Collagen peptides improve knee osteoarthritis in elderly women
A 6-month randomized, double-blind, placebo-controlled study

IAN-XIN JIANG[1], SHEN YU[1], QI-REN HUANG[2], XIAN-LONG ZHANG[1], CHANG-QING ZHANG[1], JIAN-LIE ZHOU[3]*, JANNE PRAWITT[4]*

*Corresponding authors

1. Department of Osteology, Shanghai 6th People's Hospital,
Jiaotong University, 600 Yishan Road, Shanghai 200233, PR China
2. Department of Osteoporosis, Shanghai 6th People's Hospital,
Jiaotong University, 600 Yishan Road, Shanghai 200233, PR China
3. Medical Consultant of Rousselot AP, 25/A,
18 North Cao Xi Road, Shanghai 200230, PR China
4. Rousselot BVBA, Meulestedekaai 81, B-9000 Gent, Belgium

***KEYWORDS**: Collagen peptides, osteoarthritis, joint pain, healthy ageing, and nutraceuticals*

***ABSTRACT**: As the global population gets older, joint-related health concerns are increasingly common, such as osteoarthritis causing pain and reducing mobility. Collagen peptides have been proposed as nutraceuticals to improve joint health in patients with osteoarthritis. We performed a prospective, randomized, double-blind, placebo-controlled study in elderly women with mild-to-moderate knee osteoarthritis and showed that the oral intake of collagen peptides for a duration of 6 months significantly reduces joint pain and improves physical mobility as assessed by two well-established scoring systems (WOMAC and Lysholm score). This study confirms that collagen peptides are a highly efficient nutraceutical to improve joint health which can help to maintain an active lifestyle throughout ageing.*

INTRODUCTION

Population ageing

Worldwide, the population is increasingly ageing, with a greater proportion of people getting old and old people reaching an even higher age than before. In 2009, 10% of the population were 60 years and older and this fraction is estimated to increase up to 20% by 2050 (1). This demographic development is associated with an increase in age-related diseases (2) concurrently building a strong case for the maintenance of health throughout ageing and the focus of interest of the pharmaceutical and nutraceutical industry.

Osteoarthritis

One age-related disease with rising prevalence is osteoarthritis with 10% of all men and 20% of all women over 60 years old already suffering from it today (3). Osteoarthritis is a degenerative disease of the articular cartilage in joints of the knee, hip, spine and hand. Pain, stiffness and locking of the joint are key symptoms reducing mobility and strongly impacting on the quality of life of the patient.

The hyaline cartilage of the joint consists mainly of extracellular matrix composed of collagen, proteoglycans (e.g. aggrecan) and glycosaminoglycans such as hyaluronic acid. Chondrocytes present in the cartilage maintain the

matrix in a finely-tuned turnover process balancing synthesis and breakdown. In osteoarthritis, a dysregulation of this balance leads to a shift towards degradation with a subsequent loss of cartilage. In addition to cartilage degradation, the inflammation of the lining surrounding the joint space, the synovium, as well as alterations in the bone underlying the joint cartilage, such as sclerosis and the formation of osteophytes, are involved in the pathological manifestation of osteoarthritis (4).

Currently, osteoarthritis cannot be cured and available treatment is mostly symptomatic. To treat pain, mainly analgesics and non-steroidal anti-inflammatory drugs (NSAIDs) are used, which at long-term or high-dose use may cause heavy side effects, such as gastrointestinal bleeding and cardiovascular disease (5, 6). Suggested as a safe alternative, the dietary supplements glucosamine and chondroitin sulfate have been used to treat osteoarthritis. However, a systematic, multi-centre study at a large scale did not find a general beneficial effect of glucosamine or chondroitin sulfate. Only a small subgroup of patients with moderate-to-severe pain significantly benefited from a combined treatment with glucosamine and chondroitin sulfate (7). Thus, the strong need for alternative symptom-modifying therapies has created a highly active field of research.

Collagen peptides

Collagen peptides are a specific mix of peptides of different length, obtained by the enzymatic hydrolysis of native collagen coming from animal connective tissues, with a high abundance of the amino acids hydroxyproline, glycine and proline. Used as a food ingredient, collagen peptides are proven to be safe (8) and to have a high bioavailability (9). They have been shown to exert a beneficial effect on bone and skin (10, 11) and have been proposed as a candidate therapy for osteoarthritis (12).

