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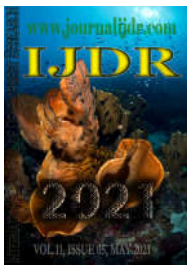

RESEARCH ARTICLE OPEN ACCESS

EFFECTS OF ADMINISTRATION OF UNNATURED COLLAGEN TYPE II (UC-II) IN KNEE OSTEOARTHRITIS: A PLACEBO-CONTROLLED, DOUBLE-BLIND RANDOMIZED CLINICAL TRIAL

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ABSTRACT

Purpose: Approximately 25% of people 55 and older have had knee pain, and about half of them have symptomatic knee osteoarthritis (OA). A new nutraceutical, undenatured collagen II (UC-II), has been studied in knee OA patients. The objective was to evaluate the effect of using undenatured type II collagen in subjects with knee OA. **Methods:** A double-blind, placebo-controlled, randomized controlled trial with 40 patients with grade I-III knee OA, randomized into collagen group (CG) and placebo collagen group (PCG). Pain, quality of life, functional capacity, muscle strength, flexibility, and joint mobility were evaluated at pre- and post-intervention. UC-II® was administered as a single daily oral dose of 40 mg for 90 days, as was placebo. **Results:** Pain decreased significantly at the end of the protocol in the CG. Bilateral quadriceps muscle strength was significantly higher in the CG participants in all reevaluations. Active and passive mobility of right knee flexion and extension increased in the CG. The CG showed greater functional gain than the PCG test at 30 days and at follow-up. WOMAC and Lequesne scores decreased significantly in all evaluations for CG compared to PCG. **Conclusions:** Administration of UC-II® improved pain, mobility, and function in participants with knee OA.

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INTRODUÇÃO

Knee osteoarthritis (OA) is a highly prevalent condition among adults, characterized by progressive destruction of the cartilage that covers knee joints, subchondral bone, and synovium. Symptoms include pain, immobility, muscle weakness, reduced function, and inability to perform daily life activities (Cibulka *et al.,* 2017) (Collins *et al*., 2018) (Cross *et al*., 2014) (Liu *et al*., 2018) (McKay *et al*., 2019). Mechanical factors for the development of knee OA include abnormal joint congruence, joint misalignment, meniscal injuries, and other joint injuries (Li *et al*., 2016) (McKay *et al*., 2019) Mills *et al*., 2019) (Steim *et al*., 2010). Diagnosis is made from signs and symptoms, and confirmation is made by radiological examination (Steim *et al*., 2010). Accurate diagnosis and prompt intervention are essential to minimize the consequences of knee OA and slow the progression of this pathology (Pereira *et al*., 2013) (Quicke *et al*., 2017) (Steim *et al*., 2010). Knee OA treatment includes using commonly recommended nonpharmacological and pharmacological therapies to reduce pain in patients, preventing reductions in functional capacity, and maintaining or increasing joint mobility (Levy *et al*., 2018). Nonsteroidal anti-inflammatory drugs are other agents commonly prescribed for this condition, but its administration often causes severe gastrointestinal and cardiovascular adverse events (Charleswortg *et al*., 2019) (Levy *et al*., 2018). The dominant collagen in articular cartilage is type II collagen, which is associated with smaller amounts of other collagen (Bagi *et al*., 2017) (Hunter *et al*., 2016) (Quicke *et al*., 2017). As collagen is the most prevalent

component of the solid matrix of articular cartilage, its supplementation has been considered a key treatment option to prevent progressive cartilage damage over time and to accelerate the healing process after OA onset (Crowley *et al*., 2009) (Gao *et al*., 2016) (Katz *et al*., 2018) (Lugo *et al*., 2016). Moreover, dietary compounds known as nutraceuticals have emerged with a supporting role in balancing anabolic and catabolic signals within the articular cartilage (Bakilan *et al*., 2016). In recent years, a new nutraceutical type, taken from chicken sternal cartilage, has been studied in patients with knee OA (Bagi *et al*., 2017). It is the undenatured type II collagen (UC-II) (Bagi *et al*., 2017). Orally administered undenatured type II collagen antigens interact with Peyer's plaques in gut-associated lymphoid tissue, preventing T-cell attack to the structural protein type II collagen in the cartilage (Gupta *et al*., 2009). This desensitization process in Peyer's plaques, also known as oral tolerance, prevents endogenous type II collagen in the cartilage to be recognized as an antigen by the immune system (Gupta *et al*., 2009). Considering this mechanism of action, UC-II may have positive effects on inflammation and deterioration of joint diseases (Gupta *et al*., 2009). The present study evaluates the effect of using undenatured type II collagen (UC-II) in subjects with knee OA.

