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UC-II Undenatured Type II Collagen for Knee Joint Flexibility: A Multicenter, Randomized, Double-Blind, Placebo-Controlled Clinical Study

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Abstract

Objective: Joint-related stress models have been used in the past to induce a standardized load on physical structures, allowing researchers to observe changes in perceived stress on joints as accurately as possible in healthy individuals. Previous studies support the efficacy of UC-II® undenatured type II collagen (''undenatured collagen'') supplementation in maintaining joint health. The purpose of this study was to assess the effect of undenatured collagen on knee flexibility in healthy subjects who experience activity-related joint discomfort (ArJD).

Methods: This randomized, double-blind, placebo (PLA)-controlled study was conducted in healthy subjects with ArJD who had no history of osteoarthritis, or joint diseases. Ninety-six ($n=96$, 20–55 years old) subjects who reported joint discomfort while performing a standardized single-leg-step-down test were randomized to receive either PLA ($n=48$) or 40 mg of undenatured collagen ($n=48$) supplementation daily for 24 weeks. Range of motion (ROM) flexion and extension were measured using a digital goniometer.

Results: At the end of the study, a statistically significant increase in knee ROM flexion was observed in the undenatured collagen group versus the PLA group (3.23° vs. 0.21°; $p=0.025$). In addition, an increase in knee ROM extension by 2.21° was observed over time in the undenatured collagen group ($p=0.0061$), while the PLA group showed a nonsignificant increase by 1.27° ($p>0.05$). Subgroup analysis by age showed a significant increase in knee ROM flexion in subjects >35 years old in the undenatured collagen supplemented group compared with PLA (6.79° vs. 0.30°; $p=0.0092$).

Conclusion: Overall, these results suggest that daily supplementation of 40 mg of undenatured collagen improved knee joint ROM flexibility and extensibility in healthy subjects with ArJD.

Keywords: undenatured collagen, joint flexibility, range of motion, knee joint function

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Introduction

Arthritis is a chronic degenerative joint disease that impacts the mobility and physical functioning in affected individuals.[1] Osteoarthritis (OA) is the most common form of arthritis that involves destruction of joint cartilage and damage to the adjacent bone. Furthermore, OA is the most prevalent joint disease in the United States and its prevalence has approximately doubled since the mid-20th century.[2] According to the Centers for Disease Control (CDC), in 2020, an estimated 32.2 million Americans were living with OA.[1,3] As common treatment of OA symptoms, prescription of oral nonsteroidal anti-inflammatory drugs (NSAIDs) is widely used. In a recent individual patient data meta-analysis, Persson et al. showed that topical NSAIDs are effective for OA pain relief.[4]

Daily life activity is characterized by differing intensities of physical activity that exert variable weight-bearing load on the joints. Joint stress caused by mechanical overload, anatomical weaknesses (e.g., unequal leg length and knock knees) or joint instability leads to localized pain and stiffness that limit joint flexibility and mobility in healthy subjects without diagnosed OA.[5] Studies have shown that even a few degrees of loss of knee range of motion (ROM) flexibility can result in altered gait patterns leading to difficulty in running and jumping.[6,7] Knee ROM is essential for daily function for athletes as well as for normal active people.[8] Interventions aimed at improving ROM have been shown to alleviate joint stiffness, increase joint mobility, and maintain joint function.

A recent investigation of Wallace et al. on long-term trends in knee OA prevalence in the United States indicated that knee OA may be more preventable than is currently supposed.[2] Therefore, preventive actions, which include joint protection by physical activity, dietary intervention, or dietary supplements, appear to be an important factor in the progression of this disease. Undenatured collagen is one such dietary supplement that could be used in subjects with activity-related joint discomfort (ArJD) to prevent possible progression of the complaints such as limited mobility. Studies have shown that undenatured collagen supplementation can improve joint mobility in OA subjects as well as in healthy subjects who experience ArJD.[9,10]

In a placebo-controlled study, Lugo et al. reported improvements in knee joint extension in healthy subjects supplemented with undenatured collagen and who experienced joint pain while performing the stepmill exercise.[10] More recently, our group validated the single-leg-step-down (SLSD) test as a reliable model to select for healthy subjects who experience ArJD, thereby allowing assessment of knee joint function in this population.[11] In this study, Schön et al. demonstrated that subjects with ArJD may show impairments of knee joint flexibility assessed by goniometry in comparison with the healthy subjects without any joint complaints. Therefore, the purpose of the current study was to evaluate the impact of undenatured collagen supplementation on joint flexibility, as measured by knee ROM flexion and extension in healthy subjects who experience ArJD on the SLSD test.

Materials and Methods

Study design

The study was performed as a prospective, multicentric, randomized, double-blind, placebo-controlled study in parallel design. This study was conducted following the guidelines for Good Clinical Practice set forth by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH E6 [R2], Nov.2016) and following the Declaration of Helsinki (E8) for treatment of human subjects in a study. This study was approved by the local ethics committee (Institutional Review Board of the Landesärztekammer Baden-Württemberg, file number F-2019-072) and the clinical trial was registered at DRKS—German Clinical Trials Register DRKS: DRKS00018792.

Subjects were screened for their eligibility after providing written informed consent. All subjects completed a medical history questionnaire at screening. Subjects were assessed for anthropometric measures and vital signs. Healthy males and females, 20–55 years old with a body mass index between 19 and 29.9 kg/m^2, were eligible to participate in the study.

All subjects had to perform sports at least two times per week, including but not limited to activities such as soccer, basketball, handball, volleyball, tennis, and running. In addition, subjects had to report reversible knee-joint discomfort during or immediately after physical activity over a period of at least 3 months. The SLSD test was further used to select subjects at the screening visit.[11,12] Only subjects who experienced a pain level of five on an 11-point Likert scale while performing between 30 and 150 steps during the SLSD test were eligible to participate.

Exclusion criteria included joint replacement of the knee, planned surgical intervention during the study duration, intra-articular therapy within the 3 months before the study initiation, and a history or presence of any medical disorders that could potentially interfere with the study, such as active cancer, cardiovascular disease (e.g., stroke and heart attack), or pregnancy and lactation. In addition, subjects with hip, spine, or foot injuries were excluded. Further exclusion criteria were smoking of more than five cigarettes per day, known hypersensitivity to eggs, chicken, or any ingredients in the products, and chronic use of pain relief medication within 30 days before the screening visits.

To reduce the effect of confounding factors, study subjects were asked to maintain their usual diet during the study duration. The use of dietary supplements that could influence joint pain, discomfort, and recovery was not allowed throughout the study. Forty-eight hours before the test days, subjects were not allowed to perform any sporting activities, such as cycling, running, or other exhaustive physical activities, such as heavy gardening or hiking. Thirty-six hours before screening and at all study visits, subjects were asked not to take any oral pain medication (e.g., aspirin and paracetamol) to avoid any possible impact of anti-inflammatory ingredients on joint discomfort or joint flexion. Subjects had to document any intake of pain medication in a diary. In addition, at each study visit, the recent intake of pain relievers was assessed.