Studies in rodents have demonstrated that radio-actively labeled collagen peptides accumulate in cartilage upon ingestion (13, 14). Since collagen is the major protein component of the extracellular matrix in cartilage, collagen peptides have been suggested to stimulate the formation of cartilage by simply providing building blocks. In addition, collagen peptides have been shown to enhance the synthesis of collagen (15) and proteoglycans (16) in primary chondrocytes as well as the secretion of hyaluronic acid from synovial fibroblasts (17) and might thus be able to actively shift the balance of cartilage turnover in osteoarthritis towards net formation.

Indeed, several clinical studies have investigated the effect of collagen peptide treatment in individuals with joint discomfort or osteoarthritis, reporting different levels of joint pain improvement upon treatment (18-21) but with conflicting results on the effect on joint function (19 - 21). The aim of the present study was to assess the effect of collagen peptide ingestion on knee joint pain and function in patients with mild-to-moderate knee osteoarthritis.

STUDY DESIGN

A prospective, single-centre, randomized, double-blind, placebo-controlled trial was conducted between January and July 2012 at the 6th People's Hospital affiliated to Shanghai Jiaotong University, China. The study protocol was approved by the hospital's ethical committee which works according to the guidelines of Good Clinical Practice and the study was registered in the hospital's database (Clinical Trial Registration No. 2011-51). All participants gave their informed consent.

Patient recruitment, inclusion criteria and treatment

Hundred women between the age of 40 and 70 presenting themselves with knee joint pain or discomfort were recruited to participate in the study. This effect size assures, at a significance level of a=0.05, a power of 90% for WOMAC score, and of more than 80% for Lysholm score. Osteoarthritis was diagnosed by x-ray and quantified using the Kellgren-Lawrence x-ray classification (22). According to the guidelines the scores were defined based on the

following symptoms: grade I - doubtful narrowing of the joint space and possible osteophytes lipping; grade II - definite narrowing of the joint space and definite osteophytes; grade III - definite narrowing of the joint space, moderate multiple osteophytes, some sclerosis and possible deformation of the bone contour; grade IV - marked narrowing of the joint space, large osteophytes, severe sclerosis and definite deformation of the bone contour. Only subjects with a Kellgren-Lawrence score of 0-I to III (excluding stage IV of severe osteoarthritis), without allergies and with normal liver and kidney function were included, who had not used nutraceuticals or analgesics within the last 6 months. Patients were randomly assigned to receive a daily oral dose of 8g collagen peptides or 8g placebo for a duration of 6 months. **The administered collagen peptide was of bovine origin,** while maltodextrin was used as placebo. Compliance was defined as the percentage of those subjects who took the treatment in agreement with the protocol guidelines of all subjects designated to the respective treatment group.

Assessment of safety parameters and treatment efficacy

Patients were examined at baseline as well as 3 and 6 months after the start of the treatment. Blood and urine were sampled for the analysis of liver and kidney parameters by standard biochemical procedures. Joint pain and function were assessed using two well-established scoring systems based on standardized questionnaires, the WOMAC (23) and the Lysholm score (24). The primary endpoint of the study was defined as the difference of the WOMAC and Lysholm score between the placebo and the collagen peptide group after 6 months of treatment. The WOMAC score evaluates 24 parameters for pain, stiffness and physical function of the joint which are recorded on a visual analogue scale with a high score indicating more severe symptoms of osteoarthritis. The Lysholm score assesses 6 parameters for knee joint function (e.g. limping, stair climbing, running, jumping). A high score is associated with a better knee function.

Statistical analysis

All values are indicated as mean with standard deviation unless indicated otherwise. Statistical analysis was performed by means of Student’s T-Test , repeated measurement ANCOVA or Fisher’s exact test. Differences were considered significant when $p<0.05$.

RESULTS

Patient characterisation and adherence

The hundred osteoarthritis patients that entered the study were equally randomized to the two treatment groups, placebo or collagen peptides.

Over the course of the study, two patients dropped out of the placebo group (for reasons of non-adherence and lateral thigh pain), and four patients dropped out of the collagen peptide group (three had already taken collagen peptides before the study and one presented septic arthritis). As shown in Table 1, there were no significant differences at baseline between both groups regarding age, height, weight, body mass index (BMI), WOMAC score and Lysholm score.