MATERIALS AND METHODS

A placebo-controlled, double-blind, randomized clinical trial was performed with 40 patients with grade I-III knee OA according to the Kelgren Lawrence radiological classification, from April 2015 to May 2019.

Evaluation Protocol: Clinical and radiological diagnosis of knee OA was made by orthopedic doctors from the orthopedics and traumatology service at a regional hospital. Once the inclusion criteria were met, patients were initially invited to sign the Informed Consent Form (Table 1). After agreeing to participate in the study, subjects were randomized by an independent researcher and referred for a functional physical evaluation performed by a blind evaluator previously trained for that purpose. He was unaware of the intervention group to which the participant belonged. Evaluations were performed at four different times within the study period. The initial evaluation occurred prior to the beginning of the therapeutic program. The second evaluation occurred after 30 days of intervention. The third evaluation occurred at 90 days, that is, after the intervention protocol ended. The fourth and last evaluation occurred six months after the intervention protocol ended. The same evaluator was responsible for performing all evaluations during the study. Initially, body mass index (BMI) was verified by measuring body weight and height. The Visual Analogue Pain Scale (VAS) was used to assess pain. Knee joint mobility was assessed using a Carci® goniometer for passive and active flexion and extension of the involved knee. Muscle strength was assessed by manual dynamometry. Maximal voluntary isometric contraction (MVIC) of the hamstrings and quadriceps of both knees was measured in kilograms (kg) using a Chataanooga® manual push-pull dynamometer. Functional capacity was assessed using the Timed Up & Go (TUG) test and the 6-Minute Walk Test (6MWT). Quality of life and functionality were assessed using the Lequesne scale and the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) questionnaire for knee osteoarthritis.

Sample Calculation: Pain was considered as the primary outcome of the study for sample calculation. Based on the study by Crowley et al. (2009), we estimated a mean initial pain of 100% in the study participants. At 90 days, mean final pain was 60% for subjects of the intervention groups, and 85% for those of the placebo groups. Using a study power of 80%, a significance level of 95%, and a sample size ratio of 1:1 for all groups, we reached the estimated number of 13 subjects for each intervention group. Believing that losses and refusals would be around 50%, we reached the initial number of 20 subjects for each of the study groups totaling 40 subjects.

Randomization: Randomization was performed by an independent researcher, that is, who did not participate in the intervention and evaluation protocols. He was responsible for providing UC-II to the researcher who would administer it to the participant. Randomization was performed through a list of random numbers provided by the EPI-Info® software. Patients were randomly divided into a collagen group (CG), with 20 participants continuously treated with UC-II®, which received posture and exercise guidance, in addition to standard analgesic and anti-inflammatory medication; and a placebo collagen group (PCG), with 20 participants continuously treated with placebo UC-II®, which also received posture and exercise guidance, and standard analgesic and anti-inflammatory medication.

Ethical Aspects: This research has been approved by the IRB of the authors affiliated institutions.

Intervention Protocol with UC-II: Both active and placebo UC-II® interventions were administered for 90 days. Each participant received a vial containing 30 capsules at different times, every 30 (thirty) days. Each UC-II® capsule contained 40 mg UC-II® standardized to 10 mg bioactive undenatured type II collagen, identified by specific registration numbers to differentiate them from placebos. Participants in both groups were instructed to take one UC-II® capsule at breakfast, for a daily dose of 40 mg UC-II®. The dose of 40 mg UC-II® is justified based on the efficacy shown in previous studies (Bakilan *et al*., 2016) (Mahmoudian *et al*., 2017). Patients in the placebo group received cornstarch capsules. Participants showing adverse effects or pregnancy would be removed from the UC-II® study group. However, no adverse event occurred in the sample studied. Participants were followed through monthly visits by the researchers and were asked about their difficulties and problems. Both the study participant and the researcher who administered the supplement were not aware of which compound was assigned to each participant, only the researcher responsible for randomization had such knowledge.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) version 23.0 was used for statistical analysis. Data were expressed as mean, median, and standard deviation. Afterwards, the normality of data distribution was analyzed using the Shapiro-Wilk test. For comparative analyses between CG and PCG, parametric data were statistically analyzed by paired Student's t-test for intragroup analysis and unpaired Student's t-test for intergroup analysis, both before and after exercise protocols. For nonparametric variables, Wilcoxon tests were used within each group, and Mann-Whitney tests were used for intergroup analysis. For intragroup analysis, we used analysis of variance for repeated measures for parametric variables, and Fisher's test for nonparametric variables. The significance level established for all statistical tests was $p<0.05$.

