammation, inhibits proinflammatory cytokine expression (Furuzawa-Carballeda, 2012); and increases type II collagen levels (Furuzawa-Carballeda, 2009); however, this reduced inflammation also requires long observation period for validation.

This study demonstrated the clinical safety of LMWCP. In a systematic review and meta-analysis summarizing all available randomized placebo-controlled trials on the safety of collagen supplements to treat

knee OA, supplements were found to be generally safe for treating OA (Liu et al., 2018). LMWCP clinical use is associated with minimal adverse effects. Clinical conditions and kidney (blood urea nitrogen and creatinine) functions are not affected (Gupta et al., 2009); and the most common adverse event is in the gastrointestinal region, characterized by fullness or unpleasant taste (Moskowitz, 2000). Collagen peptides are classified as safe foods by the European Food Safety Authority (European, Food, and Safety, Authority, 2005) and Food and Drug Administration (FDA (U.S. Food and Drug Administration)., 2003). Therefore, its efficacy and high level of safety make it an attractive agent for long-term use (Moskowitz, 2000).

Our study has some limitations. First, we did not monitor the participant food intake throughout the study. As CP is a hydrolyzed form of gelatin (Liu et al., 2015), high CP consumption in daily life in the form of desserts, bakery products, or gummy candy may affect the outcomes of this study. Second, our study had a follow-up period of three months, but other studies have had follow-up periods of six months, which is the generally accepted period for pain alleviation assessment (Wehling et al., 2017). A previous study reported that a longer follow-up period of at least 24 months may be necessary to determine any additional effects of collagen peptide intake (Song and Li, 2017). Although we did not intend to observe the long-term effect of pain relief or regeneration of articular cartilage, additional studies with a longer follow-up period are needed to validate our findings.

Our study followed a randomized, double-blind, and placebo-controlled design with a high level of evidence to evaluate the efficacy and safety of LMWCP administration in patients with early stage OA. Our study comprehensively analyzed the knee pain, subgroups of WOMAC, laboratory biomarker levels, and radiographic results, which was an improvement over previous studies. Our findings suggest LMWCP as an attractive alternative option to other analgesic or anti-inflammatory drugs that may cause adverse effects when used long-term for reducing pain in patients with early knee OA. However, longer clinical trials in larger populations are required to validate the potential beneficial effects of collagen supplementation in patients with symptomatic OA (García-Coronado et al., 2019). Moreover, our study did not verify the effect of LMWCP on modification of joints, further studies are warranted to validate our findings and elucidate the action mechanism and the effect on joint structure of the LMWCP supplement in patients with OA.

This study registered with the Clinical Research Information Service (Identifier: KCT0007584; Clinical trial to evaluate efficacy and safety of low molecular collagen peptides for joint pain Status: Approved First Submitted Date: 2022/07/19 Registered Date: 2022/07/28 Last Updated Date: 2022/07/19 (nih.go.kr)).

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## CRediT authorship contribution statement

**Hye Jun Lee:** Visualization, Writing – review & editing, Writing – original draft, Data curation, Investigation, Methodology, Conceptualization. **Do un Kim:** Visualization, Writing – review & editing. **Choon Ok Kim:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Software, Supervision, Validation, Writing – review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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*Role of funding resource*

The sponsors had no role in the design, execution, interpretation, or writing of the study (Co-author DUK, a full-time employee of the sponsor, did not participate in study design, execution, or interpretation).

*Data sharing*

Data described in the manuscript, code book, and analytic code will be made available upon request pending (The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy.)

*Ethics Statement*

This study protocol was approved by the institutional review board of Severance Hospital (Seoul, Republic of Korea; IRB number 4-2020-0320). It is also registered with the Clinical Research Information Service (Identifier: KCT0007584). This study was performed in accordance with the Declaration of Helsinki and Korean Good Clinical Practice guidelines. All subjects provided written informed consent before enrolment in the study.

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