The Effects of Collagen Peptide Supplementation on Knee Joint Health

—A Double-blind, Placebo-controlled, Randomized Trial in Healthy University Students Belonging to a Running Club—

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ABSTRACT

The objective of this study was to evaluate the effects of collagen peptide (CP) supplementation on the maintenance of knee joint condition in healthy university students belonging to a running club. Fifty-one male healthy university students were recruited from the running club of Josai University and were given a placebo or CP for 8 weeks. Subjective knee condition was evaluated by the Japanese Knee Osteoarthritis Measure (JKOM) score as the primary outcome. Secondary outcome was defined as changes in serum parameters. The total JKOM score and the JKOM II score for "pain and stiffness in the knees" were significantly lower in the CP group than in the placebo group at 4 weeks. Moreover, the JKOM III score for "knee condition in daily life" in the CP group was lower than that in the placebo group at 4 and 8 weeks of intervention. At 4 weeks, interleukin-6 and 3-methylhistidine in serum were significantly elevated in the placebo group, whereas in the CP group, they were unchanged. No adverse events were observed. In summary, CP supplementation improved knee joint condition in male healthy subjects belonging to a running club and also demonstrated the potential to suppress inflammation and reduce muscle tissue damage.

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KEY WORDS Collagen peptide, Joint condition, Running, Clinical study, JKOM

INTRODUCTION

Collagen is a major structural component of connective tissues, accounting for about 30% of the total protein content in mammals. Gelatin, a heat-denatured product of collagen, has long been used as a compo-

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Table 1 Inclusion and exclusion criteria

Inclusion
・Subject must be≥18 years of age
・Male
・Subjects who perform a training with mechanical stress
Exclusion
・Clinically significant renal, hepatic, endocrine（including diabetes mellitus), cardiac disorder
・Subjects who have serious medical history
・Known food allergy to pork and gelatin
・Subjects with a history of gastrointestinal tract surgery
・Subjects who have chronic or acute infections
・Participating in other clinical trials at the start of this study
・Collagen peptide ingestion within 3 months prior to enrollment
・Continuous ingestion of supplements in affected joint pain within 3 months prior to start enrollment
・Subjects were judged inappropriate by a doctor
・Subjects with habit of smoking or excessive alcohol consumption

nent of food due to its gel-forming ability. Collagen peptide（CP）can be formed by hydrolyzing gelatin with an enzyme. CP is widely used as supplements.[1,2] Iwai, et al. reported that several food-derived CP were identified in human blood after the oral ingestion of CP.[3] Moreover, Oesser, et al. determined the bioavailability of CP after oral administration in mice using ^{14}C-labeled CP. The distribution of labeled amino acids in cartilage was confirmed, and they were still present 192 h after administration. This means that CP are almost completely absorbed and accumulated in some tissues such as articular cartilage.[4] On the basis of these results, several clinical studies were performed to examine the joint-protective effects of CP. For example, Kumar, et al. demonstrated that the ingestion of 5 g of CP for 13 weeks significantly improved the Western Ontario and McMaster Universities Osteoarthritis Index（WOMAC）score used for the evaluation of joint pain and the visual analog scale（VAS）score, which is an indicator of pain in osteoarthritis（OA）patients.[5] Moreover, McAlindon, et al. reported that proteoglycan concentrations in hyaline cartilage of patients with mild OA increased within 13 weeks after the ingestion of 5 g of CP.[6]

Several studies have examined the effect of CP in preventing the occurrence of injuries or knee pain in healthy individuals and university athletes.[7-9] It is particularly important for athletes to avoid injuries as well as to improve their competitive skills. Endurance athletes are at particular risk for overuse injuries or exercise-related joint pain.[9] Furthermore, there are few large clinical trials to examine the effects of CP in maintaining knee joint condition in healthy subjects. In this context, more clinical trials are needed to investigate the effects of CP with the aim of improving knee pain or maintaining knee joint condition in endurance athletes or healthy individuals.

In the present study, we performed a randomized, double-blind, placebo-controlled clinical trial aimed at evaluating the effects of CP supplementation with respect to the maintenance of knee joint condition in male healthy university students belonging to a running club.

MATERIALS AND METHODS

1 Investigational products

In this study, CP derived from porcine skin（average molecular weight 3500-7000; Nitta Gelatin Inc., Japan）were used and a placebo（maltodextrin）was purchased from Matsutani Chemical Industry Co., Ltd.（Itami, Japan）. All preparations were of food grade.