RESULTS

Sixty-four (64) patients were initially selected for the study. Of these, 24 participants were excluded prior to randomization because they did not meet the eligibility criteria. All 40 participants completed the 30-day evaluation. Only one participant from the PCG dropped out of the study at the 90-day evaluation. In the follow-up evaluation, one participant from the CG was not found and two from the PCG underwent surgery, one due to a patella fracture after a bicycle fall, and another who underwent meniscectomy. The final sample thus comprised 36 participants (Figure 1). Both groups were homogeneous for anthropometric characteristics, OA grade classification, pain duration, and affected knee (Table 2). Both groups significantly reduced pain after the intervention protocol. In the CG, pain decreased from 5.60 ± 2.35 points at baseline to 2.68 ± 2.44 points at 90 days ($p=0.009$). In the PCG, in turn, pain decreased from 5.30 ± 2.36 points to 4.05 ± 2.92 points ($p=0.045$) (Figure 2). No significant changes were observed in muscle strength in any intervention group.

Table 1. Elegibility Criteria

Inclusion Criteria	• Men and women aged 40 to 75 years, with unilateral and/or bilateral grade I-III knee OA (according to the Kelgren and Lawrence radiological classification) for more than three months, confirmed by radiographic examination and clinical examination by an orthopedic doctor;
	• Ambulatory subjects;
	• Lequesne functional score greater than 4.5;
	• Availability during the study period (six months);
	• Not participating in any other knee treatments (physical/drug) during the study period;
	• Having signed the Informed Consent Form.
Exclusion Criteria	• Patients with OA greater than IV Kelgren and Lawrence classification) confirmed by an orthopedist;
	• Nonambulatory patients;
	• History of inflammatory arthropathy, septic arthritis, rheumatoid arthritis, inflammatory joint disease, uric gout, joint fracture, fibromyalgia, collagen genetic disease, neurological disorders, cognitive deficits, history of asthma, type I or II diabetes, psychiatric disorders, and symptomatic heart disease;
	• Clinical manifestations preventing the exercise to be performed.
	• Presence of skin injury;
	• Skin disease in the thigh region;
	• Urinary incontinence;
	• Pregnancy;
	• Alcoholism;
	• Cancer;
	• History of corticosteroid joint infiltration or viscosupplementation in the knee within the last three months;
	• History of knee trauma or surgery in the last six months;
	• Having three consecutive unjustified absences to physical rehabilitation.

Table 2. Characterization of the initial study sample (n=40)

	Intervention Group		
Variable	**CG (n=20)**	**PCG (n=20)**	**p value**
Age, years (mean ± SD)	60.25 ± 7.45	57.60 ± 6.96	0.252
Gender, M/F	7/13	6/14	0.736
Skin color, n (%)			0.311
White	19 (95.0)	20 (100.0)	
Black	1 (5.0)	0 (0.0)	
Occupation			0.718
Retired	5 (25.0)	8 (40.0)	
Housekeeper	4 (20.0)	4 (20.0)	
Government employee	2 (10.0)	2 (10.0)	
Professor	3 (15.0)	1 (5.0)	
Other	6 (30.0)	5 (25.0)	
Kellgren Lawrence, n (%)			0.405
Grade I	2 (10.0)	4 (20.0)	
Grade II	11 (55.0)	7 (35.0)	
Grade III	7 (35.0)	9 (45.0)	
Affected Knee, n (%)			0.481
Right	5 (31.3)	5 (41.7)	
Left	4 (25.0)	2 (16.6)	
Bilateral	7 (43.8)	5 (41.7)	
Cigarette use, n (%)			0.292
Yes	3 (15.0)	1 (5.0)	
No	17 (85.0)	19 (95.0)	
Time of pain, years (mean ± SD)	7.71 ± 9.55	6.06 ± 5.42	0.507
Weight, kg (mean ± SD)	76.33 ± 14.61	80.86± 10.55	0.268
Height, cm (mean ± SD)	166.85 ± 10.45	163.80 ± 8.33	0.314
BMI, kg / cm^2 (mean ± SD)	26.95 ± 3.84	30.01 ± 4.47	0.026

PCG = placebo collagen group; SD = standard deviation; % = percentage; M / F = male / female; KG = kilograms; cm^2 = square centimeters. CG = collagen group;