Among the 178 males and females screened, 96 subjects were randomized, and 82 subjects were identified as screen failures according to the inclusion or/and exclusion criteria (Fig. 1). The most common reasons for screen failure were too early occurrence of pain level 5 during the SLSD test (<30 repetitions) or too low pain level during the SLSD test performance (<5). There were no dropouts during the course

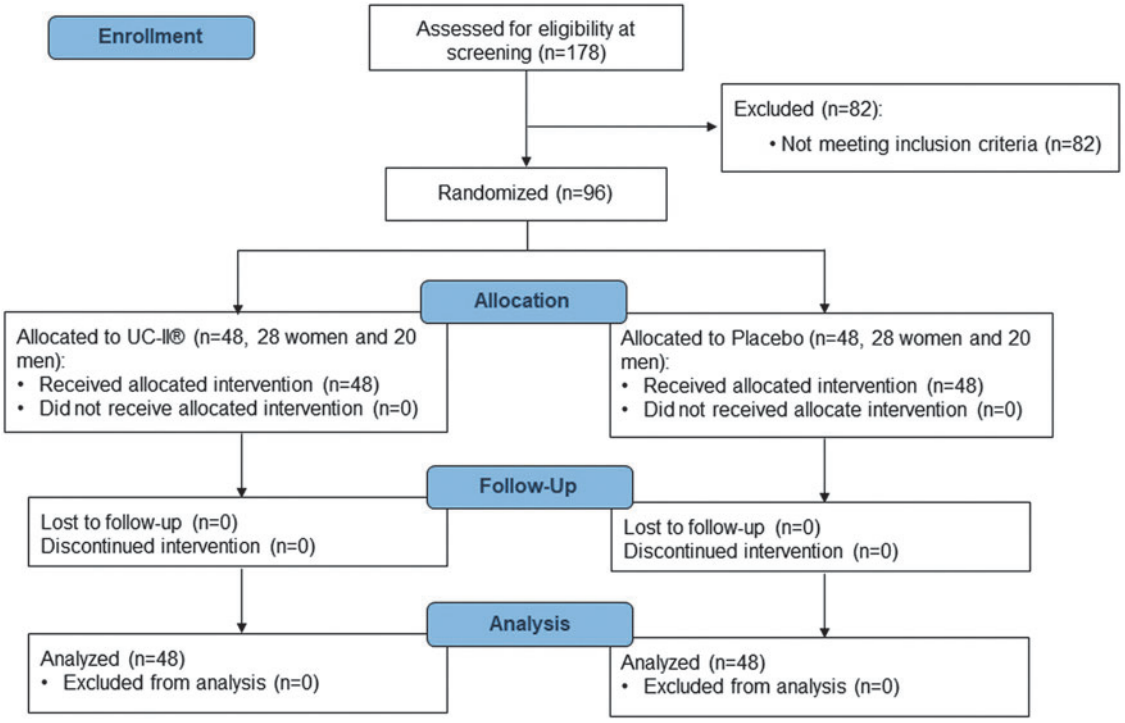

FIG. 1. CONSORT flow diagram.

of the study. The study was conducted at BioTeSys GmbH and at the Institute of Sport and Movement Science of the University of Stuttgart from September 2019 to January 2021.

The study duration was 24 weeks (6 months) with a total of seven study visits in 4-week intervals that included screening, baseline (visit one), 4 weeks (visit two), 8 weeks (visit three), 12 weeks (visit four), 16 weeks (visit five), 20 weeks (visit six), and 24 weeks (visit seven).

Knee ROM flexion was performed at each study visit using a digital goniometer. Knee ROM extension was performed at baseline, and at week 12 and 24. The assessment of study parameters was done on the leg that typically experienced more intense pain after physical activity (target knee).

Sample size and randomization

Sample size calculations were based on the results of a previously performed pilot study[11] and a study by Lugo et al.[10] Assuming an effect size of 0.636, a sample size of 40 subjects per group would provide $\sim$80% power using a significance level of 5%. Considering a dropout rate of 15%, the study was performed with 48 subjects per group. When subjects fulfilled all the inclusion criteria and none of the exclusion criteria, effectively establishing study eligibility, they were allocated randomly to one of the two study groups according to the randomization list, using consecutive counting following the schedule of their inclusion visit. The randomization was stratified by gender (male and female) in blocks of $n=4$. The randomization was performed with RandList (Datinf GmbH, Tübingen, Germany).

Investigational products

Undenatured collagen from chicken sternum (40 mg UC-II® undenatured type II collagen per day, providing ≥3% undenatured type II collagen) and PLA (containing only excipient, microcrystalline cellulose) in sensory identical capsules were supplied by Lonza Greenwood LLC. (Greenwood, SC, USA). Subjects were instructed to consume products as one capsule daily with water in the evening before bedtime. Subjects were asked to document their intake time in a diary. All investigational products were carefully stored at room temperature and in dry conditions until distributed to subjects. At visits one through six, new bottles were handed out with enough study products to last until their next visit. Compliance was calculated based on the dispensed and returned study products.

SLSD test

The SLSD test is a unilaterally performed test, which was validated in a previous study.[11] During the test, subjects had to step forward and down from a platform with adjustable height. The down limb brushed the floor with the heel and then returned back up to the platform to full knee extension. The frequency of repetitions was given by a metronome. Subjects were instructed to indicate the pain level of five on NRS-11, where 0 meant ''no pain'' and a pain of 10 meant ''worst pain possible.'' After that, the test was stopped. The number of total repetitions was documented. If no joint pain occurred after latest of at least 10 min, the stress test was stopped.

Knee ROM

The knee ROM of a joint is typically measured by the number of degrees from the starting position of a segment to its position at the end of its full range of movement. In this study, a digital goniometer (Halo Medical Devices, Sydney, Australia) was used to measure knee ROM flexion and extension.

For knee ROM flexion measurement, the axis of a goniometer was placed at the intersection of the thigh and shank at the knee joint. Subjects lay in a prone position with shanks

(lower legs) hanging free over the edge of the examination table (position 1). Position 2 was the maximal flexion of the knee joint (Supplementary Fig. S1). During the measurement of active ROM flexion, the position 2 was reached actively by the subject. While for passive ROM flexion, the position 2 was reached using slight pressure by the investigator. Knee ROM extension was measured at baseline, and at week 12 and 24. The subject sat on an examination table with shanks (lower leg) hanging vertical to the floor (position 1), and the ROM from this position to the maximal extension (position 2) was measured (Supplementary Fig. S1).

Safety

Hematology, liver enzymes, lipid profile, hsCRP, HbA1c, and kidney function parameters were assessed at screening as well as at the final visit at Synlab Medizinisches Versorgungszentrum Leinfelden-Echterdingen, Germany. Blood pressure and heart rate were evaluated at screening, and at week 12 and 24 after 5 min of rest in a sitting position. Adverse events (AEs) were documented during the study duration. Subjective rating of tolerability was assessed at week 24 using a questionnaire rating as ''well-tolerated,'' ''slightly unpleasant,'' and ''very unpleasant.'' The focus of the tolerability assessment was on any gastrointestinal events possibly linked to the intake of the study product as well as the intake regimen and size of capsule.

Statistical methods

The analysis was performed on an intent-to-treat population. Analysis of covariance (ANCOVA) with baseline value as a covariate was used to analyze the statistical differences between the groups. Changes over time within the study group were evaluated using analysis of variance (ANOVA) repeated measurements or Friedman test as appropriate. *Post hoc* analysis for comparison between baseline and each study visit was performed applying Dunnett's multiple comparison test or Dunn's multiple comparison test as appropriate. A significance level of $p < 0.05$ was used. The analysis was performed with IBM SPSS statistics 24 statistical software (Armonk, NY).

Results

Demographics and baseline characteristics

Table 1 presents the baseline characteristics of the study participants. Ninety-six subjects met the eligibility criteria and were randomized to the PLA ($n = 48$) or to the undenatured collagen ($n = 48$) group. No significant differences were observed for baseline characteristics in both the groups ($p > 0.05$).

Product compliance

Overall, intake compliance of study products was more than 99% for both the groups ($p > 0.05$).