Importantly, the level of osteoarthritis quantified using the Kellgren-Lawrence X-ray classification (Table 2) was distributed in a comparable manner without significant differences between the placebo and the collagen peptide group. Around half the patients of each group presented mild osteoarthritis in one or both knees (score I-II).

Treatment safety

At baseline as well as after 6 months of treatment, parameters of liver function (SGOT, SGPT) and kidney function

(blood urea nitrogen, serum creatinine, serum uric acid) were within the normal range for all patients with no significant differences between the placebo and the collagen peptide group neither at baseline nor after 6 months of treatment (Table 3). Compared to baseline, the improvement of liver parameters and blood urea nitrogen was significant in the collagen peptide group, even though the change from baseline was very small. Serum creatinine showed a small but significant increase from baseline in the placebo and to a lesser extent in the collagen peptide group. Overall, this result demonstrates that the treatment of osteoarthritis patients with 8g collagen peptides daily over a duration of 6 months is safe.

Treatment efficacy

The effect of the treatment on joint pain and function was evaluated by the WOMAC and the Lysholm scoring system. The WOMAC score is composed of subscale scores for pain, stiffness and physical function. As shown in Figure 1, the values of the WOMAC score decreased over time in patients treated with collagen peptides indicating a gradual improvement of joint pain and function. At 3 months of treatment, a small but already highly significant effect was visible (treatment difference of 0.002 in the placebo vs. -1.07 in the collagen peptide group, $p<0.001$) which developed into a pronounced and highly significant improvement of knee osteoarthritis after 6 months of treatment with collagen peptides (treatment difference of -0.77 in the placebo vs. -3.93 in the collagen peptide group, $p<0.001$). The difference between the WOMAC scores of the collagen peptide group and the placebo group after six months was significant for all three WOMAC subscales (Table 4), demonstrating an improvement in knee pain and stiffness as well as in physical function.

The Lysholm score which emphasizes on a comprehensive evaluation of joint function demonstrated an improvement over time by gradually increasing values in patients treated with collagen peptides (Figure 2). In line with the results of the WOMAC score a highly significant effect already detectable at 3 months of treatment (treatment difference of 0.25 in the placebo compared to 2.39 in the collagen peptide group, $p<0.001$) further increased into a clear and significant effect at 6 months of treatment (treatment difference of 0.77 in the placebo compared to 5.00 in the collagen peptide group, $p<0.001$). Thus the beneficial effect of collagen peptide treatment on joint pain and function in osteoarthritis patients could be shown independently by two different evaluation systems.

DISCUSSION

Collagen peptides are nutraceuticals used in dietary supplements and as a food ingredient offering health benefits at different levels. Their positive effect on skin physiology, increasing skin hydration and elasticity (11), stimulating synthesis of skin matrix components (25, 26) and decreasing skin collagen fragmentation (Rousselot, unpublished data), is well documented. Several *in vivo* studies have further reported a positive impact of collagen peptides on bone formation, resulting in increased bone strength and bone mineral density helping to reduce osteoporosis (10, 27, 28). Lately, collagen peptides have been vividly discussed as a symptom-modifying agent for osteoarthritis (12). Based on their application in functional foods as a bioactive ingredient they are thought to act at a rather early stage of the disease helping to prevent or delay the manifestation of osteoarthritis.

We performed a randomized, double-blind, placebo-controlled trial in elderly women to evaluate the effect of collagen peptides on the symptoms of knee osteoarthritis. Since the variability of results in other clinical trials has been attributed to the investigation of rather heterogeneous patient cohorts, we recruited only women within a defined age and BMI range, who presented with mild knee osteoarthritis. The relative homogeneity of the study groups was confirmed by the fact that over 50% of all subjects in each group presented a rather low score of I-II on the Kellgren-Lawrence scale, indicating mild osteoarthritis.

In the present study, the administration of collagen peptides at a dose of 8g/d was highly efficient to decrease joint

pain and stiffness and to improve joint function in comparison to the placebo, with a significant effect already observed after 3 months of treatment. The improvement of pain evaluated with the help of the WOMAC score is in line with the results of several other clinical studies. Benito-Ruiz *et al*. observed a decrease in pain according to two different pain scales (VAS and WOMAC) in a gender-mixed cohort with mild knee osteoarthritis (19). Another trial showed that collagen peptides performed even better than glucosamine in reducing pain in osteoarthritis patients as early as 3 months after the treatment start (20).