Notwithstanding, the CG presented a significantly higher level of right and left quadriceps strength compared to the PCG in the 30-day evaluation. This difference remained in the 90-day and follow-up evaluations ($p<0.05$) (Table 3). Active and passive range of motion (ROM) increased significant only in the CG. Regarding active ROM for the CG, there was an increase in right knee flexion in the follow-up evaluation, and in active extension of the right knee at 30 and 90 days and in the follow-up evaluation ($p<0.05$). Regarding passive ROM, there was an increase in right knee extension in all evaluations ($p<0.05$). The CG showed a better active and passive extension compared to the PCG at the end of the study (90 days) and in the follow-up evaluation ($p<0.05$) (Table 4). The CG group had a significantly shorter TUG execution time in the 30-day and follow-up evaluations. The initial score was 8.88 ± 2.60 seconds in the CG, and 11.28 ± 4.99 seconds in the PCG ($p=0.065$). After 30 days, the CG completed the test in 8.03 ± 2.90 seconds, and the PCG completed it in 10.30 ± 3.55 seconds ($p=0.033$). In the six-month follow-up evaluation, the CG performed the test in 8.01 ± 2.61 seconds, and the PCG in 11.04 ± 4.34 seconds ($p=0.015$) (Figure 3). The CG showed a significant increase in the 6MWT distance in the follow-up evaluation, with a value significantly higher than the PCG ($p=0.027$). The distance covered initially by the CG was 325.40 ± 71.70 meters, increasing to 371.0 ± 70.6 meters in the follow-up evaluation ($p=0.05$). The PCG traveled a distance of 308.23 ± 89.67 meters in the initial evaluation, and 309.60 ± 88.80 meters in the follow-up evaluation ($p=1.00$) (Figure 4). Functionality and quality of life, assessed through the Lequesne Algofunctional Questionnaire, significantly decreased in the CG compared to the PCG at all times of the study (30 days, 90 days, and six months after the intervention) ($p<0.05$). The CG reduced the initial score from 9.60 ± 2.60 points to 4.50 ± 2.80 points in the 30-day evaluation ($p=0.0001$). In the 90-day and follow-up evaluations, the score remained at 4.00 ± 3.30 points

Table 3. Results of muscle strength (MVIC) in the initial study groups, 30 days, 90 days, and six months after UC-II administration

Variable	CG	PCG	p value
Right quadriceps, kg ± sd			
Initial	24.25 ± 10.12	19.75 ± 10.59	0.178
30 days	27.35 ± 9.97	21.00 ± 9.45	0.046
90 days	27.80 ± 11.03	20.53 ± 9.17	0.032
Follow-up	27.05 ± 11.16	20.12 ± 9.71	0.050
Left quadriceps, kg ± sd			
Initial	25.35 ± 12.41	21.18 ± 11.25	0.273
30 days	28.60 ± 12.42	21.65 ± 9.40	0.050
90 days	29.73 ± 13.43	21.63 ± 11.24	0.049
Follow-up	29.21 ± 13.09	21.71 ± 10.04	0.064
Right hamstrings, kg ± sd			
Initial	12.55 ± 5.65	10.71 ± 4.29	0.255
30 days	14.58 ± 6.16	12.15 ± 5.65	0.183
90 days	14.92 ± 5.51	12.05 ± 4.66	0.089
Follow-up	13.32 ± 5.86	11.17 ± 4.19	0.221
Left hamstrings, kg ± sd			
Initial	12.35 ± 5.88	11.05 ± 5.33	0.468
30 days	14.68 ± 5.94	12.70 ± 5.65	0.292
90 days	15.25 ± 5.96	12.37 ± 4.93	0.109
Follow-up	13.68 ± 6.26	11.29 ± 3.84	0.183

CG = collagen group; PCG = placebo collagen group; kg = kilograms.

Table 4. Results of active and passive knee joint range of motion (ROM) in the initial study groups, 30 days, 90 days, and six months after UC-II administration