2 Study design

This double-blind, placebo-controlled, randomized, clinical study was carried out at Josai University. It was approved by the Ethics Committee of Josai University（No. A27-7）, registered with the University Hospital Medical Information Network（No. 000023033）, and conducted in accordance with the Ethical Guidelines for Medical and Health Research Involving Human Subjects published by the Ministry of Health, Labour and Welfare of Japan and the Declaration of Helsinki. A total of 51 male healthy university students, 18 to 21 years of age, were recruited from the running club of Josai University and were screened for eligibility using the inclusion-exclusion criteria defined in **Table 1**. Only healthy students who

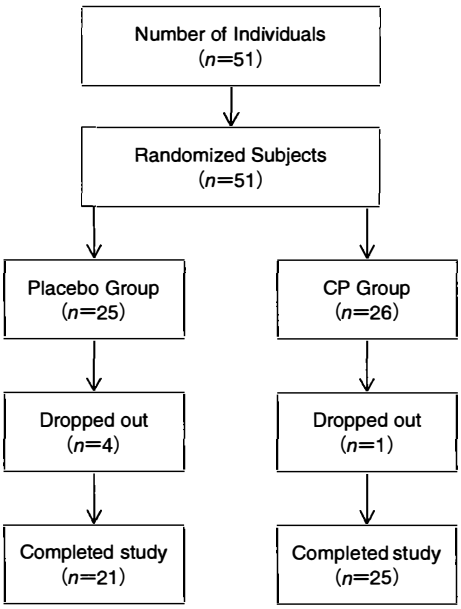

Fig. 1 The method for selecting subjects for the intervention study

Fifty-one subjects were randomly assigned to the CP or placebo group. A total of 25 subjects in the CP group and 21 subjects in the placebo group completed the study.

were diagnosed as having no OA by an attending physician participated in this study. Written informed consent was obtained from the 51 subjects who fulfilled the entry criteria and did not meet the exclusion criteria. Subjects were randomly assigned to the CP or placebo group. The subjects were advised to consume CP or placebo orally at 5 g/day in their free time. A double-blind method was adopted in which an assignment table was created using random numbers so that age, BMI, and other factors at the time of participation were not imbalanced between the two groups. However, in case of an emergency in which a serious condition occurred in the subjects, the minimum necessary information was disclosed. The intervention test period was 8 weeks. At baseline, after 4 weeks, and at the end of the intervention period, Japanese Knee Osteoarthritis Measure (JKOM) score assessment and blood testing were carried out.

3 Primary outcome

Subjective knee condition was evaluated by JKOM score at baseline and at 4 and 8 weeks of follow-up: this was taken as the primary outcome. JKOM is a self-administered questionnaire based on a patient's subjective evaluation, consisting of 25 items in four

Table 2 Demographic data of a double-blind, placebo-controlled, randomized trial in male healthy university students belonging to a running club

	Placebo	CP
	(n=21)	(n=25)
Age (y)	18.9±1.1	19.5±1.3
Height (cm)	169.2±4.1	170.6±4.6
Weight (kg)	55.9±5.4	57.6±3.7
BMI(kg/m^2)	19.8±1.1	19.5±1.3

Results are mean±SD.
CP, collagen peptide

subcategories: JKOM II (pain and stiffness in the knees; 0-32 points), JKOM III (knee condition in daily life; 0-20 points), JKOM IV (general activities; 0-20 points), and JKOM V (health condition; 0-8 points). The total JKOM score was assessed by summing the scores from subcategories, with 100 points as the maximum score.[10] In each evaluation scale, a higher value indicates a worse condition.

Knee pain was evaluated individually at baseline and at 4 and 8 weeks of follow-up, using a VAS. The VAS score represented the degree of knee pain, with a large value indicating severe knee pain.

4 Secondary outcome

Secondary endpoints were defined as changes in serum parameters. The serum parameters assessed were blood urea nitrogen (BUN), lactate dehydrogenase, creatine phosphokinase, aspartate aminotransferase, alanine aminotransferase, creatinine, tumor necrosis factor-α(TNF-α), C-reactive protein, interleukin-6 (IL-6), and 3-methylhistidine (3-MH). 3-MH corrected with creatinine was used as a marker of muscle tissue damage.

5 Statistical analysis

Data are presented as mean±standard deviation(SD). All statistical analyses were performed using SPSS version 25.0 for Mac. Mann-Whitney U-test was used to compare means between CP and placebo groups. Friedman's test was used to evaluate the change in score at each visit. P-values<0.05 were considered statistically significant. The analysis was supported by per-protocol methods.