Range of motion

Regarding the parameter ROM flexion active, no significant differences between the study groups were observed at baseline ($p > 0.05$). At the end of the study, the undenatured collagen supplemented group showed a statistically significant mean increase of 3.23° in the ROM flexion compared

Table 1. Baseline Characteristics and Safety Parameters

Details	*Undenatured collagen* (n = *48*)	*PLA* (n = *48*)
Age, year	34.5 ± 1.5	37.8 ± 1.6
BMI, kg/m^2	23.91 ± 0.43	24.29 ± 0.38
Gender, M/F	20/28	20/28
Frequency of regular sporting activity		
1×/week	0%	0%
2×/week	31%	21%
3×/week	35%	44%
>3×/week	33%	35%
Frequency of intensity of physical activity		
Low	—	2%
Moderate	21%	21%
High	79%	77%

Safety parameters

Parameter	*Undenatured collagen Baseline* (n = *48*)	*Undenatured collagen End of intervention* (n = *48*)	*PLA Baseline* (n = *48*)	*PLA End of intervention* (n = *48*)
SBP, mm Hg	126.5 ± 13.6	125.4 ± 13.9	124.8 ± 12.1	120.5 ± 11.5
DBP, mm Hg	77.1 ± 7.8	78.3 ± 9.2	77.7 ± 9	76.5 ± 9.5
Cholesterol, mg/dL				
Men	175.5 ± 24	191.9 ± 23.35	176.5 ± 37.1	186.9 ± 36.08
Women	178.6 ± 34.5	184.6 ± 32.78	186.5 ± 35.2	187.5 ± 28.37
hsCRP, mg/dL				
Men	0.9 ± 0.7	0.7 ± 0.9	1.1 ± 0.8	1.2 ± 2.0
Women	1.7 ± 1.9	2.0 ± 2.5	1.1 ± 1.3	1.1 ± 1.3

BMI, body mass index; DBP, diastolic blood pressure; PLA, placebo; SBP, systolic blood pressure.

with the mean increase of 0.21 in PLA° group (undenatured collagen, $n=48$: 95% confidence interval [CI]: 0.44– 6.02 vs. PLA, $n=48$: 95% CI: −1.67 to 2.08; $p=0.0250$). The significant difference between the undenatured collagen group and the PLA group in the ROM flexion active was observed as early as week 8 of supplementation and continued to improve significantly during the course of the study (Fig. 2a). The delta changes from baseline to 24 weeks between the groups are additionally summarized in Table 2 and Supplementary Figure S2.

ROM flexion active according to subgroup gender

Subgroup analysis was performed for knee ROM flexion active based on gender. In the subgroup of females, a statistically significant increase of 4.79° was observed in the undenatured collagen group versus a slight decrease of −0.32° seen in the PLA group (undenatured collagen, $n=28$: 95% CI: 1.21–8.36 vs. PLA, $n=28$: 95% CI: −2.85 to 2.21; $p=0.0063$) after 24 weeks of supplementation (Table 2 and Supplementary Fig. S3). The undenatured collagen group showed a significant increase in ability to flex the knee over baseline ($p<0.01$) and over time ($p=0.0332$) among the female subjects, and no such change was observed in the PLA group ($p>0.05$).

In the subgroup of men, there was a slight nonsignificant increase seen in the ROM flexion for both the groups at the end of the study (undenatured collagen, $n=20$: +1.05 vs. PLA, $n=20$: +0.95; $p>0.05$). The results of the analysis are summarized in Table 2.

ROM flexion active according to subgroup age

Subgroup analysis was performed for knee ROM flexion active based on age. In the subgroup of age >35 years, subjects in the undenatured collagen group showed a significant increase in ROM flexion than in the PLA group (undenatured collagen, $n=19$: +6.79°, 95% CI: 2.38–11.20 vs. PLA, $n=27$: +0.30°, 95% CI: −2.60 to 3.19; $p=0.0092$). In addition, the undenatured collagen group showed a significant increase in the ability to flex the knee within the group over time ($p=0.0024$) and over baseline ($p<0.01$) in subjects >35 years old, while no such change was observed in the PLA group ($p>0.05$, Table 2).

The results of the group of age >35 years are further summarized in Supplementary Figure S4. In the 20–35-year-old subgroup, there was a slight nonsignificant increase in the ROM at the end of the study in both the groups (undenatured collagen, $n=29$: 95% CI: −2.64 to 4.44 vs. PLA, $n=21$: 95% CI: −2.32 to 2.51; $p>0.05$) (Table 2).

Knee flexion ROM passive

No significant difference between the study groups was observed at baseline ($p>0.05$, Table 2). A nonsignificant increase was observed in the undenatured collagen group, while no changes were seen in the PLA group after 24 weeks of intervention (undenatured collagen, $n=48$: +2.15°, 95% CI: −0.71 to 5.00 vs. PLA, $n=48$: +0.06°, 95% CI: −2.85 to 2.97; $p>0.05$).

ROM extension

ROM extension was evaluated at visits one (baseline), four (12 weeks), and seven (24 weeks). Baseline values were comparable between both the study groups ($p>0.05$). After 24 weeks of supplementation, a slight increase in ROM extension of 2.21° was observed in the undenatured collagen group and a slight increase of 1.27° in the PLA group. Analysis of changes over baseline ($p<0.01$) and over time ($p=0.0061$) in knee ROM extension showed a significant increase in the undenatured collagen group. No such change was observed in the PLA group ($p>0.05$, Fig. 2b, Table 2).

Safety assessments

No abnormalities were reported for any of the blood biochemistry, hematology, or vital signs (Table 1). Similarly, no study product-related AEs were noted in either the undenatured collagen or the PLA group. The tolerability of undenatured collagen was rated as ''well tolerated'' by 98% of the study participants. Based on this, it is concluded that supplementation of undenatured collagen was well-tolerated over the 24-week study period.

Discussion

Joint flexibility is of utmost importance in the daily lives of athletes as well as in active people. It has been reported

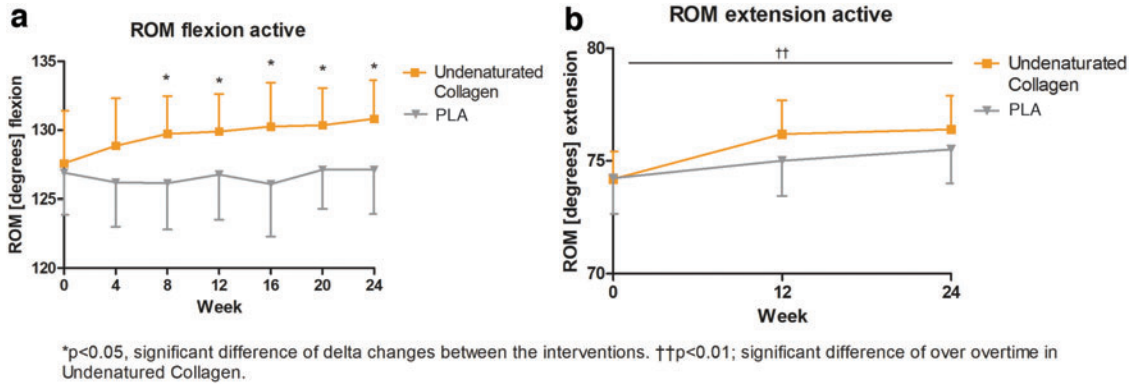

FIG. 2. Distribution of ROM results for **(a)** ROM flexion active (°); **(b)** ROM extension active (°) in the undenatured collagen group versus PLA group over the study; *line* graph with mean ± 95% CI; $^{*}p<0.05$, significant difference of delta changes between the interventions. $^{\dagger\dagger}p<0.01$; significant difference over time in undenatured collagen. CI, confidence interval; ROM, range of motion; PLA, placebo.