Variable	CG	PCG	p value
Right active flexion, degrees (mean ± SD)			
Initial	125.50 ± 10.64	116.90 ± 20.62	0.106
30 days	129.20 ± 10.87	122.65 ± 12.18	0.081
90 days	128.70 ± 12.68	124.26 ± 10.72	0.247
Follow-up	130.21 ± 8.06	123.18 ± 12.23	0.047
Left active flexion, degrees (mean ± SD)			
Initial	124.45 ± 9.68	120.30 ± 24.55	0.486
30 days	128.00 ± 12.16	125.00 ± 12.68	0.450
90 days	128.50 ± 13.63	127.53 ± 9.79	0.800
Follow-up	130.05 ± 8.38#	126.23 ± 9.62	0.212
Right passive flexion, degrees (mean ± SD)			
Initial	130.90 ± 10.08	124.75 ± 16.97	0.172
30 days	134.15 ± 7.99	131.20 ± 11.38	0.200
90 days	134.45 ± 11.04	132.00 ± 10.54	0.483
Follow-up	136.74 ± 8.52	131.94 ± 12.37	0.181
Left passive flexion, degrees (mean ± SD)			
Initial	129.85 ± 9.48	129.65 ± 16.97	0.785
30 days	131.45 ± 9.22	131.20 ± 11.39	0.940
90 days	132.60 ± 11.40	134.37 ± 9.01	0.595
Follow-up	134.32 ± 9.29	134.06 ± 9.77	0.936
Right active extension, degrees (mean ± SD)			
Initial	- 1.65 ± 3.55	- 5.40 ± 10.86	0.150
30 days	0.00 ± 0.00	- 2.25 ± 6.18	0.111
90 days	0.00 ± 0.00	- 5.21 ± 11.97	0.050
Follow-up	0.00 ± 0.00	- 4.65 ± 8.38	0.021
Left active extension, degrees (mean ± SD)			
Initial	- 1.10 ± 2.81	- 2.50 ± 5.50	0.317
30 days	0.00 ± 0.00	0.00 ± 0.00	0.200
90 days	0.00 ± 0.00	- 0.21 ± 0.92	0.311
Follow-up	0.00 ± 0.00	- 0.24 ± 0.97	0.297
Right passive extension, degrees (mean ± SD)			
Initial	- 1.50 ± 3.28	- 3.20 ± 6.62	0.310
30 days	0.00 ± 0.00	- 2.25 ± 6.17	0.111
90 days	0.00 ± 0.00	- 4.21 ± 9.47	0.050
Follow-up	0.00 ± 0.00	- 4.12 ± 7.75	0.027
Left passive extension, degrees (mean ± SD)			
Initial	- 0.60 ± 1.57	- 1.05 ± 3.09	0.564
30 days	0.00 ± 0.00	0.00 ± 0.00	1.000
90 days	0.00 ± 0.00	0.00 ± 0.00	1.000
Follow-up	0.00 ± 0.00	0.00 ± 0.00	1.000

CG = collagen group; PCG = placebo collagen group.
# p <0.05 compared to the initial assessment of the same group.

(p=0.0001) and 4.70 ± 4.10 points, respectively (p=0.0001). The PCG showed an initial score of 10.70 ± 5.00 points, which decreased to 8.70 ± 5.40 points after 30 days of nutraceutical intervention (p=0.40) and 8.50 ± 5.20 points in the 90-day evaluation (p=0.36), with 9.40 ± 6.00 points after a six-month follow-up (p=1.00) (Figure 5).

The WOMAC Questionnaire score decreased more significantly for the CG compared to the PCG in the 30-day, 90-day, and six-month evaluations (p<0.05). The CG presented an initial score of 34.90 ± 16.20 points. In the 30-day evaluation, the score decreased to 14.90 ± 10.80 points (p=0.0001). In the 90-day evaluation, the score