Table 3 Difference in the JKOM and VAS scores according to CP consumption in male healthy university students belonging to a running club

	Group	*n*	Baseline	4 week	8 week
JKOM Total Score（0-100 points）	Placebo	21	4.43±6.34	3.57±4.53	1.90±4.17#
	CP	25	3.00±4.01	1.16±1.21*	0.48±0.82#
JKOM II（Pain and stiffness in the knees, 0-32 points）	Placebo	21	1.43±3.31	1.33±2.39	0.48±2.18
	CP	25	1.04±2.68	0.16±0.47*	0.04±0.20
JKOM III（Knee condition in daily life, 0-20 points）	Placebo	21	0.86±1.96	0.57±1.12	0.67±2.20
	CP	25	0.60±1.78	0.04±0.20*	0.00±0.00*
JKOM IV（General activities, 0-20 points）	Placebo	21	1.19±1.97	0.76±1.26	0.43±0.87
	CP	25	0.64±0.95	0.60±0.96	0.24±0.60
JKOM V（Health condition, 0-8 points）	Placebo	21	0.95±0.86	0.90±1.09	0.38±0.59
	CP	25	0.72±0.84	0.36±0.57	0.20±0.41
VAS（Degree of knee pain, mm）	Placebo	21	19.5±20.7	19.0±22.7	12.2±19.1
	CP	25	18.5±20.4	8.6±15.7	18.4±26.1

Results are mean±SD.
CP, collagen peptide
*$P<0.05$（Placebo vs. CP）, #$P<0.05$（vs. Baseline）

RESULTS

1 Baseline characteristics

A total of Fifty-one male healthy university students belonging to a running club met the inclusion criteria and were randomized to the placebo（$n=25$）or CP groups（$n=26$）. Forty-six of the Fifty-one subjects completed the study correctly, representing the evaluated per-protocol population. One subject in the CP group and four in the placebo group dropped out because of individual circumstances that were not related to any side effects caused by ingesting the CP or placebo（**Fig. 1**）. Baseline demographic characteristics of the study participants（$n=46$）are shown in **Table 2**. No statistically significant differences between the CP and placebo groups were detected.

2 JKOM scores

The JKOM scores of subjects in the CP and placebo groups at baseline and at 4 and 8 weeks are shown in **Table 3**. There was no significant difference in the JKOM scores at baseline between the CP and placebo groups. However, the total JKOM score significantly decreased at 8 weeks compared with the score at baseline, in both CP and placebo groups. There were no significant changes in the scores in each of the four subcategories from those at baseline at 4 and 8 weeks in both CP and placebo groups.

The total JKOM score at 4 weeks was significantly lower in the CP group than in the placebo group. The JKOM II score for "pain and stiffness in the knees" and the JKOM III score for "knee condition in daily life" at 4 weeks in the CP group were significantly lower than those in the placebo group. Furthermore, the JKOM III score for "knee condition in daily life" in the CP group was lower than that for the placebo group at 8 weeks of intervention. There were no significant differences in the JKOM IV score for "general activities" and the JKOM V score for "health condition" between the CP and placebo groups at all-time points.

3 VAS score

The VAS scores of subjects in the CP and placebo groups at baseline, 4 weeks, and 8 weeks are shown in **Table 3**. There were no changes in the VAS scores at 4 and 8 weeks compared with that at baseline in either group. Furthermore, there was no difference between the CP group and the placebo group at all-time points.

4 Blood parameters

The changes of serum parameters are shown in **Table 4**. The serum concentration of IL-6, a cytokine that plays a central role in inflammation, significantly increased in the placebo group at 4 weeks from baseline. Serum 3-MH, a marker of muscle protein degradation,[11] increased in the placebo group at 4 weeks from baseline. There were no significant changes in IL-6 and 3-MH levels in the CP group.