Table 2. Assessment of Range of Motion Parameters at Each Study Visit

Assessment of ROM	*Baseline*	*Week 4*	*Week 8*	*Week 12*	*Week 16*	*Week 20*	*Week 24*	*Delta change after 24 weeks*	p-*Value between*[a] *groups*	p-*Value within*[b] *groups*
ROM flexion active (°)										
UC-II	127.6 (123.8–131.4)	128.9 (125.4–132.3)	129.7 (127.0–132.4)	129.9 (127.2–132.6)	130.3 (127.1–133.4)	130.4 (127.6–133.10)	130.8 (128.0–133.6)	3.23 (0.44 to 6.02)	0.025	0.2844
PLA	126.9 (123.9–129.9)	126.2 (123.0–129.4)	126.1 (122.8–129.4)	126.8 (123.5–130.0)	126.1 (122.3–129.8)	127.1 (124.3–129.9)	127.1 (123.9–130.3)	0.21 (−1.67 to 2.08)		0.9134
ROM flexion active (°) by gender										
UC-II female	128.4 (123.1–133.7)	130.5 (126.1–134.9)	131.4 (127.7–135.1)	132.1 (128.9–135.4)	131.8 (128.1–135.5)	131.8 (128.8–134.9)	133.2 (129.7–136.7)	4.79 (1.21 to 8.36)	0.0063	0.0332
PLA female	128 (123.5–132.5)	126.8 (122.5–131.0)	127.1 (122.3–131.9)	128.0 (123.1–133.0)	127.6 (122.2–133.1)	127.9 (123.6–132.2)	127.7 (122.9–132.6)	–0.32 (−2.85 to 2.21)		0.9732
UC-II male	126.4 (120.6–132.2)	126.6 (120.7–132.4)	127.5 (123.3–131.6)	126.8 (122.1–131.4)	128.1 (122.1–1340.0)	128.3 (123.2–133.4)	127.5 (122.9–132.0)	1.05 (−3.60 to 5.70)	0.7881	0.9465
PLA male	125.3 (121.3–129.3)	125.4 (120.2–130.6)	124.8 (120.1–129.5)	125.0 (121.1–129.5)	123.9 (118.6–129.2)	126.0 (122.8–129.1)	126.3 (122.4–130.1)	0.95 (−2.07 to 3.97)		0.8828
ROM flexion active (°) by age										
UC-II age 20–35	130.9 (127.0–134.9)	130.8 (127.0–134.5)	130 (126.9–133.1)	131.2 (128.5–134.0)	133.1 (130.1–136.2)	131.7 (129.0–134.3)	131.8 (129.1–134.5)	0.90 (−2.64 to 4.44)	0.6012	0.3129
PLA age 20–35	130.7 (127.3–134.0)	128.2 (122.8–133.6)	127.0 (120.5–133.4)	129.9 (125.1–134.7)	128.7 (122.4–135.0)	131.0 (127.4–134.7)	130.8 (127.1–134.4)	0.10 (−2.32 to 2.51)		0.2337
UC-II age >35	122.5 (115.1–129.8)	125.9 (119.1–132.8)	129.3 (123.9–134.7)	127.8 (122.2–133.5)	125.8 (119.1–132.3)	128.4 (122.5–134.2)	129.3 (123.2–135.4)	6.79 (2.38 to 11.20)	0.0092	0.0024
PLA age >35	124 (119.4–128.5)	124.6 (120.6–128.6)	125.5 (121.9–129.1)	124.3 (119.9–128.7)	124.0 (119.2–128.8)	124.0 (120.2–127.9)	124.3 (119.5–129.0)	0.30 (−2.60 to 3.19)		0.9462
ROM flexion passive (°)										
UC-II	146.2 (142.9–149.5)	145.7 (142.1–149.3)	148.0 (144.6–151.4)	146.5 (143.1–149.8)	147.9 (143.8–152.0)	147.7 (144.5–150.9)	148.3 (144.4–152.3)	2.15 (−0.71 to 5.00)	0.3103	0.3167
PLA	146.4 (142.9–149.9)	144.3 (140.3–148.2)	144.8 (140.8–148.8)	145.9 (142.1–149.8)	145.6 (141.0–149.8)	145.2 (141.0–150.2)	146.4 (141.9–151.0)	0.06 (−2.85 to 3.97)		0.7695
ROM extension active (°)										
UC-II	74.2 (73.0–75.4)	n.a.	n.a.	76.2 (74.7–77.7)	n.a.	n.a.	76.4 (74.9–77.9)	2.21 (0.82 to 3.60)	0.3115	0.0061
PLA	74.2 (72.7–75.8)	n.a.	n.a.	75.0 (73.4–76.6)	n.a.	n.a.	75.5 (74.0–77.0)	1.27 (−0.17 to 2.71)		0.2540

Values are means (upper and lower bound of 95% CI).
$n = 48$ in each intervention group.
[a]*p*-Value for comparison between the groups by ANCOVA.
[b]*p*-Value for within-group analysis by repeated-measures ANOVA or Freidman test as appropriate.
ANCOVA, analysis of covariance; ANOVA, analysis of variance; CI, confidence interval; n.a., not applicable; PLA, placebo; ROM, range of motion; UC-II, Undenaturated type II collagen.

that the stress on knee joints during physical activity may result in immunologic responses that mirror those seen in arthritic diseases, which can ultimately lead to a decrease in knee joint flexibility.[13] Similarly, aging has been shown to reduce knee ROM due to wear and tear exerted on the joints from daily use.[14–19] According to the CDC, an average adult loses 1° of knee flexion and extension ROM for every 10 years of age.[18] This loss in flexibility with age has been attributable, in part, to decreased activity[20] and decreased joint mobility.[18] Therefore, restoring and maintaining knee ROM are critical to keeping joints healthy.

Knee ROM is commonly used as an outcome measure in clinical studies of people with knee OA, rheumatoid arthritis (RA), and in athletes.[21,22] Steultjens et al. demonstrated that the mean knee ROM in an OA group was 19% lower than in control group.[21] In another study, McCarthy et al. reported that patients with knee OA had significantly lower knee flexion ROM than matched controls.[23] Other studies have reported that patients with knee OA demonstrate reduced knee motion during walking compared with healthy controls alongside a reduction in gait velocity.[24–26] In another study, strong correlations were found between the loss of ROM of the knee and hip joints, and disability in an elderly population.[27–29]

In the current study, a significant improvement in knee flexion was observed with undenatured collagen supplementation. An increase in knee flexion was seen as early as 8 weeks of supplementation and it continued to improve eventually reaching 3.23° at the end of the study. In a recently published pilot study, the authors reported that healthy subjects with ArJD have impaired ROM.[11] The improvement by 3° in the current study suggests that undenatured collagen supplementation may benefit to improve knee flexion in healthy subjects who are at risk of developing joint ailments down the road.

Subgroup analysis based on gender showed that females reported higher ROM flexion improvement in response to undenatured collagen supplementation than was seen in males. This gender-based difference in efficacy could be possibly attributed to the fact that females with joint disorder exhibit lower ROM,[21] through which supplementation with undenatured collagen might allow for a greater chance to see better outcomes in such a population.

A separate subanalysis based on age demonstrated that subjects aged 35 years and older experienced a higher increase in active ROM flexion in response to undenatured collagen supplementation than was observed with subjects between 20 and 35 years of age. One possible explanation for these preferential results might be due to the fact that older subjects are expected to have lower ROM to start with compared with younger subjects, and hence, one would expect that older adults could benefit to a higher extent from undenatured collagen supplementation.

A significant increase in knee extension was seen in the undenatured collagen group after 24 weeks of supplementation. This is in agreement with the previous research where supplementation of undenatured collagen for 120 days was shown to improve knee extension ROM in healthy subjects who experienced joint pain upon strenuous exercise.[10] These same subjects were also able to exercise longer before experiencing joint pain post-120 days of supplementation.[10] In the present study, an increase in knee extension, in addition to flexion, suggests that undenatured collagen supplementation could improve joint function and mobility to better support everyday activities.

With respect to passive knee flexion, no significant changes were observed between the study groups. This is not surprising as subjects in this study were healthy and hence did not have any overt restriction in their joint mobility/movement. In addition to this, considering that passive knee flexion is reached with help from the investigator, many of the study participants were able to reach passive knee flexion to the maximum extent at the beginning of the study. This could possibly explain the lack of significant change in passive knee flexion between the study groups.

Although it has been also shown in previous studies that OA subjects as well as subjects with activity-related joint discomfort may benefit from a supplementation with undenaturated type II collagen by enhancing joint mobility,[9,10] the exact mechanism of action is still not fully understood. According to animal and *in vitro* studies, it is assumed that during exercise, some processes that also occur in OA are activated, such as distribution of proinflammatory cytokines.[13,30,31] Undenatured type II collagen appears to reduce joint inflammation by acting via the gut-associated lymphoid tissue. It seems to stimulate immune cells (T cells) to produce anti-inflammatory cytokines in joints.[31] This mechanism helps to diminish inflammatory processes and to stimulate cartilage repair, which seems to be a possible mode of action of the study product.