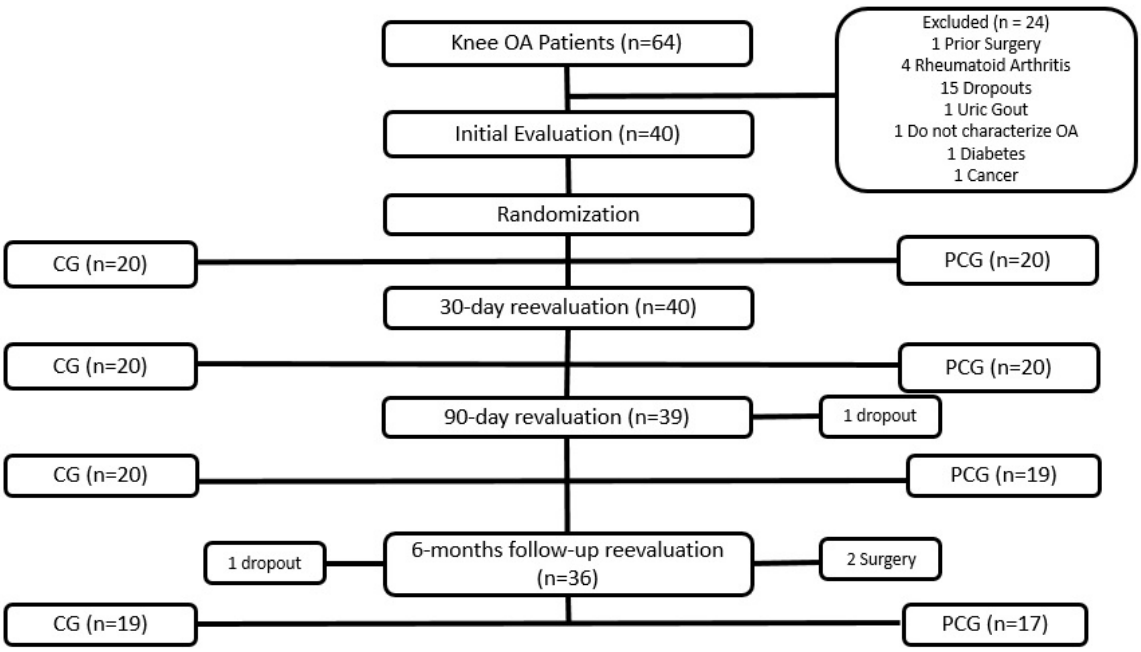

Figure 1. Study flowchart

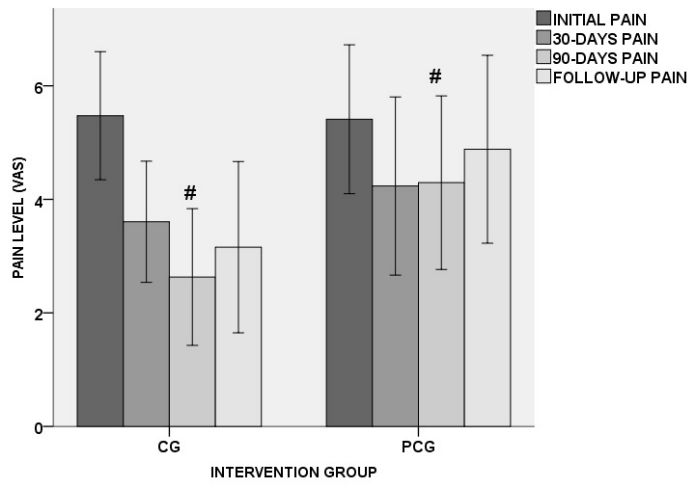

Figure 2. Assessment of pain level (VAS) in the study groups. One-way ANOVA.
 # p <0.05 compared to the initial assessment of the same group.
$ p <0.005 compared to the initial assessment of the same group.

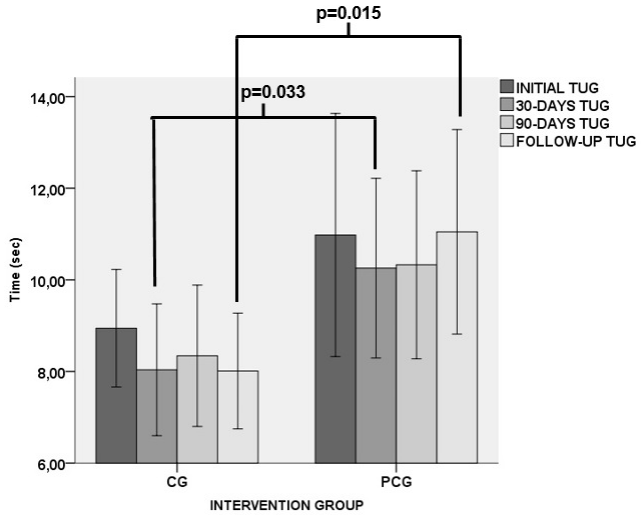

Figure 3. Timed Up & Go (TUG) test evaluation in study groups

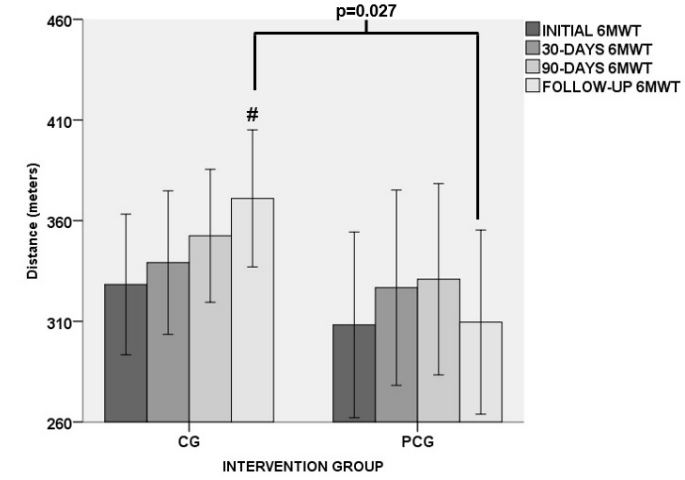

Figure 4. Distance obtained in the 6-Minute Walk Test (6MWT) in the study groups. One-way ANOVA. # p <0.05 compared to the initial assessment of the same group