As part of the safety assessment, various biochemical parameters in serum were also analyzed. BUN at 8 weeks from baseline was significantly increased in the CP group but not in the placebo group. BUN level at 4 weeks from baseline in the CP

Table 4 Difference in serum parameters according to CP consumption in male healthy university students belonging to a running club

	Group	*n*	Baseline	4 week	8 week
Inflammation markers					
TNF-α (pg/mL)	Placebo	21	0.65±0.15	0.66±0.13	0.73±0.29
	CP	25	0.62±0.16	0.64±0.14	0.70±0.18
IL-6 (pg/mL)	Placebo	21	0.50±0.32	0.80±0.64#	0.82±1.00
	CP	25	0.55±0.33	0.86±1.54	0.92±1.22
CRP (mg/dL)	Placebo	21	0.03±0.02	0.04±0.04	0.08±0.22
	CP	25	0.05±0.13	0.10±0.29	0.06±0.08
Muscle damage marker					
3-MH (nMOL/mL)	Placebo	21	3.92±0.65	4.34±0.84#	4.24±0.76
	CP	25	4.28±0.63	4.06±0.63	4.35±0.81
Safety markers					
BUN (mg/dL)	Placebo	21	15.7±3.4	16.0±3.9	18.2±3.2
	CP	25	17.4±3.8	18.0±3.0*	20.0±3.1#
LDH (U/L)	Placebo	21	240±99	236±89	242±99
	CP	25	246±89	249±75	268±95
CPK (U/L)	Placebo	21	480±358	463±353	368±171
	CP	25	482±319	552±448	569±530
Creatinine (mg/dL)	Placebo	21	0.75±0.08	0.76±0.08	0.79±0.10#
	CP	25	0.78±0.06	0.77±0.06	0.80±0.06
AST (U/L)	Placebo	21	37±18	35±16	32±8
	CP	25	39±20	41±19	45±43
ALT (U/L)	Placebo	21	30±13	28±10	28±7
	CP	25	27±15	27±13	29±15

Results are mean±SD.

CP, collagen peptide; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; CRP, C-reactive protein; 3-MH, 3-methylhistidine; BUN, blood urea nitrogen; LDH, lactate dehydrogenase; CPK, creatine phosphokinase; AST, aspartate aminotransferase; ALT, alanine aminotransferase

*$P<0.05$ (Placebo vs. CP), #$P<0.05$ (vs. Baseline)

group was significantly higher than that in the placebo group. Creatinine at 8 weeks from baseline was significantly increased in the placebo group but not in the CP group. These changes observed were not clinically significant, and the all data in both groups were showed no significant difference from the normal range. These data demonstrated the safety of CP supplementation. None of the adverse events was considered to be associated with CP or placebo supplementation.

DISCUSSION

This study was established with the aim of clarifying the effects of CP supplementation with respect to maintaining knee joint condition in healthy subjects. We conducted a randomized, double-blind, placebo-controlled clinical trial on male students belonging to a running club at Josai University. Subjective knee condition was evaluated by JKOM score as the primary outcome of this study. JKOM is a measure of subjective symptoms involving an improvement on WOMAC, a QOL evaluation system for OA patients, adapted to the lives of Japanese people; it is widely used for Japanese knee joint research.[10] JKOM consists of 25 items that cover four subcategories: JKOM II for pain and stiffness in the knees, JKOM III for knee condition in daily life, JKOM IV for general activities, and JKOM V for health condition. Patients with knee OA and individuals with pain or discomfort in the knees show higher total JKOM score or subcategory scores.

The results obtained here showed that the total JKOM score at 4 weeks was significantly lower in the CP group than in the placebo group (**Table 3**). Furthermore, the JKOM II score for "pain and stiffness in the knees" and JKOM III score for "knee condition in daily life" at 4 weeks in the CP group were also significantly lower than those in the placebo group. Our results showed that continuous CP ingestion can

improve knee joint discomfort resulting from running long distances and not caused by OA.

It has been reported that oral administrated CP is transferred to the blood in the form of di-peptides, such as Pro-Hyp, these peptides remain in a blood for a long time.[12] Due to its physiological role in the organism, Pro-Hyp is considered to be a bioactive compound. Study with labelled Pro-Hyp have shown that an amount of Pro-Hyp could be detected in skin and cartilage after orally administered [^{14}C] Pro-Hyp.[13] Importantly, orally administered Pro-Hyp inhibited phosphorus-induced loss of chondrocytes and thinning of the articular cartilage in mice. Furthermore, results from an *in vitro* study demonstrated that Pro-Hyp suppressed the hypertrophic differentiation of chondrocyte, which is involved in involved in the pathogenesis of OA and induced glycosaminoglycan synthesis by chondrocyte.[14] Pro-Hyp also stimulated cultured synovial cells to synthesize hyaluronic acid, which is present in the synovial fluid in synovial joints.[15] In addition, CP has reported to have a chondroprotective effect on OA by inhibiting the degeneration of type II collagen which is found primarily in the cartilage and the expression of matrix metalloproteinase (MMP)-13 (an important type II collagen-degrading enzyme).[16] It can be speculated that Orally administered CP suppresses the degradation of type II collagen, and CP-derived Pro-Hyp enhances the production of hyaluronic acid and glycosaminoglycans by synovial cells and chondrocytes thereby exhibiting chondroprotective action in healthy male subjects.