The current study has limitations that the reader should consider when reviewing the results. No biomarker assessment was performed in the current study to investigate the mode of action of undenatured type II collagen on joint health. This should be investigated in further studies.

Furthermore, during the study, there was a global outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), leading to the COVID-19 pandemic. For the major period of 2020, gymnasiums, fitness centers, stores, and other public places were all closed as part of a global lockdown aiming to halt the spread of the virus. Some subjects, especially those who regularly performed weight training and team sports—such as soccer and handball—could not participate in their usual physical activities or training.

However, study investigators advised the subjects to continue with their usual physical activity routines and perform alternative sport types at home or outdoors where possible to maintain consistency. Most subjects followed these recommendations; however, during the winter months in colder climates, outdoor physical activities were often not possible. Therefore, some subjects reported a reduction in the frequency of regular physical activity.

As these changes were only transient, these deviations were rated as minor. Because of the COVID-19 pandemic, some rescheduling of study visits had to be made. Nevertheless, the intake period was not interrupted as subjects were supplied with additional products.

Overall, the findings from this study underline the importance of maintaining healthy ROM, especially in subjects who already show signs of impairment. This study showed promising results for undenatured collagen supplementation as a dietary ingredient with the ability to help improve knee joint ROM flexion and extension.

Conclusions

In the current study, the effect of undenatured collagen supplementation on knee joint flexibility in subjects with ArJD was investigated. The data support that undenatured collagen UC-II is a food ingredient with the potential to positively affect function of knee joint resulting in an improvement of knee flexion assessed by goniometry, demonstrating the benefit in a population at risk. As the biomarker assessment was not performed in the current study, this should be emphasized in future research to investigate the mode of action of undenatured type II collagen on joint health.

Authors' Contributions

Conceptualization: K.K., C.S., V.J., and W.A.; formal analysis and data curation: K.K., C.S., V.J., and W.A.; writing—original draft preparation, and review and editing: K.K., C.S., W.A., and V.J.; review and editing: S.D. and Z.S. All authors have read and agreed to the submitted version of the article.

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Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

The data presented in this study are available on request from the corresponding author. The data are not publicly available due to confidentiality and ethical reasons.

Author Disclosure Statement

V.J., S.D., and Z.S. are employees of Lonza Greenwood LLC., Greenwood, SC, USA. All other authors declare no conflict of interest.

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Supplementary Material

Supplementary Figure S1
Supplementary Figure S2
Supplementary Figure S3
Supplementary Figure S4

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Research Article

UC-II® Undenatured Type II Collagen Reduces Knee Joint Discomfort and Improves Mobility in Healthy Subjects: A Randomized, Double-Blind, Placebo-Controlled Clinical Study

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ABSTRACT

Joint discomfort is a common issue in athletes and healthy, active individuals. The objective of this study was to evaluate the efficacy of UC-II® undenatured type II collagen (Undenatured Collagen) in managing knee joint discomfort and mobility in healthy subjects with Activity-related Joint Discomfort (ArJD). Subjects who reported knee pain of 5 on an 11-point Likert scale while performing a Single-Leg-Step-Down (SLSD) test were randomized to receive placebo (PLA, n=48), or Undenatured Collagen (n=48) for 24 weeks. Joint mobility was measured from the daily step number. Joint discomfort was evaluated using the Knee Injury and Osteoarthritis Outcome Score (KOOS) questionnaire, duration of pain during sporting activities and number of steps to reach defined pain levels during the SLSD test. A sub-analysis by gender showed a higher number of daily steps in males from the Undenatured Collagen group versus PLA ($p=0.0374$) after 24 weeks. In the SLSD test, the Undenatured Collagen group showed a significant change over time and baseline in the number of repetitions to reach pain 2 ($p<0.05$). Subjects in Undenatured Collagen group of 20-35 years old took more steps on the SLSD test before reporting the pain 5. A significant change over baseline for the pain duration after sports was further observed in 20-35 years old subjects ($p<0.05$). Analysis of KOOS demonstrated an improvement of subscale function in sport and recreation over time ($p=0.0009$) and of subscale quality of life over baseline ($p<0.05$) in the Undenatured Collagen group. In conclusion, the data suggests Undenatured Collagen reduces joint discomfort and improves mobility.

Keywords: Joint discomfort; Knee joint; KOOS; Step count; Physical activity; Undenatured type II collagen; Mobility, Collagen

Abbreviations: OA: Osteoarthritis; CDC: Centers for Disease Control; ROM: Range Of Motion; SLSD: Single -Leg-Step-Down; KOOS: Knee Injury and Osteoarthritis and Outcome Score; ArJD: Activity-related Joint Discomfort; GCP: Good Clinical Practice; QOL: Quality Of Life; ADL: Activities of Daily Living; ANCOVA: Analysis of Covariance; ITT: Intention-To-Treat population; LOCF: Last Observation Carried Forward; Treg: regulatory T cells; DRKS: Deutsches Register Klinischer Studien; ICH: International Council for Harmonisation SE: Standard Error

INTRODUCTION

Osteoarthritis (OA) is a major public health risk and challenge. According to the Centers for Disease Control and Prevention (CDC), one in four U.S. adults have some form of arthritis, a figure projected to reach 78 million by the year 2040 [1]. Knee OA contributes to more than $27 billion in health care costs each year [1,2]. Overall the burden of OA and complications are increasing in most countries [3].

Over the past few years, the health and wellness industry has witnessed large number of millennials participating in exercise on a regular basis as they pursue more active lifestyles including use of dietary supplements to improve their activities [4-6].

UC-II® undenatured type II collagen (Undenatured Collagen) is a joint health ingredient derived from chicken sternum cartilage, and has been shown in clinical and preclinical studies to support knee joint comfort and reduce overall joint pain by lowering markers of inflammation [7-10]. In addition, laboratory animal study results demonstrate that a clinically relevant daily dose of Undenatured Collagen can improve the mechanical function of the injured knee and prevent excessive deterioration of articular cartilage and reduces inflammation [11-13].

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Undenatured (native) Collagen is believed to exert its effects via a unique mechanism in the gut known as oral tolerance, which involves induction of T regulatory cells thereby allowing the knee joint to repair and rebuild the cartilage. The presence of active epitopes on the Undenatured Collagen are required to interact with Peyer's patches in the gut and induce oral tolerance [14-15].

Some research groups examined people with activity-related joint discomfort and showed improvement of joint pain in response to stresses from the daily life activity [16-21]. Such an approach has limitations because daily life activity is characterized by variable intensities and therefore by the variable load on the joints resulting in different levels of stress on joint-related structures. To overcome these shortcomings, stress models (joint or muscle-related) have been used to induce a standardized load on physical structures to observe changes in perceived stress as accurately as possible [19-21].

We recently reported the effect of Undenatured Collagen on improving knee Range of Motion (ROM) flexion and extension in a 24-week study in healthy subjects who experienced activity-related join discomfort [22]. The current paper reports the data from the same study specifically focused on the ability of Undenatured Collagen supplementation to improve knee joint discomfort and mobility in healthy subject with Activity-related Joint discomfort (ArJD). In addition, we also present the sub-analysis of the joint outcome measures based on age and gender.

METHODOLOGY

Study design

This study is a randomized, parallel design and double-blind two-arm study. Subjects signed the informed consent forms prior to participating in the study. Table 1 provides the summary of the study design.

We recently validated a Single-Leg-Step-Down (SLSD) test as a reliable model to induce joint discomfort [21]. Subjects with previous history of joint discomfort during sports activities, and who reported joint pain while performing the SLSD test were enrolled in the study. Joint discomfort during daily life and physical activity was evaluated using the subjective Knee Injury and Osteoarthritis Outcome Score (KOOS) questionnaire [23]. Joint mobility was measured using the daily number of steps taken as recorded by a step counter.