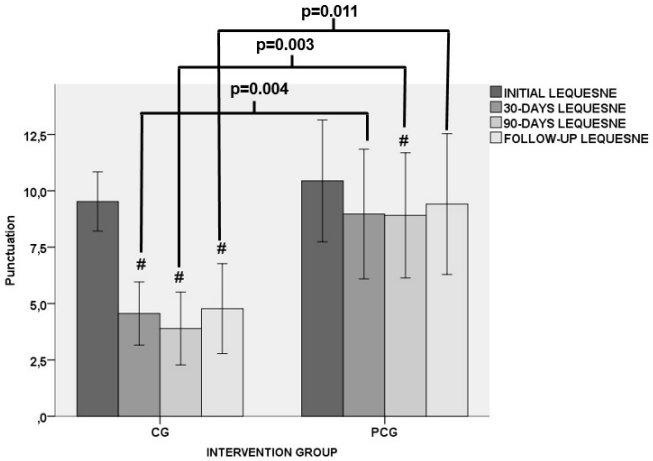

Figure 5. Lequesne Questionnaire score in the various study groups. # p <0.05 compared to the initial assessment of the same group. ANOVA for repeated measurements

decreased to 12.40 ± 12.40 points. Six months after the intervention, the score was 15.80 ± 15.80 points (p=0.001). The PCG showed an initial score of 34.90 ± 20.60 points, decreasing to 28.70 ± 19.10 points in the 30-day evaluation (p=0.700), and 26.00 ± 18.50 points in the 90-day evaluation (p=0.039). In the follow-up evaluation, the score was 29.7 ± 20.1 points (p=1.000) (Figure 6).

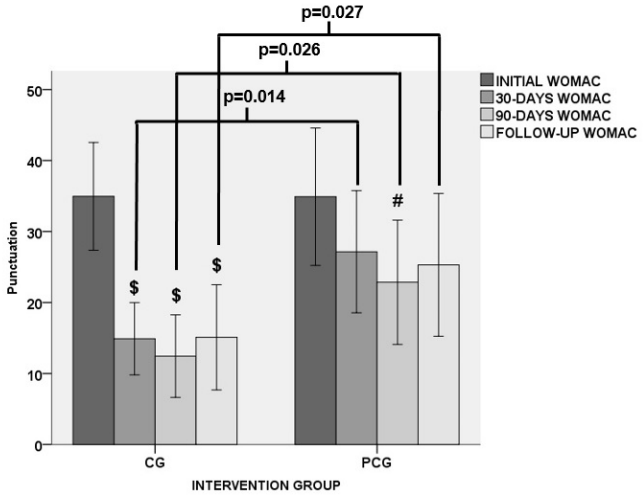

Figure 6. WOMAC Questionnaire score in the various study groups
 # p <0.05 compared to the initial assessment of the same group
$ p <0.001 compared to the initial assessment of the same group

DISCUSSION

This study investigated the therapeutic effect of nutraceutical UC-II® on symptomatology, joint mobility, muscle strength, and knee joint function in knee OA patients. Administration of UC-II® at a dose of 40 mg/day for 90 days significantly decreased joint pain at the end of the protocol. However, there was also an analgesic effect on the PCG at the end of the protocol, although less expressive than in the CG. Liu *et al.* (2018) concluded in their review that five supplements, including UC-II, showed unclear effects of clinical importance, although statistically significant. Supplementation has not shown to have clinically significant effects on medium to long-term pain, except for green-lipped mussel extract and UC-II, which have demonstrated clinically significant effects on medium-term pain reduction (Liu *et al.*, 2018). Several mechanisms probably contribute to the effectiveness of UC-II, including anti-inflammatory effects, pain reduction, preservation of mechanical function and bone quality, and supply of cartilage repair material (Scarpellini *et al*., 2008). Given the different and potentially complementary mechanism of action of UC-II, in addition to the available evidence supporting its safety and efficacy, it can be considered as a rational synergistic supplement. This collagen can be used at the dose of 40 mg per day as a second- or third-line agent as an adjunct to the fundamental protocol of nutritional intervention for OA (Bakilan *et al*., 2016). A potential mechanism of action of UC-II is likely to be mediated by T-cell reeducation regarding oral tolerance, which results in humoral and cellular immunomodulation (Bakilan *et al*., 2016).