In addition to the WOMAC score, we used the Lysholm scoring system for the assessment of joint function. The Lysholm score has been developed specifically to evaluate knee function, by integrating information on limping, stair climbing, locking, giving way of the knee during activity and the ability to squat the joint. Corresponding to the results of the WOMAC rating, the Lysholm score significantly improved over the study duration in patients treated with collagen peptides in comparison to the placebo group. This finding is remarkable since only one other study has described such an effect (20). Two other trials assessed joint function by WOMAC or Quality of Life scores but did not find an effect of collagen peptide treatment (19, 21). However, both studies used a much more heterogeneous group of subjects presenting with general joint pain, not diagnosed osteoarthritis, and performed a combined evaluation of different joints (knee, hip, spine, etc.), which might explain why no effect on joint function was observed.

The presented data provide strong evidence for the symptom-relieving effect of collagen peptides in knee osteoarthritis, but the study design does not allow to conclude on potential mechanisms of action. A recent study has investigated the effect of collagen peptides on joint structure in a small group of patients using a MRI technique which can visualise cartilage (29). The result suggests that collagen peptides increase the proteoglycan content in knee cartilage after 6 months of treatment, which is consistent with the in vitro data showing a stimulation of extracellular matrix synthesis by collagen peptides (15, 16).

Even if the differences observed after 6 months are highly significant in the current study, more investigations should be initiated in future to confirm the efficacy of collagen peptides as a protective factor of cartilage in randomized, placebo controlled clinical studies of bigger scale, and with diverse patient characteristics to overcome the current study's limitations of sex, ethnicity of the subjects and the cause and location of osteoarthritis. In addition, mechanistic and biochemical parameters (e.g. MRI, uCTX-I, uCTX-2) could be assessed, and efforts could be made to investigate the potential differences between collagen peptide products from different sources and different production processes.

The present study demonstrates a clear beneficial effect of collagen peptide treatment on joint pain and function in patients with mild knee osteoarthritis. Their safety record and demonstrated absence of side effects make collagen peptides a valuable alternative symptom-modifying treatment for osteoarthritis. Thus, they present a highly useful nutraceutical to help maintain the quality of life during ageing.

ACKLOWLEDGMENTS

The authors thank Hongshan Tan (Department of Occupational and Environmental Medicine, School of Public Health, Fudan University, Shanghai, PR China) for his support of statistical analysis.