Subjects were asked to maintain their normal activities, nutrition and lifestyle habits plus avoid taking any medications or dietary supplements for pain relief within 36 hours prior to the study visits. Additionally, on the day of study visits, subjects were instructed to consume their last meal at least one hour before the performance of the step test. During the study, subjects documented the type of frequency of their physical activity, as well as the pain level.

This study was conducted following the guidelines for Good Clinical Practice (GCP) set forth by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use International Council for Harmonisation (ICH E6 (R2), Nov 2016) and following the Declaration of Helsinki (E8) regarding the treatment of human subjects in a study. This study was approved by the local ethics committee (Institutional Review Board of the Landesärztekammer Baden-Württemberg, file number F-2019-072) and the clinical trial was registered at Deutsches Register Klinischer Studien (DRKS)-German Clinical Trials Register DRKS (ID: DRKS00018792).

Investigational products

Undenatured Collagen sourced from chicken sternum (40 mg UC-II® collagen per day, providing ≥ 3% undenatured type II collagen) was provided by Lonza Consumer Health Inc. Placebo capsule was sensory identical and contained only the excipient, microcrystalline cellulose. Subjects were asked to take one capsule of study product daily with water before bedtime. Subjects documented their intake time in a diary.

Study product compliance

Compliance was calculated based on dispensed and returned study products. In the case of missing products or no return, compliance was estimated from subject diaries.

Study population

In the current study, 178 men and women were screened for eligibility using the inclusion-exclusion criteria described in detail in Table 2. Inclusion criteria was based on subjects reporting joint discomfort of at least 5 on an 11-point Likert scale while performing between 30 and 150 repetitions during the SLSD and performing joint bearing sports (at least 2 times per week for at least 20 min). Ninety-six (96) subjects who met the eligibility were randomized to receive either Undenatured Collagen or PLA. The study was conducted at BioTeSys GmbH, Esslingen, and at the Institute of Sport and Movement Science of the University of Stuttgart from September 2019 to January 2021.

Table 1: Study Design; measures and outcomes based on visits during the period of the study.

Study activities	Screening	Baseline	Wk 4	Wk 8	Wk 12	Wk 16	Wk 20	Wk 24
Informed consent	X							
Inclusion/exclusion	X	X						
Medical history/physical exam	X							
Vital signs	X				X			X
Urine pregnancy test	X							
Questionnaires/scales		X	X	X	X	X	X	X
SLSD test	X	X	X	X	X	X	X	X
Randomization		X						
Study supplement dispensing		X	X	X	X	X	X	
Compliance			X	X	X	X	X	X
Safety blood markers	X							X

Abbrevations: Wk: Week; SLSD: Single-Leg-Step-Down

Table 2: Inclusion and exclusion criteria of the subjects.

Inclusion criteria	Exclusion criteria
1. Age: 20-55 years old	1. Joint replacement of the knee or hip, or any hip or back pain which interferes with ambulation
2. Men and women (minimum 25% of each gender)	2. Planned surgical intervention in knee and foot during the next 6 months
3. Subjects having a Body Mass Index (BMI) between 19 and 29.9 kg/m^2	3. Intra-articular therapy within the last 3 months
4. Performing joint bearing sports (at least 2 times per week for at least 20 min) and willing to maintain it during the whole study period, e.g. soccer, basketball, handball, volleyball, tennis, running etc.	4. Diagnosed OA, rheumatoid arthritis or other inflammatory, infectious or metabolic joint disorder
5. Subject is able and willing to sign the Informed Consent Form prior to screening evaluations	5. Acute knee pain at rest or during light daily life activity
6. Subject is able to communicate well with the Investigator, to understand and comply with the requirements of the study, and be judged suitable for the study in the opinion of the Investigator	6. Acute or history of musculoskeletal injury or previous severe joint injury at the knee joints (e.g. ligament rupture or surgical intervention of meniscus) if not completely recovered without remaining functional impairment and if not more than 2 years ago
7. Subject is in good physical and mental health with no existing co-morbidities as established by medical history, physical examination, electrocardiogram, vital signs, which could interfere with the current study	7. Chronic intake of supplements influencing joint-health (e.g. glucosamine, chondroitin sulphate, collagen hydrolysate, curcumin, etc. or in combination) 3 months prior to screening; Vitamin D ≤ 2,000 I.U., niacin equivalent ≤ 20 mg are permitted
8. Subject having clinically normal findings for haematology and clinical chemistry	8. Chronic intake of medication influencing joint health (e.g. corticosteroids); permitted are stable thyroid gland medication or anti-hypertensives
9. Reversible knee-joint discomfort during or immediately after physical activity over a period of at least 3 months	9. Chronic use of pain relief medications (i.e. Nonsteroidal Anti-Inflammatory Drugs (NSAIDs), analgesics, opioids, anti-depressants prescribed for painful conditions such as e.g. fibromyalgia 30 days prior to screening)
10. Joint discomfort of at least 5 on an 11-point Likert scale while performing between 30 and 150 repetitions during the Single -Leg- Step-Down test (SLSD).	10. History or presence of significant cardiovascular disease or co-morbidities (e.g. heart failure, stroke, diabetes, etc.)
11. Subjects willing to abstain from regenerative actions or pain medication after the stress test until 24h after	11. Elevated blood pressure measured during screening (≥ 140/90) in subjects under hypertensive treatment or with diagnosed hypertension
	12. Clinically significant disorders (e.g. Diabetes mellitus, neurological disorders, cancer, inflammatory bowel diseases, etc.)
	13. Smoker >5 cigarettes/day
	14. Subjects not willing to abstain from intake of analgesic, cannabinoid and/or opioid medication 36 hours prior to and during visits
	15. Vegans and vegetarians
	15. History of hypersensitivity to eggs, chicken or any ingredients in the products
	17. Anticipate problems with product consumption (e.g. unable to swallow capsules)
	18. Female patients that are pregnant or nursing (women have to agree to use appropriate contraception methods)
	19. Participation in a clinical study with an investigational product within 30 days before screening
	20. Known alcohol abuse or drug abuse
	21. Known infection of Human Immunodeficiency Virus (HIV) or hepatitis B or C

Subjects' medical history, concomitant medications use, anthropometric measurements and vital signs (blood pressure and heart rate) were recorded during the course of the study. General joint health conditions were assessed based on small orthopedic tests (knock knees, bandy legs, McMurray Test, Steinman I and Steinman II, Drawers Test, Zohlen sign). Additionally, routine blood parameters such as liver enzymes, kidney function, hematocrit, hs-CRP, HbA1c and lipid profiles were assessed for safety at baseline and the end of the study. Adverse Events (AEs) were recorded during the study period.

Joint discomfort

Steps to reach pain 5 and 2 during SLSD test: For the SLSD test Figure 1, subjects were asked to performed as many repetitions (step number) as possible until they reported the knee joint pain level of 2 (first onset) as well as of level 5 on an 11-point Likert scale, where 0 meant "no pain" and 10 meant "worst pain possible". The number of total repetitions was documented. The leg with the highest pain level after regular physical activity was defined as the target knee. At the initial screening, the SLSD test was used to assess suitability for study inclusion. At visit one (baseline), only subjects who met the inclusion criteria concerning the SLSD test were enrolled. SLSD test was performed at each visit and the number of repetitions (step number) to reach a pain level of 2 and 5 on Likert scale were recorded (Figure 2).

Figure 1: Single-leg-step-down (SLSD) test.

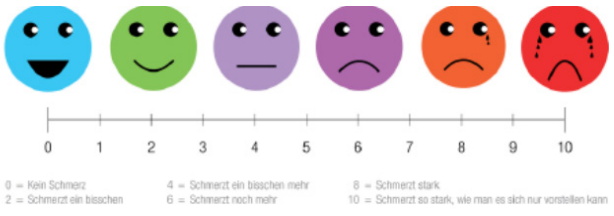

Figure 2: Likert rating scale to rate knee pain while doing the single-leg-step-down test.