The differences in total JKOM score and JKOM II score between the CP and placebo groups were significant at 4 weeks, but at 8 weeks, they became insignificant. This apparent loss of the effect of CP ingestion at the end of the study might be ascribed to the reported pain score being low at the beginning of this study. In this experiment, the mean VAS score (±SD) at baseline was 18.5 (±20.4) in the CP group and 19.5 (±20.7) in the placebo group. On the basis of previous studies, patients with VAS scores of 30 mm or less were defined as having mild pain.[17,18] It can be speculated that, for this study's subjects, the effect of CP was not obvious in the later period, possibly because the subjects were less symptomatic at that phase. However, the differences in the JKOM III score for activities of daily living between the CP and placebo groups were maintained at the end of the study. It is indicated that continuous CP ingestion leads to improvement in the activities of daily life in healthy subjects.

Another analysis was conducted to investigate whether CP ingestion affected the serum parameters of the subjects. It has been reported that inflammatory cytokines such as TNF-α and IL-6 are elevated in patients with OA.[19] In this study, the level of the inflammatory cytokine IL-6 at 4 weeks was significantly increased compared with that at baseline in the placebo group (**Table 4**). IL-6 level in the CP group did not change through the intervention, but the difference between the groups was not significant at any time point. Although the involved mechanism is not fully understood, CP are considered to have anti-inflammatory effects, as reported in several studies. CP administration to post-traumatic OA model mice showed a chondroprotective effect and further showed an anti-inflammatory response to decrease the expression of TNF, an inflammatory cytokine, in the synovial membrane.[20] Furthermore, the collagen-derived peptides Pro-Hyp and Hyp-Gly inhibited TNF-α-induced Inflammatory response in endothelial cells.[21] Based on these studies, it can be assumed that Pro-Hyp and Hyp-Gly derived from orally administered CP suppress the inflammatory response of endothelial cells, thereby exhibiting anti-inflammatory effects.

It has been reported that, in patients with knee OA, in addition to the degeneration of articular cartilage and reduction of cartilage components such as proteoglycan, the muscle mass, and muscle strength near the knees, such as at the quadriceps muscle, are reduced.[22] Against this background, in this study, we demonstrated that serum 3-MH, a marker of muscle tissue damage, at 4 weeks was significantly increased from the level at baseline in the placebo group, but CP supplementation suppressed this elevation (**Table 4**). Recently, the effects of CP on muscle tissues have been reported. For example, a combination of CP supplementation and resistance training improved body composition by increasing lean body mass and muscle strength.[23] Kitakaze, et al. also reported that the collagen-derived dipeptide (Hyp-Gly) can induce myogenic differentiation and myotube hypertrophy by activating the PI3K/Akt/mTOR pathway.[24] Moreover, Shimizu, et al. suggested that the oral administration of Pro-Hyp and Hyp-Gly resulted in the upregulation of gene expression related to muscle function in DNA microarray analysis in skin barrier dysfunction model mice.[25] Praet, et al. recently reported that oral supplementation of specific CP may accelerate the clinical benefits of a well-structured calf-strengthening and return-to-running program in Achilles tendinopathy patients.[26] Therefore, it may be

possible that CP supplementation can lead to decreased knee discomfort due to the muscle-maintenance or tendon-protective properties provided by specific peptides such as Pro-Hyp and Hyp-Gly.

There were some limitations in this study. First, JKOM is a self-managed questionnaire based on patients' subjective assessment. Although JKOM has been validated, a more objective assessment of knee condition by physicians may be needed. Second, our results cannot be extrapolated to all young Japanese men because of the limited number of participants studied here, who belonged to the running club of a particular university.

CONCLUSIONS

In conclusion, our data suggest that the continuous ingestion of CP improved knee discomfort in male healthy subjects belonging to a running club. CP supplementation was also demonstrated to have the potential to suppress inflammation and reduce muscle tissue damage. Because knee joint health is important to not only endurance athletes but also athletes specializing in other activities or elderly people, further study will be needed to explore the effects of CP supplementation on knee joint health. Overall, these findings suggest that CP are dietary supplements that can contribute to the improvement of knee joint condition that has been adversely affected by physical activities.

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