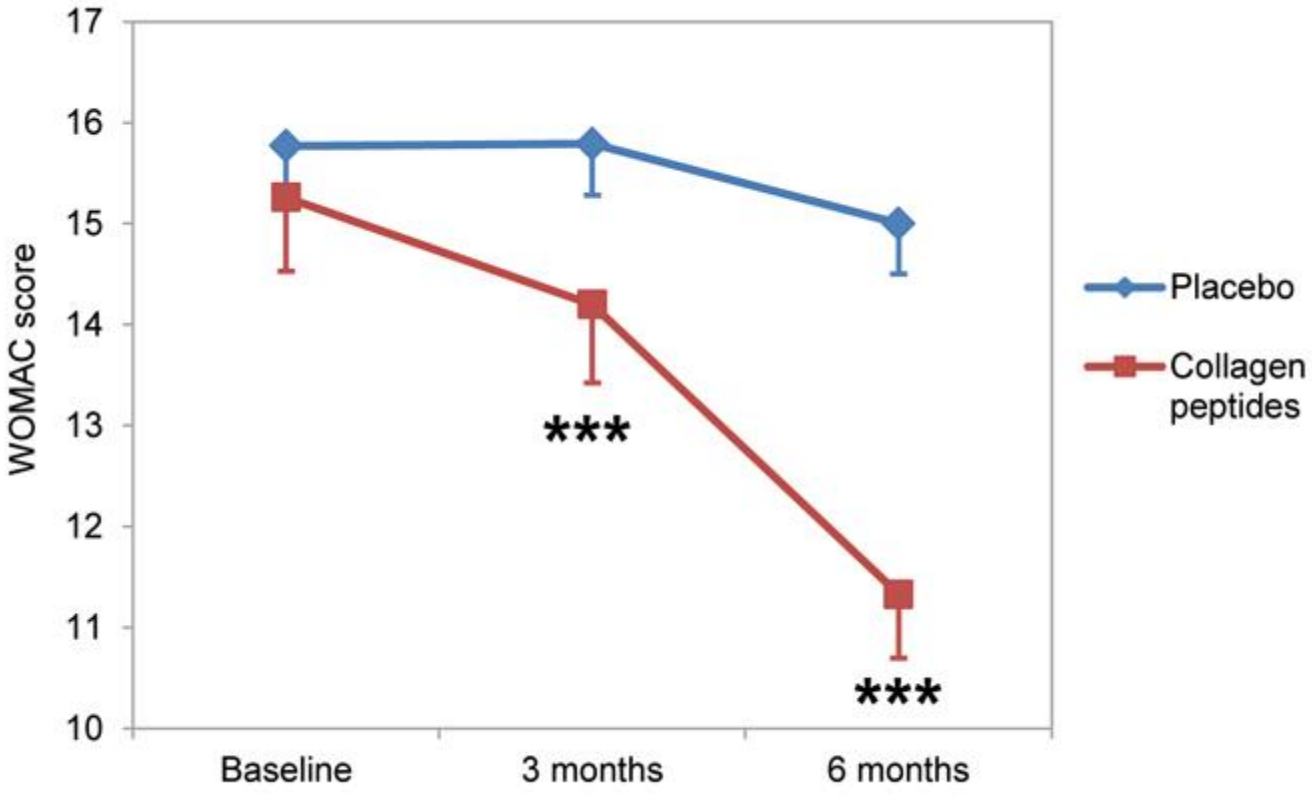

*Figure 1. Effect of collagen peptide treatment on osteoarthritis assessed by the WOMAC score. Score values at baseline, after 3 months and 6 months of treatment are presented as mean ± standard error mean. Statistical significance of differences was calculated by ANCOVA. A low WOMAC score indicates a low degree of osteoarthritis. *** p<0.001.*