Duration after sports activity

Starting with the initial screening, subjects documented the type and frequency of their activity and reported duration of joint discomfort after sports activity.

KOOS questionnaire

The KOOS questionnaire is a validated instrument used to assess subject's opinion about their knee and associated problems [23]. It consists of five subscales: (1) Pain, (2) Other symptoms, (3) Activities of Daily Living (ADL), (4) Function in sport and recreation (Sport/Rec), and (5) Knee-related Quality Of Life (QOL). Subjects are asked to consider their previous week when answering the questions. For the retrospective measurement of joint discomfort during daily life and physical activity, the KOOS was used and filled in by subjects at initial screening, visit one, visit four, and visit seven. Subscale Sport/Rec was filled in at each study visit.

Daily step count

A step counter was used between visits (OMRON Walking style IV) during the study to measure the daily steps count. The daily step count was documented in the subjects' diaries.

Statistical analysis

Sample size calculations were based on the results of a previously performed pilot study [7,21]. Assuming an effect size of 0.636, a sample size of 40 subjects per group would provide approximately 80% power at a significance level of 5%. Considering a drop-out rate of 15%, the study was performed with 48 subjects per group.

Analysis of Covariance (ANCOVA) with baseline value as covariate was used for between group comparisons. Changes over time within study groups were evaluated with repeated measures ANOVA or Friedman test as appropriate. Post hoc analysis for comparison between baseline and each study visit was performed applying Dunnett's Multiple Comparison Test or Dunn's Multiple Comparison Test as appropriate. Additionally, analysis between and within groups was assessed applying a linear mixed model with repeated measures considering baseline and gender as covariates. All analysis was evaluated in the Intention-To-Treat population (ITT). The missing data were imputed by the Last Observation Carried Forward (LOCF) method. All statistical tests were performed two-sided with p value ≤ 0.05 was considered significant. The analysis was performed with IBM SPSS statistics 24 statistical software (Armonk, NY, USA) as well as with SAS software Version 9.4 (Cary, NC, USA).

RESULTS

Baseline characteristics

There were no significant difference between the groups at baseline with respect to the age (Undenatured Collagen: 34 ± 1.5 y; PLA: 37.8 ± 1.6 y) and BMI (Undenatured Collagen: 23.91 ± 0.43 kg/m^2; PLA: 24.9 ± 0.38 kg/m^2) (p>0.05).

Daily step count

Joint mobility was measured based on the daily number of steps taken by subjects using a step counter. It is a direct determinant of posture and movements that influences daily activities.

The Undenatured Collagen supplemented group reported taking a higher number of daily steps at week 24 compared to the baseline value (+217 steps), whereas the PLA groups reported reduction in number of steps versus the baseline value (-530 steps). However, this change failed to achieve statistical significance between the groups (p=0.2113). A sub-analysis based on gender showed a significantly higher number of daily steps in males from the Undenatured Collagen group versus the PLA group over the study period (+669 steps vs. -526 steps, p=0.0374, Table 3).

Table 3: Daily step count between the study groups delta change from baseline to 24 weeks of intervention.

Daily Step Count	Undenatured Collagen	Placebo
	Mean ± SE	
Overall Gender	217.3 ± 341.5 (n=48)	-529.5 ± 348 (n=48)†
Females	-105.3 ± 448.0 (n=28)	-532 ± 537.5 (n=28)
Males*	668.8 ± 524 (n=20)	-525.7±379.7 (n=20)

Note: *p<0.05, significant difference of delta changes between the study groups; †p=0.0703 strong statistical trend

Joint discomfort

Steps to reach pain 5 during SLSD test: After 24 weeks of supplementation, the Undenatured Collagen group demonstrated an average increase in the number of steps (2.85 steps) prior to reporting pain 5, whereas the PLA group showed a slight decrease in number of steps (-0.65 steps). However, these changes were not significantly different between the groups (p>0.05). In a subgroup analysis based on age, subjects aged 20 and 35 years old in the Undenatured Collagen group showed a significant increase in number of steps change overtime (p=0.0409) and no changes were observed in PLA group during the study period (p>0.05).

Steps to reach pain 2 during SLSD test: The Undenatured Collagen group showed a non-significant increase in the number of steps to reach pain 2 (first onset of pain) than the PLA group after 24 weeks of supplementation (p>0.05). Undenatured Collagen group showed a significant increase of step number to first onset of pain over the baseline (p<0.05). Both study groups further showed a significant change overtime (p<0.05, Table 4). A significant product effect (p=0.0427) as well as a significant time effect (p=0.0013) was further observed. Table 4 summarizes the results of number of repetitions for both study groups.

Knee Injury Osteoarthritis and Outcome Score (KOOS)

The analysis of KOOS subscale data demonstrated a significant improvement of subscale function in sport and recreation over time in the Undenatured Collagen group (p=0.0009, Figure 3), while no change was observed in PLA group (p>0.05). The Undenatured Collagen group also showed improved quality of life over the study period versus the baseline (p<0.05) and no significant change observed in PLA group. Significant differences for such selected KOOS individual items as knee pain (p=0.0482), knee twisting/pivoting (p=0.0346), walking while descending stairs (p=0.0215) and walking on a flat surface (p=0.0241) were observed at visit 7 after 24 weeks of supplementation of Undenatured Collagen compared with the PLA group.

Duration after sporting activity

Subjects were instructed to document joint discomfort and duration of joint discomfort after their regular sporting activity in a daily diary. From this diary, the mean joint discomfort over 14 days before each study visit was calculated. No significance was observed between the study groups at baseline for the pain duration (p>0.05). A significant difference in the duration of pain after sporting activity over baseline was observed in Undenatured Collagen and PLA groups (p<0.05). When performing the subgroup analysis, a significant decrease of pain after sporting activity over baseline in subgroup of subjects aged 20-35y old in Undenatured Collagen group (p<0.05, Table 5) was observed, while no such significance was seen in PLA group (p>0.05).

Table 4: Steps to reach pain 5 and 2 after SLSD test (delta changes from baseline to 24 weeks of intervention).

Number of Steps to reach pain	Undenatured Collagen	Placebo
	Mean ± SE	
Steps to reach pain 5		
Overall gender	2.85 ± 4.60	-0.65 ± 4.01
Males	11.75 ± 9.76	6.15 ± 6.10
Females	-3.50 ± 3.44	-5.50 ± 5.22
Age 20-35	9.62 ± 7.03†	4.0 ± 6.37
Age >35	-7.47 ± 3.54	-4.26 ± 5.13
Steps to reach pain 2		
Overall gender	8.85 ± 4.06*†	2.81 ± 2.0†
Males	13.80 ± 9.34	5.70 ± 3.04
Females	5.32 ± 2.05	0.75 ± 2.67
Age 20-35	10.72 ± 6.59	3.76 ± 2.91
Age >35	6.00 ±2.19	2.07±2.83

Note: *p<0.05 over baseline; †p<0.05: significant change of step number over time

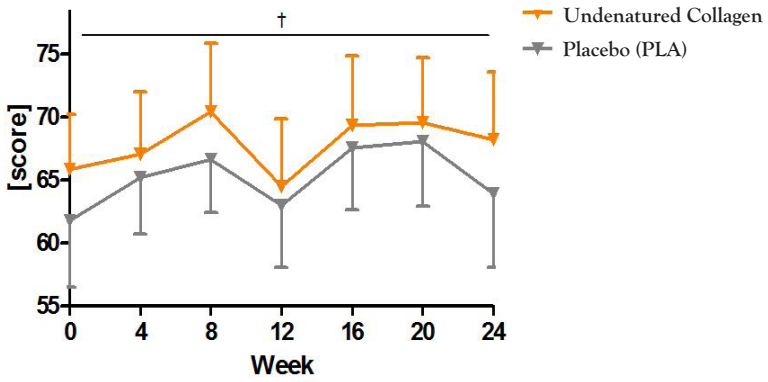

Figure 3: Distribution of KOOS Sport/Rec score between baseline and week 24 in the Undenatured Collagen and Placebo group.
Note: † p<0.05, significant change over time in Undenatured Collagen group.