Regulatory T cells secrete immunomodulatory cytokines such as IL-10 and TGF-β that inhibit antigen (type-II collagen) immune responses. This may decrease targeted amplification of the immune response to type-II collagen within the cartilage extracellular matrix, thus preventing exaggerated proinflammatory reaction of the immune system to articular cartilage under arthritic conditions (Bakilan *et al*., 2016). In the case of OA, characterized by a subclinical immune disorder and a vicious cycle of inflammatory events, UC-II may significantly decrease inflammation (Liu *et al*., 2018). Peyer's plaques filter compounds in the small intestine and serve as a switch to activate or deactivate the body's immune response (Liu *et al*., 2018). In a study with humans, UC-II has been shown to be able to turn off the targeted immune response, specifically to articular cartilage type-II collagen, without demonstrating adverse effects (Liu *et al*., 2018). In addition to the current understanding of the role of various cytokines in normal joint physiology, these findings made it possible to hypothesize that UC-II supplementation could alleviate joint discomfort and restore joint function in healthy individuals (Liu *et al*., 2018). Scarpellini *et al.* (2008) evaluated the anti-inflammatory effect of various nutraceuticals on synovial cells and observed mild or moderate suppression of IL-1β stimulated IL-8 production by MH7A cells. At the dose of 50 μg/ml, UC-II further decreased the level of IL-8 already reduced by glucosamine (Scarpellini *et al*., 2008). Results indicated that UC-II and other nutraceuticals mildly or moderately suppressed IL-1β-stimulated IL-8 production by MH7A cells. These observations suggested that these nutraceuticals exert an anti-inflammatory action (inhibition of IL-8 production) (Scarpellini *et al*., 2008).

The CG showed a significantly greater gain in active right knee flexion and extension ROM compared to the PCG at the end of the study and six months after the intervention. We also found that the CG improved left knee flexion compared to baseline. Our findings corroborate those of Lugo *et al.* (2016), in which the UC-II supplemented cohort showed a statistically significant increase in knee extension on day 120 compared to baseline and to the placebo group. Bagi *et al.* (2017) demonstrated that a 40 mg/day dose of UC-II as administered in this study, showed improved flexibility and reduced pain in arthritis patients. There was a significant difference in right and left quadriceps muscle strength in CG compared to PCG from the 30-day evaluation. Onset of muscle weakness and impaired proprioception in patients with knee OA results in limitation of daily activities, leading to worsening of postural control and predisposition to increased risk of falls (Mahmoudian *et al*., 2017) (Spinoso *et al*., 2018). Thus, one can think of muscle weakness as a result of OA and as a risk factor for its occurrence, being more than a pain responsible for biomechanical compensation (Waller *et al*., 2017). Quadriceps muscle strength is closely related to the symptoms of knee OA (Berger, 2014). Moreover, quadriceps weakness in knee OA results in reduced shock absorption capacity and joint instability further impairing neuromuscular joint control (Berger, 2014). In addition to quadriceps strength, other areas such as joint instability, functional performance, and sensorimotor deficiencies should also be focused (Berger, 2014). Improved strength in the CG corroborates the findings of Deparler *et al.* (2005), who noticed an improvement in thrust force in arthritic dogs after 90 and 150 days of UC-II treatment at 10 mg/day (Deparler *et al*., 2005). Similarly to our study, the authors observed significant pain reduction, which can be related to increased strength, since patients with less pain are more functional (Deparler *et al*., 2005). Regarding functionality, the CG showed a significant improvement compared to the PCG.

The first group scored better on the TUG test from 30 days, and on the 6MWT six months after the intervention. In the study by Lugo *et al.* (2016), the distance covered in six minutes by the group of healthy people using UC-II ranged from 505 to 522 meters. In our study, the mean distance initially traveled by the CG ranged from 293 to 363 meters, that is, below the values considered normal. Notwithstanding, after six months of UC-II administration, the distance covered ranged from 337 to 405 meters, i.e., within the values considered acceptable to the general population. Functional decline is known to be immediately noticeable in the early stages of OA, and some authors have stated that pain is largely responsible for poor performance (Resende *et al*., 2016). However, other authors argue that muscle weakness is the leading cause (Berger, 2014). The WOMAC and Lequesne functional questionnaires showed that the CG had significantly better scores than the PCG at 30 days of intervention, maintaining these better results with six months of follow-up compared to baseline. Some studies have shown the relationship between OA, pain, and functional worsening (Carlesso *et al*., 2017) (Resende *et al*., 2016). Our study has some limitations that make it difficult to extrapolate the results, such as limited sample size and six-month follow-up.

CONCLUSION

Administration of UC-II® has shown positive effects on pain improvement, knee mobility, and functionality in subjects with knee OA. However, the complex nature of OA probably requires simultaneous treatment with multiple lines of therapy to successfully treat the disease. Multimodal approach to treatment will depend on the severity and duration of OA, but should include ingredients such as UC-II, which has been shown to be safe and improve flexibility, joint pain, and overall bone and cartilage health.

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