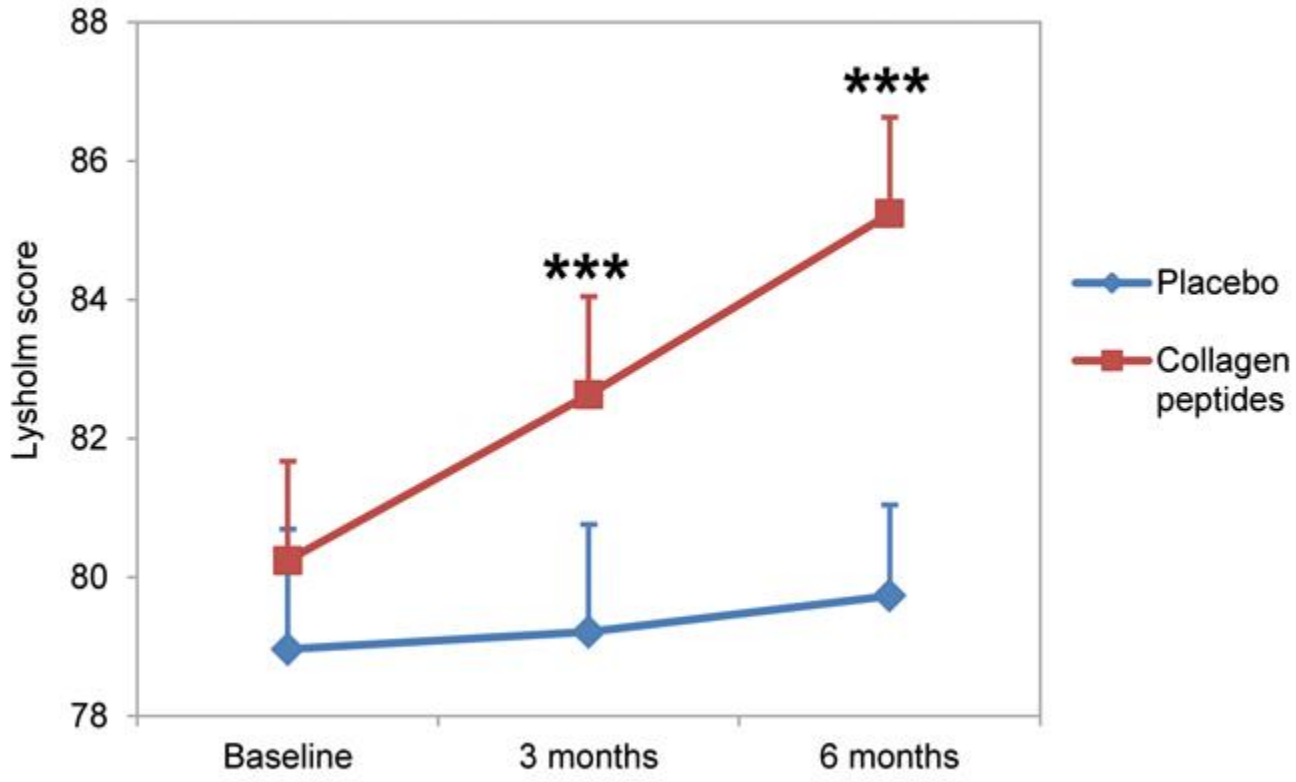

*Figure 2. Effect of collagen peptide treatment on osteoarthritis assessed by the Lysholm score. Score values at baseline, after 3 months and 6 months of treatment are presented as mean ± standard error mean. Statistical significance of differences was calculated by ANCOVA. A high Lysholm score indicates a low degree of osteoarthritis. *** p<0.001.*

	Total n=94	Placebo n=48	Collagen peptides n=46	P value
Age (y)	60.8±7.6	60.7±6.2	60.9±8.8	0.870
Height (cm)	158.3±5.7	158.5±6.0	158.0±5.5	0.722
Weight (kg)	60.8±9.0	60.3±8.8	61.3±9.3	0.607
BMI (kg/m^2)	24.3±3.2	24.0±3.3	24.5±3.1	0.497
WOMAC score	15.5±4.2	15.8±3.5	15.3±5.0	0.568
Lysholm score	79.6±10.8	79.0±9.9	80.2±11.8	0.571

Table 1. Baseline characteristics of the recruited patients. Values are presented as means ± standard deviation. Statistical significance of differences was calculated by Student's T-Test. BMI=body mass index.

Knee joint	Group	0-I	I	I-II	II	II-III	III	P value
Left	Placebo	2 (5%)	5 (11%)	19 (43%)	9 (20%)	9 (20%)	0 (0%)	0.798
	Collagen peptides	4 (8%)	5 (10%)	26 (50%)	9 (17%)	7 (13%)	1 (2%)	
Right	Placebo	1 (2%)	3 (7%)	21 (47%)	8 (18%)	12 (27%)	0 (0%)	0.673
	Collagen peptides	3 (6%)	6 (12%)	25 (48%)	9 (17%)	9 (17%)	0 (0%)	

Table 2. Kellgren-Lawrence score of the recruited patients at baseline. Statistical significance of differences was calculated by Fisher's exact test. Levels of osteoarthritis are defined as 0=None, I=Doubtful, II=Minimal, III=Moderate (22).

	Baseline			6 months		
	Placebo n=48	Collagen peptides n=46	P value	Placebo n=48	Collagen peptides n=46	P value
SGOT (IU/L)	24.0±9.7	22.5±5.8	0.956	22.5±9.7	21.5±8.9	0.592
SGPT (IU/L)	22.8±16.3	22.7±15.2	0.971	24.6±20.3	19.7±9.6	0.465
BUN (mg/dL)	4.72±1.27	4.56±0.92	0.487	4.39±0.99	4.46±0.97	0.724
Serum creatinine (µmol/L)	58.6±10.8	58.7±7.5	0.955	61.8±12.7	60.7±9.0	0.543
Serum uric acid (µmol/L)	304±76	288±65	0.265	311±71	286±65	0.074

Table 3. Parameters of liver and kidney function were measured in blood and urine at baseline and after 6 months of treatment with placebo or collagen peptides. Values are presented as means ± standard deviation. Statistical significance of differences was calculated by Student's T-Test. SGOT=serum glutamic oxaloacetic transaminase; SGPT=serum glutamic pyruvic transaminase; BUN=blood urea nitrogen.