Table 5: Duration of pain after sporting activity (min) in the Undenatured Collagen and placebo group.

Duration of Pain after regular sports (min)	Overall, N=94		Age, Mean ± SE		Age, Mean ± SE	
			20-35y		>35-55y	
	Baseline	Final visit	Baseline	Final visit	Baseline	Final visit
Undenatured Collagen, N=48	98.23 ± 16.4	66.77 ± 16.05*	96.72 ± 19.90 (N=29)	66.79 ± 19.45* (N=29)	100.50 ± 28.87 (N=19)	66.74 ± 28.32* (N=19)
Placebo, N=46†	91.24 ± 18.21	66.96 ± 15.92*	124.60 ± 35.72 (N=19)	117.30 ± 34.93 (N=19)	67.74 ± 17.46 (N=27)	31.56 ± 5.98* (N=27)

Note: *Significance over baseline; *p<0.05; †Two subjects values were more than 3 × SD above the mean at baseline. Hence these two values were excluded by the statistician; Number in parenthesis is number of subjects.

Safety

Analysis of the vital signs and routine blood parameters showed no clinically abnormal findings or product related changes. None of the reported AEs were related to the study product consumption. The tolerability of the Undenatured Collagen and PLA study products were rated as "well" by almost all subjects. The results of this study further support the tolerability of Undenatured Collagen supplementation.

DISCUSSION

Joint and bone health supplements are increasingly finding their place in the functional foods and nutraceuticals markets. Joint discomfort and joint overload are common problems not only for athletes but also in the daily lives of healthy, active people. Knee joint stress exerted by overload leads to micro-damage of the cartilage those results in localized joint pain. Stress-related joint pain can be caused by anatomical weaknesses (e.g., unequal leg length, bandy legs, small patella, etc.), mechanical overload, or unstable joints resulting from previous ligament injuries. It has also been suggested that the load on knee joints during physical exercise may result in immunological responses that mimic those seen in arthritic disease [24]. Earlier studies have shown Undenatured Collagen supplementation can moderate joint pain and stiffness in osteoarthritic subjects [8-15]. Likewise, Undenatured Collagen has been shown to improve joint function and mobility in healthy subjects who experience activity-related joint pain [7].

There is a need to develop standardized stress tests that would allow evaluation of joint health efficacy of an ingredient in healthy subjects who report ArJD. Our group recently validated a SLSD test as a reliable model to induce joint discomfort and select for participants who are healthy and have ArJD [21]. One parameter to assess joint discomfort with SLSD test is to measure the change in number of repetitions (steps) required to reach a pain of five on an 11-point Likert scale. This test also provides a good measure to study the impact of intervention on improving knee joint mobility and discomfort. Hence, an intervention that increase the number of repetitions until one reaches a defined pain level, it means the intervention is successful in reducing joint discomfort thereby it may allow subjects to move more, and move farther.

In the current study, the SLSD test was used to investigate the effect of Undenatured Collagen group supplementation on joint health in healthy subjects. After 24 weeks of supplementation, subgroup of men in Undenatured collagen group took higher number of steps on SLSD test before experiencing the pain level of 5. Regarding the subgroup analysis by age, an increase in step count to reach pain 5 was seen in subjects of age 20-35 years of the Undenatured Collagen group, whereas, in the PLA group, the increase was only minor. In subjects over 35 years old, a decrease in step count was seen at the end of the study in both the Undenatured Collagen and the placebo group. The decrease in step count observed in both groups might be attributed to the imbalance in the gender distribution with the subgroup comprising of two-third proportion of females in each group. Another possible explanation could be that younger subjects were more responsive to the Undenatured Collagen supplementation.

Interestingly, when comparing the number of repetitions performing the SLSD test to the first onset of pain (pain 2 on the Likert scale), a significant effect of Undenatured Collagen supplementation was observed (p=0.0427). This indicates that

Undenatured Collagen supplementation may increase time to the first onset of pain. This observation is in agreement with the previous study where 40 mg Undenatured collagen supplementation for 120 days was shown to extend the pain free exertion in healthy subjects with ArJD [7], where subjects performed a standardized step mill test until reaching a discomfort level of five on an 11-point Likert scale. When evaluating the results at the end of the study in Undenatured Collagen, a significant increase in time to the first onset of pain was observed over baseline [7]. Extrapolating the results of the current study to daily activity, one could say that reduction in joint discomfort translates into increased mobility or movement. This is also further supported by the results of the daily step counting, which showed that Undenatured Collagen group took more daily steps at the end of the study compared to the baseline. Undenatured Collagen group took over 700 steps more than the placebo group, which in practical terms equates to about 1/4th of a mile per day.

Additionally, the KOOS questionnaire was used to assess knee joint discomfort. When comparing the changes between the intervention groups, no differences were seen between the study groups after 24 weeks of intervention in all subscales. Nevertheless, the evaluation of the KOOS questionnaire indicated, that even if the knee pain only occurred during heavy load or sporting activities, this already had some impact on knee related quality of life plus sport and recreation. This is consistent with the observations made during the pilot study [21]. In this study, the KOOS questionnaire was identified as a good tool to differentiate between healthy people, subjects with OA, and subjects with ArJD. Significant differences could be seen between healthy and ArJD subjects in the subscales of Pain, Sport/Rec, and QOL [16]. Nevertheless, some differences were seen when evaluating individual KOOS items in the current study. Regarding individual items of KOOS activities of daily living as descending stairs or walking on a flat surface, Undenatured Collagen group showed lower joint discomfort over PLA group at 24 weeks after supplementation.

Overall, the findings from the current study confirms the previous observation [7,8] that Undenatured Collagen supplementation helps to alleviate the onset of joint discomfort. Previous studies have shown that intense physical activity or exercise may activate processes similar to that seen in arthritis such as increase in production of proinflammatory mediators [11-13]. This is an interesting observation considering the fact that Undenatured Collagen supplementation has been shown in previous studies to reduce inflammation markers and prevent degradation of articular cartilage [11]. In terms of mechanism, Undenatured Collagen is proposed to work via induction of oral tolerance because of the presence of active epitopes on the native type II collagen molecule. When consumed orally, Undenatured collagen passes through the gut and is believed to be taken up by the Peyer's patches in the small intestine, where it activates immune cells leading to generation of type II collagen specific T regulatory (Treg) cells. Treg cells then migrate through the circulation (blood stream), and when they arrive at an inflamed knee joint, they secrete anti-inflammatory cytokines, TGF- β and IL-10 that helps reduce joint inflammation and promotes cartilage repair [14-16,25-29].

A recent study reported that walking 6000 or more steps per day reduces risk of developing knee OA related mobility issues, such as difficulty getting up from a chair and climbing stairs [30]. The results further suggest that walking an additional 1000 steps per day was associated with 16% to 18% reduction in incidence of functional limitation two years later [30]. In the current study, we observed increase in daily step count with Undenatured Collagen supplementation suggesting that it may enhance joint mobility typically by increasing ambulatory activity.

The current study has some limitations, during the study there was a global outbreak of COVID-19 pandemic. Most of the sports training centers were all closed as part of a lockdown to control the spread of the virus. Hence, subjects performed alternative sport activities at home or outdoors where possible to maintain consistency during the period of the study. These changes were considered as minor deviations. Another limitation of the study is, we observed prominent changes in few parameters in subgroup analysis for the Undenatured Collagen group, however, it was still realized that the overall time and product effect favored the Undenatured Collagen compared to the PLA group.

CONCLUSION

In short, future larger size studies are warranted to further strengthen the clinically significant outcomes seen in subgroup analysis based on age and gender. In conclusion, the results of this study suggest that a daily dose of 40 mg of UC-II® Undenatured Collagen supplementation may reduce joint discomfort during daily physical activities and supports joint mobility. The Undenatured Collagen supplementation improved daily step count which may help to enhance the daily activities and health related quality of life. However, a larger sample size and longer evaluation period is necessary for future studies.

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