WOMAC subscale	Baseline			6 months		
	Placebo n=48	Collagen peptides n=46	P value	Placebo n=48	Collagen peptides n=46	P value
Pain	3.94±1.42	3.41±1.68	0.105	3.67±1.48	2.33±1.55	<0.001
Stiffness	1.40±1.38	1.27±1.36	0.650	1.29±1.27	0.71±0.87	0.012
Function	10.5±3.3	10.6±3.5	0.833	9.98±3.30	8.24±3.14	0.010

Table 4. Effect of collagen peptide treatment on the WOMAC subscale scores for pain, stiffness and function. Score values at baseline and after 6 months of treatment are presented as mean ± standard deviation. Statistical significance of differences was calculated by ANCOVA. A low WOMAC subscale score indicates a low degree of pain or stiffness, and a lower degree of difficulty in physical function.

REFERENCES

1. United Nations. Population Ageing and Development, 2009: http://www.un.org/esa/population/publications/ageing/ageing2009.htm
2. World Health Organisation. Global Health and Aging, 2011: http://www.who.int/ageing/publications/global_health.pdf
3. World Health Organisation. Chronic rheumatic conditions: http://www.who.int/chp/topics/rheumatic/en/ (last checked on Jan. 8th 2014)
4. Bijlsma J.W., Berenbaum F., et al., Lancet, 377(9783), 2115–2126 (2011).
5. Zhang W., Nuki G., et al., Osteoarthr Cartil, 18(4), 476–499 (2010).
6. Singh G., Wu O., et al., Arthritis Res Ther, 8(5), R153 (2006).
7. Clegg D.O., Reda D.J., et al., N Engl J Med, 354(8), 795-808 (2006).
8. European Food Safety Authority. Opinion of the Food Safety Authority on safety of collagen and a processing method for the production of collagen. EFSA J, 174, 1–9 (2005).
9. Ichikawa S., Morifuji M., et al., Int J Food Sci Nutr, 61(1), 52-60 (2010).
10. Guillerminet F., Beaupied H., et al., Bone, 46(3), 827-834 (2010).
11. Matsumoto H., Ohara H., et al., ITE Letters, 7(4), 386-390 (2006).
12. Van Vijven J.P., Luijsterburg P.A., et al., Osteoarthr Cartil, 20(8), 809-821 (2012).
13. Oesser S., Adam M., et al., J Nutr, 129(10), 1891-1895 (1999).
14. Kawaguchi T., Nanbu P.N., et al., Biol Pharm Bull, 35(3), 422-427 (2012).
15. Oesser S., Seifert J., Cell Tissue Res, 311(3), 393-399 (2003).
16. Schunck M., Schulze C.H., et al., International Cartilage Repair Society, Poster #189 (2007).
17. Ohara H., Iida H., et al., Biosci Biotechnol Biochem, 74(10), 2096-2099 (2010).
18. Clark K.L., Sebastianelli W., et al., Curr Med Res Opin, 24(5), 1485-1496 (2008).
19. Benito-Ruiz P., Camacho-Zambrano M.M., et al., Int J Food Sci Nutr, 60(S2), 99-113 (2009).

20. Trc T., Bohmová J., Int Orthop, 35(3), 341-348 (2011).
21. Bruyère O., Zegels B., et al., Complement Ther Med, 20(3), 124-30 (2012).
22. Kellgren J.H., Lawrence J.S., Ann Rheum Dis, 16(4), 494-501 (1957).
23. Bellamy N., Buchanan W.W., et al., J Rheumatol, 15(12), 1833-1840 (1988).
24. Lysholm J., Gillquist J., Am J Sports Med, 10(3), 150-154 (1982).
25. Ohara H., Ichikawa S., et al., J Dermatol, 37(4), 330-338 (2010).
26. Matsuda N., Koyama Y., et al., J Nutr Sci Vitaminol, 52(3), 211-215 (2006).
27. Nomura Y., Oohashi K., et al., Nutrition, 21(11-12), 1120-1126 (2005).
28. De Almeida-Jackix E., Cuneo F., et al., J Med Food, 13(6), 1385-1390 (2010).
29. McAlindon T.E., Nuite M., et al., Osteoarthr Cartil, 19(4), 399-405 (2011).